# Statistical analysis and optimization of simultaneous biological nutrients removal process in an intermittently aerated SBR

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Abstract*−*Simultaneous removal of carbon and nutrients from a synthetic wastewater in aerobic/anoxic sequence batch reactor (SBR) was investigated. The experiments were conducted based on a central composite design (CCD) and analyzed using response surface methodology (RSM). Two significant independent variables, cycle time and aeration time, were studied to analyze the process. Five dependent parameters--total COD (TCOD) removal, total nitrogen removal, total phosphorus removal, total Kjeldahl nitrogen removal and effluent nitrate concentration--were monitored as the process responses. The region of exploration for the process was taken as the area enclosed by cycle times (2, 4.25 and 6.5 h) and aeration times (30, 40 and 50 min/h) boundaries. The maximum COD (87.18%) and TKN (78.94%) removal efficiencies were obtained at the cycle time and aeration time of 6.5 h and 50 min/h, respectively. While the maximum TN (71.15%) and phosphorus (68.91%) removal efficiencies were obtained at cycle time of 6.5 h and aeration time of 40 min/h. As a result, high cycle time (6.5 h) and moderate aeration time (40 min/h) were found to be the optimal region for maximum carbon and nitrogen removal efficiencies.

Keywords: Intermittently Aerated SBR, Operating Variables, Nutrients Removal, RSM, Process Analysis, Synthetic Wastewater

## INTRODUCTION

Sequencing batch reactors were originally used for COD and phosphate removal from wastewaters [1,2]. Recent regulations over nutrient discharges to natural water systems resulted in modifications to sequential batch reactor (SBR) systems to achieve nitrification, denitrification along with COD and phosphate removal. The SBR treatment system consists of a sequencing operation including the steps of fill, react, settle, decant, and idle. When biological nutrient removal is desired, the steps in the react cycle are adjusted to provide anaerobic, anoxic and aerobic phases in certain number and sequence [3,4].

In the biological nutrient removal processes, denitrifiers and phosphate accumulating organisms (PAOs) consume the readily biodegradable COD (rbCOD). If the influent wastewater has a low BOD/ N ratio (BOD/N<9), external carbon sources such as fermented waste sludge should be added to enhance denitrification [5,6]. This results in increased operational costs. If simultaneous P and N removal is expected in a single reactor, PAOs will compete with denitrifiers for rbCOD for anaerobic P release. This competition between PAOs and denitrifiers will result in unstable biological P removal if the influent wastewater does not contain sufficient rbCOD or the aeration period is very long [7,8].

Intermittent aeration can achieve nitrogen and phosphorus removal by simultaneous nitrification and denitrification, P-uptake and Prelease in the same reactor in accordance with time cycle of aeration and non-aeration [9,10]. The intermittent aeration strategy can

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also reduce the cost of treatment operation and demand for rbCOD contained in the influent wastewater in the fill phase by minimizing the occurrence of N removal in the fill phase, so that PAOs will obtain sufficient rbCOD for anaerobic P release, which is beneficial to biological P removal [11].

Jian et al. [12] revealed that the highest nitrogen removal efficiency in an intermittent aerated submerged membrane bioreactor (SMBR) was achieved at 30-min aeration off time in a 120-min cycle. An average removal efficiency of 63.9% was obtained by Hongfang and Xiufeng [13] in an intermittent aerated SMBR treating wastewater with slight fluctuation under two different aeration on/off time. In another study, the effect of intermittent aeration on the treatment performance (simultaneous removal of C, N and P) in a pilot-scale submerged membrane bioreactor (SMBR) with intermittent aeration under four kinds of operation conditions was evaluated [14]. It showed a significant impact of the intermittent aeration on the TN removal.

In the last few years, response surface methodology (RSM) has been applied to optimize and evaluate interactive effects of independent factors in numerous chemical and biochemical processes [15-17]. The RSM is a statistical technique for designing experiment, building models, evaluating the effects of several factors, and searching optimum conditions for desirable responses and reducing number of experiments.

Despite the significant effects of intermittent aeration on the process performance, little is known about the effect on the removal efficiencies of organic matter and some other parameters, such as ammonium and phosphate. Therefore, the main objective of this study was to evaluate an aerobic/anoxic SBR with a short intermittent aeration time and cycle time favoring the condition for simul-

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taneous removal of carbon and nutrients in low COD/N ratio (COD/ N=8). The main difference between the present work and others reported to date is the way of intermittent aeration; in this study, aeration was alternately provided in each aeration phase in a cycle time. Therefore, the present work aimed to model, analyze and optimize nutrients removal from a synthetic wastewater using RSM with respect to the simultaneous effects of two independent variables: cycle time and aeration time.

## MATERIAL AND METHODS

## 1. Synthetic Wastewater (SWW)

The synthetic wastewater used throughout the study was com-**1. Synthetic Wastewater (SWW)**<br>The synthetic wastewater used throughout the study was com-<br>posed of glucose as carbon sources (870 mgl<sup>−1</sup>), KH<sub>2</sub>PO<sub>4</sub> (110 mgl<sup>−1</sup>) Fraction source source sources sources.<br>The synthetic wastewater used through<br>posed of glucose as carbon sources (870 mg<br>as phosphorus source, NH<sub>4</sub>Cl (476 mgl<sup>−1</sup>) as phosphorus source,  $NH<sub>4</sub>Cl$  (476 mgl<sup>-1</sup>) as nitrogen source and minerals including NaHCO<sub>3</sub> (600 mgl<sup>-1</sup>), KH<sub>2</sub>PO<sub>4</sub> (110 mgl<sup>-1</sup>)<br>as phosphorus source, NH<sub>4</sub>Cl (476 mgl<sup>-1</sup>) as nitrogen source and<br>minerals including NaHCO<sub>3</sub> (600 mgl<sup>-1</sup>), MgSO<sub>4</sub> (200 mgl<sup>-1</sup>), CaCl<sub>2</sub>· bosed of glacose as eareer sources (070 mg/-),  $\overline{M_2}$  of (3<br>as phosphorus source, NH<sub>4</sub>Cl (476 mgl<sup>-1</sup>), as nitrogen so<br>minerals including NaHCO<sub>3</sub> (600 mgl<sup>-1</sup>), MgSO<sub>4</sub> (200 mgl<br>2H<sub>2</sub>O (100 mgl<sup>-1</sup>), NaNO<sub>3</sub> (5 mgl<sup></sup> 2H<sub>2</sub>O (100 mgl<sup>-1</sup>), NaNO<sub>3</sub> (500 mgl<sup>-1</sup>) and FeSO<sub>4</sub> (10 mgl<sup>-1</sup>). Typical<br>composition of the synthetic wastewater was COD=1,000 mgl<sup>-1</sup>,<br>N<sub>7</sub>=125 mgl<sup>-1</sup> and P<sub>7</sub>=25 mgl<sup>-1</sup>, resulting in a COD : N : P ratio of composition of the synthetic wastewater was COD=1,000 mg<sup>-1</sup>), Ng solution of the synthetic wastewater was COD=1,000 mg<sup>-1</sup>). composition of the synthetic wastewater was COD=1,000 mg $l^{-1}$ ,  $N_{\tau}$ =125 mgl<sup>-1</sup> and P<sub> $\tau$ </sub>=25 mgl<sup>-1</sup>, resulting in a COD : N : P ratio of 1,000 : 125 : 25.

## 2. Bioreactor Configuration and Start Up

The glass bioreactor column was fabricated with an internal diameter of 10 cm and a liquid height of 31.8 cm. The effective working volume was 2,500 ml. The SBR was operated at room temperature  $(20 \pm 2 \degree C)$ . The sequence of the SBR operation was controlled by pre-programmed timers (feeding, aeration, settling and withdrawal). At the beginning of each cycle, immediately after withdrawal (earlier sequence), a pre-defined feed volume (1.25 l) was pumped into the system and the reactor contents were mixed by aeration during the reaction phase. At the end of the cycle, suspended biomass was settled and the effluent was withdrawn from the reactor. Feeding and wastewater withdrawal was done with the help of peristaltic pumps and control valve in the middle ports of the reactor. Air was introduced into the reactor with bubble air diffuser at the bottom of the reactor, and the airflow rate and aeration time was controlled

with an air flow meter and timer connected to a blower. The excess sludge was removed during the draw and idle period to control MLSS of the system. The reactor was inoculated with activated sludge taken from an aeration tank (municipal wastewater treatment plant, Kermanshah, Iran). The inoculum sludge had a sludge age of about 15 d, and a mixed liquor suspended solids (MLSS) concentration of 5,800 mgl<sup>−1</sup>. After initial dilution, 2.5 L activated sludge was seeded to the and a mixed liquor suspended solids (MLSS) concentration of 5,800 mgl<sup>-1</sup>. After initial dilution, 2.5 L activated sludge was seeded to the reactor, resulting in an initial MLSS concentration of 3,800-4,000 mgl<sup>-1</sup> in the reactor. reactor, resulting in an initial MLSS concentration of 3,800-4,000  $mgl^{-1}$  in the reactor.

## 3. Experimental Design and Mathematical Model 3-1. Variables Evaluation

Nutrient removal in BNR systems depends on a multitude of variables. Among these, six main factors which affect the nutrients removal in different reactors are cycle time, COD : N : P ratio, aeration time, biomass concentration, temperature and pH. We chose cycle time and aeration time as the independent and most critical operating factors for the following reasons:

1. The most important parameter affecting the 'cost' of biological treatment system is hydraulic cycle time, because this parameter dictates the overall system volume and mass as well as the amount of liquid held up in the system. Therefore, finding the shortest cycle time to produce the required effluent quality will result in an optimal reactor size. The range studied for cycle time is shown in Table 1.

2. Operational costs of the biological nitrogen removal process are also related to the aeration for nitrification and the recycle of nitrified liquid for denitrification. On the other hand, in the simultaneous nitrification-denitrification (SND) process, the most influential process control factor is aeration period within an applied cycle time. Thus, exploring the optimum aeration time to provide the required efficiency is of crucial importance. The range studied for the aeration time and intermittent cycling program for the aeration and settling times is presented in Table 1.

3-2. Experimental Design

The statistical method of factorial design of experiments (DOE) eliminates systematic errors with an estimate of the experimental



#### Table 1. Experimental range and levels of the independent variables

		Variables	Responses						
Run	Factorl cycle time h	Factor2 Aeration time min/h	<b>TCOD</b> removal $\%$	<b>TKN</b> removal $\%$	NO <sub>3</sub> out mg/l	TN removal $\%$	Phosphorus removal $\%$		
	4.25	40	68	62.8	9.98	55.53	60.82		
2	2	30	37	34.85	3.63	35	44		
3	4.25	30	65	51.2	1.13	51	59		
4	4.25	40	69.2	64.3	7.21	59.2	63.2		
5	6.5	40	85.2	72.56	3.11	71	67		
6	4.25	40	67.7	59.3	9.49	53.6	58.4		
7	4.25	40	71.2	67.3	9.98	61	65.2		
8	4.25	50	73	70.16	143.18	44.6	39		
9	2	50	57.64	49.6	114.07	29	35		
10	6.50	30	82	71.04	11.30	69	62		
11	4.25	40	73.2	64.2	72.54	51.1	55		
12	6.50	50	88	76.4	135.09	52	44.22		
13	2	40	49.64	45.2	17.30	42.08	39		

Table 2. Experimental conditions and results of central composite design

error and minimizes the number of experiments [18-20]. The RSM used in the present study was a central composite face-centered design (CCFD) involving two different factors, cycle time (A) and aeration time (B). The bioreactor performance in nutrients removal was assessed based on the full face-centered CCD experimental plan (Table 2). The design consisted of  $2<sup>k</sup>$  factorial points augmented by 2k axial points and a center point where k is the number of variables. The two operating variables were considered at three levels: low (−1), central (0) and high (1). Accordingly, 13 experiments were conducted with nine experiments organized in a factorial design (including four factorial points, four axial points and one center point) and the remaining four involving the replication of the central point to get good estimate of the experimental error. Repetition experiments were carried out after other experiments followed by order of runs designed by DOE as shown in Table 2. For a comprehensive analysis of the reactor, five dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, total nitrogen removal, total Kjeldahl nitrogen removal, effluent nitrate concentration and phosphorus re- moval. 3-3. Mathematical Modeling

After conducting the experiments, the coefficients of the polynomial model were calculated using the following equation, Khuri and Cornell [18]:

$$
Y = \beta_0 + \beta_1 X_i + \beta_2 X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j + \cdots
$$
\n(1)

Where, i and j are the linear and quadratic coefficients, respectively, and  $\beta$  is the regression coefficient. Model terms were selected or rejected based on the P value with 95% confidence level. The results were completely analyzed using analysis of variance (ANOVA) by Design Expert software. Three-dimensional plots were obtained based on the effect of the levels of the two factors. From these threedimensional plots, the simultaneous interaction of the two factors on the responses was studied. The experimental conditions and results are shown in Table 2.

#### 4. Bioreactor Operation

In the first stage (bioreactor start-up), after adding the prepared

inoculums, the bioreactor was operated under intermittent aeration condition at cycle time, COD : N : P ratio and aeration time of 4.25 h, 1,000 : 125 : 25 and 40 min/h, respectively. The experiments were performed at ambient temperature, 20±2 °C. This was continued until steady state condition was achieved. Intermittent effluent discharge was also provided by using a programmable control valve at the bioreactor output. Treated wastewater was intermittently discharged after each cycle time by giving the time program a programmable timer.

In the second stage, involving modeling by RSM, the SBR reactor was operated with synthetic wastewater and the experimental conditions were designed by Design Expert Software (Stat-Ease Inc., version 6.0.6) as described in Section 2.3. The results can be obtained as response surface presentations for visualization to appreciate the effect of system variables on responses.

## 5. Chemical Analysis

Chemical oxygen demand (COD), Total Kjeldahl nitrogen (TKN), nitrate, total nitrogen (TN), phosphate, total suspended solids (TSS) were determined according to standard methods [21]. For COD, a colorimetric method with closed reflux method was used. A spectrophotometer (DR 5000, Hach, Jenway, USA) at 600 nm was used to measure the absorbance of COD samples. Total Kjeldahl nitrogen (TKN) was determined by TKN meter Gerhardt model (Vapodest 10, Germany). The dissolved oxygen (DO) concentration in wastewater was determined using a DO probe. DO meter was supplied by WTW DO Cell OX 330, electro DO probe, Germany. The pH meter model HANNA-pH 211 was used to measure the pH. Turbidity was measured by a turbidity meter model 2100 P (Hach Co.).

## RESULTS AND DISCUSSION

## 1. Statistical Analysis

The ANOVA results for all responses are summarized in Table 3. As various responses were investigated in this study, different degree polynomial models were used for data fitting (Table 3). To quantify the curvature effects, the data from the experimental results

Response	The models selected to	Probability	$\mathbb{R}^2$	Adj.	Adeq.	<b>SD</b>	<b>CV</b>	<b>PRESS</b>	Probability
	describe the responses			$\mathbb{R}^2$	precision				for lack of fit
TCOD removal, %	$69.61 + 18.49A + 5.77B$ $-3.03A^2 - 3.66AB$	< 0.0001	0.99	0.98	37.08	2.11	3.09	119.48	0.65
TN removal, $\%$	$56.22 + 14.32A - 4.90B$ $-9.45B^2$	< 0.0005	0.89	0.86	22.40	3.46	6.67	244.09	0.75
Effluent nitrate, $mg/l$	$18.52 + 62.71B + 49.55B^2$	< 0.0001	0.91	0.88	13	20	48	270.72	0.97
TKN removal, %	$62.75+15.06A+6.51B$ $-4.48A^2$	< 0.0001	0.94	0.93	23.22	3.35	5.52	248.12	0.34
Phosphorous removal, %	$58.37+9.20A-7.80B$ $-11.17B2$	< 0.0008	0.83	0.78	12.59	5.35	10.05	574.26	0.21

Table 3. ANOVA results for the equations of the design expert 6.0.6 for studied responses

A: cycle time, B: aeration time, R<sup>2</sup>: determination coefficient, Adj. R<sup>2</sup>: adjusted R<sup>2</sup>, Adeq. Precision: Adequate precision, SD: standard deviation, CV: coefficient of variation, PRESS: predicted residual error sum of squares

were fitted to higher degree polynomial equations, i.e., quadratic model. In the Design Expert software, the response data were analyzed by default. The model terms in the equations are those that remained after the elimination of insignificant variables and their interactions. Based on the statistical analysis, the models were highly significant with very low probability values (<0.0008). It is shown that the model terms of independent variables were significant at the 99% confidence level. The square of correlation coefficient for each response was computed as the coefficient of determination (R2 ). It showed high significant regression at 95% confidence level. The value of the adjusted determination coefficient (adjusted  $R^2$ ) was also high to prove the high significance of the model [18].

The models adequacy was tested through lack-of-fit F-tests [19]. The lack of fit results were not statistically significant as the P values were found to be greater than 0.05. Adequate precision is a measure of the range in predicted response relative to its associated error or, in other words, a signal to noise ratio. Its desired value is four or more [20]. The value was found to be desirable for all models. Simultaneously, low values of the coefficient of variation (CV) (3.09-48) indicated good precision and reliability of the experiments as suggested by Khuri and Cornell [18], Kuehl [22] and Ahmad et al. [23]. Detail analysis on the models is presented in the following sections.

# 2. Process Analysis

## 2-1. TCOD Removal

The actual and the predicted COD removal efficiency plots are shown in Fig. 1(a). Actual values are the measured response data for a particular run, and the predicted values were evaluated from the model and generated by using the approximating function. The values of  $R^2$  and  $R^2$ adj were evaluated as 0.985 and 0.977, respectively. By applying multiple regression analysis on the experimental data, the experimental results of the CCD design were fitted with a modified quadratic model. The empirical relationship between COD removal and the two test variables in terms of coded and actual factors for COD removal is presented below: modified quadratic model. The empirical relation<br>TCOD removal and the two test variables in terms of<br>tors for COD removal is presented below:<br>TCOD removal, %=69.61+18.49A+5.77B−3.03A<sup>2</sup> TCOD removal, %=69.61+18.49A+5.77B−3.03A<sup>2</sup>-3.66AB<br>TCOD removal, %=69.61+18.49A+5.77B−3.03A<sup>2</sup>-3.66AB (COD removal, %=-26.88+19.81(Cycle time)+1.27(Aeration time)

$$
TCOD removal, \% = 69.61 + 18.49A + 5.77B - 3.03A^2 - 3.66AB
$$
 (2)

0.599 (Cycle time)<sup>2</sup>–0.163(Cycle time) (3)<br>0.599(Cycle time)<sup>2</sup>–0.163(Cycle time) (Aeration time)  $(3)$ 

As noted in Eq. (2), the main-order effects of both variables (cycle

time and aeration time) had positive impacts on COD removal efficiency, while second-order effect of cycle time and two-level interactions of the variables (AB) showed negative impacts on the response. To better understand the interaction effects of the variables on COD removal efficiency, a three-dimensional contour plot for the measured response was formed based on the model (Eq. (2)) as shown in Fig. 2(a). It was found that with a simultaneous increase in both variables, TCOD removal efficiency was increased. However, the increase in the response caused by increase in cycle time at constant values of aeration time was greater than the increase in the response resulting from increase in the aeration time at constant values of cycle time. Therefore, the most significant factor on the response is cycle time. As can be seen in Fig. 2(a), the response increased upon increasing the aeration time at lower cycle time, while at higher cycle time, aeration time had less effect on TCOD removal. It was attributed to sufficient aeration time at higher cycle time, which makes the response less dependent on aeration time in the design space studied. As a result, as the cycle time increases, less aeration time is needed [24]. It is proven by perturbation plot (Fig. 2(b)). The perturbation plot (Fig. 2(b)) also shows the comparative effects of cycle time and aeration time on TCOD removal efficiency. In the Fig. 2(b), steep curvatures in cycle time and aeration time curves show that the response was very sensitive to these factors.

The maximum TCOD removal was determined to be 87.2% at cycle time and aeration time of 6.5 h and 50 min/h, respectively. While the minimum TCOD removal efficiency (38.6%) was obtained at cycle time and aeration time of 2 h and 30 min/h, respectively. Therefore, short cycle time and low aeration time had reverse effects on the COD removal. Two reasons caused high effluent COD (low COD removal efficiency). First, microorganisms did not have sufficient time to degrade organic matter from wastewater. Second, lower amount of  $NO<sub>3</sub>$  produced from the deteriorated nitrification consumed less organic carbon source in denitrification. Diez and coworkers [25] reported similar findings.

## 2-2. TN Removal

The ANOVA results for TN removal efficiency are presented in Table 3. A reduced quadratic model describes the variation of the TN removal as a result of changes in the variables. The main effects of the variables  $(A, B)$  and second-order effect of aeration time  $(B<sup>2</sup>)$ are significant model terms. The other model terms  $(A^2, AB)$  were



Fig. 1. Predicted vs. actual values for: (a) TCOD removal, (b) TN removal, (c)TKN removal, (d) effluent nitrate, (e) phosphorus removal.

eliminated due to their large p value ( $>$ 0.05). A high value of  $\mathbb{R}^2$ (0.94) shows a very good correlation between the variables. The coded and actual regression equations for TN removal are presented as follows:

TN removal, %=56.22+14.32A-4.90B-9.45B<sup>2</sup><br>TN removal, %=−102.42+6.36 (Cycle time)

Formularies-9.45B<sup>2</sup> (4)<br>  $T = 102.42 + 6.36$  (Cycle time)<br>
+7.069(Aeration time)−0.094(Aeration time)<sup>2</sup> (5)

January, 2014



Fig. 2. (a) Response surface plot for TCOD removal with respect to cycle time and aeration time, (b) Perturbation plot for TCOD removal.



Fig. 3. (a) Response surface plot for TN removal with respect to cycle time and aeration time, (b) Perturbation plot for TN removal.

Fig. 1(b) shows the predicted versus actual values for this response. It shows good agreement between predicted and actual values. Fig. 3(a) depicts three-dimensional plot of the model for TN removal efficiency with respect to cycle time and aeration time within the design space. The figure indicates that rising cycle time provides favorable conditions for TN removal.

A reverse impact of the aeration time on TN removal was observed as the variable increased (Fig. 3(a)). At constant values of cycle times, an increase in aeration time (from 30 to 40 min/h) caused an increase in the response due to higher  $NO<sub>3</sub>$  production as well as the favored condition for denitrification resulted from enough settling time (non aeration period) [26]. Further increment in the variable (from 40 to 50 min/h) decreased the response. This was due to domination of nitrification over denitrification process, which originated from a much shortened time of settling [27]. Therefore, proper duration for aeration/anoxic phases is necessary for good nitrification and denitrification in a SBR system. It takes longer time for oxygen to drop to zero if oxygen concentration is too high in aeration phase, which might lead to incomplete denitrification. Nitrification did not proceed well under low DO. Nitrification was combetween the when DO concentration was higher than 5.0 mgl+1 aeration phase, which might lead to incomplete denitrification. Nitrification did not proceed well under low DO. Nitrification was complete when DO concentration ation, but incomplete denitrification caused effluent  $NO<sub>3</sub>$  higher than  $2.0 \text{ mg}$ <sup> $-1$ </sup> [28]. This result was in good agreement with the observations of Dong and co-workers [27].

The maximum and minimum TN removal efficiency were obtained as 71.2 and 27.5% at cycle time and aeration time of 6.5 h and 40 min/h and 2 h and 30 min/h, respectively. From Fig. 3(a), two minimum regions were obtained; one resulted from high aeration time (>40 min/h) due to domination of the nitrification process and another one obtained at low cycle times because of not enough time for nitrification process. The perturbation plot shown in Fig. 3(b) demonstrates the comparative effects of cycle times and aeration time on TN removal efficiency. An increasing linear slope in cycle time  $(A)$  and curvature of aeration time  $(C)$  shows that the response was sensitive to these two process variables with different effects.

## 2-3. TKN Removal

In a similar way, response surface analysis was performed to evaluate the effects of cycle time and aeration time on TKN removal efficiency. Statistical analysis on the experimental data showed that the coefficients of both  $B<sup>2</sup>$  and AB were statistically insignificant due to their large  $p$  value ( $> 0.05$ ). By the removal of the insignificant coefficients, the following equations in terms of coded and actual factors were obtained: to their large p value (>0.05). By the removal of the insignifi-<br>The coefficients, the following equations in terms of coded and actual<br>tors were obtained:<br>TKN removal, %=62.75+15.06A+6.51B−4.48A<sup>2</sup> (6)

TKN removal, %=62.75+15.06A+6.51B-4.48A<sup>2</sup> 
$$
(6)
$$

ctors were obtained:

\nTKN removal, %=62.75+15.06A+6.51B-4.48A<sup>2</sup> (6)

\nTKN removal, %=-7.70+14.20 (Cycle time) 
$$
+0.65
$$
(Aeration time)-0.88(Cycle time)<sup>2</sup> (7)

The predicted versus actual plot for the response is shown in Fig. 1(c). The actual values are distributed relatively close to the straight line (y=x). As noted in Table 3, a reduced quadratic model was fitted with the experimental data. The reduced quadratic interaction model shows that the main effect of cycle time (A) and aeration time (B) and second-order effects of cycle time are significant model terms. The three-dimensional plot was made as a function of cycle time and aeration time of the system. The effect of these variables is illustrated in Fig. 4(a).

The perturbation plot (Fig. 4(b)) also shows the comparative effects of cycle time and aeration time on TKN removal efficiency. In Fig. 4(b), steep curvatures in cycle time and aeration time curves show that the response of TKN removal efficiency was very sensitive to these factors. With a simultaneous increase in both variables, the TKN removal efficiency was increased, favoring the nitrification condition. By comparing the results obtained for TKN removal with TN removal (Fig. 3(a) and 4(a)), it was figured out a similar trend in



Fig. 4. (a) Response surface plot for TKN removal with respect to cycle time and aeration time, (b) Perturbation plot for TKN removal.



Fig. 5. (a) Response surface plot for effluent nitrate concentration with respect to cycle time and aeration time, (b) Perturbation plot for effluent nitrate concentration.

the responses was obtained until aeration time of about 40 min/h, indicating an appropriate proportion between nitrification and denitrification processes. While at the higher values of aeration time (>40 min/h), nitrification was the dominant process, which is confirmed by results shown in the Fig. 5. The effect of cycle time on the response was reduced by increasing aeration time due to a limited anoxic condition. The maximum TKN removal was determined to be 79.84% at cycle time and aeration time of 6.5 h and 50 min/h, respectively. While the minimum TKN removal efficiency (37.7%) was obtained at cycle time and aeration time of 2 and 30 min/h, respectively. −Similar findings were reported by Wang and co-workers [29].

2-4. Effluent Nitrate Concentration

Effluent  $NO<sub>3</sub>$  concentration is an indicator to justify the difference between TN & TKN removal efficiencies. It implies the progress in the nitrification and denitrification processes. The predicted versus actual plot for the response is shown in Fig. 1(d). The actual values are distributed close to the straight line  $(y=x)$ . From the ANOVA results, Table 3, B and  $B^2$  are significant model terms. Insignificant model terms, which have limited influence, such as A, A<sup>2</sup> and AB, were excluded from the study to improve the model. The following regression equations are the empirical models in terms of coded Francisco effluent nitrate concentration:<br>and actual factors for effluent nitrate concentration:<br>Effluent nitrate concentration, mgl<sup>-1</sup>=18.52+62.71B+ ∪.

=18.52+62.71B+49.55B<sup>2</sup> (8)

Effluent nitrate concentration, mgl<sup>−</sup><sup>1</sup> =560.5 33.4(Aeration time)+0.495(Aeration time)<sup>2</sup> (9)

The effects of A and B on the effluent nitrate concentration are shown in Fig. 5(a). The most significant factor on the response was determined to be aeration time (B). Increase in aeration time from 35 to 50 min/h resulted in an increase in effluent nitrate concentration with a main and second order effect, while an increase in cycle time did not show significant effect on the response. The increase in aeration time from 30 to 35 min/h showed less significant effect on the response due to an appropriate proportion between nitrification and denitrification processes. The perturbation plot (Fig. 5(b)) shows the comparative effects of two variables on effluent nitrate concentration. In Fig. 5(b), a steep curvature in aeration time curve shows that the response was very sensitive to this factor. The relatively flat lines of cycle time show insensitivity of the responses to change in this variable. As mentioned above, high values of aeration time and cycle time favored nitrification process. On the other hand, high value of aeration time showed a negative effect on denitrification process due to increase in oxidation and reduction potential (ORP), increasing effluent nitrate concentration. It proved the earlier discussion on TN and TKN removal efficiencies. This result was close to that reported by Dong et al. [27], Hasar et al. [30], and Scheumann and Kraume [31].

#### 2-5. Phosphorus Removal

Phosphorus can only be removed by its uptake into biomass, which can be discharged from the system as surplus sludge. Thus, a biomass with high phosphorus content is desirable for biological phosphorus removal. Removal of phosphorus in wastewater is closely dependent upon the phosphorus release in anaerobic conditions and on the subsequent uptake process of the excess phosphorus including that contained in wastewater in aerobic conditions. In the present study, as the system is intermittently aerated, a micro anaerobic environment seems to be provided in the biofloc formed in the process. Therefore, phosphorus removal efficiency was measured as a response in this study. Relatively good agreement between predicted and actual values is shown in Fig. 1(e).

From the analysis carried out (Table 3), a reduced quadratic model was selected to describe the variation of the response. The model terms,  $A$ ,  $B$  and  $B<sup>2</sup>$  are significant factors. Insignificant model terms were found to be  $A^2$  and AB that were excluded from the study to improve the model. No interactive impact of the studied variables on the response was shown. The regression equations obtained in terms of coded and actual factors for TP removal are presented below. prove the model. No interactive impact of the studied variables<br>the response was shown. The regression equations obtained in<br>ms of coded and actual factors for TP removal are presented below.<br>Phosphorus removal, %=58.37+9 the response was shown. The regression equal ms of coded and actual factors for TP removal are Phosphorus removal,  $%=58.37+9.20A-7.80B-11.1$  Phosphorus removal,  $%=-106.55+4.09(Cycle time)$ 

+8.16(Aeration time)−0.11(Aeration)<sup>2</sup> (11)

Fig. 6(a) demonstrates phosphorus removal efficiency as a func-



Fig. 6. (a) Response surface plot for phosphorus removal with respect to cycle time and aeration time, (b) Perturbation plot for phosphorus removal.

tion of cycle time and aeration time. From Fig. 6(a), an increase in cycle time caused an increase in the response. It is clear from the perturbation plot (Fig. 6(b)) that the response of phosphorus removal efficiency was very sensitive to these factors. As noted in Figs. 6(a) and (b), aeration time had a reverse impact on the response. An increase in aeration time (from 30 to 40 min/h) caused an increase in the response due to higher uptake of the excess phosphorus in aerobic conditions. Further increment in the variable (from 40 to 50 min/h) decreased the response. This was because an increase in aeration time causes a decrease in the anaerobic time when the phosphate absorbing organisms (PAOs) accumulate poly-â-hydroxy butyrate (PHB) from volatile fatty acids (VFAs) produced. In this process, glucose as the source of VFAs requires sufficient time for acidification [32,33]. Another reason for decrease in phosphorous removal at high aeration time was due to presence of nitrate, which inhibits the fermentation processes producing VFAs in the anaerobic zone.

The values of P removal efficiency may be described by the results obtained for TN removal efficiency, as almost similar operational conditions favor P and TN removal. Cycle time showed an increasing effect on both responses, while the aeration time showed a reverse impact. This is confirmed by the same operational condition obtained for the maximum and minimum values of P and TN removal efficiencies (cycle time=6.5 h and aeration time ≅40 min/ h for maximum and cycle time=2 h and aeration time ≅30 min/h for minimum).

#### 2-6. Process Optimization

With multiple responses we need to find regions where requirements simultaneously meet the critical properties, the "sweet spot." The best compromise can visually be searched by superimposing or overlaying critical response contours on a contour plot. Graphical optimization produces an overlay plot of the contour graphs to display the area of feasible response values in the factor space. The optimum region was identified based on five critical responses (COD removal, TN removal, TKN removal, effluent nitrate and phosphorus removal), whose criteria were adopted as shown in Table 4. The shaded area in the overlay plots is the region that meets the proposed criteria. Fig. 7 shows the graphical optimization, which displays the area of feasible response values (shaded portion) in the factors space. The area that satisfies the constraints is white, while the area that does not meet your criteria is gray. The optimal region enclosed by the cycle time (6, 6.5 h) and aeration time (35, 42.5 min/h) boundary.

## **CONCLUSIONS**

Nutrient removal from synthetic wastewater was successfully accomplished by using an aerobic/anoxic SBR with intermittent aeration. Comparison of predicted and experimental values revealed good correlation between them, implying that empirical models de-

Table 4. The optimization criteria for chosen response

Response	Limits	l Init
<b>TCOD</b> removal	> 80	$\%$
TN removal	>65	$\%$
<b>TKN</b> removal	>70	$\%$
Effluent $NO3$ concentration	<20	mg/l
Phosphorous removal	560	$\frac{0}{0}$



Fig. 7. Overlay plot for optimal region.

rived from RSM can be used to adequately describe the relationship between the factors and responses in nutrient removal from synthetic wastewater in aerobic/anoxic SBR. With a simultaneous increase in both variables (cycle time and aeration time), TCOD and TKN removal efficiencies were increased; however, the most significant factor for these responses was determined to be cycle time. The maximum COD (87.18%) and TKN (78.94%) removal efficiencies were obtained at the cycle time and aeration time 6.5h and 50 min/h, respectively. The optimal region was enclosed by the cycle time (6, 6.5 h) and aeration time (35, 42.5 min/h) boundary.

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