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Effects of ultrasonic pretreatment on sludge dewaterability and extracellular polymeric substances distribution in mesophilic anaerobic digestion

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Abstract

Effect of ultrasonic pretreatment on sludge dewaterability was determined and the fate of extracellular polymeric substances (EPS) matrix in mesophilic anaerobic digestion after ultrasonic pretreatment was studied. Characteristics of proteins (PN), polysaccharides (PS), excitation-emission matrix (EEM) fluorescence spectroscopy and molecular weight (MW) distribution of dissolved organic matters (DOM) in different EPS fractions were evaluated. The results showed that after ultrasonic pretreatment, the normalized capillary suction time (CST) decreased from 44.4 to 11.1 (sec·L)/g total suspended solids (TSS) during anaerobic digestion, indicating that sludge dewaterability was greatly improved. The normalized CST was significantly correlated with PN concentration (R^2 = 0.92, $p < 0.01$) and the PN/PS ratio (R^2 = 0.84, $p < 0.01$) in the loosely bound EPS (LB-EPS) fraction. Meanwhile, the average MW of DOM in the LB-EPS and tightly bound EPS (TB-EPS) fractions also had a good correlation with the normalized CST ($R^2 > 0.66$, $p < 0.01$). According to EEM fluorescence spectroscopy, tryptophan-like substances intensities in the slime, LB-EPS and TB-EPS fractions were correlated with the normalized CST. The organic matters in the EPS matrix played an important role in influencing sludge dewaterability.

Key words: anaerobic digestion; excitation-emission matrix; extracellular polymeric substances; sludge dewaterability

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Introduction

Large amount of activated sludge without proper disposal poses a great threat to ecology system in recent years (Rai et al., 2004). Sludge reduction is an effective method to alleviate this problem, and anaerobic digestion is widely used for sludge reduction. However, conventional anaerobic digestion usually takes more than 20 days to meet the regulation standard, due to the rate-limiting step of hydrolysis (Valvilin et al., 1996; Tiehm et al., 2001; Chu et al., 2002; Gronroos et al., 2005). Recently, it has been reported that the ultrasonic pretreatment could break the rate-limiting stage and shorten the digestion time (Khanal et al., 2007). The organic matters were found to transfer from the sludge flocs to aqueous phase after ultrasonic pretreatment, which resulted in the improvement of digestibility and dewaterability (Tiehm et al., 1997; Khanal et al., 2007). Whereas, Feng et al. (2009) observed that sludge dewaterability was not improved obviously, but deteriorated after ultrasonic pretreatment.

Extracellular polymeric substances (EPS) are a three-dimensional, gel-like, highly hydrated matrix, in which micro-organisms are embedded. EPS account for about 50%–80% organic matters in activated sludge and are considered important for physicochemical properties of activated sludge flocs (Frølund et al. 1996). Yu et al. (2008a) reported that sludge flocs could be stratified into different fractions by centrifugation and ultrasound methods, including slime, loosely bound EPS (LB-EPS), tightly bound EPS (TB-EPS), and pellet.

Three dimensional excitation-emission matrix (EEM) fluorescence spectroscopy and gel permeation chromatogram (GPC) could not only describe the chemical and physical properties of dissolved organic matters (DOM), but also supply qualitative analytical parameters to characterize the wastewater and effluent (Crozes et al., 1996; Yu et al., 2009). However, the relation between sludge dewaterability and EEM spectra or molecular weight (MW) has been seldom reported.

The main purposes of this study were: (1) to investigate the variations of organic substances, MW distribution and EEM fluorescence spectra in EPS matrix and the sludge flocs in mesophilic anaerobic digestion, and (2) to understand their effects on sludge dewaterablilty.

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1 Materials and methods

1.1 Activated sludge sample

Activated sludge samples were collected from the aerated basin of a municipal wastewater treatment (WWTP) in Shanghai, China. The plant treats 75,000 m^3/day of wastewater (93% domestic and 7% industrial sewage) using anaerobic-anoxic-oxic process. The samples were settled for 1.5 hr at 4°C. Then the sediments were collected from them and screened through a 1.2-mm screen to remove grit. Finally, the under-flow fractions were pretreated by ultrasound at 20 kHz and 480 W for 10 min (Yu et al., 2008a). Characteristics of the sludge are listed in Table 1.

1.2 Operation of anaerobic reactors

Figure 1 presents the schematic diagram of anaerobic digestion performed in this experiment. Anaerobic digestion was carried out using two airtight vessels with a working volume of 4.0 L, and one fed with raw sludge (unsonicated) and the other with sonicated sludge. For each vessel, 3.0 L of the sludge samples (Table 1) were used for experiment. Oxygen in the vessel was removed from the headspace by N_2 sparging for 2 min. The vessel was sealed with the rubber stopper and the temperature of the reactor was kept at 37°C. Meanwhile, pH was maintained between 6.8 and 7.5 by 6 mol/L HCl or NaOH. The sludge recirculation was adopted for a better mixture. The sludge sample of 100 mL was collected for the fractionation extraction and analysis every five days at the beginning, and then every seven days after digestion became stabilized.

1.3 Fractionation protocol

Sludge fractionation protocol was adopted according to our previously described methods (Yu et al., 2008b, 2008c). In brief, the sample was first centrifuged at 2000 $\times g$ for 15 min, and the supernatant was collected as slime. Then, the sediments were re-suspended to the original volume with buffer solution consisting of (mmol/L) Na_3PO_4 1.3, NaH_2PO_4 2.7, NaCl 6 and KCl 0.7. The suspensions were centrifuged again at 5000 $\times g$ for 15 min and the organic matters in the bulk solution were LB-EPS. The collected sediments were re-suspended with the aforementioned buffer to the original volume and extracted with ultrasonic at 20 kHz and 480 W for 10 min. The extracted suspensions were centrifuged at 20,000 ×g for 20 min. The bulk solution was collected as TB-EPS. After the TB-EPS was extracted, the residues (cells) were re-suspended with the buffer solution to the same volume and this fraction was pellet. Hence, from the surfaces to the cores of the granules, sludge flocs possessed a multi-fractioned structure consisting of slime, LB-EPS, TB-EPS, and pellet.

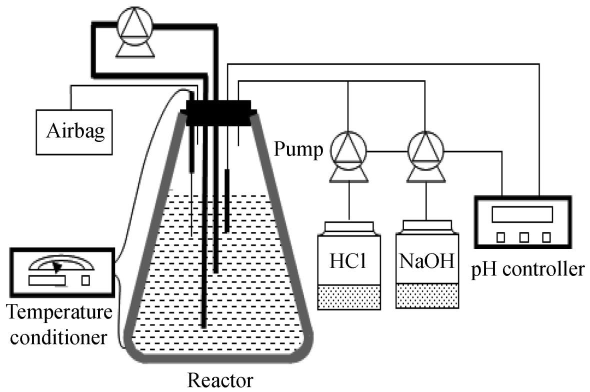

Fig. 1 Schematic diagram of anaerobic digestion.

1.4 Sludge characterization analysis

The chemical analyses were carried out in duplicates using chemicals of analytical grade. Proteins (PN) were determined by the modified Lowry method, using casein (Shanghai Sangon Biotechnology Co., Ltd., China) as standard (Frølund et al., 1995). Polysaccharides (PS) were measured by the Anthrone method using glucose as standard (Gaudy, 1962). Chemical oxygen demand (COD) and soluble COD (SCOD) were analyzed using a HACH DR/2000 spectrometer (Hach Company, USA). Three-dimensional EEM spectroscopy (F-4500, Hitachi Company, Japan) and GPC (LC-10A, Shimadzu Company, Japan) were employed to characterize the organic matters. The sludge dewaterability was characterized using a capillary suction time (CST) instrument (Model 319, Triton, UK) and the CST values were normalized by dividing them through the TSS concentration and then expressed in units of seconds per liter per gram TSS (APHA, 1998). Statistical analysis was carried out by the software SPSS version 16.0 for Windows. The software SigmaPlot 10.0 was employed to process the EEM data.

The sludge sample was filtered with a 0.45-μm filter and then diluted 50 times by distilled water for the detection of MW distribution. The mobile phase was Milli-Q water. Polyethylene glycol/oxides (with MW of 1169 kDa, 771 kDa, 128 kDa, 12 kDa, 4 kDa, 620 Da, and 194 Da) were used as reference molecules for the calculation (Yu et al., 2009).

The sample pretreatment for three-dimensional EEM was the same as that for GPC. EEM spectra were gathered with subsequent scanning emission spectra from 250 to 600 nm at 4 nm increments by varying the excitation wavelength from 200 to 500 nm at 5 nm increments. The

Table 1 Characteristics of sludge used in the experiment

Sludge	pH	COD (mg/L)	SCOD (mg/L)	TSS (g/L)	VSS (g/L)	Normalized CST ((sec·L)/g TSS)	SRF (m/kg)	PN (mg/g VSS)	PS (mg/g VSS)	Conductivity (μS/cm)
Unsonicated	6.9	24,956	219	20.13	10.31	2.3	8.3×10^{11}	80.7	30.6	12
Sonicated	7.0	23,680	7530	22.65	11.84	44.4	1.1×10^{13}	501.1	148.7	46

COD: chemical oxygen demand; SCOD: soluble COD; TSS: total suspended solids; VSS: volatile suspended solids; CST: capillary suction time; SRF: specific resistance of filtration; PN: proteins; PS: polysaccharides.

spectra were recorded at a scan speed of 1200 nm/min, with excitation and emission slit bandwidths of 10 nm. The voltage of the photomultiplier tube (PMT) was set to 700 V for low level light detection (Lu et al., 2009). The blank scans were performed at intervals of 10 analyses using double distilled water.

2 Results

2.1 Variations of COD, SCOD and TOC during anaerobic digestion

Figure 2 shows the variations of COD, SCOD, SCOD/COD and total organic carbon (TOC) of the sonicated sludge during the entire experimental period. The COD and SCOD concentrations were 23,680 and 7530 mg/L, respectively, while they decreased to 14,200 and 3170 mg/L at the end of the process (i.e., the 47th day). The ratio of SCOD/COD after ultrasonic pretreatment was kept approximately at 0.32 during the first 10 days and then dropped to about 0.20 ± 0.02 for the rest of experiment. But the ratio of SCOD/COD for the unsonicated sludge ranged between 0.01 and 0.14 (the data are not shown in the figure). The changing pattern of TOC was similar with that of SCOD (Fig. 3). Specifically, TOC concentration decreased from the initial 2300 to 250 mg/L at the end of experiment, with a degradation of 89.2%. Ultrasonic pretreatment could disintegrate the sludge flocs and improve the ratio of SCOD/COD (Chu et al., 2002; Dewil et al., 2006; Yu et al., 2008a), which would be in favor of the degradation of organic matters in subsequent mesophilic anaerobic digestion.

2.2 PN and PS during anaerobic digestion

The variations of PN and PS of the sonicated sludge during the entire experimental period are illustrated in Fig. 3. The quantity of PN in the pellet fraction contributed mostly (about 75%) to the total quantity of sludge flocs and the variations of PN in the pellet were agreed with those in the sludge flocs. From day 20, PN in the pellet fraction increased and reached to a peak value at day 34. In addition, LB-EPS fraction had the lowest PN concentration compared with other fractions. The initial PN concentration in the LB-EPS fraction was (107.5 ± 1.7) mg/g VSS, accounting for about 21% of the total EPS, whereas it declined to (7.0 ± 0.3) mg/g VSS at the end, only accounting for 2% of the total EPS.

In contrast, PS in the slime, LB-EPS and TB-EPS fractions fluctuated slightly during the entire period. However, PS in the pellet and the sludge flocs also kept unchangeable during the first 20 days, while they climbed to the maximum at day 34, it might be due to the disintegration of cells and the release of PS. Afterwards, they both decreased to the minimal level that were lower than the initial concentration. The PS variation in anaerobic digestion was consistent with the results of Chen et al. (2007) and Yu et

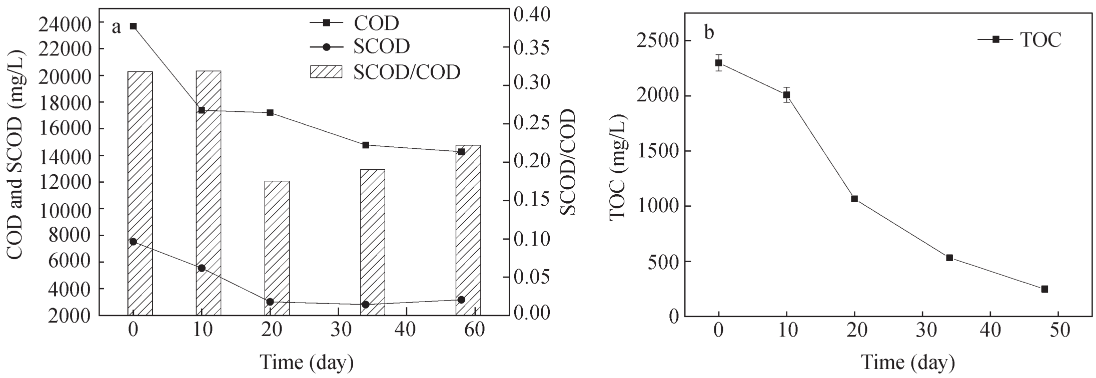

Fig. 2 Variations of COD, SCOD, SCOD/COD and TOC during anaerobic digestion.

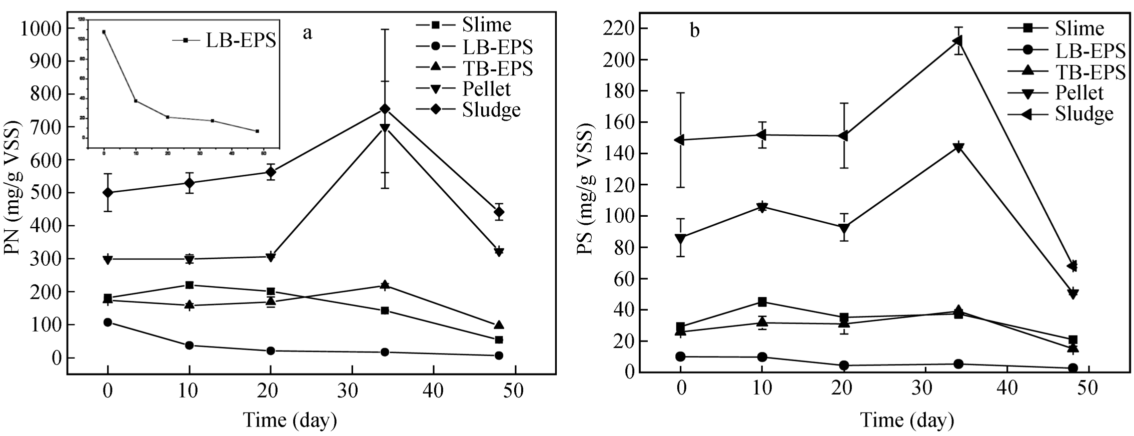

Fig. 3 Variations of PN and PS in EPS matrix and the sludge flocs during anaerobic digestion. LB-EPS: loosely bound EPS; TB-EPS: tightly bound EPS.

al. (2008a).

2.3 MW distribution of DOM in EPS matrix and the sludge flocs during anaerobic digestion

The MW distribution could be applied to further elucidate the DOM in different EPS fractions (Yu et al., 2009). The MW distribution of DOM was detected by GPC, based on selective exclusion or retention of dissolved organic molecules on gel pores as they migrated through the gel bed (Crozes et al., 1996). The technique was used in the experiment to qualitatively compare the variations of MW distribution on DOM in sludge flocs during anaerobic digestion. The GPC chromatograms of EPS matrix and the sludge flocs are shown in Fig. 4. It was found that the ranges of MW distribution became narrow during the process. And the number of peaks in the LB-EPS and pellet fractions was less than that in other fractions, indicating that there were much fewer types of materials in LB-EPS and pellet fractions. On the other hand, different fractions had different MW characteristics based on their GPC chromatogram. Each chromatogram could be divided into three regions according to the characteristics of MW. Specifically, regions I, II, and III were differentiated by MW less than 10 kDa, between 10 and 1000 kDa, and more than 1000 kDa, respectively. Meanwhile, heterogeneous peaks existed in the three regions, and the strongest peak appeared in region II, which would be due to PN, PS or extracellular enzymes (Frølund et al., 1995). All the MW present in the slime, LB-EPS, and TB-EPS fractions were more than 10 kDa, and low molecular matters were not detected in the three fractions during anaerobic digestion. The areas of peaks in region III became small and even vanished (i.e., in the LB-EPS fraction) with digestion time. Compared with region III, the areas of peaks in region II decreased during the first 10 days, and then increased in the subsequent digestion process.

2.4 Characteristic of EEM spectra of DOM in EPS matrix and the sludge flocs

EEM spectra as an advanced monitoring tool was applied to quantitatively analyze DOM in many different fields, such as water quality and pollution monitoring in rivers (Reynolds and Ahmad, 1997), specific pollutants in industrial waste water (Kuzniz et al., 2007). In this study, typical EEM fluorescence spectra in LB-EPS fraction were adopted as examples (Fig. 5). Two major peaks (peaks A and peak B) were identified in the fluorescence EEM spectra. The first main peak (peak A) was located at the excitation/emission wavelengths ($\lambda_{Ex/Em}$) of (220–230)/(320–355) nm, while the second main peak (peak B) was observed at $\lambda_{Ex/Em}$ of (270–280)/(320–335) nm. The two peaks were reported as protein-like peaks (Henderson et al., 2009), in which the fluorescence was associated with the aromatic protein-like substances and tryptophan protein-like substances.

The fluorescence intensities of peaks in the different fractions are tabulated in Table 2. Although the maximal fluorescence intensities of peak A appeared at day 15, the changing trend of the fluorescence intensity in each fraction during the process was not obvious (Table 2). Peak B intensities, which were much stronger than peak A intensities except on day 20, decreased gradually from more than 5000 to less than 3000 during anaerobic digestion, suggesting that EPS was significantly consumed during the digestion. In addition, several researchers have reported the correlations between fluorescence intensity and biological oxygen demand (BOD), TOC and dissolved organic carbon (DOC) (Chen et al., 2003). We also investigated the correlations between fluorescence intensity and DOC in each fraction, and the Pearson's coefficient is shown in Table 3. No correlation was found between peak A intensities and DOC in each fraction during anaerobic digestion. However, the peak B intensities in slime, LB-EPS, TB-EPS fractions and the sludge flocs had strong positive correlations with DOC in corresponding fraction. Since DOC could denote the dissolved EPS, there may be a correlation between the peak intensities and sludge dewaterability during anaerobic digestion, which will be discussed below.

Table 3 Pearson's coefficients between the fluorescence intensities and DOC (n = 9)

Intensity	DOC				
	Slime	LB-EPS	TB-EPS	Pellet	Sludge flocs
Peak A	0.050	0.24	0.0086	0.010	0.089
Peak B	0.79**	0.68*	0.69*	0.41	0.64*

* $p < 0.05$ (2-tailed), ** $p < 0.01$ (2-tailed).

Table 2 Parameters of peaks A and B in EPS matrix and the sonicated sludge flocs during anaerobic digestion

Time (day)	Slime		LB-EPS		TB-EPS		Pellet		Sludge flocs	
	$\lambda_{Ex/Em}$	Intensity	$\lambda_{Ex/Em}$	Intensity	$\lambda_{Ex/Em}$	Intensity	$\lambda_{Ex/Em}$	Intensity	$\lambda_{Ex/Em}$	Intensity
Peak A										
1	228/330	2631	224/330	2467	228/335	1982	228/325	2008	228/330	2137
10	232/335	1564	224/305	938	228/330	1340	232/340	858.3	228/330	1207
20	228/330	9835	220/310	3089	228/335	8630	228/340	7762	228/335	7630
34	232/320	4687	232/345	1828	228/335	5838	232/345	3366	228/335	4628
47	232/355	1075	232/350	577.5	228/325	1481	228/325	1238	228/335	1415
Peak B										
1	284/330	7881	280/335	8008	284/335	9876	280/345	5419	224/335	8181
10	276/320	7713	272/310	3462	280/330	4477	288/380	1062	280/320	6159
20	280/330	6215	276/320	1640	280/330	6445	280/335	4566	280/335	6131
34	280/335	1468	284/325	690	280/330	2883	280/335	2293	280/330	3079
47	280/345	1732	284/350	758.5	280/330	2924	280/330	2194	280/335	2573

LB-EPS: loosely bound EPS; TB-EPS: tightly bound EPS.

2.5 Sludge dewaterability during anaerobic digestion

Normalized CST could be used to evaluate the dewaterability of activated sludge. In general, the improvement of sludge dewaterability could be expressed by the decrease of normalized CST (Yu et al., 2008b). Figure 6 depicts the variations of the normalized CST during anaerobic digestion. The normalized CST of the unsonicated sludge increased from the initial 2.3 to 51.4 (sec·L)/g TSS at

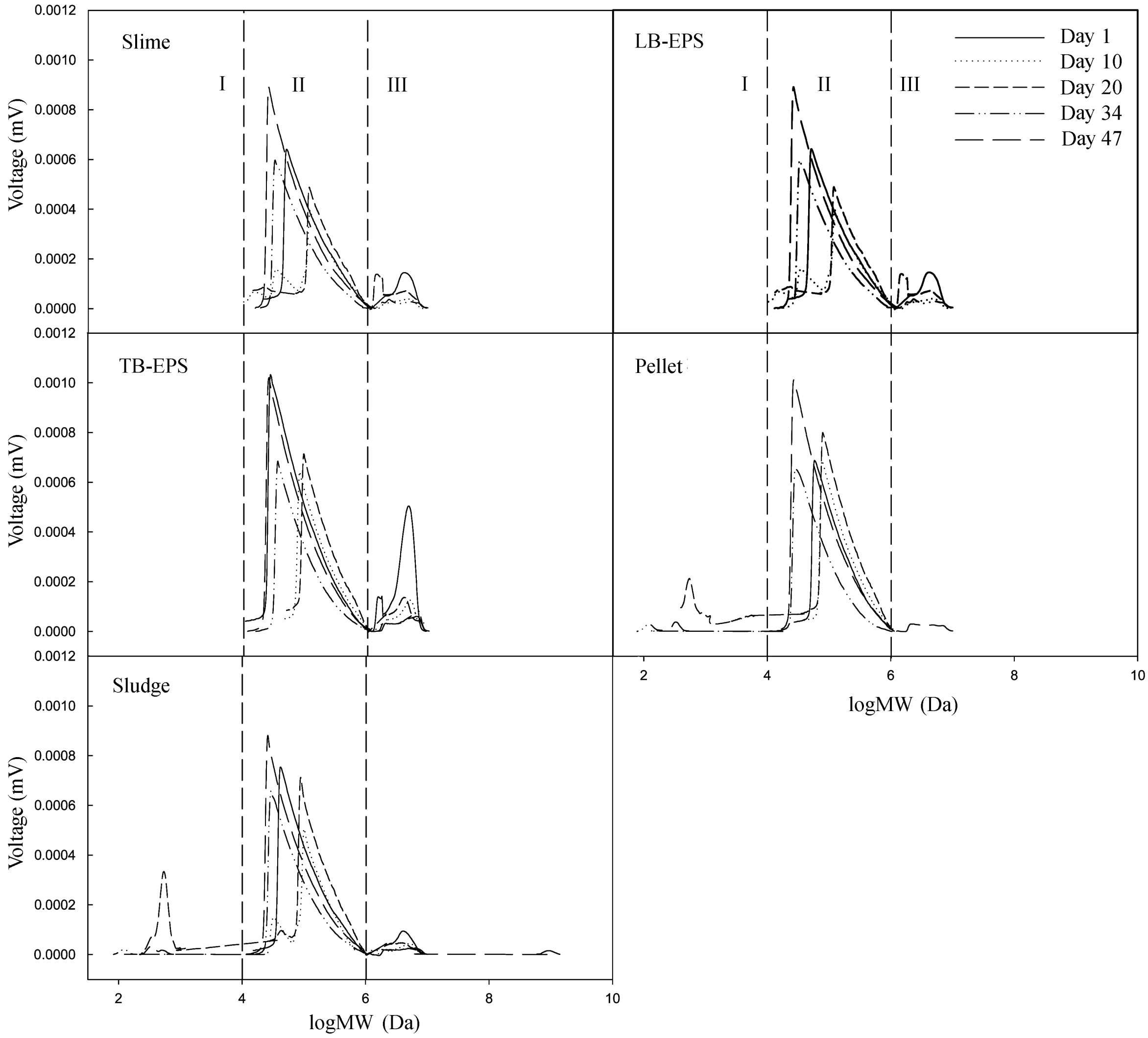

Fig. 4 Gel permeation chromatograms (GPC) of dissolved organic matters (DOM) in extracellular polymeric substances (EPS) matrix and the sonicated sludge flocs during anaerobic digestion.

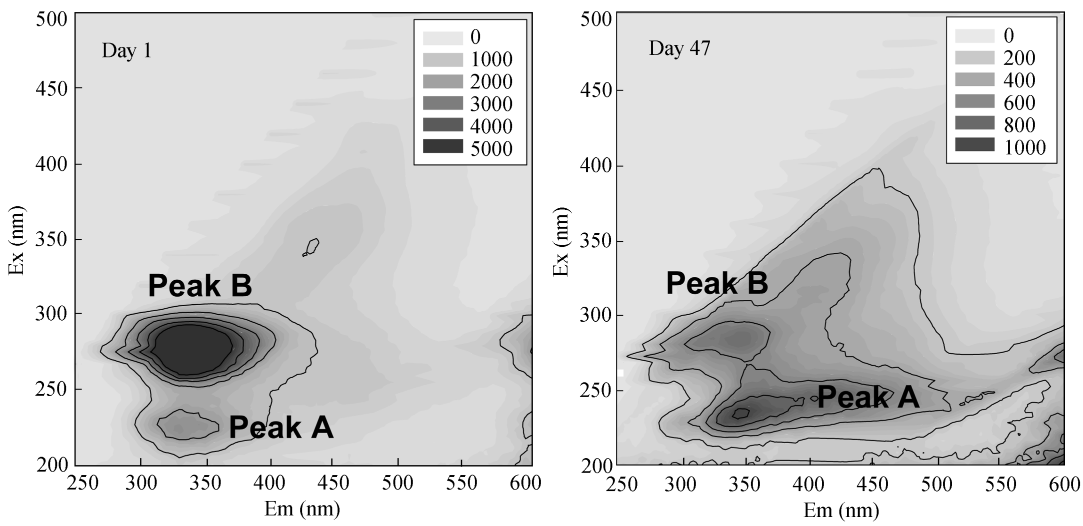

Fig. 5 Typical excitation-emission matrix (EEM) spectroscopy of loosely bound EPS (LB-EPS) fraction for the sonicated sludge.

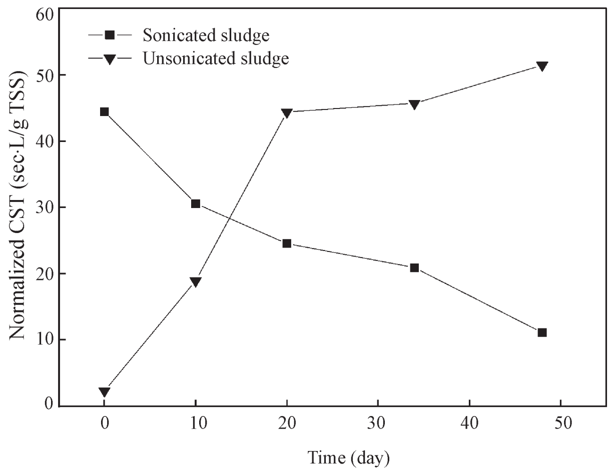

Fig. 6 Variations of the normalized CST during anaerobic digestion.

day 47, indicating that sludge dewaterability was deteriorated. Interestingly, for the sonicated sludge, the initial normalized CST increased sharply to 44.4 (sec·L)/g TSS, which was attributable to the break role of ultrasonic pretreatment (Feng et al., 2009). During anaerobic digestion, the normalized CST decreased gradually to 11.1 (sec·L)/g TSS at day 47, suggesting that sludge dewaterability was improved during anaerobic digestion after ultrasonic pretreatment. The reasons leading to the improvement of sludge dewaterability will be further discussed in following sections.

3 Discussion

3.1 Effects of PN and PS in EPS matrix and the sludge flocs on sludge dewaterability

PN and PS in EPS played a crucial role in sludge flocculation and dewaterability (Murthy and Novak, 1998; Wang et al., 2008; Shao et al., 2009). As listed in Table 4, the normalized CST of the sonicated sludge was correlated positively with PN and PN/PS in the LB-EPS fraction, and negatively with PN/PS in the pellet fraction. In addition, there was no correlation between the normalized CST and PS in other EPS fractions. It was evident that PN rather than PS in the LB-EPS fraction played an important role in sludge dewaterability during anaerobic digestion.

Table 4 Pearson's coefficients between each parameter and the normalized CST ($n = 9$)

	Sludge fractions				
	Slime	LB-EPS	TB-EPS	Pellet	Sludge
Index			PN		
CST	0.36	0.92**	0.12	0.17	0.018
Index			PS		
CST	0.014	0.18	0.13	0.02	0.01
Index			PN/PS		
CST	0.42	0.84**	0.17	0.58*	0.14
Index			Average MW		
CST	0.097	0.66**	0.90**	0.01	0.11
Index			Peak A		
CST	0.036	0.29	0.001	0.015	0.0071
Index			Peak B		
CST	0.67*	0.94**	0.61*	0.19	0.64*

* $p < 0.05$ (2-tailed), ** $p < 0.01$ (2-tailed).
CST: capillary suction time.

3.2 Correlation between MW distribution of DOM and sludge dewaterability

Yu et al. (2009) investigated the MW distribution of DOM in different EPS fractions on bioflocculability for kaolin solution, but the information about the influence of MW distribution on sludge dewaterabililty is still limited. Compared with the GPC chromatograms of EPS matrix and the sludge flocs (Fig. 4), the organic matters with small MW (< 10 kDa) only occurred in pellet and the sludge flocs. According to the extracellular enzymes distribution pattern (Yu et al., 2008a), it could be concluded that most of extracellular enzymes were embedded in the pellet, which contributed to the occurrence of small MW matters. MW declined gradually from outer fraction (i.e., slime) to inner fraction (i.e., pellet), and small MW in pellet was beneficial for the utilization of microorganism during anaerobic digestion. Otherwise, ultrasonic pretreatment could cause sludge disintegration and higher concentration of small MW EPS. In the subsequent anaerobic digestion, due to the EPS flocculation, the retention time of the peaks in region II became short, leading to the increase of MW during the first 20 days. While for region III, the MW of DOM in EPS matrix and the sludge flocs all decreased gradually during anaerobic digestion, suggesting that EPS with large MW were degraded by enzymes.

The Pearson's coefficients between the normalized CST and the average MW of DOM in EPS matrix and the sonicated sludge flocs are listed in Table 4. The normalized CST was correlated with average MW in LB-EPS (R^2 = 0.66, $p < 0.01$) and TB-EPS (R^2 = 0.90, $p < 0.01$), but there was no correlation with that in the slime, pellet and the sludge flocs. That is to say, the sludge dewaterability was only influenced by the average MW in LB-EPS and TB-EPS.

3.3 Correlations between fluorescence intensity of DOM and sludge dewaterability

As listed in Table 4, the normalized CST of the sonicated sludge was significantly correlated with peak B intensities in the slime (R^2 = 0.67, $p < 0.05$), LB-EPS (R^2 = 0.94, $p < 0.01$) and TB-EPS fractions (R^2 = 0.61, $p < 0.05$) during anaerobic digestion. Three-dimensional EEM fluorescence spectroscopy is a rapid, selective and sensitive technique. Its advantage is that information regarding the fluorescence characteristics can be acquired entirely (Sheng and Yu, 2006). Thus, peak B intensities in the slime, LB-EPS and TB-EPS fractions would be appropriate as indicators to evaluate the sludge dewaterability during anaerobic digestion.

4 Conclusions

Compared with the unsonicated sludge, the dewaterability of sludge pretreated by ultrasound was greatly improved during the mesophilic anaerobic digestion. PN in the LB-EPS fraction, PN/PS in the LB-EPS and pellet fractions, peak B fluorescence intensities and MW distribution in the slime, LB-EPS and TB-EPS fractions were all decreased during anaerobic digestion. Pearson's coefficients further showed that the normalized CST was correlated with PN and PN/PS in the LB-EPS fraction, with average MW of DOM in the LB-EPS and TB-EPS fractions, and with peak B fluorescence intensities in the slime, LB-EPS and TB-EPS fractions. Thus, the above-mentioned parameters in EPS matrix and the sludge flocs would be used to evaluate sludge dewaterability during anaerobic digestion. Ultrasonic pretreatment could change the distribution patterns of organic matters in EPS matrix and the sludge flocs, which played an important role in sludge dewaterability during the subsequent anaerobic digestion.

Acknowledgments

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