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# Process modeling and optimization of biological removal of carbon, nitrogen and phosphorus from hospital wastewater in a continuous feeding & intermittent discharge (CFID) bioreactor

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**Abstract**−We evaluated the feasibility and treatment performance of a continuous feeding and intermittent discharge (CFID) bioreactor treating real hospital wastewater with the emphasis on simultaneous carbon, nitrogen and phosphorus (CNP) removal. The experiments were based on a central composite design (CCD) and analyzed by response surface methodology (RSM). To analyze the process, three significant variables, aeration time (2-4 h), mixing time without aeration (30-90 min) and MLSS concentration (2,000-6,000 mg/*l*), were studied. Results show that an increase in aeration time increased the nitrogen and phosphorous removal efficiency. However, when the aeration time was more than 3 h, the efficiency of phosphorous removal was decreased due to insufficient acidification. A similar scenario was observed when mixing time was increased for phosphorus and nitrogen removal efficiency. MLSS had a positive effect on all the responses. Under optimal conditions, the concentrations of quality parameter in the influent in average were recorded as 586 mg COD/*l*, 296 mg $BOD_5$/*l*, 97 mgTN/*l* and 16.47 mg TP/*l*, which yields the following removal efficiencies, 95.6%, 98.3%, 88.0% and 92.0%, respectively.

Keywords: Hospital Wastewater, Simultaneous Nutrient Removal, RSM

## INTRODUCTION

Hospital wastewater is one of the major sources of surface and groundwater pollution. A hospital approximately generates about 400 to 1,200 liters of wastewater per day per bed [1]. Therefore, serious attention has been directed towards these significant volumes of wastewater which is very complex and includes several inorganic and organic components such as non-metabolized pharmaceutical compounds, antibiotics, disinfectants, anesthetics, pathogens, radioactive elements, X-ray contrast agents and other persistent and dangerous compounds [2,3]. Hospital wastewater contains high concentrations of chlorinated molecules and trace heavy metals such as mercury and silver. COD and $BOD_5$ contents of the hospital wastewaters are about 850 and 600 mg/*l*, respectively [4]. Therefore, hospital wastewater must be collected and treated before discharging it into the receiving waters.

Hospital effluents are usually discharged to the urban sewer system where they mix with other effluents and finally reach the sewage treatment plant. The quality of hospital wastewater is relatively similar to municipal wastewater. However, conventional wastewater treatment processes such as activated sludge with suspended growth could not treat hospital wastewater effectively [5,6]. Thus, alternative techniques such as integrated bioreactors (anoxic/oxic-membrane bioreactor (A/O-MBR), staged anaerobic-aerobic membrane bioreactor (MBR) and integrated anaerobic-aerobic fixed-film reactor (FFR)), activated carbon adsorption, advanced oxidation processes (AOPs), and combinations of them, may be needed to reach higher removals before the final disposal of the effluents. Furthermore, it is necessary in order to reuse the wastewater for irrigation or ground water recharge.

Removal of organic matter, nitrogen (N) and phosphorus (P) compounds are often the main aims in designing different types of wastewater treatment systems. For example, biological wastewater treatments such as anaerobic, anoxic and aerobic biological reac-

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tors are usually used in combinations to provide an efficient treatment scheme for organics and nutrient removal [7,8]. Recently, substantial attention has been focused towards simultaneous carbon and nutrient removal using compact high-rate directors in bioreactors order to satisfy the strict constraints posted with respect to space, odor, view and bio-solids production [9]. The integrated bioreactors, which are a combination of aerobic and anaerobic processes in a single reactor, has been proven as a viable alternative and could enhance overall degradation efficiency [9].

Wen et al. [10] studied the treatment of hospital wastewater using a submerged membrane bioreactor. The results showed that the removal efficiency for COD, $NH_4^+$-N, and turbidity was 80, 93 and 83%, respectively, with the average effluent quality of COD <25 mg/*l*, $NH_4^+$-N<1.5 mg/*l* and turbidity <3 NTU. Liu et al. [11] used a membrane bioreactor to treat hospital wastewater. The results obtained from this work indicated that a membrane bioreactor could remove more than 80% of the COD, BOD, TSS, and $NH_4^+$-N. BOD concentration in the effluent varied from non-detectable level to 20.6 mg/*l*. Sousa and Foresti stem composed of an UASB reactor followed by sequencing batch aerobic reactors (SBR) in treating domestic sewage. The system performance was evaluated through a bench scale set-up comprised of a 4 liter volume UASB reactor followed by two SBRs of 3.6 liters each. The HRT of 4 h in UASB was maintained constant throughout the study, while the 4 h cycles in the following sequence of fill (0.10 h), reaction (1.9 h), sedimentation (1.6 h), discharge (0.25 h); idle (0.15 h) were maintained in SBR. The combined system removed about 85% of total nitrogen through nitrification. The COD removal in UASB reactor was around 86% while in SBR around 65% of the remaining; thus, combined systems removed 95%.

Greentech Company [13] has reported a case study in Dong Thap General Hospital Vietnam. The study reported that a combination of the activated sludge system and biological contactor-ASBC has been used to treat the hospital wastewater. The results of this study showed 87.8, 71.2, 83.6 and 99.9% removal of COD, Total N, Total P and Coliforms, respectively. Asadi et al. [14] reported a study on simultaneous removal of carbon and nutrients from an industrial estate wastewater in a single up-flow aerobic/anoxic sludge bed (UAASB) bioreactor. The finding indicated that an optimum condition for more than 80% removal of COD removal and 50% of TKN can be achieved with HRT of 12 h and aeration time of 40-60 min/h. Sperling et al. 2011 [15] worked with a UASB - activated sludge system for the treatment of municipal wastewater. The removal efficiencies for COD and TKN were reported 95 and 85%, respectively.

Therefore, considering the above-mentioned scenarios and studies, we tested a continuous feeding and intermittent discharge (CFID) bioreactor as a hybrid reactor to treat hospital wastewater with an emphasis to remove carbon, nitrogen (N) and phosphorus (P) compounds. Note that aerobic, anoxic and anaerobic conditions in this single reactor were provided by intermittent aeration. The possibility to achieve high biomass concentration at a lower hydraulic retention time, without additional requirement of equipment to circulate the mixed liquor between aerobic and anaerobic compartments, will be an advantage of this reactor.

Furthermore, in this work conventional technique for the optimization of a multi-factorial system was not used. Central composite design (CCD) and response surface methodology (RSM) were applied to model and optimize the hospital wastewater bioreactor treatment. RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing process [16,17]. The main advantage of RSM is that it could reduce the number of experimental trials needed to evaluate multiple parameters and their interactions. Aeration time, mixing time (without aeration) and MLSS concentration were identified as the factors or variables. The interactions among the variables as well as their direct impacts on the nine process responses (chemical oxygen demand (COD) removal, biological oxygen demand (BOD) removal, total nitrogen (TN) removal, total Kjeldahl nitrogen (TKN) removal, organic nitrogen removal, effluent nitrite and nitrate concentrations, total phosphorus (TP) removal and TSS removal) were discussed.

## MATERIALS AND METHODS

### 1. Wastewater Source and Characterization

Samples of hospital wastewater (HWW) were obtained from Imam Reza hospital in Kermanshah, Iran. Imam Reza Hospital is one of the biggest hospitals in Kermanshah with a total of 650 beds and 1200 staff members. Imam Reza Hospital is located near the north of Kermanshah town with 1,000,0000 inhabitants. The effluent produced by the hospital is directly discharged into the combined sewage network, where it will be conveyed to the municipal wastewater treatment plant and co-treated with other urban wastewater. Over a time-period of one month, 24 hours of composite wastewater samples were taken daily. This was done to avoid large variations in concentration between the different departments. The collected samples were stored in a cold room at 4 °C. The storage technique had no observable effect on its composition. The characteristics of the HWW are shown in Table 1.

### 2. Analytical Methods

The concentrations of chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), nitrate, total nitrogen (TN), total phosphate (TP), N-organic nitrogen, MLSS and volatile suspended solids (VSS) of the system were determined using standard methods of

**Table 1. Characteristics of hospital wastewater**

| Parameter | Range |
|---|---|
| TCOD (mg/*l*) | 450-654 |
| BOD (mg/*l*) | 220-345 |
| TSS (mg/*l*) | 259-520 |
| TKN (mg/*l*) | 81-120 |
| $NH_4$-N (mg/*l*) | 18-41 |
| $NO_2$-N (mg/*l*) | 0.03-0.3 |
| $NO_3$-N (mg/*l*) | 0.08-0.36 |
| N-organic (mg/*l*) | 59-72 |
| TN (mg/*l*) | 81.1-120.7 |
| TP (mg/l) | 14-19 |
| Turbidity (NTU) | 50-71 |
| pH | 7.5-7.9 |
| Alkanity (mg/*l*) | 376-509 |

water and wastewater analysis [8]. For COD, a colorimetric technique with a closed reflux method was developed. Spectrophotometer (DR 5000, Hach, Jenway, USA) at 600 nm wavelength was used to measure the absorbance of COD samples. Total Kjeldahl nitrogen (TKN) was determined by TKN meter Gerhardt model (vapodest10), whereas for the dissolved oxygen (DO) concentration DO probe (WTW DO CellOX 330, electro DO probe, Germany) was used. The pH meter model HANNA, pH211 was used to measure the pH readings. Turbidity was measured by using a turbiditmeter model 2100p (Hach Co.).

## 3. Bioreactor Configuration and Startup

Fig. 1 shows the layout of the laboratory scale continuous feeding and intermittent discharge (CFID) up-flow bioreactor. This reactor was used for biological nutrient removal from the HWW. The Plexiglas brand bioreactor column was designed and fabricated with an internal diameter of 8 cm and a liquid height of 80 cm. The total volume of the column is 4 *l*. An automated control valve was mounted on the reactor column, at a height of 80 cm (25% of the total volume) in order to achieve the intermittent discharge. Air was introduced into the reactor at the bottom of the reactor with a bubble air diffuser. The air flow rate and aeration time were controlled by an air flow-meter and a pre-programmed timer respectively. To distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column.

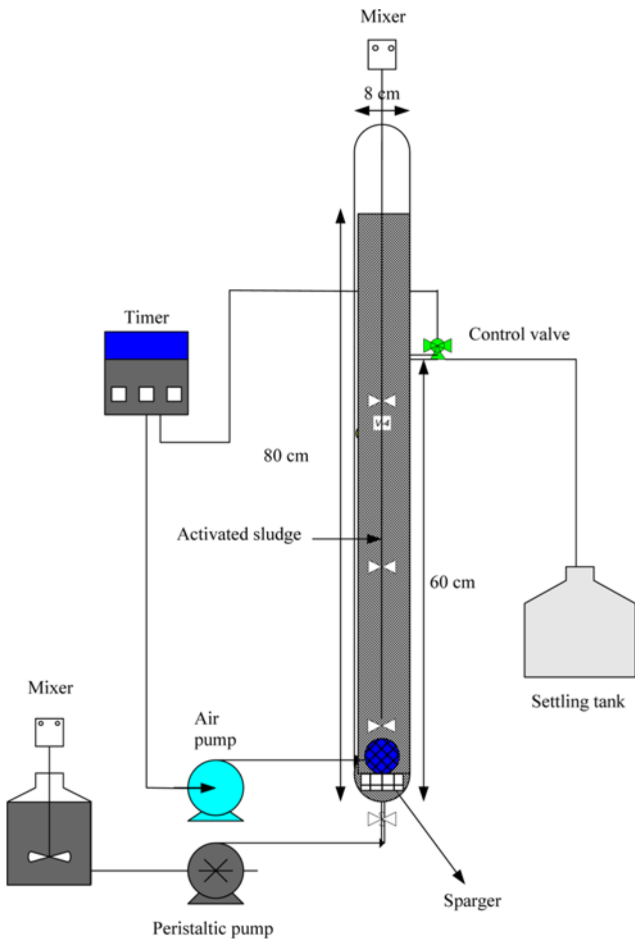



**Fig. 1. Experimental setup.**

The HWW was continuously introduced into the bioreactor from the bottom of the column. The treated effluent was occasionally discharged as supernatant at the end of the each run. The bioreactor was inoculated under room temperature (20±2 °C) with activated sludge from an aeration tank of a municipal wastewater treatment plant. The inoculums' MLSS concentration was 6.4 g/*l* and was diluted to the required initial concentrations.

The sequence of the bioreactor operation was controlled by a pre-programmed timer. Each operation cycle consists of four phases. In the first phase, the reactor was aerated based on the designed aeration time (2 to 4 h) at a constant aeration rate of 5 *l*/min. In the second phase, aeration was stopped and mixing was started for about 30 to 90 min. The third phase was settling, which lasted for 30 min and was fixed throughout the study. Finally, in the fourth phase the effluent was withdrawn for 3 min. Wastewater feeding and withdrawal were done using peristaltic pumps and control valves. Since, biomass concentration is a variable in this study, the biomass content of the reactor was maintained constantly by removing surplus biomass after each cycle.

In the first stage (bioreactor start-up), after adding the prepared inoculums, the bioreactor was operated in the batch mode with the following conditions: cycle time of 6 h ( aeration time of 4 h, mixing time (without aeration) of 90 min and settling time of 30 min). In this stage, HWW was used as feed with a COD concentration of about 600 mg/*l*. This process was continued until a steady state condition was achieved. In this condition the removal efficiency for COD, TN and TP was around 88, 76 and 83%, respectively. In the second stage, once the bioreactor reach the steady state condition, the bioreactor was operated in the continuous mode according to the three independent variables: aeration time (2-4 h), mixing time (30-90 min) and MLSS concentration of (2,000-6,000 mg/*l*). The experimental runs were designed using Design Expert Software (DOE) (Stat-Ease Inc., version 6.0) as described in Section 2.4.

## 4. Experimental Design

The DOE software creates a platform to design the experimental runs. DOE removes systematic errors, estimates experimental error and reduces the number of experiments to obtain the optimum operating conditions. Moreover, it can be used to estimate the relative significance of several affecting factors in the presence of complex interactions. When a combination of several independent variables and their interactions affects desired responses, response surface methodology (RSM) is an effective tool for optimizing the process. There are many classes of response surface designs such as central composite design, Box-Behnken design, hybrid design and three-level factorial design [18]. Among these methods the most frequently used RSM design is central composite design (CCD).

**Table 2. Experimental range and levels of the independent variables**

| Variables | Ranges and levels | | |
|---|---|---|---|
| | −1 | 0 | 1 |
| Aeration time, h | 2 | 3 | 4 |
| Mixing (without aeration), min | 30 | 60 | 90 |
| MLSS, mg/*l* | 2000 | 4000 | 6000 |

**Table 3. Experimental conditions and results**

| Run | Variables | | | Responses | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Factor 1 | Factor 2 | Factor 3 | COD rem. | BOD rem. | TKN rem. | TN rem. | N-organic rem. | Effluent nitrate | Effluent nitrite | TP rem. | TSS rem. |
| | Aeration time, h | Mixing time, min | MLSS, mg/*l* | % | % | % | % | % | mg/*l* | mg/*l* | % | % |
| 1 | 3 | 60 | 4000 | 70 | 75 | 58 | 50 | 52 | 5.26 | 3.45 | 70 | 70.3 |
| 2 | 4 | 60 | 4000 | 83 | 87 | 70 | 64.2 | 66 | 4.42 | 0.56 | 73 | 83.3 |
| 3 | 2 | 30 | 2000 | 30 | 34 | 20 | 17.5 | 16 | 1.24 | 1.38 | 26 | 48.6 |
| 4 | 4 | 30 | 2000 | 51 | 57 | 43 | 39.2 | 39 | 2 | 1.80 | 31 | 61.3 |
| 5 | 4 | 90 | 2000 | 57.6 | 63 | 44 | 37.7 | 37 | 3.24 | 3.20 | 54 | 74.3 |
| 6 | 3 | 60 | 2000 | 43.3 | 47 | 30 | 25 | 21 | 1.77 | 3.90 | 49 | 80 |
| 7 | 3 | 60 | 4000 | 69 | 74.1 | 56 | 48.8 | 51 | 6.3 | 2.7 | 69 | 72 |
| 8 | 3 | 90 | 4000 | 78 | 80 | 64 | 60 | 62.3 | 2.36 | 1.59 | 80 | 76.6 |
| 9 | 3 | 60 | 4000 | 71.8 | 76.2 | 60 | 51 | 54 | 5 | 2.6 | 71 | 71 |
| 10 | 4 | 30 | 6000 | 87.6 | 92 | 81 | 74 | 77 | 5.7 | 1.18 | 67 | 92 |
| 11 | 2 | 30 | 6000 | 70.3 | 76 | 59 | 54.6 | 55 | 2.78 | 1.97 | 58 | 74 |
| 12 | 2 | 60 | 4000 | 60.6 | 64 | 50 | 45 | 45 | 5.4 | 1.24 | 60 | 64 |
| 13 | 2 | 90 | 6000 | 77.3 | 82 | 64 | 58 | 59 | 5.7 | 0.87 | 80 | 77 |
| 14 | 3 | 60 | 4000 | 73 | 79 | 60 | 51 | 52 | 4.9 | 2.2 | 70 | 69.5 |
| 15 | 3 | 30 | 4000 | 63.6 | 68 | 55 | 42 | 48 | 3.47 | 7.44 | 60 | 63.6 |
| 16 | 3 | 60 | 4000 | 72 | 76 | 58.9 | 50.8 | 52.6 | 5.16 | 2.48 | 69 | 72 |
| 17 | 3 | 60 | 4000 | 69 | 75.1 | 59 | 51 | 52.7 | 5.2 | 2.53 | 71 | 71.7 |
| 18 | 2 | 90 | 2000 | 35 | 40 | 25 | 21 | 18 | 1.81 | 2.53 | 40 | 41 |
| 19 | 3 | 60 | 6000 | 82 | 89 | 79 | 73 | 75.6 | 5.1 | 1.04 | 81 | 91.3 |
| 20 | 4 | 90 | 6000 | 95 | 96 | 90 | 86.47 | 95.3 | 3.02 | 4.56 | 90 | 98.6 |

In the current study, CCD was used to study the three different factors: aeration time (A), mixing time (B) and MLSS concentration (C). The region of exploration for the process was enclosed by aeration time dosage (2-4 h), mixing time (30-90 min) and MLSS (2,000-600 mg/*l*) to evaluate nine different responses. The design consists of $2^k$ factorial points augmented by 2k axial points and a center point, where k is the number of variables. The ranges or levels of the parameters are shown in Table 2. The coded values of A, B and C are set at three levels: 1 (minimum), 0 (central), +1 (maximum). These three levels were assessed based on the full face-centered CCD experimental plan. Accordingly, 20 experiments were employed in this study. Repetitions were conducted according to the order of the runs designed by CCD as indicated in Table 3.

**5. Mathematical Modeling**

RSM involves screening and codification of the variables, mathematical-statistical treatment of data, and evaluation of the fitted model and determination of the optimal conditions. RSM, describes a model in the form of Eq. (1) to fit the experimental data and by optimization, the coefficients for the model were calculated. The relationship between the responses, input and the quadratic equation model for predicting the optimal variables were identified using the following:

$$Y=\beta_0+\beta_i X_i+\beta_j X_j+\beta_{ii} X_i^2+\beta_{jj} X_j^2+\beta_{ij} X_i X_j+\ldots \quad (1)$$

where, Y, i, j, $\beta$, X are process response, linear coefficient, quadratic coefficient, regression coefficient and coded independent variables, respectively. All these coefficient variables are analyzed by multiple regression analysis. Response contour plot will be generated using DOE. Model terms are selected or neglected based on the probability of error (P) value with 95% of confidence level. The results obtained from CCD were examined by analysis of variance (ANOVA). Three-dimensional (3D) plots and their respective contour plots were obtained based on the effect of the levels of the two factors while other factors can be changed by default. Therefore, the results of CCD can be presented in 3D presentations with contours. This will help to study the simultaneous interaction of the three variables on the responses. The experimental conditions and results are depicted in Table 3.

## RESULTS AND DISCUSSION

**1. Statistical Analysis**

CCD was selected to find the relationship between the process responses and the variables. Table 4 shows a complete list of the three independent variables (A, B and C) in the terms of coded and actual units, and the experimental data obtained for the nine responses ($Y_1$–$Y_9$). 20 experimental runs were performed in accordance with Table 3 by CCD.

Table 4 illustrates the reduced models in terms of coded factors with significant model terms and analysis of variance (ANOVA) results for the responses. Various responses were investigated with different degree polynomial models for data fitting (Table 4). To

**Table 4. ANOVA results for the equations of the Design Expert 6.0.6 for studied responses**

| Response | Modified equations with significant terms | Type of model | $R^2$ | Adj. $R^2$ | Pred. $R^2$ | Adeq. precision | S.D | CV | PRESS | P-value | F-value | Probability for lack of fit |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| COD removal, % | $Y_1=+71.00+10.10A+4.04B+19.53C-8.09C^2$ | Quadratic | 0.99 | 0.99 | 0.98 | 70.242 | 1.92 | 2.86 | 101.48 | <0.0001 | 25.58 | *0.3684* |
| BOD rem.% | $Y_2=+75.44+9.90A+3.40B+19.40C-7.84C^2-2.00AC$ | Quadratic | 0.99 | 0.99 | 0.98 | 62.30 | 1.92 | 2.68 | 96.79 | <0.0001 | 22.6 | *0.3627* |
| TN rem.% | $Y_3=+50.54+11.40A+5.14B+20.92C$ | Linear | 0.99 | 0.97 | 0.96 | 59.85 | 2.80 | 5.54 | 232.91 | <0.0001 | 252.7 | 0.06 |
| TKN rem.% | $Y_4=+59.09+11.00A+2.90B+21.10C-5.59C^2$ | Quadratic | 0.99 | 0.98 | 0.97 | 62.5 | 2.24 | 3.98 | 148.18 | 0.0044 | 8.39 | *0.1307* |
| N-organic rem. | $Y_5=+51.43+12.13A+3.66B+23.09C$ | Linear | 0.95 | 0.94 | 0.91 | 37.1 | 4.69 | 9.11 | 675.35 | <0.0001 | 105.3 | 0.0007 |
| Effluent nitrate | $Y_6=+4.85+1.32C-1.62B^2$ | Quadratic | 0.65 | 0.61 | 0.48 | 11.23 | 0.98 | 24.27 | 24.30 | 0.0261 | 4.75 | 0.05 |
| Effluent nitrite | $Y_7=1.7$ | Mean | - | - | - | - | - | - | - | - | - | - |
| TP rem.% | $Y_8=+70.01+5.10A+10.20B+17.60C-6.06A2-7.56C2$ | Quadratic | 0.98 | 0.97 | 0.95 | 43.19 | 2.78 | 4.40 | 245.76 | <0.0001 | 32.14 | *0.4540* |
| TSS rem.% | $Y_9=+72.54+10.49A+12.77C-10.71B2+9.84C2+3.02AB$ | Quadratic | 0.92 | 0.89 | 0.79 | 21.73 | 4.42 | 6.13 | 747.24 | 0.0110 | 6.36 | 0.05 |

quantify the curvature effects, the data from the experimental results were fitted to higher degree polynomial equations: two factor interaction (2FI), quadratic etc. The finalized model terms in the equations are those remaining after the elimination of insignificant variables and their interactions.

The values obtained from the ANOVA analysis determine the rank of the significance's degree. For each response, the *F*-value and *P*-value were computed to determine the significance of the model terms. The greater the amount of *F*-value, the smaller will be the values of 'Prob>F'. This will indicate that the corresponding models and the individual coefficients are more significant [19].

In Table 4, nine models ($Y_1-Y_9$) were developed with the following *F*-values: 25.58, 25.6, 252.7, 8.39, 105.3, 12.33, 4.75, 32.14 and 6.36. The probability values were very low (in the range of 0.0001-0.0261). This implies that the terms were significant for all the models. However, the lack-of-fit are insignificant (bigger than 0.05) for N-organic removal.

The fit of the models were further verified by the correlation coefficients, $R^2$, adjusted $R^2$ and predicted $R^2$ between the experimental and the model predicted values. Table 4 proves that the correlation coefficients, $R^2$, adjusted $R^2$ and predicted $R^2$ are near to each other and close to 1.0 except for effluent nitrate.

Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable. In all the cases, the value of adequate precision was around 11.23-70.24. This value indicates adequate model discrimination. Furthermore, low values of the standard deviation (SD) (1.8-4.69) and coefficient of variation (CV) (2.68-9.11%) indicated good precision and reliability of the experiments as suggested by Khuri and Cornell [18] and Ahmad et al. [19].

As mentioned, except for nitrate model, the other proposed models could be adequately used to describe the responses under a wide range of operating conditions. Detailed analyses on the models are presented in the following sections.

**2. Process Performance**

2-1. Carbon Removal

The mean concentration of BOD and COD of raw wastewater of 70 hospitals in Iran, in terms of wastewater strength, has been reported as 348 and 527 mg/*l*, respectively [22]. These readings are close to the results of the present study. Table 1 shows the mean concentration of $BOD_5$ and COD in the raw HWW as 296 and 586 mg/*l* (correspond to the $BOD_5$/COD ratio of 0.5), respectively. $BOD_5$/COD ratio provides a good measurement of wastewater biodegradability, whereby a $BOD_5$/COD ratio greater than 0.4 is generally accepted as biodegradable [23]. From the literature it is shown that the $BOD_5$/COD ratio for HWW is higher than 0.4. Hence, this wastewater can be categorized as biodegradable wastewater [24].

The effects of aeration time and MLSS at a constant mixing time of 90 min (without aeration) on two responses, namely COD and BOD removal efficiency, are described by empirical models in the Table 4. The trend is shown in Fig. 2(a) and 2(b).

Fig. 2(a) and 2(b) shows that the trend for COD and BOD removal efficiency was the same as the aeration time and MLSS changed from 2 to 4 h and 2,000 to 6,000 mg/*l*, respectively. The COD and $BOD_5$ removal efficiency significantly increased with increase in aeration time and MLSS. Mixing time (range 30-90 min) did not show any strong effect on the process because the changes were very small. However, the improvement of COD and BOD removal efficiency was about 8% when the mixing time was increased from 30 to 90 min. Therefore, a mixing time of 60 min was chosen as optimal condition.

Maximum COD (96.58%) and BOD (98.3%) removal efficiency were observed at the aeration time of 4 h, MLSS of 6,000 mg/*l* and mixing time of 90 min. Meanwhile, the lowest predicted removal efficiency of COD (29.24%) and BOD (32.9%) was obtained at the

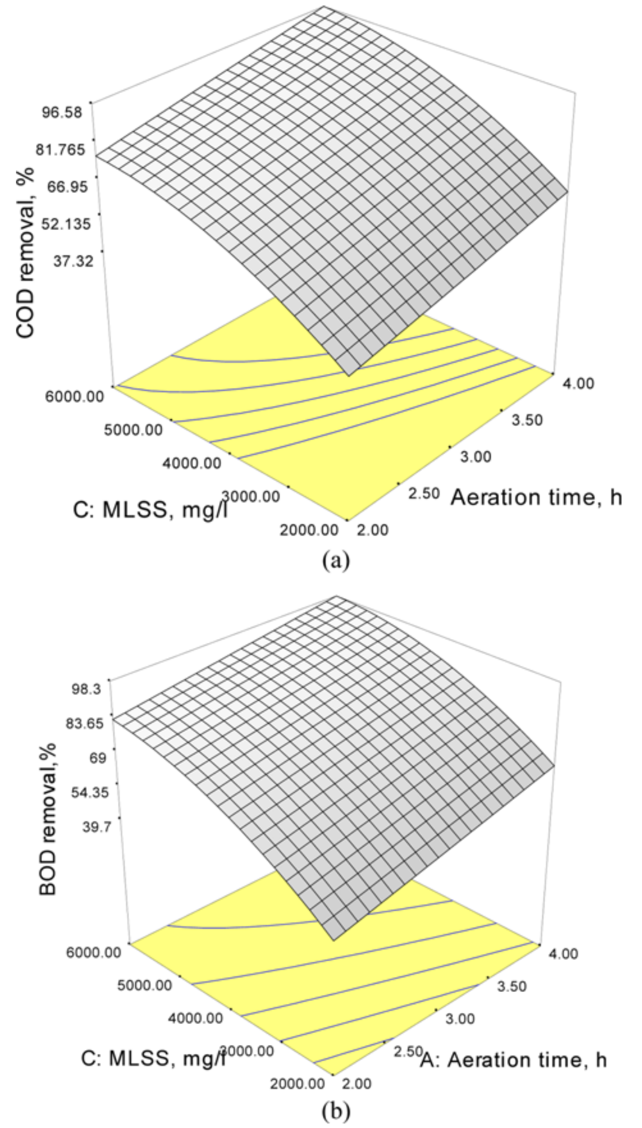



**Fig. 2. 3D surface plots for carbon removal with respect to aeration time and MLSS at constant value of mixing time (90 min); (a) COD removal, (b) BOD removal.**

aeration time of 2 h, MLSS of 2,000 mg/*l* and mixing time of 30 min. The influence of aeration time and MLSS on COD and BOD removal efficiency was more significant than mixing time, which had limited effects on both responses.

Note that the HRT in this study was in the range of 3 h (aeration time (2 h): mixing time without aeration (30 min): settling time (30 min)) to 6 h (4 h (aeration time): 90 min (mixing time without aeration): 30 min (steeling time)). The range of HRT (3-6 h) studied corresponds to organic loading rate (OLR) and feed flow rate of 10.4-20.8 g COD/d and 16-32 *l*/d, respectively. As shown in Figs. 2 and 3, at the aeration time of 2 h (corresponding to HRT of 3 h) (aeration time=2 h, non aeration time with mixing=30 min and settling time=30 min), the COD, BOD and TN removal efficiency are lower than other HRT, which was due to high organic loading rate in this condition.

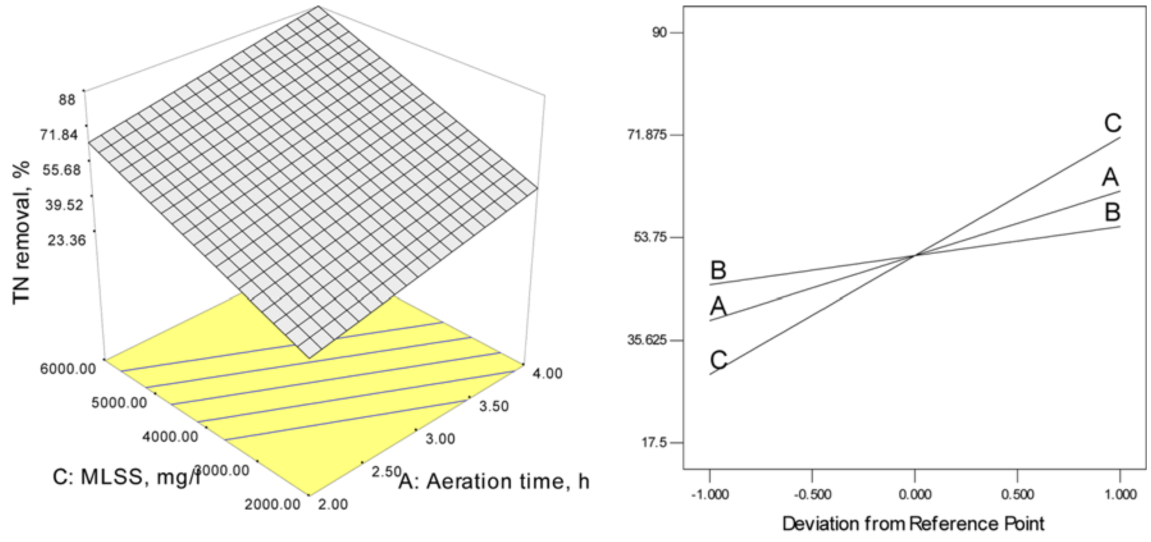
The effect of aeration time at the lower values of the MLSS was greater than those with the highest MLSS. Fig. 2 shows that the response increased upon increasing the aeration time at lower values of MLSS, and at higher MLSS, aeration time did not show any significant effect on TCOD removal. This is due to sufficient amounts of microorganisms at higher HRT, which makes the response relatively independent of aeration time or HRT. As a result, when MLSS was increased, lesser aeration time or HRT was needed. Mansouri et al. [25] reported a similar effect of the cycle time and aeration on the COD removal efficiency in the SBR reactor, so that a maximum COD removal efficiency was achieved 87.18% at the cycle time and aeration time of 6.5 h and 50 min/h, respectively. A sequencing batch flexible fiber biofilm reactor was examined for the treatment of dairy wastewater at three different OLR (0.4, 1.27 and 2.74 kg COD $m^{-3}$ $d^{-1}$) and 24 h aeration time by Abdulgader and coworkers [26]. An inverse relationship between OLR and COD removal efficiency was observed in this study. In another study, the interactive effects of initial chemical oxygen demand (CODin), MLSS and aeration time on the performance of a lab-scale sequencing batch biofilm reactor (SBBR) treating a synthetic dairy wastewater were investigated [27]. The results of this study indicated that as the aeration time and MLSS increased the COD removal efficiency was increased. Also, the reverse impact of the $COD_{in}$ on COD removal was observed as the variable increased. Kargi and Konya [26] found that stepwise increase in HRT from 5 to 15 h resulted about 40 percent increase in COD removal and the efficiency remained almost constant at larger HRT levels. Meng et al. [27] also reported similar results.

## 2-2. Nitrogen Removal

Biological nitrogen removal involves aerobic nitrification and anoxic denitrification. The nitrification process contributes to the transformation of ammonia to nitrite ($NO_2^-$), and then to nitrate ($NO_3^-$). Therefore, in a conventional treatment process, nitrogen is removed by using at least two separate reactors under different environments (two kinds of bacteria: nitrifiers and denitrifiers) [30]. The main drawbacks of conventional treatment processes are their complicated operation and high cost due to the addition of external carbon source and recycling for the effluent denitrification process. Thus, in this study, the two conditions for nitrogen removal were provided in a single reactor by implementing intermittent aeration.

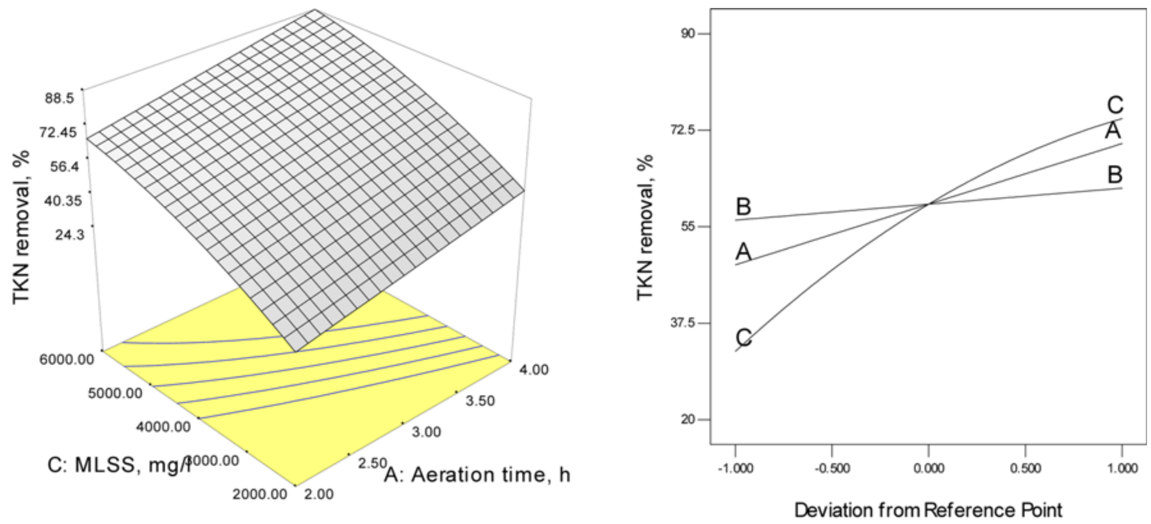
The sum of organic-N, $NH_4^+$-N-N, $NO_2^-$-N and $NO_3^-$-N could be regarded as TN. Thus, any change in the nitrogen compounds can be illustrated by TN removal efficiency. The response surface plot for TN removal was made as a function of aeration time (A) and MLSS (C) of the system, while keeping the mixing time at 90 min (as the factor had no major effect on the model). Fig. 3(a) illustrates the effect of the two variables (A and C) on TN removal efficiency. As it is obvious from Fig. 3(a), the response increased with simultaneous increasing in aeration time and MLSS. The maximum predicted TN removal (88%) was achieved when aeration time and MLSS were at the highest level (aeration time of 4 h and MLSS of 6,000 mg/*l*) with mixing time of 90 min. It is obvious from the 3D surface and perturbation plots (Fig. 3(a) and (b)) that the MLSS plays a predominant and positive effect on the TN removal.

This is because the high MLSS leads to the increase of the $NO_3^-$ and cell production as well as the favored condition for denitrifica-




(a) (b)

**Fig. 3. (a) 3D surface plot and (b) Perturbation plot for TN removal efficiency with respect to aeration time and MLSS at a constant value of mixing time (90 min).**




(a) (b)

**Fig. 4. (a) 3D surface plot and (b) Perturbation plot for TKN removal efficiency with respect to aeration time and MLSS at a constant value of mixing time (90 min).**

tion resulted from high DO consumption rate, and consequently more nitrogen compounds removed. Therefore, increasing the MLSS is more preferable than increasing aeration time or mixing time for the purpose of obtaining a high TN removal. Since settling time was constant in this study and samples were taken at the end of each cycle (aeration+non-aeration+settling) after settling, the results show the effect of all the phases (aeration+non-aeration) provided. However, it is clear that the most of denitrification reaction ($TN_{in}$-$TN_{out}$) occurred in the anoxic condition provided in the non-aeration phase. As the system is not mixed in the settling step, a small volume of the biomass at the bottom of reactor was exposed with the raw feed. So, the rate of settling step may only be related to the phosphate accumulating denitrifiers in producing PHBs by consuming readily bCOD content of the fresh feed, which is not significant.

Fig. 4(a) represents the response surface plot of the quadratic model for variation in TKN removal, as a function of aeration time (A) and MLSS (C) with a constant mixing time (90 min). The perturbation plot (Fig. 4(b)) also shows the comparative effects of variables on TKN removal efficiency. In Fig. 4(b), steep curvatures in aeration time and MLSS 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. In terms of interaction effect of aeration time and MLSS, as shown in Fig. 3(a), it is evident that the TN removal tends to reach the peak at the condi-

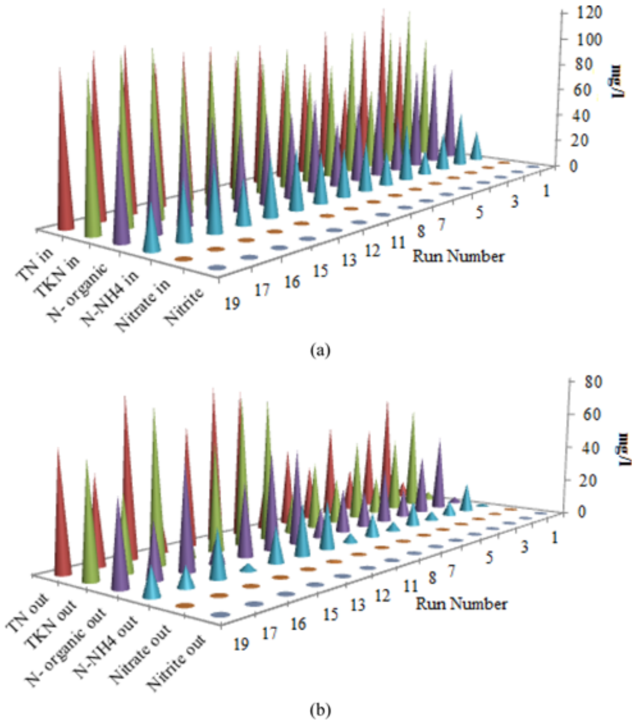



**Fig. 5. Nitrogen fractionation in (a) influent and (b) effluent under different operational conditions.**

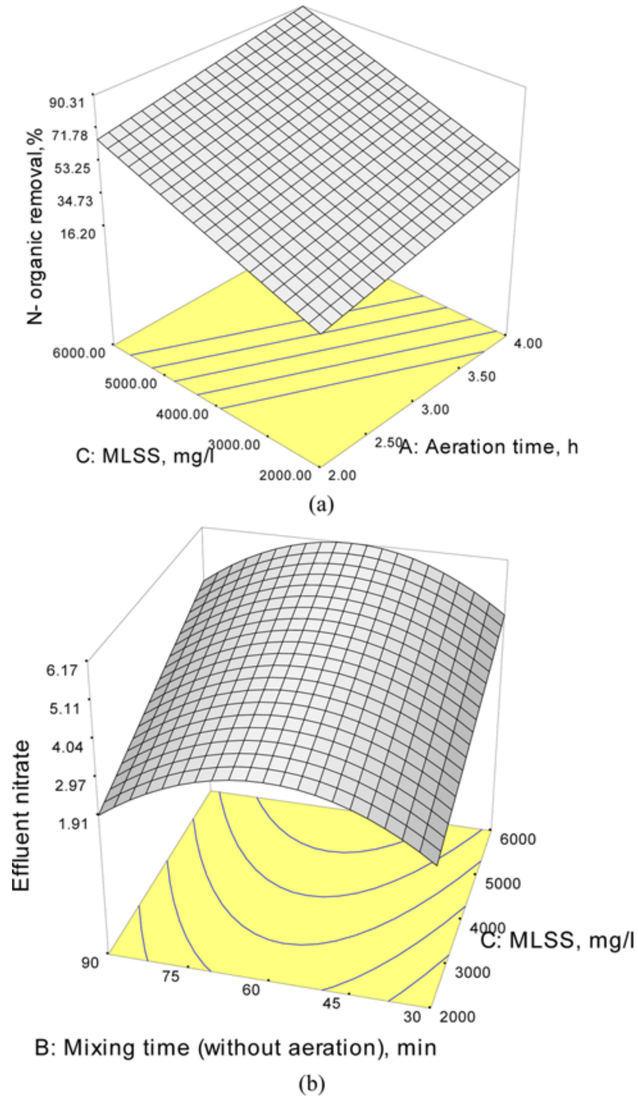



**Fig. 6. 3D surface plots for (a) N-organic removal efficiency and (b) effluent nitrate with respect to aeration time and MLSS at a constant value of mixing time (90 min).**

tion of high aeration time (4 h) and MLSS (6,000 mg/*l*). By comparing the results obtained for TKN removal with TN removal (Fig. 3(a) and 4(a)), a similar trend in the responses was obtained, indicating an appropriate proportion between nitrification and denitrification processes. The maximum TKN removal was determined to be 88.5% at aeration time, MLSS and mixing time 4 h, 6,000 mg/*l* min and 90 min, respectively.

In similar work, the performance of continuous flow intermittent decant type sequencing batch (CFID) reactor was investigated in different HRTs (22, 8 and 6 h) and dissolved oxygen (DO) patterns (0.5, 2.5-3.5 and 3.5-4.5 mg/*l*) by Khan et al. [31]. The operation under DO limiting conditions (0.5 mg/*l*) showed a suppression of nitrification and a negligible removal of $NH_4$-N disregarding the HRT. Under the operation of non-limiting DO conditions (2.5 mg/*l*) high nitrification rate constants of greater than 0.89 h-1 were observed. The highest effluent quality was observed at the 8 h HRT and 2.5-3.5 mg/*l* DO concentration. At this operational condition, the average BOD, TSS, ammonia nitrogen and fecal coliform removal efficiencies were 83, 90, 74 and 99%, respectively.

As shown in Fig. 5(a)-(b), a major fraction of the total nitrogen in influent and effluent of HWW is organic nitrogen. Imam Reza Hospital wastewater has about 65% of the organic nitrogen. To nitrify, organic-nitrogen must be converted to ammonia/ammonium. If it is not converted to ammonia/ammonium, then the organic-nitrogen will pass through the treatment plant. The origin and composition of organic in HWW are unknown. However, it is known that the organic comprises large portions of proteins, free and combined amino acids, low molecular weight (LMW) aliphaticamines, urea and amides [32]. Pehlivanoglu-Mantas and Sedlak [29] also reported that it is possible to derive a significant organic nitrogen fraction from the metabolic products which is generated by the microbes in the wastewater.

The effect of variables on the organic nitrogen removal efficiencies are shown in Fig. 6(a) as a 3D plot. The organic nitrogen removal increased significantly with simultaneous increasing aeration time and MLSS. However, when MLSS was increased, the effect of aeration time on the response was reduced. Also, from Fig. 6(a) the maximum N-organic removal was achieved at the highest aeration time and MLSS of 4 h and 6,000 mg/*l*, respectively. At this condition, maximum TN and TKN removal were also obtained. This indicates a balance between nitrification and denitrification processes. This proves that N-organic consumption was higher. An interesting finding was also observed: it is expected that the N-organic removal will decrease due to the domination of aerobic condition per anaerobic condition; however, when the aeration time was increased, N-organic removal increased for all the HRTs tested. This

**Table 5. Mass balance model for TN removal in different runs except repeating ones**

| Run | Factor 1 | Factor 2 | Factor 3 | Influent parameters | | | | | | Effluent parameters | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Aeration time, | Mixing time, | MLSS, | TKN | $NH_4$-N | $NO_2$-N | $NO_3$-N | N-org. | TN | TKN | $NH_4$-N | $NO_2$-N | $NO_3$-N | N-org. | TN | Nitrogen removal by denitrificatin | Nitrogen removal by sludge |
| | h | min | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | mg/*l* | g/d | g/d |
| 1 | 3 | 60 | 4000 | 106 | 41 | 0.19 | 0.27 | 65 | 106.46 | 44.52 | 13.32 | 3.45 | 5.26 | 31.2 | 53.23 | 1.68 | 0.31 |
| 2 | 4 | 60 | 4000 | 84 | 25 | 0.09 | 0.2 | 59 | 84.29 | 25.2 | 5.14 | 0.56 | 4.42 | 20.06 | 30.18 | 1.37 | 0.3 |
| 3 | 2 | 30 | 2000 | 94 | 35 | 0.12 | 0.21 | 59 | 94.33 | 75.2 | 25.64 | 1.38 | 1.24 | 49.56 | 77.82 | 1.2 | 0.19 |
| 4 | 4 | 30 | 2000 | 98 | 29 | 0.04 | 0.1 | 69 | 98.14 | 55.86 | 13.77 | 1.8 | 2 | 42.09 | 59.67 | 1.59 | 0.22 |
| 5 | 4 | 90 | 2000 | 100 | 40 | 0.03 | 0.2 | 60 | 100.23 | 56 | 18.2 | 3.2 | 3.24 | 37.8 | 62.45 | 1.75 | 0.14 |
| 6 | 3 | 60 | 2000 | 110 | 43 | 0.06 | 0.17 | 67 | 110.23 | 77 | 24.07 | 3.9 | 1.77 | 52.93 | 82.67 | 1.76 | 0.15 |
| 8 | 3 | 90 | 4000 | 96 | 39 | 0.12 | 0.16 | 56.72 | 96.28 | 34.56 | 13.18 | 0.15 | 2.36 | 21.38 | 38.51 | 1.54 | 0.3 |
| 10 | 4 | 30 | 6000 | 96 | 30 | 0.29 | 0.33 | 66 | 96.62 | 18.24 | 3.06 | 1.18 | 5.7 | 15.18 | 25.12 | 1.59 | 0.34 |
| 11 | 2 | 30 | 6000 | 103 | 28 | 0.26 | 0.24 | 75 | 103.5 | 42.23 | 8.48 | 1.97 | 2.78 | 33.75 | 46.99 | 1.36 | 0.47 |
| 12 | 2 | 60 | 4000 | 120 | 41 | 0.19 | 0.27 | 79 | 121.17 | 60 | 16.55 | 1.24 | 5.4 | 43.45 | 66.64 | 1.78 | 0.34 |
| 13 | 2 | 90 | 6000 | 107 | 38 | 0.18 | 0.2 | 69 | 107.38 | 38.52 | 10.23 | 0.87 | 5.7 | 28.29 | 45.1 | 1.61 | 0.41 |
| 15 | 3 | 30 | 4000 | 81 | 35 | 0.3 | 0.36 | 46 | 81.66 | 36.45 | 12.53 | 7.44 | 3.47 | 23.92 | 47.37 | 1.13 | 0.32 |
| 18 | 2 | 90 | 2000 | 103 | 41 | 0.17 | 0.08 | 62 | 103.25 | 77.25 | 26.41 | 2.53 | 1.81 | 50.84 | 81.57 | 1.56 | 0.16 |
| 19 | 3 | 60 | 6000 | 100 | 34 | 0.29 | 0.23 | 66 | 100.52 | 21 | 4.896 | 1.04 | 5.1 | 16.1 | 27.14 | 1.64 | 0.36 |
| 20 | 4 | 90 | 6000 | 95 | 23 | 0.1 | 0.14 | 72 | 86.47 | 9.5 | 6.116 | 0.36 | 3.02 | 3.384 | 8.647 | 1.48 | 0.33 |

could be due to the high nitrification rate in the high aeration time. Choi et al. [30] evaluated the performance of an intermittently aerated membrane bioreactor (IAMBR) across several COD/N ratios. Their results showed that the increase of the COD loading rate led to a higher denitrification rate and better assimilation of organic matter and nutrients. Asadi et al. [14] studied the treatment efficiency of an industrial estate wastewater with low $BOD_5$/COD ratio in an up-flow aerobic/anoxic sludge bed (UAASB) bioreactor, with an intermittent regime in aeration and discharge. In this study, simultaneous increase in the HRT and aeration time reduced TN removal efficiency. This is a result of insufficient carbon source and restriction in anoxic conditions at the higher HRT and aeration times.

2-3. Effluent Nitrite and Nitrate

Fig. 5(a) and 5(b) show the trend of nitrogen compounds in influent and effluent of the bioreactors for all the experiments except for the repeated runs. It can be concluded that the effluent nitrate and nitrite concentration are very low compared to organic and ammonium nitrogen in the raw wastewater. Fig. 6(b) shows the effect of mixing time and MLSS on effluent nitrate. It was observed that an increase in the MLSS caused an increase in the effluent $NO_3^-$ concentration. This is due to an increase in oxidation potential that favored nitrification process. Two opposite impacts of the mixing time on effluent nitrate were observed as the variable increased. Effluent nitrate concentration was enhanced with mixing time increased from 30 to 60 min. However, further increment in the mixing time from 60 to 90 min decreased the response. Note that the effluent $NO_3^-$ concentration (<7.44 mg/*l*) was low in all the experiment, indicating the appropriate proportion between nitrification and denitrification processes.

For effluent nitrite, RSM did not propose any model and the predicted $R^2$ value was negative (-0.12). A negative "Pred R-Squared" implies that overall mean can be a better predictor for this response. The mean of nitrite concentration in the influent was 1.7 mg/*l*. In this study, $NO_2$-N concentration was reported lower than 2.53 mg/*l*. The decrease of $NO_2$-N in the bioreactor could be detected with an increase in aeration time and MLSS and a decrease in mixing time. This scenario indicates that it is possible to have an alternative occurrence of nitrification and denitrification.

Table 5 shows the mass balances of nitrogen in different runs obtained by measuring the amount of nitrogen in the influent and effluent and estimating of the nitrogen content removed through the waste sludge discharge. The influent nitrogen concentration ranged from 81.1-121 mg/*l* and the major constituent was organic nitrogen. The nitrogen in the effluent after one reaction cycle consisted of $NH_4$-N; $NO_X^-$-N, organic nitrogen and nitrogen in the suspended sludge. A certain amount of sludge was removed daily from the reactor to maintain constant SRT. The amount of nitrogen removed in the waste sludge was assumed to be 0.12 mg N/mg VSS, as suggested by Metcalf and Eddy [8]. The percentage of influent nitrogen removed by denitrification and assimilation process was in the range of 59-81%, and 6-21%, respectively. The results indicated that the CFID bioreactor exhibited a high performance of TN and TKN removal, at aeration time of 4 h, non-aeration time of 1.5 h and MLSS of 6,000 mg/*l*. This could be attributed to a balance of nitrification and denitrification processes in this condition. Based on the calculation, the maximum $N_2$ production in the system was obtained in aeration time of 2 h and non-aeration time of 1 h and MLSS of 200 mg/*l*, which was due to a high TN loading in this condition. The relatively short HRTs and SRTs (see Table 3) were not favorable for nitrification because the autotrophic nitrifi-

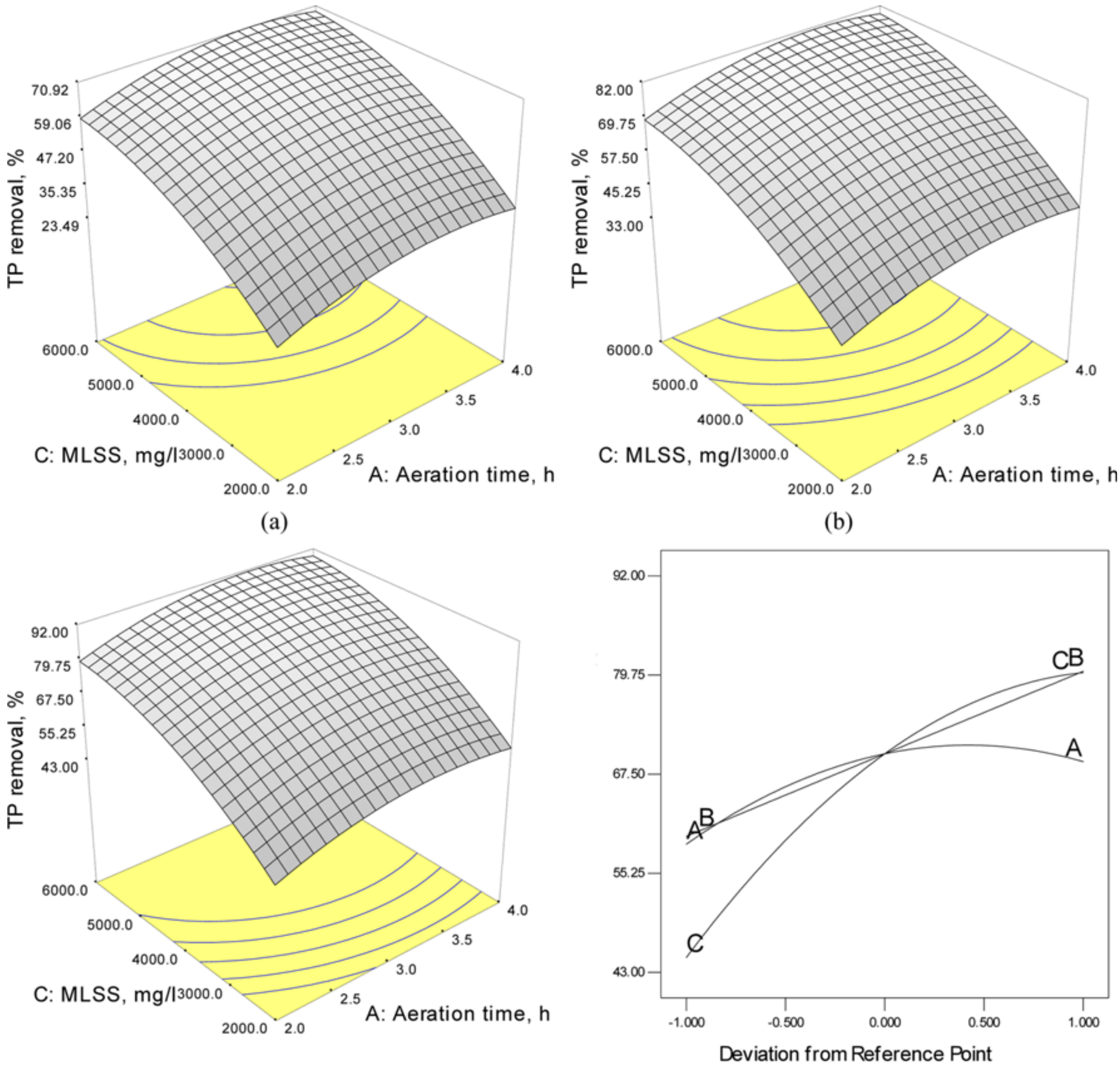



**Fig. 7. 3D surface plots for TP removal efficiency with respect to aeration time and MLSS at constant values of mixing time; (a) mixing time=30 min (b) mixing time=60 min (c) mixing time=90 min, (d) perturbation plot.**

ers are slow-growing bacteria and are washed out at low SRTs.

2-4. Total Phosphorus (TP) Removal

Based on the literature, an anaerobic/aerobic sequence is necessary to promote biological P removal. P was released in the anaerobic stage followed by an excess of P uptake in the aerobic stage. P accumulation as poly-phosphates is bigger than the P release [14]. Thus, in this study, the aerobic/anoxic/anaerobic conditions for nitrogen and phosphorus removal were provided in a single reactor by implementing intermittent aeration. Fig. 7 illustrates the overall TP removal efficiency (%) as a function of aeration time and MLSS concentration at three different mixing times. Generally, the reactor could achieve a high level of TP removal. Furthermore, an increase in MLSS and aeration time from 2 h to 3 h increased the TP removal. However, further increment in aeration time (3 h to 4 h) decreased the response. Maximum TP removal efficiency was about 92% under the following conditions: aeration time of 3 h, 6,000 mg/*l* of MLSS and a mixing time of 90 min. The lowest removal efficiency (23%) was obtained at the lowest values of all the three variables. This proves that all three variables have significant effects on TP removal efficiency, as shown in Fig. 7(d), the perturbation plot.

Fig. 7(a)-(c) shows an increase in the aeration time from 3 to 4 h causes a decrease in the anaerobic condition, whereby at this timing the phosphorus accumulating organisms (PAOs) accumulate poly hydro butyrate (PHB) from the volatile fatty acids (VFAs) produced. In this process, COD and BOD (as the source of VFAs) require sufficient time for acidification [34]. Another reason for the decrease in the phosphorous removal at high aeration time was due to the presence of nitrate, which inhibits the fermentation processes which produces VFAs in the anaerobic zone. Studies have shown that biomass subjected to using alternating anoxic and aerobic conditions (activated sludge was cycled between anaerobic and aerobic phases) would promote the accumulation of PAOs [14, 35]. Moreover, in this study, sludge recycle line was omitted, suggesting that external anoxic (or anaerobic) and aerobic conditions have been alternated for the biomass. However, results showed that PAOs existed in this single bioreactor. Thus, it can be concluded that there was an inner sludge recycle between the anoxic and oxic zone, which induces PAOs' accumulation. The liquid recycle flow was mainly dependent on the extent of activated sludge mixing. The flow was driven by concentration differences between the anoxic and oxic zones. Therefore, due to the possibility of inner recycle in the experiment, traditional denitrification could have been responsible for the efficient TN removal. These findings were in a good agreement with those obtained by Asadi et al. [14].

2-5. Effect of DO Concentration on TN and TP Removal

The dissolved oxygen (DO) concentration has significant impact on the success of the nitrogen and phosphorus removal process. DO plays a crucial role in nitrification, while it has an adverse impact on biological denitrification. The negative effects of high DO concentrations on the denitrification process depend on the amount and type of carbon source. As mentioned above, in this study, aerobic and anoxic condition were provided by intermittent aeration. During the aeration period, DO concentration in the reactor maintained in the range of 4-6 mg/*l*. Based on the obtained results, the DO concentration was sufficient for nitrification as proven by high $NO_3^-$ production and TKN removal. It is generally known that DO concentration above 1 mg/*l* is essential for complete dominated nitrification; at lower DO levels, oxygen becomes a limiting factor for the nitrification process [36]. The high TKN removal obtained in this study could be explained by the relatively complete nitrification in the duration of aeration. In the non-aeration phase, the DO concentration in the reactor was lower than 1 mg/*l* (0.1-1 mg/*l*) where the denitrification process was progressing. As presented in Table 4, TN and TKN removal efficiencies were stable and the average values increased up to 88% for both responses. Influent nitrite and nitrate levels were low, and generally nitrate in permeate was lower than that in the influent. However, a slight increase in the effluent and accumulation of nitrite in the reactor was found in the high aeration time. It meant that the reactor offered relatively complete nitrification and denitrification.

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 [25]. 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. From the results obtained and in the cycle tests carried out under normal operating conditions, denitrification was assumed to occur mainly through the nitrate pathway. Ideally, PAO could be selected to simultaneously reduce nitrate and perform anoxic dephosphatation. The low DO applied in the non-aeration period favored the development of denitrifying PAOs, which enhanced phosphate removal coupled to denitrification (denitrifying dephosphatation).

2-6. Mass Balance Analysis in the Optimal Condition

For a UAASFF reactor without biomass recycle, the rate of change in the substrate can be expressed as Eq. (2):

Accumulation=inflow-outflow-generation

$$\frac{dC}{dt}V = QC_0 - QC + r_cV \tag{2}$$

where, V is volume of the reactor (lit); Q is feed flow rate (*l*/d); $C_0$ is influent COD concentration (mg/*l*); C is effluent COD concentration (mg/*l*) and $r_c$ is the reaction rate.

Assuming first-order removal kinetics ($r_c=-KC$), Eq. (1) can be rearranged and written as follows:

$$C' + BC = \frac{Q}{V}C_0 \tag{3}$$

where and C′=dC/dt and B=k+Q/V

To solve Eq. (3) both sides of the expression are multiplied by the integrating factor $e^{Bt}$:

$$(C' + BC)e^{Bt} = \frac{Q}{V}C_0e^{Bt} \tag{4}$$

The left-hand side of the above expression can be written as a differential as follows:

$$(Ce^{Bt})' = \frac{Q}{V}C_0e^{Bt} \tag{5}$$

Integration of Eq. (5) yields

$$Ce^{Bt} = \frac{QC_0}{V\,B}e^{Bt} + K \tag{6}$$

But when t=0 and $C=C_0$, K is equal to

$$K = C_0 - \frac{QC_0}{V\,B} \tag{7}$$

Substitution of K in the Eq. (6) and its simplification yields the following expression:

$$C = \frac{QC_0}{V\,B}(1 - e^{-Bt}) + C_0e^{-Bt} \tag{8}$$

By solvng the Eq. (8) under steady-state condition (i.e., the accumulation rate is assumed to be zero (dc/dt=0)), we will have:

$$C = \frac{C_0}{\left(1 + k\frac{V}{Q}\right)} = \frac{C_0}{(1 + k\tau)} \tag{9}$$

The CFID bioreactor without recycle, as shown in the Fig. 1, receives wastewater with COD and TN concentration of 650 and 120 mg/*l*, respectively, in the optimal condition. In this condition, the flow rate is 16 *l*/d and the reactor effluent COD, TN and VSS are 0.022, 0.014 and 0.18 g/*l*, respectively.

The values of k for COD, TN and TP removal, in optimal condition (aeration time=4 h, mixing time=90 min, settling time=30 min and MLSS=6,000 mg/*l*), were computed to be 3.87, 1.2 and 1.39 $h^{-1}$, respectively, as shown in Table 6. The other results from mass balance analysis in the optimal condtion are summarized in Table 6. As shown in the table, VSS produced, COD removed, observed yield, oxygen used per unit of COD removed, TN removed and $N_2$ produced from denitrification were calculated to be 2.88 gVSS/d, 10.045 g COD/d, 0.286 g VSS/g COD removed, 0.6 g $O_2$/g

**Table 6. Kinetic parameters based on the mass balance model in the optimal condition**

| Response | Variables (optimum condition) | Influent parameters | Effluent parameters, mg/l | k, $h^{-1}$ | VSS produced, gVSS/d | COD removed, g/d | $Y_{obs}$, g VSS/g COD removed | Oxygen used per unit COD, g $O_2$/g COD | TN removed, g/d | $N_2$ produced from denitrification, g/d |
|---|---|---|---|---|---|---|---|---|---|---|
| COD | HRT=6 h<br>Aeration time=4 h<br>Mixing time=90 min<br>Settling time=30 min<br>MLSS=6000 mg/l | $COD_{in}$=650 mg/l<br>TN=120 mg/l<br>TP=19 mg/l<br>Flow rate=16 l/d | $COD_{in}$=22<br>TN=14<br>TP=2.03<br>VSS=180 | 3.87 | 2.88 | 10.045 | 0.286 | 0.6 | 1.69 | 1.33 |
| TN | | - | - | 1.2 | - | - | - | - | - | - |
| TP | | - | - | 1.39 | - | - | - | - | - | - |

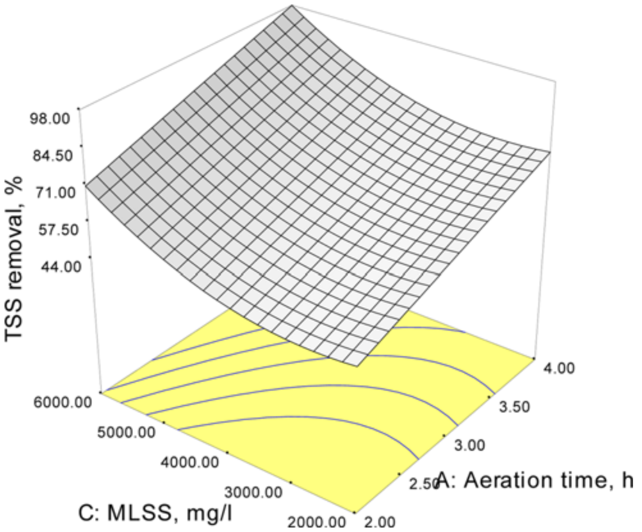



**Fig. 8. 3D surface plot for TSS removal efficiency with respect to aeration time and MLSS at a constant value of mixing time (90 min).**

COD, 1.69 g TN/d and 1.33 g/d, respectively.

2-7. TSS Removal

Fig. 8 shows the removal of TSS. More than 98% (actual value) of removal was achieved at the following conditions of reaction time (4 h), MLSS (6,000 mg/l) and mixing time (90 min). Drastic increase was observed in the TSS removal by increasing the MLSS concentration. The removal efficiency of TSS was reported low at minimum levels of reaction time and MLSS concentration and maximum level of mixing time. The high value of the biomass concentration causes an increase in the sludge volume index (SVI). In this study, high values of the SVI were found in the high values of the biomass concentration because of decrease in dissolved oxygen concentration and F/M ratio. Low-DO bulking is brought about by filamentous bacteria such as Sphaerotilusnatans. They begin to predominate when the dissolved oxygen concentration is not high enough to allow good oxygen penetration into the flock [37].

2-8. Process Optimization and Verification

Graphical optimization produces an overlay contour plot that demonstrates the feasibility area for the responses. The optimum region was identified based on nine critical responses, which were adopted as shown in Table 7. The shaded area in the overlay plots is the region that meets the proposed criteria. Fig. 9 shows the graphical optimization as a function of aeration time and mixing time

**Table 7. The optimization criteria for chosen response**

| Response | Limits | Unit |
|---|---|---|
| COD removal | >90 | % |
| BOD removal | >90 | % |
| TN removal | >80 | % |
| TKN removal | >80 | % |
| N-organic removal | >80 | % |
| Effluent nitrate | <6 | mg/l |
| Effluent nitrite | <3 | mg/l |
| TP removal | >90 | % |
| TSS removal | >90 | % |

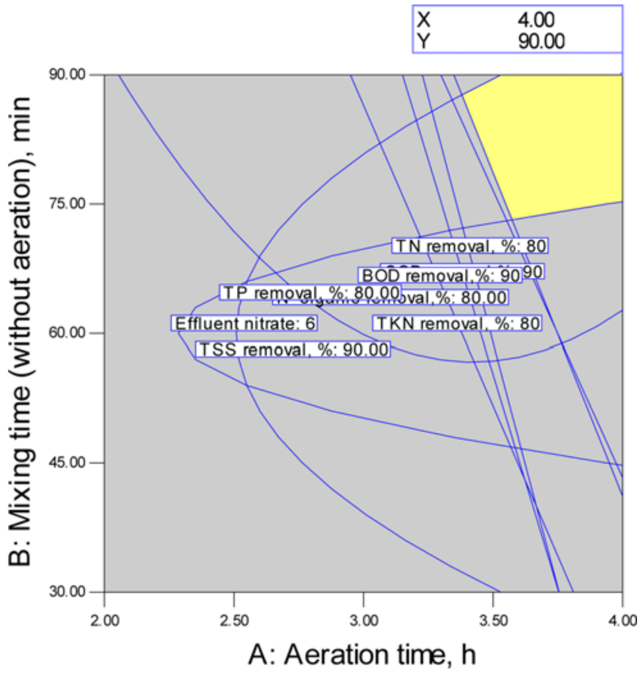



**Fig. 9. Overlay plots for the optimal regio.**

**Table 8. Verification experiments at optimum conditions**

| Run | | Conditions | Responses | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | COD removal (%) | BOD Removal (%) | TN removal (%) | TKN removal (%) | N-organic removal (%) | Effluent nitrate (mg/*l*) | Effluent nitrite (mg/*l*) | TP removal (%) | TSS Removal (%) |
| 1 | **Experimental values** | Aeration time=4 h | **96.58** | 98.3 | 88 | 88.5 | 90.3 | 4.8 | 1.7 | 89.28 | 94.9 |
| | **Model response with Cl 95%** | Mixing time=90 min | 92.8 | 95.4 | 83 | 85.6 | 89.2 | 5.1 | 1.64 | 86 | 90.6 |
| | **Error** | MLSS=6,000 mg/*l* | | | | | | | | | |
| | **Standard deviation** | | −3.9 | −2.9 | −5.6 | −3.2 | −1.2 | +6.25 | −3.5 | −3.6 | −4.5 |

with a constant value of MLSS concentration (6,000 mg/*l*). The yellow highlighted area satisfies the constraints, while the area that does not meet the criteria is gray. The optimal region enclosed by the aeration time (3.5-4 h) and mixing time (75-90 min) boundary is at the MLSS concentration of 6,000 mg/*l*. To verify the accuracy of the models, a point within the optimum region was chosen (conditions shown by flags in Fig. 9). The bioreactors were operated accordingly to compare the actual responses with the predicted values. Table 8 presents the results of this experiment conducted within the optimum regions (Fig. 9). The accuracy of the optimum conditions, found for each response from the DOE experiments, was tested using standard deviation. As a result, the experimental findings were in close agreement with the prediction of the model.

## CONCLUSION

A continuous feeding up-flow bioreactor with an intermittent regime in aeration and discharge has been successfully designed, fabricated and operated for simultaneous removal of carbon, nitrogen and phosphorus (CNP) from HWW. It was found that, the bioreactors could achieve high CNP removal efficiency in a short duration. The RSM results demonstrated the effects of the operating variables as well as their interactive effects on the responses. Experimental findings were in close agreement with the prediction of the model. Besides, the intermittent aeration process was the key factor in achieving biological nutrients removal in a single bioreactor. This system could achieve high process performance in terms of COD (95%), BOD (98.3%), TN (88%), and TP (92%) removal with a shorter HRT.

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