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Contribution of stratified extracellular polymeric substances to the gel-like and fractal structures of activated sludge

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ABSTRACT

The gel-like and fractal structures of activated sludge (AS) before and after extracellular polymeric substances (EPS) extraction as well as different EPS fractions were investigated. The contributions of individual components in different EPS fractions to the gel-like behavior of sludge samples by enzyme treatment were examined as well. The centrifugation and ultrasound method was employed to stratify the EPS into slime, loosely and tightly bound EPS (LB- and TB-EPS). It was observed that all samples behaved as weak gels with weak-link. TB-EPS and AS after LB-EPS extraction showed the strongest elasticity in higher concentrations and highest mass fractal dimension, which may indicate the key role of TB-EPS in the gel-like and fractal structures of the sludge. Effects of protease or amylase on the gel-like property of sludge samples differed in the presence of different EPS fractions.

1. Introduction

Three-dimensional, gel-like, highly hydrated extracellular polymeric substances (EPS) is considered to act as a biological glue, which is of significant importance in binding floc components together, containing polysaccharides (PS) and proteins (PN) as dominant components together with some lipid, nucleic acids, and humic-like substances (HS) (Nielsen et al., 1996). Some researchers stated that a significant proportion of the EPS was hydrophobic, such as PN, amino acids and lipids, while PS was hydrophilic (Dignac et al., 1998; Jorand et al., 1998). PS are composed of long chains of monosaccharide units bound together by glycosidic bonds with linear to highly branched structure. They can readily mix in water because of the presence of the strong electrostatic interactions and hydrogen bond forces. While PN consisting of one or more chains of amino acid residues and lipids, a group of molecules, including phospholipids, monoglycerides and others, are non-polar and less readily bound with water (Raszka et al., 2006). On the other hand, the hydrophobic EPS was involved in the formation and organization of microbial aggregates (Jin et al., 2003; Liu et al., 2004), which maintains the structural and functional integrity of flocs/aggregates (Mu and Yu, 2006). Both the hydrophobic and hydrophilic components of EPS need to be accounted for the dewatering process

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(Raszka et al., 2006; Mikkelsen and Keiding, 2002). Recently, EPS stratification has generated great research interest. Yu and co-workers (Yu et al., 2008, 2009) employed a novel EPS fractionation approach, the centrifugation and ultrasound approach, to stratify the EPS into different fractions including slime, loosely and tightly bound EPS (LB- and TB-EPS), and found that PN and PN/PS in the slime layers markedly influenced the sludge dewaterability as well as the TB-EPS fraction possessed higher flocculating rate to kaolin suspension in comparison with the other EPS fractions. In addition, Li and his co-workers (Li and Yang, 2007, 2009) employed a heat extraction method to fractionate EPS into LB- and TB-EPS and suggested that LB-EPS had a negative effect on bioflocculation and sludge dewatering.

Wastewater sludge is a non-Newtonian fluid, which possesses both viscous and elastic properties, namely, also called viscoelastic (VE) properties (gel-like structure) originating from EPS (Legrand et al., 1998), which can be measured by dynamic testing where samples are subjected to oscillatory motion (Steffe, 1996; Ayol et al., 2006). Due to the existence of negatively charged macromolecules, EPS have been previously reported to provide cohesion and gel-like behavior to the floc structure (Keiding et al., 2001; Wilén et al., 2003). Seviour et al. (2009a) also revealed that EPS are responsible for the gel-like properties of the granules, which can be referred to as hydrogels. EPS components, like exopolysaccharides or glycosides were the gelling agent in aerobic granules, confirmed to be important in determining the gel-like properties of sludge as well (Seviour et al., 2009a). In addition, Yuan and Wang (2013) found that at a TSS content of 54 g/L, AS after LB-EPS extraction exhibited the strongest gel-like structure. The result that TB-EPS may be the key fraction to the gel-like properties of AS was also obtained when an assumption was proposed to the formula derivation of rheological parameters for identifying the contribution of stratified EPS to the gel-like properties of AS. However, the direct experimental evidences for the contribution of stratified EPS to the gel-like behavior of AS at different TSS contents are still absent.

On the other hand, EPS can be formed from simply aggregated polymeric chains, or their structure can be reinforced by cross-linking (Dursun, 2007). Several studies on the gel-like properties of EPS have also been reported (Seviour et al., 2009b; Lewin, 1956; Moreno et al., 2000). Seviour et al. (2009b) observed that the sol-gel transition of the granule EPS occurred at pH 9.0 to 12.0. At pH < 9 the granule EPS exhibited as a strong gel. The transition to a weak gel for the EPS from sludge flocs was found at pH 4.0 to 5.0. Seviour et al. (2010) also described the full structure of the exopolysaccharide, indicating that the gel-forming properties of granule EPS was attributed to a key exopolysaccharide. In addition, PN and PS are both able to form gels under very specific structural, stereochemical and environmental conditions (Seviour et al., 2009b). The result that the exopolysaccharides produced by green algae (Lewin, 1956) or from cyanobacterium *Anabaena* sp. (Moreno et al., 2000) behaved as a weak gel was obtained by rheological measurements. It has ever been reported that gel-like behavior is significant in restricting water mobility (Poxon and Darby, 1997). The gel structure of EPS results in fairly tenacious retention of water within the sludge (Steffe, 1996). Hence, it's may be relatively easier to dewater after weakening or destroying the gel-like structure of the EPS or the AS. However, as the aforementioned research was performed based on EPS as a whole, the gel-like behavior of different EPS fractions is rather limited.

Aside from rheological characterization of the structure of wastewater sludge, fractal structure characterization is another method commonly used to describe sludge properties (Li and Ganczarczyk, 1990; Wu et al., 2002; Wang et al., 2011). An important parameter used to describe the fractal structure of the sludge is the mass fractal dimension, D_f. Generally, D_f could indicate the floc density, floc strength, and the compactness/looseness of flocs/aggregate structures (Wang et al., 2009). While fractal analysis is widely used in studies on sludge flocs/aggregate structures (Wu et al., 2002), an apparent lack of knowledge about the influence of different EPS fractions on the AS and about the EPS themselves with respect to the fractal structure remains.

The main objective of the present study is to explore the gel-like behavior at different TSS contents and fractal structure of the AS before and after EPS extraction as well as different EPS fractions. The contributions of individual components in different EPS fractions to the gel-like behavior of sludge samples by enzyme treatment were examined as well. The present study also proposes a conceptual sludge model to elucidate the significance of EPS on the AS structure. In this case, the EPS layer which mainly contributes to the gel-like structure of AS would be indentified, and would become a key fraction to be weakened during the dewatering and drying process. Therefore, these results obtained will provide further information on the influence of EPS on the dewatering properties of the AS.

2. Materials and methods

2.1. *Characteristics of the AS*

The AS was sampled from a municipal wastewater treatment plant in Beijing, China. Sludge samples kept on crushed ice were transferred to the laboratory within 2 h and immediately passed through a 1.2 mm sieve. Filtered samples were subsequently stored at 4 °C. Table 1 lists the main characteristics of the AS. All of the tests were conducted within one week. The pH was measured using a pH meter (PB-10, Sartorius Stedim Biotech Co., Ltd., Beijing, China). Conductivity was measured using a conductivity meter (EC215, Beijing

Table 1 – Characteristics of the AS.

pH	Conductivity (mS/cm)	TSS (g/L)	VSS (g/L)	VSS/TSS (%)	COD (mg/L)	SCOD (mg/L)	Zeta potential (mV)
6.73 ± 0.02	1.46 ± 0.01	8.86 ± 0.30	6.13 ± 0.21	69.15 ± 0.03	6020.0 ± 32.4	78 ± 5	−18.9 ± 1.1