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Optimization of Parameters Responsible for the Rate of Gas Generation Through Mixed Anaerobic Digestion

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ABSTRACT

A key source of renewable energy, biogas (methane) was generated in the anaerobic mixed digestion of floral waste along with the combinations of other substrates. The present study has focused on the treatment of floral waste by anaerobic mix digestion along with co wastes named as canteen waste (CW), dairy waste (DW), and yard waste (YW) by using cow dung (CW) and sewage sludge (SS)as an inoculum. The concept of mixed digestion is used in this work by using different substrates with the main substrate as floral waste. The substrates are added with co substrates in a ratio of 2:1. Three types of comparative studies are carried out by making different combinations of substrates by keeping floral waste common in every combination. Different parameters responsible for the quantity of methane gas resulting from anaerobic digestion are optimized using design expert software's response surface methodology (RSM). A specially designed laboratory-scale model is used which is attached with a biogas analyser to continuously measure and analyse the generated biogas. A total of 45 experiments were carried out on the predicted conditions for different combinations. Parameters such as pH, temperature, and food to microorganism ratio have been chosen as independent variables. Daily biogas generation and cumulative biogas generation were recorded. COD removal efficiency recorded after eight days was in the range of 75–85%. Good interactions have been occurred among the independent variables chosen for the generation of biogas. Highest results were observed at optimum conditions (with pH = 7.2, F/M ratio = 2, T = 37°C). The cumulative biogas yield resulting from an experiment was 7.2L/kg VS. The average VS removal of 62–73% and TS removal of 45–55 % were recorded.

Keywords: anaerobic digestion, floral waste, biogas, optimization, RSM.

INTRODUCTION

Solid waste sources include industries, agricultural works, worship places, domestic waste, institutional wastes, etc. This organic waste is rich in carbon content and has major problems with its disposal [1]. India is a religious country and has lots of worship places in the form of temples, churches, gurudwaras, and mosques. Approximately 300 tonnes/day of floral waste is generated [2]. As a symbol of devotion and worship, people approximately offer100 g of flowers per person per day, garlands, coconut, and milk to the deities in the temples. But on the next day, these offerings get converted into huge humps of solid waste. In India, approximately two million tons of floral waste is generated and thrown every day after religious ceremonies, which could be recycled completely. If we consider the large pilgrimage city Haridwar from India, more than 2500 people come to Haridwar during the off-season. But during auspicious peak days and Chardham Yatra this crowd increases terrifically. This count reaches five lakhs per day during particular occasions like Navaratri, Purnamasi, and and Baisakhi and about one crore during Kavan Yatra. One more fact noted is not only in the temple, but people offer flowers to the Ganga river directly, which directly disturbs the river's ecology, creating a threat to living aquatic microorganisms. Studies have shown that 10 tons/day of flowers are disposed of from the temple or to the river. Still, currently, there is no proper strategy for monitoring and increasing responsiveness among the people for the disposal of this waste efficiently. [1]studied 40% of flowers get sold and 40 % remain unsold, which directly goes to garbage. From 40 % of sold flowers, approximately half amount is used for miscellaneous use, including decoration purposes. The other half is used as an offering which another day reaches water bodies. The holy places do not have a suitable disposal method for flower waste.It is dumped randomly in open public areas or thrown into the water bodies, harming the environment and society [3]. The present scenario shows that approximately 40 % of solid waste is directly dumped on the ground, producing harmful leachates that find their way to groundwater during rainfall or deep infiltration. This waste has the high potential to create all types of pollution, i.e., soil, water, and air. Unless given proper treatment, it will affect the environment and directly affect living being's health [4]. The most commonly used flowers for worship include marigolds, rose, hibiscus, jasmine, lotus, astar, and Kanher. Flowers have wide industrial applications and can be converted into useful value-added products, including fertilizers, fuels, colorful dyes, biogas, organic acids, pigments, food products, biosurfactants production, sugar syrups, agarbatties, etc.[1,2]. Rose and marigold flowers are mostly used for offerings as well as decoration purposes. Thus, these flowers are planted every season. This work has focused on the anaerobic digestion of floral waste with other substrates.

Anaerobic digestion AD is a well-known process used to treat organic wastes since ancient times, Which includes stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, respectively [5]. It is a process in which anaerobic bacteria break down organic material, leaving behind valuable byproducts like biogas[2,3,].All operations occur in the absence of oxygen, yielding 70-80% methane, carbon dioxide 20-30 %, and other gases in minimal amounts. The volume of methane produced decides the effectiveness of the digestion process. The potential of methane production can be measured per total solids of the substrate added and for liquid per biomass COD digestion. Biogas is considered one of the chief sources of green energy that has many benefits. It helps in considerable volume reduction and reduction of organic content of the waste by replacing fossil resources, which reduces further environmental pollution [9]. Many researchers have used various substrates from agricultural wastes like Maize straw, Wheat straw, Rice husk, etc., for biogas production [9,13]. Few researchers used water hyacinth, dry leaves, and Prosopis juliflora seeds for biogas production through anaerobic digestion [15]. Recently, many researchers have been using this technology to treat many organic solids such as organic wastes resulting from food industries, animal wastes, industrial wastes, crop residues, etc. [16]. Biomethane potential is different for each substrate. Instead of a single substrate, two substrates can be added to the same digester to enhance the efficiency of the co-digestion process, which promises a superior methane yield and an increased rate of methane production [8,9,17]. Flowers are famous for their fragrance and beauty that contain essential minerals, vitamins, carbohydrates, proteins, lipids, and essential oils, which increases their tremendous potential for reuse and recycling options. By carrying out preliminary studies and experiments, it is clear that floral waste has a high potential to generate biogas[10].Marigold flowers developed 4.36 g/kg of biogas in 5 days[16]. From the literature reviewed, it is observed that significantly less work has been carried out on floral waste. Also, the comparative study by combining floral waste with single, double and triple co substrates and its analysis for the enhancement of biogas is not done up till now. Many worship places run Anna Kshetra, where they offer food to visitors. A lot of food waste is generated from these places. As we have focused on trash generated from worship places, an attempt has been made to select the co substrates generated at these same worship places, including food waste, dairy waste, yard waste, etc. This work aims to optimize results using a RSM's central composite design [19]. pH, temperature, and food to microorganism ratio were chosen as three independent variables. And daily biogas production in L/kg, cumulative biogas generation in L/kg, and percentage of methane content were recorded as the responses. The resulting responses were compared with the experimental results.

MATERIALS AND METHODS

Feed stocks and inoculums collection

To maintain the beauty and holiness of the worship places, care must be taken to treat each and every type of waste generated here. This work has focused on trash generated from these worship places. Also an attempt has been made to select the co substrates generated at these same worship places, including food waste, dairy waste, yard waste, etc. shown in Figure 1.

The primary feedstock called the substrate used for anaerobic digestion in this study is floral waste (FW). Other feed stocks used for mix digestion include canteen waste (CW), yard waste (YW), and dairy waste. Cow dung (CD) and digested sewage from Noble gas exchange, Talegaon, and Pune were used as an inoculum. Floral waste is collected from the different worship places and flower vendors nearby these worship places in Pune city. Canteen waste (CW) is collected from the College of Engineering Pune canteen. Dairy waste (DW) is collected from the Sonali dairy, Balewadi Pune. Inoculums were stored in the refrigerator until used for the experiment purpose. All the collected wastes except DW were sun-dried and then used for experiment purposes.

Preparation of feed mixture

Substrates and co substrates were washed with water for the removal of dirt, and after that, it was kept in water for one hour. Then a slurry is made with tap water by grinding the materials into fine powder form. Three different comparative studies have been carried out (explained in another section). After making a homogeneous paste with water, all the substrates were taken in proper proportion and mixed with inoculums. Other parameters like F/M ratio, pH, and temperature are kept different for each set of experiments. It is done as per the requirement of each group and configuration given by the central composite design of design expert software.

Pre-treatment of feedstock

Treatments given to the feedstock before feeding in the digester are called pre-treatment. Pre-treatment improves the AD process of organic waste enhancing biogas production. The major benefit of pre-treatment is that it breaks the lignin layer, which protects the cellulose and hemicelluloses, making the organic material easily accessible to the microorganisms and thus improving the digestion process. Pre-treatment also helps to decrease cellulose's crystalline nature, which increases its porosity. [20] Studied, using 1.0 g of $NaHCO_3$/g VS has improved the cumulative methane yield by11.2% to 29.7%. [21] reported, alkaline pre-treatment was given to the substrates by using NaOH solution. Initially, trial experiments were conducted to decide the time of pre-treatment. Substrates are soaked in four NaOH solutions having concentrations 1%, 2%, 3%, and 4%,

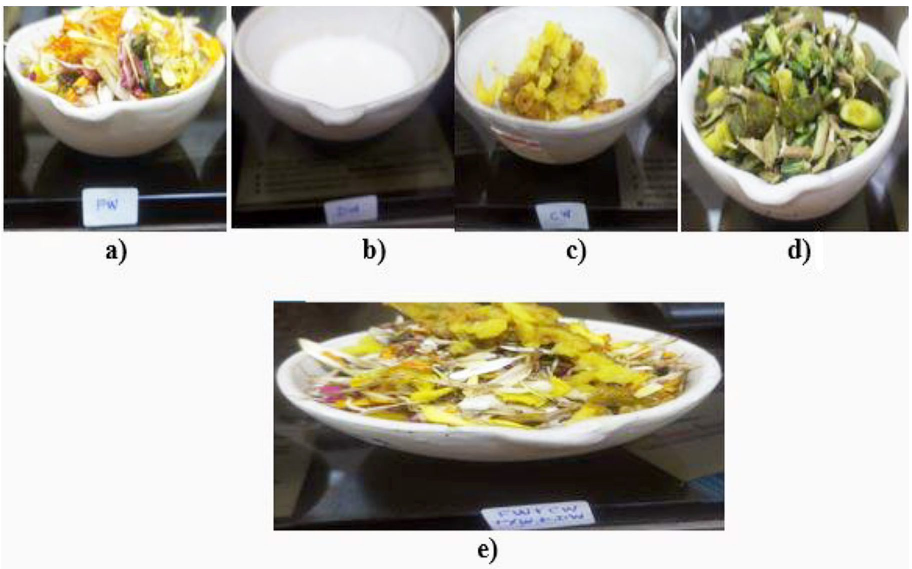

Figure 1. Photographs of substrates used for experimental analysis: a - flower waste b - dairy waste, c- canteen waste, d – Yard waste, e - mixed waste

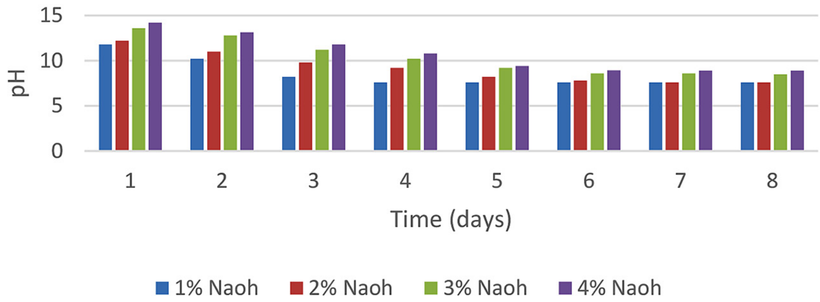

Figure 2. pH variations w.r.t. NaOH doses and duration

and a drop in pH (Fig. 2) is noted. pH decreases in the initial two days and becomes almost constant on the 3rd day. Due to desirable results, selected 1% NaOH pre-treatment for three days.

Experimental setup

A special, unique experimental setup has been customized and assembled for this study.

Figure 3. Photograph of experimental setup for biogas generation through anaerobic digestion

Figure 4. Photographs of components of experimental setup; (1) digesters on shaking platform, (2) gas measurement unit (water displacement technique), (3) gas collection bags, (4) biogas filled bags, (5) biogas analyser

Complete experimental set up is shown in Figure 3. Figure 4 shows the individual components of experimental set up which includes:

1. Digester – it was accompanied by eight bottles of borosil glass with a volume of 1liter, in a series of 2×4.
2. Temperature controller – this unit has a heating capacity of ambient to 60 degree centigrade, and cooling will be below ambient up to 10 degree centigrade. It was operated smoothly by the P I D controller with a timer facility of a minimum of 999 minutes having a countdown facility.
3. Shaker/agitator – the digester was mounted on a continuous vibrating shaker with a linear shaking facility on/off the system. The whole system will be under constant agitation when the batch is on to generate methane.
4. Gas collection and measurement system – the water displacement technique is used for gas collection. Every bottle inside the digester was attached to a one litre fully graduated glass vessel kept inverted in water separately with the connecting silicon tubing of 7 mm dia. Each of these glass vessels had an air-tight rubber cap and one outlet with an on/off valve for the gas to collect in a container.
5. Gas analyser – finally, the outlet of the gas collection system is attached to a gas analyser where analysis of biogas is done for the presence of various gases in different percentages.

The bio methane potential (BMP) tests were performed in a 1.5 L digester with a functional volume of 1000ml, keeping 500ml headspace for gas generation. Degasification is carried out by purging nitrogen gas for 2–3 minutes which ensures the anaerobic condition in the digester. The earlier pH was kept to 6.5–7.0 using 0.1 normal NaOH and HCl solutions. The water displacement technique is used to measure and collect the biogas. Collected biogas was analysed with a gas analyser for the existence of methane, carbon dioxide, and hydrogen sulphide in various percentages. Initial experiments were carried out 37°C [22] for 7 days. A shaking assembly continuously mixed the content of each bottle.

Analytical methods

COD, moisture content (MC), total solids (TS), and volatile solids (VS) of the substrates were measured by using standard methods (APHA 2005) [23]. pH is calculated by using a pH meter. A Scanning Electron Microscope, (SEM) is used to study the surface characteristics such as topography and composition of the substrates [24]. Characteristics of substrates and inoculums are given in Table 1.

Comparative analysis

The quantity of biogas produced was monitored by performing three different comparative studies. In comparison-I, four different sets were run under which substrates were treated individually, combined with 2, 3, and mixed all four, respectively. Comparison I includes 13 experiments. In type II comparison, alkaline pretreatment was given to the substrates which has shown better result in comparison-I by using a 1% NaOH solution for three days. Then it is send to anaerobic digestion. The results of anaerobic digestion of pre-treated and untreated samples are compared. Same combination which has shown maximum result in comparison-I study, again chosen for comparison-III. RSM, Response surface methodology was chosen for the optimization of the selected parameters (details are given in section 2.7). Optimization of design is carried out by central composite design. 20 experimental sets were designed amounting to twenty experimental data points shown in Table 2.

Table 1. Characterization of raw substrates and inoculum

Parameters	Cow dung	FW	CW	DW	YW
pH	8.2	4.81	5.58	7.2	5.1
MC (%)	81.2	83.65	74.47	87.35	71.23
TS (%/TS)	18.8	16.35	25.53	17.37	28.77
VS (%/TS)	12.42	96.32	92.25	97.14	94.53
COD (g/L)	152	72	32	36	10.15
N (%/TS)	03	2.23	2.68	3.82	2.3
C (%/TS)	36	50.93	59.47	23.22	73
C/N	12	22.84	22.19	6.07	31.73

Table 2. Experimental trial runs

No. of run	Parameter 1	Parameter 2	Parameter 3
	A (pH)	B (Temperature in °C)	C (F/M ratio)
1	7.25	18.18	1.75
2	10.5	45	0.5
3	7.25	35	3.85
4	4	45	3
5	7.25	35	0.35
6	4	25	3
7	10.5	45	3
8	10.5	25	0.5
9	7.25	51.82	1.75
10	4	45	0.5
11	12.72	35	1.75
12	4	25	0.5
13	7.25	35	1.75
14	10.5	25	3
15	7.25	35	1.75
16	7.25	35	1.75
17	1.78	35	1.75
18	7.25	35	1.75
19	7.25	35	1.75
20	7.25	35	1.75

Statistical analysis and optimization by response surface methodology (RSM)

Optimization of biogas generation from the mixed digestion process is carried out using Design Expert software (version 13, Stat-Ease, Inc., USA) under different operating conditions. In RSM, collection of mathematical and statistical techniques useful for modelling and analysing problems is carried out by selecting several variables that influence the response of interest. The objective is to optimize this response. Parameters such as (A) – pH, (B) – temperature, and (C) – food to micro-organisms ratio have been chosen as independent variables. Average biogas production (ABP, L/kg/d) is the dependent variable. Twenty experimental sets were performed for three independent variables. Basic information on software is given in Table 3. Minimum to maximum range for the selected independent variables pH, temperature, and food to microorganisms ratio are shown in Tables 4. Following Equation (1) describes a quadratic model mathematically that

Table 3. RSM model information

RSM version	13.0.12.0
Type of study	RS, response surface
Type of design	CCD, central composite design
Design model	Quadratic
Sub type	Randomized
No of run	20

Table 4. Minimum to maximum range of parameters

Factor	Name	Units	Type	Sub-type	Minimum	Maximum	Low coded	High coded	Mean	Std. Deviation
A	pH	-	Numeric	Continuous	1.78	12.72	-1 ↔ 4.00	+1 ↔ 10.50	7.25	2.76
B	Temperature	°C	Numeric	Continuous	18.18	51.82	-1 ↔ 25.00	+1 ↔ 45.00	35.00	8.48
C	F/M ratio		Numeric	Continuous	0.3500	3.85	-1 ↔ 0.50	+1 ↔ 3.00	1.78	0.9959

was used to fit the response (Y) correlating with the variables (A, B, and C).

$$Y=\beta_0+\beta_1A+\beta_2B+\beta_3C+\beta_{11}A^2+\beta_{22}B^2+\beta_{33}C^2+\beta_{12}AB+\beta_{23}BC+\beta_{13}AC \quad (1)$$

where: Y is the response variable (average biogas production per day, L/kg/d, VS added), β0, β1, β2, β3 represent the coefficients of the linear expression, β11, β22, β33 represent quadratic coefficients, β12, β23, β13 represent interaction coefficients. Analysis of variance, ANOVA is performed to analyse the effect of the variables A, B, and C along with their relationship with the response variable, Y. Also, the adequacy of the model is checked through regression coefficients (R^2) and adjusted regression coefficients (R_{adj}^2) which denotes the value of the modelling equation. Fisher test (F-test) established the statistical analysis based on p values having confidence level of 95%.

RESULTS AND DISCUSSION

Comparative study I – biogas generation

It was observed that individually every substrate has the potential to produce an average of 96–250 ml of biogas daily when treated separately. As readily available methanogens were added through inoculum collected from digestate from anaerobic digester, from first day biogas generation was observed. For the initial 3–4 days, maximum gas production was observed, on 2nd or 3rd day, it was at its peak, and after 4–7 days, it slowed down. After 8–10 days, it almost stopped [25]. Set ups were run for 15 days. Cumulative readings of 8 days have been noted for all setups of experiments carried out [22] as, after 8 days it was almost zero gas production was there. The average biogas production of 8 days from floral waste was 184 ml/day. When combined with another substrate, floral waste, the primary substrate being kept common in every combination (FW+

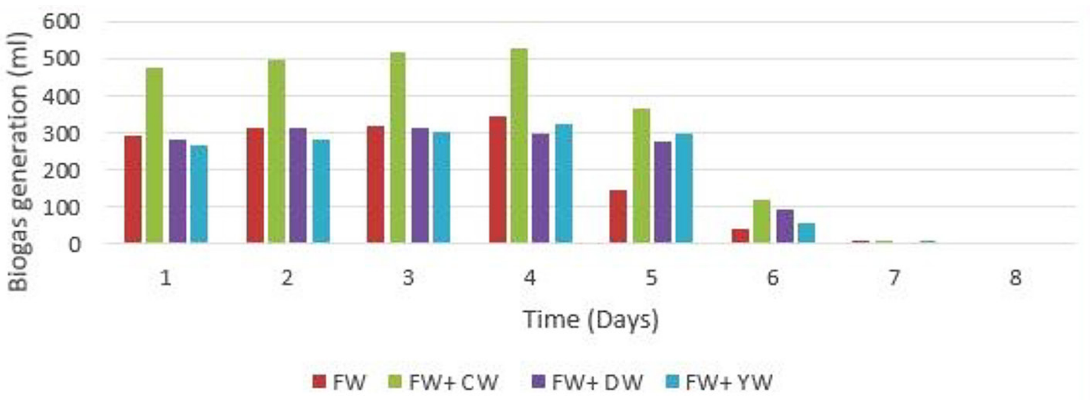

Figure 5. Biogas generation comparison of mono and co-digestion

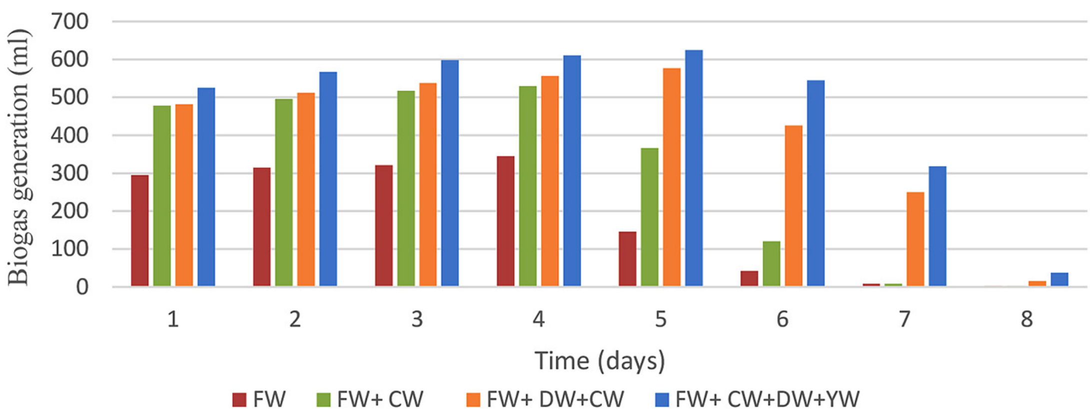

Figure 6. Biogas generation comparison of mono, co, tri and mixed digestion

CW, FW+ DW, FW+ YW), has increased daily average biogas production from 184ml to 314.63 ml, which is 70% more than mono digestion (Fig. 5). Biogas production improved more when FW was combined with the other two substrates instead of single (FW+CW+DW, FW+CW+YW, FW+DW+YW). This combination of 3 substrates has increased average biogas production from 314.63 ml to 419.50 ml, which is 33.33 % more than the combination of two substrates. Results were further enhanced in mixed digestion, where the primary substrate, FW, is mixed with the three other substrates (FW+ CW+DW+YW). The maximum biogas production through mixed digestion was 478.25 ml, which is 14 % more than the combinations of three substrates (Fig. 6). A total of 13 experiments were carried out for the first comparative study. Biogas analysed for its composition showed methane 66–72%, Carbon dioxide 28–34% and traces of hydrogen sulphide.

Comparative study II – pre-treatment

The type I comparison concluded that mixed digestion had better results than the first three sets. The fourth set has been chosen for further Type II comparative study. Here in the Type II comparison, alkaline pre-treatment was given to the substrates by using a 1% NaOH solution for three days. The three days chemical pre-treatments with 1% NaOH was found to be very effective and improved the volume of biogas yield from floral waste mixed with the other three substrates. The same untreated combination showed a biogas yield of 478 ml. At the same time, NaOH pre-treated sample shows an average biogas yield of 642 ml which is 34.20% more than that of untreated waste (Fig. 7). Cumulative biogas production was 5.13 L/kg VS added.

Statistical analysis by response surface methodology

The design of experiments (DOE) was used to optimize parameters responsible for biogas generation, and analysed the effects of the three independent factors on the biogas generation volume. A central composite design (CCD), three factors (three-level-three, n=3) of RSM, response surface methodology was used. A complete run of 20 experimental data points have designed and analysed, shown in Table 5. A considerable increase was observed when the experiments were performed under controlled conditions for various combinations suggested by RSM with an ABG of 1103.25 ml/kg shown in Table 5. A centre run measured evaluation of lack of fit and experimental inaccuracy. An efficient relationship was experienced between the dependent or response variable (Y) and all the independent variables (A, B, and C). The average biogas generation per day (ABG, L/kg/d) was chosen as the output response. A reasonable hypothesis was observed between the independent parameters and their interaction with a 95% confidence level. The data point normality was expressed by plotting the residual normality plots shown in Figure 8. Figure 9 shows the graph of predicted versus actual values of the response.

Interactions amongst pH, temperature F/M ratio and its effect on biogas generation

By keeping one variable constant and varying the other two, three-dimensional surface plots have

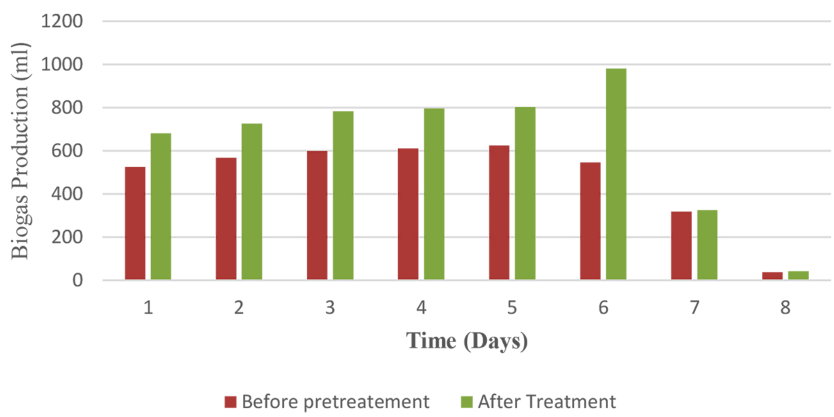

Figure 7. Biogas generation comparison before and after pre-treatment

Table 5. Experimental trial runs with responses

No. of run	Parameter 1	Parameter 2	Parameter 3	Response
	A (pH)	B (temperature in °C)	C (F/M ratio)	ABG (ml/kg)
1	7.25	18.18	1.75	658
2	10.5	45	0.5	887
3	7.25	35	3.85	840
4	4	45	3	435
5	7.25	35	0.35	655
6	4	25	3	160
7	10.5	45	3	1350
8	10.5	25	0.5	425
9	7.25	51.82	1.75	1256
10	4	45	0.5	487
11	12.72	35	1.75	1240
12	4	25	0.5	286
13	7.25	35	1.75	2110
14	10.5	25	3	726
15	7.25	35	1.75	2110
16	7.25	35	1.75	2110
17	1.78	35	1.75	0
18	7.25	35	1.75	2110
19	7.25	35	1.75	2110
20	7.25	35	1.75	2110

been drawn (Figures 10, 11, and 12). Good interaction has been seen between pH, F/M ratio, and temperature. Higher ABG of 2110 ml was recorded at pH=7.25, T = 35, and F/M ratio = 1.75 (Table 6).

An increase in biogas generation has been observed with the increase in pH and temperature up to a certain extent. But after that, it drops the production. The optimum pH for higher gas production volume was 7.25. Ranges of pH from 1.78-to 12.72 were tested, and it was noted that the rate of gas production went on increasing with an increase in pH up to 7.8. Further increased pH dropped the rate of gas production. The reason behind is that lower pH values produce more acidic matter, and volatile fatty acids get deposited in the digester, which lowers the degradation of materials. Increased pH value improves the growth of methanogenic bacteria, thus improving the gas generation rate. Hence, the drop-off in optimal pH value causes a greater adverse impact on ABG than a little higher pH. A proper F/M ratio maintains the balance of substrate (food) and inoculums

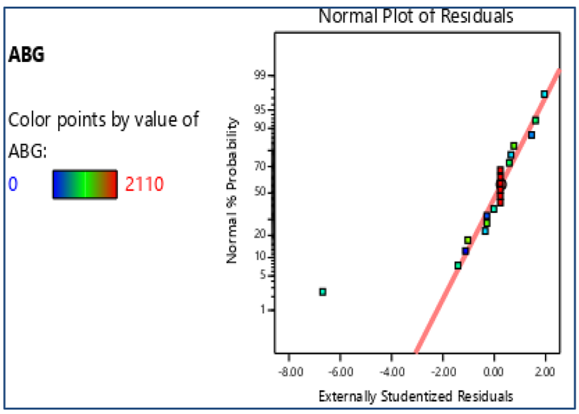

Figure 8. Normality plot of residuals

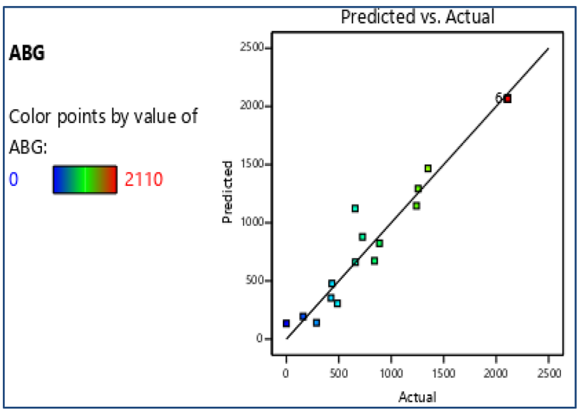

Figure 9. Predicted vs. Actual response

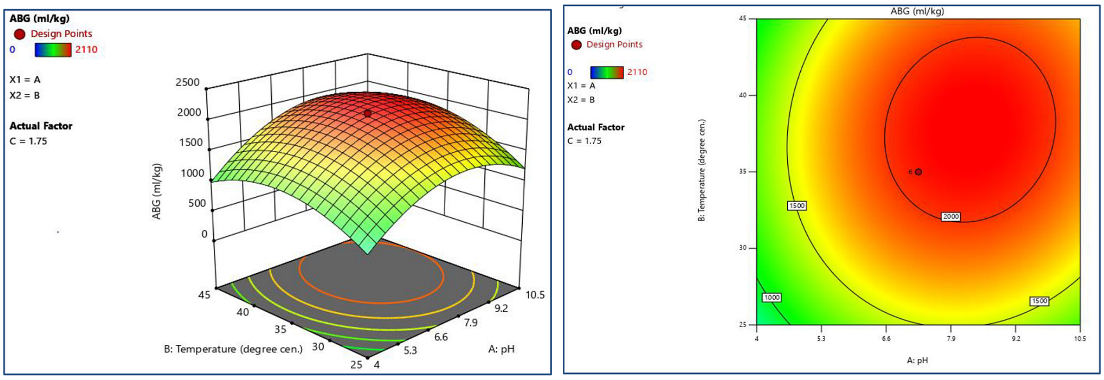

Figure 10. Interaction between pH and temperature

Table 6. Response (ABG) by RSM

Response name	Unit	No. of observations	Minimum response	Maximum response	Mean	Std. Deviation
R1 (ABG)	ml/kg	20.00	0	2110	1103.25	757.52

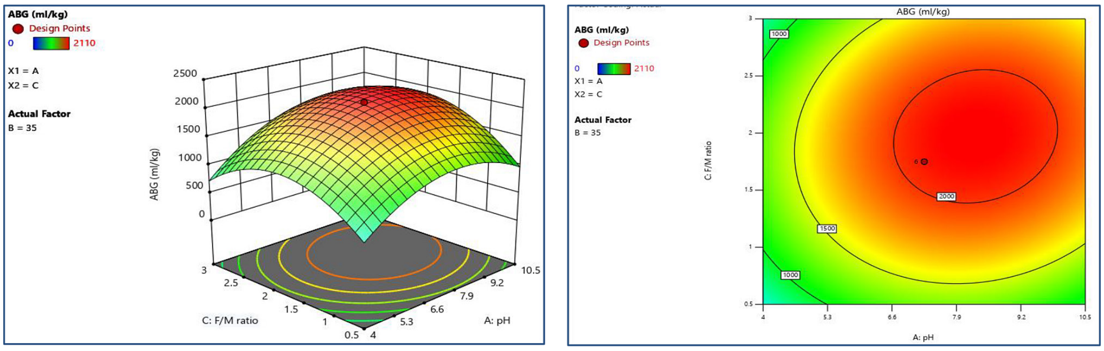

Figure 11. Interaction between pH and F/M ratio

(micro-organisms) in the digester. A small F/M ratio indicates a small amount of micro-organisms results in incomplete digestion of organic material. Whereas a higher ratio indicates, too much organic material for the bacteria to digest also results in the formation of a scum layer in the digester. A ratio of 1.75 was observed as promising, giving the highest methane generation.

The results of this study (maximum biogas generation per day: 2110 mL/g-VS added, having 67–70% methane: 1413–1500 mL CH_4/g-VS added) was compared with the results of co digestion

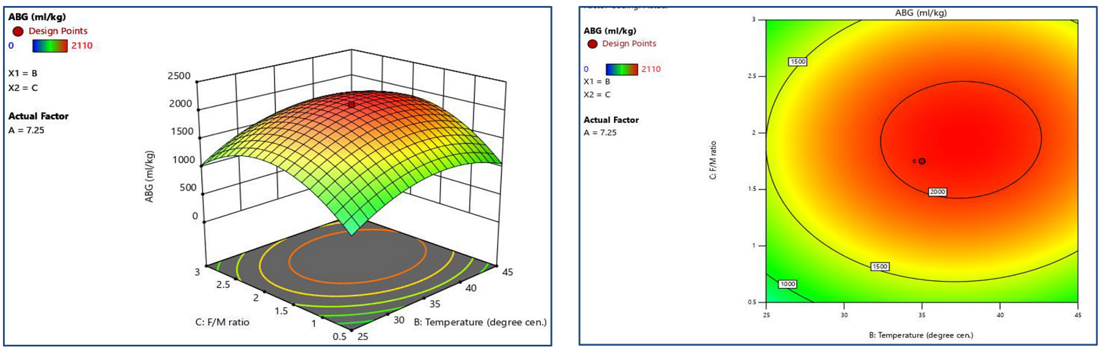

Figure 12. Interaction between temperature and F/M ratio

study of other researchers using different substrates (Table 7) shows that input response (pH, Temperature, and F/M ratio) parameters and co-substrates CW, DW and YW noticeably improved the efficiency of digestion process. The polynomial model for biogas generation was described after applying the DOE analysis to experimental data, given by the following Equation (2)

$$Y = 2065.62 + 300.54A + 188.01B + 173.82C + 76.25 AB + 117.75 AC + 29.50BC - 503.30 A^2 - 384.82B^2 - 597.09C^2 \quad (2)$$

According to Equation (2), the variables A, B, and C had favourable effects due to their positive coefficient (300.54, 188.01, and 173.82 respectively). At the same time, the interaction effects of the three variables had different effects on biogas generation. Multiple linear regressions were used in the quadratic model to compute the regression coefficients and minimize the sum of squares of the process. Anova results showed that the model is significant and can be used to navigate the design domain. In the present study, each parameter was statistically significant as per the p value being $p < 0.0001$[2]. The F-value of Model 30.07 implies the model is significant. P values less than 0.05 indicate model terms are effective (Table 8). Difference between actual and predicted R^2 values is less than 0.2, thus it indicates that predicted R^2 is in reasonable agreement with actual R^2. Adequate precision measures the signal-to-noise ratio, which is expected to be greater than 4. For our model, the ratio was 13.858, which indicates a fair signal. So to navigate the design space, this model can be used. Also, if the R^2 values are close to 1, it shows the model's reasonable adjustment to the experimental data. In this present study, the R^2 value was 0.96, indicating that the model is highly significant.

Volatile solids (VS) and total solid (TS) reduction

Efficiency of digestion process is assessed on the basis of volatile and total solids removal percentage. Significant volatile solids and total solid reduction were recorded. Further, it was more remarkable for mix digested waste than mono treated waste. The average VS removal of 42–70% and TS removal of 32–70% were recorded. The combination of four substrates FW+CW+DW+YW showed the maximum VS (69.71%) and TS (69.99%) removal. TS removal achieved by mix digestion is 5.42% greater than that achieved by two substrates and 39.29% more than that of mono digestion. Whereas VS removal achieved by mix digestion is 10.7% greater than that achieved by two substrates and 28.94% more than that of mono digestion (Table 9). Although there is drastic difference in VS and TS reduction achieved through mix digestion than that of mono digestion, mixing of 3 and 4 substrates showed near about same VS and TS reduction.

Table 7. Response (ABG) by RSM

Primary substrate	Co-substrate	Inoculum used	Methane generation (ml/g–VS added)	Digestion process	Duration of digestion (days)	Reference
Flowers	--	Cow dung	568 ml CH4/g Vs		14 days	[25]
Teff straw		seed sludge	248.8 mL/g TS	Batch process	50 days	[26]
Rice straw	food waste	Cow dung	323.78 mL/g-VS added,	Batch process	35 days	[27]
Tannery solid waste,	sludge	-	274 mL/g-VS added,	Batch process	50 days	[5]
agricultural solid waste	Animal dung	-	386.3 NL/kg VS		30 days	[20]
food waste	waste activated sludge	-	326.3 mL CH4 g−1 VSS	Batch process	20 days	[28]
Fruit waste	Vegetable and yard waste	Buffalo dung	0.56–0.62 L/kg VS added respectively.	Batch process	40 days	[29]
Floral waste	Food waste, dairy waste, yard waste	Sludge from anaerobic digester and cow dung	1413-1500 ml CH4/ g-VS added,	Batch process	14 days	This study

Table 8. ANOVA for quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.051E+07	9	1.168E+06	30.07	< 0.0001	significant
A-pH	1.234E+06	1	1.234E+06	31.77	0.0002	
B-Temperature	4.828E+05	1	4.828E+05	12.42	0.0055	
C-F/M ratio	3.375E+05	1	3.375E+05	8.69	0.0146	
AB	46512.50	1	46512.50	1.20	0.2996	
AC	1.109E+05	1	1.109E+05	2.85	0.1220	
BC	6962.00	1	6962.00	0.1792	0.6810	
A^2	3.692E+06	1	3.692E+06	95.01	< 0.0001	
B^2	2.154E+06	1	2.154E+06	55.42	< 0.0001	
C^2	3.386E+06	1	3.386E+06	87.15	< 0.0001	
Residual	3.885E+05	10	38854.85			
Lack of Fit	3.885E+05	5	77709.69			
Pure Error	0.0000	5	0.0000			
Cor Total	1.090E+07	19				

It is observed that biogas generation increases with the higher reduction of dry matter. COD removal is an indirect measure of bio methane generation. The larger the COD removal, the greater the amount of gas production. COD removal efficiency recorded after eight days was 85–96% for an F/M ratio of 1.75. High removal of COD in this study indicates the high digestion of organic substrate, resulting in the most elevated biogas formation.

SEM and EDX analysis

High-resolution images analysed the surface morphology of all the substrates through scanning electron microscopy, SEM (FE-SEM, model sigma IV). Elemental identification and quantitative composition are made by energy dispersive X-ray analyser, (EDX)[30].

SEM and EDX were employed for both raw and treated waste (biochar). The applied alkaline pre-treatment method shredded the lignocellulose present, breaking down the surface in a significant level to damage the intact substrates' structure [31]. This causes cutting of the biomass in length size, providing a large surface area for microorganisms to feed, and enhancing biogas production. SEM images of biomass shown in Figures 13, 14, 15 and 16 show the morphology of floral and other refuse. Raw floral and other substrates showed a fibrous, plate-like structure with a smooth surface high in organic matter. Detailed structural analysis indicated the presence of pores in between the fibrous structures of the biomass. The morphology of mixed waste biochar after decomposition, and degradation of lingo cellulosic material, showed a uniform particle size of 4–8 mm with porous crystalline structures and rough texture. It is observed that after digestion the structure of digested substrate get degraded into small units. In The EDX analysis

Table 9. Characterization of substrates and inoculums after feeding

Digester No.	Combinations	Before digestion		After digestion			
		% TS	% VS	% TS	% TS reduction	% VS	% VS reduction
1	FW +I	19.40	96.33	13.5	30.42	56.79	41.05
2	FW +CW+I	20.30	92.55	7.25	64.29	37.68	59.29
3	FW +DW+I	21.35	96.60	11.5	46.13	45.29	53.12
4	FW +YW+I	20.63	94.19	14.2	31.18	52.23	44.55
5	FW +CW+DW+I	20.20	92.67	6.25	69.06	28.58	69.16
6	FW+CW+DW+YW+I	23.64	90.81	7.16	69.71	27.25	69.99

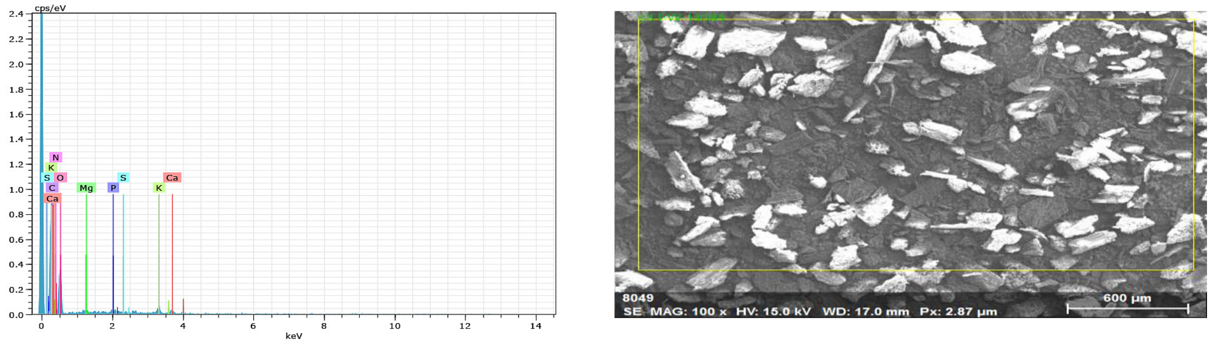

Figure 13. (a) Floral waste EDX analysis (b) Floral waste SEM image

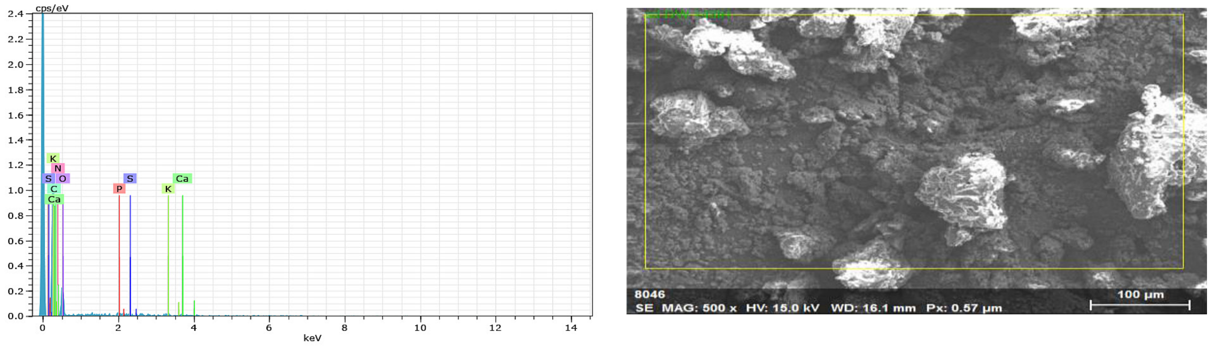

Figure 14. (a) Dairy waste EDX analysis (b) dairy waste SEM image

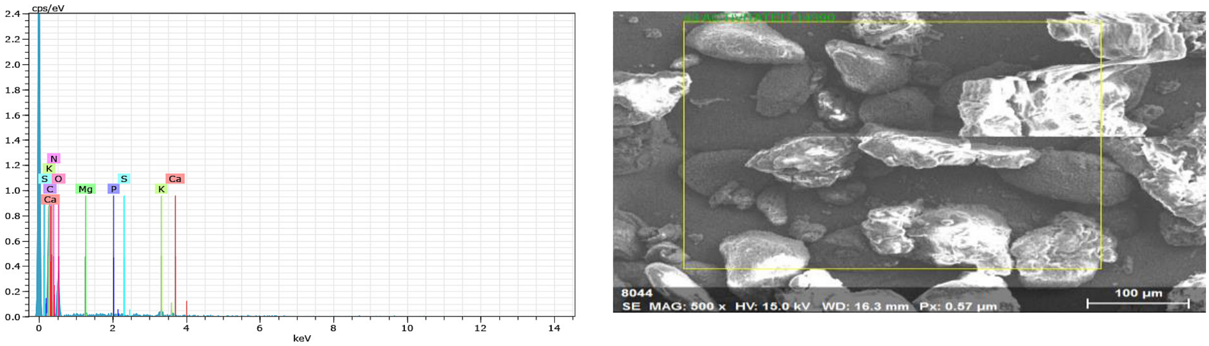

Figure 15. (a) Canteen waste EDX analysis b) Canteen waste SEM image

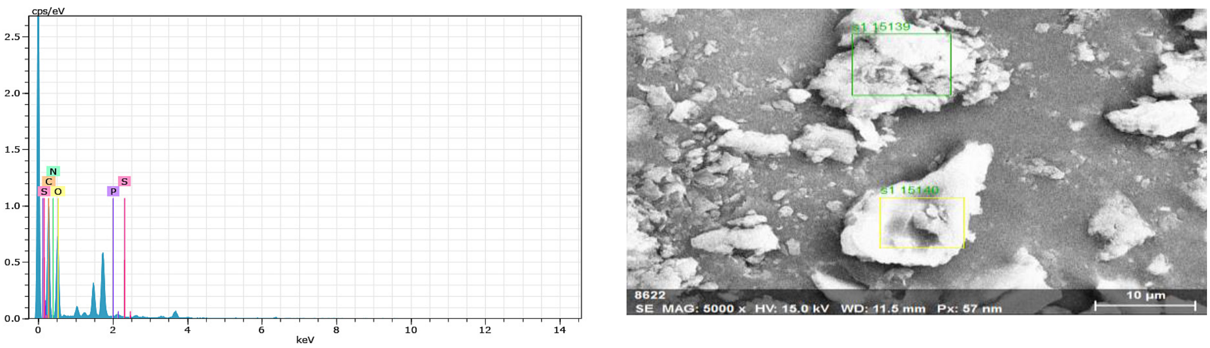

Figure 16. (a) Biochar of mixed waste EDX analysis (b) biochar of mixed waste SEM image

of floral and other refuse and its biochar revealed the presence of various major elemental components like C, O, K, Ca, Mg, P, S, and N in different amounts, which improved the importance of obtained biochar as a high potential material.

CONCLUSIONS

The concept of mixed digestion was adopted for this study and showed the enhanced level of biogas generation. Statistical optimization along with pre-treatment techniques significantly improved biogas production. Gas generation was found to increase by 40% and 53% when treated flower waste with 3 co-substrates compared to mono and co-digestion, respectively. In the second set of comparisons, alkaline pre-treatment was given to the substrates, and the results were compared with untreated waste. The mixed pre-treated wastes produced cumulative biogas of 7.2 l/k, which was 45 % and 40% more than that of untreated mono and untreated co-digestion. Further results were observed to be more improved on optimization of parameters. Optimization study showed significant interaction amongst the controlling parameters, and showed average biogas production per day of, 1103 ml/g, for the optimal condition: pH 7.25, Temperature 35°C and F/M ratio 1.75. The results revealed a generous interaction between the selected independent variables and response biogas yield. Structural composition of substrates improved the biodegradability of the substrate, leading to increased biogas yield from the floral waste. Three days of alkaline pre-treatment was the optimum time for lingo cellulosic compounds of floral waste. It was pre-treated with 1% NaOH for 3 days and produced a total biogas yield of 1498.66 mL/kg with 72% methane content. The result of, reflected in the change in structural composition, improved biodegradability and enhanced biogas yield from floral waste and other substrates. The mixed digestion concept adopted in this study balances the nutrition for the microbial population. It adjusts the reactor's pH by improving the reactor's buffering capacity during the whole process.

This study explores the concept of mixed digestion and its impact on biogas production, focusing on the co-digestion of flower waste with multiple substrates and the effect of pre-treatment techniques. Key highlights include:

- Enhanced biogas generation – mixed digestion with three co-substrates led to a biogas production increase of 40% compared to mono-digestion and 53% compared to standard co-digestion.
- Alkaline pre-treatment – substrates treated with 1% NaOH for three days exhibited an improvement in biogas yield, producing 7.2 L/kg of cumulative biogas, which was 45% and 40% more than untreated mono- and co-digestion, respectively. The pre-treated waste also resulted in 1498.66 mL/kg of biogas with 72% methane content.
- Statistical optimization – optimization of the process parameters (pH 7.25, temperature 35°C, F/M ratio 1.75) yielded an average biogas production of 1103 mL/g/day, demonstrating significant interaction between the selected variables (pH, temperature, and F/M ratio).
- Structural improvement – alkaline pre-treatment improved the biodegradability of floral waste by altering its structural composition, enhancing overall biogas yield.
- Balancing nutrition for microbes – mixed digestion helps balance nutrients, enhance microbial activity, and maintain pH stability throughout the digestion process, leading to optimized biogas generation.

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