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BIOLOGICAL TREATMENT OF A SYNTHETIC DAIRY WASTEWATER IN A SEQUENCING BATCH BIOFILM REACTOR: STATISTICAL MODELING USING OPTIMIZATION USING RESPONSE SURFACE METHODOLOGY

In this study, the interactive effects of initial chemical oxygen demand (COD$_{in}$), biomass concentration and aeration time on the performance of a lab-scale sequencing batch biofilm reactor (SBBR) treating a synthetic dairy wastewater were investigated. The experiments were conducted based on a central composite design (CCD) and analyzed using response surface methodology (RSM). The region of exploration for treatment of the synthetic dairy wastewater was taken as the area enclosed by the influent comical oxygen demand, COD$_{in}$ (1000, 3000 and 5000 mg/l), biomass concentration (3000, 5000 and 7000 mg VSS/l) and aeration time (2, 8 and 18 h) boundaries. Two dependent parameters were measured or calculated as response. These parameters were total COD removal efficiency and sludge volume index (SVI). The maximum COD removal efficiencies (99.5%) were obtained at COD$_{in}$, biomass concentration and aeration time of 5000 mg COD/l, 7000 mg VSS/l and 18 h, respectively. The present study provides valuable information about interrelations of quality and process parameters at different values of the operating variables.

Keywords: sequencing batch biofilm reactor (SBBR); dairy wastewater; statistical modeling; response surface methodology (RSM).

The dairy industry is one of the major sources of water pollution in the all countries, which generates strong wastewaters characterized by high BOD$_5$ and COD values representing their high organic content. Therefore, an appropriate treatment approach is required prior to disposal into receiving water bodies. So far, several biological treatment systems have been used such as activated sludge system, anaerobic pond, oxidation pond, trickling filter, and combined attached and suspended growth systems [1].

The sequencing batch reactor (SBR) process possesses many advantages over the continuous activated sludge processes and has been widely used in practice [2]. The SBR system might be suitable to treat milk industry wastewater because of its ability to reduce nitrogen compounds by nitrification and denitrification [3-5], but the SBR system still has some disadvantages such as high excess sludge produced and high sludge volume index [6]. The SBR can be combined with biofilm growth on the surface of a support material, originating the sequencing batch biofilm reactor (SBBR). In SBBR systems, high concentrations of biomass can be maintained independently of settling characteristics of the biological aggregates and hydraulic retention time of the reactor [7].

Sirianuntapiboon and co-workers reported that a SBBR system could increase COD removal efficiency, improve sludge quality, reduce the amount of excess bio-sludge, and also reduce acclimatization period of the system. Startup of the SBBR system was 2-3 days faster than the SBR system in reaching steady state [8]. Another study carried out on SBBR

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proved that biofilm configured sequencing batch reactor (SBR) showed comparatively higher efficiency to the corresponding suspended growth systems [9]. Sulfate removal efficiency of 20% was also observed due to the prevailing anoxic microenvironment during the sequence phase operation and the existing internal anoxic zones in the biofilm [9].

Treatability of dairy wastewater with influent COD concentration of 427-1384 mg l^{-1} was evaluated with the help of a cross flow medium trickling filter [10]. A decrease in COD removal efficiency was observed when hydraulic loading rate increased. In another study, a SBR system was examined for treatment of milk industry wastewater. The optimal removal efficiency of the SBR system with milk industry wastewater was noted at a low OLR of 80 g BOD_5 m^{-3} d^{-1}. The COD and BOD removal efficiencies of the SBR system were about 5-7% higher than conventional SBR under the same organic loading condition [3]. In an earlier work published elsewhere [11], the dairy wastewater treatment process in a SBR was analyzed and optimized. The optimum conditions (where more than 90% and 70 ml/g, respectively for COD removal and SVI were achieved) were determined to be more than 4200 mg/l for MLVSS and 72 ml/g for SVI.

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for analyzing the effects of several independent variables on the response [10]. RSM has an important application in the process analysis and optimization as well as the improvement of existing design. This methodology is practical as it arises from experimental methodology, which includes interactive effects among the variables and, eventually, it depicts the overall effects of the parameters on the process [12]. Its greatest applications have been in industrial research, particularly in situations where a large number of variables influence the system feature. This feature termed as the response and normally measured on a continuous scale, represents the most important function of the system [13,14].

This study aims to study the treatability of a stimulated dairy wastewater in a SBBR, model the effect of three variables (influent COD, biomass concentration and aeration time) on the performance of the reactor and optimize the system using response surface methodology (RSM).

MATERIALS AND METHODS

Synthetic dairy wastewater (SDW)

The synthetic dairy wastewater (SDW) was prepared in the laboratory using dry milk powder supplied from BIOMIL (www.behdashtkar.com/pages/en//biomil-plus.htm).

The used milk powder was composed of proteins (12.5 g/1000 g powder), carbohydrate (54 g/100 g), fat (28 g/100 g), and inorganic matters (3 g/100 g, including Na 175 mg, K 480 mg, Ca 340 mg, Cl 300 mg, P 190 mg, Mg 41 mg, Fe 6 mg, Zn 3.8 mg, Cu 400 µg, Mn 30 µg, Se 10.5 µg, etc.). SDW samples were prepared based on the three different concentrations of COD_{in} (1000, 3000 and 5000 mg/l). The actual COD values have been verified each time before initiation of experimental work.

Bioreactor configuration and start up

The schematic of the experimental setup used is shown in Figure 1. This glass bioreactor was fabricated with length, width and height 10, 10 and 30 cm, respectively. The effective working volume (total liquid volume excluding volume of the plastic media) was 2 l. The voidage of the packed-bed reactor was 90% and the specific surface area of the packing material was 500 m^2/m^3. Fifty percent of the reactor volume was filled by the packing media. The SBBR was operated under room temperature (24±2 °C). The reactor was inoculated with an activated sludge taken from an aeration tank (municipal wastewater treatment plant, Kermanshah, Iran). The inoculums had a total suspended solids (TSS) concentration of 5.8 g/l. Biofilm development on the packing was done by feeding the SBBR with a 3000 mg O_2 l^{-1} of SDW and aeration time of 10 h.

The sequence of the SBBR operation was controlled by a pre-programmed timer. Each operation cycle was comprised of four phases. In the first phase, the reactor was fed during 30 min; in the second phase, the feeding was stopped and aeration began (at constant aeration rate, 4 l/min); the third phase, settling, lasted 20 min; and finally in the fourth phase, the effluent was withdrawn during 5 min. At the beginning of each cycle, immediately after withdrawal (earlier sequence), a pre-defined feed volume (1 l) was pumped into the system. At the end, biomass (including suspended and attached biomass) settled and effluent was withdrawn from the reactor. As the biomass concentration was a variable in this study, biomass content of the reactor was maintained constant by removing surplus biomass after each cycle.

Wastewater feeding and withdrawal were done with the help of peristaltic pumps and control valve in the middle ports of the reactor. The air was introduced into the reactor with bubble air diffusers at the bottom of the reactor, and the air flow rate and aeration time was controlled with an air flow-meter and a timer that

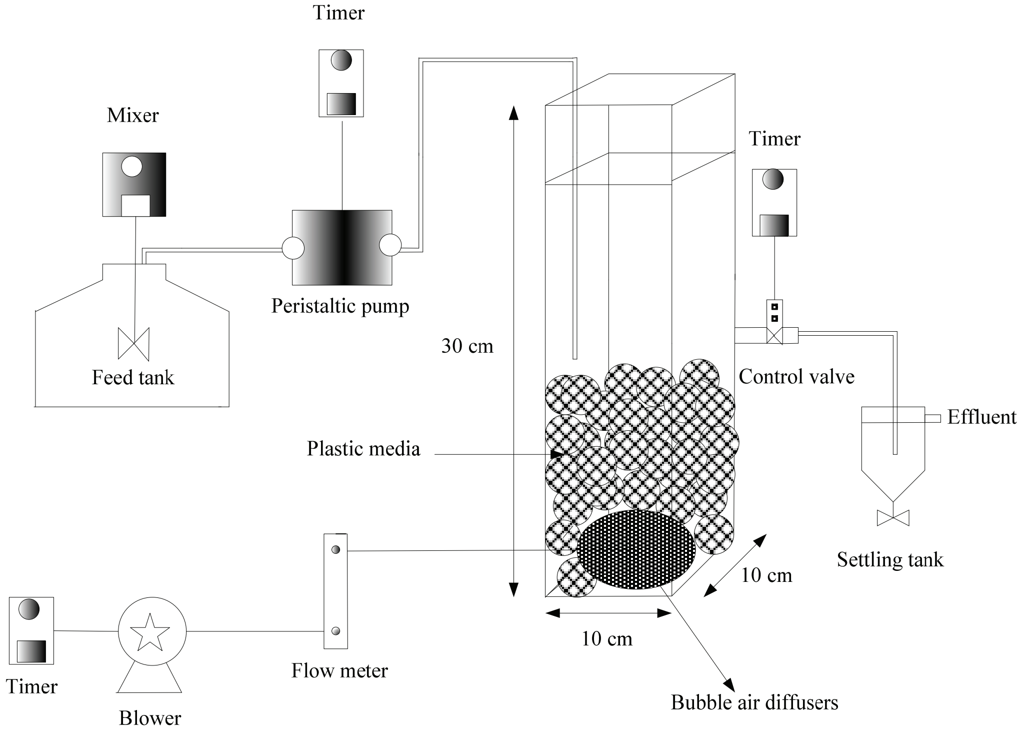


Figure 1. Experimental setup.

connected to blower. The excess sludge was removed during the draw and idle period to control biomass concentration of the system. Different biomass concentrations in the SBBR were supplied by adding the acclimatized suspended sludge. It is noted that the biomass reported in this paper consists of attached biofilm and volatile suspended solids.

Experimental design and mathematical model

Variables evaluation

Biological wastewater treatment of dairy industries depends on a multitude of variables. Among these, six main factors (which affect the efficiency of reactor) are aeration time, COD_{in}, biomass concentration, type of packing, temperature, and pH [15-19]. In this study, COD_{in}, biomass concentration and aeration time were chosen as independent and the most critical operating factors due to the following reasons:

1. Dairy wastewater is distinguished by relatively high BOD and COD contents, so that, COD concentration of dairy wastewater ranging from about 1000 to more than 5000 mg/l [20]. Therefore, in this study, the performance of a SBBR treating a synthetic dairy wastewater was examined in the above-mentioned range (Table 1).

2. Biomass concentration was varied to determine the optimum conditions that could achieve maximum COD removal and minimum sludge volume index (SVI).

3. The most important parameter affecting the “cost” of biological treatment system is aeration 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 aeration time to produce the required effluent quality will result in an optimal cost. The range studied for aeration time is 2-18 h as shown in Table 1.

Table 1. Experimental range and levels of the independent variables

Variables	Range and levels		
	-1	0	1
COD_{in}, mg/l	1000	3000	5000
Biomass concentration, mg/l	3000	5000	7000
Aeration time, h	2	10	18

Design of experiment

The statistical method of factorial design of experiments (DOE) eliminates systematic errors with an estimate of the experimental error and minimizes the number of experiments [21]. The RSM used in the present study was a central composite face-centered design (CCFD) involving three different factors, COD_{in}, biomass concentration and aeration time. The bioreactor performance in dairy wastewater treatment was assessed based on the full face-centered CCD experimental plan (Table 2). The design consisted of 2^k

Table 2. Experimental conditions and results of central composite design

Run no.	Variable			Responses	
	Factor 1	Factor 2	Factor 3	COD removal, %	SVI, ml/g
	$\mathrm{COD_{in}}$, mg/l	Biomass concentration, mg/l	Aeration time, h		
1	3000	5000	10	95.67	76.36
2	3000	7000	10	96.67	85.32
3	5000	3000	18	93.00	74.93
4	1000	5000	10	95.00	81.91
5	3000	5000	18	97.33	81.31
6	3000	5000	10	94.00	78.41
7	3000	5000	10	93.00	75.00
8	1000	3000	2	52.00	87.69
9	1000	7000	18	97.00	104.43
10	5000	3000	2	45.00	90.00
11	3000	5000	10	97.00	74.00
12	3000	3000	10	86.67	74.85
13	3000	5000	10	98.5	83.00
14	1000	7000	2	55.00	115.0
15	1000	3000	18	98.00	88.16
16	5000	5000	10	93.00	67.56
17	5000	7000	2	64.00	107.72
18	3000	5000	2	61.67	75.34
19	3000	5000	10	94.50	65.00
20	5000	7000	18	99.50	90.02

factorial points augmented by 2*k* axial points and a center point where *k* is the number of variables. The three operating variables were considered at three levels namely, low (-1), central (0) and high (1). Accordingly, 20 experiments were conducted with 15 experiments organized in a factorial design (including 7 factorial points, 7 axial points and 1 center point) and the remaining 5 involving the replication of the central point to get good estimate of experimental error. In order to carry out a comprehensive analysis of the reactor, 2 dependent parameters were either directly measured or calculated as response. These parameters were COD (TCOD) removal and SVI.

Mathematical modeling

After conducting the experiments, the coefficients of the polynomial model were calculated using the following equation [22]:

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

where *i* and *j* are the linear and quadratic coefficients, respectively, and β 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 and their respective contour plots were obtained based on the effect of the levels of the three factors. From these three-dimensional plots, the simultaneous interaction of the three factors on the responses was studied. The experimental conditions and results are shown in Table 2.

Chemical analysis

The concentrations of COD, volatile suspended solids (VSS) and sludge volume index (SVI) of the system were determined by using standard methods for the examination of water and wastewater [23]. The sludge volume index (SVI) is the volume in millimeters occupied by 1 g of a suspension after 30 min settling. SVI was measured according to Standard Methods (2710 D) [23].

The dry weight of the attached biofilm per unit wetted surface area of packing was evaluated by drying the removable section of packing before and after the biofilm attachment, at a temperature of 80 °C for 24 h. The difference between the weight measurements was divided by the wetted surface area of the packing.

RESULTS AND DISCUSSION

Statistical analysis

The ANOVA results for all responses are summarized in Table 3. In order to quantify the curvature effects, the data from the experimental results were fitted to higher degree polynomial equations i.e. quadratic. In the Design Expert software, the response data were analyzed by default. Some raw data might not be fitted and transformations which apply a mathematical function to all the response data might be needed to meet the assumptions that make the ANOVA valid.

The model terms in the equations are those 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 (<0.0001). It is shown that the model terms were significant at the 99% confidence level. The square of correlation coefficient for each response was computed as the coefficient of determination (R^2). 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 [22]. The predicted *versus* actual plot for the eight responses is shown in Figure 2. It shows that the actual values are distributed close to the straight line ($y = x$) with relatively high values of R^2.

The models adequacy was tested through lack-of-fit F-tests [24]. 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 4 or more [25]. The value was found to be desirable for the all models. Simultaneously, low values of the coefficient of variation (CV) (1.98-84.25) indicated good precision and reliability of the experiments as suggested by Ahmad *et al.* [26]. Detail analysis on the models is presented in the following sections.

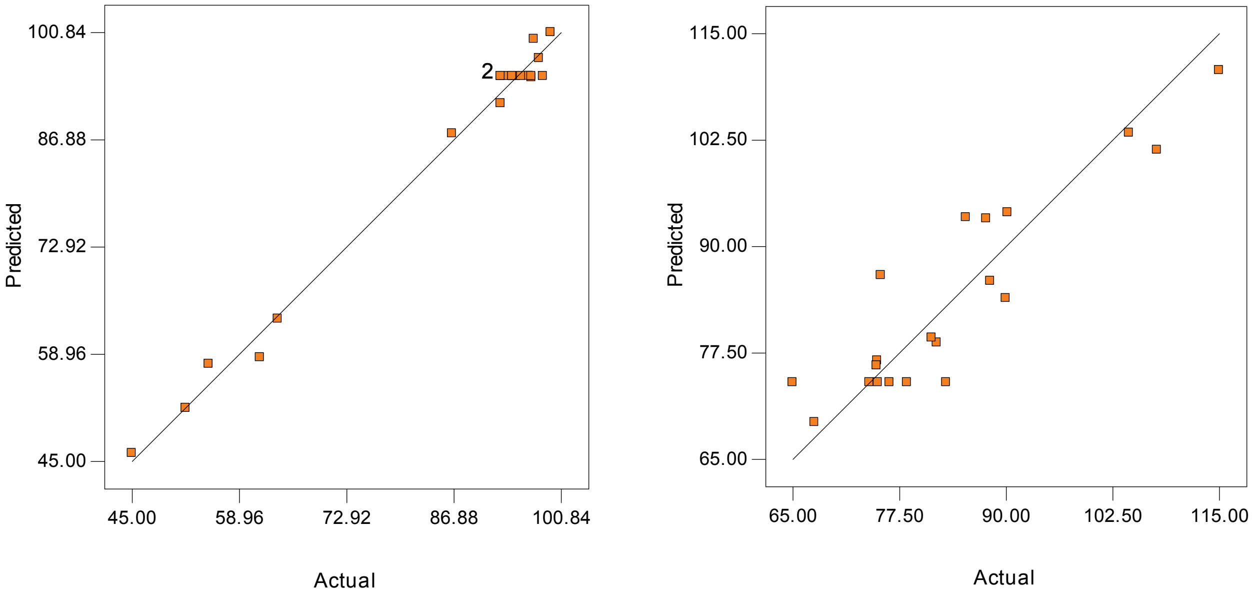
Process analysis

COD removal

In order to investigate the effects of the variables studied on COD removal efficiency, dependency of this response to the variables was analyzed and modeled. From the analysis carried out (Table 3), a reduced quadratic model was selected to describe

Table 3. ANOVA results for the equations of the Design Expert 6.0.6 for studied responses; modified quadratic model (R^2: determination coefficient, SD: standard deviation, CV: coefficient of variation, PRESS: predicted residual error sum of squares)

Response	Probability	R^2	Adjusted R^2	Adequate precision	SD	CV	PRESS	Probability for lack of fit
COD removal efficiency	<0.0001	0.9901	0.9855	42.23	2.19	2.57	175.20	0.4176
SVI	<0.0001	0.8282	0.7668	12.04	6.27	7.48	1247.3	0.4578



(a) (b)

Figure 2. Actual versus predicted values for (a) COD removal efficiency, (b) SVI.

the variation of the response. The model terms, B, C, B^2, C^2, AB and BC are significant factors. Other model terms are not significant (with a probability value larger than 0.05). In order to simplify the model, these model terms (A, A^2 and AC) were eliminated. The regression equation obtained in terms of coded factors for COD removal is presented in the below:

$$\text{COD removal (\%)} = 95.15 + 3.75B + 20.72C - 3.74B^2 - 15.91C^2 + 2.94AB - 2.06BC \quad (2)$$

In Eq. (2), A, B and C correspond to independent variables of COD_{in}, biomass concentration and aeration time, respectively. As it is noted in Eq. (2), the main-order effects of biomass concentration and aeration time had positive impacts on the response while second-order and two-level interactions effects of these variables had negative impacts on the response.

ANOVA results of the quadratic model presented in Table 3 indicate that the model can be used to navigate the design space. In order to confirm the selected model, the diagnostic plots such as the predicted *versus* actual value and normal probability plot of the studentized residual provided by the Design Expert software (ver. 6.0.7) were prepared to judge the model adequacy. In Figure 2a, the values of R^2 and adjusted R^2 were evaluated as 0.99 and 0.98, respectively, showing a very good agreement between the predicted and actual data.

In order to gain a better understanding of the interaction effects of variables on COD removal efficiency, two and three dimensional contour plots for the measured response were formed based on the model (Eq. (2)). Figures 3a-3c shows the plots of the model for variation in COD removal as a function of COD_{in} (A) and biomass concentration (B) with three different aeration time (2, 10 and 18 h). As can be seen in Figures 3a-3c, almost the same trends were found as the aeration time changed from 2 to 18 h. It is clear from the figures and Eq. (2) that the most significant factor on the response is aeration time. So that, as the aeration time increased the response was increased. It should be noted that the aeration time did not have significant effect on the response at the values more than 10 h. It is proven by perturbation plot (Figure 4a). The perturbation plot shows the comparative effects of the variables on the response. A steep curvature in aeration time, C curve, shows that the response was very sensitive to this factor. The comparatively semi-flat A and B curves show less sensitivity of the COD removal efficiency to alter with respect to a change in aeration time. In other words, the COD_{in} and biomass concentration (in the range studied) have no major function in the treatment process when comparing aeration time. The response surface plot of COD removal efficiency at aeration time 2 h (Figure 3a) showed that an increase biomass concentration yielded an increase in the response while increase in the COD_{in} did not have significant effect on the response. The value of COD removal predicted by the model reached its highest level at 63% when COD_{in}, biomass concentration and aeration time were 5000 mg/l, 7000 mg/l and 2 h, respectively. As noted in Figures 3b and 3c, a reverse impact of increasing biomass concentration on COD removal was observed as the variable increased. In Figures 3a and 3b, an increase in MLSS (from 3000 to 6000 mg/l) caused an increase in the response, while at higher biomass concentration (from 6000 to 7000 mg/l), an increase in the variable decreased the response. The maximum modeled COD removal was 101.3% (with standard deviation of 2.19) at COD_{in}, biomass concentration and aeration time 5000 mg/l, 6000 mg/l and 18 h, respectively. Meanwhile, the minimum predicted response (46.03 %) was obtained at COD_{in}, biomass concentration and aeration time of 5000 mg/l, 3000 mg/l and 2 h, respectively.

A sequencing batch flexible fibre biofilm reactor has been 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 [20]. An inverse relationship between OLR and COD removal efficiency has been observed in this study [20]. The SBBR system achieved a higher COD removal compared with the previous studies [3,27,28] due to the high amount of biomass in the reactor volume as a combination of attached and suspended biomass. This should be noted that the plastic media used had a high specific surface area providing a much higher support area per unit of reactor volume.

Sludge volume index (SVI)

As the SBBR system was operated under relatively high concentration of biomass (3000-7000 mg/l), therefore in addition of biological activity of the sludge, its physical characteristic was of high significance. Many researchers recognize SVI as the best parameter characterizing sludge settling properties. SVI is also a good indicator of sludge bulking. A proper SVI value, especially below 100 mg/l, is of major im in the activated sludge systems [29].

ANOVA results for SVI are shown in Table 3. A reduced quadratic model was fitted with the experimental data (Eq. (3)). The reduced quadratic model shows that the main effect of the variables and second-order effects of biomass concentration (B) and aeration time (C) are significant model terms on SVI.

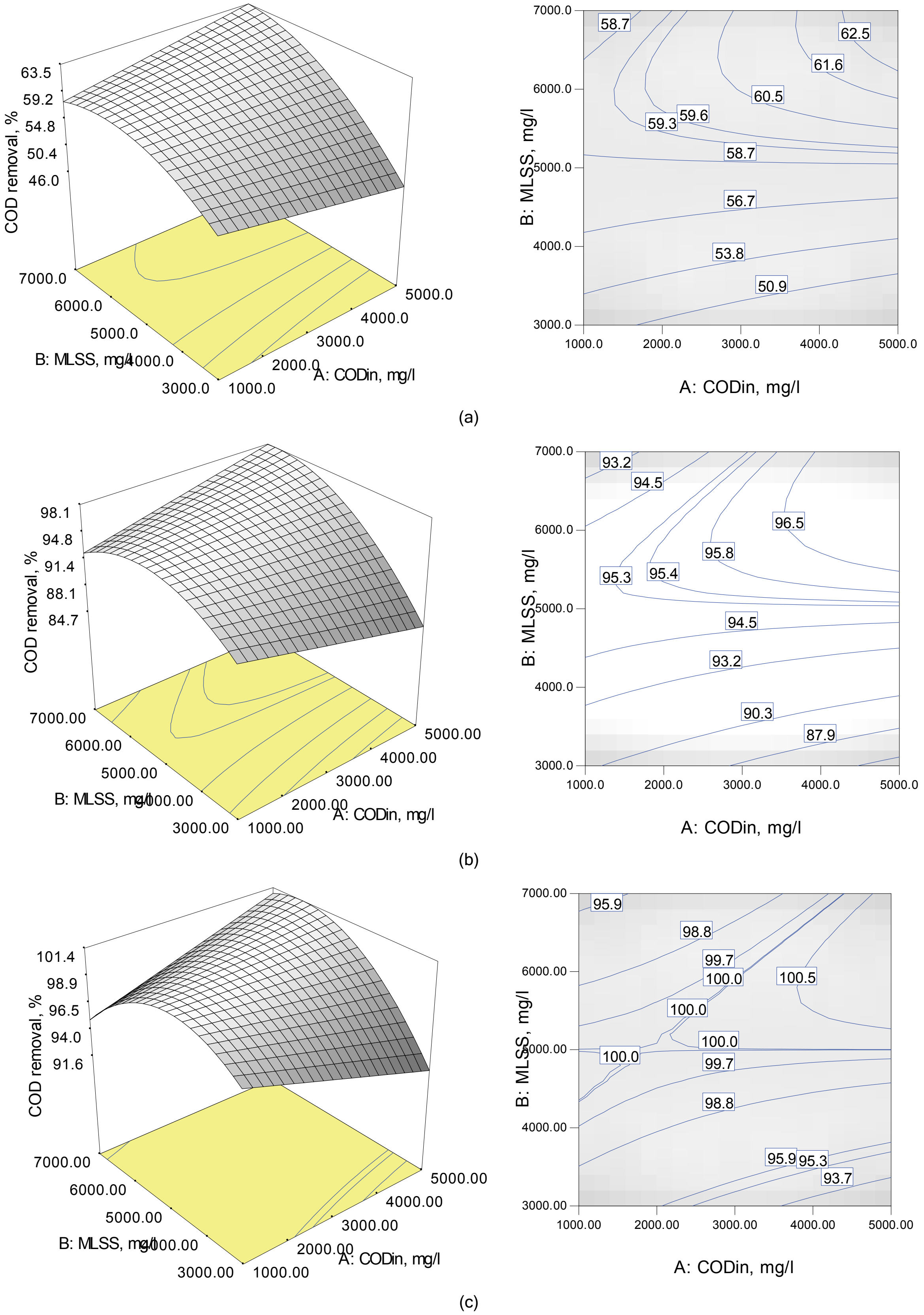


Figure 3. 3D and contour plots for COD removal efficiency with respect to COD_{in} and biomass conc. at constant value of aeration time; aeration time: a) 2, b) 10 and c) 18 h.

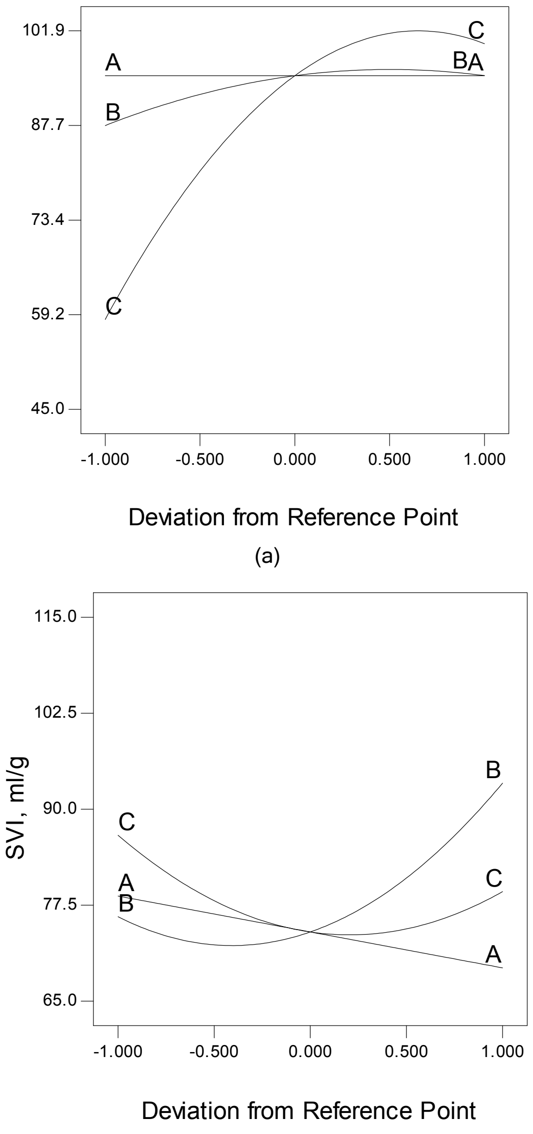


Figure 4. Perturbation plots; a) COD removal efficiency, b) SVI.

Other model terms such as A^2, AB, AC and BC are not significant (with a probability value larger than 0.05). Therefore, these model terms were excluded from the study to improve the model.

$$\text{SVI (mg/l)} = 74 - 4.68A + 8.70B - 3.67C + 10.68B^2 + 8.93C^2 \quad (3)$$

Figure 2b shows the predicted *versus* actual values for this response. It shows a relatively good agreement between predicted and actual values. The two- and three-dimensional contour plots were made as a function of *A* and *C* of the system, with three different B in Figure 5. From the results, there was no major interaction between the three independent factors *(AB*, *AC* and *BC*) on SVI in the design space.

As can be seen in the Figure 5, the same trends were found as the biomass concentration changed from 3000 to 7000 mg/l. It is clear from the perturbation plot (Figure 4b) that the most significant factor was determined to be biomass concentration. Increase in the biomass concentration resulted in an increase in SVI with main and second order effects. Although, biomass concentration did less significant effect on the SVI in the ranges between 3000 to 5000 mg l^{-1} but high value of the biomass concentration (7000 mgl^{-1}) causes an increase in the SVI. In this study, high values of the SVI were found at the high values of the biomass concentration because of decrease in dissolved oxygen concentration and F/M ratio (0.24 gCOD/gVSS.d). Low-DO bulking is brought about by filamentous bacteria such as *Sphaerotilus natans*. They begin to predominate when the dissolved oxygen concentration is not high enough to allow good oxygen penetration into the floc [30].

A reverse impact of increasing aeration time on the SVI was observed as the variable increased. As depicted in Figures 4b and 5, an increase in the aeration time (up to 10 h) at a constant value of COD_{in} yielded a decrease in the response. Whereas by progressing aeration time, increase in the SVI was started due to a decrease in F/M ratio. As noted in Figures 5a-5c, increase in COD_{in} at a constant value of aeration time (from 2 to 10 h) did not have significant effect, while at aeration time longer than 10 h showed a reducing effect on the response.

The SVI values obtained in this study (74-108 mg/l) were rather low compared to the results reported by other authors, demonstrating that the SBBR as integrated fixed-film activated sludge takes the advantages of both attached and suspended growth systems [30-32].

Process optimization

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 two critical responses (COD removal and SVI), which criteria were adopted as shown in Table 4. The shaded area in the overlay plots is the region that meets the proposed criteria. Figure 6 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 yellow, while the area that does not meet the criteria is gray. The optimal region enclosed by the COD_{in} (1500-5000 mg/l) and biomass concentration (5000-6000 mg/l) boundary at the aeration time of 7 h.

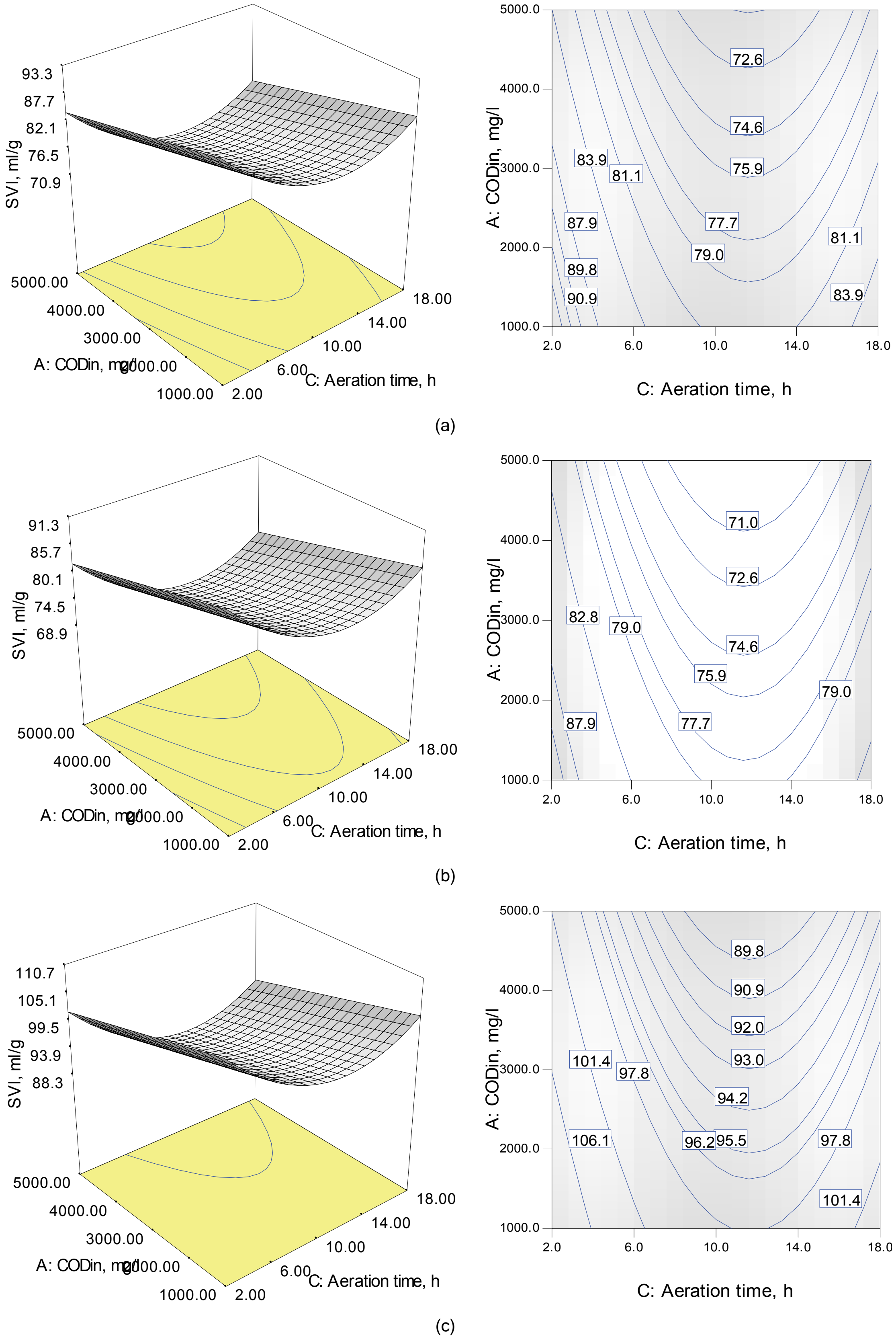


Figure 5. 3D and contour plots for SVI with respect to COD$_{in}$ and aeration time at constant value of biomass concentration; biomass concentration: a) 3000, b) 5000 and c) 7000 mg/l.

Table 4. The optimization criteria for chosen responses

Response	Limits	Unit
TCOD removal efficiency	>85	%
SVI	<80	ml/g

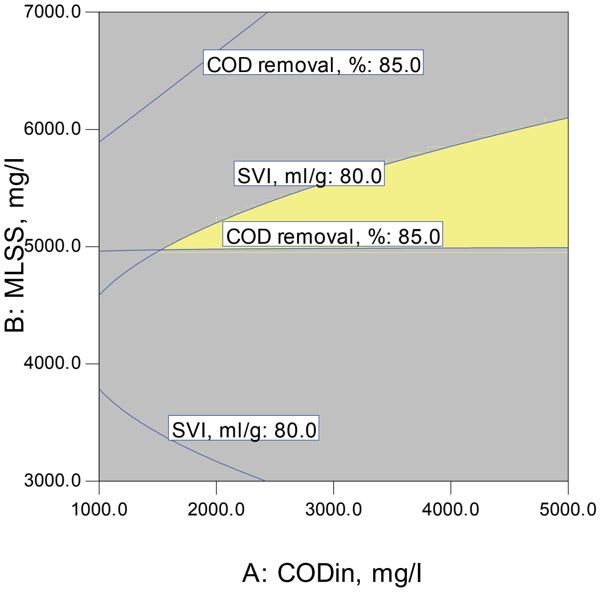


Figure 6. Overlay plots for optimal region, biomass concentration vs. COD$_{in}$ at aeration time of 7 h.

CONCLUSIONS

The SBBR was a successful, reliable and promising biological treatment system to achieve high COD removal efficiency from a synthetic dairy wastewater in a short period of time. The most effective operational factors on the COD removal and SVI were determined to be aeration time and biomass concentration, respectively. The optimal region obtained from the models enclosed by the COD$_{in}$ (1500-5000 mg/l) and biomass concentration (5000-6000 mg/l) boundary at aeration time of 7 h.

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NAUČNI RAD

BIOLOŠKA OBRADA MODELA OTPADNE VODE MLEKARE U SEKVENCIONOM ŠARŽNOM REAKTORU SA BIOFILMOM: STATISTIČKO MODELOVANJE OPTIMIZACIJOM POMOĆU METODE POVRŠINE ODZIVA

U ovom radu istraživani su interaktivni efekti početne hemijske potrošnje kiseonika (HPK_{in}), koncentracije biomase i aeracionog vremena na performance laboratorijskog sekvencionog šaržnog reaktoru sa biofilmom (SŠRB) u kome je obrađivana sintetička otpdna voda mlekare. Korišćen je centralni kompozitni dizajn eksperimenata, koji su analizirani metodom površine odziva. Oblast istraživanja obrade sintetičke otpadne vode je bila ograničena vrednostima ulaznog HPK_{in} (1000, 3000 i 5000 mg/l)), koncentracije biomase (3000, 5000 ind 7000 mg/l) i aerationog vremena (2, 8 i 18 h). Merena su ili izračunavana dva zavisna parametra kao odziv. Ovi parametri su bili ukupna efikasnost izdvajanja HPK i indeks zapremine mulja (IZM). Mskismalna efikasnost izdvajanja HPK (99,5%) je dobijena pri HKP_{in}, koncentraciji biomase i aeracionog vremena od 5000 mg O_2/l, 7000 mg/l i 18 h, redom. Ovo istraživanje daje vredne informacije o kvalitetu i parametrima procesa pri različitim operativnim promenljivima.

Ključne reči: ekvencioni šaržni reaktor sa biofilmom; otpadna voda mlekare; statističko modelovanje; metoda površine odziva.