



Roles of extracellular polymeric substances in enhanced biological phosphorus removal process



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ABSTRACT

Enhanced biological phosphorus removal (EBPR) process is known to mainly rely on the ability of phosphorus-accumulating organisms to take up, transform and store excess amount of phosphorus (P) inside the cells. However, recent studies have revealed considerable accumulation of P also in the extracellular polymeric substances (EPS) of sludge, implying a non-negligible role of EPS in P removal by EBPR sludge. However, the contribution of EPS to P uptake and the forms of accumulated extracellular P vary substantially in different studies, and the underlying mechanism of P transformation and transportation in EPS remains poorly understood. This review provides a new recognition into the P removal process in EBPR system by incorporating the role of EPS. It overviews on the characteristics of P accumulation in EPS, explores the mechanism of P transformation and transportation in EBPR sludge and EPS, summarizes the main influential factors for the P-accumulation properties of EPS, and discusses the remaining knowledge gaps and needed future efforts that may lead to better understanding and use of such an EPS role for maximizing P recovery from wastewater.

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1. Introduction

Recovering phosphorus (P) from wastewater is not only central in resolving the water pollution problem but also essential for meeting the sustainability of future P resources (Gilbert, 2009; Hao et al., 2013; Mihelcic et al., 2011). As an efficient and low-cost P removal technology, enhanced biological phosphorus removal (EBPR) process has been adopted in wastewater treatment plants worldwide (Hao and van Loosdrecht, 2003; Oehmen et al., 2007), and offers great promise to recovery the P resource if appropriately integrated with subsequent extraction/purification steps (Yuan et al., 2012). The success of EBPR process relies mainly on a group of selectively enriched heterotrophic bacteria—phosphorus-accumulating organisms (PAOs)—that take up P excessively (Seviour et al., 2003) and are then removed from the liquid streams. It is conventionally considered that the accumulated P by PAOs is transformed to polyphosphate (polyP) and stored inside the cells (Mino et al., 1998; Oehmen et al., 2007). However, recent studies reveal that extracellular polymeric substances (EPS) component of sludge also play a role in this process. Thus, the mechanisms of P

removal in EBPR process may need a re-examination.

In activated sludge, the microbial cells are enclosed by a matrix of EPS, a permeable hydrogel layer with good adsorption ability (Sheng et al., 2010). EPS play multiple roles in microbial aggregation (Liu et al., 2010), carbon source/nutrition conservation (More et al., 2014), and cells protection (Li and Yu, 2014). In addition, as an inevitable pathway of mass transfer between cells and environments, EPS also pose influences on the microbial metabolism and contaminant removal behaviors (Sheng et al., 2010), including the uptake and release of P in EBPR process. The accumulation of a significant amount of P in sludge EPS have been recently demonstrated in laboratory investigations, but the concentrations and existing forms of such EPS-bounded P varied substantially in different studies (Cloete and Oosthuizen, 2001; Liu et al., 2006; Oosthuizen and Cloete, 2001; Zhang et al., 2013b). For example, Wang et al. (2013b) observed a relatively lower content of 0.024 mg-P/mg-EPS in a granular sludge system, while a much higher value of 0.35 mg-P/mg-EPS in activated sludge was reported by Zhang et al. (2013a). Moreover, Zhang et al. (2013a) observed the coexistence of orthophosphates (orthoP), pyrophosphate (pyroP) and polyP in EPS, with composition and structure substantially different from the intracellular P. Choi et al. (2011) found that the P dynamics during the anaerobic-aerobic cycles and the species were significantly affected by the metal ions in the liquid phase.

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Apparently, the P in EPS have diverse origins and forms, which are further complicated by the different influent characteristics, process conditions and the extraction methods adopted. Given the contribution of EPS in P removal by sludge, it is of interest to get insights the roles of sludge EPS in P release and uptake processes. Other important but unknown questions include: how the P species and dynamics in EPS are linked with the intracellular PAO metabolism and biologically-induced precipitation? what are the major factors governing the P-accumulation performance of EPS?

This review attempts to look for the answers and provide a new recognition into the EBPR process. First, the state-of-art progresses in understanding the characteristics of P contents in EPS are summarized; Then, the mechanism of P transformation and transport in microbial EPS and the major influential factors for P uptake by EPS are discussed; Lastly, the remaining knowledge gaps and the future research directions are identified. It is hoped that this review will lead to a more comprehensive and realistic knowledge of EBPR process, as well as an improved design and optimization of EBPR process to better adapt to future resource-oriented wastewater treatment schemes.

2. Characteristics of P accumulation in EPS of EBPR sludge

2.1. P content in EPS

While it is well established that EBPR process is mainly attributed to the activities of PAO cells in storing P as intracellular poly-P (Oehmen et al., 2007), there are increasing evidences showing that EPS are critically involved in EBPR process by serving as a P reservoir. When studying the EBPR sludge using scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS), Cloete and Oosthuizen (2001) discovered a significant accumulation of P in sludge EPS. The abundant presence of P in the EPS matrix was further confirmed by Zhang et al. (2013b). As illustrated in Fig. 1a large number of white and spherical P-rich granules were clearly observed within the PAO cells of sludge, indicating the formation of intracellular poly-P granules (Boonfueng et al., 2009; Schönborn et al., 2001). Meanwhile, a considerable amount of P was also found in the EPS gel (Fig. 1d), and its content seemed to be highly correlated with the Ca^{2+} strength.

To quantify the P content in EPS, Li et al. (2010) extracted EPS from EBPR sludge at the end of aerobic phase. They found that the P contents in the extracted EPS were 0.06–0.09 mg/mg-EPS, accounting for 13% of the total P (TP) accumulation in sludge. This value was comparable with that (6.6–10.5%) reported by Zhang et al. (2013b). In both studies, the CER extraction method was adopted, which allows an efficient EPS extraction with negligible influence by intracellular P species (Zhang et al., 2013b).

Despite of the different EPS extraction method adopted that may affect the EPS content determination (Zhang et al., 2013b), a preliminary comparison of several individual studies shows that activated sludge EPS seems to have relatively higher specific P contents than the granular sludge counterpart (0.09–0.35 versus 0.024–0.071 mg/mg-EPS), likely associated with the more EPS production in granules (Table 1). Thus, the much higher total EPS contents of granules also render them a larger share in P accumulation (i.e., EPS accounted for up to 45.4% of the TP accumulation in granular sludge). Anyway, these studies indicate that the P accumulation in EPS of EBPR sludge is non-negligible.

2.2. P species in EPS

In contrast to the long-standing viewpoint that orthoP is the dominant P species in EPS. Zhang et al. (2013a, 2013b) recently discovered that polyP and pyroP were more abundantly present in

the EPS matrix of EBPR sludge. The ^{31}P nuclear magnetic resonance (NMR) spectroscopy shows apparently higher contents of polyP and pyroP than orthoP (reflected by the higher peak intensity) in the extracted EPS during the entire anaerobic-aerobic cycle (Fig. 2), suggesting possibly a stronger affinity between EPS and multi-chain P over orthoP. The average chain length of polyP in EPS, calculated from the ratio of peak area between the middle and end groups (Turner, 2004) and after calibration of the extraction-induced degradation, was estimated to be around 10 (Zhang et al., 2013a). The existence of multi-chain P in EPS of EBPR sludge was also convinced by Wang et al. (2014). The FTIR spectra of EPS from EBPR granular sludge showed a sharp band at 1151 cm^{-1} , associated with an asymmetric stretching vibration of P–O in pyroP group, while no peaks were observed in the EPS of conventional activated sludge (Fig. 3).

2.3. Dynamics of extracellular P accumulation/release

Zhang et al. (2013a) investigated the dynamics of TP contents (normalized to the sludge SS content) in the EBPR sludge ($\text{TP}_{\text{sludge}}$), bulk solution ($\text{TP}_{\text{solution}}$), cells (TP_{cell}) and EPS (TP_{EPS}) during an anaerobic-aerobic cycle. The profiles of $\text{TP}_{\text{solution}}$ and $\text{TP}_{\text{sludge}}$ exhibited a typical EBPR pattern, with a considerable transfer of TP from sludge to liquid phase in the anaerobic phase and an opposite flow in the aerobic phase (Fig. 4A). The TP_{EPS} showed a similar variation trend to the $\text{TP}_{\text{sludge}}$ (Fig. 4B), indicating that EPS were also involved in the P uptake and release process. Especially, the $\text{TP}_{\text{sludge}}$ increased from 64.15 to 93.78 mg/g-SS during the aerobic period, among which the TP_{EPS} increased from 0.93 to 6.79 mg/g-SS.

Notably, the TP_{EPS} and all the P species in EPS exhibited a slight increase in the initial anaerobic phase (Fig. 5). In the meantime, a considerable increase in EPS was also observed in the initial anaerobic phase (Zhang et al., 2013a), likely due to the sudden shift from aerobic to anaerobic environment that stimulated microbial EPS production as a self-protection mechanism (Sheng et al., 2005; Ye et al., 2013; Zheng et al., 2014). Given the fact that soluble organics in the influent have little contribution to EPS, the drastically increased EPS in this short period should be mainly originated from the sludge cells. In addition, both the $\text{TP}_{\text{solution}}$ and TP_{EPS} increased, suggesting that TP was released from the cells at the anaerobic beginning. The more rapid increase of EPS than P thus resulted in the slight decrease of specific TP contents in EPS. After about 10 min, the EPS content started to decline (Zhang et al., 2013a), possibly due to EPS hydrolysis under anaerobic conditions (Xie et al., 2012).

2.4. Main forms of extracellular P in EBPR sludge

Microbial EPS have plenty of negatively charged functional groups, such as carboxyl, phosphoryl, sulfhydryl, phenolic and hydroxyl groups, which can easily bind with metal cations and form mineral fractions of EPS (Bourven et al., 2011; Li and Yu, 2014; Sheng et al., 2013). The accumulation of these metal cations in EPS matrix would inevitably affect the forms of P species during their transportation through the EPS matrix (Barat et al., 2005). The complexation of P with extracellular metal cations of EBPR sludge has been well demonstrated (Hill et al., 1989; Li et al., 2010). Wang et al. (2014) revealed that the stoichiometric ratios between K^+ , Mg^{2+} and Ca^{2+} and polyP in EPS were in line with the cations/ $\text{TP}_{\text{uptake}}$ ratios, implying that polyP might predominantly exist in EPS by forming complex with the EPS-bounded metals. Especially, Ca^{2+} ions can cross-bridge the negatively charged bacterial polysaccharides (Patrauchan et al., 2005) and play important roles in the architecture of microbial aggregates (Lin et al., 2012).

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