Influence of Process and Operational Factors on a Sequencing Batch Reactor (SBR) Performance Treating Stimulated Dairy Wastewater

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Abstract A bench scale aerobic sequencing batch reactor (SBR) was evaluated in terms of its potential to treat synthetic dairy wastewater. The 2-l plexiglass bioreactor was supplied with oxygen via a fine bubble air diffuser, fed with synthetic dairy wastewater under various operational conditions. To analyze the process, three significant independent variables — influent chemical oxygen demand (COD), mixed liquor volatile suspended solids (MLVSS), and aeration time — were assessed. Three dependent process and quality parameters (as process responses) were also evaluated: total COD removal efficiency, sludge volume index (SVI) and final pH. The experiments were based on a central composite design (CCD) and analyzed using response surface methodology (RSM). The treatment was limited to the following concentration regimes: COD (1000, 3000 and 5000 mg/l), MLVSS (3000, 5000 and 7000 mg/l) and aeration time (2, 10 and 18 h). Maximum COD removal efficiency (of 96.5%) was obtained for an influent with the following characteristics: CODin: 3000 mg/l, MLVSS 5000 mg/l, and aeration time of 18 h. The study demonstrated the capability of aerobic SBRs for high COD removal from dairy industrial wastewater. Easy operation, low cost, and minimal sludge bulking condition were some of advantages of the SBR system as an option for biological treatment of medium-strength industrial wastewater. The present study provides valuable information about relationships between quality and process parameters for different values of operating variables.

Key words: COD removal, Sequencing batch reactor (SBR), Synthetic dairy wastewater

1 INTRODUCTION

Water management in the dairy industry has been well documented but effluent production and disposal remain problematic (Srinivasan et al., 2009). Several biological treatment systems have been developed: the activated sludge system, anaerobic ponds, oxidation ponds, trickling filters, and combined trickling filters (Department of Industrial Works, 2001; Garrido et al., 2000). However, each system had disadvantages (Ince, 1998; Metcalf and Eddy, 2003; Rusten et al., 1993).
Aerated lagoons require high surface areas and have the disadvantage of fluctuating effluent quality. Anaerobic ponds produce foul odors due to \( \text{H}_2\text{S} \) and \( \text{NH}_3 \) emissions. Due to its high removal efficiency, the activated sludge system was selected to treat dairy industry wastewater (Zayed and Winter, 1998) but this has the disadvantage of high energy consumption and frequent raising and bulking of biosludge in the clarifier (Sirianuntapiboon and Tondee, 2000).

Biological processes based on a suspended sequencing batch reactor (SBR) are effective for organic carbon removal in domestic and industrial wastewater (Mohseni-Bandpi and Bazari, 2004).

SBRs are often operated under high total solids concentration conditions, compared with operating conditions associated with conventional wastewater treatment plants. This enables a reduction in SBR reactor volumes, thus decreasing investment costs for the treatment plant (Sirianuntapiboon and Yommee, 2006). The main advantages of such a system include easy operation, low costs and the capacity to handle hydraulic fluctuations. There is also no need for settling tanks and sludge recycling and organic loading does not cause any significant variation in removal efficiency (Kolb and Wildere, 1997; Keudel and Dichtl, 2000).

The statistical method of response surface methodology (RSM) has been proposed to include the influences of individual factors as well as their interactive influences. RSM, which is a technique for designing experiments, helps researchers to build models, evaluate the effects of several factors, and achieve optimum conditions for desirable responses, in addition to reducing the number of experiments required for this type of research (Zinatizadeh et al., 2009).

The purpose of this study was to determine the treatability of dairy wastewater in a SBR and to evaluate the effects of three significant independent variables — viz. influent chemical oxygen demand (COD), mixed liquor volatile suspended solids (MLVSS), and aeration time — on the system performance.

2 MATERIALS AND METHODS

2.1 Composition of synthetic dairy wastewater

Powdered milk, mixed with water to make up a standard concentration, was assumed to be a suitable representative of dairy processing wastewater. The powdered milk used in this study was supplied by BIOMIL, www.behdashtkar.com/pages/en/biomil-plus.htm).

The ingredients of the milk powder were as follows: proteins 12.5 g/1000 g powder, carbohydrate 54 g/1000 g powder, fat 28 g/1000 g powder, inorganic matter 3 g/1000 g powder. The stimulated dairy wastewater used in the study had a chemical oxygen demand (COD) of 5g/l and pH of 6.8–7.5.

2.2 Bioreactor design and operation

A lab-scale SBR, with a volume of 2 l, was constructed from plexiglass (Fig. 1). The glass bioreactor dimensions were 10×10×30 cm (length, width and height, respectively). The effective working volume (total liquid volume) was 2000 ml. The sequence of the SBR operation was controlled by pre-programmed timers (for feeding, aeration, settling and withdrawal). At the beginning of each cycle, immediately after withdrawal (earlier sequence), a pre-defined feed volume (1 l) was pumped into the system and the liquid in the reactor volume was aerated during the reaction phase. At the end of the cycle, suspended biomass (VSS) settled and effluent was withdrawn from the reactor. Feeding and wastewater withdrawal were undertaken with the help of peristaltic pumps and a control valve in the middle part of the reactor. Air was introduced into the reactor by means of 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 timer that connected to a blower. Excess sludge was removed during the draw and idle period to...
control the MLSS of the system. The reactor was inoculated with activated sludge taken from an aeration tank (municipal wastewater treatment plant, Kermanshah, Iran). The sludge age of the inoculum was about 15 d, and had an MLSS concentration of 5.8 g/l. The bioreactor feed was synthetic dairy wastewater. In the first phase, the reactor was operated for 2 weeks at a COD concentration of 1400 mg/l (Metcalf and Eddy, 2003) in order to attain steady state conditions. The second phase of the experiment was run under different operational conditions, designed using a statistical program based on a two-factorial design. Experimental conditions and the range of independent variables are described in Table 1.

![Fig. 1 A view of experimental setup in the study.](image)

**Table 1** Experimental conditions and ranges of independent variables.

<table>
<thead>
<tr>
<th>Independent factors</th>
<th>Level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>COD in (mg/l)</td>
<td>1000</td>
</tr>
<tr>
<td>MLVSS (mg/l)</td>
<td>3000</td>
</tr>
<tr>
<td>Aeration time (h)</td>
<td>2</td>
</tr>
</tbody>
</table>
2.3 Experimental design

The RSM used in the present study was a central composite face-centered design (CCFD) involving three different factors: COD in, MLVSS concentration, and reaction time. The assessment of COD removal efficiency of the bioreactor was based on the full face-centered CCD experimental plan (Table 2). The design consisted of $2^k$ 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 nine experiments organized in a factorial design (including seven factorial points, seven axial points and one center point) with the remaining five involving the replication of the central point to obtain a reliable estimate of experimental error (Khuri and Cornell, 1996). In order to carry out a comprehensive analysis of the reactor, three dependent parameters were either directly measured, or calculated as a response. These parameters were total COD (TCOD) removal, sludge volume index (SVI) and pH (Metcalf and Eddy, 2003).

<table>
<thead>
<tr>
<th>Run no.</th>
<th>A: COD in, mg/l</th>
<th>B: MLVSS, mg/l</th>
<th>C: aeration time, h</th>
<th>COD removal, %</th>
<th>SVI, ml/g</th>
<th>pH</th>
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<tr>
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<td>94.20</td>
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<td>94.00</td>
<td>106.42</td>
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<td>7000</td>
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<td>95.67</td>
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<td>5000</td>
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<td>74.80</td>
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<td>3000</td>
<td>18</td>
<td>88.20</td>
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<td>5000</td>
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<td>94.50</td>
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<td>50.00</td>
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<td>18</td>
<td>94.00</td>
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<td>92.00</td>
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<td>10</td>
<td>94.30</td>
<td>81.70</td>
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<td>5000</td>
<td>2</td>
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<td>3000</td>
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<td>18</td>
<td>96.67</td>
<td>83.12</td>
<td>6.5</td>
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<td>20</td>
<td>3000</td>
<td>5000</td>
<td>10</td>
<td>94.80</td>
<td>74.00</td>
<td>6.0</td>
</tr>
</tbody>
</table>
2.4 Chemical analysis
The concentrations of COD, MLSS and MLVSS were determined, using standard methods for the examination of water and wastewater (APHA, 1995). For COD, a colorimetric technique with a closed reflux method was developed. A spectrophotometer (DR 5000, Hach, Jenway, USA) was used to measure the absorbance of COD samples at 600 nm. Dissolved oxygen (DO) concentration in wastewater was determined using a DO probe. The DO meter was supplied by WTW DO Cell OX 330, electro DO probe, Germany. A HANNA-pH 211 model pH meter was used to measure the pH.

3 RESULTS AND DISCUSSION
3.1 Statistical analysis
ANOVA results for all responses are summarized in Table 3. Polynomial models using a number of different degree models were used for data fitting (Table 3). To quantify the curvature effects, the data from experimental results were fitted to higher-degree polynomial equations, i.e. two factor interaction (2FI), quadratic models, and so on. Response data were analyzed by default using Design Expert software. The terms of the model in equations are those that remained after the elimination of insignificant variables and their interactions.

Based on statistical analysis, the models were found to be highly significant, with a probability of <0.0001, and the independent variables were significant at the 99% confidence level. The square of the correlation coefficient for each response was computed as the coefficient of determination (R²). It showed high significant regression at the 95% confidence level. The value of the adjusted determination coefficient (adjusted R²) was also high, indicating the high significance of the model (APHA, 1995). The model adequacy was evaluated using lack-of-fit F-tests (Montgomery, 1991). 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; in other words, a signal-to-noise ratio. Its desired value is 4 or more. This value was found to be desirable for all models. Simultaneously, low values of the coefficient of variation (CV) (2.56–6.22) indicated good precision and reliability of the experiments as suggested by Masonet et al. (2003) and Ahmad et al. (2005). Detailed analysis on the models is presented in the text that follows.

3.2 COD removal
To investigate the effects of the variables studied on TCOD removal efficiency, dependency of this response on the variables was analyzed and modeled. By applying multiple regression analysis on experimental data, the experimental results of the CCD design were fitted with a modified quadratic model. The empirical relationship between COD removal and the three test variables, in terms of coded factors for COD removal, is expressed as follows:

\[
\text{COD removal } \% = +93.3 + 1.6A + 4.2B + 2.8C - 4.92B^2 - 16.92C^2 - 3.45AC - 2.0BC \quad (\text{Eq. 1})
\]

As noted in Eq. 1, the main-order effects of the variables (aeration time, COD\textsubscript{in} and MLVSS) had positive impacts on COD removal efficiency while second-order effects of MLVSS and aeration time and two-level interactions of the variables (AC and BC) showed negative impacts on the response. The model was able to identify the effects of binary combination of the two independent factors. Fig. 2 shows the variable interaction for all responses. The non-parallel curvatures of Fig. 2a and b imply that there is a relatively strong interaction between the aeration time and other variables (COD\textsubscript{in} and MLVSS). As the result from these strong interactions, AC and BC appeared as the significant terms in Eq. 1.
Table 3 ANOVA results for the equations derived using Design Expert 6.0.6, for the studied responses.

<table>
<thead>
<tr>
<th>Response</th>
<th>Modified equation with significant terms</th>
<th>Probability</th>
<th>R²</th>
<th>Adj. R²</th>
<th>Adeq. Precision</th>
<th>SD</th>
<th>CV</th>
<th>Probability for lack of fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD removal efficiency, %</td>
<td>93.44+1.6A+4.2B+2.84C+4.92B²–16.92C²–3.45AC–2BC</td>
<td>&lt;0.0001</td>
<td>0.99</td>
<td>0.98</td>
<td>43.4</td>
<td>2.11</td>
<td>2.56</td>
<td>0.052</td>
</tr>
<tr>
<td>SVI</td>
<td>75.9-6.5A+7.2B+10.3B²+6.8C²–3.3BC</td>
<td>&lt;0.0001</td>
<td>0.87</td>
<td>0.83</td>
<td>14.2</td>
<td>5.25</td>
<td>6.22</td>
<td>0.066</td>
</tr>
</tbody>
</table>


According to the model, the operating condition for the maximum COD removal was predicted in the region of higher aeration time and MLVSS concentration and middle COD<sub>in</sub>. The major diagnostic plots (Fig. 2c and d) are used to determine the residual analysis of the response surface design, ensuring that the statistical assumptions fit the analysis data. Fig. 1c displays the normal probability of the residuals, to verify whether the standard deviations between the actual and the predicted response values follow a normal distribution (Lee, 2005). The results illustrated in Fig. 2c convey the general impression of a normal distribution of underlying errors, since the residuals fall near to a straight line; thus, there is no clear indication of non-normality of experimental results. The plots of residual versus predicted responses are illustrated in Fig. 2d. All points of experimental runs were scattered randomly within the constant range of residuals across the graph, i.e. within the horizontal lines at the point of ±3.0. This implies that the models proposed are adequate and that the constant variance assumption was confirmed. Reliability and adequacy of empirical models from respective responses were confirmed when the actual values obtained from experimental studies were compared with estimated values from regression models (Fig. 2e). Responses from experimental results fitted well within an acceptable variance range when compared to the predicted values from respective empirical models. The perturbation plot (Fig. 2f) also shows comparative effects of the variables on TCOD removal efficiency. In Fig. 2f, steep curvatures in the aeration time curve shows that the response was very sensitive to this factor. Aeration time is therefore the most significant factor affecting the response.

To gain a better understanding of the interaction effects of variables affecting COD removal efficiency, two- and three-dimensional contour plots for the measured response were formed, based on the model (Eq. 1) as shown in Fig. 3. A simultaneous increase in COD<sub>in</sub> and MLVSS (Fig. 3a) also indicates that COD removal efficiency was increased. However, the increase in the response caused by an increase in MLVSS at constant values of COD<sub>in</sub> was greater than the increase in the response resulting from an increase in COD<sub>in</sub> at constant values of MLVSS. As seen in Fig. 3a–c, the response increased as COD<sub>in</sub> increased at lower aeration time (2 h), while at a higher aeration time, from 10 to 18 h, the COD<sub>in</sub> had a less important effect on COD removal. This was attributed to sufficient aeration time, which makes the response independent of COD<sub>in</sub> in the design space studied.

The maximum TCOD removal was determined to be 99 % at COD<sub>in</sub>, MLVSS and aeration time of 1000 mg/l, 5000 mg/l and 18 h, respectively. The minimum TCOD removal efficiency (39%) was obtained at COD<sub>in</sub>, MLVSS and aeration time of 1000 mg/l, 5000 mg/l and 30 min/h, respectively.
Fig. 2 (a) Interaction graph between MLVSS and aeration time. (b) Interaction graph between COD$_{in}$ and aeration time. (c) Normal probability plot of residual for COD removal. (d) Plot of residual versus predicted response for COD removal. (e) Predicted vs. actual values plot for COD removal. (f) Perturbation plot for COD removal.
Fig. 3 Response surface and counter plots for COD removal efficiency with respect to COD\textsubscript{in} and MLVSS at constant values of aeration times of (a) 2 h, (b) 10 h, (c) 18 h.
3.3 Sludge volume index (SVI)

Development of a flocculent biomass requires the proper proportion of floc-forming and filamentous bacteria. Many authors recognize SVI as the best parameter characterizing sludge settling properties. SVI is also a good indicator of sludge bulking. A suitable SVI value, particularly below 100 ml/g, is of major importance when using activated sludge as a water treatment option (Metcalf and Eddy, 2003).

The ANOVA results for SVI are presented in Table 3. By applying multiple regression analysis on experimental data, results from CCD design tests were fitted to a modified quadratic model. From the analysis, the main effects of COD in (A) and MLVSS (B) and second-order effects of MLVSS (B) and aeration time (C) and the two-level interactions of (B) and (C) are significant model terms. Other model terms were not significant (with probability values greater than 0.05). The terms C, A^2, AB and AC were thus eliminated. The following regression equation is the empirical model in terms of coded factors for SVI:

\[
\text{SVI, ml/g} = +75.9 - 6.5A + 7.2B + 10.3B^2 + 6.8C^2 - 3.3BC \\
\text{(Eq. 2)}
\]

This empirical model was well fitted to experimental results, as the high value of R^2 (0.87) and Adj-R^2 (0.83) provided a good explanation of the reliability of regression models for SVI, as shown in Table 3. The actual and the predicted SVI plot are shown in Fig. 4, which indicates a good agreement between predicted and actual values.

Fig. 5 shows the response surface and contour plots of the model for various SVI levels, as a function of MLVSS (B) and aeration time (C) with three different COD in (1000, 3000 and 5000 mg/l). As can be seen in Fig. 5a–c, the same trends were found as the COD in changed from 1000 to 5000 mg/l. It is apparent from the response surface plots (Fig. 5) that aeration time and MLVSS gave reverse effects on the SVI. As depicted in Fig. 4a–c, an increase in aeration time from 2 to 10 h at a constant value of COD in yielded a decrease in the response. Progressing aeration time resulted in a slight increase in SVI due to a decrease in food available to microorganisms (F/M) ratio. MLVSS also showed a similar effect as that of aeration time on the SVI. An increase in MLVSS from 3000 to 5000 mg/l resulted in a decrease in the response but a further increment in MLVSS caused a slight increase in SVI.

A proper SVI value, particularly below 100 ml/g, is of major importance to activated sludge methodology. Janczukowicz and co-workers (2001) observed a tendency towards sludge bulking in the Olsztynek Wastewater Treatment Plant when SVI increased from 127 to 258 ml/g. Palm and Jenkins (1980) also reported that sludge with an SVI of over 150 ml/g is often classified as bulking sludge. These authors also pointed out the risks associated with too-low SVI in conventional wastewater treatment systems. They found that quick-settling sludge (of SVI less than 70 ml/g) can be the reason for turbid effluent, caused by weakly structured and small, flocs.

Maximum values of the SVI were 110, 103.4 and 96.9 ml/g at COD in values of 1000, 3000, 5000 mg/l, respectively. The high values of SVI in lower COD in have resulted from a low food to microorganism (F/M) ratio. It is clear from the perturbation plot (Fig. 6) that the most significant factors affecting the SVI is MLVSS, confirming that the microbial population is an important factor affecting microbial integrity in flocs.
**Fig. 4** Predicted vs. actual values plot for SVI.

**Fig. 5** Response surface plots for SVI with respect to aeration time and MLVSS at constant values of COD: (a) COD = 1000 mg/l, (b) COD = 3000 mg/l, (c) COD = 5000 mg/l.
3.4 Final pH
For design and operation of a treatment system, the optimum pH condition is required for growth of the bacteria of interest. For treated effluents discharged to the environment, the allowable pH range usually varies from 6 to 8.5 (Metcalf and Eddy, 2003). In this study, the effluent pH was monitored throughout the experiments and remained in the range of 6–6.5.

3.5 Process optimization
With multiple responses we need to find regions where requirements simultaneously meet the critical properties required by the system. The best compromise can be determined by visual searching, i.e. by superimposing or overlaying critical response contours on a contour plot. Graphical optimization produces an overlay plot of contour graphs, thereby displaying the area of feasible response values in the factor space. Fig. 7 shows the graphical optimization, which displays the area of feasible response values (shaded portion) in the factors space. The identification of the optimum region was based on two critical responses (COD removal, SVI).

The shaded area on the overlay plots is the region that meets the proposed criteria (COD removal efficiency greater than 90 %, and SVI between 70 and 100 ml/g). The MLVSS values greater than 4200 mg/l at any COD∞ in the range examined (1000–5000 mg/l), was determined as the optimum region.

4 CONCLUSIONS
The SBR that we developed was a successful, reliable and promising biological treatment system that, in a short period, achieved high COD removal efficiency from dairy wastewater with concentrations of 1000, 3000 and 5000 mg COD/l. RSM results demonstrated the effects of the operating variables as well as their interactive effects on the responses. The most effective operational factors on the bioreactor performance, in terms of removing COD, were determined to be aeration time and MLVSS. The optimum conditions (that attained removal rates of more than 90% and 70 ml/g, for COD and SVI, respectively) were determined to be more than 4200 mg/l for MLVSS and 72 ml/g for SVI.
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تأثیر فاکتورهای راهبردی و فراآیندی بر عملکرد یک راکتور هوازی نابایوسته متواالی (B/SR) تصفیه فاضلاب سنتی صنایع لبی

چکیده در این مطالعه، عملکرد یک راکتور متواالی نابایوسته (SBR) در مقیاس آزمایشگاهی جهت تصفیه فاضلاب لبی سننی مورد بررسی قرار گرفت. این راکتور از جنس پلاستیک گلاس و به حجم 2 لیتر ساخته شد. هواهایی از طریق دیفونزور هوا با حبابهای بزرگ و خرواهای دیگر با فاضلاب سننی تحت شرایط راهبردی متغیرات انجام گرفت. به منظور تحلیل فرآیند، سه متغیر مستقل شامل COD، MLVSS و زمان هواهایی و سه پارامتر وابسته فراآیندی و کیفی به عنوان پایه فراآیندی مورد مطالعه قرار گرفتند. شاخص حجمی لجس و pH نهایی پایه‌ها فراآیندی بودند که در این مطالعه ارزیابی شدند. آزمایش‌ها بر اساس طرح ترکیب مرکزی (CCD) و با استفاده از روش پایخ سطحی (RSM) اندازه و مدال‌سازی شدند. حدوده ناحیه مورد آزمایش برای تصفیه فاضلاب سننی برابر 1000 تا 5000 میلی گرم در لیتر و برای زمان هواهایی (MLVSS) برابر 500 تا 10000 میلی گرم در لیتر. COD سه ساعت در انظار گرفته شد. این سه سناری نتیجه نشان می‌داد که نتیجه‌های آزمایش‌های آزمایشگاهی باعث درک بهتری از فاز‌های مختلف راکتورهای انرژی‌منش از طریق سنجش و آزمایش سنجشی تأثیر گرفت. در مورد تغییرات pH، اثر فراآیندی کیفی و فراآیندی در مقایسه متغیرات و فراآیندی را به صورت متفاوتی ثبت کرد. کلمات کلیدی: حذف COD، راکتور متواالی نابایوسته (SBR)، فاضلاب لبی سننی