Extracellular polymeric substances and dewaterability of waste activated sludge during anaerobic digestion

Fenxia Ye, Xinwen Liu and Ying Li

ABSTRACT

Anaerobic digestion of waste activated sludge was conducted to gain insight into the mechanisms underlying change in sludge dewaterability during its anaerobic digestion. Unexpectedly, the results indicated that sludge dewatering properties measured by capillary suction time only deteriorated after 10 days of anaerobic digestion, after which dewaterability recovered and remained stable. The loosely bound extracellular polymeric substance (LB-EPS) content increased three-fold after 20 days of anaerobic digestion, and did not change significantly during the remaining 30 days. The tightly bound EPS (TB-EPS) content reduced slightly after 20 days of anaerobic digestion, and stabilized during the last 30 days. Polysaccharides (PS) and proteins (PN) content in LB-EPS increased after 10 days of anaerobic digestion. However, PS and PN contents in TB-EPS decreased slightly. The relationship analysis showed that only LB-EPS correlated with dewaterability of the sludge during anaerobic digestion.

Key words | anaerobic digestion, capillary suction time (CST), dewaterability, extracellular polymeric substances (EPS), waste activated sludge

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INTRODUCTION

In recent years, as the wastewater treatment industry has developed in China, the problem of waste activated sludge (WAS) has been introduced. In 2009, around 32 million tons of wet sludge was generated in China and the sludge yield is increasing by 10% per year. WAS must be treated and disposed of properly and may account for up to 30–60% of total plant operating costs. The ultimate disposal of WAS has been, and continues to be, one of the most serious problems faced by wastewater utilities.

Anaerobic digestion is a widely accepted process for reducing sludge volume and inactivating pathogens that cause odor and biogas production. However, this process alters the structural properties of the sludge drastically, resulting in higher resistance to solid/liquid separation. Significant differences can be observed between activated and digested sludge for dewatering characteristics including filterability, settleability and conditioner requirements. Previous researchers have reported that anaerobic digestion leads to poorer dewatering properties (Bruss *et al.* 1993; Novak *et al.* 2003). Treatment of digested sludge, especially conditioning and dewatering, are costly operations in treatment plants.

Although it is known that digestion makes dewatering less effective, the reasons for dewatering deterioration following anaerobic digestion remain unclear. Bruss *et al.* (1993) attribute the poorer dewaterability to the reduction of iron and the release of discrete bacteria into solution. Poxon & Darby (1997) studied the relationship between extracellular polyanions (largely polysaccharides) and dewaterability, concluding that colloidal polyanions were not a major contributor to the poor dewaterability of digested sludge. Murthy *et al.* (1999) found that biopolymers were released from activated sludge floc into solution during aerobic and anaerobic digestion, and these biopolymers would cause deterioration of sludge dewatering properties.

Extracellular polymeric substances (EPS) are the major component of the activated sludge flocs. The gel structure of EPS leads to fairly tenacious retention of water within the sludge. This limits the solids concentrations that can be achieved in dewatering, producing adverse effects on transportation, landfilling, and incineration costs. However, the precise role of EPS usually appeared ambiguous. An increase in the level of EPS present causes the sludge to become more difficult to dewater (Mikkelsen & Keiding 2002). The deterioration of sludge properties is usually accompanied by the release of the biopolymer from the floc (Murthy *et al.* 2000). However, there are findings that

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capillary suction time (CST) decreased as the amount of EPS increased (Jin *et al.* 2004), and no simple relationship between the quantity of EPS present and the sludge dewaterability was observed (Poxon & Darby 1997). A related problem is that the EPS apparently leads to significant increases in the amount of chemical conditioner required (Novak *et al.* 2003). There are some other concerns with EPS's ability to increase viscosity, thus leading to higher pumping costs.

The aims of this study are to investigate the EPS during anaerobic digestion of sludge, to determine if the EPS content including its chemical components might be the underlying cause for the deterioration of dewaterability, and to lead to a better understanding of the changes that occur in the sludge during anaerobic digestion. An enhanced fundamental understanding of the change of EPS in sludge during anaerobic digestion could have many practical benefits, such as better methods for the selection of conditioning chemicals, reduction of conditioning chemical costs, improved operational practices for dewatering processes, and modifications to the processes that favorably alter sludge dewaterability.

MATERIALS AND METHODS

WAS samples

The WAS samples were collected from a concentrated tank of a municipal wastewater treatment plant in Ningbo, China. The sludge was not conditioned with any conditioner.

Sludge anaerobic digestion

Anaerobic digestion of the sludge samples (5 L) was carried out using an airtight vessel with a working volume of 8 L. Oxygen in the vessel was removed from the headspace by purging with N_2 for 2 min. The vessel was sealed with a rubber stopper. The sludge sample of 100 mL was collected for analysis every 5 days. The CST, the loosely bound EPS (LB-EPS) and the tightly bound EPS (TB-EPS) contents and chemical constituents of the treated sludge were investigated.

Sludge filterability and dewaterability test

Both CST and specific resistance to filtration (SRF) have been widely used to determine dewaterability of the activated sludge. SRF and the normalized CST were correlated strongly (Ge *et al.* 2011). The CST test was chosen due to the simple equipment and procedure of measurement. However, the normalized CST is feasible because the solids concentration should be taken into account during CST measurement. The CST was measured by a CST instrument (Triton, Model 304M, Essex, UK) using method 2710G of *Standard Methods* (APHA 1998) with a CST paper purchased from Triton Electronics Ltd. The CST for distilled water was stable at 11 s. The test was made in triplicate with a relative standard deviation of 5%.

Extraction of EPS and determination of components of EPS

The sludge sample was fully mixed prior to commencing the extraction procedure. EPS extraction was carried out using a sonication/thermal extraction process according to Li & Yang (2007).

Analytical methods

The suspended solids (SS) content was determined according to *Standard Methods* (APHA 1998). The LB-EPS and TB-EPS extractions were analyzed for total organic carbon (TOC), protein (PN) and polysaccharide (PS). TOC was measured by a TOC analyzer (Elementar, Liqui TOC/TNb, Elementar Analysensysteme GmbH, Hanau, Germany), and expressed as milligrams of TOC per gram of SS. Proteins were determined from an adaptation of the Lowry method (Frølund *et al.* 1995) using casein (Shanghai Sangon Biotechnology Co., Ltd, Shanghai, China) as the standard. PS were determined using the anthrone method with a glucose standard. All samples were made in triplicate.

Statistical analysis

Statistical analysis was carried out with SPSS software version 11.0 for Windows (SPSS, Chicago, IL, USA). Correlations were considered statistically significant at a confidence interval ($p < 0.05$).

RESULTS AND DISCUSSION

Variation of the dewaterability of the sludge during anaerobic digestion

Total suspended solids (TSS) and volatile suspended solids (VSS) were generally employed as sludge digestion indicators. The TSS and VSS contents decreased from the initial 13.16 and 8.52 g/L to 7.05 and 3.89 g/L at 30 days of digestion, respectively (Figure 1). The reduction ratios of TSS and VSS were 46.3 and 54.3%, respectively. Anaerobic conditions solubilized the particulate component of the WAS into soluble form, resulting in a reduction of sludge volume. The reductions of TSS and VSS contents over time indicated the effect of anaerobic digestion on sludge solid reduction and stabilization. However, after 30 days, the TSS and VSS contents did not significantly change.

Changes in dewatering properties for WAS undergoing anaerobic digestion are shown in Figure 2. The initial normalized CST value was approximately 3.88 s·L/g SS. The sludge filtering dewaterability deteriorated after anaerobic digestion. The normalized CST value of the sludge gradually increased and remained relatively constant after 30 days of anaerobic digestion. At the end of 50 days of anaerobic digestion, the anaerobically digested sludge had a normalized CST of 6.23 s·L/g SS. These observations suggested that the sludge might undergo acidogenesis followed by methanogenic digestion. Dewatering is expected to be difficult for the acidogenic sludge and less difficult for the stabilized sludge after thorough digestion. The results were consistent with the findings by Xu *et al.* (2011) who also reported that the normalized CST for unsonicated sludge increased after anaerobic digestion, suggesting that anaerobic digestion had a negative effect on sludge dewaterability.

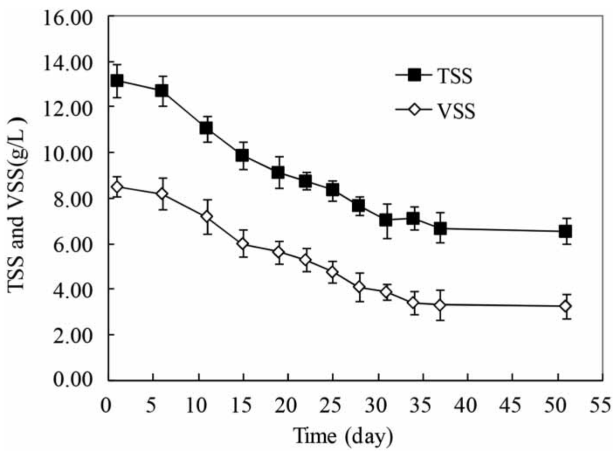

Figure 1 | Changes in TSS and VSS of WAS during anaerobic digestion.

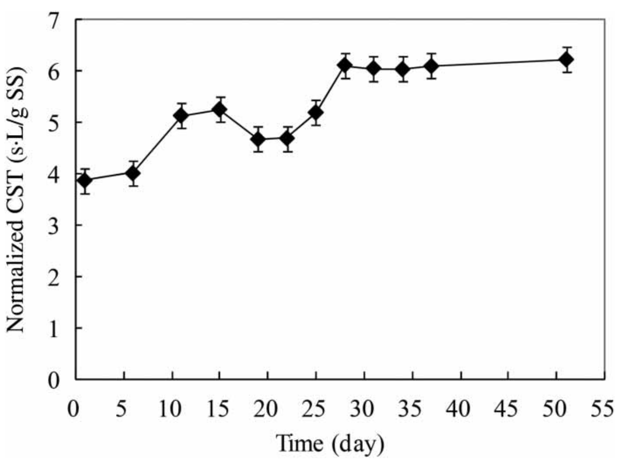

Figure 2 | Changes of normalized CST of WAS during anaerobic digestion.

Variation of the EPS content and components of the sludge during anaerobic digestion

Prior researches have shown that biopolymers are released from the sludge flocs into solution during anaerobic digestion and these biopolymers will cause deterioration of sludge dewatering properties (Bruss *et al.* 1993; Novak *et al.* 2003). However, the change of biopolymer in the sludge during anaerobic digestion has not been evaluated. The goal of this study was to investigate the change of EPS content and its chemical constituents in the sludge during anaerobic digestion and to determine the impact these materials have on dewaterability of the sludge.

The LB-EPS content increased significantly after the sludge was digested anaerobically. The LB-EPS content increased about 3.3 times at 20 days of anaerobic digestion (Figure 3(a)), and did not significantly change afterwards. However, the TB-EPS content decreased over the first 10 days of anaerobic digestion, then increased and finally stabilized (Figure 3(b)). Novak *et al.* (1999) also found that the amount of EPS increased after anaerobic digestion. When the sludge was exposed to undesirable anaerobic conditions, the sludge flocs were broken and cells destroyed, causing intercellular materials to be released. The TB-EPS possibly transformed from a tightly bound layer of the floc to a loosely bound layer. As a result, the content of LB-EPS increased greatly with a reduction of TB-EPS over the first 20 days of anaerobic digestion. However, the reason that TB-EPS content did increase after 20 days of anaerobic digestion remains unclear. It may be due to the pellet of sludge disintegrating after further digestion. The results suggested that solubilization of EPS from the sludge flocs was not complete when the sludge underwent anaerobic digestion, and EPS may not be the only or main parameter in understanding the dewatering behavior.

In Figure 4, the changes of PS and PN in LB-EPS and TB-EPS of the sludge are shown. Both PS and PN were

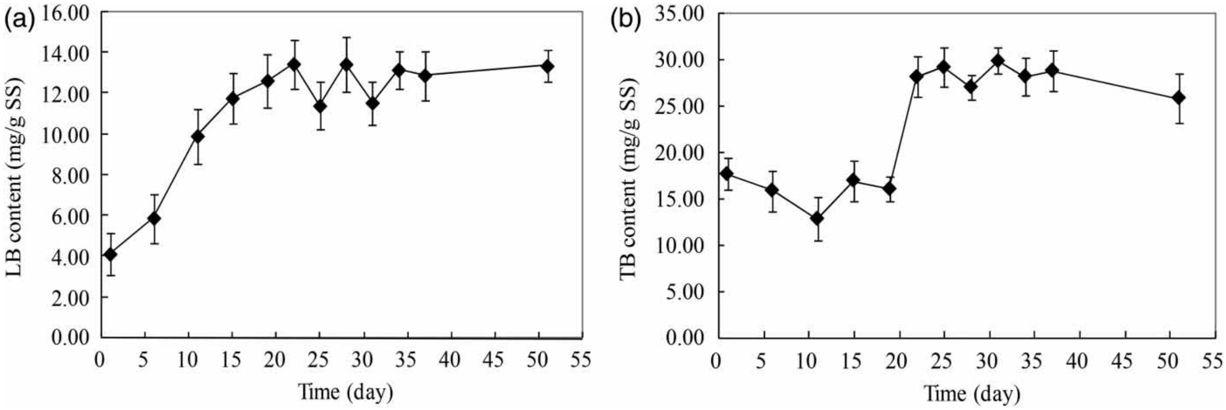

Figure 3 | Changes in LB-EPS and TB-EPS of WAS during anaerobic digestion.

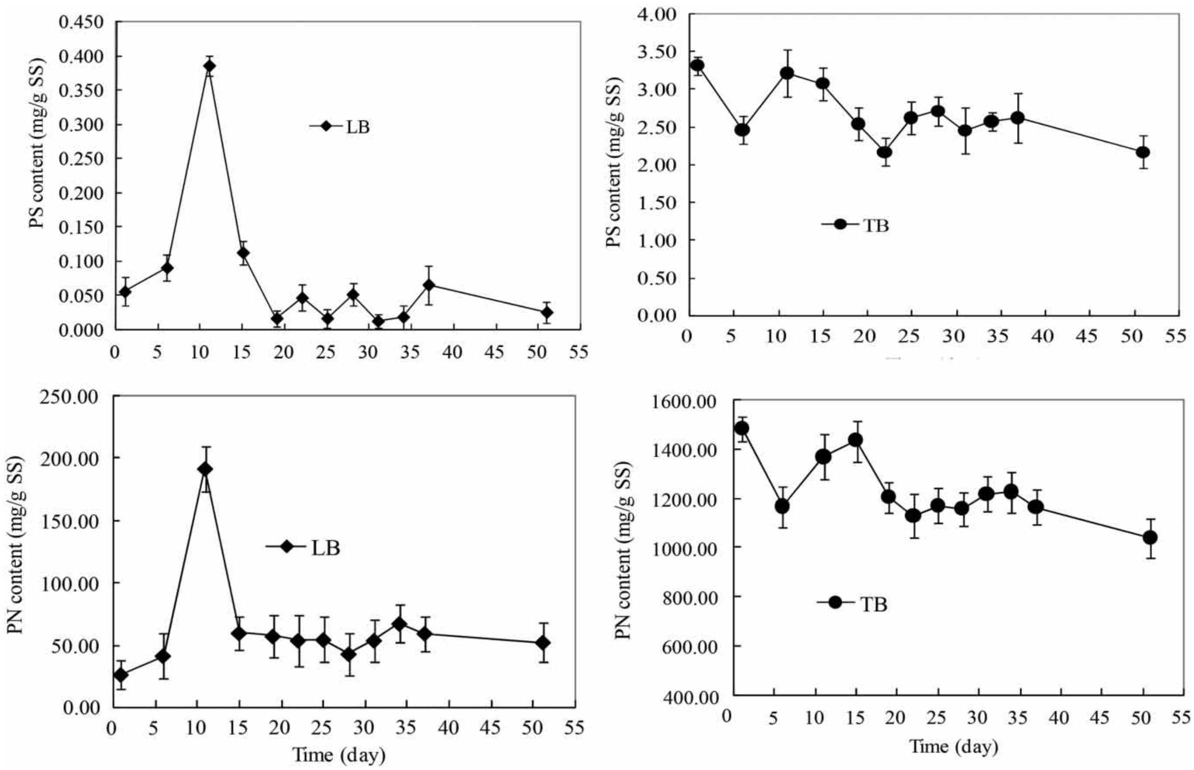

Figure 4 | Changes in PS and PN contents in LB-EPS and TB-EPS during anaerobic digestion.

mainly distributed in the tightly bound fractions (TB-EPS) and less in the LB-EPS. The contents of PS and PN in TB-EPS were approximately 60 times and 25 times greater than those in LB-EPS, respectively. PS in LB-EPS increased greatly before day 10. However, after 10 days of anaerobic digestion, PS content decreased and did not significantly change thereafter. PS in TB-EPS decreased gradually with time, but only slightly when compared to the value before anaerobic digestion. The trends for protein content change in LB-EPS and TB-EPS were similar to those of PS in LB-EPS and TB-EPS. The ratio difference of PN/PS in LB-EPS and TB-EPS is more striking. The ratio of PN/PS in LB-EPS increased dramatically after 15 days of anaerobic digestion, but the ratio of PN/PS in TB-EPS did not change significantly during the whole digestion period. The protein concentration was around 450–500 times greater than the polysaccharide concentration in LB-EPS and TB-EPS over the first 15 days of digestion. However, in LB-EPS, after 15 days, proteins were more than 2,000 times the level of PS. The results are in agreement with other works (Novak *et al.* 2003). It was surprising that PN contents were much higher than PS contents both in LB and TB. The results suggest that the protein might release more easily than polysaccharide, or decompose much more slowly than polysaccharide in LB-EPS. We are currently investigating the reason.

The ratio of PN/PS in LB-EPS increased greatly after 20 days of anaerobic digestion, but the ratio in TB-EPS did not change significantly (Figure 5). The results are in agreement with other reports (Houghton *et al.* 2001; Novak *et al.* 2001).

Relationship between physicochemical property parameters

The precise role of EPS on dewaterability usually appeared ambiguous. An increase in the level of EPS caused the sludge to become more difficult to dewater (Mikkelsen & Keiding 2002). However, there are findings that the CST decreased as the amount of EPS increased (Jin *et al.* 2004), and no simple correlation between the quantity of EPS present and the sludge dewaterability was observed (Poxon & Darby 1997). To understand the action of anaerobic digestion, the essential role played by EPS needs to be understood.

The establishment of a relationship between sludge dewaterability and the EPS content will enable decisions to be made that ensure maximum dewaterability of the digested sludge. The association between EPS and dewaterability indicated that only LB-EPS was positively correlated with normalized CST (Pearson's correlation coefficient (R^2) and *p*-values were 0.658 and 0.04, respectively). No correlation between the other parameters and the normalized CST was found. The findings were consistent with other research results (Li & Yang 2007; Ye *et al.* 2012) and were corroborated by Murthy & Novak (1999) who found that the PS in digested sludge were small and had not much impact on the dewatering rate. No correlation was found between TB-EPS and dewaterability with measurements of the SRF (Li & Yang 2007; Yang & Li 2009).

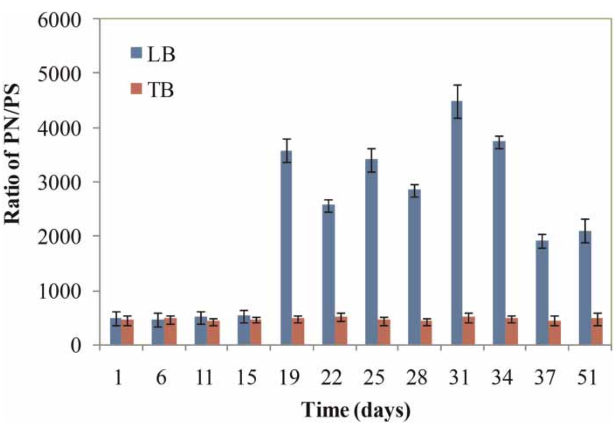

Figure 5 | Changes of ratio of PN/PS in LB-EPS and TB-EPS during anaerobic digestion.

CONCLUSIONS

The following conclusions may be drawn from this study:

(1) The LB-EPS content increased significantly after the sludge was digested anaerobically in the first 20 days, and then stabilized. However, the TB-EPS content decreased in the first 10 days, and then increased and finally stabilized.
(2) PS and PN in LB-EPS increased greatly in the first 10 days of anaerobic digestion and then decreased and finally reached a steady state. PS in TB-EPS decreased gradually with time, but only slightly. The ratio of PN/PS in LB-EPS increased dramatically after 15 days of anaerobic digestion, but the ratio of PN/PS in TB-EPS did not change significantly during the entire digestion time.
(3) Only LB-EPS was positively correlated with normalized CST, which suggested that the great increase of LB-EPS during anaerobic digestion caused the deterioration of dewaterability of sludge.

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REFERENCES

APHA 1998 *Standards Methods for the Examination of Water and Wastewater*, 20th edn. American Public Health Association/ American Water Works Association/Water Environment Federation, Washington, DC, USA.

Bruss, J. H., Christensen, J. R. & Rasmussen, H. 1993 Anaerobic storage of activated sludge: effect on conditioning and dewatering performance. *Water Sci. Technol.* **28** (1), 350–357.

Frølund, B., Palmgren, R., Keiding, K. & Nielsen, P. H. 1995 Extraction of activated sludge biopolymers by a cation exchange resin. *Water Res.* **56**, 216–230.

Ge, P., Fenxia, Y. & Ying, L. 2011 Comparative investigation of parameters for determining the dewaterability of activated sludge. *Water Environ. Res.* **83**, 667–671.

Houghton, J. I., Quarmby, J. & Stephenson, T. 2001 Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer. *Water Sci. Technol.* **44** (2–3), 373–379.

Jin, B., Wilén, B. M. & Lant, P. 2004 Impacts of morphological, physical and chemical properties of sludge flocs on dewaterability of activated sludge. *Chem. Eng. J.* **98**, 115–126.

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 Res.* **41**, 1022–1030.

Mikkelsen, L. H. & Keiding, K. 2002 Physico-chemical characteristics of full scale sewage sludges with implication to dewatering. *Water Res.* **36**, 2451–2462.

Murthy, S. N. & Novak, J. T. 1999 Factors affecting floc properties during aerobic digestion: implications for dewatering. *Water Environ. Res.* **71** (2), 197–202.

Murthy, S. N., Novak, J. T., Holbrook, R. D. & Sukovitz, F. 2000 Mesophilic aeration of autothermal thermophilic aerobic digestor (ATAD) biosolids to improve plant operation. *Water Environ. Res.* **71**, 476–483.

Novak, J. T., Agerbak, M. L., Sørensen, B. L. & Hansen, J. A. 1999 Conditioning filtering and expressing waste activated sludge. *J. Environ. Eng.* **125**, 816–824.

Novak, J. T., Muller, C. D. & Murthy, S. N. 2001 Floc structure and the role of cations. In: *Proceedings Sludge Management Entering the Third Millenium – Industrial, Combined, Water and Wastewater Residues, Taipei, Taiwan*, pp. 35–39.

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 Res.* **37**, 3136–3144.

Poxon, T. L. & Darby, J. L. 1997 Extracellular polyanions in digested sludge: measurement and relationship to sludge dewaterability. *Water Res.* **31**, 749–758.

Xu, H. C., He, P. J., Wang, G. Z., Shao, L. M. & Lee, D. J. 2011 Anaerobic storage as a pretreatment for enhanced biodegradability of dewatered sewage sludge. *Bioresour. Technol.* **102**, 667–671.

Yang, S. & Li, X. 2009 Influences of extracellular polymeric substances (EPS) on the characteristics of activated sludge under non-steady-state conditions. *Proc. Biochem.* **44**, 91–96.

Ye, F. X., Liu, X. W. & Li, Y. 2012 Effects of potassium ferrate on extracellular polymeric substances (EPS) and physicochemical properties of waste activated sludge. *J. Hazard. Mater.* **199–200**, 158–163.

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