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Understanding the role of extracellular polymeric substances in an enhanced biological phosphorus removal granular sludge system

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HIGHLIGHTS

- EPS plays an important role in the biological phosphorus removal granular system.
- There is obvious accumulation of (K^+, Mg^{2+} and Ca^{2+}) in EPS.
- EPS is the storage buffer in the EBPR process.

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ABSTRACT

The role of extracellular polymeric substances (EPS) in the enhanced biological phosphorus removal (EBPR) process was investigated in a P-accumulating granular sludge system by analyzing the distribution and transfer of P, K^+, Mg^{2+} and Ca^{2+} in the sludge phase, EPS, and the bulk liquid. In the sludge phase, about 30% P, 44.7% K^+, 27.7% Mg^{2+}, 28% Ca^{2+} accumulated in the EPS at the end of aeration. The rate of P, K^+, Mg^{2+} and Ca^{2+} released from the EPS matrix into the bulk liquid in the anaerobic phase was faster than the rate they were adsorbed from the bulk liquid into the EPS in the aerobic phase. P, K^+, Mg^{2+} and Ca^{2+} were retained in EPS before transferring into the phosphorus accumulating organisms (PAOs). These results suggest that EPS play a critical role in facilitating the accumulation and transfer of P, K^+, Ca^{2+} and Mg^{2+} between PAO cells and bulk liquid.

1. Introduction

Enhanced biological phosphorus removal (EBPR), as a cost effective and environmentally friendly process, has been widely employed in wastewater treatment plants (WWTPs) (Mielczarek et al., 2013). It is generally accepted that EBPR technology is based on the phosphorus accumulating organisms (PAOs) with the "luxury" phosphorus uptake ability, and then phosphorus would be removed in the form of polyphosphate (poly-P) (storing in the PAO cells) by withdrawing the excess sludge (Oehmen et al., 2007). Moreover, phosphorus removal induced by biological phosphorus precipitation and EPS adsorption in EBPR systems has also been reported (Maurer et al., 1999; Cloete and Oosthuizen, 2001).

EPS, located on the bacterial cell surface, are the major components of sludge in biological wastewater treatment system and their potential effect on the phosphorus removal in EBPR system has been reported. Cloete and Oosthuizen (2001) found EPS roughly contained 27–30% of total phosphorus in an EBPR system by using scanning electron microscopy (SEM) combined with energy dispersive spectrometry (EDS). Li et al. (2010) demonstrated that the phosphorus in extracted EPS accounted for 13% of the total phosphorus in another EBPR activated sludge system. Recently, Zhang et al. (2013a) found that orthophosphate, pyrophosphate and polyphosphate were the main species present in EPS by using ^{31}P nuclear magnetic resonance (NMR) spectroscopy while they together accounted for about 6.6–10.5% of the total phosphorus in the EBPR flocculent sludge. More recently, a new EBPR metabolic model incorporating the EPS contribution was proposed (Zhang et al., 2013b). The above studies indicate that adsorption of phosphorus in EPS is non-negligible, which can enhance the phosphorus removal efficiency. In addition, the extracellular phosphorus removal would alleviate the PAOs' demand for organic carbon sources and maintain the biological phosphorus removal system stable. However, detailed information on the effects of EPS in the EBPR system is still unclear, such as the phosphorus transfer process among PAO cells, EPS, and the bulk

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liquid. In addition, it needs to explore whether the cations (K^+, Mg^{2+} and/or Ca^{2+}) could accumulate in the EPS matrix, since these cations usually co-transport with phosphorus in the EBPR system (Wu et al., 2006; Choi et al., 2011).

Moreover, the metal cation/P ratios are inconsistent in different studies, and the role of Ca^{2+} on the phosphorus transformation process is still uncertain although magnesium and potassium have been confirmed to be essential in the EBPR process. Nguyen et al. (2008) reported that the respective interactions of Ca^{2+} and Mg^{2+} with EPS was different, and Ca^{2+} is more preferentially bound with EPS than Mg^{2+}. It is predicted that the different research results on the cations/P may be caused by the different EPS adsorption effect on the metal cations and the unclear role of Ca^{2+} in the EPBR process might be also associated with the EPS effects. Therefore, it is necessary to investigate the EPS adsorption effect on the metal cations (K^+, Mg^{2+} and Ca^{2+}).

Up to now, little information is available on the role of EPS in phosphorus removal in EBPR granular sludge system. Aerobic granular sludge technology is a novel alternative for wastewater treatment and offers several operational and economic advantages over conventional floccular-sludge systems (Adav et al., 2008). During the past few years, P-accumulating granules have been developed to overcome the sludge bulking and secondary P release in a clarifier and maintain a stable and reliable P removal efficiency (Lin et al., 2003; Wu et al., 2010; Bassin et al., 2012). It is well known that EPS play an important role in the formation of aerobic granules. As sticky materials, EPS form a three-dimensional matrix in which bacteria and other particles are embedded in a compact structure. Many researchers found that EPS content in aerobic granules is much higher than that in conventional activated sludge (Zhang et al., 2007; McSwain et al., 2005). Besides, aerobic granular sludge system has a relatively longer sludge age compared with the conventional activated sludge (Winkler et al., 2012), which would facilitate the accumulation of phosphorus in EPS matrix. Therefore, the EPS adsorption of phosphorus in EBPR granular sludge system would be different from the previous researches in activated sludge system.

Therefore, in this study, an EBPR granular sludge system was applied to investigate the phosphorus accumulation in EPS matrix, including the transfer process of P, K^+, Mg^{2+} and Ca^{2+} among the microbes, EPS, and bulk liquid as well as the adsorption of metal cations by EPS. This study provides important information on the precise role of EPS in the bio-phosphorus removal process so as to improve our understanding on phosphorus removal in biological systems.

2. Methods

2.1. EBPR granular sludge system operation

The P-accumulating granules were cultivated in a sequencing batch reactor (SBR) with a working volume of 6 L. The SBR was operated automatically under anaerobic and aerobic conditions alternatively with 6 h per cycle, consisting of 5 min feeding, 85 min anaerobic mixing, 210 min aeration, 40 min settling and 15 min discharging and 5 min idle. The exchange ratio was 50%, resulting in a hydraulic retention time (HRT) of 16 h. Fine air bubbles for aeration were supplied through a dispenser at the bottom of the reactor with an airflow rate of 1.6 L/h. Temperature was kept at 23–25 °C. pH and dissolved oxygen (DO) concentration were monitored online by pH/oxi 340i meter (WTW, Germany) during the operation.

2.2. Media

A synthetic wastewater with 400 mg/L COD, 15.0 mg/L NH_4^+-N, 6 mg/L PO_4^{3-}-P, 7.46 mg/L K^+, 2.44 mg/L Mg^{2+} and 11.23 mg/L Ca^{2+} was used as the feeding influent. The details of the synthetic wastewater composition were shown as follows (per liter tap water): CH_3CH_2COONa 0.37 g, NH_4Cl 0.057 g, KH_2PO_4 0.026 g, $MgSO_4{\cdot}7H_2O$ 0.025 g, $CaCl_2$ 0.02 g, $NaHCO_3$ 0.2 g and 1 mL trace solution. The composition of the trace elements solution was described in Ni et al. (2009).

2.3. Sampling and chemical analysis

To analyze the variations of K^+, Mg^{2+}, Ca^{2+} and TP concentration in bulk liquid, EPS, and the sludge (including EPS and microorganisms) during one cycle, samples were taken at 15–30 min intervals at the anaerobic phase and 30–60 min intervals at the aerobic phase. At every sampling time, 4 mL and 10 mL mixtures were taken from the reactor with 5 mL and 15 mL centrifuge tubes for water sampling and EPS extraction, respectively. The 4 mL-volume sample was centrifuged at 4000 rpm for 3 min, and then the supernatant was filtered through 0.45 μm acetate filters. The residue sludge pellet was washed twice with deionized water gently avoiding the loss of sludge fragments and frozen at −20 °C. After lyophilized, the weighted dry sludge sample was digested with aquaregia–perchloric. Finally, the concentrations of K^+, Ca^{2+}, Mg^{2+}, TP in supernatants and digestion solutions were analyzed by inductively coupled plasma emission spectrometer (Perkin Elmer, Wellesley, MA, USA, Optima 2000). Two parallel samples were prepared according to the same procedure.

2.4. EPS extraction and analysis

The EPS of sludge samples were extracted using heating extraction method, one of the extraction method used by Adav and Lee (2008) and Chen et al. (2010). 10 mL mixed liquor sampled from the reactor at the given time point was washed three times with deionized water, followed by centrifugation (4 °C, 2100×*g* for 10 min). After the supernatant was discharged, the sludge pellet was re-suspended to their original volume with deionized water and homogenized. Then the sludge mixture was transferred to a centrifuge tube, and heated in a water bath at 80 °C for 30 min. During the heating process, the mixture was shaken in every 10 min. Afterwards, the mixture was centrifuged (12,000×*g*, 4 °C) for 10 min and the supernatant was filtrated through 0.45 μm cellulose acetate membranes. The filtrate was used as EPS extraction solution for polysaccharides, protein, TP, and metal cations (K^+, Mg^{2+} and Ca^{2+}) analysis.

The EPS prepared for the test of Ca^{2+} and Mg^{2+} binding to EPS was extracted using the cation exchange resin (CER) method (Frølund et al., 1996; Sheng et al., 2008; Liu et al., 2014). 10 mL mixed liquor taken at the end of anaerobic phase was washed three times with deionized water, followed by centrifugation (4 °C, 2100×*g*, 10 min). After the supernatant was discharged, the sludge pellet was re-suspended to their original volume with deionized water and homogenized. Afterwards, the mixture was transferred to an extraction beaker and the cation exchange resin (DOWEX MARATHON C, Na^+-form, 20–50 mesh, Sigma–Aldrich) was added to the suspension with a dosage of 70 g resin/g volatile suspended solids (VSS). And then the sludge/CER suspension was stirred at 600 rpm and 4 °C for 12 h. After the 12-h extraction, the suspension was centrifuged (12,000×*g*, 4 °C) for 10 min and then the supernatant was filtered through 0.45 μm cellulose acetate filters. The filtrate was used as the EPS fraction and freeze-dried for binding test.

The polysaccharides and proteins in the extracted EPS solution were analyzed using phenol–sulfuric acid method and modified Lowery method, respectively. The concentrations of K^+, Ca^{2+}, Mg^{2+}, TP in the EPS solution were determined as previously mentioned.