A comparative study of an up-flow aerobic/anoxic sludge fixed film bioreactor and sequencing batch reactor with intermittent aeration in simultaneous nutrients (N, P) removal from synthetic wastewater
Amir Mohammad Mansouri and Ali Akbar Zinatizadeh

ABSTRACT
The performance of two bench scale activated sludge reactors with two feeding regimes, continuous fed (an up-flow aerobic/anoxic sludge fixed film (UAASFF) bioreactor) and batch fed (sequencing batch reactor (SBR)) with intermittent aeration, were evaluated for simultaneous nutrients (N, P) removal. Three significant variables (retention/reaction time, chemical oxygen demand (COD): N (nitrogen): P (phosphorus) ratio and aeration time) were selected for modeling, analyzing, and optimizing the process. At high retention time (>6 h), two bioreactors showed comparable removal efficiencies, but at lower hydraulic retention time, the UAASFF bioreactor showed a better performance with higher nutrient removal efficiency than the SBR. The experimental results indicated that the total Kjeldahl nitrogen removal efficiency in the UAASFF increased from 70.84% to 79.2% when compared to SBR. It was also found that the COD removal efficiencies of both processes were over 87%, and total nitrogen and total phosphorus removal efficiencies were 79.2% and 72.98% in UAASFF, and 71.2% and 68.9% in SBR, respectively.

Key words | comparative study, nutrients removal, response surface methodology, sequencing batch reactor, wastewater treatment

INTRODUCTION
Eutrophication of an enclosed water area is caused by contaminants, especially chemical oxygen demand (COD), nitrogen and phosphorus. Long-term accumulation of nutrients will cause eutrophication and influence the quality of water resource (Martín et al. 2013; Pirsaheb et al. 2014; Huang et al. 2015; Fang et al. 2016).

As environmental pollution is considered as a major challenge facing the world (Ahmadi et al. 2017) and removal of nutrients from the effluent of wastewater plants is an important factor in environmental sustainability, therefore, biological treatment of wastewaters has become an established pollution control technology, and several reactor configurations and modifications have been studied (Wang et al. 2013; Mansouri et al. 2014; Dong et al. 2015; Wan et al. 2016). Biological wastewater treatment methods and especially activated sludge (AS), which consists of a stirred and aerated flocculated suspension of a mixed bacterial population that comes into contact with wastewater, are the most commonly used processes in wastewater treatment and they have been successfully applied for different types of wastewater (Ma et al. 2009; Mohammadi et al. 2012; Sharafi et al. 2014; Ahmadi et al. 2015).

In most wastewater treatment systems, biological nitrogen and phosphate removal are usually integrated in a single sludge system. However, the combined enhanced biological phosphate removal and conventional biological nitrogen removal (nitrification and denitrification) processes can face problems, since COD is often a limiting factor for phosphate removal and denitrification (Bassin et al. 2012; Pirsaheb et al. 2015a, 2015b). In the biological nutrient removal processes, denitrifiers and phosphate accumulating organisms (PAOs) consume the readily biodegradable COD (rbCOD). If the influent wastewater has a low biochemical oxygen demand (BOD)/N ratio (BOD/N < 9), external carbon sources such as fermented waste sludge should be added to enhance denitrification (Ra et al. 2000; Li et al. 2010).
..., which increases the operating costs. If the simultaneous P and N removal is expected to be achieved in a single reactor, PAOs will compete with denitrifiers for rbCOD in the wastewater because both denitrification and phosphate release require organic carbon. This competition between PAOs and denitrifiers will result in unstable biological P removal if the influent wastewater does not contain sufficient rbCOD, or the aeration period is very long (Arun et al. 1988; Kargi & Konya 2007). The limitation of COD can be overcome when organisms capable of performing simultaneous denitrification and anoxic phosphorus uptake are present in the treatment system (Van Loosdrecht et al. 1998; Wang et al. 2009).

One of the emerging technologies suitable for accomplishing simultaneous nitrogen and phosphate removal in a single reactor unit is intermittent aeration (Zhang et al. 2006; Zhang et al. 2017). Intermittent aeration can achieve nitrogen and phosphorus removal by simultaneous nitrification and denitrification (SND), P-uptake and P-release in the same reactor in accordance with the time cycle of aeration and non-aeration (Chiemchaisri 1993; Belli et al. 2017). This strategy can also reduce the cost of the treatment operation, and demand for rbCOD contained in the influent wastewater in the fill phase by minimizing the occurrence of N removal in the fill phase, so that PAOs will obtain sufficient rbCOD for anaerobic P release, which is beneficial to biological P removal (Orhon et al. 2005).

Jian et al. (2008) revealed that the highest nitrogen removal efficiency in an intermittent aerated submerged membrane bioreactor (SMBR) was achieved in 30-min of aeration of time in a 120-min cycle (Jian et al. 2008). An average removal efficiency of 63.9% was obtained by Hongfang and Xiufeng in an intermittent aerated SMBR treating wastewater with slight fluctuation under two different aeration on/off time (Hongfang & Xiufeng 2008). An intermittently aerated membrane reactor was applied at laboratory scale by Huidong et al., and in this study for recovery of copper ions from wastewater using a hollow fiber supported emulsion liquid membrane was studied. The extraction rate increases with the increase of feed phase pH, carrier concentration, hydrogen ion concentration in the stripping phase, and effective hollow fiber area (Huidong et al. 2013).

The sequencing batch reactor (SBR) has been applied as one alternative biological nutrient removal (BNR) technology because its process is simple to operate and very flexible for combining nitrogen and phosphorus removal. Its cycle format can be easily modified at any time to offset changes in process conditions, influent characteristics or effluent objectives (Pochana & Keller 1999). However, SBR has a potential deficit in that poor clarification and a turbid effluent are associated with it. To overcome these drawbacks and improve system performance, in the present study, a novel up-flow aerobic/anoxic sludge fixed film (UAASFF) reactor as a hybrid reactor, which is a combination of an AS and an immobilized cell or fixed film (FF) reactor, was established and applied as a single treatment unit for carbon, nitrogen and phosphorus removal, and also the process performance of this reactor was compared with SBR. The possibility to achieve high biomass concentration, no requirement for additional equipment to circulate the mixed liquor between aerobic and anoxic compartments and, consequently, the application of low hydraulic retention time (HRT) are the advantages of this reactor. Although the removal of nitrogen and phosphorus in various reactors has been examined in several studies, the investigation of their simultaneous removal in a single bioreactor is limited. In conventional BNR systems, both N and P removal require COD, which is often the limiting substrate in the incoming wastewater. Making the best use of the available COD for N and P removal is one of the objectives of the current research and development efforts in BNR design and operation.

Furthermore, the conventional technique for the optimization of a multi-factorial system is to deal with one factor at a time. However, this type of method is time consuming and also does not reveal the alternative effects among the components. Therefore, in this study, central composite design (CCD) and response surface methodology (RSM) have been applied to the modeling and optimization of CNP removal in these bioreactors. RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing process (Ghorbani et al. 2008). The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. The factors (variables) studied in this study were retention/reaction time, COD:N:P ratio and aeration time. The interactions among the variables as well as their direct impacts on the process responses (COD removal, total nitrogen (TN) removal, total Kjeldahl nitrogen (TKN) removal, effluent nitrate concentrations and total phosphorus (TP) removal) are discussed.

MATERIALS AND METHODS

Synthetic wastewater

Synthetic wastewater (SWW) was prepared based on the three different COD:N:P ratios (1,000:250:50, 1,000:83.3:35 and...
The SWW was composed of glucose as a simple carbon source, KH₂PO₄ as a phosphorus source, NH₄CL as a nitrogen source and mineral nutrients such as MgSO₄, FeSO₄, CaCl₂ and NaHCO₃. Chemical composition and properties of each type of SWW for the UAASFF bioreactor are shown in Table 1.

### Bioreactor configuration and start-up

A laboratory-scale UAASFF bioreactor (as presented in graphical abstract) was used in this study. The glass bioreactor column was fabricated with an internal diameter of 5.2 cm and a liquid height of 122 cm. The working volume (total liquid volume excluding the volume of the pall rings in the fixed bed section) was 2,500 ml. The column consisted of three sections: bottom, middle and top. The bottom part of the column, with a height of 80 cm, was operated as an up flow AS reactor; the middle part of the column, with a height of 25 cm, was operated as a FF reactor; and the third part is just for continuous feeding with the intermediate discharge. The middle section of the column was packed with a plastic media (supplied by Jiang Xi Transung Chemical Packing Co., China). The voidage of the packed-bed reactor was 85.45% and the specific surface area of the packing material was 500 m²/m³. The UAASFF bioreactor was operated under room temperature (20 ± 2 °C). In order to distribute the feed uniformly in the reactor, an influent liquid distributor was mounted at the base of the column. The air was introduced into the reactor with two bubble air diffusers at the bottom of the reactor, and the airflow rate and aeration time were controlled with an air flow meter and timer that were connected to a blower. The excess sludge was removed during the draw and idle period to control the MLSS of the system.

Both bioreactors were inoculated with AS taken from an aeration tank (municipal wastewater treatment plant, Kermanshah, Iran). The inoculum sludge had a sludge age of about 15 d and an MLSS concentration of 5.8 g/l. After initial dilution, 2.5 L of AS was seeded to the bioreactor, resulting in an initial MLSS concentration of 4 ± 1 g/l in the bioreactor.

For both bioreactors, in the first stage (bioreactor start-up), after adding the prepared inoculums, the bioreactors were set with the following conditions: HRT of 6 h, aeration time of 4 h, mixing time (without aeration) of 90 min and settling time of 30 min. In this stage, SWW was used as feed with a COD concentration of about 1,000 mg/l. This program was continued until a steady state condition was achieved (about 4 weeks). In the second stage, once the bioreactors reached the steady state condition, the bioreactors were operated according to Table 2. For all the experiments presented in Table 3, the reactors were operated until the steady state removal of CNP was achieved.

### Table 1 | Composition of the SWW used in this study for UAASFF bioreactor: (A) COD:N:P – 1,000:50:20, (B) COD:N:P – 1,000:83.3:35, and (C) COD:N:P – 1,000:50:20

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<th>Composition</th>
<th>A (g/L)</th>
<th>B (g/L)</th>
<th>C (g/L)</th>
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### Experimental design and mathematical model

#### Variables evaluation

Nutrients removal in BNR systems depends on a multitude of variables. Among these, the six main factors that affect the nutrient removal in different reactors are HRT, COD:N:P ratio, aeration time, biomass concentration, temperature and pH. In this study, HRT, COD:N:P ratio and
aeration time were chosen as independent; they are the most critical operating factors for UAASFF for the following reasons:

1. The most important parameter affecting the ‘cost’ of biological treatment systems is HRT, 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 HRT to produce the required effluent quality will result in an optimal condition. The range studied for HRT is shown in Table 2.

2. Operational costs of the biological nitrogen removal process are also related to the aeration for nitrification and the recycling of nitrified liquid for denitrification. On the other hand, in the SND process, the most influential process control factor is the aeration period within an applied HRT. Thus, exploring the optimum aeration time to provide the required efficiency is of crucial importance. The range studied for HRT is shown in Table 2.

3. Microbial degradation of any wastes depends on the amount of carbon, nitrogen, and phosphorus available for their activity. Too little or too much nitrogen cause inhibition in the biological activity and growth of the bacteria. On the other hand, the denitrification intensity depends on carbon availability. The carbon to nitrogen ratio in the influent should be high enough to denitrify all nitrates that arise in the nitrification process. The treatment of various wastewaters has been studied by many researchers, but they have not presented any common agreement on the COD/N/P ratio that supports most of the useful microorganisms. However, in the present study, the ratio was chosen to be in the range of 1,000:250:50 to 1,000:50:20 (Table 2), so that the ratio in many different wastewaters including municipal

<table>
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<th>Experimental range and levels of the independent variables</th>
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<td>Variables</td>
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</tr>
<tr>
<td>COD:N:P ratio</td>
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<tr>
<td>Aeration time(min/h)</td>
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</table>

| SBR | Range and levels |
| Variables | |
| Cycle time | 2 | 4.25 | 6.50 |
| Aeration time(min/h) | 30 | 40 | 50 |

<table>
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<th>Aeration strategy in different aeration time</th>
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<tr>
<td>30 min/h</td>
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<td>40 min/h</td>
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<td>50 min/h</td>
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which was automatically controlled by a time relay. During the anoxic stage, the reactor was continuously stirred. For the UAASFF bioreactor, the discharge of wastewater occurred in the last minute of non-aeration time.

In this study, three different aeration strategies (30 min/h, 40 min/h and 50 min/h) were used for simultaneous CNP removal.
### Table 3 | Experimental conditions and results of CCD for UAASFF and SBR bioreactors

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<th>NO₃</th>
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**UAASFF**

**Variables**

- **Factor 1**: HRT
- **Factor 2**: Aeration time
- **Factor 3**: COD:N:P

**Responses**

- **COD Re.**
- **TKN Re.**
- **NO₃ Eff.**
- **TN Re.**
- **P Re.**

**SBR**

**Variables**

- **Factor 1**: HRT
- **Factor 2**: Aeration time

**Responses**

- **COD Re.**
- **TKN Re.**
- **NO₃ Eff.**
- **TN Re.**
- **P Re.**
wastewater is covered (Mason et al. 2003). In the batch experiments, cycle time and aeration time were chosen as independent and the most critical operating factors. Of course, in order to examine the performance of the two bench scale AS systems, the COD:N:P ratio was placed at 1,000:125:25.

Experimental design

The factorial design of the experiments (DOE) eliminates systematic errors with an estimate of the experimental error, and minimizes the number of experiments (Kuehl & Kuehl 2000; Bingöl et al. 2015). The Design Expert software (version 6.0.7) was used for the statistical DOE and data analysis. In this study, the CCD and RSM were applied to optimize the three most important operating variables (HRT, COD:N:P ratio and aeration time) for UAASFF and two important variables (cycle time and aeration time) for SBR.

The bioreactor performance in the nutrient removal was assessed based on the full face-centered CCD experimental plan (Table 3). The design consisted of $2^k$ factorial points augmented by 2k axial points and a center point where k is the number of variables. The operating variables were considered at three levels, namely low (-1), central (0) and high (1). Accordingly, 20 experiments (based on three variables) were conducted for UAASFF (nine experiments organized in a factorial design (including seven factorial points, seven axial points and one center point) and the remaining five involving the replication of the central point) and 13 experiments (two variables) were conducted for SBR (nine experiments organized in a factorial design (including four factorial points, four axial points and one center point) and the remaining four involving the replication of the central point) to get a good estimate of the experimental error. Repetition experiments were carried out after the other experiments followed by the order of runs designed by DOE as shown in Table 3. In order to carry out a comprehensive analysis of the reactor, five dependent parameters were either directly measured or calculated as response. These parameters were total COD (TCOD) removal, TN removal, TKN removal, effluent nitrate concentration and phosphorus removal.

Mathematical modeling

After conducting the experiments, the coefficients of the polynomial model were calculated using the following equation (Shahrezaei et al. 2012):

$$Y = \beta_0 + \beta_iX_i + \beta_jX_j + \beta_{ij}X_iX_j + \beta_{i2}X_i^2 + \beta_{j2}X_j^2 + \beta_{ij}X_iX_j + \ldots$$  \hspace{1cm} (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 were obtained based on the effect of the levels of the variables. From these three-dimensional plots, the simultaneous interaction of the three factors for UAASFF and two factors for SBR on the responses was studied. The experimental conditions and results are shown in Table 3.

RESULTS AND DISCUSSION

Statistical analysis

The relationship between the three variables (HRT, aeration time and COD:N:P ratio) for the UAASFF bioreactor and two variables (cycle time and aeration time) for the SBR with the five important process responses (COD removal, TN removal, TKN removal, effluent nitrate concentration and phosphorus removal) were analyzed using RSM.

Significant model terms are desired to obtain a good fit in a particular model. The CCD shown in Table 4 allowed the development of mathematical equations where predicted results (Y) were assessed as a function of the retention/reaction time (A) aeration time (B) and COD:N:P ratio (C) and calculated as the sum of a constant, two first-order effects (terms in A and B), one interaction effect (AB) and two second-order effects (A^2 and B^2) according to Equation (1). The results obtained were then analyzed by ANOVA to assess the ‘goodness of fit’. Equations from the first ANOVA analysis were modified by eliminating the terms found to be statistically insignificant. Table 4 illustrates the reduced models in terms of coded factors and also shows other statistical parameters. Data given in this table demonstrate that all the models were significant at the 5% confidence level since P values were less than 0.05. The lack of fit (LOF) F-test describes the variation of the data around the fitted model. If the model does not fit the data well, this will be significant. The large P values for LOF (>0.05) presented in Table 4 (PLOF) show that the F-statistic was insignificant, implying significant model correlation between the variables and process responses. The $R^2$
The ANOVA values for TCOD removal efficiency in both systems are shown in Table 4. From the analysis, the main coefficient gives the proportion of the total variation in the response predicted by the model, indicating the ratio of the sum of squares due to regression (SSR) to the total sum of squares. A high $R^2$ value, close to 1, is desirable and a reasonable agreement with adjusted $R^2$ is necessary. A high $R^2$ coefficient ensures a satisfactory adjustment of the quadratic model to the experimental data. Adequate precision (AP) compares the range of the predicted values at the design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination (Idris et al. 2006). Besides, AP values higher than four (Table 4) for all the responses confirm that all predicted models can be used to navigate the design space defined by the CCD. The coefficient of variance (CV) as the ratio of the standard error of estimate to the mean value of the observed response defines the reproducibility of the model. A model normally can be considered reproducible if its CV is not greater than 10%. According to Table 4, the only model that falls short in terms of reproducibility is the model for effluent nitrate concentration in both systems.

### Process analysis

#### COD removal

The ANOVA values for TCOD removal efficiency in both systems are shown in Table 4. From the analysis, the main and second-order effects and the two-level interactions of retention/reaction time and aeration time (A, B, A² and AB) are significant model terms in both systems; the COD: N:P (C) ratio also had a little effect on COD removal in the UAASFF bioreactor. Other model terms are not significant (with a probability value greater than 0.05). Therefore in order to simplify the model, these model terms (B², C², and BC) were eliminated. The regression equation shown in Table 4 demonstrates an empirical model in terms of coded factors for COD removal. The influent COD concentration was constant at about 1,000 mg/l during all the experiments.

Figure 1 shows the response surface plots of the models for variation in the COD removal as a function of retention/reaction time and aeration time (B) with a constant COD:N:P ratio 1,000:125:25 in both systems. As can be seen in Figure 1(a) and 1(b), the same trends were found as the retention/reaction time and aeration time changed from 2 to 6.5 h and 30 to 50 min/h respectively. The COD removal increased remarkably with an increase in retention time, favoring the condition reducing the organic loading rate (OLR). At high retention/reaction time (>6 h), both bioreactors showed comparable removal efficiencies and elimination capacity, but at lower HRT (<4.25 h), the UAASFF bioreactor showed a better performance with higher removal efficiency (22–28%) than the SBR.
The UAASFF bioreactor showed a sharp drop in removal efficiency and elimination capacity at high loading rates. It was observed that when aeration time increased, the COD removal increased significantly for both systems. When aeration time was increased from 30 to 50 min/h, the COD removal increased from 65 to 84.7% for the UAASFF bioreactor and from 37 to 63% for SBR at lower HRT. This showed that the rate of substrate removal at lower retention/reaction time was higher for the UAASFF bioreactor compared to the SBR, indicating that UAASFF bioreactors are much more susceptible to changes in the COD concentration in this condition. The effect of retention/reaction time at lower values of the aeration time was greater than those with higher aeration times. Normally, for aerobic treatment systems, a higher F/M ratio leads to higher organic loading, which in turn places a greater stress on the system; this generally results in a low efficiency of substrate removal and low oxygen utilization. The interaction showed that retention/reaction time and aeration time played an important role in TCOD removal in both systems. In the figures, the response was increased upon increasing the aeration time at lower retention/reaction time, while at higher retention/reaction times, the HRT aeration time did not show a significant effect on TCOD removal. This was attributed to sufficient aeration time at higher HRT, which makes the response independent of aeration time in the design space studied. As a result, as the retention/reaction time increases, less aeration time is needed. Kargi and Konya found that a stepwise increase in HRT from 5 to 15 h resulted in about a 40 percent increase in COD removal, and the efficiency remained almost constant at larger HRT levels. Meng et al. also reported similar results (Kargi & Konya 2007; Meng et al. 2009).

The maximum values of the response were obtained as 91.27% and 87.18% for UAASFF and SBR bioreactors, respectively. The COD removal efficiency is highest in the UAASFF bioreactor, probably due to good mixing, the continuous availability of nutrients and biomass growth in the packed bed in the middle part of the UAASFF bioreactor. The availability of nutrients also enables a shorter acclimation time for the UAASFF than the SBR.

It must be mentioned that the HRT for the UAASFF bioreactor was in the range of 3–6 h. The range of studied HRT corresponded to the food to microorganism ratio (F/M) and feed flow rate of 0.39–2.34 g COD/g VSS.d and 3.96–7.92 l/d, respectively. These observations indicate that UAASFF bioreactor is less vulnerable to high OLR as compared to SBR, allowing UAASFF to sustain a higher organic removal. High organic loading enables downsizing of reactors, and a better rate of acclimation allows for early start-ups and rapid recovery from shock, both being desirable from the practical standpoint.

**TN removal**

The ANOVA results for TN removal efficiency for UAASFF and SBR bioreactors are presented in Table 4. Reduced quadratic models describe the variation of the TN removal as a result of changes in the variables in both bioreactors. The main effects of the variables (A, B) and second-order effect of aeration time (B²) are significant model terms for the SBR bioreactor but in the UAASFF bioreactor
two-level interactions of the variables (AB), as well as these model terms, had a significant impact on TN removal. The other model terms were eliminated due to their large p value (>0.05).

In order to gain a better understanding of the interaction effects of the variables on TN removal efficiency, three-dimensional contour plots for the measured response were formed based on the regression equations for UAASFF and SBR bioreactors (Table 4) as shown in Figure 2. The figures indicate that rising retention/reaction time provides favorable conditions for TN removal. As noted in Figure 2(a) and 2(b), the increase in TN removal caused by an increase in retention/reaction time at the lower values of the aeration time (40 min/h) was greater than that at the higher values of the aeration time (>40 min/h). So, the changes in the TN removal at an aeration time of 30 min/h (the lowest limit) for the UAASFF and SBR were 34% and 43%, respectively, while for an aeration time greater than 40 min/h the value was negligible for UAASFF and 33% for SBR. The denitrification process occurred under anoxic conditions with enough NO\textsubscript{3}\textsuperscript{-}/C\textsubscript{0}\textsubscript{3} where the aeration time was relatively low. Therefore, at the low aeration times, as HRT increases more NO\textsubscript{3}\textsuperscript{-} is generated from the nitrification process and sufficient time is conversely provided for denitrifying the nitrate to nitrogen gas, resulting in more TN removal.

A reverse impact of the aeration time on TN removal was observed as the variable increased in both systems (Figure 3(a) and 3(b)). However the reverse impact of the aeration time for the UAASFF was greater than for the SBR. At low HRTs, an increase in aeration time (from 30 to 40 min/h) caused an increase in the response due to higher NO\textsubscript{3}\textsuperscript{-} production; also, the favored condition for denitrification resulted from a high dissolved oxygen (DO) consumption rate. A further increment in the variable (from 40 to 50 min/h) decreased the response. This was due to the domination of nitrification over the denitrification process, which originated from the much shortened time of settling in both systems. Therefore, TN removal was greatly influenced by the operating DO concentration (aeration time). On the basis of these considerations, SND have gained significant attention, because high DO concentrations inhibit the denitrification process, which causes an accumulation of nitrate in the reactor. On the other hand, at lower DO concentrations, the nitrification process is inhibited and the denitrification process is enhanced. Therefore, the DO level is a factor critical to the SND process. It must be maintained at an appropriate level in the SND reactor. Zhao et al. pointed out that DO must be available for the nitrifiers, but must not exceed a certain level at the same time for the denitrifiers and SND (Zhao et al. 1999).

The regression equation for the UAASFF clearly indicated that COD:N:P (C) did not have any significant effect on the response, therefore this bioreactor can be used at a wide range of COD:N:P ratios (1,000:50:20 to 1,000:250:50). This was due to intermittent aeration time. In the simultaneous P and N removal bioreactors, PAOs will compete with denitrifiers for rbCOD for anaerobic P release. This competition between PAOs and denitrifiers will result in unstable biological P removal if the influent wastewater does not contain sufficient rbCOD or the aeration period is very long. Therefore, in the two bioreactors, in order to avoid this problem, a conventional operation

![Figure 2](https://iwaponline.com/wst/article-pdf/76/5/1044/450615/wst076051044.pdf)
can be changed to intermittent aeration. The intermittent aeration strategy can also reduce the cost of the treatment operation and the demand for rbCOD contained in the influent wastewater in the fill phase by minimizing the occurrence of N removal in the fill phase, so that PAOs will obtain sufficient rbCOD for anaerobic P release, which is beneficial to biological P removal.

From Figure 2(a) and 2(b), two minimum regions were obtained; one resulted from high aeration time (>40 min/h) due to the domination of the nitrification process, and the other was obtained at low HRTs because of more OLR.

Response surface plots in Figure 2(a) indicate optimum points to be at about HRT 6.5 and an aeration time of 38 min/h for the UAASFF bioreactor. Likewise, Figure 2(b) demonstrates that the optimum removal occurred at around a cycle time of 6.5 h and aeration time of 40 min/h for the SBR. Removal efficiencies are found to reduce when moving away from these points of aeration time, meaning that either an increase or decrease in any of the tested variables results in a decline of the responses. The TN removal efficiency in the UAASFF bioreactor was calculated to be in the range of 45–79.21%, while the TN reduction achieved by the SBR system varied between 27–71%.

TKN removal

In a similar way, response surface analysis was performed to evaluate the effects of the variables on TKN removal efficiency. In order to investigate the effects of the variables studied on TKN removal efficiency, the dependency of this response to the variables was analyzed and modeled. By applying multiple regression analysis on the experimental data, the experimental results of the CCD design were fitted with a two factor interaction (2FI) model for the UAASFF and a modified quadratic model for the SBR. The empirical relationship between TKN removal and the variables for both bioreactors in terms of coded factors are presented in Table 4. The high regression coefficient values of 0.93 and 0.94 indicate that the regression models were an appropriate representation of TKN removal efficiency within the defined experimental zone for the UAASFF and SBR bioreactors, respectively. As is noted in Table 4, the main-order effects of the HRT and aeration time had positive impacts on TKN removal efficiency in both reactors, while two-level interactions of the variables (AB) and the second-order effect of cycle time (A²) showed negative impacts on the response for the UAASFF and SBR, respectively.

The response surface plots for UAASFF and SBR are shown in Figures 3(a) and 4(b), respectively. The plots are approximately the same shape. It was found that with a simultaneous increase in both variables (A and B), the TKN removal efficiency increased in both systems, favoring the nitrification condition. By comparing the results obtained for TKN removal with TN removal (Figures 2(a), 3(a) and 3(b)), it was figured out that a similar trend in the responses was obtained until aeration time of about 40 min, indicating an appropriate proportion between nitrification and denitrification processes. While at the higher values of
aeration time (>40 min/h), the nitrification process was the dominant process, which is confirmed by the results shown in Figure 4. The effect of cycle time on the response was reduced by increasing the aeration time due to a limited anoxic condition.

The maximum TKN removal for UAASFF was determined to be 79.2% at an HRT and aeration time of 6.5 h and 30 min/h, respectively. Also, the maximum TKN removal efficiency (37.6%) for SBR was obtained at an HRT and aeration time of 6.5 and 50 min/h, respectively.

**Effluent nitrate**

Effluent NO$_3^-$ concentration is an indicator to justify the difference between TN & TKN removal efficiencies. It implies the progress in the nitrification and denitrification processes. Therefore, in this study, the effluent NO$_3^-$ concentration was measured to verify the TN and TKN removal data. The ANOVA statistics for both the UAASFF and SBR bioreactors are shown in Table 4. ANOVA results of these quadratic models indicated that these quadratic models could be used to navigate the design space. The modified quadratic model (Table 4) for the UAASFF bioreactor shows that the main effect of HRT (A) and aeration time (B), two-level interaction HRT and aeration time (AB) and the second order effect of aeration time had a positive impact on effluent nitrate concentration, while the COD:N:P ratio (C) and BC had a negative impact on the response. However, for the SBR it shows that the most significant factor on the response was the aeration time. The retention time (B), on its own, did not appear to have a direct effect on effluent nitrate concentration in the SBR. From Figure 4, the negative effect of an increase in the aeration time on effluent nitrate was dominant at an aeration time lower than 40 min/h, while at values higher than 40 min/h, B and B$^2$ terms became dominant and demonstrated an increasing effect on effluent nitrate.

The response surface for both systems had a nearly stationary region between aeration times of 30 and 40 min/h. A near stationary region is defined as a region where the surface slopes (or gradient along the variable axis) are small compared to the estimate of the experimental error. The response surface was sharply increased as the aeration time increased from 40 to 50 min/h. Of course, the effect of increasing the aeration time in the SBR was much more than the UAASFF bioreactor. By visualizing these results, it can be said that the aeration time was the key factor on the effluent nitrate in both systems, while HRT played only a supportive role for the response in the range of variables tested in this study. It proved the earlier discussion on TN & TKN removal efficiencies. It was observed that with a simultaneous increase in retention/reaction time and the aeration time (by comparing Figure 4(a) and 4(b)), the effluent nitrate concentration was increased. As mentioned above, high values of aeration time and HRT favored the nitrification process. On the other hand, a high value of aeration time showed a negative effect on the denitrification process due to an increase in the oxidation and reduction potential, increasing the effluent nitrate concentration. This result was close to that reported by Dong et al. and Hasar et al. (Hasar et al. 2002; Dong et al. 2009).

![Figure 4](https://iwaponline.com/wst/article-pdf/76/5/1044/450615/wst076051044.pdf)

*Figure 4* | Response surface plots for effluent nitrate concentration: (a) with respect to HRT and aeration time at constant value of COD:N = 1,000:125:25 in UAASFF bioreactor, (b) with respect to cycle time and aeration time for SBR.
According to the models and figures, the minimum level for nitrate was predicted to be around the middle level of the aeration time, corresponding to the maximum level of TN removal. The maximum effluent nitrate for the UAASFF and SBR was determined to be 30.35 and 133.2 mg/l respectively.

**Phosphorus removal**

Phosphorus can only be removed by its uptake into biomass, which can be discharged from the system as surplus sludge. Thus, a biomass with high phosphorus content is desirable for biological phosphorus removal. Removal of phosphorus in wastewater is closely dependent upon the phosphorus release in anaerobic conditions and on the subsequent uptake process of the excess phosphorus, including that contained in wastewater in aerobic conditions. This basic information indicates that the control of anaerobic (or anoxic) and aerobic conditions are of great importance to biological phosphorus removal. In the present study, as the system is intermittently aerated, a micro anaerobic environment seems to be provided in the biofilm formed in the process. On the other hand, the biofilm formed in the middle part of the UAASFF bioreactor favors the development of anaerobic conditions while aeration is not supplied. Therefore, biological phosphorus removal would be possible in such a system with intermittent aeration.

From the analysis carried out (Table 4), reduced quadratic models were selected to describe the variation of the response for UAASFF and SBR. From the ANOVA results (Table 4) for UAASFF bioreactor, A, C, A², C² are significant model terms. Insignificant model terms, which have limited influence, such as B, B², AB, AC, and BC, were excluded from the study to improve the model. Therefore, the COD/P ratio did not show any significant effect in the design space studied.

The model terms, A, B and B² are significant factors for SBR. Insignificant model terms were found to be A² and AB, which were excluded from the model. No interactive impact of the studied variables on the response was shown.

Figure 5 demonstrates phosphorus removal efficiency as a function of retention/reaction time and aeration time at a constant value of COD:N:P = 1,000:125:25 for the UAASFF and SBR. From Figure 5(a), an increase in HRT caused an increase in the response. It is clear from Figure 5(a) that the response of phosphorus removal efficiency was very sensitive to these factors. Figure 5(b) depicts the response surface plot for phosphorus removal with respect to the cycle time and aeration time. In Figure 5, the response increased upon increasing the cycle time and decreasing the aeration time.

As noted in Figure 5(a) and 5(b), the aeration time had a reverse impact on the response. An increase in the aeration time (from 30 to 40 min/h) caused an increase in the response due to higher uptake of the excess phosphorus in aerobic conditions. Further increment in the variable (from 40 to 50 min/h) decreased the response. This was because an increase in the aeration time (C) causes a decrease in the anaerobic time when the phosphate accumulating organisms (PAOs) accumulate poly-β-hydroxy butyrate (PHB) from the volatile fatty acids (VFAs) produced. In this process, glucose as the individual source of
VFAs requires sufficient time for acidification (Hasar et al. 2002). Another reason for the decrease in the phosphorus removal at high aeration time was the presence of nitrate, which inhibits the fermentation processes, producing VFAs in the anaerobic zone.

Based on the above consideration, the biological phosphorus removal was proportionally accomplished depending on the concentration of organic compounds in the influent, HRT, as well as anoxic/aerobic (aeration time) control in the system, where the aeration time was a major controlling factor in determining the performance of the biological phosphorus removal. In this study, the influent COD/TP ratio was varied from 20 to 50 for the UAASFF and 25 for the SBR, indicating a wide range of COD:TP. However, the long aeration time control in both bioreactors restricted the phosphorus elimination such that the level of aeration time was considered as the limiting factor to implement simultaneous biological nitrogen and phosphorus removal (Fu et al. 2009). During the long-term operation of the SBR and UAASFF, the alternating aerobic/anoxic situation was provided by periodically switching off the aeration. It is known that when the bioreactors are aerated, the outer portion of the AS flocs and biofilm are aerobic while the inner portion of the flocs and biofilm will be anoxic and/or anaerobic (Tchobanoglous et al. 2014). Different biochemical reactions, such as COD heterotrophic biodegradation, nitrification, denitrification, and phosphorus uptake and release by polyphosphate accumulating organisms, occurred in the alternating aerobic/anoxic cycle. The DO concentration in the biofilm reactor in diethyl phthalate and diallyl phthalate removal from synthetic wastewater. Bioresource Technology 183 (0), 129–135.


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CONCLUSION

The UAASFF bioreactor proved to be a suitable bioreactor with higher nutrient removal efficiency than the SBR. At high HRT, both bioreactors showed comparable removal efficiencies and elimination capacity, but at lower HRT, the UAASFF bioreactor showed a better performance with higher removal efficiency than the SBR. The UAASFF bioreactor is more susceptible to changes in the COD concentration, and is less vulnerable to high OLR compared to the SBR. TN removal was greatly influenced by the operating aeration time, so that is a critical factor for the SND process. It must be maintained at an appropriate level in the SND reactor.

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