Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2016.1141372

57 (2016) 24240–24256
November

Taylor & Francis
Taylor & Francis Group

Optimization of several hydrodynamic and non-hydrodynamic operating parameters in treatment of synthetic wastewater containing wheat starch in a sequencing batch reactor (SBR) using response surface methodology

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Received 9 February 2015; Accepted 5 January 2016

ABSTRACT

Synthetic wastewater containing wheat starch was treated using sequencing batch reactor (SBR) system working with bio sludge extracted from cow dung. In the first stage, full factorial design was used to investigate the impact of four hydrodynamic variables on process responses and optimum condition was detected. In the second stage, the most important hydrodynamic factor together with four different factors comprised a new design based on the response surface methodology and central composition design. Removal efficiency of starch (COD removal), effluent, and sludge quality were assessed as process responses. Impeller diameter and reactor geometry did not show strong impact on responses. Both designs showed superior results in predicting SBR performance. The analyses revealed that interactions between variables are crucially important and should be accounted in wastewater management. Moreover, it was attained that independent factors have diverse effects on responses which intensifies the necessity of optimization on all dependent parameters. In the optimum condition of the second design, COD removal of 92%, TSS of 38 mg/L, and SVI of 57 ml/g were obtained.

Keywords: Synthetic starch wastewater; SBR; RSM; Full factorial design; Optimization

1. Introduction

Starch processing plants produce tons of dilute wastewater with high COD, BOD, and SS [1] and as a source of contamination that can be harmful for environment [2]. Different varieties of physical [3–5], biological [6–12], and hybrid [13,14] treatments have been used to treat starch-containing wastewater. Moreover, utilization of sequencing batch reactor (SBR) in bio-hydrogen production from starch feedstock has been reported [15].

Wheat provides more than 20% of the world population calories which were produced 611 million tons per year between 2004 and 2007 and used as feed, food, seed, and industrial utilization [16]. Less than 6% of total wheat is used as raw material for industrial starch production. Wheat wastewater is generated in both washing and extraction processes. About 20 m^3 wastewater yields from production of one ton of starch, which reveals the indispensability of applying effective treatment processes. In comparison with other treatment systems such as anaerobic treatment plants and membrane technology, aerobic biological treatment plants are proved promising due to their

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operational and economical advantages such as sufficient sludge production rate, operational flexibility, and high nutrient removal [17]. SBR is a modified activated sludge procedure which combines activated sludge benefits with robustness, cost effectiveness, flexibility, single basin operation, shock loading control, no sludge loss, simplicity, and high efficiency [18,19].

In most experiments, classical methods were used to determine the influence of multiple factors on treatment effectiveness which include changing one factor while the other factors are fixed at certain points. Design of Experiment (DoE) methods change all the factors in some levels and after fitting the best curve on the data points, the equation will be used to find optimum points. In addition, the interactions between factors can be observed and their importance will be reported [20].

There have been few studies on biological treatment of wastewater using response surface methodology (RSM). Zinatizadeh et al. studied the effect of three operating parameters (influent COD, biomass concentration, and aeration time) on COD removal and sludge volume index (SVI) for treatment of synthetic dairy wastewater using a sequencing batch bio-film reactor [21]. They reported that cycle time (2–18 h) was the most effective factor. In another investigation, Asadi et al. reported that high HRT has a decreasing effect on SBR performance [22]. In both reports, the optimum conditions were introduced. Aziz et al. used RSM in a SBR for treatment of landfill leachate [23]. It has been stated that low-aeration rate was sufficient to remove certain parameters.

Qian Feng et al. examined the effect of variable turbulence conditions on an SBR system. An increase subsequent to a decrease in SVI was occurred when the characterized dissipation rate increased. The effect of some other hydrodynamic factors is discussed in literature [24].

Although few reports are attainable in the literature examining the effect of hydrodynamic and other factors on COD removal and sludge characteristics, to our knowledge, an absence of an extensive report on a detailed investigation on the effect of hydrodynamic and non-hydrodynamic effects and a comparison between them looks conspicuous.

The general objective of the research is to evaluate SBR performance for treating starch-containing synthesized wastewater. Special attention is devoted to the study of the effect of several hydrodynamic and non-hydrodynamic parameters and their interactions on treatment efficiency and optimum conditions of the process by employing statistical DoE methods.

2. Materials and methods

2.1. Experimental setup and operation

In the first part of the experiment, two bench-scale reactors with different geometries were constructed as SBRs for the treatment of synthetic starch-containing wastewater. Cylindrical and cubic reactors were used in order to investigate the effect of reactor geometry on SBR performance. The area of the propellers was independent of the geometry. The reactors were made of glass (6 mm thick) and the base areas of SBRs were equal. Height, total volume of reactors, and working volumes were 40 cm, 16 L, and 8 L, respectively. Each reactor was filled with 2 L of settled sludge, 2 L of water, and 4 L of synthetic wastewater and at the first set of experiments, 4 L of supernatant was removed after settling of microorganisms, meaning that exchange volume ratio was fixed at 50%. The exchange ratio of the reactors is considered as one of the variables at the second set of experiments and was adjusted according to DoE. Cycle time was fixed in 18 h in the first experiment, including 17 h aeration, 30 min sedimentation, 5 min filling, and 25 min decanting and idle condition. The mixed liquor was aerated homogeneously using a pair of aerators (SEA STAR Ltd, ACD-800 model, China) and round flat diffusers were located at the bottom of the reactors. Aeration continued in both filling and reaction phases in order to maintain dissolved oxygen concentration above 2 mg/L. Aeration velocity was adjusted according to experimental condition. Two gear motors (NSM, 60-KTYZ Model, 50 Hz, Iran, 50 and 110 rpm) were used to blend the mixed liquor. All experiments were carried out at an optimum temperature of 25˚C. There is to ensure microbial aggregation and settle ability will be improved by adding calcium ions to the reactor [25,26], therefore, egg shell was used as a source of calcium. Egg shells were washed thoroughly, heated in 60˚C, and ground to acquire a white powder. 2 mg of egg shell powders was used for every mg/L of mixed liquor suspended solids (MLSS). The supplementation of calcium lowered the SVI from 145 to less than 100 L/mg after five cycles. In the first experiment, two impellers with different diameters were used to examine the effect of impeller diameter on SBR performance. Thus far, the impact of this factor has never been investigated. Schematic of the reactors and their parts are presented in Fig. 1.

In the second part of the experiment, optimum conditions of the first experiment were used except for the aeration rate which again was one of the variables. The aeration rate was selected for the second part to assess its importance in comparison with other influential non-hydrodynamic factors. Cycle time was

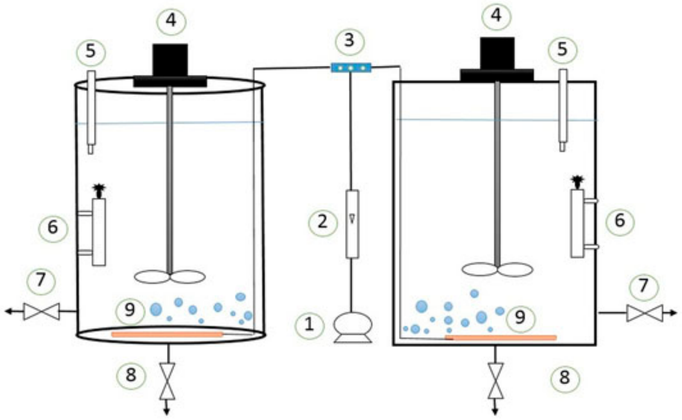

Fig. 1. Two geometries used in the study: cubic and cylindrical.
Notes: (1) air pump, (2) flow meter, (3) gas distributor, (4) mixer, (5) pH and DO meter, (6) heater, (7) treated wastewater discharge valves, (8) bio-sludge discharge valve, and (9) diffuser stones.

considered as one of the variables, therefore, aeration time varied according to cycle time. Times needed for filling, settling, decanting, and idle were identical to designed experiments of previous part. COD and exchange ratio of the cycles varied according to the design of experiments.

2.2. Synthetic wastewater composition

Starch was the sole carbon source and its concentration was identified by input COD. Urea 140 mg/L, NH_4Cl 80 mg/L, K_2HPO_4 20 mg/L, $MgSO_4.7H_2O$ 50 mg/L, and trace element solution with the concentration of 2 ml/L (2 ml of trace elements solution for each liter of synthetic wastewater) were added to develop synthetic wastewater. Trace element solution consisted of the following components: $ZnSo_4{\cdot}7H_2O$ 2 mg/L, $FeSO_4{\cdot}7H_2O$ 1 mg/L, $MnCl_2{\cdot}2H_2O$ 0.5 mg/L, and $CuSO_4{\cdot}5H_2O$ 0.5 mg/L. These concentrations of the materials were opted according to the ratio of the elements in the real wastewater and the necessary concentrations for biomass growth. Tap water was conserved for several minutes before making synthetic wastewater solution in order to avoid adding undesirable suspended solids.

2.3. Bio-sludge preparation

It is prevalent to collect microbial culture from biological wastewater treatment plants. However, in this study, cow dung was used as the source of bio sludge. Cow dung was washed, the concentrated leachate gained was passed through a sieve, and permeate was transferred to SBR. After 3 days, aggregation of bio-sludge seeds was observable. COD was raised in three stages from 300 to 600 mg/L and then to 2,000 mg/L on the day of 20. After 30 days, microbial culture was acclimatized and was ready to employment. The end of acclimatization period was detected by constant MLSS at 8,000 mg/L which is shown in Fig. 2 and constant SVI. In acclimatization period, no bacteria were removed deliberately; however, a small portion of bacteria was washed out by supernatant in decant period. In the beginning of the first set of experiments, 2 L of suspended sludge was used in 8 L volume reactors, meaning that MLSS kept constant at 2,000 mg/L at the start of subsequent experiments. Excess sludge was removed in the end of react stage; hence, the MLSS at the beginning of each cycle remained constant. The same method was used in the second set of experiments except that the MLSS was fixed at 3,000 mg/L at the beginning of every run. This concentration is neither very low so as to allow micro-organisms to perform well nor very high to cause a rapid depletion of the nutrients. MLSS shows the concentration of suspended solids in the mixed liquor, while the MLVSS indicates the concentration of the micro-organisms. Owing to minute quantities of solids in input and output wastewater, MLSS can be replaced by MLVSS in study of biomass concentration.

2.4. Analytical methods

Output COD and TSS measurement were performed on clear supernatant attained after sedimentation. Sludge-settled volume at 30 min (SV30), SVI, and MLSS were carried out on mixed liquor in the end of the reaction stage. To state the matter differently, the MLSS indicated the concentration of solids in the mixed liquor, mostly consisted of bio sludge, while

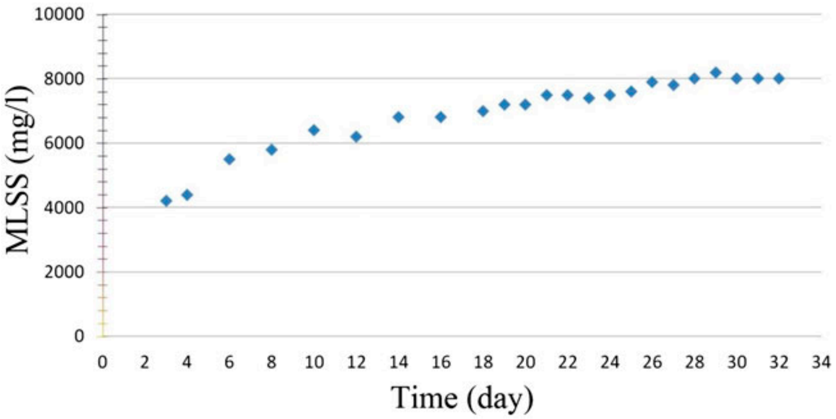

Fig. 2. MLSS concentration in acclimatization period.

TSS presented the concentration of solids in the supernatant. SVI is defined as the volume of settled sludge (ml) occupied by 1gr dry sludge solids after 30 min of settling in a 1,000 mL graduated cylinder which can be defined as the ratio of SV30 to MLSS. Filtered and unfiltered COD (unfiltered reported), TSS, SVI, and MLSS were analyzed according to standard methods for the examination of water and wastewater [27]. Inlet samples were analyzed for pH and input COD if necessary. pH and DO of the mixed liquor were monitored by electrode located in the reactor. Sludge retention time (SRT) also known as sludge age was evaluated as the ratio of MLSS to the sludge wasted everyday. SRT was altered by the amount of sludge produced in the reactor and is not presented. In certain circumstances, if the concentrations were higher than upper limit of the measuring methods, samples were diluted using distilled water. DO and temperature were measured by DO meter (AZ-8403, China) and pH was measured by pH meter (Behineh Ltd, SAT 2002 model, Iran).In the second part of the experiment, for measurement of COD removal, input COD was considered as the concentration of COD in reaction tank, instead of COD concentration in the influent. This alteration was carried out due to the variable exchange ratios. Exchange ratio controls the volume of discharged supernatant, input wastewater, and also the extent of dilution of organic matter in the moment of entrance to the tank. The alternative definition for input COD was introduced to overcome this shortcoming.

2.5. Experimental design and data analysis

The DoEs, analysis of acquired data, and optimization were performed via the Design Expert Software (version 7.0). In the first stage of this work, two-level full factorial design (2 K) was conducted. Agitation rate (A), respiration rate (B), impeller diameter (C), and reactor geometry (D) were selected as independent variables. The geometry was considered as categorical factor, while the remaining factors were numerical. According to factorial design, 16 experiments (8 experiments in cylindrical reactor and 8 experiments in cubic reactor) were proposed. Factorial design is an effective and common design used to estimate main effects and their interactions for screening factors and discovering important ones. The levels and range of variables investigated by two-level factorial are given in Table 1.

In the second stage, RSM together with central composition design (CCD) was used. This method appraises every factor in five levels including $-\alpha$, –1, 0, +1, $+\alpha$. In this work, alpha value was fixed at 1.0 (face centered) to avoid irrational points (e.g. below zero cycle time or input COD). Two blocks were opted due to the fact that experiments executed in two similar reactors, both with optimum geometry attained from previous stage. Five factors (four new factors including input COD (A), pH (B), exchange ratio (C), and cycle time (E), together with aeration rate (D) as the most significant factor obtained by full factorial design) were selected for this stage which resulted in 53 experiment consisted of 32 factorial point, 10 axial points, and 11 center points in two blocks. The level and range of each independent factor used by this method are presented in Table 2.

Final COD, MLSS, and SVI were studied as dependent factors in full factorial design, whereas MLSS was replaced by TSS and final COD by COD removal in the RSM design. In the first stage, the MLSS variation was achieved to be minor; therefore, TSS was replaced with MLSS in order to investigate the quality of the treated wastewater in the matter of the

Table 1
Types and levels of factors for the FFD

Factor	Type	Unit	Low	High
Agitation rate (A)	Numeric	rpm	50	110
Aeration rate (B)	Numeric	L/min	2.25	9
Impeller diameter (C)	Numeric	cm	8	14
Reactor geometry (D)[a]	Categoric	–	Cylindrical	Cubic

[a]Reactor geometry has no low/high levels and the types are interchangeable.

Table 2
Levels of experimental factors for the RSM

Parameter	Unit	Low	Mean	High
Input COD	mg/L	400	2,200	4,000
pH	–	4	7	10
Exchange ratio	–	0.1	0.4	0.7
Aeration rate	L/min	1	5	9
Cycle time	h	4	14	24

concentration of solids. The obtained data were used to ascertain the coefficient of model fitted to experimental points. The quadratic equations were conducted in RSM design that is described by following equation [23]:

$$y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

where y is the response, β_i, β_{ii}, and β_{ij} are the regression coefficient of linear, second-order, and interaction terms, respectively. i and j are linear and quadratic coefficients and x_i and x_j are the variables, while ε demonstrates the error.

The fitted models were appraised by coefficient of determination. The value of R^2 always increases by increase in model terms, irrespective of their significance to the model [28]. In order to tackle this problem, R^2_{adj} is used. Unlike R^2, R^2_{adj} value decreases as non-significant terms are added to the model. R^2_{pre} illustrates the amount of variation in new data explained by model. R^2, R^2_{adj}, and R^2_{pre} in the vicinity of 1.0 and close to each other ensure an appropriate fitting. Adequate precision indicates the signal to noise ratio which contrasts the range of the predicted values at the design points to the average predicted errors. Ratios greater than four show adequate model discrimination. Model was diagnosed by certain plots and analysis of variance (ANOVA) to confirm model adequacy. Model terms were assessed using Fisher's statistical test (F-value) and probability (p-value) with 95% confidence. p-value less than 0.05 implies significance of the factor. Lack of fit is the variation of the data around the fitted model. A small lack of fit F-value (p-value > 0.05) is desirable. After model diagnosis, three-dimensional plots were attained from the fitted model and were used to study the effect of single factors and their interactions on responses. In the full factorial design, Pareto charts were presented. This chart is helpful in recognizing the significance of every effect which comprises three different areas. The area above the Bonferroni limit indicates absolutely important effects. Effects located below t-value limit are of no account. Effects which are situated between Bonferroni and t-value limits are conceivably important factors.

3. Results and discussion

3.1. Full factorial design

Full factorial design was used to determine effect of four hydrodynamic factors including agitation rate (A), aeration rate (B), impeller diameter (C), and reactor geometry (D), on responses. Final COD of supernatant, MLSS, and SVI were measured as responses.

Analysis of variance (ANOVA) and certain plots and graphs illustrated model credibility of separate response factors. Effects could be first-order effects, interaction effects, or second-order effect of factors. Pareto charts for three distinctive responses are presented in Fig. 3.

ANOVA results were used to assess the accuracy of the fit as well as important parameters and effects. Model p-value was less than 0.01 for all three results showing fit model suitability. There was only 0.12% chance that MLSS Model F-value occurred due to noise (the chance for final COD and SVI were 0.02 and 0.01%, respectively). For each response, the predicted R-square was in reasonable agreement with R-square and adjusted R-square and close to 1.0, representing model reliability. None of R-square, adjusted R-square, and predicted R-square

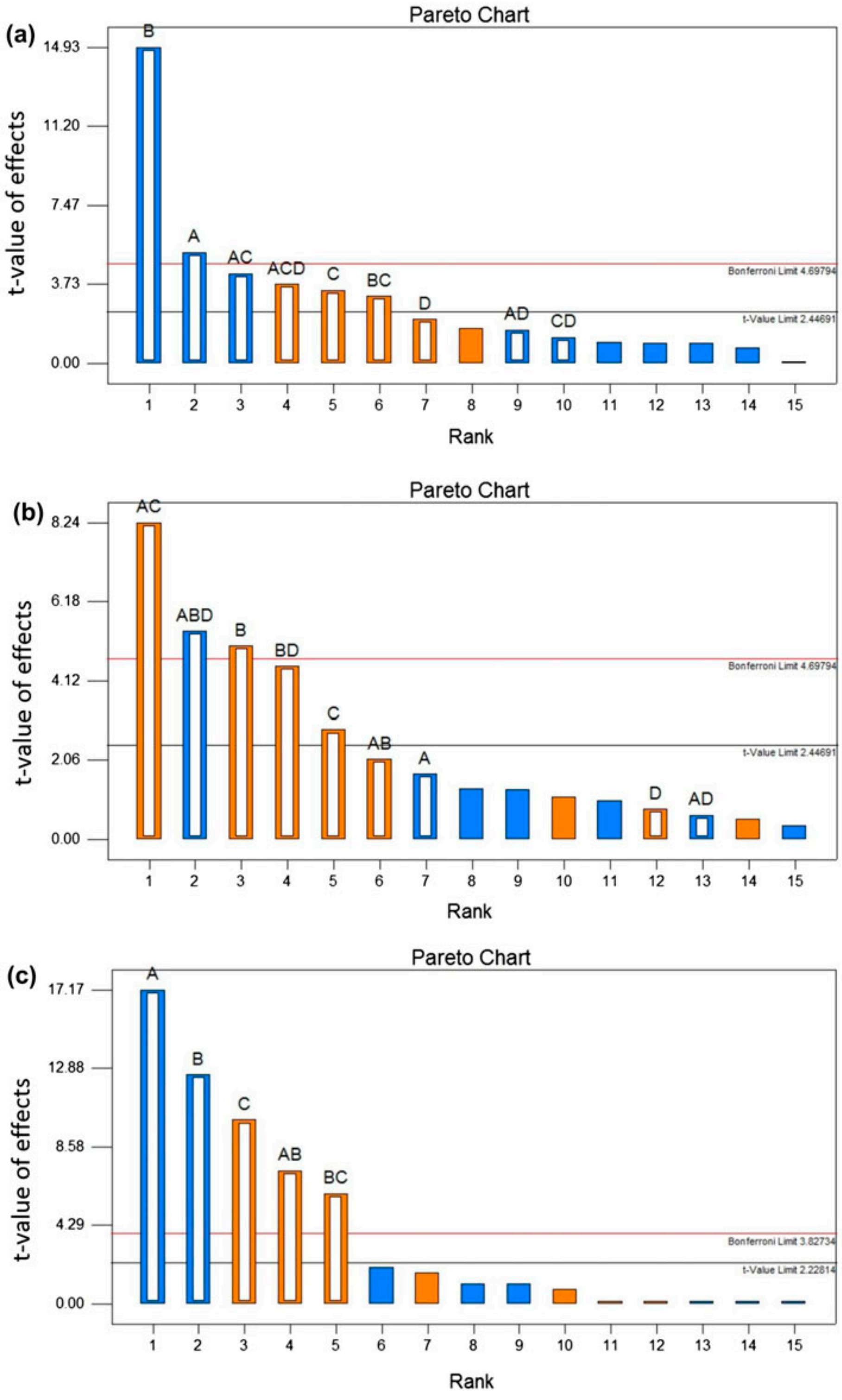

Fig. 3. Pareto chart of the full factorial design: Final COD (a), MLSS (b), and SVI (c).

values were less than 0.74. Adequate precision for every result was greater than 4.0 to ensure adequate model inequity in design spaces. The least adequate precision value of 13.392 was related to MLSS. Important statistical parameters of ANOVA are available in Table 3.

Table 3
ANOVA results for responses of the full factorial design

Response	Model *p*-value	Model *F*-value	R^2	R^2_{adj}	R^2_{pre}	Adeq. precision
Final COD	<0.001	34.73	0.9812	0.9529	0.8661	17.437
MLSS	0.001	17.69	0.9637	0.9092	0.7417	13.392
SVI	<0.001	128.41	0.9847	0.9770	0.9607	32.456

Significant effects are determined using *p*-values. These values confirm important effects acquired by Pareto chart. Greater *F*-values are related to more important effects. Selected effects and fit model equations of dependent variables are arranged in Table 4.

Final equations are presented in coded terms. In coded equations, input factors can vary between the minimum (−1) and maximum (+1) values defined by the design. These equations are not recommended for larger or altered domains of input factors.

Predicted vs. actual plots is a functional graphical device to examine the accuracy of final model in predicting actual response values at data points. The points should be placed in the neighborhood of 45-degree line and split evenly by it. Fig. 4 depicts predicted vs. actual plots for response factor.

It appears that equations are perfectly capable of predicting response values. Other diagnostic plots (e.g. normal plot, residual vs. predicted, and residual vs. runs) were checked (not presented here). Accordingly, there was no reason to suspect the fitted models. These models were used to achieve graphs in order to study impact of factors and their interactions on result factors.

3.1.1. Final COD

ANOVA table shows that B (aeration rate) is by far the most significant effect. Some other parameters and interactions are also considered as important effects. Final COD varied between 67 and 163 (mg/L) and the average value of 115.25 for all 16 experiments meaning a maximum, minimum, and average COD removal of 96.6, 91.8, and 94.2%, respectively. Effects of agitation rate and impeller diameters for both reactor geometries are presented in Fig. 5. Since the reactor geometry is a categorical factor, two plots were drawn for cubic and cylindrical geometries. Fig. 5 shows that in cubic reactor, final COD decreases by increase of impeller diameter and agitation rate. However, the trend is different for cylindrical reactor. As it can be seen, interaction between these two factors affects final COD.

It appears from Fig. 6 that additional aeration can significantly decrease the final COD. Similarity of plots in two reactors reveals that reactor geometry is not as important as other parameters. The effect of aeration rate is fully discussed in the RSM design.

It is noteworthy that in every plot shown here, two factors are shown in two horizontal axis and the other numerical factors are fixed at their mean values.

3.1.2. MLSS

The average value of MLSS for experiments and maximum to minimum ratio were 2,393 (mg/L) and 1.89, respectively. Increase in MLSS is associated with bacterial growth and aeration improves the circumstance for better bacterial growth. Low F/M ratio favors the situation by producing reduced levels of excessive bio sludge and lowering the SVI [29–31]. From one point of view, biomass production is believed to be beneficial and also necessary, as it is needed to maintain a certain amount of bio sludge in the reactor, otherwise, the bio sludge would be washed out after several cycles. Nevertheless, bio-sludge disposal comprises up to 60% of the total cost of treatment which emphasizes on the fact that

Table 4
Final equations for responses of the full factorial design

Response	Equation in terms of coded factors
Final COD	$115.25 - 9.13\text{ A} - 26.0\text{ B} + 6.0\text{ C} - 7.38\text{ AC} + 5.5\text{ BC} + 6.5\text{ ACD}$
MLSS	$2393.13 + 164.38\text{ B} + 93.13\text{ C} + 269.38\text{ AC} + 146.88\text{ BD} - 176.88\text{ ABD}$
SVI	$83.31 - 6.92\text{ A} - 5.06\text{ B} + 4.06\text{ C} + 2.94\text{ AB} + 2.44\text{ BC}$

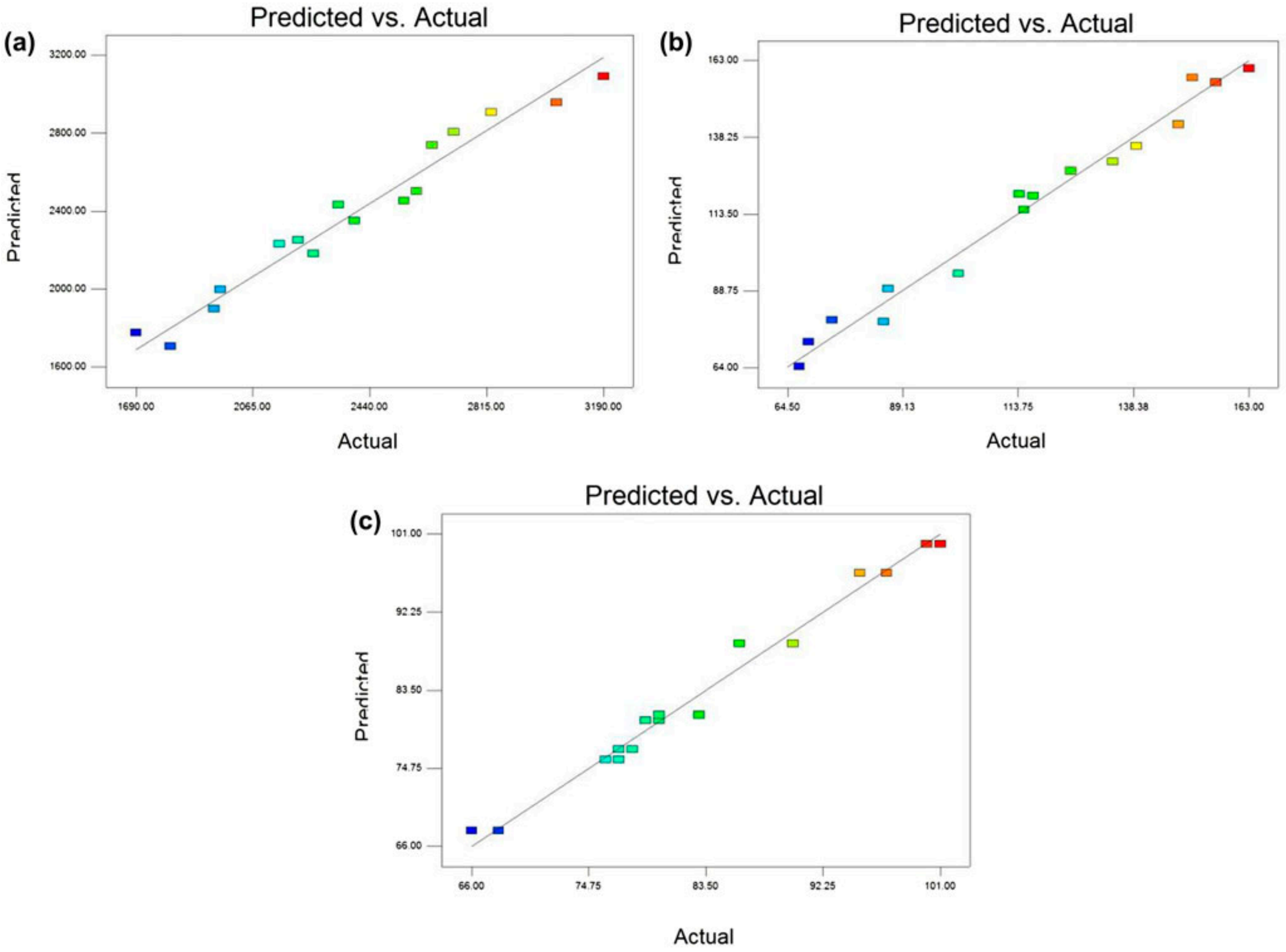

Fig. 4. Predicted vs. actual plots for responses of full factorial design: Final COD (a), MLSS (b), and SVI (c).

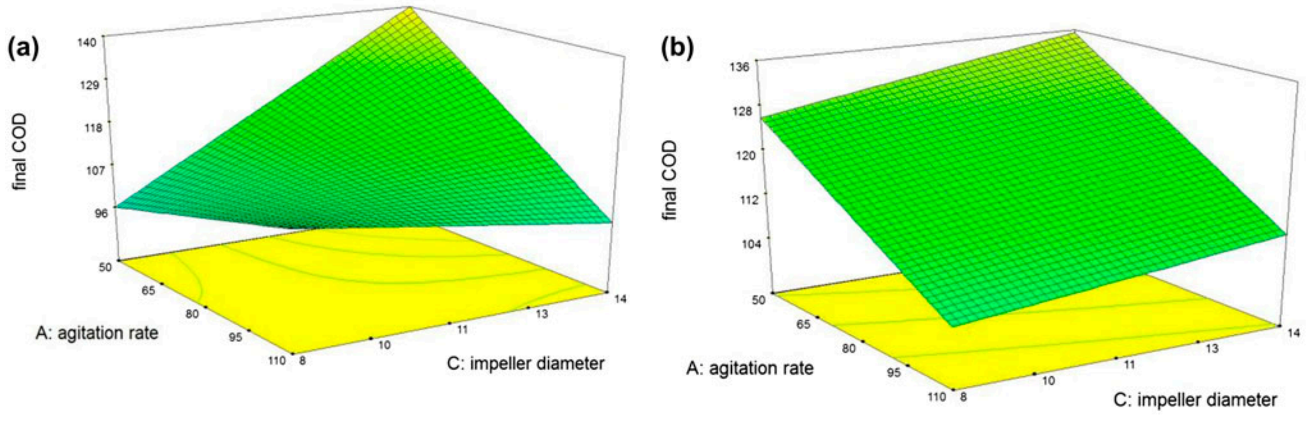

Fig. 5. Final COD vs. agitation rate and impeller diameter: (a) cylindrical and (b) cubic.

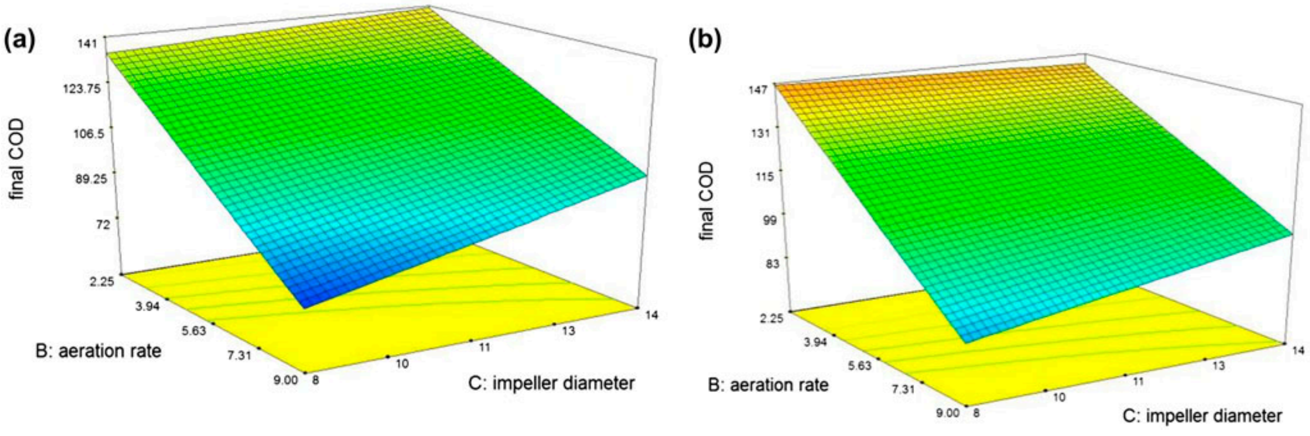

Fig. 6. Final COD vs. aeration rate and impeller diameter: (a) cylindrical and (b) cubic.

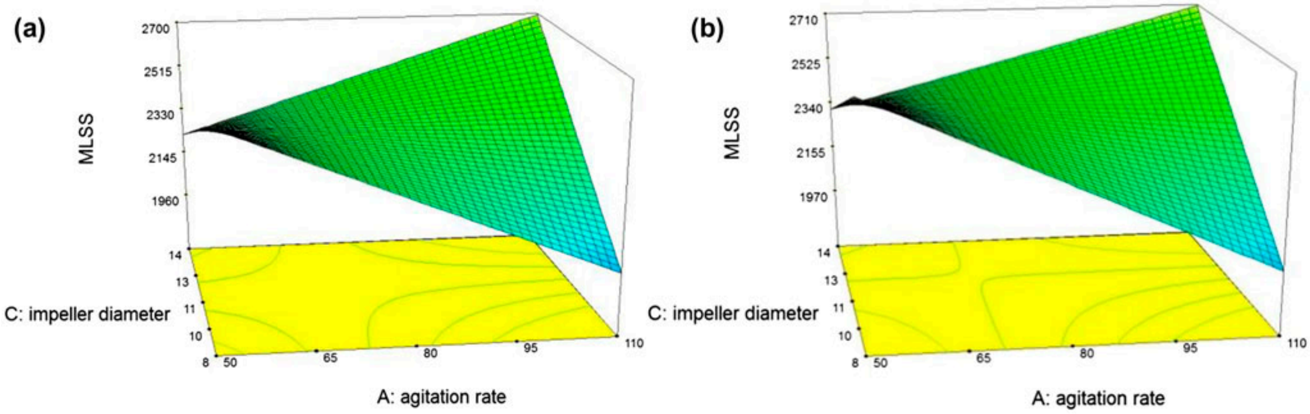

Fig. 7. MLSS vs. agitation rate and impeller diameter: (a) cylindrical and (b) cubic.

excessive bio-sludge production should be avoided [32,33]. The dependency of MLSS on agitation rate and impeller diameter is given in Fig. 7. It is shown that none of these parameters should be used alone to determine the dependency of MLSS on them owing to diverse trend in maximum and minimum values of the independent factors. ANOVA table confirms this reason as AC (interaction of aeration rate and impeller diameter) has the most important effect.

3.1.3. SVI

SVI is widely used as a measure of bio-sludge settleability in settle stage. High settleability is desirable in the view of the efficacy on bio-sludge washout. Bio-sludge washout deteriorates wastewater quality and as a consequent can pollute the environment. A low-SVI value is an indicator of high settleability and low bio-sludge washout. Average SVI value was 83.31 (ml/g) and highest and lowest values were 101 and 66 (ml/g), respectively. The average value obtained in this study is not as appropriate and beneficial as values from granular sludges [8]. High agitation rate and aeration rate favors reduction in SVI, which is shown in Fig. 8.

Both plots in Fig. 8 indicate that smaller impeller leads to lower levels of SVI. Fig. 9 indicates the effect of aeration rate and impeller diameter on SVI. Resemblance between two plots in Fig. 9 signifies the fact that reactor geometry is of little importance. ANOVA table suggests that agitation rate, aeration rate, and impeller diameter are three influential effects; however, the effect of impeller diameter is seen to be secondary.

3.1.4. Optimization

In this section, Design Expert software was utilized to find the optimum experimental conditions to

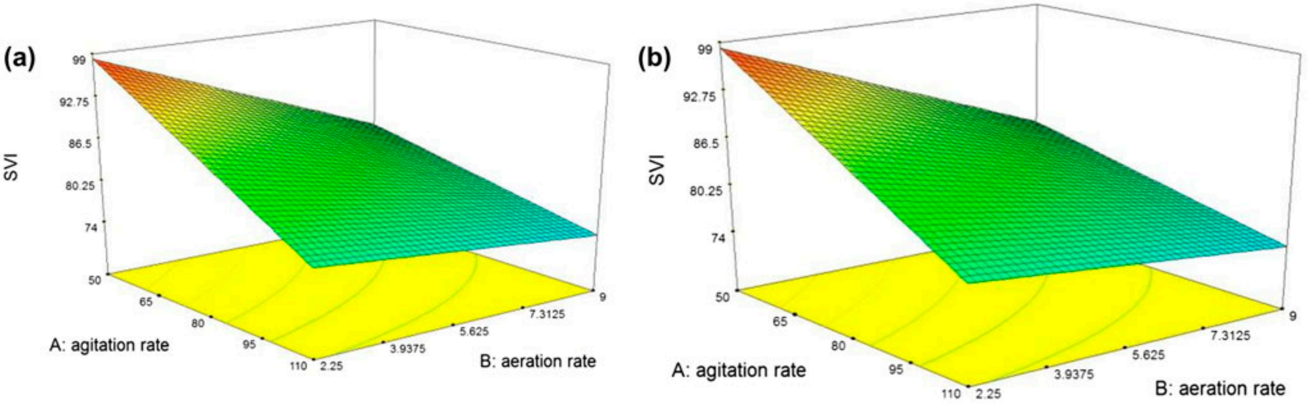

Fig. 8. SVI vs. agitation rate and aeration rate: (a) cylindrical and (b) cubic.

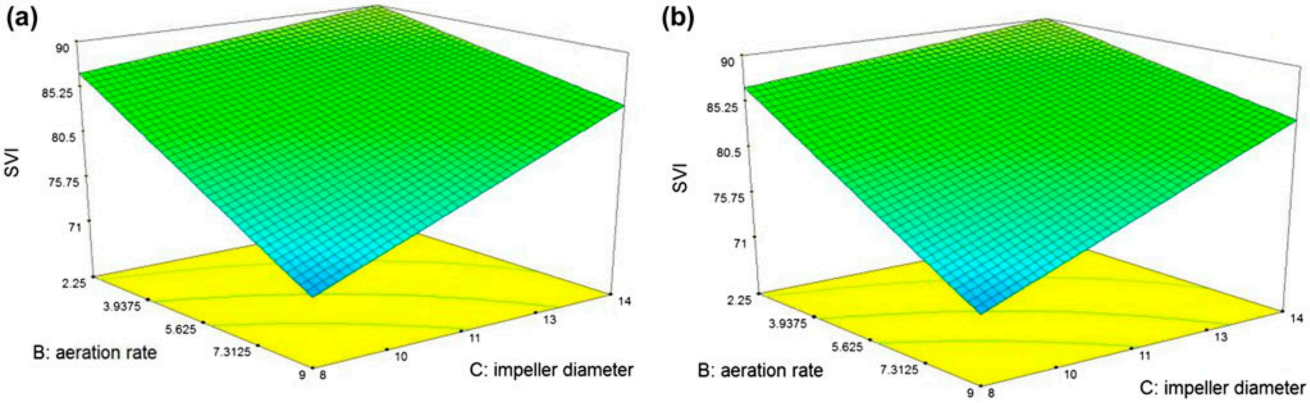

Fig. 9. SVI vs. aeration rate and impeller diameter: (a) cylindrical and (b) cubic.

achieve the best results in the matter of process responses. This procedure attempts to contemplate every possible solution in order to achieve the most desirable points. Preferable condition consists of low-final COD and SVI. As mentioned before, MLSS should not exceed certain levels. To satisfy latter condition, MLSS was adjusted between 2,000 and 2,400 mg/L. Table 5 shows chosen variables and predicted responses in optimum condition.

According to the software, optimum condition occurred at the agitation rate of 110 rpm, aeration rate of 9 L/min, impeller diameter of 8 cm, and cubic geometry of the reactor. In addition, the Design Expert software predicted responses using model equations which resulted in the 72.50 mg/L, 2,183 mg/L, and 67.75 ml/g for the Final COD, MLSS, and SVI, respectively. Three distinct experiments were carried out at predicted optimum condition and the results were

Table 5
Optimum condition for the full factorial design

	Agitation rate	Aeration rate	Impeller diameter	Geometry	Final COD	MLSS	SVI	Desirability
Optimum point	110 rpm	9 L/min	8 cm	Cubic	72.5014	2182.5	67.7503	0.946

compared with predicted responses. Discrepancy between predicted and obtained values of responses was less than 10%, meaning that the optimization was successful.

Aeration rate was selected as the most significant factor and along with four other factors were used in an RSM design in the second part of the experiment. According to optimum condition, agitation rate of 110 rpm, cubic geometry, and impeller with 8 cm diameter was used in the second part of experiments. There was evidence that shows effect of another geometry type (length/diameter ratio) on SBR performance is negligible [34]. As the agitation rate increases, mass transfer amplifies which would be beneficial. New experiments were performed in optimum conditions of the first experiment, except for the aeration rate which was opted as a variable.

3.2. RSM design

CCD was performed to investigate dependency of three process responses on five numeric variables: input COD (A), pH (B), exchange ratio (C), aeration velocity (D), and cycle time (E). Response surface design was made up of 2^5 factorial points, 10 axial point together with 8 replications of the center point in blocks 1 (first reactor) and 3 replications of the center point in block 2 (second reactor).

ANOVA table was used to determine model accuracy and considerable effects using *p*-values. Effects with *p*-values smaller than 0.05 were regarded as significant model terms. Model *F*-values of 51.76, 21.86, and 23.67 for COD removal, TSS, and SVI, in addition to *p*-value less than 0.001 for all three responses proved fit mode precision. Lack of fit *F*-values of more than 0.05 indicated model capability of predicting correlation between dependent and independent factors. R^2, R^2_{adj}, and R^2_{pre} correlation coefficients of process responses were greater than 0.9338, 0.8911, and 0.7673, respectively. By noticing the ANOVA results shown in Table 6, adequate precisions are satisfactorily larger than 4.0.

For each process response, a quadratic equation was developed considering main effects, second-order effects, and interactions as represented in Table 7. Insignificant effects were removed from the equations.

Fig. 10 shows predicted vs. actual plots. Proximity of the points to the 45-degree line points out the potential capability of predicting data points. Normal probability plot of residuals along with the plot of residuals vs. predicted values, blocks, and run order were examined and were totally adequate.

3.2.1. COD removal

In RSM design, influent COD was varied in order to study its effect on process responses, including COD removal. According to COD removal as defined earlier, it varied between 49 and 92%, while the average value was 73.47%. ANOVA table shows cycle time (E), exchange ratio (C), input COD (A), and aeration velocity (D) were significant main effects. Fig. 11 represents effect of different variables on COD removal. Four interactions (AD, AE, BE, and CE) and one second-order effect (B^2) were other significant effects. Second-order effect was considered to be responsible for non-linear behavior of the plots.

Near the center of the defined domain for pH, a maximum in the COD removal curve is observable. This case implies that there would be an optimum pH, in which the COD removal is in its maximum values. pH in the range of 7.1–8.0 was proposed in the literature [35] and, similar to other biological systems, lower pH values showed less COD removal [36]. Acidic condition reduces the energy balance in both sides of the micro-organisms' cell membranes and hinders their proliferation. Excessive alkalinity is also harmful for micro-organisms [35]. pH is reported not to be a critical factor in determination of SBR performance [37]. Based on the results of this study, however, it is clear that first- and second-order interactions of pH can be significant, hence, should be taken into account.

As can be detected from plots, aeration rate below 5 L/min can influence the COD removal. As the aeration rate increases, the COD removal increases. However, for more intense aerations, the COD removal is independent of the aeration rate. Hu et al. [38] showed

Table 6
ANOVA results for responses of the RSM

Response	Model *p*-value	Model *F*-value	R^2	R^2_{adj}	R^2_{pre}	Adeq. precision
COD removal	<0.0001	51.76	0.9709	0.9522	0.9083	25.780
TSS	<0.0001	21.86	0.9338	0.8911	0.7673	18.183
SVI	<0.0001	23.67	0.9385	0.8989	0.7798	18.915

Table 7
Final equations for responses of the response surface methodology

Response	Equation in terms of coded factors
COD removal	$81.55 - 2.79A - 1.76C + 0.97D + 11.47E - 1.19AD + 1.00AE + 1.31BE - 2.37CE - 5.53B^2$
TSS	$69.58 + 4.12A + 28.82C + 6.47D - 5.16\ BC - 5.78BD + 3.59DE + 14.77D^2$
SVI	$67.33 - 8.29A + 18.59C - 8.76D - 3.35E + 7.69AD + 3.31AE + 3.06BD - 4.06CD - 2.94CE + 26.68C^2 + 15.68D^2$

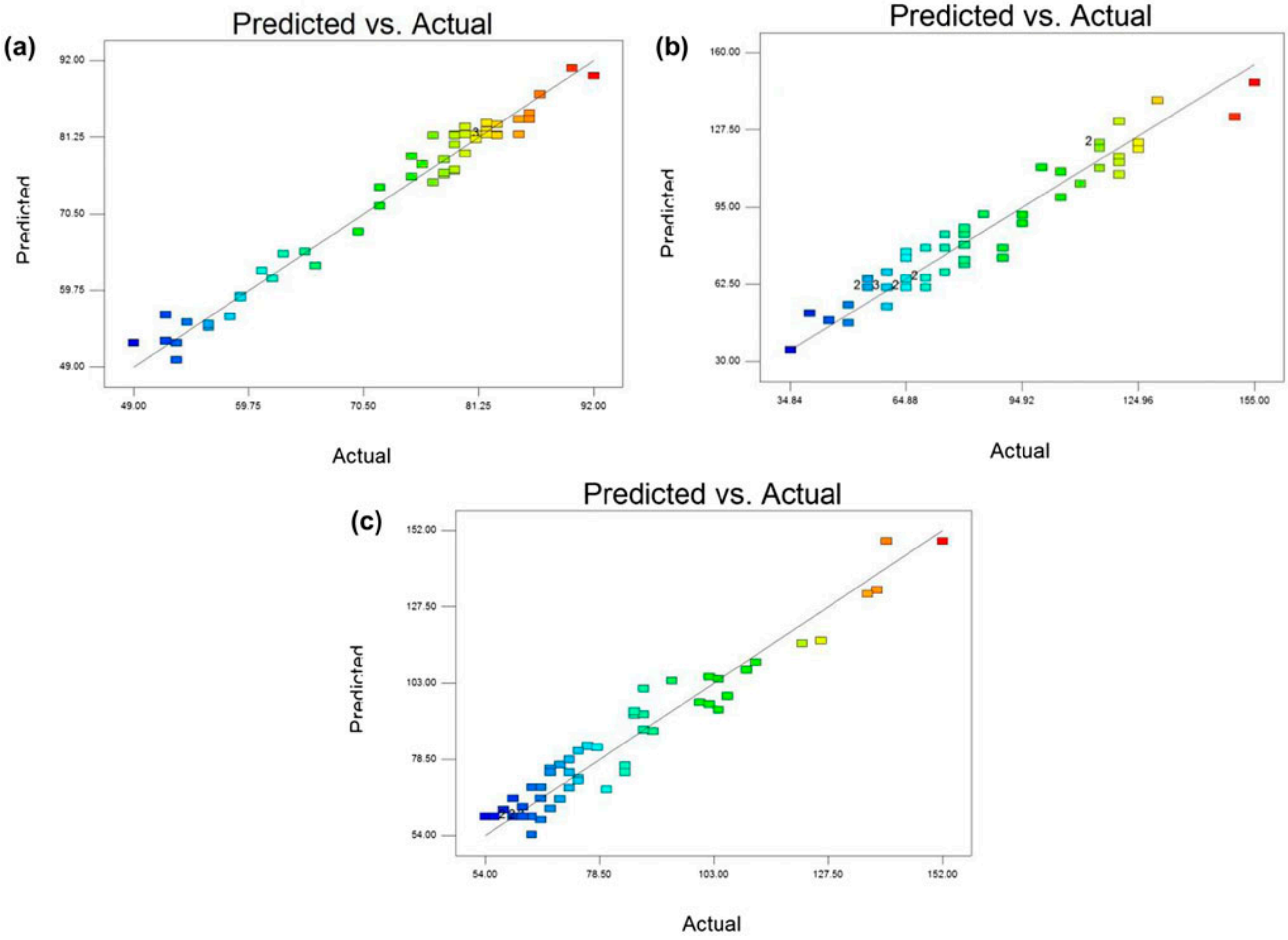

Fig. 10. Actual plots for responses of full factorial design: Final COD (a), MLSS (b), and SVI (c).

effluent COD was higher in 20 L/h than 40 and 160 L/h and higher air fluxes resulted in rapid removal. As was expected for biological systems, addition of organic matter in feed wastewater generally results in increased final COD and decreased COD removal. Rajasimman et al. [39] used low-density biomass support to treat starch wastewater in a fluidized bed bioreactor and concluded higher air velocity and lower initial COD are beneficial to COD removal efficiency. High levels of initial COD may cause organic overload which is associated with microbial incapacity to consume organic compounds.

High levels of cycle time (E) are advantageous in the matter of the COD removal. In another work, Moussavi et al. [40] concluded that reduced aeration time (which is connected to reduced cycle time) abates

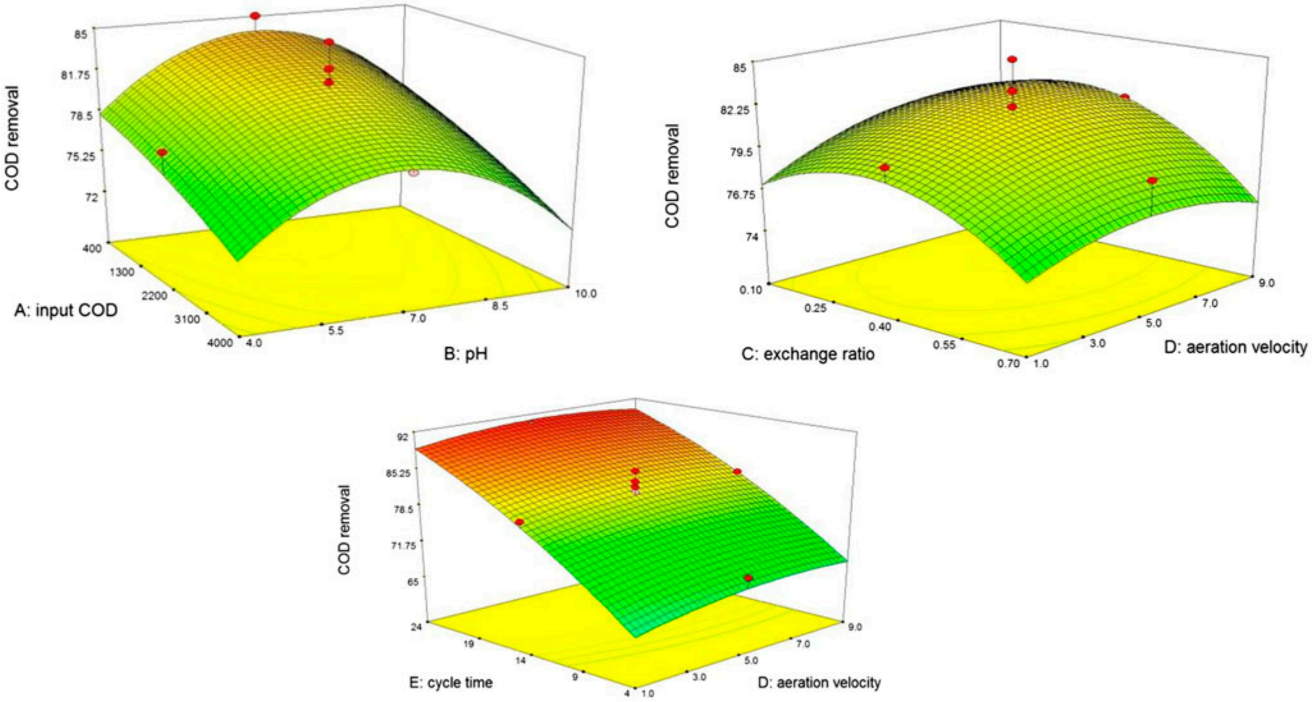

Fig. 11. Effect of variables on COD removal.

COD removal; however, aeration time of greater than 15 h is of no avail.

Hydraulic retention time is supposed to affect the organic matter removal which can be altered by changing exchange ratio and cycle time (CE). Previous findings state that COD removal increases in higher HRTs [41], whereas some authors reported increasing HRT could be ineffective or even decrease removal efficiency [42]. Sirianuntapiboon et al. [29] investigated the impact of organic loading rate (OLR) on COD removal. Increment of OLR is related to high initial COD concentration or reduction in HRT, which, as can be seen from Fig. 11, results in lower COD removal.

By noticing the experimental data table which is not presented in the current paper, for the 4 h cycles (3 h for reaction phase), about 60% of organic matter will be removed while in another 20 h of reaction (in a 24 h cycle), the COD removal will increase only about 20%. This substantiates the fact that reaction rate decreases by the time. Supplementary experiments were conducted at fixed intervals of 20 min (which are not presented here) demonstrated that the reaction rate is faster at the beginning of the cycle and most of the organic matter is removed at first 3 h of reaction. This result is in agreement with first-order kinetics proposed for bacterial wastewater treatment in SBRs [43].

3.2.2. *TSS*

TSS value varied enormously between an adequate lower value of 35 and an inappropriate high value of 155 (mg/L) which indicates the necessity of achieving the best experimental condition in order to reduce the TSS. Bio-sludge washout is considered as the most important factor which provokes high levels of TSS in supernatant liquid in settle phase. Increase in settling time helps bio sludge to settle more. Nevertheless, in this study, settle time was fixed at 30 min and effect of other factors was studied. 30-min settling time allowed the reactor to maintain two phases, while the settled bio-sludge height was less than decant valves height which means no settled bio sludge left the tank.

Effect of variables on TSS is presented in Fig. 12. According to ANOVA table, exchange ratio (C), input COD (A), and aeration velocity (D) are most significant effects, respectively. BC, BD, and D^2 are other important factors. By increase in exchange ratio, TSS markedly increases. pH did not profoundly alter TSS values and mean values showed lower TSS values. As input COD increases, TSS decreases until a certain point and after that minimum, the trend in TSS curve reverses. The raise in TSS values can be explained by micro-organism adaptation to lower input COD and time they required to adapt themselves to new

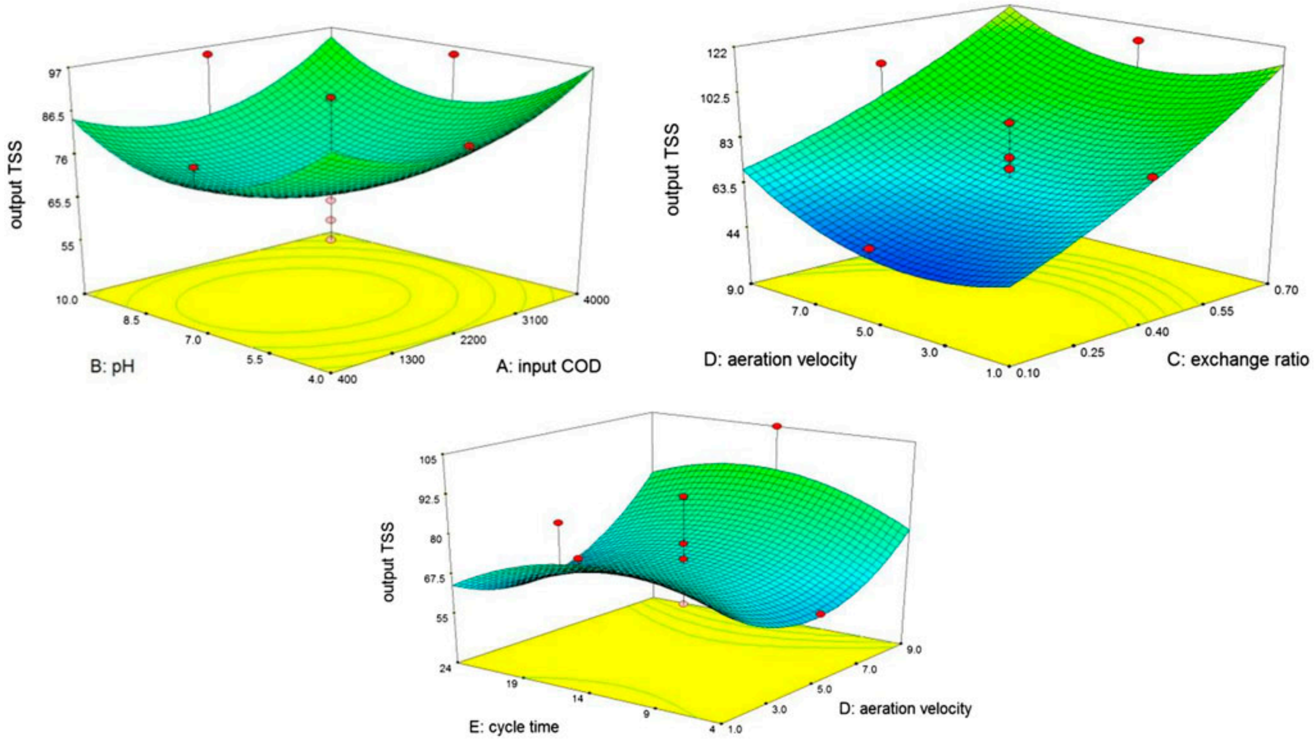

Fig. 12. Effect of variables on output TSS.

environment (after several cycles). It is reported that TSS value decreased (TSS removal increased) in medium OLRs. From another point of view, organic overload deteriorates supernatant quality [42].

TSS value greatly depends on aeration rate. An optimum aeration rate of around 5 L/min highlights the fact that excessive aeration is not favorable. Variation of TSS by cycle time was opposite of COD which means there was a maximum point. Substrate deficit is announced to be suitable for filamentous growth and sludge bulking [44]. Therefore, these conditions should be prevented so as to diminish output TSS. By increase in exchange ratio, TSS markedly increased which can be explained by the fact that in higher exchange ratios, a higher amount of treated wastewater is drained and as a consequence, low-settling sludge will leave the tank by supernatant.

3.2.3. SVI

Minimum and maximum SVI values obtained were 46 and 152 ml/g, respectively. Average value for all experiments was 87.39 ml/g. As stated by *F*-values, exchange ratio (C), input COD (A), and aeration velocity (D) were considered as the most significant effects and pH was of little importance. AD, AE, BD, CD, CE, C^2, and D^2 were other important effects.

The changes in SVI by operational variables are shown in Fig. 13. The least SVI values were recorded at moderate levels of pH. High cycle time decreases SVI which can be related to the fact that high starvation period approves of granulation and prevents sludge bulking [40]. It was reported that a certain starvation time raises the possibility of establishment of denser structure with better settleability. It is mentioned that starvation has profound impact on zeta potential and hydrophobicity of bio sludge and allows micro-organisms with more extracellular polymer substances to develop [45–47].

Aeration velocity of 4 L/min was considered as the optimum velocity. In higher aeration rates, SVI slightly increased and in lower rates, the increase was drastic. High aeration is beneficial for development biomass aggregation and sludge sedimentation. Shear force which is caused by aeration exerts a direct effect on polysaccharide production and assists settleability [48]. In this work, all aeration rates provide DO larger than 2 mg/L and high shear stress would make a frictional force on the surface of the flocs which separates filamentous bacteria from the flocs to washout. The remaining bio sludge can settle easier which identifies by lower SVI values. However, it is also mentioned that high DO is related to low bio-sludge production [49] which in turn leads to high SVI.

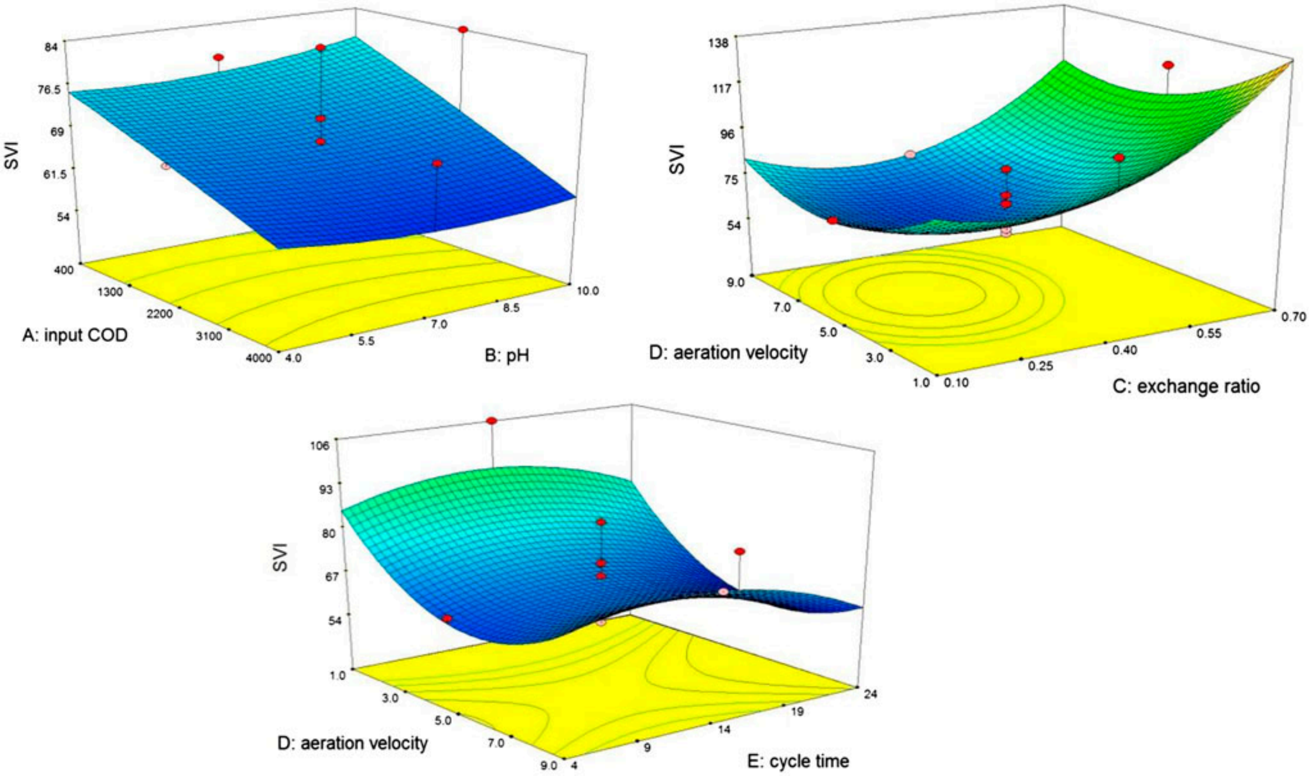

Fig. 13. Effect of variables on SVI.

SVI value decreased by an increase in input COD which is in line with previous findings [50]. High-specific COD loading rate results in high bio-sludge growth, high MLSS concentration, and low SVI value. High exchange ratio was destructive which can be interpreted as low settleability. In low exchange ratios, SVI changes were not noticeable and exchange ratio of 0.2 resulted in the best and the least SVI. In long-term experiments, high-exchange ratio and short cycle time (low HRT and high OLR) lead to filamentous bacterial washout, growth, and dominance of non-filamentous bacteria after a number of cycles which benefits settleability [45,51]. However, in short-term experiments similar to experiment conducted in this work (consisted of one cycle for new environment), high-exchange ratio damages settleability and increases TSS and SVI values.

3.2.4. Optimization

Design Expert was performed to achieve optimum condition for SBR operation. Superior results were defined as high COD removal, low TSS, and low SVI. All three factors have given the same importance. The best operational system and predicted results are presented in Table 8.

Three additional experiments were carried out at proposed variables and results were recorded and

Table 8
Optimum condition for the response surface methodology

	Input COD (mg/L)	pH	Exchange ratio	Aeration rate (lit/min)	Cycle time (hr)	COD Removal (%)	Output TSS (mg/L)	SVI (ml/g)	Desirability
Optimum point	1,703	7.1	0.18	5.1	24	92.87	38.68	57.44	0.930

compared with predicted values. Difference of less than 10% validated optimization procedure. Average quantities of obtained results were 89%, 40 mg/L, and 60 ml/g for COD removal, TSS, and COD, respectively. In the optimum conditions, the predicted effluent COD and TSS is presented as 187.33 and 40 mg/L. These values are capable of meeting Iran department of environment effluent discharge standards for agricultural uses. The upper limit for effluent COD and TSS concentrations are 200 and 100 mg/L, respectively [52].

4. Conclusion

The treatability of starch-containing synthesized wastewater was studied using an SBR system. The following conclusions were drawn from this work:

(1) The SBR system demonstrated overall stability and high organic removal efficiency (closely 90%) in treating synthetic starch-containing wastewater.

(2) Impeller diameter and reactor geometry (cylindrical and cubic) were two novel variables which were studied in the full factorial design. The conclusion drawn from the first study showed high-agitation rate, smaller impeller, and cubic geometry led to better results.

(3) In the second part of the experiments, optimum condition for better COD removal and lower TSS and SVI consisted of input COD = 1,703 mg/L, pH 7.1, exchange ratio = 0.18, aeration rate = 5.1 L/min and cycle time = 24 h. In the optimum condition, 89% of input COD in tank was removed and output TSS and SVI were 40 mg/L and 60 ml/g, respectively.

(4) DoEs was remarkably successful in predicting the effects of variables on responses. Further experiments performed in optimum condition confirmed these findings.

Acknowledgment

The authors gratefully acknowledge the cooperation and financial support of Ferdowsi University of Mashhad.

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