Enhancement of Biological Nutrient Removal in an Alternating Anaerobic-Aerobic Sequencing Batch Reactor: Optimization of Anaerobic and Aerobic Hydraulic Retention Times

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Abstract. Biological phosphorus removal (BPR), possesses the significant advantages of low operational costs and little environmental impacts, is an economical and sustainable process to remove P by recycling the activated sludge through anaerobic and aerobic processes. For the BPR system, the hydraulic retention times (HRT) in aerobic and anaerobic processes are the most significant controlling parameters which can directly affect the effluent quality, especially the effluent phosphorus removal efficiencies. In this study, six lab-scale sequencing batch reactors (SBRs) were operated to conduct the single-factor experiments. 13 experimental runs designed by a 2-factor and 5-level response surface methodology (RSM) using Central composite design (CCD) were used to optimize the relationship between anaerobic HRT (X1, h) and aerobic HRT (X2, h) and two most important responses, COD removal efficiency (CRE, Y1, %) and P removal efficiency (PRE, Y2, %). High squared regression coefficients R2 (> 0.99) and adjusted R2 (> 0.99) indicated a high degree of correlation between the predicted and actual responses, which means that the model could fit the response well. Experimental validation by operating under the optimal combination of the two operational HRTs were conducted. Good correlation between the predicted and experiments values provides confidence in the obtained models.

1. Introduction

Biological phosphorus removal (BPR) is one of the most economical and sustainable processes to remove P from wastewater achieved by recirculating activated sludge through anaerobic and aerobic processes [1-3]. Although many satisfactory results have already been reported in lab- and pilot-experiments [4,5], its practical implementation still faces many challenges, both in scientific research and practical engineered application. The hydraulic retention times (HRT) in aerobic and anaerobic process are the most significant controlling parameters which can directly affect the wastewater treatment, especially the phosphorus removal efficiency. On the other hand, the design of HRT also imposes a significant effect on the infrastructure and operational costs in an engineered bioreactor [6]. However, in terms of the investigations from previous studies, the controlling of the HRTs in anaerobic and aerobic reactions changed widely. In consideration of the controlling aerobic and anaerobic HRTs in SBRs during BPR process, Long et al. [7] used a 4 h anaerobic HRT and a 7 h aerobic HRT with an influent chemical organic demand (COD) of 600 mg/L and P of 30 mg/L, the specific controlling conditions were12 h per cycle: a 4 h anaerobic period, 7 h of aeration, a 50 min settlement, 5 min of decanting, and 5 min of idling. Zhang et al. [8] controlled a 2 h anaerobic HRT and a 170 min aerobic HRT with an influent COD of 214 mg/L and P of 20 mg/L. While almost
similar controlling strategies of 2 h anaerobic and 3 h aerobic period were conducted when an influent COD/P of 300 mg/L/12 mg/L were performed [9]. Furthermore, Yang et al. [10] used a 2 h anaerobic HRT and a 4 h aerobic HRT with different influential loadings: COD/P of 150 mg/L/6 mg/L; COD/P of 300 mg/L/13 mg/L; and COD/P of 400 mg/L/20 mg/L. From the literatures in the previous studies, it could be seen that the systematic analyses on why their anaerobic and aerobic HRTs in SBR are chosen and whether they are the optimal HRTs are not clearly stated in these papers. Thus, the controlling and optimization of the HRTs in aerobic and anaerobic processes are significant for mechanism study and engineering application.

The original single factor optimization method cannot account for the mutual effects of all the factors involved and requires a large number of experiments [11]. Response surface methodology (RSM) is a useful statistical technique for researching complex variable processes based on the Central composite design (CCD), which can effectively describe the interactions between independent experimental factors and response parameters [12]. By using a two-factor and five-level CCD, batch experiments on the study of the optimization of the HRTs in anaerobic and aerobic processes were conducted. The objective of this paper is to optimize the HRTs in different anaerobic and aerobic of the SBRs to achieve improved nutrient removal efficiencies during biological wastewater treatment process.

2. Material and Methods

2.1. Sludge cultivation and seed microorganisms

In this study, the applied bench-scale sequencing batch reactors (SBRs) are designed with a diameter of 15 cm, a height of 35 cm. The working volume of the SBR is 5.0 L. The schematic diagrams of SBR is shown in Fig. 1. Each SBR was inoculated to maintain a mixed liquor suspended solids (MLSS) of 4000 mg/L with activated sludge from a municipal wastewater treatment plant in Harbin. The temperature controlled in SBRs were 20.0 ± 0.5 °C. The constant temperature for the SBRs was maintained by a precision thermostatic bath circulator with the digital temperature controller (Ningbo Tianheng Instrument factory, Ningbo, China). Air was supplied from the bottom of the reactor by aerators and the dissolved oxygen (DO) concentration was maintained at 2-6 mg/L during aerobic phase. The DO concentration in each SBR was measured and controlled precisely by a DO probe with a DO industrial intelligent controller (SUP-DM2800, Hangzhou Sinomeasure automation Technology Co., LTD, China). Electric mechanical stirrer was performed to prevent sludge settling, which were run constantly except the time for settling, feeding and decanting: 40 min of sludge settling period, 15 min of decanting period, and 5 min of idling idle phase. The anaerobic period and aerobic period were performed in Table 1. Six identical SBRs with different anaerobic and aerobic HRTs during operation was operated to conduct the single-factor experiments. During operation, the pH was maintained at 7.50 ± 0.05 by two automatic titration units (SC-200A, ChangSha Sichen Instrument Technology Co., LTD, China) dosing 1 M HCl and NaOH to avoid the phosphate precipitation.

During operation period, the synthetic wastewater used as the influent of the tested SBRs were as follows: 256.4 mg/L sodium acetate (in chemical oxygen demand), 38.2 mg/L NH4Cl, 21.95 mg/L KH2PO4, 40 mg/L CaCl2, 75 mg/L MgSO4. After about 2 months’ cultivation, the sludge characteristics and effluent concentrations in the SBRs were maintained in steady states. Each measurement was performed in triplicate.

| Parameters                              | #1     | #2     | #3     | #4     | #5     | #6     |
|-----------------------------------------|--------|--------|--------|--------|--------|--------|
| Effective volume of SBRs (L)            | 5.00   | 5.00   | 5.00   | 5.00   | 5.00   | 5.00   |
| DO in anaerobic tank (mg/L)             | –      | –      | –      | –      | –      | –      |
| DO in aerobic tank (mg/L)               | 2-6    | 2-6    | 2-6    | 2-6    | 2-6    | 2-6    |
| HRT in anaerobic tank (h)               | 0.5    | 1.0    | 1.5    | 2.0    | 2.5    | 3.0    |
| HRT in aerobic tank (h)                 | 2.0    | 3.0    | 4.0    | 5.0    | 6.0    | 7.0    |
| HRT in setting tank(h)                  | 2.0    | 2.0    | 2.0    | 2.0    | 2.0    | 2.0    |
| MLSS in the main reactor (mg/L)         | 4000   | 4000   | 4000   | 4000   | 4000   | 4000   |
2.2 Central composite design and statistical analysis

RSM is a mathematical and statistical technique used to analyse the mutual relationships between the response and the independent variables [13], which were widely used to optimize operating parameters for a system. Furthermore, this optimization method is expected to describe the entire effects of the selected parameters on the process [12]. The results obtained from the orthogonal experiments are therefore analysed using RSM. In this study, 13 experimental runs designed by a two-factor and five-level RSM using CCD were used to optimize the relationship between anaerobic HRT ($X_1$, h) and aerobic HRT ($X_2$, h) and the most important response, COD removal efficiency (CRE, $Y_1$, %) and P removal efficiency (PRE, $Y_2$, %). Eq. (1) showed the relationship between the uncoded and coded values:

$$ x_i = \frac{X_i - X_i^*}{\Delta X_i} $$  \hspace{1cm} (1)

The second-order polynomial model is presented in Eq. (2):

$$ Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 $$  \hspace{1cm} (2)

where $x_i$ is the coded value of the independent variable; $X_i$ is the uncoded value of the independent variable; $X_i^*$ is the uncoded value of the independent variable at the centre point; $\Delta X_i$ is the step change value; $Y$ is the response variable; $\beta_0$, $\beta_1$, $\beta_2$, $\beta_{12}$, $\beta_{11}$, and $\beta_{22}$ represent the regression coefficients from the experimental data.

| Table 2 Experimental ranges and levels of the independent variables. |
|---------------------------------------------------------------|
| Independent variables | Low | High | -alpha | +alpha |
| Anaerobic HRT (h), $x_1$ | 1   | 2    | 0.792893 | 2.20711 |
| Aerobic HRT (h), $x_2$ | 3   | 5    | 2.58579 | 5.41421 |
2.3. Analytical methods
During the operation period, the measurements of COD, the soluble chemical oxygen demand (SCOD), MLSS, mixed liquor volatile suspended solids (MLVSS), sludge volume index (SVI), total phosphorus (TP), \( \text{PO}_4^{3-} \)-P were measured in accordance with the standard methods [14]. DO and pH were measured by probes (Germany WTW Company pH/Oxi 340i main engine, pH meter, Germany). Before examination, the collected samples were filtered through 0.45 µm filters for analyses. Analyses of COD, SCOD, MLSS, MLVSS, SVI, TP, \( \text{PO}_4^{3-} \)-P were determined every day. All the experiments were performed in triplicate. All the experiments were conducted at room temperature.

3. Results and discussion

3.1. Analysis of single factor test results
Six lab-scale SBRs (Table 1) performed under 20 ± 0.5 °C generally reached steady-state after one-month inoculation. In order to provide a basis for the subsequent orthogonal experiments, single-factor experiments were used to study the optimal operating ranges of HRTs in anaerobic and aerobic processes.

The results of the single factor tests shown in Fig. 2a indicated that the CRE and PRE in effluents were altered by varying the anaerobic and aerobic HRTs in SBRs. In Fig. 2a, enhanced CRE and PRE were observed with the increases in anaerobic HRT from 0.5 h to 1.5 h, then decreased from 2.0 h to 3.0 h. For the anaerobic HRT in SBR, 1.5 h was considered to be the best anaerobic HRT to control. In Fig. 2b, results showed that enhanced CRE and PRE were showed with an increased aerobic HRT of 4 h. Results of this study demonstrated that the anaerobic and aerobic HRTs were indeed significant parameters in COD and P removal during BPR process. Results obtained in this study demonstrated that when the anaerobic HRT was controlled from 4.2 h to 4.8 h, and the aerobic HRT was controlled from 1.35-1.45 h, better COD removal efficiency could be obtained from an alternating anaerobic and aerobic SBR system. According to the observation, a higher HRTs controlled in the alternating anaerobic/aerobic SBR might deteriorate the effluent COD. This phenomenon might be induced by the production of soluble microbial products (SMP) and biomass-associated products (BAP), more BAP production had significant effects on higher COD concentrations in effluent [15].

![Figure 2](image_url). Single factor test results of the CRE and PRE under different anaerobic HRT and aerobic HRT in SBRs

3.2. Optimization of operating variables and their reciprocal analysis
The design matrix and results obtained based on the experimental CCD design are showed in Table 3. Linear fitting of the predicted and the actual data (a) CRE (%) and (b) PRE (%) (Fig. 3) demonstrated that there is a good agreement between the predicted and the actual data.
Figure 3. Linear fitting of the predicted and the actual data (a) CRE (%) and (b) PRE (%).

Table 3 Response surface CCD and experiments.

| Actual values of parameters | Run | $X_1$ | $X_2$ | $Y_1$ | $Y_2$ |
|-----------------------------|-----|-------|-------|-------|-------|
| 1                           | 1   | 1.5   | 5.41421| 93.92 | 97.23 |
| 2                           | 1.5 | 4     | 94.34 | 97.53 |
| 3                           | 1.5 | 4     | 94.34 | 97.53 |
| 4                           | 1.5 | 4     | 94.34 | 97.53 |
| 5                           | 2   | 3     | 91.23 | 96.23 |
| 6                           | 1.5 | 4     | 94.34 | 97.53 |
| 7                           | 1.5 | 2.58579| 89.71 | 95.98 |
| 8                           | 0.792893| 4    | 93.33 | 92.32 |
| 9                           | 2.20711| 4    | 93.21 | 96.23 |
| 10                          | 1   | 5     | 94.32 | 94.44 |
| 11                          | 2   | 5     | 93.67 | 96.89 |
| 12                          | 1   | 3     | 91.21 | 93.12 |
| 13                          | 1.5 | 4     | 94.34 | 97.53 |

Second-order polynomial models for coded responses of $Y_1$ (CRE, %) were established as described in Eq. (3):

$$Y_{coded} = +94.34 - 0.100X_1 + 1.44X_2 - 0.17X_1X_2 - 0.52X_1^2 - 1.25X_2^2$$  \(3\)

Second-order polynomial models for actual responses of $Y_1$ (CRE, %) were established as described in Eq. (4):

$$Y_{actual} = +62.26916 + 7.36503X_1 + 11.91052X_2 - 0.33500X_1X_2 + 2.07499X_1^2 - 1.24626X_2^2$$  \(4\)

Table 4 shows the analysis of variance for the experimental model equations to examine the significance and the adequacy of the second-order polynomial equation. $P<0.0001$ indicated a high significance of the corresponding variable. A high squared regression coefficient, $R^2$ of 0.9981 and adjusted $R^2$ of 0.9967 indicated a high degree of correlation between the predicted and actual responses, indicating that the model could fit the response well. To represent the interaction between the independent variables and determine the optimal levels of each independent variable for observing the optimal response levels, the 2D contour curves and 3D response surface plots were depicted in Fig.
4. Results obtained in this study demonstrated that when the anaerobic HRT was controlled from 4.2 h to 4.8 h, and the aerobic HRT was controlled from 1.35-1.45 h, better COD removal efficiency could be obtained from an alternating anaerobic and aerobic SBR system. It could be observed that a higher HRTs controlled in SBR might deteriorate the effluent COD, which might induce by more BAP generated as the major effluent components at a long HRT and thus had significant effects on higher COD concentrations in effluent [15]. This conclusion was consistent with the single-factor experiments in this study.

Table 4 Analysis of variance (ANOVA) results for the response surface quadratic mode.

| Source          | Statistics | Sum of squares | df | Mean Square | F-value | P-value |
|-----------------|------------|----------------|----|-------------|---------|---------|
| Model           |            | 28.44          | 5  | 5.69        | 719.61  | < 0.0001 significant |
| $x_1$           |            | 0.080          | 1  | 0.080       | 10.11   |
| $x_2$           |            | 16.54          | 1  | 16.54       | 2093.07 |
| $x_1 x_2$       |            | 0.11           | 1  | 0.11        | 14.20   |
| $x_1^2$         |            | 1.87           | 1  | 1.87        | 236.86  |
| $x_2^2$         |            | 10.80          | 1  | 10.80       | 1367.07 |
| Residual        |            | 0.055          | 7  | 7.903E-003  |
| Lack of Fit     |            | 0.055          | 3  | 0.018       |
| Pure Error      |            | 0.000          | 4  | 0.000       |
| Cor Total       |            | 28.49          | 12 |             |

Figure 4. The effect of the anaerobic and aerobic HRTs and the response $Y_1$ (CRE, %): (a) 2D contour curves and (b) 3D response surface plots.

Second-order polynomial models for coded responses of $Y_2$ (PRE, %) were established as described in Eq. (5):

$$Y_{coded} = +97.53 + 1.39 x_1 + 0.47 x_2 + 0.16 x_1 x_2 + 1.69 x_1^2 - 0.53 x_2^2$$

(5)

Second-order polynomial models for actual responses of $Y_2$ (PRE, %) were established as described in Eq. (6):

$$Y_{actual} = +65.7825 + 24.43233 x_1 + 5.20350 x_2 - 0.33 x_1 x_2 + 6.77998 x_1^2 - 0.53000 x_2^2$$

(6)

The analysis of variance for the experimental model equations to examine the significance and the adequacy of the second-order polynomial equation was showed in Table 5. P<0.0001 demonstrated a high significance of the corresponding variable. $R^2$ of 0.9960 and adjusted $R^2$ of 0.9932 proved that the
high degree of correlation between the predicted and actual responses, indicating that the model could fit the response well. In Fig. 5, the contour 2D curves and the response surface 3D plot were depicted to represent the interaction between the independent variables and to determine the optimal levels of each independent variable for observing the optimal response levels.

Table 5 Analysis of variance (ANOVA) results for the response surface quadratic mode.

| Source     | Statistics | Sum of squares | df | Mean Square | F-value | P-value  |
|------------|------------|----------------|----|-------------|---------|----------|
| Model      |            | 37.90          | 5  | 7.58        | 350.10  | < 0.0001 significant |
| $x_1$      |            | 15.37          | 1  | 15.37       | 710.05  |          |
| $x_2$      |            | 1.76           | 1  | 1.76        | 81.10   |          |
| $x_1 \times x_2$ | | 0.11          | 1  | 0.11        | 5.03    |          |
| $x_1^2$    |            | 19.99          | 1  | 19.99       | 923.16  |          |
| $x_2^2$    |            | 1.95           | 1  | 1.95        | 90.26   |          |
| Residual   |            | 0.15           | 7  | 0.022       |         |          |
| Lack of Fit|            | 0.15           | 3  | 0.051       |         |          |
| Pure Error |            | 0.000          | 4  | 0.000       |         |          |
| Cor Total  |            | 38.05          | 12 |             |         |          |

Figure 5. The effect of the anaerobic and aerobic HRTs and the response $Y_2$ (PRE, %): (a) 2D contour curves and (b) 3D response surface plots.

3.3. Validation of the models

From Eqs. (4) and (5), the optimal actual values of $x_1$ and $x_2$ were determined to be 1.4 h and 4.6 h, and the maximum predicted COD was 94.78%; and a maximal PRE of 97.89% was obtained when the anaerobic HRT and aerobic HRT were controlled at 1.7 h and 4.4 h. To validate the optimal combination of the two operational HRTs, the optimal conditions predicted by RSM was used to test the predictive model. Triplicate tests under the optimized condition were conducted. Good correlation between the predicted and experiments values provides confidence in the obtained models.

4. Conclusion

In this study, 13 experimental runs designed by a 2-factor and 5-level RSM using CCD were conducted to design and study the optimal levels of the anaerobic HRT and aerobic HRT in an alternating anaerobic/aerobic SBR system. A good agreement between the predicted and the actual data were observed. For the established models, the second-order polynomial models for actual responses of $Y_1$ (CRE, %) and $Y_2$ (PRE, %) were obtained. Results demonstrated that when the anaerobic HRT was controlled from 4.2 to 4.8 h and the aerobic HRT was controlled from 1.35 to 1.45
h, better COD removal efficiency could be obtained from an alternating anaerobic and aerobic SBR system. A high HRT controlled in anaerobic and aerobic process might deteriorate the effluent COD due to more BAP production in effluent. High $R^2$ and adjusted $R^2$ (>0.99) demonstrated that a high degree of correlation between the predicted and actual responses, which further indicated that the model could fit the response well. To validate the optimal combination of the two operational HRTs, the optimal conditions predicted by RSM was used to test the predictive model. Good correlation between the predicted and experiments values provides confidence in the obtained models.

5. References
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