Improvement of an integrated system of membrane bioreactor and worm reactor by phosphorus removal using additional post-chemical treatment

Jia Liu, Wei Zuo, Yu Tian, Jun Zhang, Hui Li and Lipin Li

ABSTRACT

A membrane bioreactor (MBR) coupled with a worm reactor (SSBWR) was designed as SSBWR-MBR for sewage treatment and excess sludge reduction. However, total phosphorus (TP) release caused by worm predation in the SSBWR could increase the effluent TP concentration in the SSBWR-MBR. To decrease the amount of TP excreted, chemical treatment reactor was connected after the SSBWR-MBR to remove the excess phosphorus (P). The effects of chemical treatment at different time intervals on the performance of the SSBWR-MBR were assessed. The results showed that a maximum TP removal efficiency of 21.5 ± 1.0% was achieved in the SSBWR-MBR after chemical treatment. More importantly, a higher sulfate concentration induced by chemical treatment could promote TP release in the SSBWR, which provided further TP removal from the SSBWR-MBR. Additionally, chemical oxygen demand (COD) removal efficiency of the SSBWR-MBR was increased by 1.3% after effective chemical treatment. In the SSBWR-MBR, the chemical treatment had little effects on NH3-N removal and sludge production. Eventually, chemical treatment also alleviated the membrane fouling in the SSBWR-MBR. In this work, the improvement on TP, COD removal and membrane fouling alleviation was achieved in the SSBWR-MBR using additional chemical treatment.

INTRODUCTION

A membrane bioreactor (MBR) combined with a worm reactor is an advanced process for sewage treatment and excess sludge reduction (Tian et al. 2012). However, worm predation could release a significant amount of phosphorus (P) (Tamis et al. 2011). Due to the limited capacity of MBR for P removal (Abegglen et al. 2008), it was suggested that P release in the worm reactor might increase the effluent P concentration in the combined system. It was found that discharging P into surface water stimulated eutrophication in water bodies (Jiang et al. 2008). Therefore, it was necessary to reduce the effluent P concentration in the combined system.

As the effective supplement of a biological wastewater treatment system, chemical treatment methods for P removal, using metal salts, were reliable and well-established processes (Ebbers et al. 2015). The three main metal salts available for chemical treatment were calcium, aluminum and iron. Aluminum (Al) was chosen due to its ability to cluster complex P and its insensitivity to redox potential fluctuations in sediments (Auvray et al. 2006). Besides, higher sulfate concentration could stimulate P release (Caraco et al. 1989). To remove the excess P from the worm reactor, Al2(SO4)3·18H2O as the chemical coagulant was selected in this study.

The main drawbacks induced by the chemical treatment were the increase in sludge volume (Aguilar et al. 2002), particle size of mixed liquor (Song et al. 2008) and operating cost. To avoid these problems, chemical treatment reactor as external device was connected after the combined system to remove the excess P. Thus, the sludge volume was not affected by the chemical treatment. The particle size of mixed liquor was related to the soluble microbial products (SMP) and extracellular polymeric substances (EPS). Since the EPS and SMP might be affected by the Al3+, the changes in SMP and EPS were studied in the combined system.

In this study, an MBR combined with a worm reactor was developed for total phosphorus (TP) removal. The aim
of this work was to study the influence of chemical treatment on the performance of the combined system, including wastewater treatment, sludge production and membrane fouling. In addition, the potential influencing factors of TP release were analyzed.

**MATERIALS AND METHODS**

**SSBWR-MBR and additional post-chemical treatment**

In this study, an MBR combined with the worm reactor (SSBWR) as the SSBWR-MBR (R1) was connected with the chemical treatment reactor (Figure 1). Another SSBWR-MBR (R2) was operated as a control system. All the process zones of the R1 and R2 were operated in parallel and in the same way. The working volume of the combined MBRs and SSBWRs were 40 L and 39 L, respectively. The same hollow fiber microfiltration membrane modules were submerged into the combined MBRs, respectively. The texture and material on the structure of membrane modules were polyvinylidene fluoride. Effective surface area and normal pore size of each membrane modules were 0.2 m² and 0.2 μm, respectively. The R1 and R2 systems were fed with the same synthetic wastewater and its composition was the same as the previous study (Tian et al. 2012). Chemical oxygen demand (COD), NH₃-N, TP concentrations of the influent were 355.3 mg/L, 26.2 mg/L and 8.4 mg/L, respectively. The operation conditions of the combined MBRs and SSBWRs were 400 L and 39 L, respectively. The temperature and DO were controlled at 25 ± 2°C and 1.0 – 1.5 mg/L, respectively. The pH was controlled at 7.0 ± 0.2. The HRT of the combined MBRs and SSBWRs was 5 h and 1 h, respectively. The SRT of the combined MBRs and SSBWRs was uncontrolled (Liu et al. 2014) and controlled (Tian et al. 2010) (Table 1). The MLSS concentration of the combined MBRs was controlled at 9,000–10,000 mg/L. Due to the combination of the SSBWRs, the sludge retention time (SRT) of the MBRs was uncontrolled (Liu et al. 2014). The chemical treatment reactor was used to receive the supernatant of the SSBWR in the R1. The propeller at a slow stirring rate for 0.5 h was installed at the reactor to assure adequate mixture of the mixed liquor. The coagulant (Al₂(SO₄)₃·18H₂O) was selected for the chemical treatment. The mole ratio of Al³⁺: P (1.5) was introduced as the optimum ratio to estimate the influence of chemical treatment on TP removal (Boake 2006). Based on the supernatant TP concentration in the supernatant of the SSBWR in the R1, the propeller at a slow stirring rate for 0.5 h was installed at the reactor to assure adequate mixture of the mixed liquor. The coagulant (Al₂(SO₄)₃·18H₂O) was selected for the chemical treatment. The mole ratio of Al³⁺: P (1.5) was introduced as the optimum ratio to estimate the influence of chemical treatment on TP removal (Boake 2006).

**Table 1** | Operation conditions of the R1 and R2 during the whole experiment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Combined MBRs</th>
<th>SSBWRs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time (days)</td>
<td>1–98</td>
<td>1–98</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>25 ± 2</td>
<td>25 ± 2</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>1.8–4.2</td>
<td>1.0–1.5</td>
</tr>
<tr>
<td>HRT (h)</td>
<td>5</td>
<td>–</td>
</tr>
<tr>
<td>SRT (days)</td>
<td>Uncontrolled</td>
<td>1</td>
</tr>
<tr>
<td>pH</td>
<td>7.0 ± 0.2</td>
<td>–</td>
</tr>
<tr>
<td>MLSS (mg/L)</td>
<td>9,000–10,000</td>
<td>3,000–4,000</td>
</tr>
</tbody>
</table>

HRT: hydraulic retention time; DO: dissolved oxygen.
SSBWR of the R1, the quantity of coagulant was calculated. After mixing, the reaction solution in the chemical treatment reactor was settled for 0.5 h, and the clarified supernatant was recycled into the SSBWR of the R1.

In this study, the chemical treatment at different time intervals was performed on the SSBWR of the R1. Thus, the experimental process of the R1 was divided into four stages: stage 1 (chemical treatment once every 24 h), stage 2 (chemical treatment once every 48 h), stage 3 (chemical treatment once every 72 h) and stage 4 (chemical treatment once every 96 h). In the R1, chemical treatment was connected with the integrated system of the MBR and the SSBWR when the stable operating of the system was achieved. Simultaneously, the R2 as the control system was also operated under the stable state.

Extraction of SMP and EPS

SMP samples were extracted from the mixed liquor of the combined MBRs and SSBWRs, respectively, through centrifugation (5,000 rpm, 5 min) and filtration (0.45 μm, cellulose acetate membrane). After centrifugation, the supernatant of mixed liquor was discarded. The remaining sludge precipitation was suspended in deionized water. Then, the mixed liquor was put in a water bath (80 °C, 30 min). After centrifugation (5,000 rpm, 10 min) and filtration (0.45 μm, cellulose acetate membrane), the EPS sample was collected from the mixed liquor.

Extraction of TP in the sludge

After drying and grinding to a fine powder, each sludge sample (0.5 g) was placed in a 150 mL conical flask. According to the method reported previously (Li et al. 2016), the sludge sample in each flask was digested. After cooling down to room temperature, each flask was added 5 mL distilled water to heat until the white smoke was last for 10 min. Then, to each flask was added 5 mL of 10% hydrochloride acid and 5 mL distilled water to heat until the concentrated solution was obtained. After cooling down to the room temperature, the concentrated solution was filtered into a 25 mL colorimetric tube using the filter paper (diameter 11 cm, pore size 30–50 μm) and constant-volumed with 2% nitric acid.

Enzymatic activities

The method described in the literature was applied for monitoring the activities of alkaline phosphatase and acid phosphatase (Goel et al. 1998). The sludge samples were taken from the SSBWRs on days 1, 6, 12, and 18. The substrate (0.1% of p-nitrophenylphosphate disodium salt) and buffer (0.2 mol/L of carbonate/bicarbonate buffer for alkaline phosphatase activity and 0.2 mol/L of HAc/Ac for acid phosphatase activity) was incubated for 30 min at 37 °C. Then, 1 mL of sludge sample was added to the 1 mL of substrate and 2 mL of buffer for incubation (37 °C, 30 min). After incubation, the samples were centrifuged and the supernatant retained. After that, the alkaline phosphatase and acid phosphatase activities of the sludge were calculated according to the absorbance at 410. In this experiment, all the sludge samples were fresh.

Analytical methods

The TP concentration was measured by the inductively coupled plasma (ICP) analyzer (OPTIMA 5300DV, PerkinElmer, USA). MLSS, total suspended solids (TSS), volatile suspended solids (VSS), COD and NH3-N were monitored based on the standard methods (APHA). Specific resistance to filtration (SRF) of the sludge was observed by the Buchner funnel test. Protein and carbohydrate in SMP and EPS were measured by Lowry method (Lowry et al. 1951) and phenol-sulphuric method (Dubois et al. 1956), respectively. SMP samples from the SSBWRs were critically examined by the three-dimensional excitation-emission matrix (EEM) fluorescence spectroscopy. Additionally, the sludge yield was calculated using the method reported previously (Tian et al. 2012).

RESULTS AND METHODS

TP removal from the combined system

During the operation, the MLSS concentrations of the combined MBRs were controlled at 9,000–10,000 mg/L. Due to the presence of the SSBWRs, no sludge was harvested purposefully from the R1 and R2. Thereby, the effluent of the combined MBRs was the only path for TP removal from the R1 and R2. In this work, the effects of chemical treatment at different time intervals on the TP removal performance of the R1 were analyzed (Figure 2). The obtained results were compared to those from the R2 operated in parallel.

Changes in TP removal efficiencies (i.e. \(\frac{P_{\text{influent}} - P_{\text{MBR-effluent}}}{P_{\text{influent}}} \times 100\)) of the R1 and R2, as well as the supernatant TP concentrations in the SSBWRs were
monitored (Figure 2). In the R1, the maximum TP removal efficiency of 21.5 ± 1.0% was achieved in the stage 1 (chemical treatment once every 24 h), when the supernatant TP concentration of the SSBWR was 11.5 ± 1.4 mg/L. Additionally, TP release of the R2 was observed when the supernatant TP concentration of the SSBWR was 38.5 ± 1.8 mg/L. In the SSBWR of the R1, the supernatant TP concentration was increased with increasing chemical treatment time intervals. Correspondingly, TP removal efficiency of the R1 was decreased gradually and reached 5.2 ± 0.6% in the stage 4 (chemical treatment once every 96 h). It was concluded that increasing the time interval of the chemical treatment reduced P removal of the integrated system.

The TP removal efficiency of the SSBWR-MBR was highly associated with the supernatant TP concentration in the SSBWR. In the worm reactor, P could be released during sludge degradation (Tamis et al. 2011). Due to the recirculation of non-consumed sludge containing a great amount of P, the effluent TP concentration in the SSBWR-MBR was correspondingly increased. In the R1, a large amount of P in the SSBWR was reduced effectively by chemical treatment, resulting in the improvement on TP removal efficiency of the R1. Thus, it was necessary to control the TP concentration in the SSBWR.

Stimulation of TP release by sulfate

In this study, the influencing factors of TP removal were investigated. Due to the chemical treatment, Al³⁺ was removed from the SSBWR by chemical precipitation. However, a large amount of sulfate was recycled to the SSBWR with the recirculation of the clarified supernatant. Results showed that the supernatant TP concentration in the SSBWR of the R1 was increased with increasing sulfate concentration (Figure 3(a)). Additionally, the supernatant TP concentration in the SSBWR of the R2 was maintained at 40.5 ± 1.8 mg/L, the sulfate concentration was also stable at 42.5 ± 3.6 mg/L (Figure 3(a)). Reportedly, a higher sulfate concentration could stimulate the P release (Caraco et al. 1989). Thus, the increased TP concentration in the SSBWR of the R1 might be related to higher sulfate concentration caused by the chemical treatment.

According to the above results, the chemical treatment once every 24 h was considered as a more desirable method for TP removal from the R1. Therefore, the stage 1 (chemical treatment once every 24h) was selected to further investigate the influencing factors of TP removal. The results showed that the TP in the sludge of SSBWR in the R1 was 67.6% lower than that in the sludge of SSBWR in the R2 (Figure 3(b)). A lower TP concentration in the sludge of SSBWR in the R1 was suggested to be related to the increased TP release. Additionally, the alkaline phosphatase and acid phosphatase were related to P release (Goel et al. 1998). It was found that the activities of acid phosphatase in the SSBWR of the R1 were 81.3% higher than that in the SSBWR of the R2 (Figure 3(c)). The higher activities of acid phosphatase in the SSBWR of the R1 could promote TP release, causing more P removal from the R1 by chemical treatment.

In this study, the average value of pH was 6.5 in the SSBWR of the R1, while it was 7.3 in the SSBWR of the R2 (Figure S1, available with the online version of this paper). It was reported that a great amount of NH⁴⁺ was released in the SSBWR (Tian et al. 2012), which could hydrolyze and release H⁺ in the solution. Additionally, PO₄³⁻ could hydrolyze and release OH⁻. Due to the chemical treatment, a great amount of PO₄³⁻ was removed from the SSBWR of the R1. Thus, the hydrolysis of PO₄³⁻ was prone to generate more H⁺. The acidity of solution would be strengthened, accounting for the pH decrease in the SSBWR of the R1 after chemical treatment. It was found that bacteria could change the synthesis and activity of proteins in response to environmental variation (Olson 1993). Therefore, an increase in the activities of acid phosphatase might be related to the changes of pH.

Effects of chemical treatment on COD, NH₃-N removal and sludge reduction of the combined system

In this study, COD and NH₃-N removal efficiencies of the R1 and the R2 were also studied (Figure 4). In stage 1...
Figure 3  (a) TP and sulfate concentrations in the supernatant of SSBWRs in the R1 and R2; (b) TP concentrations in the sludge of SSBWRs in the R1 and R2; (c) enzymatic activities in the sludge of SSBWRs in the R1 and R2.
(chemical treatment once every 24 h), a maximum COD removal efficiency of 94.8 ± 0.5% was achieved in the R1, which was 1.4% higher than that in the R2. Increasing time interval of the chemical treatment could increase the supernatant COD concentration of the SSBWR, resulting in lower COD removal efficiency of the R1. The results indicated that COD removal efficiency of the R1 could be improved when the chemical treatment was performed on the R1 once every 24 h.

Throughout the investigation, little difference in NH₃-N removal performance was observed between the R1 and the R2 (Figure 4(b)). In addition, the sludge yield of the R1 and the R2 were studied to evaluate the effects of chemical treatment on sludge reduction. During the experiment process, the average sludge yield was 0.032 kg VSS/kg COD_removed in the R2, while it was 0.031 kg VSS/kg COD_removed in the R1. Therefore, chemical treatment had little effect on the sludge production in the R1.

Effects of chemical treatment on membrane fouling in the combined system

Chemical treatment once every 24 h was more effective in removing P from the R1. In this study, the effects of chemical treatment on membrane fouling was studied in stage 1 (chemical treatment once every 24 h). The results indicated that the average TMP increase rate of the R1 was 31.4% lower than that of the R2, indicating that the membrane fouling in the R1 could be alleviated by chemical treatment (Figure S2, available with the online version of this paper). In MBRs, the sludge mixed liquor was the object to filtration. The foulants produced by the sludge mixed liquor was on the membrane surfaces, which affected the membrane fouling significantly. Thus, the sludge properties in the combined MBRs of the R1 and R2 were observed, such as SMP, EPS and particle size (Table 2). Compared with the combined MBR of the R2, proteins and polysaccharides of the SMP in the combined MBR of the R1 were decreased by 15.6% and 11.1%, respectively (Table 2). It was possibly due to the residual Al³⁺ entering the combined MBR of the R1 with the non-consumed sludge recirculation. The flocculation caused by the residual Al³⁺ decreased protein and polysaccharides concentrations of SMP, resulting in a lower fouling property of SMP. Additionally, there was little change in the proteins and polysaccharides of EPS in the combined MBRs of the R1 and R2. It was also found that particle size of the sludge was increased from 327.8 μm in the combined MBR of the R2 to 375.4 μm in the combined MBR of the R1 (Table 2). Reportedly, floc size and density could be increased by Al³⁺ based on divalent cation bridging theory (Sobeck & Higgins 2002), resulting in the resistance to shear (Li 2005). The increased particle size would improve the sludge resistance to shear in the combined MBR of the R1. To further indicate the sludge filterability, the SRF of sludge was measured (Table 2). The results found that the SRF of the sludge in the combined MBR of the R1 was

<table>
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<tr>
<th>Items</th>
<th>Combined MBR of the R1</th>
<th>Combined MBR of the R2</th>
</tr>
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<tbody>
<tr>
<td>SRF (10¹⁶S²/g)</td>
<td>3.27</td>
<td>3.44</td>
</tr>
<tr>
<td>Particle size (μm)</td>
<td>375.4</td>
<td>327.8</td>
</tr>
<tr>
<td>SMP-proteins (mg/L)</td>
<td>6.5</td>
<td>7.7</td>
</tr>
<tr>
<td>SMP-polysaccharides (mg/L)</td>
<td>7.2</td>
<td>8.1</td>
</tr>
<tr>
<td>EPS-proteins (mg/L)</td>
<td>45.9</td>
<td>45.7</td>
</tr>
<tr>
<td>EPS-polysaccharides (mg/L)</td>
<td>27.3</td>
<td>27.5</td>
</tr>
</tbody>
</table>

Figure 4 | (a) Changes in COD removal efficiency of the R1 and R2 and COD concentrations in the supernatant of the SSBWRs in the R1 and R2; (b) NH₃-N removal efficiency of the R1 and R2 and COD concentrations in the supernatant of the SSBWRs in the R1 and R2.
4.9% lower than that in the combined MBR of the R2, indicating the improvement of the sludge filterability in the combined MBR of the R1. Membranes were prone to fouling as a result of interactions between mixed liquor and membrane. The above results confirmed the membrane fouling in the R1 could be alleviated by the effective chemical treatment.

Additionally, proteins and polysaccharides of the SMP and EPS in the SSBWRs of the R1 and R2 were detected.

<table>
<thead>
<tr>
<th>Major compositions of SMP and EPS in the SSBWRs of the R1 and R2 in the stage 1 (chemical treatment once every 24 h)</th>
</tr>
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<tbody>
<tr>
<td><strong>SSBWR of the R1</strong></td>
</tr>
<tr>
<td>SMP</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Polysaccharide</td>
</tr>
</tbody>
</table>

**Figure 5** | (a) Three-dimensional EEM fluorescence spectra of the SMP in the SSBWR of the R1; (b) three-dimensional EEM fluorescence spectra of the SMP in the SSBWR of the R2.
The proteins and polysaccharides of the SMP in the SSBWR of the R1 were 73.9% and 15.6% lower than that in the SSBWR of the R2, respectively (Table 3), which might be related to the flocculation caused by chemical treatment. Additionally, the proteins and polysaccharides of the EPS in the SSBWR of the R1 were 22.5% and 12.1% higher than that in the SSBWR of the R2. It was reported that microbial metabolism could be affected by pH variations, such as synthesis of proteins, many kinds of storage material and release of metabolic products (Yu & Fang 2003). In this study, the average pH value was 6.5 in the SSBWR of the R1, while it was 7.3 in the SSBWR of the R2 (Figure S1). Thus, the increase in proteins and polysaccharides of the EPS in the SSBWR of the R1 might be related to lower pH induced by chemical treatment.

To date, increasing attention had been paid to the SMP, likely due to the importance of SMP to membrane fouling (Evenblij & Van der Graaf 2004; Geng & Hall 2007). It was found that compared with the carbohydrate fraction, the protein fraction in SMP was more important to the membrane fouling (Tian et al. 2013). To further analyze the protein of SMP, EEM fluorescence was carried out. Peak A (Ex/Em 275/304 nm, tryptophan protein-like substances) (Li et al. 2014), peak B (280/356 nm, soluble microbial byproduct-like material), peak C (225/354 nm, fulvic acid-like materials) and peak D at Ex/Em of 225/305 nm (simple aromatic proteins such as tyrosine) (Chen et al. 2005) were shown (Figure 5). The results indicated that compared with the fluorescence intensity of SMP in the SSBWR of the R2 (Figure 5(b)), the peaks A, B and C of SMP were diminished and the peak D was vanished (or undetectable) in the SSBWR of the R1 (Figure 5(a)). It was found that fulvic-like substances (Peak C) and soluble microbial byproduct-like material (Peak B) could pass the membranes more easily, but protein-like substances including tryptophan protein-like substances (Peak A) and aromatic protein-like substances (Peak D) could be retained or captured by the membranes more readily, leading to serious membrane fouling (Wang et al. 2009). Therefore, it was inferred that the membrane fouling potential of SMP in the SSBWR of the R1 would be further weakened after chemical treatment.

CONCLUSIONS

In this study, chemical treatment using Al₂(SO₄)₃·18H₂O was a great additional supplement to the SSBWR-MBR to remove the excess P. Up to 21.5 ± 1.0% of TP removal efficiency was achieved in the SSBWR-MBR after chemical treatment. The TP release was increased at higher SO₂⁻ concentration caused by chemical treatment, which favored TP removal from the SSBWR-MBR. Additionally, the supernatant COD concentration in the SSBWR was decreased by 40.9% after effective chemical treatment. Meanwhile, the COD removal efficiency of the SSBWR-MBR was improved. The changes in the TMPs and sludge properties indicated that the chemical treatment could alleviate the membrane fouling in the SSBWR-MBR.

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