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Role and significance of extracellular polymeric substances from granular sludge for simultaneous removal of organic matter and ammonia nitrogen

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HIGHLIGHTS

- The metal elements in EPSs play an important role in the granulation of sludge.
- FTIR showed that functional groups of different EPSs form clearly varied.
- Contents of metal elements in EPSs increased with the increase of particle size.
- Existence of metal elements changed zeta potential in EPSs.

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ABSTRACT

This study analyzed the organics and content of metal ions in extracellular polymeric substances (EPSs), tightly (TB-EPSs) and loosely (LB-EPSs) bound EPSs of granular sludge with simultaneous removal of organic matters and ammonia nitrogen, studied the dynamic variation of metal ions in EPSs from granular sludge with different particle sizes and the change of zeta potential before and after cation exchange resin (CER) treatment. Results showed, with particle size increasing, the protein content gradually increased, the content of polysaccharide basically unchanged; the content of Ca, Mg, K, Na and Zn also increased, whereas others did not show a consistent regularity. The existence of metal ions reduced zeta potential of EPSs. The existence of metal ions helped to the adhesion among granules, in order to form a granule with bigger particle size.

1. Introduction

Recently, aerobic granular sludge technology with the characteristics of high sludge concentration, good settleability, and tight structure has become the focus of attention compared with the traditional sludge flocs. As a promising technology, aerobic granular sludge technology has been widely used for processing all kinds of wastewaters, such as domestic sewage, wastewater with high concentration of organics and ammonium nitrogen (Tay et al., 2001, 2002; Kim and Seo, 2006).

Extracellular polymeric substances (EPSs) containing large amounts of organic compounds such as proteins, polysaccharides, humic acid, nucleic acid, and phospholipid (Li and Yang, 2007) widely exist as microbial secretions in the interior and surface of biological flocs. EPSs have the following functions: enriching the nutrients in the environment, storing energy substrate, and resisting the harm from fungicide and toxic substances. In addition, these materials can change the settleability of sludge flocs, hydrophobicity of the sludge surface, and have important influence on the formation of granular sludge (Li and Yang, 2007; Zhang et al., 2007). In recent years, large amounts of researches on the mechanism of organics in EPSs in granulation have been made. These researches included the content and composition of organics, and proportion of these compositions (Li and Yang, 2007; Tay et al., 2002; Zhang et al., 2007). Functional groups in organics react with metal elements, which then successfully remove the metal elements (Guibaud et al., 2009). In fact, a few researchers have studied the mineral fraction in EPSs of flocs and granules, including Ca, Mg, Pb, Ag, Fe, Zn, etc. (Leppard et al., 2003; Yu et al., 2009; d'Abzac et al., 2010; Bourven et al., 2011; Wang et al., 2014). Mineral fractions can affect the related properties of EPSs; for example, the

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presence of manganese oxide and iron hydroxide will enhance the ability of EPSs in absorbing lead and chromium (Guibaud et al., 2009). However, as far as we know, there have been no literatures reporting the respect on mineral fractions in EPSs of aerobic granular sludge, especially with short-cut nitrification.

EPSs can be divided into two parts, TB-EPSs and LB-EPSs (Li and Yang, 2007; Liang et al., 2010), which have big differences in sludge flocculation, sedimentation, dehydration, and so on.

The property of sludge as well as content and composition of EPSs will change on the formation of granular sludge, study these changes will help to study the formation mechanism of granular sludge. Meanwhile, with an extended running time, the particle size of the granular sludge increases gradually (Kim and Seo, 2006). In this process, flocs were changed to granular sludge; and small particle size was converted to bigger one. The change of relevant indicators with different particle sizes in the same system was studied, which can also help to understand the formation of granular sludge. The aim of this study is to analyze mineral fractions in EPSs, LB-EPSs, and TB-EPSs through the determination of organic and mineral fractions in EPSs from granular sludge with simultaneous removal of organic matter and ammonia nitrogen. The infrared (IR) spectral characteristics of EPSs from the granular sludge with different particle sizes and effects of different cations concentration in the EPSs of the granular sludge with different particle sizes on change of zeta potential reveal the role of organic and mineral fractions in EPSs in granulation. This study improves our understanding on the role of EPSs in granulation.

2. Methods

2.1. Experimental sludge

The experimental sludge used was the aerobic granular sludge with simultaneous removal of organic matter and ammonia nitrogen taken in a steady lab run for more than one year (Yan et al., 2014). Characteristics of aerobic granular sludge and characteristics of influent and effluent are summarized in Table 1. The initial average diameter of the granules was 0.70 mm (Yan et al., 2014), and gradually developed into granules with larger particle sizes. The mature granular sludge was tawny, its outline was spheroidic or round, its boundary was clear-cut, and it had good settleability. The mass of particle size that was more than 0.5 mm accounted for 85% of total mass of aerobic granular sludge, as such, only the granular sludge in this range was investigated in this study. Granular sludge samples went through nylon meshes of 12, 20, 32 mesh successionally, the particle size was divided into three levels of more than 1.40 mm, 0.85–1.40 mm, and 0.5–0.85 mm.

2.2. EPSs, LB-EPSs and TB-EPSs extraction method

2.2.1. EPSs extraction method

EPSs were extracted using heating method (Brown and Lester, 1980). In a typical procedure, 100 mL of sample was filtered through a nylon mesh and then diluted with deionized water to its original volume. The diluted sample was heated (80 °C, 10 min), centrifuged (8000×*g*, 20 min), and then filtered through 0.22 μm cellulose membrane filters. The obtained EPS extracts were stored at −20 °C for further analysis.

2.2.2. LB-EPSs and TB-EPSs extraction method

LB-EPSs and TB-EPSs were extracted according to literatures (Li and Yang, 2007; Liang et al., 2010) and were modified appropriately. The specific method is as follows: 100 mL of sample was filtered through a nylon mesh, and then diluted with deionized water to its original volume; the mixture was centrifuged (5000×*g*, 15 min), the supernatant was extracted as LB-EPSs; the residue was then diluted with deionized water to its original volume, and was treated according to the EPSs extraction method for TB-EPSs.

2.3. Determination of the existing form of the metal elements in EPSs

A total of 100 mL of sample was filtered through a nylon mesh, and then diluted with deionized water to its original volume. The mixture was heated (80 °C, 10 min), and then strong acid cation exchange resin (CER) was added into the mixture (70 g of CER per 1 g of VSS). The mixture was placed at 4 °C for 1 h, and then centrifuged (8000×*g*, 20 min) for further analysis of metal element contents in EPS and zeta-potential.

2.4. Analysis method

The analyses of mixed liquid suspended solids (MLSS), volatile suspended solid (VSS), the sludge volume index (SVI), Chemical Oxygen Demand (COD), ammonia nitrogen, nitrite, nitrate were performed in accordance to standard methods (APHA, 1998). The measurement of pH was carried out with a pHS-3C precision pH meter (Shanghai Weiye Co., Ltd., China). Contents of mineral elements were determined by inductively coupled plasma optical emission spectrometry (Perkin Elmer Optima 8300 ICP-OES Spectrometer, USA). Particle size distribution of the granular sludge was determined in accordance to the sieve method described by Laguna. Polysaccharide (PS) concentration was determined using the Anthrone method (Frølund et al., 1996) and protein (PN) measurement was performed using a modified Lowry method (Lowry et al., 1951). UV absorbance was measured with a UV-1800 spectrophotometer (Shimadzu Co., Tokyo, Japan). Lastly, zeta potential was determined using the zeta-potential analyzer (Malvern Instruments Ltd., Zetasizer Nano Z, UK).

The metal elements in EPSs were determined according to literature (d' Abzac et al., 2010). A total of 3 mL of EPSs, LB-EPSs and TB-EPSs were digested through the addition of 2 mL of hydrogen peroxide for 24 h. Then 3 mL of nitric acid and 1 mL of perchloric acid were added to the solution. Digestion was again performed after another 24 h. After the second digestion, samples were diluted with ultrapure water to 50 mL and then filtered through 0.22 μm cellulose nitrate membrane filters. Inductively coupled plasma optical emission spectrometry (ICP-OES, Perkin

Table 1
Characteristics of aerobic granular sludge, influent and effluent.

Sludge properties	Numerical value	Parameters	Characteristics of influent and effluent	
			Average influent	Average effluent
MLSS (mg/L)	5.75 ± 0.19	COD (mg/L)	235.31 ± 28.36	34.72 ± 16.89
VSS (mg/L)	4.60 ± 0.21	Ammonia nitrogen (mg/L)	238.62 ± 21.54	3.25 ± 2.24
SVI (ml/g)	24.92 ± 1.50	Nitrite (mg/L)	0.11 ± 0.48	107.42 ± 35.57
		Nitrate (mg/L)	0.03 ± 0.01	18.73 ± 4.55
		pH	7.37 ± 0.46	6.74 ± 0.58