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Specific component comparison of extracellular polymeric substances (EPS) in flocs and granular sludge using EEM and SDS-PAGE

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HIGHLIGHTS

- Flocs, aerobic and anaerobic granular sludge were selected for specific EPS comparison.
- Protein (PN) contents in LB-EPS and TB-EPS of granular sludge were higher than that of flocs.
- PN significantly contributed to the formation of granular sludge via surface charge adjustment.
- Proteins with high molecular weight favored the sludge granulation.
- Aromatic protein-like and tryptophan protein-like substances were more abundant in the granular sludge.

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ABSTRACT

Extracellular polymeric substances (EPS) plays an important role in the formation of bioaggregates such as flocs, biofilm and granular sludge. However, the role of their specific components in sludge flocculation and granulation is still unclear. Three sludge samples including the flocs, aerobic and anaerobic granular sludge were investigated in this study and the specific components in different EPS structures of loosely bound-EPS (LB-EPS) and tightly bound-EPS (TB-EPS) were analyzed. Results showed that the protein (PN) contents in LB-EPS and TB-EPS of the aerobic and anaerobic granular sludge were 33.6 ± 9.7 and 96.8 ± 11.9, 27.1 ± 2.8 and 61.6 ± 4.2 mg g^{-1} VSS, respectively, which were both higher than the flocs of 8.5 ± 1.5 and 43.1 ± 2.7 mg g^{-1} VSS. But the polysaccharide (PS) contents in the three sludges were all about 30 mg g^{-1} VSS. The analysis of sludge surface charge indicated that they had a linear correlation with the PN content, which implied that PN significantly contributed to the formation of granular sludge. The results of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) demonstrated that the molecular weight of PN in flocs was mainly distributed in 14.3–66.2 kDa, while it was 20.1–97.4 kDa in the granular sludge, which indicated that the proteins with high molecular weight favors the sludge granulation. According to the three-dimensional fluorescence (EEM) results, the aromatic protein-like and tryptophan protein-like substances were more abundant in the granular sludge than that in flocs, suggesting they are the key components in the structural stability of granular sludge.

1. Introduction

Biological treatment is one of the most widely used wastewater treatment processes due to the advantages of being more efficient and less costly, and no hazardous impact on the ambient environment than other physicochemical methods. The microbial aggregates including flocs, biofilm and granules, as the dominant elements in bioreactors, have been applied in different wastewater treatment systems (Martinez et al., 2004; Pijuan and Yuan, 2010; Coma et al., 2012). Compared to the conventional sludge flocs, the granular sludge has an excellent settling property for enabling high biomass retention and dense microbial structure for withstanding high-strength organic wastewater and its shock loading (Liu and Tay, 2004).

In recent years, the extracellular polymeric substances (EPS) have proved to play a crucial role in the formation and structural stability of bioaggregates (Li and Yang, 2007; Sheng et al., 2010). It has been reported that sludge cells have a double layered EPS structure: loosely bound EPS (LB-EPS) and tightly-bound EPS (TB-EPS) (Liu and Fang, 2003). Previous studies indicated that these

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two subfractions of the EPS have different effects on the sludge granulation, and the related conclusions are still contradictory (Li and Yang, 2007; Liu et al., 2010). For example, Li and Yang (2007) suggested that LB-EPS was more closely related to the sludge flocculation and settleability; Yu et al. (2010) found that TB-EPS contributed more to the cell adhesion. This can be attributed to their specific components which are rarely studied before, as well as their special spatial distribution. In addition, the key components which contribute more to the sludge aggregation and structural stability have not been well elucidated. The continuous argument on the role of extracellular proteins and polysaccharides is just a good example (Xie et al., 2010; Zhang et al., 2007). McSwain et al. (2005) found that the stability of granules was dependent on a protein core. However, Seviour et al. (2012) found that polysaccharide was in favor of the gel forming and formation of granular sludge.

The development of new techniques, such as Atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR) and Proteomics, has made it possible to analyze the natural organic matters (NOM) and EPS more precisely to access their chemical compositions and functions (Badireddy et al., 2010; Seviour et al., 2010). Among the above advanced techniques, three-dimensional excitation–emission matrix (EEM) fluorescence spectroscopy is a rapid, selective and sensitive technique which could be useful for addressing the chemical and physical properties of fluorescence compounds like proteins and humic acids in EPS (Sheng and Yu, 2006; Wang et al., 2009). Besides, some techniques like SDS-PAGE and gel permeation chromatography (GPC) are applied for the identification of the molecular weight of the matters in EPS (Zhang et al., 2007). Therefore, it is available to apply these techniques to figure out the possible key components of EPS in the sludge aggregation.

In this study, three kinds of sludge including activated sludge (flocs), aerobic and anaerobic granular sludge were selected, and the objectives are to analyze the difference of the specific EPS components between flocs and granular sludge through EEM and SDS-PAGE technologies, in order to reveal the key components of EPS in the formation of microbial aggregates especially granular sludge and then provide more information on the stable operation strategy of biological wastewater treatment process.

2. Materials and methods

2.1. Sludge samples

Three sludge samples were studied in this study. The activated sludge (flocs) was sampled from an aeration tank in the QiGe Municipal Wastewater Treatment Plant, Hangzhou, China. The aerobic granular sludge was collected from a running SBR reactor treating the simulated domestic wastewater in our laboratory. The anaerobic sludge was derived from a pilot-scale upflow anaerobic sludge blanket reactor of Jingxing paper mill, Jiaxing, China. Table 1 shows the physical properties of the sludge samples.

Table 1
The physical properties of sludge samples.

Index	Flocs	Aerobic granule	Anaerobic granule
MLVSS/VSS	0.56	0.78	0.82
SVI (mL g^{-1})	79.9	20.0	24.8
Size(mm)	0.027	0.34	0.28
EPS (mg g^{-1} VSS)	83.9 ± 1.5	170 ± 24.4	110.0 ± 1.1
Contact angle	29.5 ± 2.8	32.4 ± 2.3	32.8 ± 2.6
Surface charge (meq g^{-1} SS)	−0.076 ± 0	−0.010 ± 0	−0.026 ± 0.003

2.2. Sludge index analysis

SVI (Sludge Volume Index), MLSS (Mixed liquor suspended solids) and MLVSS (Mixed liquor volatile suspended solids) were measured according to Standard Methods (APHA, 1985). The average size of the sludge samples was determined by a dynamic particle image analyzer (QICPIC-LIXELL, SYMPATEC GmbH Co., Germany). Contact angle measurement was carried out with a video contact angle meter (OCA20, DATAPHYSI company, German) with a previous method (Liao et al., 2001).

2.3. Sludge surface charge

For granular sludge, the sludge samples were homogenized before the determination of cell surface properties including surface charge here and contact angle above. The net SC of the sludge samples was measured by colloidal titration. An excess amount of Poly (diallyldimethylammonium Chloride) Solution (2.5 mL) (Wako, Japan) (0.0025 N) was added to the diluted sludge samples, followed by back-titration with potassium polyvinyl sulfate (Wako, Japan) (0.0025 N) to a colorimetric endpoint indicated by Toluidin Blue. The titration was terminated when electrical neutrality was reached, as indicated by the change of color from blue to pink. An equal volume of Poly (diallyldimethylammonium Chloride) in distilled water was used as blank. The SC was expressed as milliequivalents per gram of MLSSs of negative colloidal charge [meq g^{-1} MLSS]. The equation was given below.

$$\text{Charge (meq g}^{-1}\text{ MLSS)} = 1000(A - B)N/XV$$

where A was the volume of PVSK added to the sample (mL), B was the volume of PVSK added to the blank (mL), N was the normality of PVSK solution used (0.0025 N), V was the volume of the sample(mL), X was the biomass concentration of the sample (g L^{-1}).

2.4. EPS extraction and analysis

The thermal extraction process as a common method was selected and modified to extract the LB-EPS and TB-EPS (Li and Yang, 2007). The sludge samples were washed with distilled water twice before extraction, and the aerobic and anaerobic granule sludge were homogenated with a glass homogenizer. Then the sludge pellet was resuspended in a 0.05%w/w NaCl solution that had a similar conductivity and was immediately sheared by a vortex mixer for 1 min, and then centrifuged at 6000 rpm for 10 min to separate solids and supernatant. The collected supernatant was regarded as the LB-EPS of the sludge sample. The residual sludge pellet left in the centrifuge tube was resuspended in 0.05% NaCl solution, then heated at 80 °C for 45 min, and finally centrifuged at 20 000 rpm for 20 min to collect the supernatant regarded as TB-EPS of the sludge sample.

Protein (PN) was determined by an adaptation of the Lowry method (Frolund et al., 1995). Casein was used as the standard. Polysaccharide (PS) was determined using the phenol-sulfuric acid method with a glucose standard.

2.5. EEM fluorescence spectroscopy

The method (Sheng and Yu, 2006) was modified for EEM measurement. All EEM spectra were measured using a luminescence spectrometry (LS-55, Perkin-Elmer Co., USA). In this study, EEM spectra were collected with subsequent scanning emission spectra from 300 to 550 nm at 0.5 nm increments by varying the excitation wavelength from 200 to 400 nm at 10 nm increments. Excitation and emission slits were maintained at 4 nm and the scanning speed was set at 1200 nm min^{-1} for all the measurements. A