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The roles of loosely-bound and tightly-bound extracellular polymer substances in enhanced biological phosphorus removal

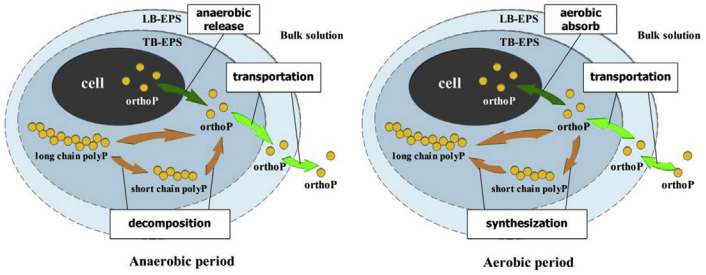
Xiangyu Long, Ran Tang*, Zhendong Fang, Chaoxin Xie, Yongqin Li, Guang Xian

Department of National Defense Architectural Planning & Environmental Engineering, Logistic Engineering University, Chongqing, 401311, China

HIGHLIGHTS

- P of EBPR activated sludge was mainly stored in TB-EPS.
- TB-EPS played an more important role than microbial cell in EBPR process.
- LB-EPS transported and retained orthoP during EBPR process.
- Microbial cell directly anaerobically released or aerobically absorbed orthoP.
- TB-EPS not only transferred and detained orthoP, but also directly participated in biological phosphorus accumulation.

GRAPHICAL ABSTRACT



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ABSTRACT

Extracellular polymeric substances (EPS) have be founded to participate in the process of enhanced biological phosphorus removal (EBPR), but the exact role of EPS in EBPR process is unclear. In this work, the roles of loosely-bound EPS (LB-EPS), tightly-bound EPS (TB-EPS) and microbial cell in EBPR were explored, taking the activated sludge from 4 lab-scale A/O-SBR reactors with different temperatures and organic substrates as objects. It was founded that the P of EBPR activated sludge was mainly stored in TB-EPS, but the P of non-EBPR activated sludge was primarily located in microbial cell. The P release and uptake of EBPR activated sludge was attributed to the combined action of TB-EPS and microbial cell. Furthermore, TB-EPS played an more important role than microbial cell in EBPR process. With the analysis of ^{31}P NMR spectroscopy, both polyP and orthoP were the main phosphorus species of TB-EPS in EBPR sludge, but only orthoP was the main phosphorus species of LB-EPS and microbial cell. During the anaerobic-aerobic cycle, the roles of LB-EPS, TB-EPS and microbial cell in transfer and transformation of P in EBPR sludge were obviously different. LB-EPS transported and retained orthoP, and microbial cell directly anaerobically released or aerobically absorbed orthoP. Importantly, TB-EPS not only transported and retained orthoP, but also participated in biological phosphorus accumulation. The EBPR performance of sludge was closely related with the polyp in TB-EPS, which might be synthesized and decomposed by extracellular enzyme.

1. Introduction

Eutrophication has become one of the most important and worldwide water quality problems, and phosphorus (P) removal

* Corresponding author. Department of National Defense Architectural Planning & Environmental Engineering, Logistic Engineering University, University Town, Shapingba District, Chongqing, 401311, China.
E-mail address: tangranhncq@126.com (R. Tang).

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from wastewater has been considered as a crucial strategy to control eutrophication. (Xie et al., 2017; Zhao et al., 2016a, 2016b).

Enhanced biological phosphorus removal (EBPR) is one of the most economical and sustainable processes to remove P from wastewater, widely applied in numerous full-scale wastewater treatment plants (WWTPs) (Oehmen et al., 2007; Zheng et al., 2014; Lanham et al., 2014; Chen et al., 2016). 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 (polyP) (storing in the PAOs) by withdrawing the excess sludge (Acevedo et al., 2012). However, many studies revealed that extracellular polymeric substances (EPS) that were widely distributed in microbial aggregates (Sheng et al., 2010), took part in the process of P removal. Cloete and Oosthuizen (2001) observed that EPS roughly contained 27%–30% of total phosphorus (TP) of the sludge floc based on an X-ray scanning electron microscope and energy dispersive spectrometer. Borovec et al. (2010) reported that the P content of EPS was 52% of TP of the benthic cyanobacterial mat(CBM)by using Ruttenberg method for chemical fractionation. Wang et al. (2014) founded that the P content of EPS was 30%–45.4% of TP of EBPR granular sludge. Moreover, Zhang et al. (2013a, 2013b) demonstrated the coexistence of orthophosphate (orthoP), pyrophosphate (pyroP) and polyP in the EPS of EBPR sludge using the ^{31}P nuclear magnetic resonance (NMR), and found the higher contents of polyP and pyro-P than orthoP (reflected by higher peak intensity from ^{31}P NMR spectroscopy) in the EPS. Therefore, EPS not only stored large amounts of phosphorus, but also participated in the process of biological phosphorus accumulation (Li et al., 2015). Importantly, the role of EPS in EBPR process is non-negligible and should be considered in the mechanisms of P removal.

In activated sludge, the microbial cells are embedded by EPS, which can be divided into the tightly-bound EPS (TB-EPS) located in inner layer of sludge floc and the loosely-bound EPS (LB-EPS) existing in outer layer of sludge floc (Han et al., 2013). Thus, the transportation of P between bulk solution and PAOs must pass through LB-EPS and TB-EPS, but the characteristics of transfer and transformation of P in EBPR sludge (including LB-EPS, TB-EPS and microbial cells) is still unclear. Moreover, the contents, compositions and properties of TB-EPS and LB-EPS are different (Geyik and Çeçen, 2015; Zhao et al., 2016a, 2016b), and their influences on the flocculation, sedimentation and dewatering of activated sludge were also significantly different (Li and Yang, 2007; Yang and Li., 2009). Thereby, the contributions and roles of TB-EPS and LB-EPS in biological phosphorus removal should be dissimilar. In order to acquire more information about the exact role of EPS in EBPR, it is necessary to fractionate EPS into TB-EPS and LB-EPS.

In biological treatment systems like EBPR, type of organic substrate and temperature that have substantial effects on microbial community and metabolism, are important factors of biological phosphorus removal (Oehmen et al., 2007). Acetate and propionate are the typical volatile fatty acids (VFAs) in sewage or municipal wastewater (Wanga et al., 2012; Mielcarek et al., 2015.), which are key carbon sources for EBPR process. Temperatures higher than 20 °C were generally reported as unfavorable to EBPR process, so EBPR technique might not be suitable for tropical climates with temperatures above 30 °C (Whang and Park, 2002; Liau et al., 2015; Sayi-Ucar et al., 2015). Furthermore, the contents and species of P in LB-EPS and TB-EPS as well as their roles in biological phosphorus removal might also be influenced by the type of organic substrate and temperature. However, the informations about these were not presented in literature.

This study aimed to investigate the roles of LB-EPS and TB-EPS in EBPR. For this purpose, four lab-scale A/O-SBR reactors with different temperatures (20 ± 1 °C or 35 ± 1 °C)and organic substrates (acetate or propionate) were operated at steady-state conditions. Through fractionating activated sludge into LB-EPS, TB-EPS and microbial cells, the contents and distributions of P in activated sludge from the four reactors during the anaerobic-aerobic cycle were compared to identify the contributions of LB-EPS, TB-EPS and microbial cell in biological P removal. At the same time, the ^{31}P NMR was employed to analyze the species of P in LB-EPS, TB-EPS and microbial cell, in order to discuss whether LB-EPS and TB-EPS were all involved in the process of biological P accumulation. Furthermore, characteristics of transfer and transformation of P in EBPR sludge were discussed, so as to understand the roles of LB-EPS and TB-EPS in EBPR.

2. Materials and methods

2.1. Culture of activated sludge

The activated sludge of four lab-scale A/O-SBR reactors was fed with synthetic wastewater, adopting sodium acetate or sodium propionate as the sole carbon source. COD: N: P of the synthetic wastewater was 100: 5: 5, and the pH was approximately 7.0, of which the trace elements are shown in Table S1. The reactors had a working volume of 15 L and were operated for 2 cycles every day, adopting instantaneous filling wastewater. Each cycle lasted for 12 h, involving a 4-h anaerobic period, 7 h of aeration, a 50-min settlement, 5 min of decanting, and 5 min of idling. The temperatures of the 4 reactors were controlled at 20 ± 1 °C or 35 ± 1 °C. The solid retention times (SRTs) were approximately 20 d, with discharging the mixed liquid every day. The dissolved oxygen (DO) concentrations at the end of the aeration stage were 3.0–5.0 mg/L, through regulating gas flow. The mixed liquid suspended solids (MLSS), sludge volume index (SVI) and chemical oxygen demand (COD) and total phosphorus (TP) of effluent were monitored every day. After the values of the parameters kept relatively stable for 2 months, experimental study was carried out.

2.2. Extraction of LB-EPS and TB-EPS

2.2.1. Extraction of LB-EPS

The modified method of sonication was used to extract LB-EPS (Han et al., 2013). The 21 kHz sonication (JY90-II; Scientz Bioscience Co., Inc., Ningbo, China) was used to act on the 40 mL sludge (VSS was between 7500 mg/L and 8500 mg/L) after once centrifugation and re-suspension. Subsequently, the treated sludge was centrifuged at 0 ± 2 °C and 43000 RCF, and the twice centrifugal supernatant was designated as LB-EPS. The sonication probe area was 0.28 cm^2, and the ultrasonic power density was 1 W/mL and the process time was 6 min. The duty cycle during the sonication process was 50%.

2.2.2. Extraction of TB-EPS

After extracting LB-EPS, the centrifugal precipitation was re-suspended to 40 mL. Then, the modified method of cation exchange resin (CER) was used to extract TB-EPS (Frølund et al., 1996). The 001 × 7 gel-type CER (20–40 mesh, Suqing, Jiangsu, China) was employed to process the 40 mL mixed liquid, of which the additional amount was 100 g of CER/g VSS, and the reaction time was 30 min. Then, the CER was intercepted using nylon mesh with a 250-μm pore diameter. Finally, the filtered mixed liquid was centrifuged at 0 ± 2 °C and 43000 RCF, and the twice centrifugal supernatant was designated as TB-EPS.