Effect of Cellulase and Protease Pretreatment on Dewaterability of Waste Activated Sludge from Paper Mill

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The feasibility of cellulase and protease pretreatment to improve the dewaterability of waste activated sludge from papermaking (WASP) was evaluated. Dewatering properties such as capillary suction time (CST), dry solids content of the sludge cakes from the specific resistance of filtration (SRF), and compression were measured to quantify the effects of cellulase and protease in sludge dewatering. The changes in the amounts of proteins (PN) and polysaccharides (PS) in tightly bound extracellular polymeric substances (TB-EPS) was found to be the most important parameter with respect to sludge dewatering. Further study, through nitrogen adsorption, verified the large change in the average pore width and surface area. Therefore, the disruption of TB-EPS and the change in the inner structure of WASP granules are the fundamental reasons for the enhanced dewaterability.

Keywords: Waste activated sludge from papermaking (WASP); Dewaterability; Cellulase; Protease; Tightly bound extracellular polymeric substances (TB-EPS)

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INTRODUCTION

Disposal of residue wastes from pulp and paper mills is a paramount issue facing the world today. The pulp and paper industry in China annually produces more than nine million tons of paper sludge (oven dry), which mainly consists of paper sludge, waste activated sludge, advanced treatment paper sludge, and de-inked paper sludge (Wu 1999; Cong *et al.* 2011). A common characteristic of paper sludge is the very high moisture content (Zhang *et al.* 2012). Such sludge is usually thickened, raised to a high dry solids contents using a belt filter press, and then typically sent to the landfill. Because of legislation and increased taxes, landfills are quickly being eliminated as a final destination for paper sludge. Common alternative disposal methods include incineration or recycling utilization, including the preparation of carbonized paper sludge (Kanno *et al.* 2007) and activated carbon adsorbents (Littrell *et al.* 2002; Kang *et al.* 2006) from paper sludge. The most important problem before ultimate disposal is to reduce the moisture content of the sludge. Dewatering is therefore required prior to the ultimate disposal of paper sludge.

Conditioning treatment before dewatering of sludge, which is generated either from municipal or industrial wastewater treatment plants, is the key technology to obtain better dewaterability of sludge. In this regard, many conditioning approaches, such as microwave (Wojciechowska 2005), ultrasonication (King and Forster 1990; Quarmby *et al.* 1999; Dewil *et al.* 2006; Zhang and Wan 2012), acid pretreatment (Mahmood and Elliott 2007), chemical additive conditioning (Kuutti *et al.* 2011; Luo *et al.* 2013; Niu *et*

al. 2013), ozone oxidation treatment (Park *et al.* 2003), surfactant conditioning (Pan *et al.* 2000), electro-chemical pretreatment (Mahmoud *et al.* 2011; Yuan *et al.* 2011), electrolysis (Zhen *et al.* 2013), and enzymatic treatment (Knapp and Howell 1978; Luo *et al.* 2011; Parmar *et al.* 2001; Roman *et al.* 2006; Yu *et al.* 2013) have been applied to enhance sludge dewaterability. Among the existing techniques, enzymatic treatment is considered a promising technology because of its potential to disrupt EPS, thus enabling the release of bound and intercellular water in sludge flocs (Wawrzynczyk *et al.* 2007), but also because it has no adverse effects on the environment. Enzyme conditioning sludge will become increasingly more attractive as an option as the enzyme prices continue to drop. In our previous study (Zhang *et al.* 2013a,b), a novel technique, *e.g.* enzyme-assisted dewatering, was set up to condition the paper sludge. Yet, no studies have been carried out to explore the effect of the cellulase and protease pretreatment on the dewaterability of waste activated sludge from papermaking (WASP).

The specific objective of this research was to investigate the principles of cellulase and protease pretreatment for improving the dewaterability of WASP. Selected properties, such as capillary suction time (CST), dry solids content of the filtered sludge cakes from the specific resistance of filtration (SRF), and pressed sludge cakes were investigated to evaluate the sludge dewaterability. The main composition of the tightly bound EPS, specific surface area, and pore size of sludge granules were also investigated to ascertain the exact roles they play in WASP dewatering. It is expected that this study can provide some fundamentals that will be beneficial in further exploration of new advanced technologies for paper sludge dewatering.

EXPERIMENTAL

Materials

The WASP used in this study was collected from the wastewater treatment plant (Fig. 1) at Shan Dong Sun Paper Industry Joint Stock Company, Ltd., China.

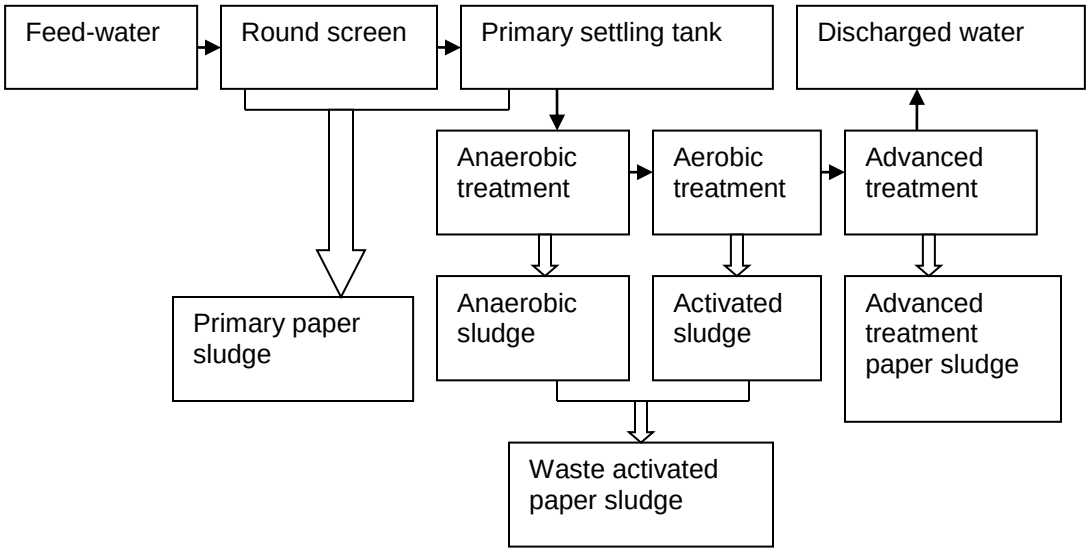

Fig. 1. Flow diagram of Shan Dong Sun Paper Industry wastewater treatment process

The collected sample was transferred to the laboratory within 4 h after sampling and stored at 4 °C. Within 48 h of collection, the sludge was prepared for experimentation. The characteristics of the original WASP include a water content of 97.0%, pH of 7.3, dry solids content (DSC) after SRF of 23.8%, DSC after press of 35.2%, and the fiber content (FC) of 1.21%.

Neutral cellulase (enzyme activity 20,000 IU/g) and neutral protease (enzyme activity 100,000 IU/g) used in this study were provided by Sukahan (Weifang) Biochemical Industry Company, Ltd., China. All chemical reagents were of analytical grade and used as received without further purification. All solutions were prepared freshly in deionized water prior to use.

Methods

Enzyme pretreatment process

The enzyme was added to 100 mL of WASP. Conditioning pretreatment was carried out at 30 °C at a constant rotation speed of 100 rpm.

Dewaterability measurement

Sludge dewaterability was evaluated with capillary suction time (CST), dry solid content of the filtered sludge cakes from the specific resistance of filtration (SRF), and pressed sludge cakes. CST was measured with a CST apparatus TR04-304M (Triton, England) fitted with a 1.8-cm-diameter cylinder and standard filter paper (Whatman No. 17). Sludge suspension (100 mL) was poured into a 9-cm standard Buchner funnel in the SRF apparatus, and a constant vacuum pressure of 0.0709 MPa was applied for 10 min. A laboratory compression test apparatus designed for the press filtering experiment was introduced in literature (Nomura *et al.* 2007). A piston was placed onto the paper sludge sample (filtered sludge cakes) by four respective layers of Whatman No. 1 filter paper on the top and bottom, with a constant pressure of 0.53 MPa for 4 min. The dry solids content of the filtered sludge cakes from SRF and pressed sludge cakes was measured after drying at 105 °C for 24 h.

EPS extraction

The EPS extraction protocol in this article was modified based on the research of Luo *et al.* (2005). Briefly, 50 mL of sludge suspension was centrifuged at 6,000 g for 5 min. After the supernatant was discarded, buffer solution (2 mM Na_3PO_4, 4 mM NaH_2PO_4, 9 mM NaCl, 1 mM KCl, at pH of 7.0) was added to restore the volume to 50 mL and 0.3 mL of formaldehyde and 50 mL (0.04 M) of sodium hydroxide were added. The solution was stirred with a magnetic stirrer at 300 rpm for 1 h, and the solution was centrifuged at 6,000 g for 10 min and filtered with a 0.2-μm membrane. The organic matter in the filtrate was regarded as tightly bound EPS (TB-EPS).

EPS analysis

The concentration changes in proteins (PN) and polysaccharides (PS) in TB-EPS were analyzed by referring to the literature (Zhang *et al.* 2013b). The PN concentration was determined by the ultraviolet visible absorption method, and its absorbance was measured at 280 nm. The PS concentration was determined by the anthrone-sulfuric acid colorimetry method, and its absorbance was measured at 620 nm.

Specific surface area measurement

The samples were placed into a freezer (-16 °C) for 24 h to freeze-dry. The Brunauer-Emmett-Teller (BET) surface area and Barret-Joyner-Halenda (BJH) pore distribution of the samples were obtained from a nitrogen adsorption apparatus (V-Sorb2800p, Gold APP, China). All samples were degassed at 150 °C prior to measurements.

Other analysis methods

The water content was estimated following standard methods (APHA 1998). The pH was tested by a pH meter (HM-30V, TOA, Japan). The fiber content (FC) in sludge was measured by cleaning the sludge with deionized water in a 100-mesh screen, as introduced in the literature (Zhang *et al.* 2012); the unscreened oven dry fiber ratio to oven dry sludge was defined as the fiber content.

RESULTS AND DISCUSSION

Optimal Enzyme Conditioning Time

To optimize the cellulase and protease pretreatment condition for WASP dewatering, a series of experiments was conducted with different conditioning times applied for the same temperature (30 °C) and enzyme dosages (1% to oven-dry sludge). Simultaneously, a control experiment (without the addition of enzyme) was conducted in the laboratory. Preliminary laboratory tests indicated that the CST of samples pretreated with cellulase or protease were larger than the control sample at every conditioning time (Table 1). These results showed a slight decrease in the sludge filterability, which is mostly related to free or capillary water in the sludge after enzyme pretreatment. Because a large CST usually implies poor sludge filterability, it cannot be directly used to evaluate the bound water in the sludge (Chen *et al.* 1996).

The DSC of the filtered sludge cakes after SRF decreased with increasing conditioning time, with and without the enzyme added in the sludge suspension. As presented in Table 1, when the conditioning time increased from 2 to 4, 6, 8, and 10 h, the DSC of the filtered sludge cakes after SRF declined to around 1.7, 1.7, 3.4, and 4.6% for the control samples, 1.2, 1.2, 1.2, and 2.5% for cellulase, and 0, 0.7, 1.7, and 5.4% for protease, respectively. These results imply a remarkable deterioration in WASP dewaterability. Additionally, the DSC of the pressed sludge cakes increased slightly at first, and then decreased sharply at the conditioning time of 10 h. These results indicate that WASP dewaterability will sharply decrease as the conditioning time of WASP reaches 10 h, with and without the enzyme added in the sludge suspension.

The DSC of the filtered sludge cakes after SRF (cellulase or protease pretreatment) was slightly higher than that of the control when the conditioning times increased from 2 h to 4 h, 6 h, and 8 h (except at 10 h), which produced low dry solids content of 18.7 and 16.3% for cellulase and protease in comparison with 18.8% for the control. However, the DSC of the pressed sludge cakes (cellulase or protease pretreatment) was remarkably higher than that of the control during the whole conditioning time. These results indicate that the DSC of the filtered sludge cakes after SRF are closely related to the free or capillary water in sludge, and the DSC of pressed sludge cakes is closely related to the bound water in sludge. Cellulase or protease pretreatment may be conducive to the release of bound water into free water in sludge

flocs by disrupting EPS or cells. Therefore, the released free water in sludge plays a key role during the pressure change from 0.0709 to 0.53 MPa. This ultimately results in higher dry solids content of the pressed sludge cakes (cellulase or protease pretreatment) in comparison with that of the control. The FC in sludge changed somewhat after cellulase or protease pretreatment, indicating a slight relationship between the FC in sludge and enzyme pretreatment.

Table 1. Dewatering Characteristics of WAPS at Different Conditioning Times

Time (h)		2	4	6	8	10
Control	CST (s)	135	147	147	145	146
	DSC after SRF (%)	23.4	21.7	21.7	20.0	18.8
	DSC after press (%)	35.6	35.6	36.0	32.6	30.3
	FC (%)	1.21	1.20	1.20	1.20	1.20
Cellulase	CST (s)	135	160	169	148	156
	DSC after SRF (%)	24.2	23.0	23.0	23.0	18.7
	DSC after press (%)	38.0	38.3	39.0	39.3	35.1
	FC (%)	1.20	1.19	1.19	1.19	1.19
Protease	CST (s)	140	155	157	146	150
	DSC after SRF (%)	24.0	24.0	23.3	22.3	16.3
	DSC after press (%)	39.7	40.5	39.1	39.0	34.2
	FC (%)	1.21	1.20	1.20	1.20	1.20

Optimal Enzyme Dosages

To optimize the cellulase and protease dosages for WAPS dewatering, a series of experiments was conducted with different enzyme dosages applied for the same temperature (30 °C) and conditioning time (4 h). The dry solids content of the filtered sludge cakes from SRF and pressed sludge cakes (cellulase or protease pretreatment) were higher than the control when the enzyme dosages increased from 0.25 to 1.0% (Table 2). The CST of the sludge suspension was lower at the enzyme dosage of 0.5% compared to that of sludge with other enzyme dosages. In addition, the DSC of the pressed sludge cakes was higher at the enzyme dosage of 0.5%. In the course of the enzyme conditioning process, superior dewaterability was obtained when cellulase and protease dosages were both 0.5%. The FC in sludge changed slightly with increasing enzyme dosage.

Table 2. Dewatering Characteristics of WAPS at Different Cellulase and Protease Dosages

Dosage (%)		0	0.25	0.50	0.75	1.0
Cellulase	CST (s)	147	146	144	146	160
	DSC after SRF (%)	21.7	22.7	23.8	22.2	23.0
	DSC after press (%)	35.6	37.4	42.8	40.7	38.3
	FC (%)	1.20	1.20	1.19	1.19	1.19
Protease	CST (s)	147	138	134	141	155
	DSC after SRF (%)	21.7	23.7	23.5	24.3	24.0
	DSC after press (%)	35.6	39.5	43.8	43.1	40.5
	FC (%)	1.20	1.20	1.20	1.20	1.20

The Role of Different TB-EPS Fractions

Proteins and PS, as the major EPS components (Frolund *et al.* 1996), were measured in this investigation, and the corresponding results are given in Fig. 2. Figure 2a illustrates the effect of different enzyme dosages on the relative PN content in TB-EPS. The PN content in TB-EPS fraction decreased in proportion to the increase of protease dosages. However, the PN content in TB-EPS fraction slightly increased with increasing cellulase dosage. In contrast, the PS content in the TB-EPS fraction decreased with increasing cellulase dosage. The PS content in the TB-EPS fraction decreased sharply at first with increasing protease dosage, and then increased slightly when the protease dosage was greater than 0.5% (Fig. 2b). The sharp decrease in PN or PS in the TB-EPS imply that the enzyme pretreatment may decompose TB-EPS, which covers the surface of the cell, functioning as a protective barrier for bacteria inside the WAPS flocs, and further effectively disrupts the bacterial cells trapped in the sludge flocs. As a result, a large quantity of the bound water in EPS could thus be released into the sludge suspension, resulting in an increase in the dry solids content of the filtered sludge cakes.

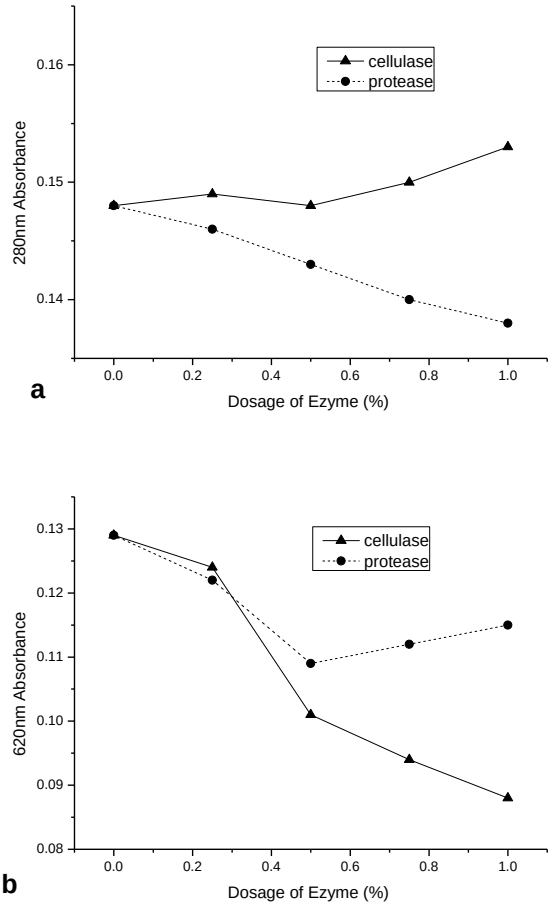

Fig. 2. Change in concentration of (a) PN and (b) PS in TB-EPS at different enzyme dosages

Specific Surface Area and Pore Size of WAPS Granules

The effects of particle size distribution on the dewatering of organic sludge generated from municipal wastewater treatment plants is reported in the literature (Olboter and Vogelpohl 1993). Little information on the inner structure of sludge floc is

available in the literature. The inner floc structure should be taken into consideration for a full understanding of the dewatering properties of WASP. Therefore, the specific surface area and adsorption pore size of freeze-dried WASP granules obtained from the control and enzyme pretreatment at the optimal condition (30 °C, 0.5% enzyme dosage, and 4 h) were measured in a V-Sorb2800p nitrogen adsorption apparatus. The BET and Langmuir surface areas of freeze-dried WASP granules (cellulose- and protease-treated) were greater than those of the control (Table 3). In contrast, the BJH and total average adsorption pore widths of WASP after enzyme pretreatment were lower than the control. These results conclusively show that enzyme pretreatment has a significant effect on the inner structure of WASP granules. In addition, the BJH and total average adsorption pore widths of WASP after protease pretreatment were lower than those after cellulase pretreatment and the control, which leads to a lower BJH adsorption cumulative volume. In conclusion, the enzyme pretreatment caused a decrease in the average pore width and an increase in the surface area of sludge granules, which subsequently caused a significant increase in the dry solids content of the pressed sludge cakes. Sludge dewaterability was closely relative to the specific surface area and pore width of WAPS granules.

Table 3. Specific Surface Area and Pore Size Distribution of the Samples

	Control sample	Cellulase-treated sample	Protease-treated sample
BET surface area (m^2/g)	2.47	3.26	4.22
Langmuir surface area (m^2/g)	3.26	4.44	5.90
Single point adsorption total pore volume (cm^3/g)	0.0557	0.0641	0.0515
BJH adsorption cumulative volume (cm^3/g)	0.0584	0.0671	0.0534
BJH desorption cumulative volume (cm^3/g)	0.0599	0.0685	0.0555
Total adsorption average pore width (nm)	90.36	78.60	48.78
BJH adsorption average pore width (nm)	50.53	49.23	33.64
BJH desorption average pore width (nm)	35.39	30.64	21.38

CONCLUSIONS

1. Cellulase and protease pretreatments can promote WAPS dewaterability. The optimal enzyme pretreatment conditions were 0.5% dosage for 4 h.
2. Dewaterability was mostly related to the concentrations of PN and PS in TB-EPS, average pore width, and surface area. Therefore, the disruption of TB-EPS and the change in the inner structure of WAPS granules are the fundamental reasons for the enhanced dewaterability.

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