Effect of Staged Dissolved Oxygen Optimization on In-situ sludge Reduction and Enhanced Nutrient Removal in an A²MMBR-M System

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Abstract. Redundant excess sludge production and considerable non-standard wastewater discharge from existing activated sludge processes are facing more and more challenges. The investigations on lower sludge production and higher sewage treatment efficiency are urgently needed. In this study, an anaerobic/anoxic/micro-aerobic/oxic-MBR combining a micro-aerobic starvation sludge holding tank (A²MMBR-M) system is developed. Batch tests on the optimization of the staged dissolved oxygen (DO) in the micro-aerobic, the first oxic, and the second oxic tanks were carried out by a 3-factor and 3-level Box-Behnken design (BBD). The optimal actual values of $X_1$, $X_2$, and $X_3$ were DO$_1$ of 0.3-0.5 mg/L, DO$_2$ of 3.5-4.5 mg/L, and DO$_3$ of 3-4 mg/L. After the optimization tests, continuous-flow experiments of anaerobic/anoxic/oxic (AAO) and A²MMBR-M systems were further conducted. Compared to AAO system, a 37.45% reduction in discharged excess sludge in A²MMBR-M system was achieved. The COD, TN, and TP removal efficiencies in A²MMBR-M system were respective 4.06%, 2.68%, and 4.04% higher than AAO system. The A²MMBR-M system is proved a promising wastewater treatment technology possessing enhanced in-situ sludge reduction and improved effluent quality. The staged optimized DO concentrations are the key controlling parameters for the realization of simultaneous in-situ sludge reduction and nutrient removal.

1. Introduction
Activated sludge process, recognized as the widespread sewage treatment process for domestic, municipal and industrial wastewater treatment are the crucial method for sewage treatment [1]. However, redundant excess sludge yields from the existing activated sludge processes worldwide are facing more and more challenges [2], [3]. In terms of the study of Guo et al. [4], sludge reduction processes/technologies can be classified into two classifications: post sludge reduction through chemical, physical, biological or the combined treatment methods [5], [6]; and in-situ activated sludge reduction during sewage treatment process [7-10]. Thus, for the purpose of reducing the produced excess sludge yield at source, investigations on in-situ activated sludge reduction sewage treatment processes/technologies are urgently needed. On the other side, nowadays, the existing WWTPs in China, for instance, confront with considerable non-standard wastewater discharge [11]. Apparently, just blindly reduce the produced sludge yield is too blunt, also may deteriorate the quality of effluent as well as the operation of a sewage treatment plant, thus the study on lower sludge production and higher sewage treatment efficiency should be took into consideration in future researches. During wastewater treatment processes, the operating parameter of the dissolved oxygen (DO) is proved extremely significant both for the performances of nutrient removal efficiencies and the production yield of the excess sludge [12-15]. In consideration of the effect of DO on chemical
oxygen demand (COD), Janus and Ulanicki [16] predicted a slight increase in soluble microbial products (SMP) with increased DO concentration with the CES-ASM3 model, the production of SMP is the major components in effluent organic matters. Research from Holakoo et al. [17] found that the mixed liquor SMP concentrations increased with increases in DO concentrations. In the proposed staged aerated process of from the study of Yoo et al. [18], the optimal maximum DO concentration for nitrogen removal was determined to be around 2.0-2.5 mg/L. Wang et al. [19] found that the optimum DO concentration for nitrogen removal was 0.50 mg/L. Guo et al. [20] compared with high DO of 3 mg/L and lower DO of 0.4-0.8 mg/L in SBR, results showed that a simultaneous nitrification and denitrification was carried out in low DO. It was concluded that by selecting a proper DO level is not only of benefit to the biological nitrogen removal technology, but also favorable to sludge population optimization. Katie et al [21] showed that simultaneous nitrification and denitrification via nitrite succeeded between 0.4-1.6 mg/L DO concentration in a pilot-scale-modified anaerobic-anoxic-oxic reactor. On the basis of the analyses above, a staged aerated and optimized DO concentrations applied are the key control parameters investigated for the realization of simultaneous nutrient removal and sludge reduction.

In this study, the anaerobic/anoxic/oxic (AAO) system which is the most widely used wastewater treatment technology due to the simultaneous biological nutrients (carbon, nitrogen, and phosphorus) removal without any chemicals, is applied. An improved AAO system, an anaerobic/anoxic/micro-aerobic/oxic-MBR combining a micro-aerobic starvation sludge holding tank (A²MMBR-M) system, possessing good performances of enhanced sludge reduction and improved effluent quality, is investigated in this study. By using a 3-factor and 3-level Response surface methodology, batch tests on the study of the optimized DO concentrations in different reaction compartments were conducted. The objective of this paper is to optimize the DO concentrations in different reaction compartments of the A²MMBR-M system to achieve remarkable sludge reduction effect and improved nutrient removal efficiency.

2. Material and methods

2.1. Sludge cultivation

Before conducting the batch tests and the continuous-flow experiments, the activated sludge, taken from Harbin sewage treatment plant was inoculated into two identical AAO systems (Fig. 1a) for 2 months sludge cultivation. After 2-month sludge cultivation, the characteristics and biophase of the activated sludge in the two AAO systems were maintained in stable state. One AAO system was modified into an A²MMBR-M system (Fig. 1b), the other AAO system was performed as the control system. The composition of the synthetic wastewater used in these two AAO systems was as shown in the study of Yang et al. [22]. The specific operation and control parameters for the AAO systems were showed in Table 1.
Fig. 1. The schematic diagrams of (a) an anaerobic/anoxic/oxic (AAO) system and (b) an anaerobic/anoxic/micro-aerobic/oxic-MBR combining a micro-aerobic starvation sludge holding tank (A²MMBR-M) system.

Table 1 Operation and control parameters for the AAO and the A²MMBR-M systems

| Control parameters                        | Unit       |
|-------------------------------------------|------------|
| Effective volume of anaerobic tank        | L          |
| Effective volume of anoxic tank           | L          |
| Effective volume of oxic tank             | L          |
| Effective volume of setting tank          | L          |
| DO value in the first oxic tank           | mg/L       |
| DO value in the second oxic tank          | mg/L       |
| HRT in anaerobic tank                     | h          |
| HRT in anoxic tank                        | h          |
| HRT in oxic tank                          | h          |
| Synthetic wastewater flow                 | L/h        |
| Nitrate recycling line                    | %          |
| Sludge return line                        | %          |
| Mixed liquor suspended solids (MLSS)      | mg/L       |

2.2. Experiment design and operation

Before conducting the continuous-flow experiments, 17 batch tests were designed and carried out to optimize the DO concentrations in different reaction compartments by using a 3-factor and 3-level Response surface methodology. The schematic diagram of batch reactors was shown in Fig. 2. The composition of the synthetic wastewater used in batch tests were same as is applied in the continuous-flow experiments.

After obtaining the optimized staged DO concentrations, the lab-scale continuous-flow A²MMBR-M system was performed (Fig. 1b), while the other AAO system was performed as the control (Fig. 1a).
For the A²MMBR-M system, the effective volume of the micro-aerobic tank was 8 L, and the HRT in the micro-aerobic tank was performed at 1.6 h. The effective volumes of the first oxic and the second oxic tanks were respective 8 L and 8 L, with 1.6 h HRT in each compartment. For the micro-aerobic starvation sludge of the A²MMBR-M system, the HRT and the DO concentration were maintained at 9 h and 0.5 mg/L. During experiments period, effluent samples taken from the AAO and the A²MMBR-M systems were collected to examine the production of the excess sludge yield and the efficiencies of the nutrient removals including COD, nitrogen, and phosphorus. Before examination, the collected samples were filtered through 0.45 µm filters for analyses. All the experiments were performed in triplicate. All the experiments were conducted at room temperature.

![Diagram of batch reactors for the A²MMBR-M system](image)

Fig. 2. The schematic diagram of batch reactors for the A²MMBR-M system

2.3. Box-behnken design and statistical analysis

Response surface methodology (RSM), a mathematical and statistical technique is always used to analyze the mutual relationships between the response and the independent variables [23], and applied to identify the optimal operating conditions for a system. The important parameters of dissolved oxygen (DO) concentrations in the micro-aerobic tank, the first oxic tank, and the second oxic tank were respective the DO₁ (X₁, mg/L), DO₂ (X₂, mg/L), and DO₃ (X₃, mg/L). The most important response was the observed sludge production yield (Y₁obs) (g·MLSS/g·COD·d), COD removal efficiencies (% Y₂), TN removal efficiencies (% Y₃), and TP removal efficiencies (% Y₄). A 3-factor and 3-level RSM using Box-Behnken design (BBD) were introduced to design tests to study the interrelationships and the optimal levels for these operating parameters. The ranges levels of the three independent variables were presented in Table 2. The relationship between the uncoded and coded values is shown in Eq. (1):

\[ x_i = \frac{X_i - X_i^*}{\Delta X_i} \]  

Eq. (2) shows a second-order polynomial model:

\[ Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{22} x_2^2 + \beta_{23} x_2 x_3 + \beta_{33} x_3^2 \]

where Y is the response variable; Xᵢ is the uncoded value of the independent variable; Xᵢ* is the uncoded value of the independent variable at the center point; and ΔXᵢ is the step change value; xᵢ is the coded value of the independent variable; β₀, β₁, β₂, β₃, β₁₁, β₁₂, β₁₃, β₂₂, β₂₃, β₃₃, and β₂₃ represent the regression coefficients from the test data.

2.4. Analytical methods

In this study, the chemical oxygen demand (COD), soluble chemical oxygen demand (SCOD), mixed liquor suspended solids (MLSS), total phosphorus (TP), and total nitrogen (TN) were measured in
accordance with standard methods [24]. ORP and dissolved oxygen (DO) values were monitored by a portable ORP/DO meter with ORP and DO probes (Germany WTW Company ORP/Oxi 340i main engine, Germany). The analyses of TP, TN, and NH₄⁺-N were carried out every other day. The measurements of COD and MLSS were tested daily. The daily produced excess sludge yields were calculated.

Table 2 Experimental ranges and levels of the independent variables.

| Independent variables | Range and levels |
|-----------------------|------------------|
| DO₁ (mg/L), x₁       | 0.1 - 0.5        |
| DO₂ (mg/L), x₂       | 2 - 6            |
| DO₃ (mg/L), x₃       | 2 - 6            |

3. Results and discussion

3.1. Optimization of operating variables and their reciprocal analysis

The design matrix and results obtained based on the experimental BBD design are showed in Table 3.

Table 3 Response surface BBD and experiments.

| Run | Actual values of parameters | Response |
|-----|-----------------------------|----------|
|     | X₁  | X₂  | X₃  | Y₁  | Y₂  | Y₃  | Y₄  |
| 1   | 0.1 | 4   | 6   | 0.22 | 89.12 | 84.33 | 91.76 |
| 2   | 0.3 | 6   | 6   | 0.24 | 88.38 | 86.76 | 92.58 |
| 3   | 0.5 | 6   | 4   | 0.21 | 90.87 | 91.29 | 94.24 |
| 4   | 0.1 | 6   | 4   | 0.2  | 90.45 | 83.46 | 91.35 |
| 5   | 0.3 | 4   | 4   | 0.16 | 95.21 | 89.76 | 93.81 |
| 6   | 0.3 | 2   | 2   | 0.12 | 92.94 | 81.52 | 91.44 |
| 7   | 0.5 | 2   | 4   | 0.15 | 94.56 | 91.16 | 93.36 |
| 8   | 0.3 | 4   | 4   | 0.16 | 95.21 | 89.76 | 93.81 |
| 9   | 0.5 | 4   | 2   | 0.15 | 93.58 | 90.82 | 94.66 |
| 10  | 0.3 | 4   | 4   | 0.16 | 95.21 | 89.76 | 93.81 |
| 11  | 0.1 | 4   | 2   | 0.13 | 93.67 | 82.94 | 89.78 |
| 12  | 0.3 | 2   | 6   | 0.18 | 92.78 | 87.52 | 92.14 |
| 13  | 0.3 | 4   | 4   | 0.16 | 95.21 | 89.76 | 93.81 |
| 14  | 0.5 | 4   | 2   | 0.23 | 89.47 | 92.67 | 94.82 |
| 15  | 0.3 | 6   | 2   | 0.19 | 94.29 | 88.77 | 92.15 |
| 16  | 0.3 | 4   | 4   | 0.16 | 95.21 | 89.76 | 93.81 |
| 17  | 0.1 | 2   | 4   | 0.14 | 94.52 | 83.65 | 90.02 |

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

$$Y_{coded}=+0.16+6.250E-003x₁+0.031x₂+0.035x₃+0.000x₁x₂-2.500E-003x₂x₃-2.500E-003x₃x₁+7.500E-003x₁²+7.500E-003x₂²+0.015x₃²$$

(3)

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 indicates a high significance of the corresponding variable. A high squared regression coefficient, $R^2$ of 0.9763 indicates a high degree of correlation between the predicted and actual responses, indicating that the model could fit the response well. In Fig. 3, 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 4 Analysis of variance (ANOVA) results for the response surface quadratic mode.

| Source | Statistics |
|--------|------------|
|       |            |
Second-order polynomial models for coded responses of $Y_2$ (COD removal efficiencies, %) were established as described in Eq. (4):

$$Y_{obs2} = 95.21 + 0.09x_1 - 1.35x_2 - 1.84x_3 - 1.44x_4 - 1.62x_5 - 0.99x_6^2 - 2.13x_7^2$$

(4)

Second-order polynomial models for coded responses of $Y_3$ (TN removal efficiencies, %) were established as described in Eq. (5):

$$Y_{obs3} = 89.76 + 3.95x_1 + 0.8x_2 + 0.9x_3 - 2.00x_4 - 1.96x_5 - 1.66x_6^2$$

(5)

Second-order polynomial models for coded responses of $Y_4$ (TP removal efficiencies, %) were established as described in Eq. (6):

$$Y_{obs4} = 93.81 + 1.77x_1 + 0.42x_2 + 0.41x_3 - 0.11x_4 - 0.46x_5 - 0.45x_6^2 - 1.12x_7^2 - 0.61x_8^2$$

(6)

For the other three significant responses, p values are all significant proving that a high significance of the corresponding variable with the three responses. Furthermore, $R^2 = 0.9596$ for $Y_2$, $R^2 = 0.9703$ for $Y_3$, and $R^2 = 0.9828$ for $Y_4$ demonstrate a high degree of correlation between the predicted and actual responses. On the basis of the results, the obtained second-order polynomial models were adequate and significant. Based on Eqs. (3) to (6), the optimal actual values of $X_1$, $X_2$, and $X_3$ were calculated and determined to be $DO_1$ of 0.3-0.5 mg/L, $DO_2$ of 3.5-4.5 mg/L, and $DO_3$ of 3-4 mg/L for
simultaneously obtaining lower sludge production and good performances of nutrient removal. The three optimal DO$_1$, DO$_2$, DO$_3$ in the three compartments in the A$^2$MMBR-M system chosen as the optimal staged DO levels.

3.2. Performances on in-situ sludge reduction and nutrient removal

In comparison with the performances of AAO system, continuous-flow studies on the impact of the optimal staged DO concentrations (the three optimal DO$_1$, DO$_2$, DO$_3$ in the three compartments) on sludge reduction and nutrient removal in the continuous-flow A$^2$MMBR-M system were further discussed. As depicted in Fig. 4a, the average $Y_{obs}$ values for the AAO and the A$^2$MMBR-M systems were respective 0.26 and 0.17 g-MLSS/g-COD-d, indicating a 37.45% reduction in discharged excess sludge in A$^2$MMBR-M system. As for the removal efficiencies in COD, TN and TP in AAO system (COD, TN, and TP removal efficiencies were respective 90.35%, 87.73%, and 90.29%), the COD, TN, and TP removal efficiencies in A$^2$MMBR-M system were 94.02%, 90.08%, and 93.94% in Figs. 4b-d. Although the AAO system is the most widely used wastewater treatment technology due to the simultaneous biological nutrients removal without any chemicals. Compare to AAO system, both higher sludge reduction and better nutrient removal efficiencies could be observed in the A$^2$MMBR-M system, implied that the staged DO optimization applied in A$^2$MMBR-M system benefit for the organic substrate competition between phosphorus accumulating organisms and denitrifying bacteria [12], [13]. In light of the previous studies [14], [15], higher nitrogen and phosphorus removal by nitrification, denitrification, and denitrifying phosphorus removal were positively correlated to the staged optimized DO concentrations in the A$^2$MMBR-M system. Furthermore, a micro-aerobic starvation sludge holding tank introduced in the sludge return line would induce the energy uncoupling metabolism [23], and hence lower sludge production would be obtained in A$^2$MMBR-M system. Then the returned low micro-molecule compounds from 9 h micro-aerobic starvation treatment would further increase the organic matters removal efficiencies [22]. Therefore, possessing good performances of nutrient removal and lower excess sludge production, the A$^2$MMBR-M system is proved a promising wastewater treatment process. The staged optimized DO concentrations applied in A$^2$MMBR-M system are the significant controlling parameters for realizing the simultaneous in-situ sludge reduction and enhanced nutrient removal.

![Fig. 4. Changes in (a) $Y_{obs}$, (b) COD removal efficiencies, (c) TN removal efficiencies, and (d) TP removal efficiencies in AAO and A$^2$MMBR-M systems.](image)

4. Conclusion
In this study, a 3-factor and 3-level BBD was introduced to design and study the optimal levels for the staged DO$_1$, DO$_2$, DO$_3$ in the three micro-aerobic, the first oxic, and the second oxic tanks of the A$^3$MMBR-M system on sludge reduction and nutrient removal. Based on the equations obtained in Eqs. (3) to (6), the optimal actual values of $X_1$, $X_2$, and $X_3$ were calculated and determined to be DO$_1$ of 0.3-0.5 mg/L, DO$_2$ of 3.5-4.5 mg/L, and DO$_3$ of 3-4 mg/L. After obtaining the optimized staged DO levels by BBD, comparative studies on the performances of sludge reduction and nutrient removal in AAO and A$^3$MMBR-M systems were further conducted. In comparison of the AAO system, a 37.45% reduction in discharged excess sludge in A$^3$MMBR-M system was obtained. The removal efficiencies of COD, TN, and TP for the A$^3$MMBR-M system were respective 94.02%, 90.08%, and 93.94%, which increased by 4.06%, 2.68%, and 4.04% comparing with the performances of AAO system. On the basis of the results obtained in the batch and the continuous-flow experiments, a staged aerated and optimized DO concentrations applied are the key controlling parameters for the realization of simultaneous nutrient removal and sludge reduction.

5. References

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