

# Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge

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### ABSTRACT

Laboratory experiments on the activated sludge (AS) process were carried out to investigate the influence of microbial extracellular polymeric substances (EPS), including loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), on biomass flocculation, sludge settlement and dewaterability. The heat EPS extraction method was modified to include a mild step and a harsh step for extracting the LB-EPS and TB-EPS, respectively, from the sludge suspension. Six lab-scale AS reactors were used to grow AS with different carbon sources of glucose and sodium acetate, and different sludge retention times (SRTs) of 5, 10 and 20 days. The variation in the bioreactor condition produced sludge with different abundances of EPS and different flocculation and separation characteristics. The sludge that was fed on glucose had more EPS than the sludge that was fed on acetate. For any of the feeding substrates, the sludge had a nearly consistent TB-EPS value regardless of the SRT, and an LB-EPS content that decreased with the SRT. The acetate-fed sludge performed better than the glucose-fed sludge in terms of bioflocculation, sludge sedimentation and compression, and sludge dewaterability. The sludge flocculation and separation improved considerably as the SRT lengthened. The results demonstrate that the LB-EPS had a negative effect on bioflocculation and sludge-water separation. The parameters for the performance of sludge-water separation were much more closely correlated with the amount of LB-EPS than with the amount of TB-EPS. It is argued that although EPS is essential to sludge floc formation, excessive EPS in the form of LB-EPS could weaken cell attachment and the floc structure, resulting in poor bioflocculation, greater cell erosion and retarded sludge-water separation.

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# 1. Introduction

The activated sludge (AS) process is the most common biological process that is used in wastewater treatment. Sludge flocculation transforms microbial cells into aggregates, which regulates the performance of biomass-water

separation and is thus crucial to the overall treatment result of the AS process. Microbial extracellular polymeric substances (EPS) are major components of the AS floc matrix (Frølund et al., 1996). They are believed to act as the glue that binds cells together to form sludge flocs. Many investigations have been carried out on the role of EPS in bioflocculation and

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sludge settleability (Nagaoka et al., 1996; Bura et al., 1998; Wilén et al., 2003). However, the results of previous studies are often inconsistent, with research showing that EPS content and bioflocculation are positively (Urbain et al., 1993) or negatively (Goodwin and Forster, 1985; Liao et al., 2001) correlated or even that there is no correlation at all (Chao and Keinath, 1979). Some investigators have suggested that the composition (Goodwin and Forster, 1985) and properties (Liao et al., 2001) of EPS, rather than the quantity, are more important in sludge flocculation. Therefore, the exact effects of EPS on bioflocculation still need to be identified.

Bacteria in the AS suspension and floc matrix are likely to have a dynamic double-layered EPS structure of loosely bound EPS (LB-EPS) diffused from the tightly bound EPS (TB-EPS) that surrounds the cells (Poxon and Darby, 1997). The LB-EPS in AS flocs may function as the primary surface for cell attachment and flocculation. However, most previous experimental work on EPS has not specifically addressed the role of LB-EPS in cell adhesion in AS. In fact, methods of EPS extraction commonly consist of thorough washing followed by harsh extraction (Liu and Fang, 2003), and the EPS that was measured in many previous studies was actually the total EPS or TB-EPS. Thus, little information is available that distinguishes the proportions of the two types of EPS and their possibly different effects on the surface properties of biomass. EPS is believed to be essential to sludge floc formation, but excessive EPS in the form of LB-EPS may deteriorate cell attachment and weaken the floc structure, which results in poor sludge-water separation.

In this laboratory study, the heating extraction method was modified to include a mild extraction step for extracting the LB-EPS and a harsh extraction step for extracting the TB-EPS from a sludge suspension. Six batch reactors were operated under different conditions to grow AS with different flocculation and compression characteristics. The objectives of the experimental work were to examine the abundance of TB-EPS and LB-EPS in the sludge produced under different feeding and growth conditions, to correlate the EPS contents and the sludge flocculation and separation behaviour, and to determine the respective effects of LB-EPS and TB-EPS on the sludge flocculation, sedimentation and dewatering properties.

## 2. Materials and methods

### 2.1. AS reactors

Six 2-L beakers were used as AS reactors, and were placed in parallel on a paddle mixer (PB-700, Phipps & Bird). Each reactor contained a sludge suspension of 1.5 L. The AS reactors and their operation have been previously described (Li and Yuan, 2002; Li and Leung, 2005). In brief, the reactors were seeded with sludge collected from a full-scale municipal wastewater plant, the Stanley Sewage Treatment Works in Hong Kong. The sludge in the reactors was well suspended by the paddles mixing at 35 rpm and continuous aeration from the bottom through stone air diffusers. The reactors were fed once a day with synthetic wastewater that contained a carbon source, urea, potassium phosphate and other inorganic salts for microbial growth, in accordance with the basic recipe that is given in the Environmental Engineering Process Laboratory Manual of AEESP (2001). In three of the AS reactors, glucose was used as the carbon source, and for the other three reactors sodium acetate was the carbon source. The chemical oxygen demand (COD):N:P ratio was kept at 100:5:1 for both the glucose-fed and the acetate-fed sludge. The reactors were operated at room temperature (water temperature: 20–22 °C), and the pH of the sludge suspensions was in the range of 6.5-7.5.

Three different sludge wastage ratios were used for the AS reactors, resulting in different sludge retention times (SRTs) of 5, 10 and 20 days for each type of the substrates (Li et al., 2003; Li and Leung, 2005). The reactors G-5, G-10 and G-20 were fed on glucose-based wastewater and the reactors A-5, A-10 and A-20 on acetate-based wastewater with the indicated SRTs. The sludge concentrations in all of the reactors were maintained at a similar level of around 2000 mg/L in mixed liquor suspended solids (MLSS) by adjusting the strength of the feeding substrates. All of the reactors reached a steady state after three times of their respective SRT, after which the fluctuations in the major daily monitoring parameters, such as MLSS, sludge volume index (SVI) and the COD in the effluent, were less than 20% for a week or so (Table 1). Sludge samples were then collected from the reactors before daily feeding for bioflocculation characterisation and EPS

# Table 1 – Characteristics of the sludge and effluent from the activated sludge reactors with different substrate carbon sources and SRTs

	Glucose as the carbon source			Sodium acetate as the carbon source		
	SRT = 5 d	SRT = 10 d	SRT = 20 d	SRT = 5 d	SRT = 10 d	SRT = 20 d
Effluent COD (mg/L)	73.2±4.5	65.1±6.8	78.9±3.3	85.6±6.2	63.6±4.5	65.4±5.5
SVI (mL/g)	$51.0 \pm 3.6$	$36.8 \pm 2.4$	$34.3 \pm 1.7$	43.2±1.9	$34.6 \pm 2.3$	$32.4 \pm 2.1$
ESS (mg/L)	$49.0 \pm 1.4$	$31.5 \pm 9.2$	$25.5 \pm 3.5$	$20.0 \pm 0.1$	$20.2 \pm 0.2$	$10.6\pm0.8$
SRF (m/kg)	$(5.5\pm0.7) imes10^{13}$	$(1.8\pm0.5) imes10^{10}$	$(1.4\pm0.4) imes10^{10}$	$(5.0\pm5.7) imes10^{12}$	$(1.7\pm0.2) imes10^{11}$	$(1.6\pm0.4) imes10^{10}$
Sludge viscosity (mPa · s)	$1.30 \pm 0.08$	$1.05 \pm 0.03$	$1.04 \pm 0.05$	$1.55 \pm 0.2$	$1.04 \pm 0.02$	$1.00 \pm 0.08$
Effluent viscosity (mPa·s)	$1.05 \pm 0.03$	$1.02 \pm 0.04$	$1.02 \pm 0.03$	$1.03 \pm 0.06$	$1.01 \pm 0.05$	$1.00 \pm 0.04$
ζ-potential (mV)	$-25.3 \pm 0.4$	$-20.5 \pm 1.3$	$-19.0 \pm 3.6$	$-28.9 \pm 1.1$	$-25.2 \pm 3.4$	$-19.2 \pm 1.9$
Mean floc size (µm)	$109.7 \pm 2.1$	91.2±1.5	93.1±3.8	$135.3 \pm 3.1$	$120.2 \pm 2.8$	103.7±3.2

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