

Investigation of anaerobic side-stream phosphorus recovery and its effect on the performance of mainstream EBPR subjected to low-consumption

Juan Ma, Ruichun Yang, Xiaojun Yu, Yuantian Zhao, Qianqian Sang, Fangjun Wang and Yongzhi Chen

ABSTRACT

In this study, phosphate-rich supernatant at the end of anaerobic phase was extracted by a certain side-stream ratio for chemical precipitation to investigate the optimal conditions for phosphorus recovery. The effect of side-stream reaction on the performance of the mainstream enhanced biological phosphorus removal (EBPR) system was also explored. The experiment was carried out in a sequencing batch reactor (SBR) operated in an alternating anaerobic/aerobic mode with dissolved oxygen controlled at $1.0 \text{ mg} \cdot \text{L}^{-1}$. The results showed that the optimum magnesium source, temperature, stirring speed and reaction equilibrium time for side-stream phosphorus recovery were: $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 25°C , 150 rpm and 20 min, respectively. It was also observed that the average phosphorus removal efficiency of the mainstream system maintained as high as 90.7% during the side-stream extraction period despite insufficient time for phosphate uptake under limited dissolved oxygen condition and phosphate deprivation of polyphosphate-accumulating organisms (PAOs). Besides, the sludge settling performance of the mainstream EBPR system decreased with no sludge loss. Afterwards, phosphorus removal and sludge settling performance were restored with dismissing side-stream phosphorus recovery. This study suggested that side-stream extraction of anaerobic supernatant from a mainstream EBPR subjected to low dissolved oxygen conditions for chemical phosphorus recovery was feasible and environmentally friendly.

Key words | anaerobic supernatant, chemical precipitation, EBPR, side-stream phosphorus recovery

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INTRODUCTION

Phosphorus (P) is one of the indispensable elements of lives, and it is also a non-renewable, non-replaceable and limited resource on earth. Recently explored and economically feasible global reserves may be depleted within only a few generations. Compared with the cycle of carbon and nitrogen in nature, the cycle of P differs in a straight line way from land to sea (Rittmann *et al.* 2011). In recent years, human inappropriate activities have enabled quantities of phosphorus to be discharged into natural waters, causing a severe environmental problem (Rittmann *et al.* 2011; Shaddel *et al.* 2019). The world is in a dilemma between extreme shortage of phosphorus resources and eutrophication caused by excessive phosphorus discharged into water bodies.

To solve the problem of eutrophication by phosphorus, various biological and chemical methods have been

adopted in wastewater treatment to meet the strict P regulation in effluent. Enhanced biological phosphorus removal (EBPR) process is an economical and environmentally friendly process for removing phosphorus from wastewater (Smolders *et al.* 1994). In EBPR, P is stored in the form of intracellular polyphosphate (poly-P) by polyphosphate-accumulating organisms (PAOs) and discharged as wasted sludge for further treatment (Smolders *et al.* 1994). Meanwhile, chemical precipitation was also regarded as an efficient means for P removal. In this process, $\text{Fe}^{2+}/\text{Fe}^{3+}$ or Al^{3+} based salts such as FeCl_2 , FeCl_3 and $\text{Al}_2(\text{SO}_4)_3$ were extensively used. However, the methods mentioned above only focus on the removal of phosphorus. Now that a phosphorus crisis is coming, the recovery of phosphorus from wastewater has been paid

more and more attention by scholars and governments all over the world.

The earlier engineering application of phosphorus recovery from wastewater was the Phostrip phosphorus recovery process in the 1960s, which was essentially a combination of biological phosphorus removal and chemical phosphate precipitation (Szpyrkowicz & Zilio-Grandi 1995). In addition, a side-stream offline sedimentation from the anaerobic tank was set up in the Biologisch-Chemische-Fosfaat-Stikstofverwijdering (BCFS) process to realize phosphorus recovery, in which anaerobic supernatant was introduced into the phosphorus sedimentation tank through side-stream extraction. After the chemical precipitation reaction, the supernatant was returned to the contact tank for subsequent biological reaction. Since the phosphate concentration of the anaerobic tank was the highest in the process, high precipitation efficiency could be achieved by dosing a small amount of chemical agent into the anaerobic tank. The off-line operation could prevent chemical precipitation from mixing with microorganisms, making phosphorus recovery easier (Barat & Loosdrecht 2006). At the same time, a portion of the influent phosphorus was removed by chemical precipitation, which reduced the load and relatively increased the influent C/P ratio of the subsequent biological phosphorus removal process (Hao & van Loosdrecht 2006). As a result, chemical phosphorus recovery could enhance biological phosphorus removal (Kato *et al.* 2006). The most common method is to precipitate phosphate minerals from wastewater, such as struvite (magnesium ammonium phosphate hexahydrate, $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) (Shaddel *et al.* 2019).

The formation of struvite requires three kinds of crystal ions in view of its chemical element composition; that is, Mg^{2+} , NH_4^+ and PO_4^{3-} , respectively. Whereas the supernatant extracted from the EBPR system at the end of the anaerobic phase was rich in PO_4^{3-} , NH_4^+ , only a magnesium source was needed for chemical precipitation. Besides, struvite precipitation is easy to operate. For the phosphate-rich supernatant and the phosphate-rich anaerobic digestion solution, it is only necessary to adjust the pH to the optimum condition and add the appropriate amount of magnesium salt into the solution to obtain the struvite recovered product. In addition, struvite has the advantages of being composed of primary macronutrient (nitrogen and phosphorus), and being a high-quality slow-release fertilizer that can be used directly as precipitated (Shaddel *et al.* 2019).

Some reports (Stratful *et al.* 2001; Jaffer *et al.* 2002; Kofina & Koutsoukos 2005) mainly discussed the influence of pH value, molar ratio of crystal ions, reaction time, reaction temperature as well as reaction intensity on the

formation of struvite and the removal efficiency of nitrogen and phosphorus, while Lv *et al.* (2014) studied the effect of side-stream phosphorus chemical phosphorus recovery on the mainstream biological phosphorus removal system in anaerobic phosphorus release supernatants. Additionally, the effect of dissolved oxygen (DO) on biological treatment was also significant. Chen *et al.* (2014) found that aeration in an EBPR system was the maximum energy consumption unit. Excessive aeration could cause system failure and effluent failed to meet the discharging standard, etc. Nevertheless, low DO operation of biological treatment system was reported not only to be in favor of increasing the biomass of the system but also to improve the oxygen transfer efficiency (Abbassi *et al.* 2000). Moreover, stable and efficient phosphorus removal performance could be achieved under low aeration conditions (Liu & Wang 2013). Therefore, if wastewater treatment plants could be operated stably under low DO concentration by controlling the biological aeration amount, energy consumption and operating costs were expected to be reduced at least about 10%. However, few studies reported stable side-stream phosphorus recovery in biological phosphorus removal systems operating under low DO condition and analysis of phosphorus recovery products.

In view of the analysis mentioned above, an anaerobic side-stream chemical precipitation with mainstream EBPR subjected to low DO was proposed in this study to recover phosphorus from wastewater. In this process, a certain proportion of phosphate-rich supernatant at the end of the anaerobic stage was extracted to the side-stream pool for phosphorus recovery. After chemical reaction, the supernatant of the precipitation tank was returned to the mainstream system to participate in the subsequent aerobic reaction and complete the biological phosphorus removal process. This study aimed to determine the optimal conditions for efficient phosphorus recovery with mainstream EBPR operated under a low-consumption condition. The impact of anaerobic side-stream phosphorus recovery on the performance of the mainstream system was also investigated. Meanwhile, X-ray diffraction (XRD), scanning electron microscopy (SEM) and energy dispersive spectroscopy (EDS) were employed for qualitative analysis of the phosphorus recovery product.

MATERIALS AND METHODS

Experimental set-up and operation

A laboratory-scale sequencing batch reactor (SBR) with a working volume of 4.2 L was employed for mainstream

enhanced biological phosphorus removal in this study. The SBR was operated in an alternating anaerobic-aerobic mode during the 80 days' experiment. An aeration pump was used for supplying oxygen in the aerobic stage, and the aeration amount was adjusted by the air flowmeter. The DO concentration of the reactor during the aerobic stage was controlled by a computer system programmed by Labview software. The reactor temperature was set at 22 ± 1 °C. DO and pH were monitored online by WTW multi 3,420 meter from Germany. The sludge retention time (SRT) was controlled at approximately 28 d through wasting 150 ml mixed liquor at the end of the aerobic period. The hydraulic retention time (HRT) was 24 h. The schematic diagram of the process is shown in Figure 1.

The SBR was operated three times per day with 8 hours for one cycle, including: 6 min filling period; 120 min anaerobic phase; 300 min aerobic phase; 54 min settling phase and 6 min withdrawing period. Experiments were conducted in three operational stages.

Stage I: SBR was operated for enhanced biological phosphorus removal without side-stream extraction for 23 days in a steady state, displaying an excellent EBPR performance.

Stage II: In this stage, a phosphorus recovery phase was introduced into the EBPR to investigate the impact

of side-stream extraction on the performance of the main-stream system. The duration of each phase differed from that of stage I, including: filling period, 6 min; anaerobic phase, 90 min; precipitation and settling, 80 min (in this phase, sludge settled for 15 min after anaerobic phosphorus release. Then the phosphate-rich supernatant was extracted by peristaltic pump with side-stream ratio of 1/3); aerobic phase, 250 min; settling phase, 54 min and withdrawing period, 6 min. In the side-stream phosphorus recovery tank, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ or $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was added as the magnesium source for chemical precipitation. The pH of the side-stream phosphorus recovery tank was adjusted to 8.5–9.5 with $1.0 \text{ mol} \cdot \text{L}^{-1} \text{NaOH}$ to promote the formation of phosphorus recovery products. Besides, different temperatures (20, 25, 30 °C) and stirring speeds (120, 150, 180 rpm) were controlled to determine the optimal conditions for phosphorus recovery products formation. The whole stage lasted for 46 days.

Stage III: Side-stream phosphorus recovery operation was terminated to confirm whether EBPR performance could be restored after side-stream extraction. The operational strategy was the same as stage I. This stage lasted for 11 days.

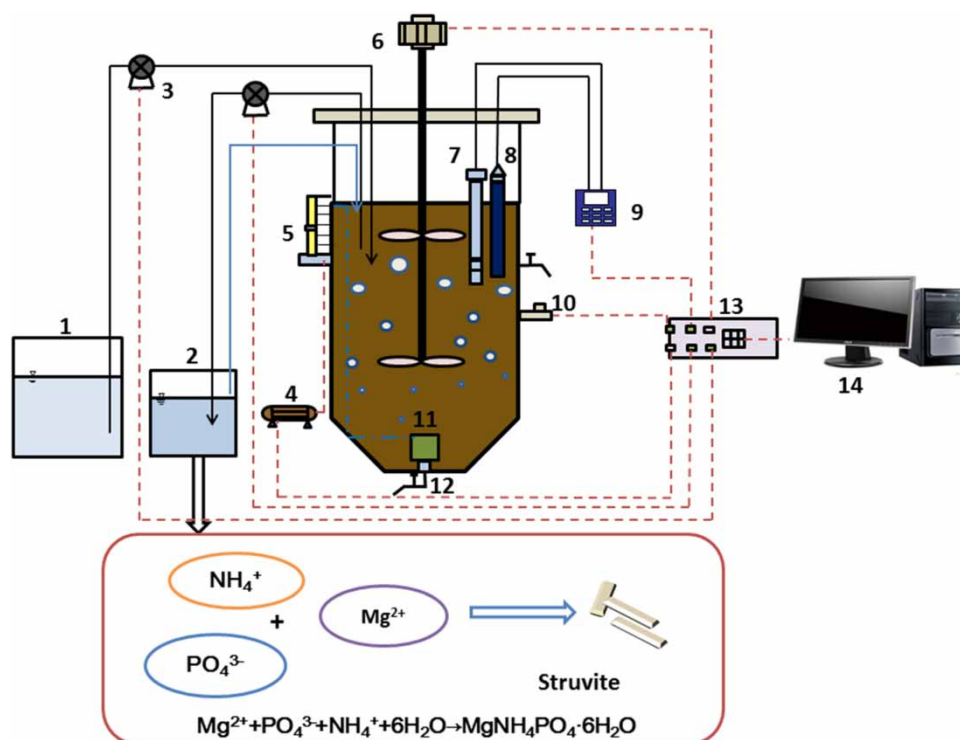


Figure 1 | Schematic diagram of the process. 1. Influent tank 2. side-stream precipitation tank 3. peristaltic pump 4. air compressor 5. air flow meter 6. mixer 7. DO sensor 8. pH sensor 9. WTW multi 3,420 meter 10. effluent valve 11. diffuser 12. sludge valve 13. control system 14. computer.

Sludge and wastewater

The SBR was seeded with the sludge from a wastewater treatment plant located in Lanzhou (China). Compositions of synthetic wastewater adopted in this study were shown as the following in mg L^{-1} : 400 COD (CH_3COONa), 8 $\text{PO}_4^{3-}\text{-P}$ (KH_2PO_4), 40 $\text{NH}_4^+\text{-N}$ (NH_4Cl). Concentrate (1 L) was composed of 80 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 45 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 21 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10 g yeast extract and 61 g peptone. Trace elements (1 L) were composed of 5.51 g citric acid, 4.03 g hippuric acid, 3.03 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.5 g H_3BO_3 , 0.12 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.06 g KI, 0.24 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.06 g $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.3 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.06 g $\text{CoCl}_6 \cdot \text{H}_2\text{O}$, 10 g EDTA, 0.06 g $\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$, 0.06 g $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. To meet the growth needs of microorganisms, 1 mL concentrate and 1 mL trace elements solution were added to 1 L of synthetic wastewater.

Sampling and analytical methods

The routine parameters for analysis in this study of the main-stream system included chemical oxygen demand (COD), HAc, ammonia nitrogen ($\text{NH}_4^+\text{-N}$), nitrite ($\text{NO}_2^-\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$), phosphate ($\text{PO}_4^{3-}\text{-P}$), mixed liquor suspended solids (MLSS), and mixed liquor volatile suspended solids (MLVSS). Influent and effluent of the SBR were sampled every cycle for chemical analysis, and an activated sludge sample was grabbed every day for concentration measurement. When the SBR operation reached a steady state, a track analysis of the entire cycle was carried out for each test, in which mixed liquor samples (10 ml) were withdrawn from the SBR during the operating cycle. For the analysis of the side-stream phosphorus recovery reaction, as the magnesium source was dosed into the side-stream supernatant, samples were taken at 0 min; 10 min, 20 min, 30 min, 40 min, 50 min and 60 min; concentrations of $\text{PO}_4^{3-}\text{-P}$ and $\text{NH}_4^+\text{-N}$ were detected. Phosphorus recovery products were filtered by 0.45 micron filter membrane and dried in an oven at 30°C for 48 h. After that, the products were qualitatively analyzed by XRD (PANalytical, The Netherlands), the operating conditions of XRD were 40 kV, 40 mA, using Cu $K\alpha$ radiation over the 2θ ranging $10\text{--}80^\circ$. The products were also characterized by Scanning Electron Microscope and Energy Dispersive Spectrometer. Daily analyses of COD, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$, $\text{NO}_3^-\text{-N}$, $\text{PO}_4^{3-}\text{-P}$, MLSS and MLVSS were performed in accordance with the standard methods (APHA 1998). HAc was regularly monitored by a Shimadzu high performance liquid chromatograph (Kyoto, Japan) equipped with an Alltech Previal Organic Acid

Column. DO, ORP and pH were measured online using DO, ORP and pH meters (WTW 340i, WTW Company), respectively.

Estimation of side-stream phosphorus recovery efficiency in stage II

In stage II, the phosphorus recovery efficiency of the side-stream was calculated by means of Equation (1). P_1 was the concentration of phosphate in the supernatant of the precipitation reaction, and P_2 was the phosphate concentration at the end of the anaerobic phase.

$$\text{Phosphorus recovery efficiency} = \frac{P_2}{P_1} * 100\% \quad (1)$$

RESULTS AND DISCUSSION

Determination of the optimum reaction conditions for side-stream precipitation

Reaction conditions, such as pH value (Stratful *et al.* 2001), type of magnesium source or concentrations of ions (Stratful *et al.* 2001; Jaffer *et al.* 2002; Matsumiya *et al.* 2010; Stolzenburg *et al.* 2015), equilibrium time (Stratful *et al.* 2001), reaction temperature (Babić-Ivančić *et al.* 2002; Rahman *et al.* 2014), stirring speed (Kofina & Koutsoukos 2005), and so on, could influence the process of chemical precipitation and even the characteristics of the recovered product. As long as the reaction parameters are controlled at the optimum condition, the expected phosphorus recovery products would be achieved.

Limited by the solubility product constant (K_{sp}), chemical precipitation could occur only if the product of crystallized ions concentration exceeded K_{sp} . As the concentration of crystallized ions was higher, chemical precipitation proceeded much more easily and the precipitation time could also be shortened. In this study, magnesium ammonium phosphate ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$, struvite) was the expected recovery product. Therefore, when the phosphate-rich supernatant was extracted into the chemical precipitation tank, a certain proportion of magnesium source was added for phosphorus recovery. From the chemical composition of $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$, the molar ratio of magnesium, ammonia and phosphate was 1:1:1 theoretically. Considering the adequacy of the crystallized ions, the molar ratio of the actual reaction was larger than the theoretical value. Stratful *et al.* (2001) concluded that for

the formation of struvite and removal of more phosphate, excessive magnesium salt was needed. Therefore, the molar ratio of Mg^{2+} and PO_4^{3-} in this study was 1.2:1 with pH ranging from 8.5 to 9.5. Figure 2 shows the influence of the magnesium source, temperature and stirring speed on the side-stream precipitation.

Determination of optimal magnesium source

In previous studies, different magnesium salts were selected for MAP recovery. Jaffer *et al.* (2002), Matsumiya *et al.* (2010), and Stolzenburg *et al.* (2015) selected MgCl_2 , seawater, and MgO as magnesium sources respectively. It was concluded that less MgCl_2 was used as the magnesium source added in the chemical reaction due to its higher solubility. When seawater was applied as the magnesium salt, the dosage was larger. And, when MgO was adopted as the magnesium salt, its dissolution occurred simultaneously with struvite precipitation. $\text{Mg}(\text{OH})_2$ was also added as the magnesium source for producing MAP without adjusting pH since $\text{Mg}(\text{OH})_2$ itself is alkaline. Nevertheless, Mg^{2+} concentration and pH could not be controlled separately due to the low solubility of $\text{Mg}(\text{OH})_2$ and larger dosage required (Stratful *et al.* 2001).

In this study, $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ with higher solubility were selected for chemical precipitation to determine the optimal magnesium source. Figure 2(a) shows the variation of phosphate concentration by the two magnesium salts. From Figure 2(a), when the precipitation reaction proceeded to 10 min, the phosphate removal rates of the two magnesium sources were similar. When the reaction continued until 20 min, the removal efficiency of phosphate was greater with $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ added. Comparatively, the phosphate removal rate of side-stream reaction with $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was higher than that with $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$.

However, SO_4^{2-} in the supernatant of the chemical precipitation tank returned to the mainstream EBPR system after the side-stream reaction completed could impose an impact on the biological process, because most microorganisms could reduce inorganic sulfates to sulfides, which have an inhibitory effect on the biological chemical reaction. Therefore, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ was only employed as a control group in the optimal magnesium source determination test. $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ was selected as the magnesium source in this study for the following experiments.

Determination of the optimum reaction temperature

Struvite crystallization is a chemical reaction, and the rate of reaction always increases with the temperature. Rahman *et al.* (2014) found that increasing the temperature from 25 °C to 30 °C could greatly improve the growth rate of struvite formation. In another study, Babić-Ivančić *et al.* (2002) studied the effect of high temperature on the size, shape and type of crystals. Besides, different chemical reactions need different activation energies. As the temperature increases, the number of activated molecules increases, resulting in the decrease of activation energy. Generally, the lower the activation energy of the reaction is, the faster the reaction rate will be.

Variation of the PO_4^{3-} -P level in the side-stream precipitation tank at different temperatures (20 °C, 25 °C and 30 °C) is depicted in Figure 2(b). It can be seen that the PO_4^{3-} -P removal efficiency is approximately similar in spite of the highest at 30 °C. The NH_4^+ -N removal efficiency also showed a maximum value at 30 °C (data not shown). Temperature had an influence on the effluent PO_4^{3-} -P concentration of the side-stream reaction and there was a difference between 20 °C and 30 °C. However, the amount of supernatant extracted during the anaerobic phase was

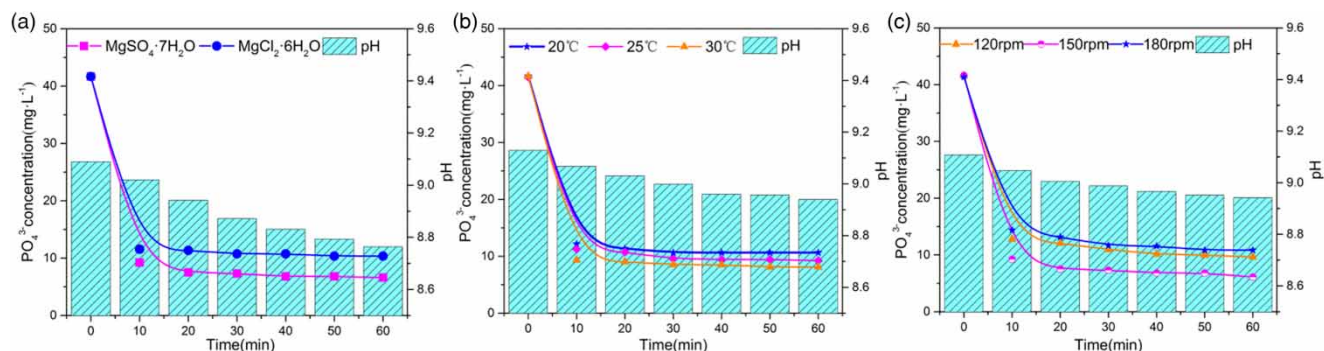


Figure 2 | Determination of optimal conditions for side-stream reaction. (a) magnesium source (b) reaction temperature (c) stirring speed. All of the pH values in the three figures were under optimal conditions ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 25 °C, 150 rpm).

only 1/3 of the liquid and the effluent of the side-stream tank after chemical precipitation was returned to the mainstream system, the remaining phosphorus could be removed biologically during the aeration phase. Besides, from Figure 2(b), there was little difference in the phosphorus removal rate between 25 °C and 30 °C, but the solution of the side-stream reaction came from the mainstream reactor and the temperature of the mainstream reactor was controlled at around 22 °C. Therefore, it was more reasonable to choose 25 °C instead of 30 °C as the temperature of the side-stream reaction for energy saving.

Determination of the optimal stirring speed

Whether stirring or not being stirred and stirring intensity would affect the removal performance of phosphorus and ammonia was investigated. Based on the previous investigation of the optimal magnesium source and temperature for the side-stream precipitation reaction in this study, a test for determining the optimal stirring intensity was conducted. The range of stirring speeds was selected to ensure a uniform suspension in the chemical precipitation tank. Figure 2(c) shows the change of $\text{PO}_4^{3-}\text{-P}$ concentration in the side-stream reactor at three stirring speeds of 120, 150 and 180 rpm, respectively.

Higher speeds increased the touch frequency of crystal-crystal, crystal-impeller, and crystal-crystalliser surface collisions, thereby causing higher rates of secondary nucleation (Frawley et al. 2012). However, faster stirring speed resulted in a larger hydraulic shear effect, making it impossible for the colliding ions and small crystals to bond firmly, and the small crystals could not be formed continuously. From Figure 2(c), when the stirring speed was 150 rpm, the phosphorus removal rate was highest and the phosphate residue in the precipitation tank was lowest. Therefore, the optimal stirring speed was determined in this study to be 150 rpm.

Determination of the optimum reaction equilibrium time

From Figure 2, it can be seen that the equilibrium was achieved after nearly 20 min reaction no matter what magnesium source, temperature or stirring speed was employed. Also, few products were formed in the next 40 min. Stratful et al. (2001) reported that the removal efficiency of PO_4^{3-} , NH_4^+ and Mg^{2+} reached the maximum and the crystal size grew to 0.8 mm when the reaction time was 60 min. Considering the sufficient time for the subsequent aerobic phase, the side-stream phosphorus recovery time was determined as 20 min.

Characterization and analysis of phosphorus recovery products

Under the optimal reaction conditions, white flocculent precipitate was produced at the end of the chemical reaction. The recovered product was filtered, dried and prepared for the characterization by XRD and SEM-EDS. Figure 3 shows the XRD pattern of the phosphorus recovery product under optimal conditions. It was found that the XRD spectrum generated by the crystal was similar to the expected phosphorus recovery product (struvite) reported in the previous literature (Rittmann et al. 2011; Rahman et al. 2014). The peak positions of XRD generated by the crystal were roughly consistent with those of the standard struvite. Moreover, the peak intensity of the XRD pattern was different from that of the standard. These results suggested that other substances formed in the process of chemical precipitation influenced the peak intensity and caused the peak position to shift slightly. Figure 4 shows the SEM-EDS images of phosphorus recovery products under optimal reaction conditions. SEM analysis demonstrated that the irregularly-shaped crystals were round and granular with an even size. And the EDS elemental analysis showed that the phosphorus recovery products contained P, O, Mg and a smaller amount of N elements. Combined with XRD results, the recovered product was assumed to be impure magnesium ammonium phosphate.

Analysing of the impurity of the produced product (struvite), it is possible that ions in the anaerobic supernatant of the EBPR system under alkaline conditions hindered the formation of struvite. In this study, the pH value of the side-stream reaction was adjusted to 8.5–9.5.

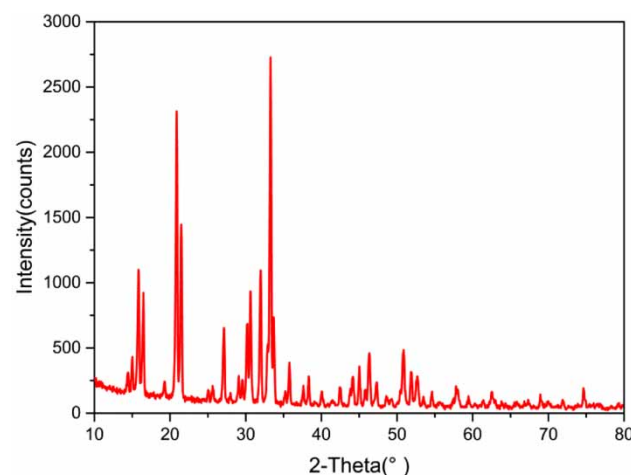


Figure 3 | XRD of the phosphorus recovery products.

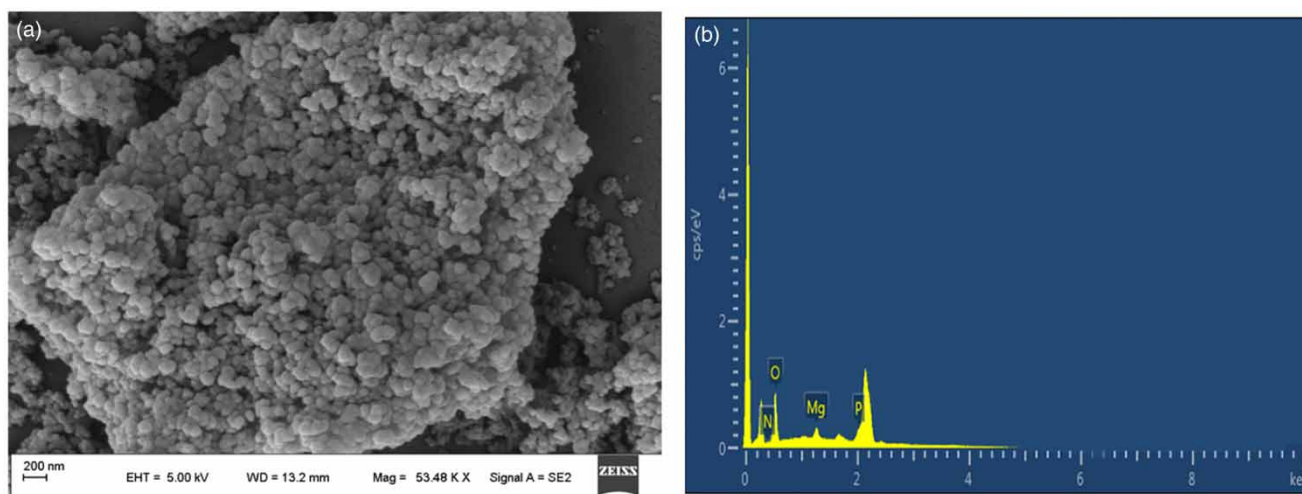


Figure 4 | SEM-EDS images of the products under optimal phosphorus recovery conditions. (a) SEM (b) EDS.

In the previous study, however, Mg^{2+} was reported to form $\text{Mg}(\text{OH})_2$ precipitate as pH of the solution increased (Lee et al. 2003). Besides, the decrease of NH_4^+ concentration caused by its volatilization to free ammonium (FA) was not beneficial for phosphorus recovery. What is more, Lee et al. (2003) found that when pH was 8.5–11, PO_4^{3-} and Mg^{2+} in solution preferentially formed magnesium phosphate rather than magnesium ammonium phosphate because the K_{sp} of magnesium phosphate was much lower than that of magnesium ammonium phosphate. Thus, even if the pH value of the solution was in the optimal range for producing struvite, the formation of other substances would prevent the formation of high-purity magnesium ammonium phosphate. In addition, N/P molar ratio was an important factor affecting the formation of struvite. In this paper, only Mg/P (molar ratio) of the side-stream precipitation reaction was controlled. The N/P molar ratio in the mixed solution was only about 0.62, which was much lower than that required to form struvite crystals. Therefore, the struvite formed in this study was not very pure. And it was calculated that the average phosphorus recovery efficiency was 70.3% in stage II.

Effect of side-stream phosphorus recovery on the performance of mainstream EBPR system

In this study, the side-stream precipitation reaction and the mainstream phosphorus removal were two independent and interrelated processes. On the aspect of reaction, chemical precipitation and biological reaction were independent of each other. The solution of the side-stream reaction

came from the mainstream EBPR system and the supernatant was returned to the EBPR after the precipitation reaction, which caused more ions to be introduced into the mainstream system. Therefore, the side-stream reaction had a certain impact on the mainstream system. Besides, continuous extraction of anaerobic phosphate-rich supernatant meant phosphorus deprivation for the mainstream system resulted in the reduction of poly-P content in the biomass. Thus, the phosphorus release and uptake capacity of PAOs in the mainstream system decreased. Furthermore, thanks to the alkaline addition, the pH of the solution was higher than that without the side-stream reaction, causing an impact on the biological phosphorus uptake process.

Effect of side-stream phosphorus recovery on pollutant removal performance of mainstream EBPR

Phosphorus and total nitrogen removal performance of the mainstream EBPR can be seen in Figure 4. During the first stage, the mean effluent phosphate concentration of the mainstream system was lower than $0.2 \text{ mg} \cdot \text{L}^{-1}$, and the phosphorus removal efficiency was 97.9%. In terms of nitrogen removal, ammonium was completely oxidized and nitrate dominated the effluent. Total nitrogen removal efficiency was 74.8%. The effluent met standard A of the national discharge of pollutants for municipal wastewater treatment plants (GB18918-2002, China) stably. The system displayed an excellent phosphorus and nitrogen removal performance.

As the process reached steady state and the phosphorus removal performance was stable, the system was operated

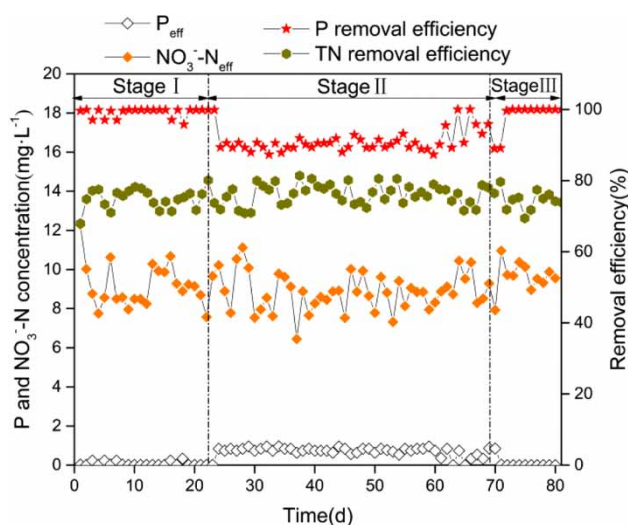


Figure 5 | Removal of $\text{PO}_4^{3-}\text{-P}$ and TN in different stages.

for the second stage. In stage II, the phosphorus removal performance of the mainstream system declined slightly, and the average phosphorus removal efficiency was 90.7%. The decrease of phosphorus removal in this stage was attributed to three aspects: (1) time for phosphate uptake was not sufficient due to the introduction of the side-stream reaction; (2) pH increase and the addition of inorganic ions returned from the side-stream reaction supernatant had an impact on the biological process; (3) owing to the phosphorus removal

by chemical precipitation, the content of intracellular poly-P in PAOs was low. Then, the release of phosphate induced by the hydrolysis of poly-P in the next cycle was suppressed, thereby reducing the uptake of phosphate and inhibiting the biological phosphorus removal activity. These results suggested that continuous anaerobic phosphorus deprivation could worsen the EBPR system (Lv *et al.* 2014). Therefore, on the 70th day of the experiment, the side-stream extraction was terminated and the system was operated as stage I. From Figure 5, the phosphorus removal performance of the whole system was restored in only 2 days. It could also be seen that side-stream phosphorus recovery had little effect on total nitrogen removal in the mainstream system. Besides, the effluent COD was lower than $50 \text{ mg} \cdot \text{L}^{-1}$ in the mainstream system during the whole experimental period, meeting standard A of the national discharge of pollutants for municipal wastewater treatment plants (GB18918-2002, China). From typical cycle analysis (data in Figure 6), the removal of COD mainly occurred in the anaerobic phase. The influent volatile fatty acids (VFAs) were absorbed by PAOs and synthesized in the microbial body in the form of poly- β -hydroxyalkanoic acid (PHAs). Moreover, side-stream phosphorus recovery reaction was operated at the end of the anaerobic phase, causing little effect on the utilization of organic matter by microorganisms in the mainstream EBPR system. Consequently, even though the side-stream phosphorus

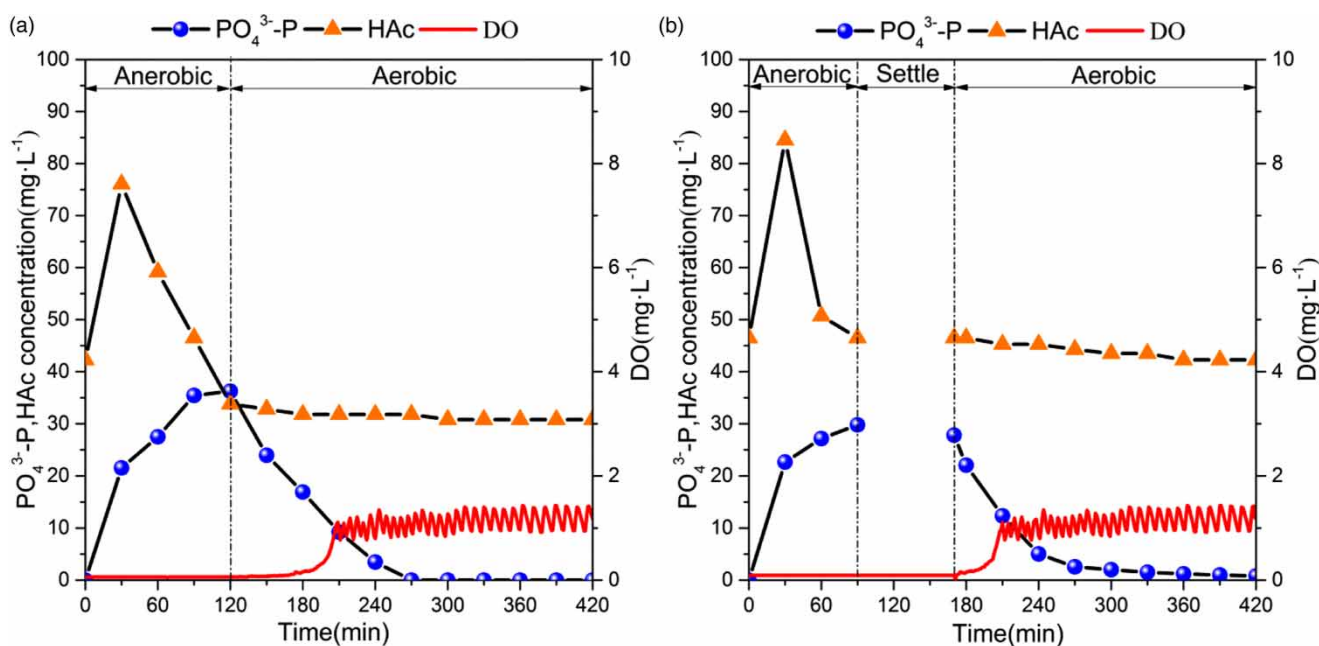


Figure 6 | Changes of phosphorus and HAC in typical cycles (a) stage I, (b) stage II.

recovery process was introduced in stage II, the removal of COD in the mainstream system remained at a high level.

Phosphorus release and uptake capacity of the sludge of mainstream EBPR

Figure 7 shows the phosphorus release and uptake capacity of the mainstream system during three stages. From Figure 7, the amount of phosphorus release and uptake in stage I was found to be the highest, which was consistent with the excellent phosphorus removal performance shown in Figure 5. In stage II with side-stream phosphorus deprivation, the capacity of phosphorus release and uptake decreased, demonstrating that side-stream chemical phosphorus recovery had an impact on the phosphorus removal performance in the mainstream system. Based on previous studies (Lv et al. 2014), after the introduction of side-stream phosphorus recovery, the glycogen-accumulating organisms (GAOs) population increased and PAOs decreased, and the population variation of PAOs and GAOs indicated that the metabolic pathway changed from polyphosphate-accumulating metabolism (PAM) to glycogen-accumulating metabolism (GAM). Therefore, the decrease of phosphorus removal performance in the side-stream phase could be attributed to the increase of GAOs population. Besides, the growth inhibition of PAOs due to phosphate deprivation by the side-stream extracting supernatant at the end of the anaerobic phase resulted in a lower phosphorus removal capacity in the poly-P restricted system. In stage III, phosphorus release and uptake of the sludge kept decreasing compared with the previous stages due to the long-term low DO operation. However, phosphorus removal performance recovered rapidly after 2 days, restoring operation, and the phosphorus was almost completely removed.

Phosphorus release and HAC uptake in the typical cycle of mainstream EBPR

The ratio of anaerobic P release/HAC uptake is an appropriate indicator of the relative populations of PAOs and GAOs or the metabolic shift between PAOs and GAOs (Saunders et al. 2003). When the ratio of phosphate release/HAC uptake during the anaerobic phase of the system is less than 0.4, the metabolic shift of PAOs or population change from PAOs to GAOs would occur (Saunders et al. 2003). In this study, the phosphate release/HAC uptake ratio of the sludge was 0.77 P mmol/HAC C mmol in stage I, which was within the range of values (0.48–0.80 P mmol/C mmol) reported in the previous studies (Zhou et al.

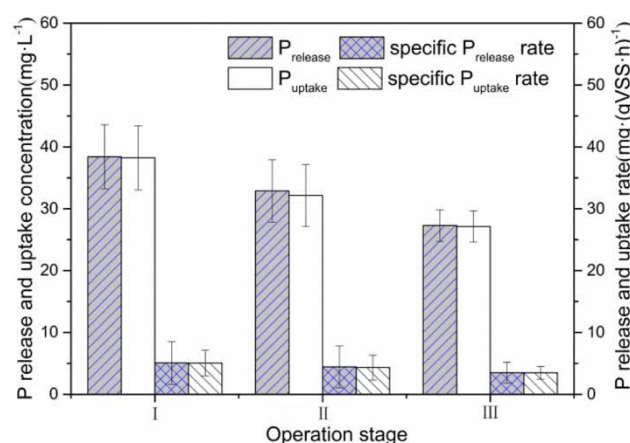


Figure 7 | Phosphorus release and phosphorus uptake in three operating stages.

2008). Even in stage II with side-stream extraction, the P release/HAC uptake value still kept as high as 0.73 P mmol/HAC C mmol, meaning metabolic shift or population change did not occur in the system although side-stream extraction was a phosphorus deprivation for the mainstream system. This result could also be proved by the rapid restoration of phosphorus removal performance of the mainstream system within a short time.

Effect of side-stream phosphorus recovery on sludge characteristics of main stream EBPR

The characteristics of sludge and poly-P content in different experimental stages are shown in Figure 8. It was observed that the MLVSS remained stable and sludge concentrations were 3,027, 2,971 and 3,123 mg · L⁻¹, respectively. The poly-P content in each stage was estimated mathematically by the method proposed by Welles et al. (2016). Figure 8 also shows that poly-P content decreased slowly over the whole experiment. Previous literatures reported that poly-P content could be reduced by two means; namely, decanting the supernatants of the anaerobic phase at short-term exposure (Zhou et al. 2008) or reducing the phosphorus feeding concentration (Welles et al. 2016). In this study, phosphate-rich supernatant at the end of the anaerobic phase of the EBPR system was extracted in a certain proportion. Thus, the poly-P content in the biomass was reduced after long-term extraction. SVI could characterize the sedimentation performance of sludge. In stage II, sludge sedimentation performance was poor and SVI increased to two times that in stage I. In order to prevent sludge loss resulting from further deterioration of sludge settling performance, a recovery stage was performed and the operation mode was

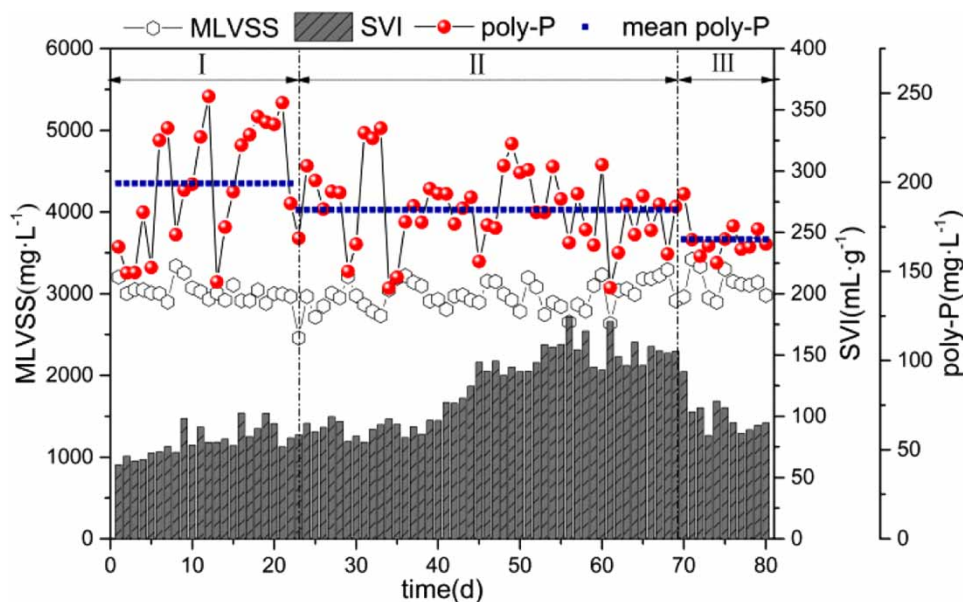


Figure 8 | Sludge characteristics and poly-P content in different experimental stages.

the same as the first stage. Afterwards, SVI decreased and the sludge settling performance increased.

Recovery of EBPR performance in stage III

In stage III, side-stream phosphorus extraction was terminated, and EBPR was operated in an alternating anaerobic and aerobic mode. It can be seen from Figure 5 that phosphorus removal performance of the system was restored and the phosphorus removal efficiency of the mainstream system increased to 99.0%. In Figure 8, sludge settling performance was improved and poly-P content also showed an increasing trend in stage III, indicating that the performance of the EBPR system could be restored to the level without side-stream extraction after terminating phosphorus deprivation.

Advantages and feasibility of the anaerobic side-stream phosphorus recovery from an EBPR subjected to low DO

In this study, the anaerobic side-stream phosphorus recovery process was operated with low consumption. It could be concluded that, although the removal performance of phosphorus in the mainstream system decreased slightly during the side-stream phosphorus recovery stage, the average removal efficiency was still stably higher than 90%, and a certain phosphorus recovery efficiency was achieved. Therefore, this process could solve the contradiction between the shortage of phosphorus resource and water eutrophication.

The recovery product (magnesium ammonium phosphate) could be used as slow-release fertilizer. Furthermore, this process was operated under low DO conditions, which could reduce energy consumption and operation costs. It could impose certain social, economic as well as environmental benefits. As a result, to extract anaerobic supernatant from a mainstream EBPR subjected to low DO for phosphorus recovery was feasible and environmentally friendly.

CONCLUSIONS

In this study, the optimal conditions for the side-stream chemical phosphorus recovery of an EBPR under low DO were investigated. $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ was selected as the optimum magnesium source. Also, the optimal reaction temperature, stirring speed and equilibrium time were determined at 25 °C, 150 rpm, and 20 min, respectively. Through XRD characterization and SEM-EDS analysis, phosphorus recovery product was detected as impure magnesium ammonium phosphate. Besides, side-stream reaction was found to have an impact on the performance of the mainstream EBPR owing to the aerobic phase being shortened, pH and inorganic ions being increased as well as phosphorus being deprived of PAOs. However, a high efficiency with 91.0% phosphorus removed was achieved in the mainstream system and a sound efficiency with

70.3% phosphorus recovered was obtained in the side-stream reaction tank. Moreover, sludge settling and phosphorus removal performance could be restored rapidly with the termination of side-stream extraction. Thus, it is feasible and environmentally friendly to implement anaerobic side-stream phosphorus recovery in a mainstream EBPR subjected to low consumption.

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