



Effect of ultrasonic pretreatment on anaerobic digestion and its sludge dewaterability

Huacheng Xu, Pinjing He*, Guanghui Yu, Liming Shao

State Key Laboratory of Pollution Control and Resource Reuse, Key Laboratory of Yangtze River Water Environment, College of Environmental Science and Engineering, Tongji University, Shanghai 200092, China. E-mail: chenghuaxu@163.com

Received 23 December 2010; revised 18 March 2011; accepted 22 March 2010

Abstract

To investigate the effect of ultrasonic pretreatment on anaerobic digestion and sludge dewaterability and further to probe into the influencing factors on sludge dewaterability, sludge flocs were stratified into four fractions: (1) slime; (2) loosely bound extracellular polymeric substances (LB-EPS); (3) tightly bound EPS (TB-EPS); and (4) EPS-free pellets. The results showed that ultrasonic pretreatment increased the anaerobic digestion efficiency by 7%–8%. Anaerobic digestion without ultrasonic pretreatment deteriorated the sludge dewaterability, with the capillary suction time (CST) increased from 1.42 to 47.3 (sec-L)/g-TSS. The application of ultrasonic pretreatment firstly deteriorated the sludge dewaterability (normalized CST increased to 44.4 (sec-L)/g-TSS), while subsequent anaerobic digestion offset this effect and ultimately decreased the normalized CST to 23.2 (sec-L)/g-TSS. The dewaterability of unsonicated sludge correlated with protein ($p = 0.003$) and polysaccharide ($p = 0.004$) concentrations in the slime fraction, while that of sonicated sludge correlated with protein concentrations in the slime and LB-EPS fractions ($p < 0.05$). Fluorescent excitation-emission matrix analysis showed that the fluorescence matters in the LB-EPS fraction significantly correlated with sludge dewaterability during anaerobic digestion.

Key words: anaerobic digestion; capillary suction time; extracellular polymeric substances; sludge dewaterability; ultrasonic pretreatment

DOI: 10.1016/S1001-0742(10)60618-3

Citation: Xu H C, He P J, Yu G H, Shao L M, 2011. Effect of ultrasonic pretreatment on anaerobic digestion and its sludge dewaterability. *Journal of Environmental Sciences*, 23(9): 1472–1478

Introduction

Increasing quantities of excess sludge have been produced in wastewater treatment plants (WWTPs), which generally accounts for 50%–60% of the total construction and operation costs of a WWTP (Appels et al., 2008; Wei et al., 2003). Sludge dewatering and anaerobic digestion are two widely used methods in recent years. Although anaerobic digestion had many advantages such as biomass stabilization and biogas production, it may adversely affect the sludge dewaterability (Dewil et al., 2006). Previous publications about anaerobic digestion were focused on the improvement of digestion performance and biogas production by physical means such as ultrasonic pretreatment. For example, Tiehm et al. (1997) demonstrated that ultrasonic pretreatment improved the sludge reduction from 45.8% to 50.3% during 22 days of anaerobic digestion. Mohammed et al. (2009) found that sludge reduction increased from 44% (control) to 66% (ultrasonic pretreatment) after 50 days of anaerobic digestion. And Onyeche et al. (2002) showed that the application of ultrasonic pretreatment

could significantly enhance the biogas production. However, the information about the dewaterability of digested sludge (especially after ultrasonic pretreatment) and the influencing factors of sludge dewaterability during anaerobic digestion is hardly mentioned.

Sludge dewaterability has been reported as correlating with many factors, such as particle size distribution (PSD), extracellular polymeric substances (EPS) concentrations, proteins and/or polysaccharides. PSD was first reported as being the key factor in controlling activated sludge dewaterability (Lawler et al., 1986); then the EPS concentrations were thought to play a major role (Houghton and Stephenson 2002; Kang et al., 1989), with the significant relationship ($p = 0.01$) between the sludge filtrability and the EPS concentrations. Recently, Yu et al. (2008a) and Shao et al. (2009) found EPS composition rather than EPS concentration affecting sludge dewaterability by applying a novel EPS fractionation approach. For example, Shao et al. (2009) reported that sludge dewaterability was negatively affected by soluble proteins ($R < -0.63$, $p < 0.01$) and soluble proteins/polysaccharides ($R < -0.67$, $p < 0.01$) of sludge flocs. However, the above results were

* Corresponding author. E-mail: solidwaste@tongji.edu.cn

jesc.ac.cn

both limited to activated sludge or anaerobically digested sludge ignoring ultrasonic pretreatment, thus it is of great necessary to investigate and compare the dewaterability of anaerobically digested sludge with and without ultrasonic pretreatment.

The aims of this study were: (1) to investigate the effect of ultrasonic pretreatment on anaerobic digestion and its sludge dewaterability, and (2) to probe into the influencing factors on sludge dewaterability during anaerobic digestion.

1 Materials and methods

1.1 Sludge sample

The activated sludge sample was collected from the aerated basin of a WWTP in Shanghai, China. The plant treats 75,000 m³/day of wastewater (93% from domestic and 7% from industrial origin) using anaerobic-anoxic-oxic process. The collected sludge was transported to the laboratory within 30 min after sampling and then settled for 1.5 hr at 4°C with supernatant decant. The sludge sediment was collected and subsequently screened through a 1.2-mm sieve to remove grit. The sludge had an initial pH of 8.0, total suspended solid (TSS) of (13.6 ± 0.3) g/L and volatile suspended solid (VSS) of (6.9 ± 0.1) g/L.

1.2 Ultrasonic pretreatment

The sludge sample was equally divided into two parts. One part was pretreated by ultrasound and denoted as sonicated sludge, while the other without pretreatment was named as unsonicated sludge. The ultrasonic reactor (FS-600, Shanghai Sonxi Co., Ltd., China) equipped with a transducer (diameter of 13 mm) was applied. The transducer was immersed 10 mm into 50 mL sludge samples, and the temperature was maintained at about 25°C via an ice water bath to prevent possible temperature effects. The detailed operating conditions of the ultrasonic pretreatment were described elsewhere (Yu et al., 2008b).

1.3 Anaerobic digestion

Two airtight reactors with the total volume of 4.0 L were applied, one fed with unsonicated sludge and the other with sonicated sludge. For each reactor, 3.0 L of the sludge sample was added and then inoculated with anaerobic sludge sampled from an upflow anaerobic sludge bed with the volume ratio of inoculum to feed 0.05. Oxygen in the reactors was removed by nitrogen gas (N₂) sparging for 2 min. The reactors were then sealed with rubber stoppers and placed in an incubator (SPX-250B, Shanghai, China) at (37 ± 1)°C. The digested liquor was well mixed using peristaltic pumps (YZ1515, LanGe, Hebei, China). The sludge pH was adjusted between 6.8 and 7.5 by 6 mol/L HCl and NaOH solutions during the whole anaerobic digestion.

1.4 Sludge structure and stratification protocol

Sludge stratification protocol was adopted according to the methods of Yu et al. (2008a, 2009). In brief, EPS

in sludge flocs is composed of soluble EPS (i.e., slime) and bound EPS. The latter exhibits a dynamic double-fractioned structure and can be classified as loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) based on the extraction methodology. After the EPS is extracted, the cells in the residue form a pellet. Hence, from the loosely bound surface to the core, the sludge floc possesses a multi-fractioned structure consisting of slime, LB-EPS, TB-EPS, and EPS-free pellet.

1.5 Analytical methods

Proteins were determined by the modified Lowry method using casein (Shanghai Sangon Biotechnology Co., Ltd., China) as the standard (Lowry et al., 1951). Polysaccharides were measured by Anthrone method with glucose as the standard (Gaudy, 1962). Sludge dewaterability was obtained with a capillary suction time (CST) instrument (Model 319, Triton, UK) equipped with an 18-mm diameter funnel and Whatman no. 17 chromatography-grade paper. To measure the dewaterability potential of sludge flocs, the CST values were normalized by dividing them by the initial TSS concentration and then expressed in units of seconds per liter per gram TSS (Yu et al., 2008a). The particle size of sludge flocs was determined by an EyeTech instrument (Ankersmid, USA) with a 300-mm lens which enables the particle measurement in the range of 0.1–1000 μm. Fluorescent excitation-emission matrix (EEM) spectra were collected with subsequent scanning emission (Em) spectra from 250 to 600 nm at 5 nm increments by varying the excitation (Ex) wavelength from 200 to 500 nm at 4 nm increments, the Ex and Em slits were maintained at 10 nm and the scanning speed was set at 1200 nm/min for all the measurements. The software SigmaPlot 9.0 was employed for handling the EEM data, and the statistical analysis was obtained by the software SPSS version 11.0 for Windows (SPSS, USA). Pearson's correlation coefficient (*R*) was used to evaluate the linear correlation between two parameters. The correlation was considered statistically significant at a 95% confidence interval (*p* < 0.05).

2 Results and discussion

2.1 Anaerobic digestion performance with and without ultrasonic pretreatment

TSS and VSS were usually applied as sludge digestion indicators (Yu et al., 2008b). The reduction ratios of TSS and VSS for sonicated sludge were 52.6% and 55.2%, and those for unsonicated sludge were 45.4% and 47.9% respectively, indicating that ultrasonic pretreatment could obviously enhance anaerobic digestion performance. In addition, compared with unsonicated sludge, more biogas was produced for sonicated sludge during the process (data not shown).

The results of previous studies about ultrasonic pretreatment followed by anaerobic digestion were similar to this study. Tiehm et al. (1997) demonstrated that the VSS removal of ultrasonic sludge (3.6 kW, 31 kHz, 96 sec)

could reach to 50.3% after 22-day anaerobic digestion, comparing with the control of 45.8%. Mohammed et al. (2009) found that the TSS removal of ultrasonic sludge (20 kHz, 31,500 J/g-TSS) increased from 44% to 66% after 50-day anaerobic digestion. And Bougrier et al. (2005) reported that ultrasonic pretreatment could enhance 25% of biogas production. The disruption of cell wall caused by ultrasonic pretreatment led to the release of intracellular materials, which improved the digestion performance and biogas production (Chu et al., 2002).

2.2 Variations of sludge dewaterability during anaerobic digestion

Although many investigators had shown that ultrasound could improve anaerobic digestion performance, information on the sludge dewaterability during anaerobic digestion with and without ultrasonic pretreatment has not been reported. Figure 1 shows the variations of sludge dewaterability during anaerobic digestion. The normalized CST for unsonicated sludge increased from the initial 1.42 to 18.9 (sec-L)/g-TSS on day 8 and approached the plateau value till day 14; afterwards, it increased up to 47.3 (sec-L)/g-TSS at the end of digestion. This phenomenon showed that the dewaterability of the unsonicated sludge was deteriorated during anaerobic digestion, which would be due to the destruction of sludge flocs and the release of cations/biopolymers during anaerobic digestion (Na et al., 2007; Wilen et al., 2000).

When applying ultrasonic pretreatment, the normalized CST immediately increased to 44.4 (sec-L)/g-TSS, showing a detrimental effect of ultrasonic pretreatment on sludge dewaterability. Interestingly, it gradually decreased to 23.2 (sec-L)/g-TSS after 15 days of digestion and approached the plateau value till the end of test, suggesting that the subsequent anaerobic digestion had a positive effect on sludge dewaterability. The results demonstrated that sludge dewaterability with and without ultrasonic pretreatment exhibited different variations during anaerobic digestion: it was improved for sonicated sludge but deteriorated for unsonicated sludge.

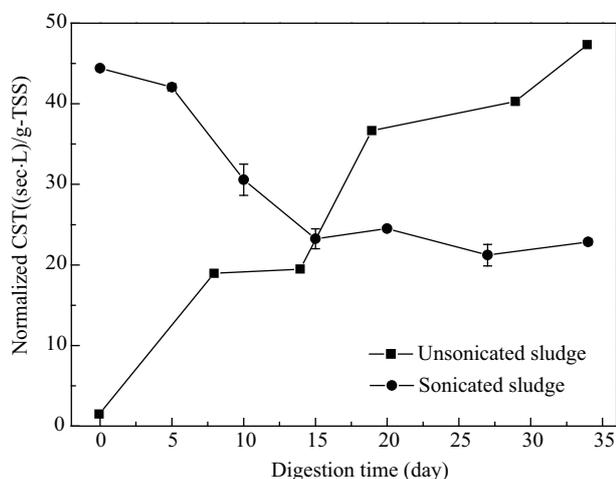


Fig. 1 Variations of sludge dewaterability during anaerobic digestion.

2.3 Variations of organic matters in different fractions during anaerobic digestion

Proteins and polysaccharides were the main EPS compositions and significantly influenced the sludge dewaterability (Dignac et al., 1998). Thus, the variations of proteins and polysaccharides during anaerobic digestion were investigated in this study.

As shown in Fig. 2, organic matters both for sonicated and unsonicated sludge were mainly distributed in the tightly bound fractions (TB-EPS and EPS-free pellet), less in the loosely bound fractions (slime and LB-EPS). Specifically, for unsonicated sludge, 87.8% of proteins and 90.4% of polysaccharides were distributed in the tightly bound fractions, while only 62.1% of proteins and 73.9% of polysaccharides were detected in these fractions for sonicated sludge. The distribution patterns of organic matters in different fractions indicated that ultrasonic pretreatment could cause organic matters transform from the tightly bound fractions to the loosely bound fractions.

The initial concentrations of protein and polysaccharide in the loosely bound fractions for the unsonicated sludge (Fig. 2a and c) were 121 and 17 mg/g-VSS, respectively, which both increased linearly during the whole digestion process. While the protein concentrations in the loosely bound fractions for sonicated sludge (Fig. 2b) decreased from the initial 290 to 161 mg/g-VSS during the digestion, and the polysaccharide concentrations (Fig. 2d) did not show noticeable change. The variations of organic matters in the loosely bound fractions were apparently different between the sonicated and unsonicated sludge during anaerobic digestion.

Sludge dewaterability was previously reported to be influenced by EPS properties. Specifically, Novak et al. (2003) found that the dewaterability of digested sludge was directly affected by the amount of biopolymers released into the solution. Mikkelsen and Keiding (2002) noted that sludge dewaterability was improved by increasing the EPS concentrations, especially the protein concentrations. In addition, Li and Yang (2007) reported that the loosely bound parts of sludge flocs significantly influenced the sludge dewaterability. However, owing to the complicated EPS matrix, these conclusions did not identify which parts and/or which compositions played a more significant role in sludge dewaterability. By applying the novel stratification protocol of sludge flocs, this study attempted to investigate the specific composition of sludge flocs on sludge dewaterability. Pearson's correlation coefficients are summarized in Table 1. For unsonicated sludge, the normalized CST positively correlated with protein ($p = 0.003$) and polysaccharide ($p = 0.004$) concentrations in the slime fraction, and there was no correlation ($p > 0.06$) with protein or polysaccharide concentrations in other fractions. While for sonicated sludge, the normalized CST only positively correlated with protein concentrations ($p < 0.05$) in the loosely bound fractions. Combined with Fig. 1, Fig. 2 and Table 1, the dewaterability improvement of anaerobically digested sludge would be attributed to the reduction of organic matters (mainly proteins) in the

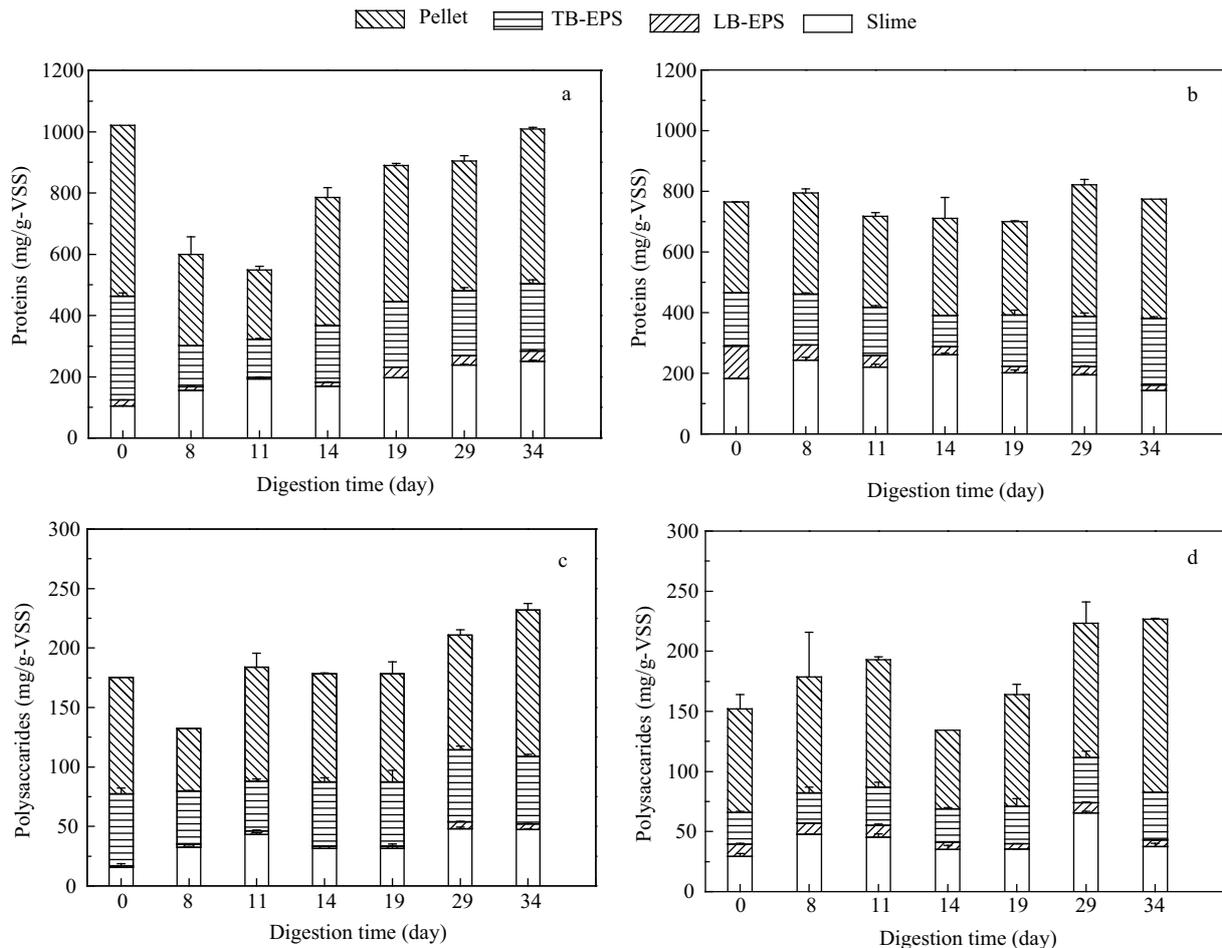


Fig. 2 Evolutions of organic matters during anaerobic digestion. (a) proteins for unsonicated sludge; (b) proteins for sonicated sludge; (c) polysaccharides for unsonicated sludge; (d) polysaccharides for sonicated sludge.

Table 1 Pearson correlation coefficients between the normalized CST and proteins/ polysaccharides

Parameter	Fractions of sludge flocs	Unsonicated sludge		Sonicated sludge	
		<i>R</i>	<i>p</i>	<i>R</i>	<i>p</i>
Proteins	Slime	0.928	0.003	0.875	0.046
	LB-EPS	0.311	0.497	0.877	0.002
	TB-EPS	-0.496	0.258	0.501	0.169
	Pellet	-0.319	0.486	-0.436	0.201
Polysaccharides	Slime	0.912	0.004	0.080	0.837
	LB-EPS	0.728	0.064	0.325	0.394
	TB-EPS	-0.206	0.658	0.130	0.740
	Pellet	0.437	0.326	0.297	0.438

loosely bound fractions.

2.4 Particle size distribution (PSD) of sludge during anaerobic digestion

Figure 3 presents the variations of PSD for sonicated and unsonicated sludge during the digestion. The mean particle size of the unsonicated sludge gradually decreased from the initial 124.5 to 41.2 μm after 34 days of digestion (Fig. 3a). The results were quite consistent with those of Mahmoud et al. (2006) who reported that anaerobic digestion led the transformation of bigger flocs into smaller ones.

Ultrasonic pretreatment significantly disintegrated the sludge flocs, with the mean particle size decreased from

124.5 to 7.5 μm . Interestingly, before decreasing to 3.6 μm at the end of digestion, the mean particle size firstly increased and reached to 23.8 μm on day 15, indicating the re-flocculation of sludge particles during the initial anaerobic digestion. The occurrence of re-flocculation would be attributed to the release of intracellular polymers (Biggs and Lant, 2000; Gonze et al., 2003).

The analysis of Pearson's correlation demonstrated that the normalized CST correlated with the mean particle size for the unsonicated sludge ($p = 0.029$), but there was no correlation ($p > 0.6$) for the sonicated sludge. Lawler et al. (1986) reported that the dewaterability of digested sludge was highly correlated with the particle size. The results obtained in this study clearly showed that the conclusion of Lawler et al. (1986) may be appropriate only for the digested sludge without ultrasonic pretreatment.

2.5 EEM spectra of EPS during anaerobic digestion

EEM spectrum was applied in this study to investigate the EPS fluorescence property during anaerobic digestion. Based on the results of Sections 2.3 and 2.4 and to further probe into the correlations of EEM spectra and sludge dewaterability, EEM spectra of the slime and LB-EPS fractions for the sonicated sludge are chosen and depicted in Figs. 4–5. Two main protein-like peaks could be identified in the EEM spectra. The first main peak (Peak

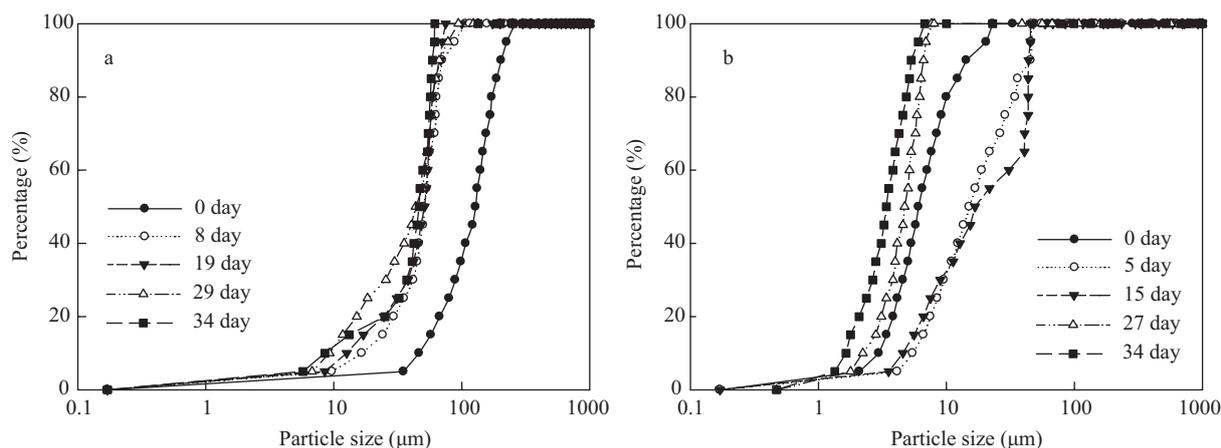


Fig. 3 Variations of PSD during anaerobic digestion. (a) unsonicated sludge; (b) sonicated sludge.

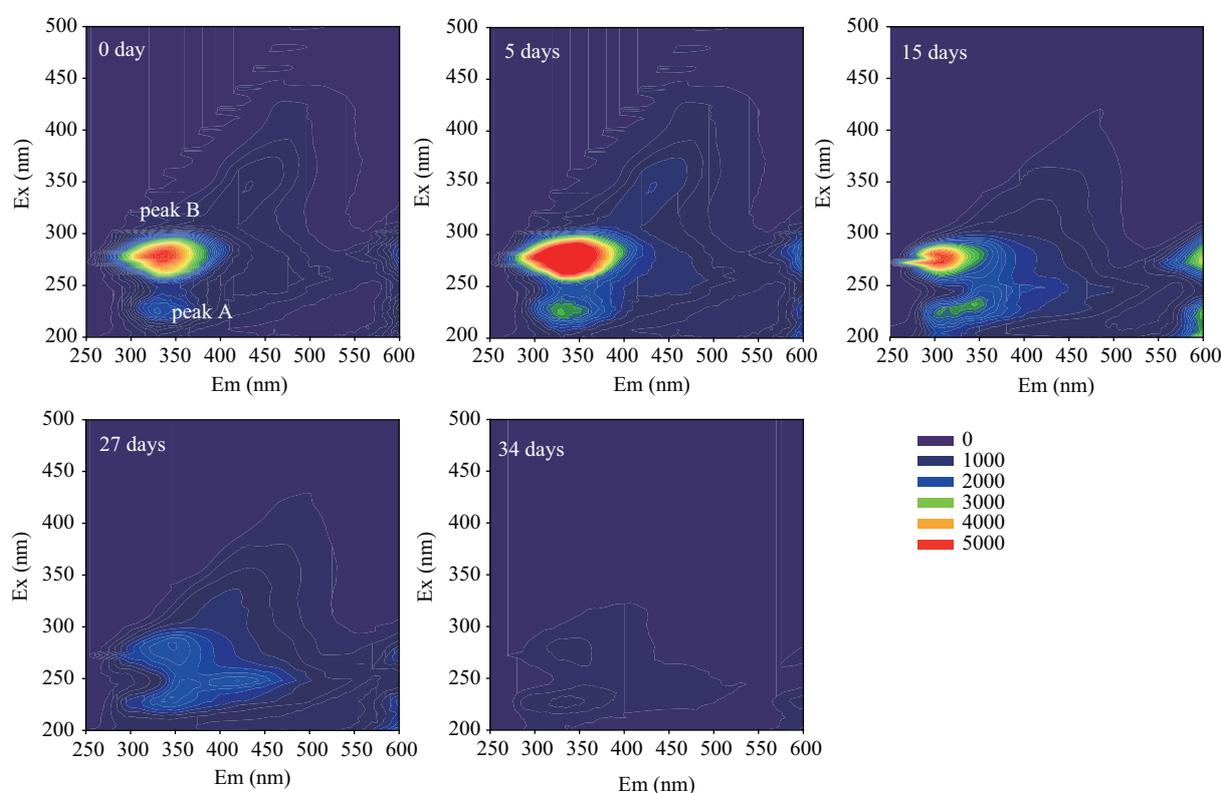


Fig. 4 Fluorescence EEM of the slime fraction for sonicated sludge.

A) was located at Ex/Em wavelength of 224–236/320–350 nm, while the second main peak (Peak B) was observed at Ex/Em of 272–288/320–350 nm. Meanwhile, peak intensities in Fig. 4 were generally higher than those in Fig. 5, indicating that the slime fraction had higher protein concentrations than the LB-EPS fraction, which was consistent with the results of Fig. 2. The peak intensities of the slime fraction slightly increased and peaked on day 15 and then gradually decreased along with the time (Fig. 4), while those of the LB-EPS fraction continuously decreased and reached the minimum at the end of digestion (Fig. 5). The variations of peak intensities were also consistent with the concentration changes of organic matters (Fig. 2).

In the past few years, EEM was widely used to characterize the organic matters in sludge EPS, river water and landfill leachate, which at low concentration was directly proportional to fluorescence intensity (Baker, 2002; Chen

et al., 2003). However, to our knowledge, not much data exist about the application of EEM to evaluate the dewaterability of digested sludge. The analysis of Pearson's correlation in this study showed that the normalized CST correlated with the fluorescence intensity of the LB-EPS fraction ($p < 0.05$). That is to say, the fluorescent matters in the LB-EPS fraction were primary contributors to sludge dewaterability, while those in the slime fraction contributed less.

Sludge dewaterability was highly correlated with the organic matters in EPS matrix, while the chemical analysis of organic matters was time consuming and cumbersome (Lowry et al., 1951; Gaudy 1962), thus it was expected to develop a more convenient method to characterize the dewaterability. As fluorescence EEM is a reagentless technique (Henderson et al., 2009), it may be applied as a potential monitoring tool for dewaterability evaluation

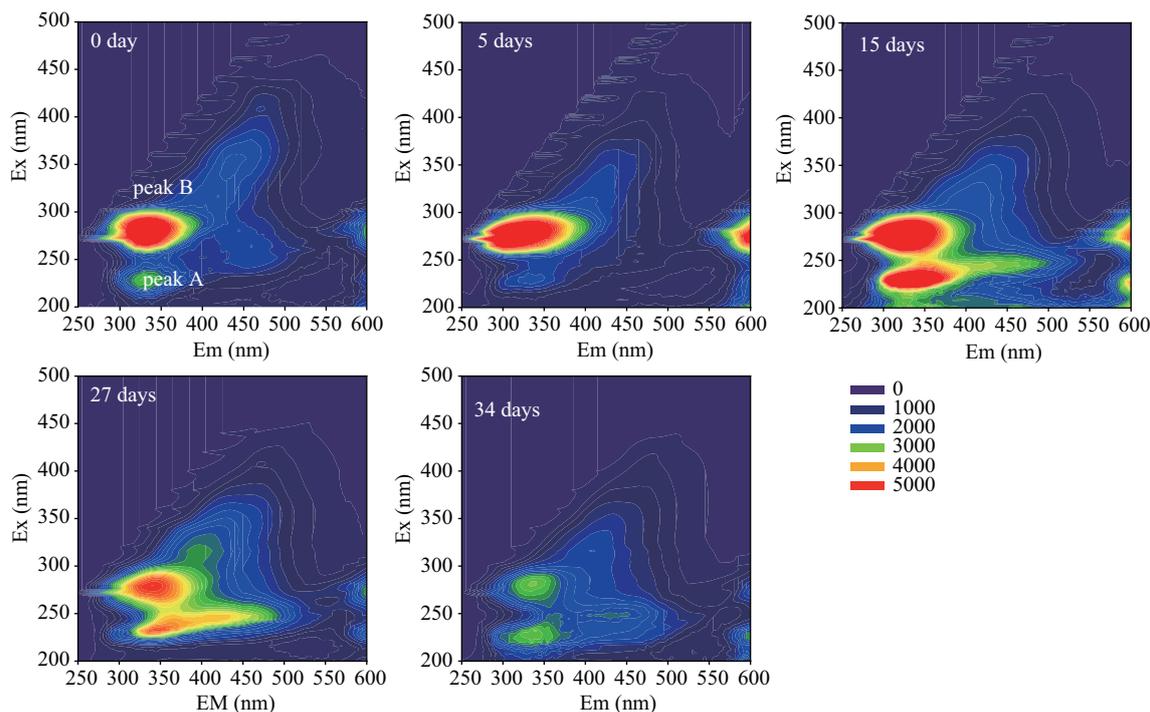


Fig. 5 Fluorescence EEM of the LB-EPS fraction for sonicated sludge.

during anaerobic digestion. The eventual goal of future studies will be to obtain accurate information by approaches such as fluorescence EEM combined with parallel factor (PARAFAC) analysis to elucidate in detail the possibility.

3 Conclusions

Ultrasonic pretreatment improved the digestion performance and biogas production. The dewaterability of anaerobic sludge without ultrasonic pretreatment was continuously deteriorated during the whole process. Ultrasonic pretreatment firstly deteriorated the sludge dewaterability, whereas subsequent anaerobic digestion offset this effect and ultimately improved it. Therefore, ultrasonic pretreatment followed by anaerobic digestion was a suitable sludge treatment route for simultaneously improvement of digestion performance and sludge dewaterability. The dewaterability of unsonicated sludge correlated with protein and polysaccharide concentrations in the slime fraction, while those of sonicated sludge correlated with protein concentrations in slime and LB-EPS fractions. EEM approach may be applied as a potential monitoring tool for rapidly evaluating sludge dewaterability during anaerobic digestion.

Acknowledgments

This work was supported by the National Key Project for Water Pollution Control (No. 2008ZX07316-003) and the Program of Shanghai Subject Chief Scientist (No. 10XD1404200).

References

Appels L, Baeyens J, Degreve J, Dewil R, 2008. Principles and potential of the anaerobic digestion of waste-activated

sludge. *Progress in Energy and Combustion Science*, 34(6): 755–781.

Baker A, 2002. Fluorescence excitation-emission matrix characterization of river waters impacted by a tissue mill effluent. *Environmental Science & Technology*, 36(7): 1377–1382.

Biggs C A, Lant P A, 2000. Activated sludge flocculation: on-line determination of floc size and the effect of shear. *Water Research*, 34(9): 2542–2550.

Bougrier C, Carrere H, Delgenes J P, 2005. Solubilisation of waste activated sludge by ultrasonic treatment. *Chemical Engineering Journal*, 106(2): 163–169.

Chen W, Westerhoff P, Leenheer J A, Booksh K, 2003. Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. *Environmental Science & Technology*, 37(24): 5701–5710.

Chu C P, Lee D J, Chang B V, You C S, Tay J H, 2002. “Weak” ultrasonic pre-treatment on anaerobic digestion of flocculated activated biosolids. *Water Research*, 36(1): 2681–2688.

Dewil R, Baeyens J, Goutvrind R, 2006. Ultrasonic treatment of waste activated sludge. *Environmental Progress*, 25(2): 121–128.

Dignac M F, Urbain V, Rybacki D, Bruchet A, Snidaro D, Scribe P, 1998. Chemical description of extracellular polymers: implication on activated sludge floc structure. *Water Science & Technology*, 38(8-9): 45–53.

Gaudy A F, 1962. Colorimetric determination of protein and carbohydrate. *Industrial Water Wastes*, 7: 17–22.

Gonze E, Pillot S, Valette E, Gonthier Y, Bernis A, 2003. Ultrasonic treatment of an aerobic activated sludge in a batch reactor. *Chemical Engineering and Processing*, 42(12): 965–975.

Henderson R K, Baker A, Murphy K R, Hambly A, Stuetz R M, Khan S J, 2009. Fluorescence as a potential monitoring tool for recycled water systems: a review. *Water Research*, 43(4): 863–881.

Houghton J I, Stephenson T, 2002. Effect of influent organic content on digested sludge extracellular polymer content

- and dewaterability. *Water Research*, 36(14): 3620–3628.
- Kang S, Kishimoto M, Shioya S, Yoshida T, Suga K, Taguchi H, 1989. Dewatering characteristics of activated sludge and effect of extracellular polymer. *Journal of Fermentation and Bioengineering*, 68(2): 117–122.
- Lawler D F, Chung Y J, Hwang S J, Hull B A, 1986. Anaerobic digestion: effects on particle size and dewaterability. *Journal of Water Pollution Control Federation*, 58: 1107–1117.
- Li X Y, Yang S F, 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research*, 41(5): 1022–1030.
- Lowry O H, Rosebrough N J, Farn A, Randall R, 1951. Protein measurement with the folin phenol reagent. *Journal of Biological Chemistry*, 193(1): 265–275.
- Mahmoud N, Zeeman G, Gijzen H, Lettinga G, 2006. Interaction between digestion conditions and sludge physical characteristics and behaviour for anaerobically digested primary sludge. *Biochemical Engineering Journal*, 28(2): 196–200.
- Mikkelsen L H, Keiding K, 2002. Physico-chemical characteristics of full scale sewage sludges with implication to dewatering. *Water Research*, 36(10): 2451–2462.
- Mohammed R S, Audrey P, Magali C, Christophe D, 2009. Pre-treatment of ctivated sludge: Effect of sonication on aerobic and anaerobic digestibility. *Chemical Engineering Journal*, 148(2-3): 327–335.
- Na S, Kim Y U, Khim J, 2007. Physiochemical properties of digested sewage sludge with ultrasonic treatment. *Ultrasonics Sonochemistry*, 14(3): 281–285.
- Novak J T, Sadler M E, Murthy S N, 2003. Mechanisms of floc destruction during anaerobic and aerobic digestion and the effect on conditioning and dewatering of biosolids. *Water Research*, 37(13): 3136–3144.
- Onyeche T I, Schlafer O, Bormann H, Schroder C, Sievers M, 2002. Ultrasonic cell disruption of stabilised sludge with subsequent anaerobic digestion. *Ultrasonics*, 40(1-8): 31–35.
- Shao L M, He P P, Yu G H, He P J, 2009. Effect of proteins, polysaccharides, and particle sizes on sludge dewaterability. *Journal of Environmentl Science*, 21(1): 83–88.
- Tiehm A, Nickel K, Neis U, 1997. The use of ultrasound to accelerate the anaerobic digestion of sewage sludge. *Water Science & Technology*, 36(11): 121–128.
- Wei Y S, Houten R T, Borger A R, Eikelboom D H, Fan Y B, 2003. Minimization of excess sludge production for biological wastewater treatment. *Water Research*, 37(18): 4453–4467.
- Wilen B M, Keiding K, Nielsen P H, 2000. Anaerobic deflocculation and aerobic reflocculation of activated sludge. *Water Research*, 34(16): 3933–3942.
- Yu G H, He P J, Shao L M, He P P, 2008a. Stratification structure of sludge flocs with implications to dewaterability. *Environmental Science & Technology*, 42(21): 7944–7949.
- Yu G H, He P J, Shao L M, Zhu Y S, 2008b. Extracellular proteins, polysaccharides and enzymes impact on sludge aerobic digestion after ultrasound pretreatment. *Water Research*, 42(8-9): 1925–1934.
- Yu G H, He P J, Shao L M, 2009. Characteristics of extracellular polymeric substances (EPS) fractions from excess sludges and their effects on bioflocculability. *Bioresource Technology*, 100(13): 3193–3198.