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A. Ramesh · Duu-Jong Lee · S. G. Hong

Soluble microbial products (SMP) and soluble extracellular polymeric substances (EPS) from wastewater sludge

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Abstract Laspidou and Rittmann (Water Research 36:2711–2720, 2002) proposed that the soluble extracellular polymeric substances (EPS) are identical to soluble microbial products (SMP) in sludge liquor. In this paper, we compared the physicochemical characteristics of the SMP and soluble EPS from original and aerobically or anaerobically digested wastewater sludge. The surface charges, particle sizes, residual turbidities of polyaluminum chloride (PACl) coagulated supernatant, and chemical compositions of the SMP and soluble EPS containing suspensions were used as comparison index. Experimental results revealed that the particles in SMP and soluble EPS fractions extracted from original wastewater sludge, before and after digestion, were not identical in all physicochemical characteristics herein measured. The current test cannot support the proposal by Laspidou and Rittmann (Water Research 36:2711–2720, 2002) that SMP is identical to the soluble EPS from a wastewater sludge.

Keywords Biological sludge · Soluble microbial products · Extracellular polymeric substance · Comparison

Introduction

Sludge liquor consists of living cells and microbial products, including extracellular polymeric substances (EPS), inert biomasses, and soluble microbial products (SMP) (Laspidou and Rittmann 2002). The EPS are microbial products located on or outside cell surfaces that aggregate cells into flocs or granules, provide resistance to surrounding toxins, accumulate enzymes for cell use, and facilitate cell–cell communication (Wingender et al. 1999).

The EPS are a complex mixture of proteins, carbohydrates, acid polysaccharides, lipids, DNA, and humic acid substances that surround cells and create a matrix of microbial flocs and films (Liao et al. 2001). Early studies identified polysaccharides as the most abundant components in EPS (Costerton et al. 1981), while in biofilm systems (Nielsen et al. 1997) and in activated sludge (Dignac et al. 1998) proteins are the most abundant component. The EPS are further differentiated into extractable EPS, the EPS fraction bound tightly with solid surfaces, and soluble EPS (also called slime polymers), the fraction able to move freely between sludge flocs and surrounding liquor (Rosenberger and Kraume 2002). Other classification paradigms have separated EPS into "loosely bound" and "tightly bound" fractions (Poxon and Darby 1997). Leung (2003) determined that most extraction approaches described in the literature effectively extract both types of fractions. Li et al. (2004) identified a correlation between the amount of loosely bound EPS and the flocculation and sedimentation features of activated sludge.

The SMP are soluble cellular components secreted by cells (Namkung and Rittmann 1986; Noguera et al. 1994; Rittmann et al. 1994; de Silva et al. 2000). Most researches treated EPS and SMP independently, as if no relationship existed. For example, Costerton et al. (1978, 1981), Nielsen et al. (1997), Sutherland (1994), Hsieh et al. (1994), Nelson et al. (1996), and Wingender et al. (1999) analyzed EPS and active biomass, whereas Furumai and Rittmann (1992), Namkung and Rittmann (1988), and Speitel et al. (1987) examined the interactions between SMP, biomass, and inert biomass. Laspidou and Rittmann (2002) observed that soluble EPS are SMP in sludge liquor. Restated, soluble EPS was equivalent to loosely bound EPS, which in turn, was the same as SMP (mixed liquor).

There exists no standard extraction methods to differentiate various fractions of EPS from biomass; hence, the categories such as soluble or bound EPS are an

A. Ramesh · D.-J. Lee (✉)
Department of Chemical Engineering,
National Taiwan University,
Taipei, 10617, Taiwan, Republic of China
e-mail: djlee@ntu.edu.tw
Tel.: +886-2-23625632
Fax: +86-2-23623040

S. G. Hong
Department of Chemical Engineering and Materials Sciences,
Yuan-Ze University,
Taoyuan, 330, Taiwan, Republic of China

operationally defined scheme depending on the measurement method used. However, if soluble EPS was the same as SMP in sludge, their physicochemical characteristics should be identical regardless of the origins of biomass or the methods of extraction. Furthermore, it is the response of sludge, such as dewaterability, digestibility, and others, subjected to numerous pretreatments or posttreatment that is of primary concern. This study extracted the SMP and soluble EPS from wastewater sludge before and after aerobic (25 °C) or anaerobic treatments (35 °C), using a methodology proposed by Leung (2003) and Li et al. (2004), and compared the physicochemical characteristics of the extracts.

Experimental

The test sample was collected from the return sludge stream at the wastewater treatment plant for the Neili Bread Plant, Presidential Enterprise, Taoyuan, Taiwan. The chemical oxygen demand and suspended solids data of the supernatant drawn from the sludge, measured via EPA Standard Methods, were 22.6 and 14.3 mg l^{-1}, respectively. The percentage weight of dried solids in the sludge sample was 0.83 % w/w, determined by weighing and drying at 102 °C.

Part of the original sludge was placed in an aerated basin, keeping dissolved oxygen >2 mg l^{-1} for 1 month with no external nutrients at 25 °C. Only the supernatant of the original sludge was added periodically to replenish the evaporation loss. This sludge is regarded as aerobically digested in this work. Another equal part of original sludge was placed in an anaerobic basin kept at 35 °C for 1 month without adding any nutrients. The yielded sludge is termed as anaerobically digested sludge herein.

The supernatant of each of the original and aerobically or anaerobically digested sludge samples was first separated from solid phase by centrifugation at 6,000×*g* for 10 min. Two volumes of acetone was added to the produced supernatant and maintained at 4 °C for 24 h to precipitate soluble substances. The collected precipitate was called the SMP.

The dewatered cake after the above-mentioned centrifugation was resuspended in a 0.85 % w/w NaCl solution with several glass beads, and then sonicated at 20 kHz and 330 W l^{-1} for 2 min, shaken horizontally at 120 rpm for 10 min, and sonicated again under the same power for an additional 2 min. The liquor was centrifuged at 8,000×*g* for 10 min to separate solids and supernatant. The supernatant was added to two volumes of acetone and maintained at 4 °C for 24 h to precipitate soluble substances. The collected precipitate was called the soluble EPS.

Chemical coagulation tests using PACl as coagulant were performed in standard jar testers. The 1,000-mg l^{-1} PACl solution, with 11 % available Al_2O_3, was slowly injected into the agitated water sample at 200 rpm for 1 min, followed by 50 rpm for 8.5 min.

The compositions of the SMP and soluble EPS were compared using a Fourier-transform infrared (FTIR) spectrophotometer (Perkin Elmer 1760, England; sample/KBr=1/100, 4000-400 cm^{-1} at 4 cm^{-1} resolution for 100 cycles), and surface charge and floc size were also compared using a zetasizer (Zetasizer 3000 HS type A, Malverin, England). Suspension samples were collected and filtered using a 0.45-μm filter. Fluorescence excitation-emission matrix (EEM) spectra were acquired for filtered samples with a Cary Eclipse fluorescence spectrophotometer (Varian, Palo Alto, CA, USA). Excitation wavelengths were increased incrementally from 200 to 400 nm at 10 nm intervals, whereas emission wavelengths were detected at 1 nm steps from 250 to 500 nm.

Results

Zeta potentials and turbidities

Figure 1 presents the zeta potentials and residual turbidities of the suspensions containing SMP or soluble EPS as a function of PACl dose. The zeta potentials of SMP and soluble EPS of original sludge were −14 and −38 mV, respectively, which were neutralized at PACl of 0.5 and 0.4 mg l^{-1} as Al, respectively (Fig. 1a). The residual turbidities of SMP and EPS suspensions were all around

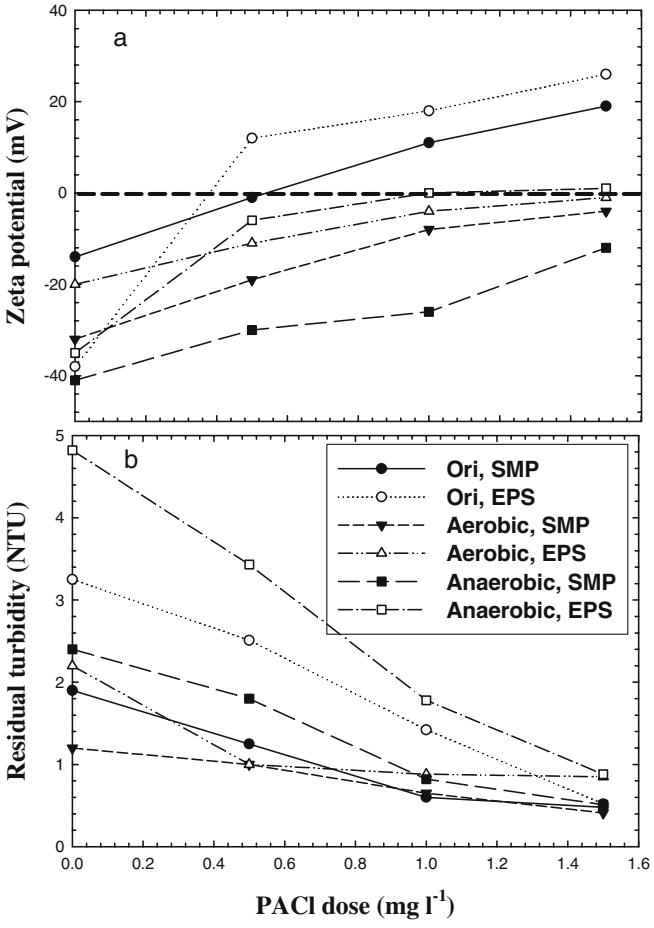

Fig. 1 Zeta potentials (**a**) and residual turbidities (**b**) of fine particles in wastewater sludge samples

2.2 nephelometric turbidity units, which decreased and then leveled off with dosed PACl at >1 mg l^{-1} as Al (Fig. 1b).

Aerobic and anaerobic digestion would yield more negatively charged SMP but less negatively charged EPS compared with the original sludge (Fig. 1a). However, more PACl was needed to neutralize the surface charges of the digested particles than that needed for original sludge. The residual turbidities of digested sludge declined monotonically with increasing PACl dose, followed by anaerobically digested > original > aerobically digested sludge (Fig. 1b).

Figure 2 presents the size distributions of SMP and soluble EPS suspensions. The SMP of original sludge exhibited monodispersed distribution of size ranging 100–200 nm. The soluble EPS in original sludge had bidispersed distributions: 200–400 nm and 700–2000 nm. Hence, the size of the soluble EPS was larger than that of the SMP.

Aerobic and anaerobic digestion would yield more negatively charged SMP, but less negatively charged soluble EPS.

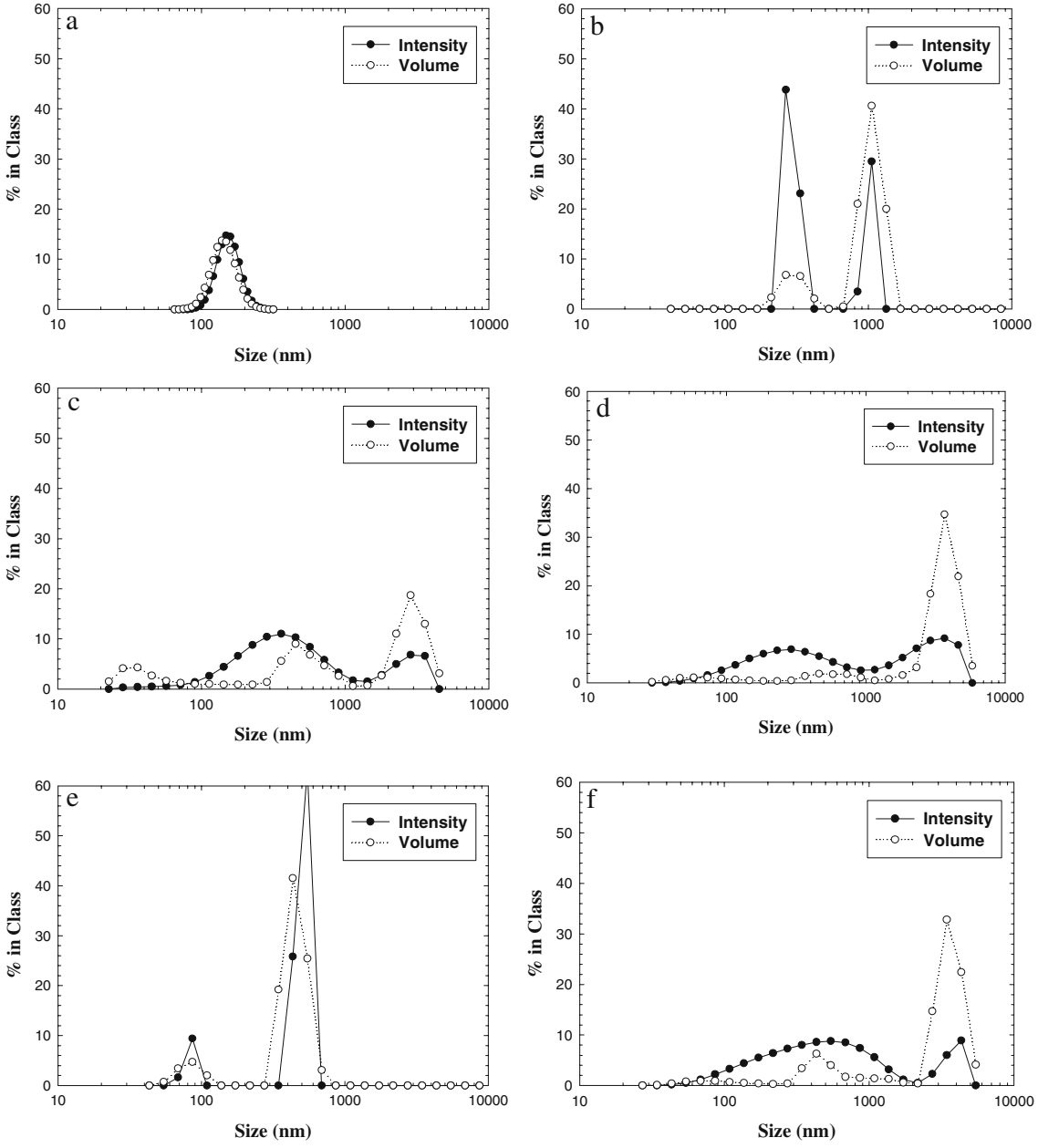

Fig. 2 Size distributions of SMPs and soluble EPS extracted from wastewater sludge. **a** SMP from original sludge; **b** EPS from original sludge; **c** SMP from anaerobic digested sludge; **d** EPS from anaerobic digested sludge; **e** SMP from aerobic digested sludge; **f** EPS from aerobic digested sludge

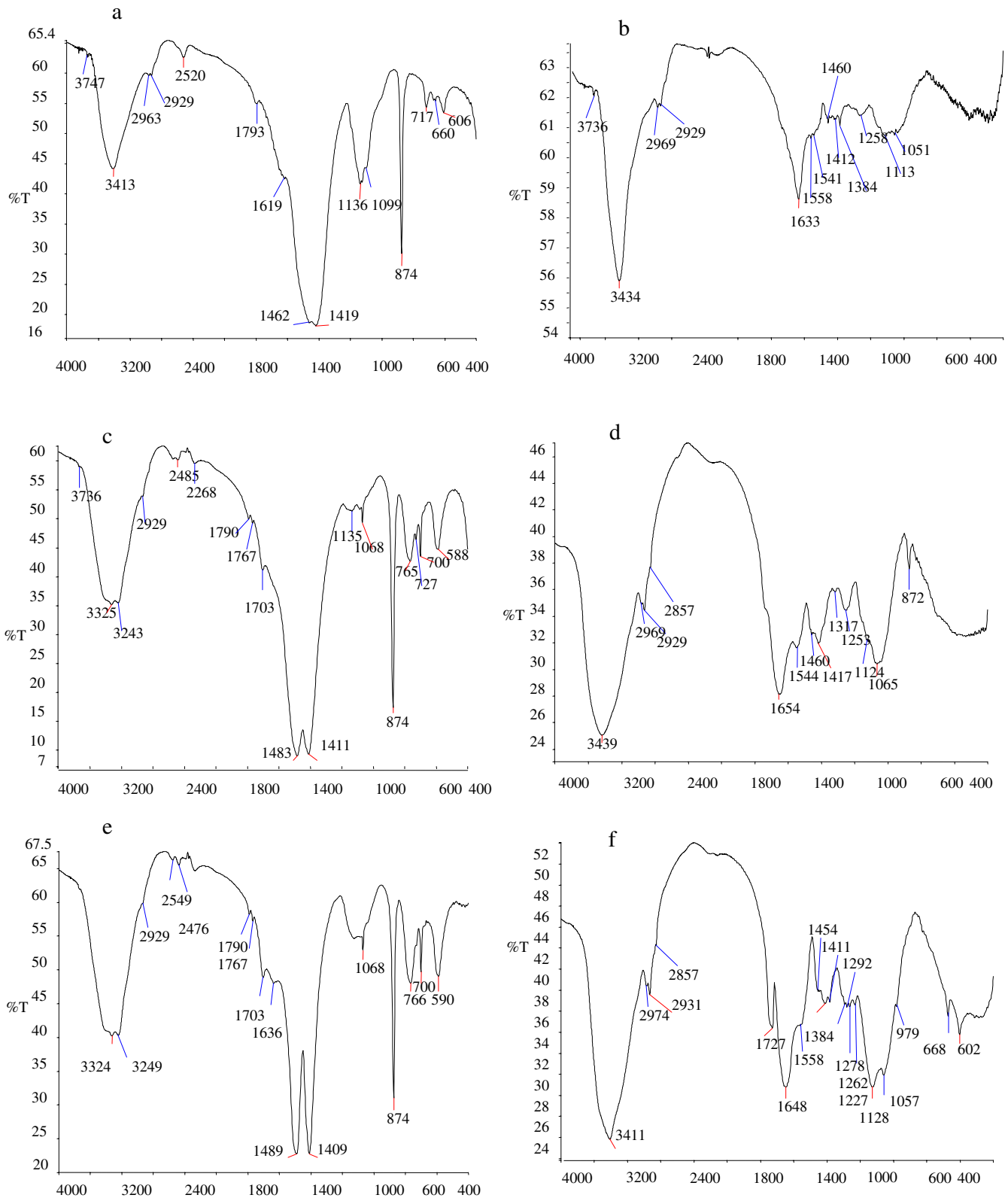

Fig. 3 The IR spectra of SMPs and soluble EPS extracted from wastewater sludge. **a** SMP from original sludge; **b** EPS from original sludge; **c** SMP from anaerobic digested sludge; **d** EPS from anaerobic digested sludge; **e** SMP from aerobic digested sludge; **f** EPS from aerobic digested sludge

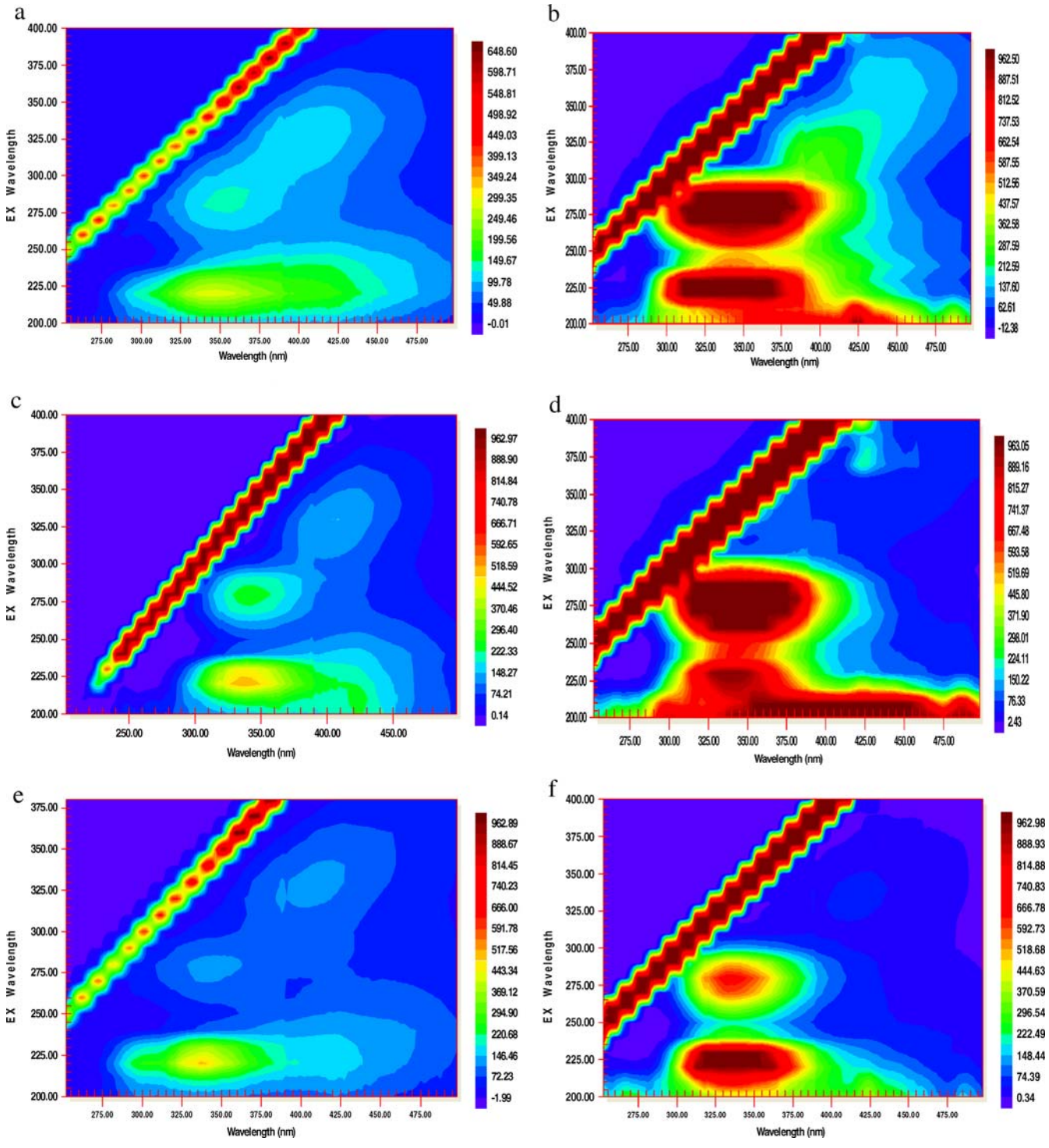

Fig. 4 Excitation-emission matrix (EEM) spectra of SMPs and soluble EPS extracted from wastewater sludge. **a** SMP from original sludge; **b** EPS from original sludge; **c** SMP from anaerobic digested sludge; **d** EPS from anaerobic digested sludge; **e** SMP from aerobic digested sludge; **f** EPS from aerobic digested sludge

IR spectra

Figure 3 presents the IR spectra of SMP and soluble EPS. According to Hung et al. (1995), the sludge mainly consists of proteins (amino acids), fat (aliphatic ester), and polysaccharides (ether). Characteristic IR adsorption peaks are summarized as follows.

The large absorption band near 3,430 cm^{-1} is from the stretching vibration of both hydroxyl (of the polysaccharides) and amino groups (of the proteins). Bands near 2,929 and 2,857 cm^{-1} reflect the existence of aliphatic chains.

The shoulder near 2,969 cm^{-1} indicates the presence of a small amount of $–CH_3$. The absorption bands near 1,790; 1,760; and 1,700 cm^{-1} are possibly attributed to the carboxylic acid, ester group (of lipids), and ketone groups. Peaks around 1,650 and 1,540 cm^{-1} are correlated with the amide I (C(N)=O) amide II (C–N + N–H) groups in proteins. The bands near 1,460 cm^{-1} are produced by the CH_2 groups of aliphatic chains. The band near 1,418 cm^{-1} is a result of the N–C–H deformation in the proteins. The band near 1,250 cm^{-1} is from the asymmetric stretching vibration of C–O–C ester in fat or in the cellulose. The band near 1,100 cm^{-1} is attributed to the combined effects of C–N stretching vibrations of primary and secondary amines and the C–O stretching from the cellulose and from the fat. Bands at 600–900 cm^{-1} denote the existence of unsaturated bonds in the sample.

From the bands near 3,430; 2,929; and 1,090 cm^{-1}, all SMP and EPS samples contained polysaccharides, while the EPS contained more polysaccharides than SMP. Based on bands near 1,760 and 1,254 cm^{-1}, both SMP and EPS contained a mediate amount of lipids. Conversely, the characteristic bands for proteins, including those near 1,650 and 1,450 cm^{-1}, were present in EPS but were totally absent in SMP samples. The SMP is mainly composed of polysaccharides, some lipids, and certain amount of unsaturated compounds and nitrogen-containing substances (but not amides). The soluble EPS contains polysaccharides, lipids, and proteins.

After aerobic or anaerobic digestion, the band for SMP near 3,413 cm^{-1} separates into two distinct bands near 3,325 and 3,250 cm^{-1}, corresponding to the presence of primary amine. The peaks near 1,100 cm^{-1} for SMP declined, while those near 1,760 cm^{-1} increased in intensity accordingly, indicating the disintegration of polysaccharides into esters. For EPS, the unsaturated compounds appeared, evidenced by the peaks that emerged at 600–900 and at 1,727 cm^{-1}. No clear evidence existed for the degradation for EPS after digestion.

EEM spectra

Figure 4 shows the EEM spectra for the SMP and EPS suspensions. Three peaks were noticeable in the EEM spectra, namely Ex/Em =220/340 nm (peak 1), 280/345 nm (peak 2), and 335/405 nm (peak 3). According to the classification scheme by Chen et al. (2003), these three peaks are located in regions II (aromatic proteins II), IV (soluble microbial by-product-like), and V (humic substance-like), respectively. All EEM peak intensities were normalized using the Rayleigh scattering intensity at their corresponding excitation wavelengths. The relative intensities of EEM peaks for all samples are in the following order: peak #1>#2>#3 and EPS>SMP. After digestion, all peaks declined in relative intensities, with aerobic digestion being the more effective means to degrade organic compared with the anaerobic digestion.

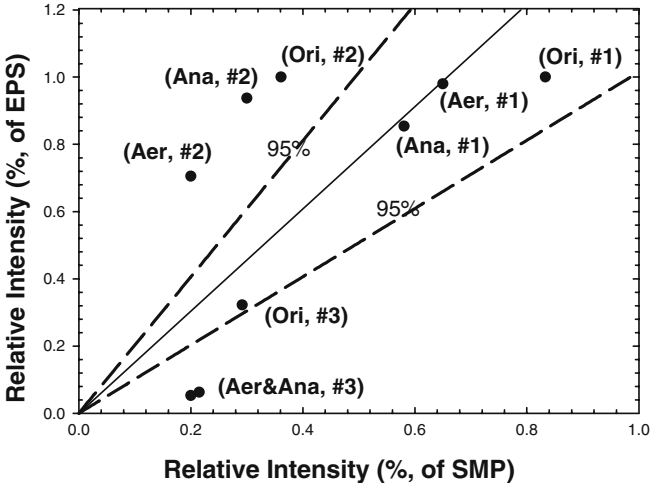

Fig. 5 Relative intensities of EEM peaks for SMP and EPS of samples before and after digestion. *Ori* Original, *Aer* aerobic, *Ana* anaerobic

Discussion

The above-mentioned tests revealed that the particles in SMP and soluble EPS fractions extracted from original wastewater sludge, before and after digestion, were not identical in zeta potentials, particle size, response to PACl coagulation, and in chemical compositions probed using FTIR and EEM spectra. The soluble EPS contained mainly polysaccharides, lipids, and proteins. On the other hand, the particles in SMP were noted to be smaller in size, less negative in surface charge, containing almost no proteins but relatively more humic substances compared with the soluble EPS.

After digestion, particles of large size were produced, while those in SMP became more negative in surface charge and those in EPS presented less negative surface charge. Moreover, primary amine and ester were yielded in SMP, while unsaturated compounds were produced in EPS. The EEM spectra revealed no significant correlation between the characteristic peaks intensities of SMP and EPS samples (Fig. 5).

The proposal by Laspidou and Rittmann (2002) that soluble EPS are SMP in sludge liquor could be applicable only to the microbial product fractions of sludge, while the present study probed all compositions in extracted suspension regardless of their origins. However, digestions, which are biological processes, had not yielded identical product distributions of tested samples on SMP and soluble EPS fractions. Therefore, based on the extraction scheme proposed by Li and coworkers, the currently tested samples cannot support the proposal by Laspidou and Rittmann that SMP is identical to the soluble EPS in wastewater sludge.

Conclusions

In this work the soluble microbial products (SMP) and soluble extracellular polymeric substances (EPS) were extracted from original and digested wastewater sludge and

compared in their particle sizes, surface charges, and residual turbidities after PACl coagulation, and in chemical compositions by a Fourier-transform infrared (FTIR) spectrophotometer and a fluorescence excitation-emission matrix (EEM) spectra.

The SMP contained small particles (100–200 nm) of negative charges, while the soluble EPS comprised two groups of particles of larger sizes (200–400 and 700–2,000 nm) and more negative charges. Dosing with PACl could neutralize the surface change and reduce residue turbidities of both suspensions, but at different magnitudes. The soluble EPS contained mainly polysaccharides, lipids, and proteins, while the SMP had no proteins but more humic substances compared with the soluble EPS.

Aerobic and anaerobic digestion yielded more negatively charged SMP, but less negatively charged EPS compared with the original sludge, both required more PACl than that for original sludge to neutralize the surface charges of the particles. After aerobic or anaerobic digestion, primary amines and some esters appeared in SMP suspensions. For EPS, certain unsaturated compounds were noted after digestion. No correlation between the EEM peak intensities of SMP and EPS samples could be identified.

Hence, this work revealed that the suspensions containing extracted SMP and soluble EPS present distinct characteristics on size, surface charge, and chemical compositions for original and digested, and for original and PACl-coagulated samples. Based on the extraction scheme proposed by Li et al. (2004), the current tests cannot justify the proposal of Laspidou and Rittmann (2002) that SMP are identical to the soluble EPS from wastewater sludge.

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