



# Response surface modeling and optimization of upflow anaerobic sludge blanket reactor process parameters for the treatment of bagasse based pulp and paper industry wastewater

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#### ABSTRACT

The interactive effects of influent chemical oxygen demand (COD<sub>in</sub>), hydraulic retention time (HRT), and temperature on the performance of an upflow anaerobic sludge blanket reactor, operated in continuous mode, were studied for the anaerobic biodegradation of bagasse effluent from pulp and paper industry. Experiments were conducted based on Box–Behnken design and analyzed using response surface methodology. COD<sub>in</sub> (4,400–6,800 mg/l), HRT (15–27 h), and temperature (20–40 °C) were the operating variables considered for this study. Three dependent parameters viz., percentage of COD removal, COD removal rate, and biogas production were either directly measured or calculated as response. Analysis of variance showed a high coefficient of determination value ( $R^2$ ) of 0.9990 for percentage COD removal, 0.9960 for COD removal rate, and 0.9953 for biogas production thus ensuring a satisfactory fit of the second-order polynomial regression model with the experimental data. Maximum values of percentage COD removal (84.3%), COD removal rate (230.9 mg/lh), and biogas production (21.2 l/d) were observed at optimum COD<sub>in</sub>, HRT, and temperature of 6212 mg/l, 23 h, and 35 °C, respectively.

*Keywords:* Bagasse effluent; UASB reactor; Response surface methodology; Optimization; Percentage COD removal; Biogas

# 1. Introduction

Of the chemical process industries, pulp and paper industry is the one that requires a large quantity of water and entails highly polluting processes. Most of the pulp and paper industries use wood as the major raw material [1] and bagasse as an alternative potential raw material for the production of paper because of the limited availability of wood. Bagasse is the fibrous mass remaining after the extraction of juice from sugarcane and is a byproduct from the sugar industry. The government of India encourages the use of bagasse as the raw material for paper to minimize deforestation.

Besides few advantages in using bagasse for making pulp, there are some practical difficulties in the form of collection and storage of bagasse because of its seasonal availability. To preserve the bagasse

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quality, it is stored under wet condition by spraying water, which consumes huge volume of water and generates an effluent, so-called storage effluent [2,3]. Several washing steps are carried out before pulping, which generates washing effluent. The black liquor the effluent from pulping section—containing a large amount of cooking chemicals (sodium hydroxide and sodium sulfate)—is sent to chemical recovery plant where chemicals are recovered and reused. Before papermaking, the pulp is bleached due to which an effluent containing adsorbable organic halogens and inorganic chloride compounds is released. The wastewater from papermaking section contains particulates, organic compounds, inorganic dyes, etc.

The effluents from bagasse storage and washing sections have similar characteristics and are mixed together to form a waste stream called bagasse effluent. Compared with the wastewater produced from other sections, the bagasse effluent has high chemical oxygen demand (COD), for which the biological treatment (anaerobic) methods will be more suitable [4]. Activated sludge process, aerated lagoon, anaerobic lagoon, stabilization ponds, etc., are some of the conventional biological treatment systems currently used in the pulp and paper industry for reducing the COD and biochemical oxygen demand (BOD) of the effluent. In recent years, the anaerobic process, especially carried out in the upflow anaerobic sludge blanket (UASB) reactor, is being adopted to treat the wastewater having relatively higher organic pollutants due to its demonstrable performance and cost saving advantage. UASB system has been successfully applied to treat wastewater from vegetable processing industry, distillery, petrochemical industry, etc., [5-8]. Various treatment methods have been used recently for the treatment of bagasse effluent. Chinnaraj and Venkoba Rao [9] conducted experiments for the anaerobic treatment of bagasse based pulp and paper industry effluent and reported that the overall COD removal was 80-85%. In their study, 520 l of biogas production per kg COD removed was achieved with 5.75 kg COD  $m^{-3}$  d<sup>-1</sup> of organic loading rate and 20 h HRT. Srinivasan and Murthy [10] studied the treatment of bagasse based pulp mill effluent using a white rot fungus and reported that the maximum color removal efficiency of 82.5% was obtained with an optimal glucose and ammonium chloride concentrations of 15 and 0.5 g/l, respectively, at a pH of 4.5. Sharari et al. [11] investigated the treatment of bagasse effluent using a white rot fungus (Phanerochaete chrysosporium) and found that 98.7% of BOD, 98.5% of COD, and 111 mg/l Pt-Co of color removal was achieved with an optimum temperature of 35°C, biomass concentration of 552 mg/l, and pH of 6. From the literature, it

was found that most of the researchers successfully used white rot fungus for the treatment of bagasse based pulp and paper industries effluent. However, very limited work has been carried out for the treatment of bagasse effluent using UASB reactor [9] and none of the authors developed a model to predict the percentage COD removal, COD removal rate, and biogas production. Response surface methodology—a collection of mathematical (statistical) techniques-is useful for developing, optimizing and understanding the performance of complex systems [12]. This technique conforms closely to practical results compared with theoretical models as it arises from experimental methodology which includes interactive effects of the variables [13]. In this study, the Box-Behnken design was employed to develop a suitable model for describing the interactive effects of COD<sub>in</sub>, HRT, and temperature on the performance of UASB reactor for the anaerobic biodegradation of bagasse effluent from pulp and paper industry.

# 2. Materials and methods

#### 2.1. Experimental setup

The schematic diagram of the UASB reactor used for this study is shown in Fig. 1. The UASB reactor was made of acrylic plastic with 0.1 m internal diameter (ID) and 1.4 m total height of which 1.04 m from the bottom formed the reaction zone with a working capacity of 8.2 l. Five sampling ports, separated with a distance of 0.2 m, were provided along the height of the reactor above 0.2 m from the bottom. The reactor was operated under different mesophilic conditions (20, 25, 30, 35, and  $40^{\circ}$ C), and the temperature was maintained using external heater and was monitored by a thermometer inserted at the side of the reactor. Above, the reacting zone of the reactor was a threephase separator (acrylic cylindrical section with 0.14 m ID and 0.36 m height) for separating and collecting biogas. The three-phase separator consisted of first separating zone, second separating zone, and settling zone. The biogas was separated in the first settling zone through the inverted funnel section; residual air bubble along with wastewater was separated in the second separating zone; the accumulated biogas, without disturbing the settling zone, was discharged through the pipe. Then, the wastewater was sent to the settling zone (where the precipitation of exiguous suspended solids results) formed by the slot between the sloping plates and inverted funnel. Thus, the three-phase separator allowed solids to be retained in the reactor and the biogas to be separated. The wastewater was continuously fed to the reactor through the



Fig. 1. UASB reactor.

base using a peristaltic pump, and the treated effluent was collected at the top. A distributor was mounted at the base of column to ensure the uniform feeding of the effluent to the reactor. A water displacement setup was connected at the top of the reactor to measure the volume of biogas produced [14].

#### 2.2. Bagasse effluent

The bagasse effluent used in this study (the characteristics of which are summarized in Table 1) was collected from a pulp and paper industry located in Erode, Tamil Nadu, India, and stored in airtight plastic cans at  $4^{\circ}$ C before the treatment.

Table 1 Characteristics of bagasse effluent

Characteristics	Value
pH	4.5–5.5
Soluble COD (mg/l)	6 <i>.</i> 800
Volatile fatty acids (mg/l)	3,500
Suspended solids (mg/l)	1,200
Total suspended solids (mg/l)	2,100

#### 2.3. Seed sludge

The seed sludge (with a total volatile solids concentration of 14,500 mg/l) was taken from an anaerobic lagoon in the pulp and paper industry.

### 2.4. Reactor start-up and operation

The reactor was inoculated with 1.6 kg of anaerobically digested wet sludge. After a rest period of 27 h, effluent was added to the reactor until the volume of the mixture became 4.11 (50% of working volume of the reactor) and it was allowed to rest for another 24 h [15]. Samples of the supernatant liquid were collected and analyzed, and 95% COD removal was observed. Then, the whole volume of reactor (8.21) was filled with effluent and allowed to rest. Samples were collected and analyzed periodically until the COD removal reached 93% after which the effluent was continuously fed using peristaltic pump. The steadystate performance was evaluated under different influent COD (COD<sub>in</sub>) concentrations (4,400–6,800 mg/l), temperatures (20-40°C), and hydraulic retention time (HRT) (15-27 h). Adjustment of the reactor pH was not necessary as it remained nearly constant (6.9-7.2) throughout the experiment and was suitable for anaerobic microbes.

#### 2.5. Experimental design

After the start-up, the reactor was operated at different  $\text{COD}_{\text{in}}$ , HRT, and temperature conditions. In order to analyze and model the interactive effects of the three variables ( $\text{COD}_{\text{in}}$ , HRT, and temperature) on the responses, the Box–Behnken design with three factors at three levels was applied using Design-Expert 8 (Table 2 gives the parameters and the operating ranges covered). Each independent variable was coded at three levels between -1 and +1 in the ranges determined by the preliminary experiments of  $\text{COD}_{\text{in}}(X_1)$ : 4,400–6,800 mg/l, HRT ( $X_2$ ): 15–27 h and temperature ( $X_3$ ): 20–40 °C. By the Box–Behnken method, a total number of 15 experiments, including three center

Table 2				
Coded and uncod	led process	parameters	and th	eir levels

	Factors x	Level			
Variable (unit)		-1	0	+1	
COD <sub>in</sub> (mg/l)	$X_1$	4,400	5,600	6,800	
HRT (h)	$X_2$	15	21	27	
Temperature (°C)	$X_3$	20	30	40	

points were carried out, and the experimental conditions of design run and corresponding results (responses) are presented in Table 3. First four columns of Table 3 show run number and experimental conditions of the runs. Performance of the process was evaluated by analyzing the responses Y, where Ydepends on the input factors  $x_1, x_2, ..., x_k$ . The relationship between the response and the input process parameters is described as

$$Y = f(x_1, x_2, ..., x_k) + e \tag{1}$$

where *f* is the real response function its format being unknown and *e* is the error which describes the deviation that can be included by the function *f*. The results were analyzed using  $R^2$ , ANOVA, and response plots [16].

A non-linear regression method was used to fit the second-order polynomial equation to the experimental data and to identify the relevant model terms using statistical software. A quadratic model, which also includes the linear model, can be described as

$$Y = \beta_0 + \sum_{j=1}^k \beta_j X_j + \sum_{j=1}^k \beta_{jj} X_j^2 + \sum_i \sum_{k=2}^k \beta_{ij} X_i X_j + e_i$$
(2)

where *Y* is the response;  $X_i$  and  $X_j$  are variables (*i* and *j* range from 1 to *k*);  $\beta_0$  is the model intercept

coefficient;  $\beta_{j}$ ,  $\beta_{jj}$  and  $\beta_{ij}$  are interaction coefficients of linear, quadratic, and the second-order terms, respectively; *k* is the number of independent parameters (*k* = 4 in this study); and *e<sub>i</sub>* is the error [17].

#### 2.6. Analytical methods

For measuring biogas generation by water displacement method, the vent pipes of the reactor were connected with 5 l tank. The total amount of gas produced was calculated by measuring the displacement volume of water. Biogas composition was measured by injecting 1 ml of gas through gas chromatograph equipped with thermal conductivity detector and data acquisition system with computer interface. The column temperature was initially maintained at 40°C for 3.5 min, followed by automatic temperature increase at a rate of 20°C/min until it reached 180°C. The injector and detector temperatures were 150 and 200°C, respectively. The carrier gas (hydrogen) was supplied at a flow rate of 40 ml/min [18,19].

Volatile fatty acids of the effluent were identified and quantified using a gas chromatograph equipped with flame ionization detector using helium as the carrier gas at a flow rate of 30 ml/min. The column temperature was programed to be maintained between 150 and 220 °C. The parameters such as COD, BOD, pH, total suspended solids and total dissolved solids, were analyzed as per standard methods [20].

Table 3						
Experimental	design	with ex	perimental	and	predicted	values

				Percentage COD removal		COD removal rate (mg/lh)			Biogas production (l/d)			
Run order	COD <sub>in</sub> (mg/l)	HRT (h)	Temperature (ºC)	Y <sub>exp</sub> (%)	Y <sub>pre</sub> (%)	% error	Y <sub>exp</sub> (%)	Y <sub>pre</sub> (%)	% error	Y <sub>exp</sub> (%)	Y <sub>pre</sub> (%)	% error
1	4,400	21	40	89.3	88.83	0.53	187.1	183.63	1.86	19.235	19.286	-0.26
2	5,600	21	30	83.3	83.30	0.00	222.1	222.10	0.00	19.997	19.997	0.00
3	6,800	27	30	78.9	79.00	-0.13	198.7	198.04	0.33	16.108	16.460	-2.19
4	4,400	21	20	68.9	69.08	-0.25	144.4	141.10	2.29	9.846	9.539	3.12
5	5,600	27	40	89.3	89.38	-0.08	185.2	182.56	1.42	18.075	17.416	3.64
6	4,400	15	30	82.5	82.40	0.12	242.0	242.66	-0.27	22.952	22.600	1.53
7	5,600	21	30	83.3	83.30	0.00	222.1	222.10	0.00	19.997	19.997	0.00
8	6,800	21	20	55.6	56.08	-0.85	180.0	183.47	-1.93	9.944	9.894	0.51
9	5,600	21	30	83.3	83.30	0.00	222.1	222.10	0.00	19.997	19.997	0.00
10	6,800	15	30	70.3	69.90	0.57	318.7	312.59	1.92	25.556	24.948	2.38
11	6,800	21	40	78.3	78.13	0.22	253.5	256.80	-1.30	22.825	23.133	-1.35
12	4,400	27	30	89.8	90.20	-0.45	146.3	152.41	-4.18	13.997	14.606	-4.35
13	5,600	15	40	79.1	79.68	-0.73	295.3	298.11	-0.95	28.124	28.426	-1.07
14	5,600	27	20	67.8	67.23	0.85	140.6	137.79	2.00	8.994	8.692	3.35
15	5,600	15	20	60.1	60.03	0.12	224.4	227.04	-1.18	13.505	14.164	-4.88

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# 3. Results and discussion

# 3.1. Statistical analysis

Experiments were carried out to study the effect of  $X_1$ ,  $X_2$ , and  $X_3$  on the responses  $Y_1$  (percentage COD removal),  $Y_2$  (COD removal rate), and  $Y_3$  (biogas production) according to design matrix and are listed in Table 3. As various responses were investigated in this study, polynomial models of different degrees were used for data fitting (Table 4). In order to quantify the curvature effects, the data from the experimental results were fitted to four higher degree polynomial equations viz., linear, two factor interaction (2F1), quadratic, and cubic models. Sequential model sum of squares and model summary statistics were employed to decide on the adequacy of various models to represent percentage COD removal, COD removal rate, and biogas production. Cubic model was found to be aliased and cannot be used for further modeling of experimental data. Though the p values for all three responses were in the acceptable range for both linear and two factor interaction (2F1) models, the adjusted  $R^2$  and predicted  $R^2$  values were found to be low (refer Table 4), however, and hence, these two models were eliminated. On the other hand, the quadratic model exhibited low p values (less than 0.0001), high adjusted  $R^2$ , and predicted  $R^2$  values and was chosen for further analyses.

#### 3.2. Fitting of second-order polynomial equation

The second-order polynomial equation with interaction terms was used to fit the experimental data and to identify the relevant model terms. The final equations obtained in terms of uncoded factors for percentage COD removal  $(Y_1)$ , COD removal rate  $(Y_2)$ , and biogas production ( $Y_3$ ) are presented in Eqs. (3)–(5), respectively.

$$\begin{split} Y_1 &= -44.62188 + 8.13542 \times 10^{-3} X_1 + 1.23264 X_2 \\ &+ 5.53042 X_3 + 4.51389 \times 10^{-5} X_1 X_2 + 4.79167 \\ &\times 10^{-5} X_1 X_3 + 0.010417 X_2 X_3 - 1.38021 \\ &\times 10^{-6} X_1^2 - 0.026042 X_2^2 - 0.082875 X_3^2 \end{split}$$

$$\begin{split} Y_2 &= -\,109.69653 + 0.083986\,X_1 - \,14.78333\,X_2 \\ &+\,15.37417\,X_3 - 8.43750 \times 10^{-4}X_1X_2 + 6.41667 \\ &\times\,10^{-4}X_1X_3 - \,0.10958\,X_2X_3 - 5.48611 \times 10^{-6}X_1^2 \\ &+\,0.33958\,X_2^2 - 0.22950\,X_3^2 \end{split}$$

$$\begin{split} Y_3 &= -\,37.61445 + 7.04688 \times 10^{-3} X_1 - 0.69676 \, X_2 \\ &+ 2.75578 \, X_3 - 1.71181 \times 10^{-5} X_1 X_2 + 7.27500 \\ &\times 10^{-5} X_1 X_3 - 0.023075 \, X_2 X_3 - 7.13802 \times 10^{-7} X_1^2 \\ &+ 0.019003 \, X_2^2 - 0.035066 \, X_3^2 \end{split}$$

The significance of regression coefficients was analyzed using ANOVA [21], and the results for percentage COD removal, COD removal rate, and biogas production are presented in Tables 5 and 6. The large value of *F* (568.71 for percentage COD removal, 138.74 for COD removal rate, and 117.13 for biogas production) indicates that most of the variations in the response can be explained by the regression equations. The associated *p* value is used to estimate whether *F* is large enough to indicate statistical significance. The lower values of Prob > *F* (<0.05) show that the model terms are significant and that of probability p (-0.0001) indicate that the model terms are significant at 95% probability level, and hence, the model is statistically significant [22]. The ANOVA indicated that the equation adequately represents the relationship between the response (percentage COD removal, COD removal rate, and biogas production) and the significant variables. The model gave coefficients of determination (*R*<sup>2</sup>) values of 0.9990, 0.9960, and 0.9953, and adjusted *R*<sup>2</sup> auges of 0.9997, 0.9888, and 0.9868 for percentage COD removal, COD removal, COD removal rate, and biogas production, respectively. Higher values of *R*<sup>2</sup> and adjusted *R*<sup>2</sup> support a strong correlation between observed and predicted values. "Adeq Precision" measures the signal-to-noise ratio, and a ratio greater than four is desirable [23]. Therefore, the ratios of 75.68, 38.92, and 36.16 for the models of percentage COD removal, COD removal, COD removal, *X*<sub>1</sub> × *X*<sub>2</sub>, *X*<sub>1</sub> × *X*<sub>3</sub>, *X*<sub>2</sub> × *X*<sub>3</sub> for CDD removal rate, and biogas production, respectively, indicate that adequate signals for the models can be used to navigate the design space (Table 6). The ANOVA results show that 
$$X_1$$
,  $X_2$ , or  $X_2$ ,  $X_1$  ×  $X_3$ ,  $X_2$  ×  $X_3$  for COD removal rate, and biogas production are -44.62188, -109.69653 and -37.61445%, respectively, and that these values are independent of the factors set in the experiment. The ANOVA shows that the model chosen to

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Source	Sum of squares	Degree of freedom	Mean square	F value	$\operatorname{Prob} > F$	Remark
Sequential m	nodel sum of squares	for percentage COD rem	oval			
Mean	89,675.74	1 0	89,675.74			
Linear	1,297.27	3	432.42	17.93	0.0002	
2F1	3.31	3	1.10	0.034	0.9911	
Quadratic	260.52	3	86.84	284.72	< 0.0001	Suggested
Cube	1.53	3	0.51	$6.366 \times 10^{7}$	< 0.0001	Aliased
Residual	0.000	2	0.000			
Sequential m	nodel sum of squares	for COD removal rate				
Mean	$6.752 \times 10^5$	1	$6.752 \times 10^{5}$			
Linear	34.358.03	3	11.452.68	35.28	< 0.0001	
2F1	557.71	3	185.90	0.49	0.6966	
Ouadratic	2.861.75	3	953.92	31.53	0.0011	Suggested
Cube	151.27	3	50.42	$6.366 \times 10^{7}$	< 0.0001	Aliased
Residual	0.000	2	0.000			
'Sequential n	nodel sum of squares	for biogas production				
Mean	4,829.52	1	4,829.52			
Linear	408.80	3	136.27	23.30	< 0.0001	
2F1	10.78	3	3.59	0.54	0.6703	
Ouadratic	51.33	3	17.11	38.30	0.0007	Suggested
Cube	2.23	3	0.74	$6.366 \times 10^7$	< 0.0001	Aliased
Residual	0.000	2	0.000			
Source	Std. dev.	Predicted $R^2$	Adjusted R <sup>2</sup>	$R^2$	PRESS	Remark
Model summ	nary statistics for perc	centage COD removal				
Linear	4.91	0.7045	0.7839	0.8302	461.68	
2F1	5.72	0.3937	0.7065	0.8323	947.35	
Ouadratic	0.55	0.9844	0.9973	0.9990	24.40	Suggested
Cubic	0.000		1.0000	1.0000		Aliased
Model summ	nary statistics for COI	O removal rate				
Linear	18.02	0.8061	0.8802	0.9059	7,352.87	
2F1	19.41	0.6096	0.8610	0.9206	14,809.06	
Ouadratic	5.50	0.9362	0.9888	0.9960	2,420.28	Suggested
Cubic	0.000		1.0000	1.0000	,	Aliased
Model summ	nary statistics for biog	as production				
Linear	2.42	0.7353	0.8269	0.8640	125.22	
2F1	2.59	0.5088	0.8019	0.8868	232.40	
Quadratic	0.67	0.9245	0.9868	0.9953	35.74	Suggested
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1.000

Table 4 Models tested for % COD removal, COD removal rate and biogas production

between the actual and predicted values of Y, and it can be inferred that the residuals for the prediction of each response are minimum, supporting that the results of ANOVA are correct.

# 3.3. Effect of various parameters on percentage COD removal

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Fig. 2 shows a good convergence between the experimental and predicted values of the percentage COD removal, and it is seen that the developed model is adequate because the data points lie close to the diagonal line. The effects of HRT and  $\mbox{COD}_{\mbox{in}}$  on percentage COD removal are shown in Fig. 3. A significant increase in percentage COD removal with increase in HRT was observed up to 24 h, beyond which the effect of HRT became insignificant at constant COD<sub>in</sub>, whereas a slight decrease in percentage COD removal was detected when COD<sub>in</sub> was increased up to 6,200 mg/l, beyond which it decreased

Aliased

1.0000

4350

Cubic

Source	Sum of squares	Degree of freedom (df)	Mean square	<i>F</i> value	$\operatorname{Prob} > F$
ANOVA for re	esponse surface models a	applied for percentage COD ren	noval		
Model	1,561.10	9	173.46	568.71	< 0.0001
$X_1$	280.84	1	280.84	920.80	< 0.0001
$X_2$	142.81	1	142.81	468.21	< 0.0001
$X_3$	873.62	1	873.62	2,864.33	< 0.0001
$X_1X_2$	0.42	1	0.42	1.39	0.2922
$X_1X_3$	1.32	1	1.32	4.34	0.0918
$X_2X_3$	1.56	1	1.56	5.12	0.0730
$X_1^2$	14.59	1	14.59	47.82	0.0010
$X_2^2$	3.25	1	3.25	10.64	0.0224
$X_{3}^{2}$	253.60	1	253.60	831.47	< 0.0001
Residual	1.53	5	0.31		
Lack of fit	1.53	3	0.51		
Pure error	0.000	2	0.000		
Cor total	1,562.62	14			
ANOVA for re	esponse surface models a	applied for COD removal rate			
Model	37,777.49	9	4,197.50	138.74	< 0.0001
$X_1$	6,675.90	1	6,675.90	220.67	< 0.0001
$X_2$	20,971.52	1	20,971.52	693.19	< 0.0001
X <sub>3</sub>	6,710.61	1	6,710.61	221.81	< 0.0001
$X_1X_2$	147.62	1	147.62	4.88	0.0782
$X_1X_3$	237.16	1	237.16	7.84	0.0380
$X_2X_3$	172.92	1	172.92	5.72	0.0623
$X_1^2$	230.44	1	230.44	7.62	0.0398
$X_2^2$	551.82	1	551.82	18.24	0.0079
$X_{3}^{2}$	1,944.75	1	1,944.75	64.28	0.0005
Residual	151.27	5	30.25		
Lack of fit	151.27	3	50.42		
Pure error	0.000	2	0.000		
Cor total	37,928.75	14			

 Table 5

 ANOVA for response surface models for percentage COD removal and COD removal rate

Table 6 ANOVA for response surface models for biogas production

Source	Sum of squares	Degree of freedom (df)	Mean square	F value	Prob > F
Model	470.91	9	52.32	117.13	< 0.0001
$X_1$	8.83	1	8.83	19.76	0.0067
$X_2$	135.82	1	135.82	304.05	< 0.0001
$X_3$	264.16	1	264.16	591.34	< 0.0001
$X_1X_2$	0.061	1	0.061	0.14	0.7274
$X_1X_3$	3.05	1	3.05	6.82	0.0475
$X_2X_3$	7.67	1	7.67	17.16	0.0090
$X_{1}^{2}$	3.90	1	3.90	8.73	0.0317
$X_{2}^{2}$	1.73	1	1.73	3.87	0.1064
$\bar{X_{3}^{2}}$	45.40	1	45.40	101.64	0.0002
Residual	2.23	5	0.45		
Lack of fit	2.23	3	0.74		
Pure error	0.000	2	0.000		
Cor total	473.14	14			

rapidly at constant HRT. This is confirmed by the Eq. (3) which shows that HRT has more influence on the response  $(Y_1)$  than  $COD_{in}$ . The lowest percentage COD removal was predicted to be 51.21% at the highest COD<sub>in</sub> (corresponding to HRT of 15 h, temperature of 20°C, and COD<sub>in</sub> of 6,800 mg/l), while the experimental value was 51.9%. The main reason for the relatively poor percentage COD removal at COD<sub>in</sub> of 6,800 mg/l was the accumulation of suspended solids in the reactor which also lead to the washout of sludge. It was noticed that after the sludge washout, the reactor efficiency was observed to be constant [25,26]. At constant COD<sub>in</sub>, the percentage COD removal increased with increase in temperature up to 35°C and decreased with further rise in temperature (Fig. 4), whereas slight decrease in percentage COD removal was observed when COD<sub>in</sub> was increased under constant temperature. From Fig. 5, it is observed that the increase in temperature significantly increases the percentage COD removal up to 35°C at constant HRT, beyond that it decreases. A slight increase in percentage COD removal was observed when HRT was increased under constant temperature [27].

#### 3.4. Effect of various parameters on COD removal rate

Fig. 6 represents a very good agreement between the experimental and predicted values of COD removal rate, and it is seen that the developed model is adequate because the data points lie close to the diagonal line. From Fig. 7, it is clear that the COD removal rate increases with increase in  $COD_{in}$ and decrease in HRT. The highest COD removal rate was predicted to be 328.47 mg/lh at the highest



Fig. 3. Effect of  $\mbox{COD}_{\rm in}$  and HRT on percentage COD removal.

COD<sub>in</sub> (corresponding to HRT of 15 h, temperature of 35°C, and COD<sub>in</sub> of 6,800 mg/l), while the experimental value was 346.3 mg/lh. It might be attributed to higher amount of biomass in the form of granules due to larger volume of the reaction zone. Fig. 8 shows that a significant increasing trend in COD removal rate with the increase in temperature up to 35°C at constant COD<sub>in</sub> and a decreasing trend for further temperature rise, whereas slight increase in COD removal rate was observed when COD<sub>in</sub> was increased under a constant temperature. From Fig. 9, it was observed that the increase in temperature significantly increases the COD removal rate up to 35°C at constant HRT, beyond which it decreases. The decrease in COD removal rate was observed when HRT was increased under constant temperature.



Fig. 2. Actual vs. Predicted values of percentage COD removal.



Fig. 4. Effect of  $\text{COD}_{in}$  and temperature on percentage COD removal.



Fig. 5. Effect of HRT and temperature on percentage COD removal.



Fig. 6. Actual vs. predicted values of COD removal rate.



Fig. 7. Effect of COD<sub>in</sub> and HRT on COD removal rate.

#### 3.5. Effect of various parameters on biogas production

Fig. 10 shows a very good agreement between the experimental and predicted values of biogas production, and it is seen that the developed model is adequate because the data points lie close to the diagonal line. The biogas production increased significantly with decrease in HRT at constant COD<sub>in</sub>, whereas a slight increase in biogas production was observed when  $COD_{in}$  was increased up to 6,200 mg/l, beyond that it decreased under constant HRT (Fig. 11). The biogas production per gram of COD removal increased with increase in HRT up to 24 h and later no significant effect. It might be due to the limitation of feed mass transport in the granules as the concentration of particulate COD is more at higher COD<sub>in</sub>. However, it showed an increasing trend in the biogas production when COD<sub>in</sub> increased at a fixed HRT, indicating that the reaction rate increases with an increase in COD<sub>in</sub> owing to high biodegradability of the substrate and enough microbial community. Fig. 12 shows that a significant increasing trend in biogas production with the increase in temperature up to 35°C at constant COD<sub>in</sub> and decreases further, whereas a slight increase in biogas production was observed when COD<sub>in</sub> was increased up to 6,200 mg/l beyond that it decreases under constant temperature. From Fig. 13, it was observed that the increase in temperature significantly increases the biogas production up to 35°C at constant HRT, beyond that it decreases. The slight decrease in biogas production was observed when HRT was increased under constant temperature.

# 3.6. Selection of optimal levels and estimation of optimum response characteristics

The process variables were optimized to obtain maximum percentage COD removal, COD removal



Fig. 8. Effect of  $\mbox{COD}_{in}$  and temperature on COD removal rate.



Fig. 9. Effect of HRT and temperature on COD removal rate.



Fig. 10. Actual vs. predicted values of biogas production.



Fig. 11. Effect of COD<sub>in</sub> and HRT on biogas production.



Fig. 12. Effect of  $\text{COD}_{in}$  and temperature on biogas production.



Fig. 13. Effect of HRT and temperature on biogas production.

rate, and biogas production. A second-order polynomial model obtained in this study was applied for each response to obtain specified optimum conditions. In order to optimize the process variables, the following constraints were taken as follows:  $COD_{in}$ (4,400–6,800 mg/l), HRT (15–27 h), and temperature (20–40 °C). The percentage COD removal, COD removal rate, and biogas production must be as high as possible. Applying the methodology of desired function, the solution was obtained for fulfilling this criteria. The solution was 6,212 mg/l of  $COD_{in}$ , 23 h of HRT, and 35 °C of temperature in order to obtain 84.3 percentage of COD removal, 230.9 mg/l h of COD removal rate, and 21.2 l/d of biogas production, respectively.

### 4. Conclusions

The UASB reactor was found to be successful for biological treatment process to achieve high percentage COD removal in a short period of time. The effects of operating parameters viz., COD<sub>in</sub>, HRT, and temperature on percentage COD removal, COD removal rate, and biogas production were elucidated. Increase in COD<sub>in</sub> decreases the percentage COD removal, increases COD removal rate, and biogas production at constant HRT and temperature. Increase in HRT increases the percentage COD removal but decreases COD removal rate and biogas production at constant COD<sub>in</sub> and temperature. Similarly, increase in temperature increases the percentage COD removal, COD removal rate, and biogas production at constant HRT and COD<sub>in</sub> up to 35°C. A Box-Behnken design was successfully employed for experimental design and analysis of results for maximizing the percentage COD removal, COD removal rate, and biogas production. Analysis of variance showed a high coefficient of determination value  $(R^2)$  of 0.9990 for percentage COD removal, 0.9960 for COD removal rate, and 0.9953 for biogas production, ensuring a satisfactory fit of the second-order polynomial regression model to the experimental data. Maximum values of percentage COD removal (84.3%), COD removal rate (230.9 mg/l h), and biogas production (21.2 l/d) were observed at optimum COD<sub>in</sub>, HRT, and temperature of 6,212 mg/ l, 23 h, and 35°C, respectively.

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