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Studies on biogas production by anaerobic process using agroindustrial wastes

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Abstract

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This study investigated the effect of factors namely temperature, pH, substrate concentration on sago and tannery effluents by the anaerobic digestion process for biogas production. Response surface methodology with the Central Composite Design (CCD) experiments verified that the biogas production rates were mainly affected by operating temperature, pH, and substrate concentration. The experiments were carried out by two distinct effluents at different organic loading rate under mesophilic range of temperature 31–33°C. Co-digestion was carried out for a period of 21 days. The gas produced was measured by the liquid displacement system. Meanwhile, the highest biogas yields – 80% of CH_4 and 20% of CO_2 –produced in the combined effluent were confirmed by the Gas Chromatography (GC) analysis.

Keywords: energy recovery; effluent; methane; sago; tannery; effluents

Bioenergy is an important form of renewable energy. Stored in a biological material such as wood, manure, straw and other agricultural products, bioenergy is one of the key options for short and medium term to mitigate Green House Gas (GHG) emissions and replace fossil fuels (Benjamin, Sovacool 2012). It can be used to generate heat, electricity and produce transport fuel (Taherzadeh, Karimi 2008; Singh, Prena 2009; Tricase, Lombardi 2009). Each year, about 590–880 million t of methane are exhausted worldwide into the atmosphere through microbial activity and about 90% get from biogenic sources (Environmental Protection Agency 2010).

The tannery effluent arises from tanneries, which causes toxicity to plants and other forms of biotic and abiotics (Pang et al. 2008; Chandra et al. 2010; Goel et al. 2010). Anaerobic digestion is doubtless the most suitable method for the treatment of high strength effluents. The favors of anaerobic treatment are widely reported by many workers (Demirer et al. 2000; Solera et al. 2002). The Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) contents of many high strength effluents from food, fermentation, beverage, and pulp and paper industries can be successfully diminished by anaerobic digestion. About 80% of tanneries are engaged in chrome tanning process (Hema et al. 2010; Mohan et al. 2010). The potential effluent cause soil and water pollution owing to the discharge of untreated effluent (Belay 2010; Wei 2010).

The effluents from agro-based industries cause environmental pollution when left untreated. These nutrients, said to be rich wastes, become a good source of organic fertilizer if properly treated. India ranks fifth in the total tapioca (*Manihot esculenta* Crantz) production after Brazil, Zaire, Nigeria and Indonesia. In India, tapioca is grown in an area of 3.05 million ha with an annual production of 5.8 million t of fresh tubers. There are about 1,000 sago fac-

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tories in Tamilnadu. In the sago industry, the tapioca tubers are processed into sago and starch. The fibrous residue left out after starch production is called 'thippi' and the effluent coming out from the settling tank are two major wastes from sago industry. On average, 30,000 to 40,000 l of effluent/t of sago processing are released into the nearby rivers, lakes and lands. The untreated effluent causes a serious threat to the environment and affects the life of people around the industrial area (Tapas et al. 1996; Clesceri et al. 1998; Romano, Zhang 2008).

Anaerobic co-digestion is the simultaneous biodegradation of distinct wastes in a reactor to establish positive synergism in the digestion medium (Mata-Alvarez et al. 2000). Merits of co-digestion include: balancing suitable ratio between required nutrients, diluting potential toxic compounds (He et al. 2008) supplying buffering capacity, sharing the equipments, establishing required moisture content and easing the handling of wastes (Mshandeta et al. 2004). In addition, anaerobic co-digestion is advantageous, if the amount of a single waste generated at a particular site is not sufficient to make anaerobic digestion cost-effective (Parawira et al. 2004). There are many studies in literature regarding the anaerobic co-digestion of various wastes which covers: food industry wastes (Carucci et al. 2005), animal manure (Gungor-Demirci, Demkrer 2004; Murto et al. 2004), municipal solid waste (Umetsu et al. 2006), wastewater sludge (Hartmann, Ahring 2005), fish wastes (Zupancic et al. 2007) and algal sludge (Yuan et al. 2011).

Response Surface Methodology (RSM) is a collection of mathematical and statistical techniques for modeling analysis in which a response of interest is influenced by several variables and the objective is to optimize this response (Montgomery et al. 2001). The objective of this study was to investigate the effect of temperature, pH and effluent concentration for an anaerobic digestion process to produce biogas in a batch scale and then optimize the biogas production process using response surface methodology with a central composite design.

MATERIAL AND METHODS

Sample collection and analytical methods. Sago effluent was collected from sago industry at the Namakkal district in Tamilnadu, India and likewise tannery effluent was collected at the Ranipet district in Tamilnadu, India. Sago and tannery effluent pH measurement was monitored using a glass electrode pH meter. Following factors were determined: total solids (TS) and volatile solids (VS), total suspended solids (TSS) and total dissolved solids (TDS), COD, BOD at 27°C for 3 days, free ammonia as NH_3, total organic carbon (TOC), total Kjeldahl nitrogen (TKN), sulphate as SO_4, sulphide as S, chloride as Cl, total alkalinity as $CaCO_3$, oil and grease, according to Clesceri et al. (1998). The liquid displacement method was used to collect and measure the biogas produced. The biogas was determined by a Gas Chromatograph GEOL GC mate (Hewlett Packard, USA).

Sludge collection and their activity. The digested sludge was collected from a primary anaerobic digester at Sewage Treatment Plant at Chennai, India. The sludge had methanogenic activity which is discussed later.

In this test, activity was not determined directly as the substrate utilization rate; rather, the methane production rate was noted. The higher the methane production rate, the higher the activity. Sludge sample of 2.08 g VS was placed in a serum flask of 500 ml (130 ml of sludge) with water (270 ml, preferably saturated with nitrogen) added to a level of 3 cm from the top of the flask. Then 5 ml of the stock solution of acetic acid was added. The rubber stop was placed and the flask was connected to the liquid displacement system. The serum flask for the blank (containing only water, in the same volume of the liquid in the serum flask containing the sludge sample) was also connected to a liquid displacement system. The volume of the 1.5% NaOH salt solution in the liquid displacement system of the blank corresponded to the volume of the liquid displacement system, which is connected with the serum flask that contains the sample Fig. 1.

The first reading of gas production was performed after one day (overnight incubation). This reading is the 'zero reading'. The volume of displaced 1.5% NaOH salt solution is not only the result of gas production but also of the realization of equilibrium between liquid displacement system and ambient pressure. Thus, the amount of liquid produced in the zero reading is not included in the calculation of the methanogenic activity. After the zero reading, reading was performed three times a day. Before every reading, the sludge flask was mixed thoroughly. The liquid displaced by the blank was measured for every reading.

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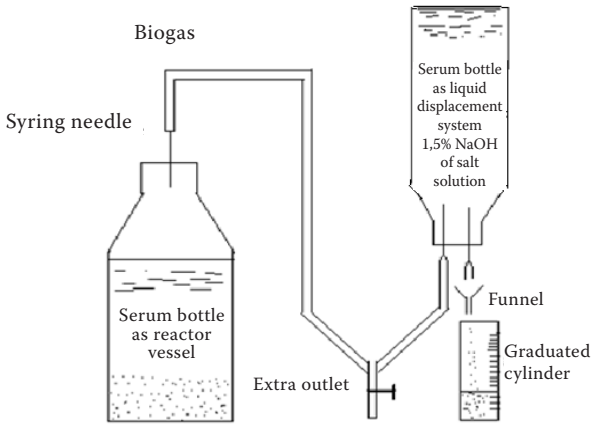

Fig. 1. Schematic diagram for methanogenic activity test and reactor setup

Methane production sludge = displaced liquid by sample – displaced liquid by blank. After every reading the accumulated methane production was calculated. The batch reactor was kept under anaerobic condition at ambient temperature (28–38°C) for a period of time to produce methane from the sludge along with acetic acid used as a substrate. At the first feeding (5 ml acetic acid) the total produced methane was 250 ml. The generated methane gas after the second feeding (5 ml acetic acid) was found to be 350 ml. The experiments were continued until the gas generation flow rate decreased remarkably. Totally, 600 ml of methane was produced in both feed 1 and 2 as shown in Fig. 2. After ceasing the reading the exact sludge amount in the serum flask was determined by measuring the TS and VS content of the sludge. A graph is to be prepared with *X*-axis for a time and *Y*-axis the cumulative gas production.

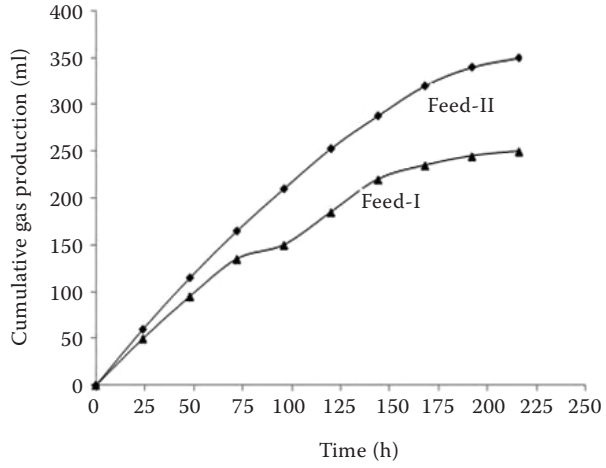

Fig. 2. Methane production during methanogenic activity: Feed-I (250 ml) and Feed-II (350 ml)

Experimental Setup. For the experimental design a Central Composite Design was used for Response Surface Methodology. The batch tests were carried out in 500 ml serum bottles. In all three batch reactors (R1, R2, and R3) equal quantity of sludge (130 of sludge with 100 ml of water saturated with nitrogen) was added. The effluent (substrate) was added into the reactor. The concentrations of effluent were changed reactor to reactor, which is tabulated in Table 1. Then the bottles were tightly closed with rubber septa. The bottles were kept at mesophilic temperature. The hydraulic retention time (HRT) of biogas fermentation was around 21 days.

Optimization by central composite design. The factors influencing the Biogas production, COD, BOD and TOC were optimized using the Response Surface Methodology (RSM) and the important class of second order design called Central Composite Design (CCD) was studied. Optimization studies were carried out by considering the effect of three variables such as temperature, substrate concentration and pH. A full factorial CCD leading to 20 runs of experiments was conducted to determine the effect of these parameters. The independent variables chosen in this study were coded according to the Eq. (1) as follows:

$$x_i = \left[\frac{X_i - X_0}{\Delta X}\right] \quad (1)$$

where:

x_i – dimensionless coded value of the i^{th} independent variable

X_0 – value of X_i at the center point

ΔX – step change value

The behavior of the system is explained by the following second-order polynomial model (Eq. 2):

$$Y = [b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{11}x_1^2 + b_{22}x_2^2 + + b_{33}x^2_3 + b_{12}x_1x_2 + b_{23}x_2x_3 + b_{31}x_3x_1] \quad (2)$$

where:

Y – predicted response (biogas production, COD, BOD and TOC)

x_1, x_2, x_3 – code forms of the input variables such as pH, temperature and substrate concentration, respectively

b_0 – constant

b_1, b_2, b_3 – linear coefficients

b_{11}, b_{22}, b_{33} – quadratic coefficients

$b_{12,} b_{23}, b_{31}$ – cross-product coefficient

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Table 1. Sample within batch reactor at various concentration

Sample No.	Anaerobic batch reactor (500 ml) serum flask	Effluent (ml)	
		Tannery	Sago
1	Reactor 1 (130 ml of sludge (2.03 VS) + 100 ml H_20 (N saturated)	75	–
2	Reactor 2 (130 ml of sludge (2.03 VS) + 100 ml of H_2O (N saturated)	–	75
3	Reactor 3 (130 ml of sludge (2.03 VS) + 100 ml of H_2O (N saturated)	75	75

A statistical program package (Minitab-15, USA) was used for regression analysis of the data obtained and to estimate the coefficients of the regression equations. Analysis of Variance (ANOVA) was applied for graphical analysis of the data in order to obtain interaction of process variables with the response. The quality of fit of polynomial model equations was expressed by the coefficient of determination R^2.

RESULTS AND DISCUSSION

Optimization studies and experimental design analysis

The batch runs were performed with the experiments designed through CCD to visualize the effect of individual factors on the responses.

ANOVA results of these quadratic models are represented in Tables 2–5 indicating that these quadratic models can be used to navigate the design space.

The ANOVA (Table 2) gives a linear and square term in the second order polynomial model as significant ($P < 0.05$) and adequate to represent the relationship between biogas production (ml) and substrate concentration, temperature and pH. The R^2 value 0.955 for biogas production, points to the accuracy of the model. The R^2 value provides a measure of how much variability in the observed response values can be explained by the experimental factors and their selected parameter interactions. The R^2 value should be between 0 and 1. The closer is the R^2 value to 1.00, the stronger is the model and the better it predicts the response. The model *F*-value of 1.69 for biogas production implied that the chosen model is highly significant. Values of Predicted > *F* less than 0.05 indicated that the model terms are significant.

The ANOVA (Table 3) gives a linear and square terms in the second order polynomial model as highly significant ($P < 0.05$) and adequate to represent the relationship between COD and substrate concentration, temperature and pH. The R^2 value 0.988 for the reduction of COD on biogas production, point to the accuracy of the model. The measure of R^2 value provides the accuracy of the selected parameters on the chosen model. The R^2 value always lies between 0 and 1. The closer is the R^2 value to 1.0, the stronger is the model for the predicted response of COD reduction. The model *F*-value of 0.99 for COD implies that the model is significant. Values of Predicted > *F* less than 0.05 indicated that the model terms are significant.

The ANOVA (Table 4) gives a linear and square terms in the second order polynomial model highly as significant ($P < 0.05$) and adequate to represent the relationship between Biochemical Oxygen Demand (BOD mg/l) and substrate concentration, temperature and pH. The regression coefficient R^2 value was found to be 0.93 BOD reductions on biogas production. The point to the accuracy of the model was selected based on the regression coefficient value. The R^2 value provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. The R^2 value is always between 0 and 1. The closer is the R^2 value to 1.00, the stronger is the model and the better it predicts the response. The model *F*-value of 3.60 for BOD implied that the model is significant. Values of Predicted > *F* less than 0.05 indicated that the model terms are significant.

The ANOVA (Table 5) gives a linear and square terms in the second order polynomial model highly significant ($P < 0.05$) and adequate to represent the relationship between Total Organic Carbon (TOC mg/l) and substrate concentration, temperature and pH. The R^2 value 0.99 for TOC reduction on biogas production, points to the accuracy of the model. The R^2 value provides a measure of how much variability in the observed response values can be explained by the experimental factors and their interactions. The R^2 value is always between 0 and 1. The closer is the R^2 value to 1.0, the stronger is the model which predicts the response of TOC reduction. The model *F*-value of 192.64 for TOC

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Table 2. Analysis of variance for biogas production (mg/l)

Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Regression	9	18,045.2	18,045.2	2,004.7	1.87	0.040
Linear	3	5,287.4	5287.4	1,765.9	1.72	0.049
Square	3	12,361.3	12,361.3	4,124.4	3.98	0.038
Interaction	3	373.4	373.4	124.5	0.12	0.945
Residual Error	10	10,265.7	10,265.7	1,026.6	–	–
Lack-of-Fit	5	6,470.1	6,470.1	1,290.3	1.69	0.158
Pure Error	5	3,793.3	3,793.3	758.7	–	–
Total	19	28,272.3	–	–	–	–

DF – difference; Seq SS – sequential sum of squares; Adj SS – adjacent sum of squares; Adj MS – adjacent mean square; *F* – factorial; *P* – predictor

Table 3. Analysis of variance for chemical oxygen demand (COD, mg/l)

Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Regression	9	2,733.81	2,733.81	320.83	2.31	0.043
Linear	3	590.83	590.83	199.24	1.49	0.050
Square	3	2,214.30	2,214.30	738.10	5.52	0.017
Interaction	3	32.50	32.50	10.83	0.08	0.969
Residual Error	10	1,336.02	1,336.02	133.60	–	–
Lack-of-Fit	5	665.19	665.19	133.04	0.99	0.504
Pure Error	5	670.83	670.83	134.17	–	–
Total	19	4,078.52	–	–	–	–

for abbreviations see Table 2

Table 4. Analysis of variance for biochemical oxygen demand (BOD, mg/l)

Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Regression	9	14,114.1	14,114.1	1,585.1	1.75	0.047
Linear	3	4789.5	4789.5	1,612.5	1.78	0.144
Square	3	8,649.7	8,649.7	2,883.2	3.39	0.050
Interaction	3	843.4	843.4	281.1	0.33	0.803
Residual Error	10	8475.1	8,475.1	851.2	–	–
Lack-of-Fit	5	6,651.1	6,651.1	1,330.2	3.60	0.093
Pure Error	5	1,846.0	1,846.0	369.2	–	–

for abbreviations see Table 2

Table 5. Analysis of variance for total organic carbon (TOC, mg/l)

Source	DF	Seq SS	Adj SS	Adj MS	*F*	*P*
Regression	9	257.468	257.468	28.6075	6.93	0.003
Linear	3	34.944	34.944	11.6479	2.82	0.093
Square	3	215.839	215.839	71.9464	17.42	0.001
Interaction	3	6.685	6.685	2.2283	0.54	0.666
Residual Error	10	41.310	41.310	4.1310	–	–
Lack-of-Fit	5	41.096	41.096	8.2193	192.64	–
Pure Error	5	0.213	0.213	0.0427	–	0.001

for abbreviations see Table 2

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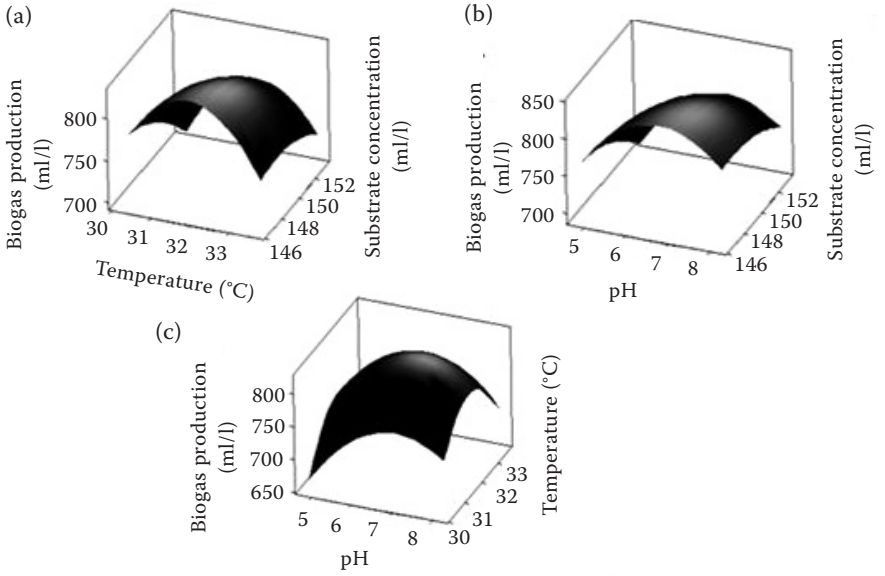

Fig. 3. Surface plot of biogas production vs (a) substrate and temperature, (b) substrate and pH and (c) pH and temperature

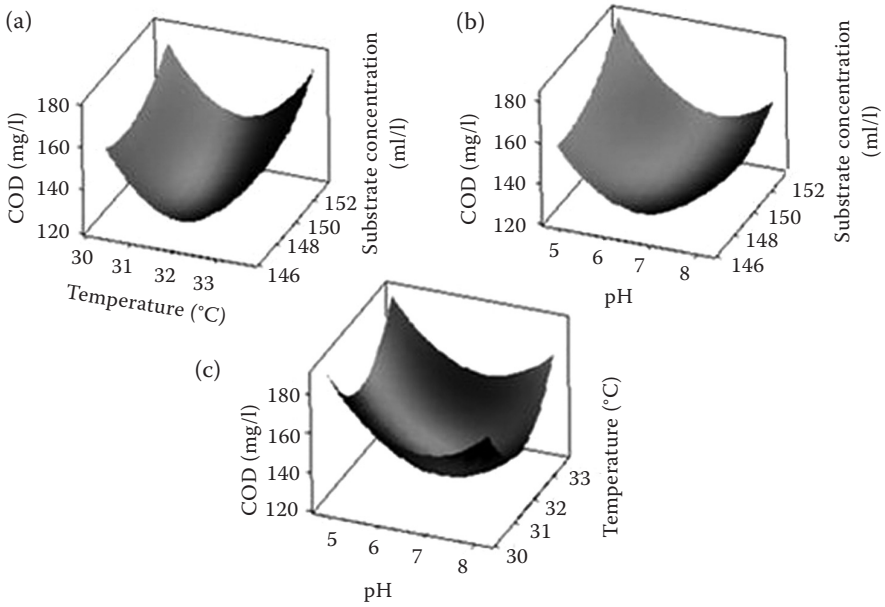

Fig. 4. Surface plot of chemical oxygen demand (COD) vs (a) substrate concentration and temperature, (b) substrate concentration and pH and (c) temperature and pH

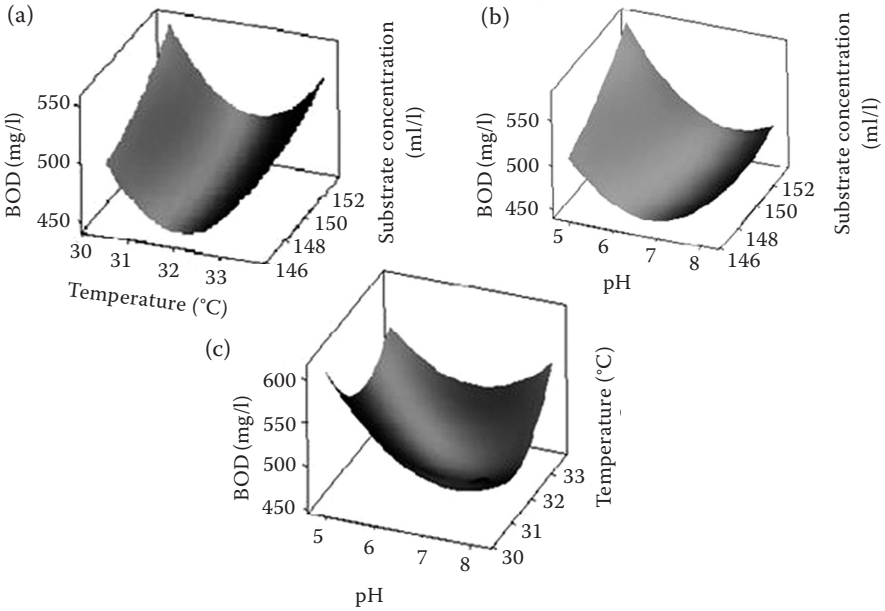

Fig. 5. Surface plot of biochemical oxygen demand (BOD) vs (a) substrate concentration and temperature, (b) pH and substrate concentration and (c) temperature and pH

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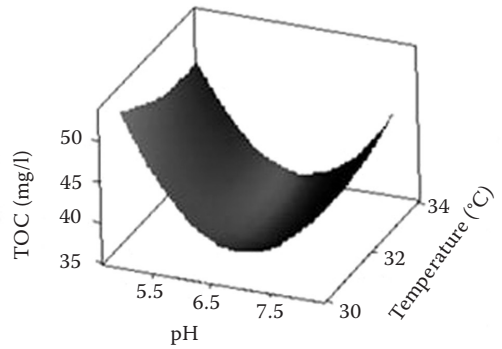

Fig. 6. Surface plot of total organic carbon vs pH and temperature

implied that the model is highly significant. Values of Predicted > *F* less than 0.05 indicated that the selected model terms are significant.

Surface plot

The surface plots of biogas production, COD, BOD and TOC are shown in Figs 3–6, respectively. From these plots, it was inferred that the max. level of biogas, 840 ml and the max. reduction of COD, BOD and TOC, 118, 438, and 36.2 mg/l, respectively, were obtained at pH 6.5, temperature 32°C and substrate concentration 150 ml/l.

In Fig. 3a at low and high temperature, the effluent produced lower amount of biogas but the max. amount of biogas was produced at mid value of temperature (32°C), Similarly, at low and high substrate concentration very low amount of biogas was produced. At the mid value of substrate concentration (150 ml/l) produced maximum amount of biogas 840 ml. Fig. 3b showed that the lower amount of biogas was produced at low and high value of pH. The max. amount of biogas (840 ml) was produced at the mid value of pH (6.5). Similarly, the max. substrate was utilized effectively at the mid value of 150 ml/l of concentration. Fig. 3c shows that at low and high value of pH and temperature lower amount of biogas was produced but at mid value of pH (6.5) and temperature (32°C) max.amount of biogas yield (840 ml) was obtained.

In Fig. 4a at low and high value of temperature, the reduction of COD was very low. The max. amount of COD (118 mg/l) was reduced at the mid value of temperature (32°C). Similarly, at low and high substrate concentration the amount of produced biogas was very low, hence the reduction of COD was very low as well. The max. production of biogas was obtained at mid value of substrate concentration. Hence, the high COD reduction was achieved at 150 ml/l concentration. As Fig. 4b shows, at low and high value of pH, the COD reduction was very low. The max. amount of COD reduction was achieved at the mid value of pH (6.5). Similarly, at the mid value of substrate concentration, the digestion was good and hence, the max. COD reduction was observed. Fig. 4c shows that at low and high values of pH and temperature, the reduction of COD was lower. The highest COD reduction was achieved at the mid value of pH and temperature of 6.5, 32°C, respectively,

In Fig. 5a at low and high value of temperature, the reduction of BOD was very low. The max. amount of BOD (438 mg/l) was reduced at the mid value of the temperature (32°C). Similarly, at low and high substrate concentration the produced biogas was very low and hence the reduction of BOD was very low as well. The max. production of biogas (840 ml) was obtained at mid value of substrate concentration. Hence, the high BOD (438 mg/l) reduction was achieved at 150 ml/l substrate concentration. Fig. 5b shows that at low and high value of pH, the BOD reduction was very low. The max. amount of BOD reduction was attained at the mid value of pH (6.5). Similarly, at the mid value of substrate concentration, the digestion was good and hence, the max. BOD reduction was observed. Fig. 5c shows that at low and high value of pH and temperature, the reduction of BOD was lower. The highest BOD reduction was attained at the mid value of pH and temperature of 6.5, 32°C, respectively.

Fig. 6 shows that at low and high value of pH and temperature, the reduction of TOC was lower. The highest TOC (36.2 mg/l) reduction was attained at the mid value of pH and temperature, which is 6.5 and 32°C, respectively. In this condition, the substrate was utilized effectively and biogas yield was very high.

Likewise, we have given a comparative plot for each parameter such as biogas production, BOD, COD and TOC and an error bar for each parameter is drawn. The bars are shown in Fig 7.

Gas chromatography

Biogas was produced from the anaerobic co-digestion of the effluent. It was measured in liquid displacement system. Volume of biogas was measured by volume of water displaced in gradu-

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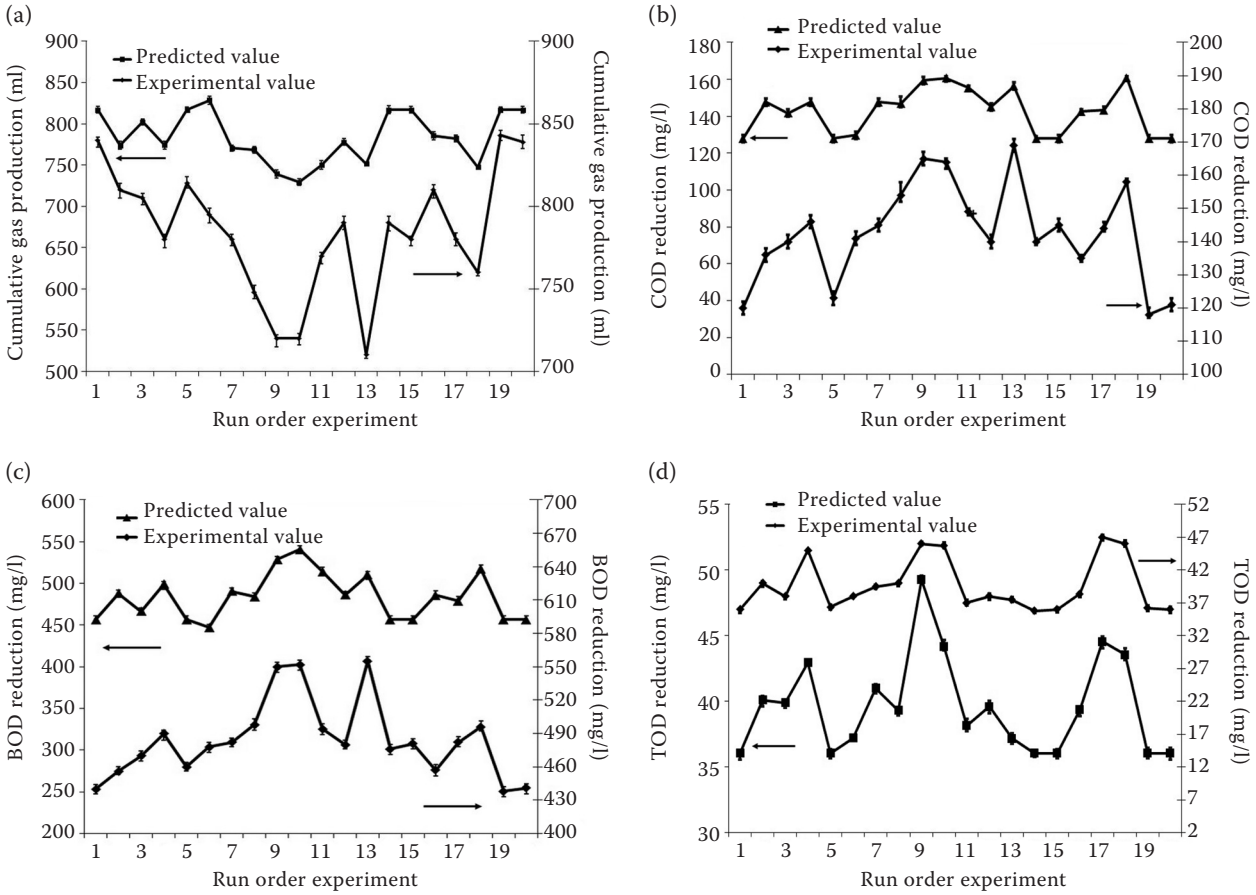

Fig. 7. Expected and predicted value of kinetic parameters of (a) biogas production, (b) COD reduction, (c) BOD reduction and (d) TOC reduction

Table 6. Composition of biogas for the sago with tannery effluents as substrate

Sample No.	Retention time (min)	Molecule	Composition (%)
1	0.02	CO_2	20
2	1.3	CH_4	80

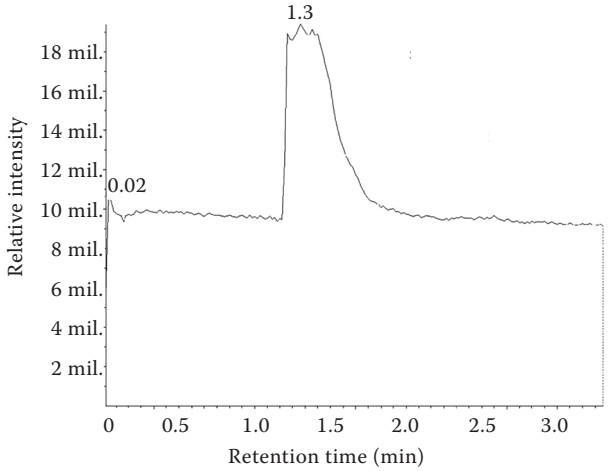

Fig. 8. Gas chromatogram of biogas produced from the sago on tannery effluent as the substrate (mil. – millions)

ated measuring jar. The JEOL GC mate instrument (Hewlett Packard, USA) was used for the analysis of bugs. JEOL GC mate instrument parameters were: injection temperature 220°C, temperature range 40–100°C, rate of temperature 2°C /min and helium gas was used as carrier gas. The column of JEOL GC mate HP5 (Hewlett-Packard, USA) was used. The biogas analysis was done at IIT, Madras, India. The gas composition was CH_4 80% and CO_2 20% as shown in Table 6 and Fig 8.

CONCLUSION

Effect of temperature, pH and substrate concentrations on fermentative biogas production by co-

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digested sample was studied in batch experiments and the optimization of fermentative biogas production process was conducted by response surface methodology with a central composite design. The following conclusions could be written.

The RSM was used to evaluate the effect of temperature, pH, substrate concentration and hydraulic retention time on sago with tannery effluent in addition to obtain the corresponding optimum condition. From these surface plots, it was inferred that the max. level of biogas, 840 ml and the max. reduction of COD, BOD and TOC, 118, 438, and 36.2 mg/l respectively, were obtained at pH 6.5, temperature 32°C and substrate concentration 150 ml/l. The findings show that 36 mg/l TOC was nearby to the predicted value (36.0435) under optimum condition at 32°C with 150 ml/l of substrate concentration and hydraulic retention time of 21 days, the biogas produced somewhat higher in experimental value (840 ml) considered with the predicted value (807.046 ml). The experiment confirmed that the optimum biogas produced was close to the value estimated by RSM analysis. The experimental biogas production values close to equal to the predicted values were obtained through the RSM, under the CCD. Hence, the selected model was the best model and the final deduced equation can be used for the evaluation of biogas production under any experimental conditions. It can be concluded that RSM is useful for the prediction of biogas production level of industrial effluent through anaerobic digestion. Therefore, co-digested effluent could be a potential to enhance anaerobic digestion and enrich biogas yield.

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