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Effect of protease and cellulase on the characteristic of activated sludge

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ABSTRACT

Sludge dewatering is a key part of sludge disposal since it can greatly reduce the volume of sludge and thus improve the treatment effect for handling and disposing. This study investigated the potential benefits of enzymatic pretreatment on activated sludge dewatering with protease and cellulase as a protein and polysaccharide degrading enzyme, respectively. Capillary suction time (CST) and the solid content after centrifugation were used to evaluate sludge dewatering. The particle size distribution, extracellular polymeric substances (EPS) content, infra-red (IR) spectrometry and microscope image were determined in an attempt to explain the observed changes in sludge dewaterability. The results indicated that adding protease and cellulase separately leads to an increase in CST and the increased value is higher with protease. Protease and cellulase both promote the degradation of protein and polysaccharide in the solids of activated sludge, leading to a smaller particle diameter and poorer dewaterability. However, due to the limited effects on the protein and polysaccharide content, the difference in sludge dewaterability is not large. Compared to the control, enzymatic pretreatment had no obvious effect on sludge IR spectrometry, while there was a detectable structure difference at a colloidal scale.

1. Introduction

Activated sludge processes are important technologies for the treatment of wastewater, but these biological processes generate large amounts of waste activated sludge. Sludge dewatering is of paramount importance in wastewater treatment systems as it reduces sludge volume and, consequently, the cost of transporting sludge to its ultimate disposal site. Moreover, dewatered sludge is generally much easier to handle. Nevertheless, sludge dewatering remains one of the most expensive and most poorly understood wastewater treatment processes [1].

The processes most often used for dewatering biological sludge are sedimentation, filtration and centrifugation. In most cases, biological sludge must be conditioned prior to dewatering to ensure proper process performance. In practice, organic or inorganic chemicals are used, such as alum, iron (III) chloride, iron (II) sulfate, and polyelectrolyte. Since the 1990s, enzymes were added to sludges as conditioners by researchers. Some researches found that the dewaterability of sludge was improved [2–5] by adding an enzyme or enzymatic product (containing carbohydrase, lipase and proteinase) with an exception being one published by Hijjins and Novak [6].

Researchers believed that enzyme product additions improved the degradation of extracellular proteins and polysaccharides, which have been responsible for poor dewaterability [5]. However, there were some researchers who thought that extracellular polymeric substances (EPS), being the third major component of sludge flocs, might have an influence on sludge dewaterability due to the high level of hydration of the polymer surrounding bacterial cells and their role in flocculation [7]. Sanin and Vesilinel [8] found that the dewaterability of sludge was adversely affected to a great degree by the extraction of EPS. The research performed by Mikkelsen and Keiding shows that EPS has a positive effect on floc stability and filterability [9,10]. With high EPS content, sludge had a lower shear sensitivity and a lower degree of dispersion.

Conclusions from these previous studies remain controversial, so further studies are necessary to assess whether enzymatic pretreatment ultimately improves activated sludge dewatering. Since proteins and carbohydrates are usually found as the major components of sludge EPS [7,11], this study investigated the effects of enzymes on activated sludge dewaterability using protease and cellulase as protein and polysaccharide degrading enzymes, respectively. Furthermore, this test determined which part of EPS has the leading role in improving sludge dewatering and reviewed the mechanism behind the observed change in sludge dewaterability.

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2. Materials and methods

2.1. Materials

Activated sludge was collected from a municipal wastewater treatment facility in Jinan, Shandong province, China. This facility uses an activated sludge process to treat 220,000 liquid tons of wastewater daily. The collected sludge was immediately transferred to the lab and stored in a plastic container at 4 °C prior to use. The solid concentration and pH of the sludge were 0.65% and 6.9, respectively.

Protease and cellulase were bought from Sigma Company. Protease (P6110-50 mL) is extracted from strain *Aspergillus orzae* and enzyme activity is 500 μ/g. Cellulase (C2605-250 mL) is from strain *Aspergillus* sp. with activity of 1000 μ/g.

2.2. Experimental procedure

Enzymes were added to 250 mL activated sludge samples according to the concentration of 500 activity units per 100 mL sludge. The sludge suspensions were maintained at 35 °C in a water bath. Distilled water was added to the control sample to match the volume of the enzyme solution added.

The dewaterability of the samples was assessed in terms of CST and solid content. The EPS concentrations, particle size distribution, infra-red spectra and microbe characteristic were also measured to aid our investigation into the mechanism behind the observed changes in sludge dewaterability.

3. Analytical methods

3.1. Capillary suction time (CST) measurement

CST analysis was carried out according to the standard method [12] with multipurpose filtration equipment (Triton-W.R.C. Multipurpose Filtration 319) and standard filtration paper (Whatman International Ltd., Maidstone England).

3.2. Solid content

After 2 h, the treated samples were centrifuged for different times at 1900 rpm. After discarding the supernatant, the solids were measured to determine the appropriate centrifugation time. The samples treated with enzymes were centrifuged at 1900 rpm for the determined length of time. Sludge samples without supernatant were transferred to trays and the solids concentration measurements were performed at 105 °C.

3.3. Particle size distribution

The particle size distribution of the control sample and the enzymatic pretreatment samples was measured with a laser particle size analyzer (Coulter LS130, Langley Ford Instruments Division of Coulter Electronics of New England, Inc.).

3.4. Infra-red (IR) spectrometry

Samples were dried at 105 °C and compacted to form a powder. The weight of each sample was about 10 mg. The IR-spectra were then determined using a spectrum AVATAR370 FT-IR (Thermo Nicolet).

3.5. EPS extraction and analysis

Many methods have been proposed for extracting EPS, including various physical and chemical extractions [13–15]. The results showed that the efficiency of EPS extractions from activated sludge was, in descending order: formaldehyde + NaOH, EDTA, cation exchange resin and the control [13]. However, IR analysis showed that all chemical extraction methods contained EPS. The contamination for EPS extracted by formaldehyde + NaOH could be interpreted as specific bands and/or the results of a formaldehyde and EPS reaction [13]. Therefore, in this study, NaOH was only used to extract EPS from the samples. The addition of NaOH increases the pH, resulting in the dissociation of acidic groups in EPS and repulsions between the negatively charged EPS. The EPS solubility in water also increases and thus allows more EPS to be extracted [16]. The supernatant EPS was measured after centrifugation at 2000 rpm for 20 min [17]. Protein and polysaccharide concentrations were determined spectrophotometrically using a UV/visible spectrophotometer (UV–vis spectrophotometer 756MC). Protein was stained with Coomassie Brilliant Blue G-250, and its absorbance was measured at 595 nm. Polysaccharide was stained with anthrone, and its absorbance was measured at 625 nm.

3.6. Microscopy image

The samples were rinsed with distilled water and fixed with 2.5% (v/v) glutaraldehyde solution for 24 h. The fixed samples were then rinsed for 2 h using 0.1 mol/L phosphate buffer and fixed with osmium acid for 1.5 h. They were then washed with redistilled water to remove osmium acid. The samples were then dehydrated by sequential immersion in increasing concentration of ethanol to remove final traces of water. The dehydrated sludges were then dried in a CO_2 atmosphere under critical conditions. The subsequent samples were coated with platinum, and examined using a scanning electron microscope (S-570, Japan).

4. Results and discussion

4.1. Effects of enzymes on CST

The CST is a measure of the rate of water release from sludge and it has long been established as a practical and empirical method for the determination of sludge dewaterability [18,19]. It has widespread use because it is simple and allows a quick comparison of the filterability of sludges [20]. The effects of protease and cellulase on the CST of activated sludge at the same enzymatic activity are shown in Fig. 1.

The CST of activated sludge was 9.8 s and the CSTs of sludges with added enzymes increased with time. During the first 5 h, CST of the sludge with protease increased quickly and it reached 13.0 s at the 5th hour. The control sample and the samples with added cellulase did not increase very much, only 10.2 s and 10.6 s, respectively. After 5 h, the CST increase of sludge with protease continued,

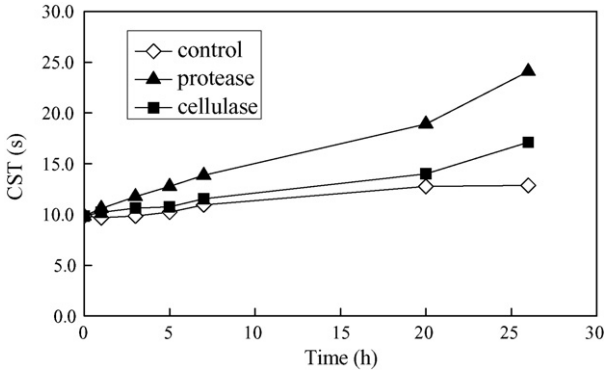

Fig. 1. Effects of protease and cellulase on the dewaterability of activated sludge.

reaching 26.7 s at the 26th hour. The sludge with cellulase and the control sample also increased and reached 11.5 s and 16.2 s at the 24th hour, respectively. It was determined that both protease and cellulase led to the CST increasing in the case of activated sludge, while the effect of protease was greater than that of cellulase at the same concentration.

The data on the effects of protease and cellulose on CST of activated sludge shows a similar tendency to the research results of Higgins and Novak [6] on enzymatic pretreatment on activated sludge. In their study, the degradation of the exocellular protein by pronase resulted in deflocculation of the suspension and poor dewatering, and no appreciable change was observed with addition of a polysaccharide degrading enzyme. The result was explained using a model of bioflocculation proposed by them. In that model, lectinlike proteins bind polysaccharides that are cross-linked to adjacent proteins. The cross-linking of polysaccharides and cation bridges acts to stabilize the biopolymer network. Because proteins connect to cells directly, the degradation of protein by protease has a greater effect on sludge dewatering than that of polysaccharides degraded by cellulase.

4.2. Effects of enzymes on solid content

Fig. 2(a) is the measurement results of sludge solid contents after centrifugation for different times at 1900 rpm. The solid content of samples was increased as centrifugation time was increased. After 60 min, the increasing value was not obvious. So in this study, 60 min of centrifugation was chosen to compare the solid content of the samples with or without enzymatic pretreatment for different hours. The results were recorded in Fig. 2(b). As reaction time was increased, the solid content of the samples with added enzymes was reduced. There was a larger decrease with the samples treated with protease. The result was a reduction of solid content of 0.01%. The small change in solid concentration indicated that protease and cellulase have no obvious effect on sludge dewatering in terms of solid concentration after centrifugation.

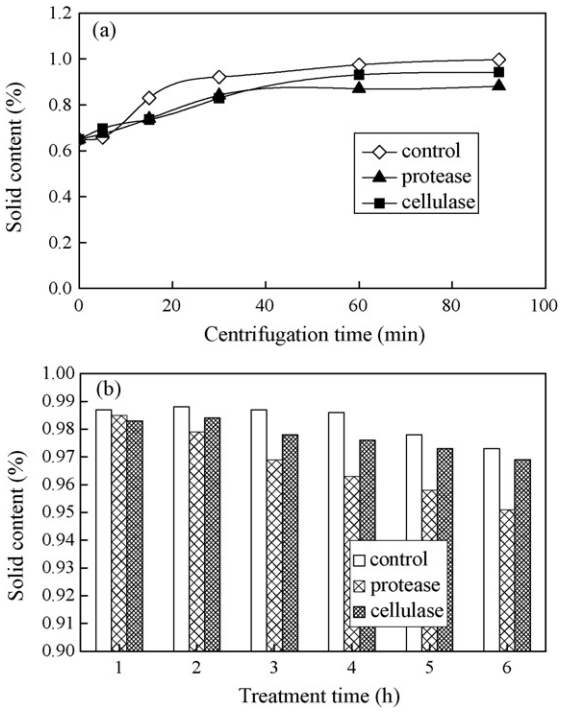

Fig. 2. Effects of protease and cellulase on the solid concentration: (a) results of sludge solid contents after centrifugation for different times at 1900 rpm; (b) comparison of the solid concentration of samples with enzymatic pretreatment for different hours after centrifuged 60 min at 1900 rpm.

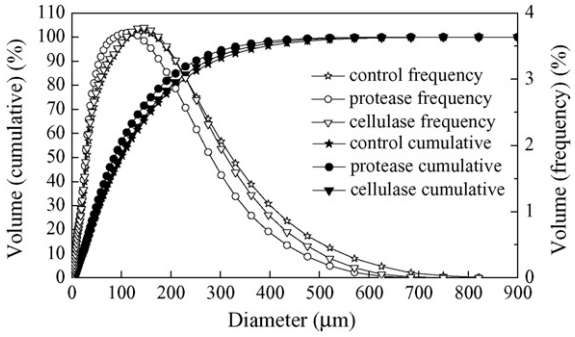

Fig. 3. Differential and cumulative volume percent vs. particle diameter of activated sludge with or without enzymatic pretreatment.

4.3. Effect of enzymes on particle size distribution

The size distribution of enzymatic pretreatment samples and control samples is shown in Fig. 3. The mean apparent diameter of activated sludge was between 3 and 800 μm and 50% of the volume consisted of flocs with a mean apparent diameter of less than 99.5 μm (median diameter Ø 50 = 99.5 μm). The average diameter was 132.6 μm. Pretreatment with protease and cellulase had a measurable effect on the granulometric distribution. The floc size distribution curve was shifted to the small size classes, with a median of 85.6 μm and average of 114.5 μm for the sludge treated with protease and 96.42 μm and 126.6 μm for the sludge treated by cellulase.

Particle sizes have an effect on sludge dewatering properties [21,22]. Karr and Keinath [23] thought that smaller particle sizes are associated with poor dewatering. In many researches using ultrasound to treat activated sludge or digested sludge, a similar conclusion was drawn that the dewaterability became poorer owing to the decreasing particle size [24,25]. In this study, both protease and cellulase had the effect of decreasing the particle size of activated sludge. Therefore, the dewatering became poorer. However, this effect was not significant, so the negative change on sludge dewaterability was not great. Compared with these two enzymes, the effect of protease on particle size was a little greater than the effect of cellulose on it. As a result, the CST of samples with protease conditioning increased much more than that with cellulase and control samples.

4.4. Effect of enzymes on IR spectrometry

Fig. 4 presents the IR-spectra of the activated sludge samples with or without enzymatic pretreatment. The analysis of IR-spectra shows the presence of numerous functional groups. Several intense characteristic bands can be attributed to functional groups present in proteins and in polysaccharides. Some less intense bands show carboxylic groups under acid or basic salt form. They suggest, when they are combined with other bands observed, the presence of uronic acid (notably with the bands characteristic of sugars) and of humic substances (CH_2, phenolic groups). Some bands observed in the "fingerprint" region could be attributed to the phosphate group which is one of the functional groups of which nucleic acids are composed. The presence of CH_2 and of carboxylic groups should indicate the presence of lipids. The functional groups corresponding to the peaks observed on IR-spectra are summarized in Table 1.

The different functional groups observed in the sludge sample without enzymes agree basically with the results of some researchers [14,26–28]. However, the two absorption bands

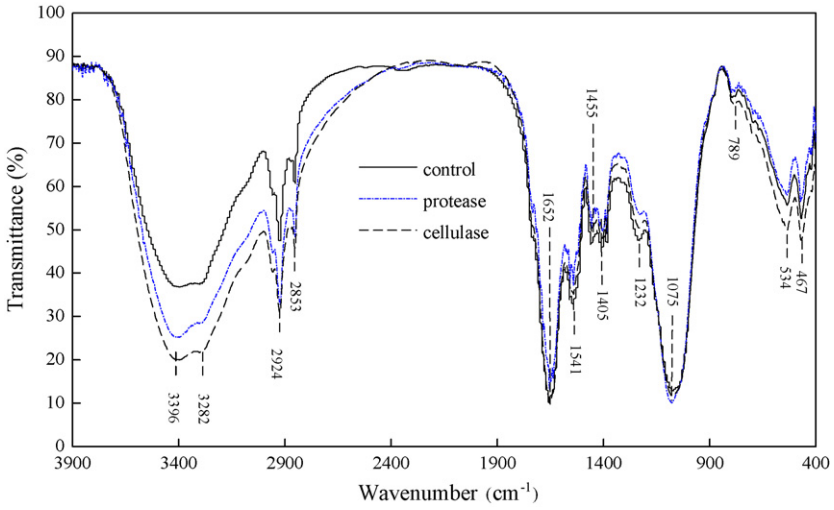

Fig. 4. IR-spectra of activated sludge after protease and cellulase pretreatment.

Table 1
Main functional groups in TWAS observed with IR-spectra.

Wave number (cm^{-1})	Vibration type	Function type
3750–3000	Stretching vibration of OH	OH into polymeric compounds
2926 ± 10	Asymmetric stretching vibration of CH_2	
2853 ± 10	Symmetric stretching vibration of CH_2	
1640–1660	Stretching vibration of C=O Stretching vibration of C–N	Amide I (peptidic bond)
1550–1560	Stretching vibration of C–N Deformation vibration of N–H	Amide I (peptidic bond) Amide II (peptidic bond)
1455	Deformation vibration of CH_2	
1400–1410	Stretching vibration of C=O Deformation vibration of OH	Carboxylates Alcohols and phenols
1240	Deformation vibration of C=O Stretching vibration of OH	Carboxylic acid Phenols
1030–1150	Stretching vibration of C–O–C	Polysaccharides
<1000	"Fingerprint" zone Several bands visible	Phosphate or sulfur functional groups

556 cm^{-1} and 471 cm^{-1} were much clearer in our detection using sludge samples directly than those of other researches detecting EPS extracted from sludge with principally physical methods. The reason is some groups of molecules present in lower proportions in solutions of EPS, such as lipids or nucleic acids, are more difficult to detect with IR-spectra. The agreement of our results with others' results showed that it was feasible to turn activated sludge samples into powder to detect their IR-spectra and compare organic chemicals.

Table 2
Main chemical composition.

Family	Subfamily identification
Protein	PR PR-OAS-M PR-M-PS PR-L
Polysaccharide	PS-PR-OAS PS-PR-OA PS
Lipid	LI

PR, protein; PS, polysaccharide; OA, organic acid; OAS, organic acid salt; M, mineral phase; LI, lipid.

Referring to the database provided by Garnier et al. [29] which is based on various activated sludges with high-pressure size-exclusion liquid chromatography and infra-red microscopy, the chemical groups identified in our study were protein, polysaccharide and lipid. The subfamilies of protein were pure protein, protein-organic acid salt-mineral phase and protein-lipid. For polysaccharide, they were polysaccharide-protein-organic acid

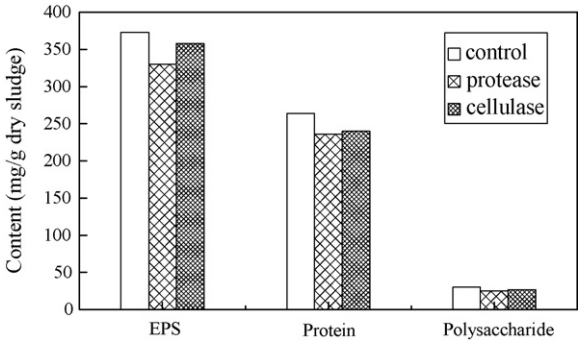

Fig. 5. Changes in bound EPS and protein and polysaccharide of activated sludge after enzymatic pretreatment.

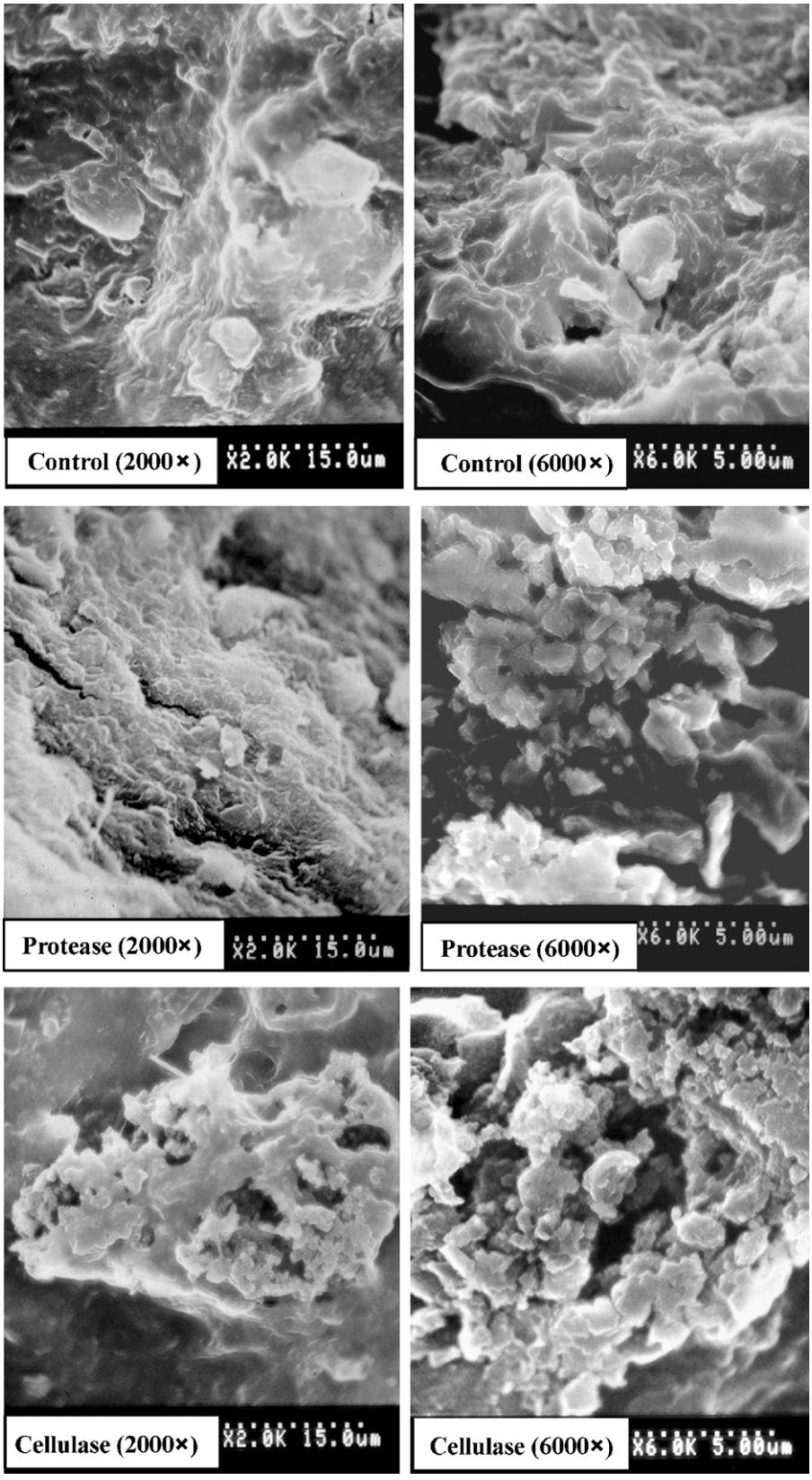

Fig. 6. Scanning electron microscope image of samples with or without enzymatic pretreatment.

salt, polysaccharide-protein-organic acid and pure polysaccharide. As for mineral phase, it was a carbonate phase and involved in organic association with some subfamilies of protein where the protein part was dominant. The chemical composition of the activated sludge is presented in Table 2. Compared to the control sample, the wave numbers of samples with or without enzymatic pretreatment, which have peak absorption, had no detectable change.

4.5. Effect of enzymes on EPS

Protein and carbohydrate are usually found as the major components of sludge EPS [7,11], although there is no agreement on which of these components has a higher proportion. Some research supported the opinion that polysaccharide has a major role in bioflocculation [1,30,31], while others argued that protein has the major role [6,9,15,32–34]. In our study, the EPS of the activated sludge was 373 mg/g (dry sludge), and the protein had the highest proportion which accounted for 70.78%, while polysaccharide was 8.04%.

The changes of EPS and its major composition in solid sludge after enzymatic pretreatment for 12 h are recorded in Fig. 5. Compared to the control sample, the EPS content of samples with enzymes decreased, but the quantity of decrease was not great. The EPS in the control sample was 373 mg/g (dry sludge), and 330 and 358 mg/g (dry sludge) for treated samples by protease and cellulase, respectively. It was protease that resulted in the greater effect on the content changes of EPS, including protein and polysaccharide in solid sludge.

The bond EPS decrease after pretreatment with enzymes was in accord with the poorer dewaterability of enzymatic pretreatment samples. It indicated that EPS in solid sludge played a positive role in sludge dewatering, and its degradation may lead to poorer dewaterability. However, owing to the limited effect of enzymes in this experiment, the dewaterability difference between samples with enzymatic pretreatment and control sample was not obvious.

4.6. Effect of enzymes on floc morphology

The different structures of samples observed with a scanning electron microscope are shown in Fig. 6. From those figures, it could be seen that the original sludge displayed a cluster of mass and there was slimy matter covering the surface. There were also biomass granules distributed among the glutinous mass. After pretreatment with protease, the glutinous mass of the activated sludge appeared to be less uniform and displayed much smaller fiber-like matter. For the sample with cellulase, the granules of the activated sludge clearly changed, forming a structure with many pores and there was nearly no glutinous mass to be found. The results indicated that enzymatic pretreatment resulted in the degradation of protein and polysaccharide in the activated sludge and affected the sludge structure, thus leading to the floc dispersal.

5. Conclusions

The addition of protease and cellulase led to an increase in CST of activated sludge and the increased value is higher with protease. Decreasing EPS concentration in sludge solids and shifting to smaller particle size were the major reasons for the observed changes in sludge dewatering. Since the effect of protease on EPS and particle size is greater than that of cellulase, the CST of sludge treated with protease increased much more than that with cellulase.

The IR-spectrometry did not show obvious changes of peak absorption compared to the control sample. This means no organic chemicals degraded completely even though both protease and cellulase led to degrading of protein and polysaccharide of EPS. SEM images revealed structural changes at the colloidal level, so, the enzymatic pretreatment could affect the activated sludge structure at a colloidal scale.

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References

[1] J.H. Bruus, P.H. Nielsen, K. Keiding, On the stability of activated sludge flocs with implications to dewatering, Water Res. 26 (12) (1992) 1597–1604.
[2] C.G. Carlson, A.B. Platmanufactur, S. Malmo, Improved filtration of biosludges by enzyme treatment, Filtr. Sep. 16 (1) (1979) 82–86.
[3] L. Thomas, G. Jungschaffer, B. Sproessler, Improved sludge dewatering by enzymatic treatment, Water Sci. Technol. 28 (1) (1993) 189–192.
[4] M. Barjenbruch, O. Kopplow, Enzymatic, mechanical and thermal pretreatment of surplus sludge, Adv. Environ. Res. 7 (3) (2003) 715–720.
[5] A. Ayol, Enzymatic treatment effects on dewaterability of anaerobically digested biosolids—I: performance evaluations, Process. Biochem. 40 (7) (2005) 2427–2434.
[6] M.J. Higgins, J.T. Novak, Characterization of exocellular protein and its role in bioflocculation, J. Environ. Eng.-ASCE 123 (5) (1997) 479–485.
[7] J.I. Houghton, J. Quarmby, T. Stephenson, Municipal wastewater sludge dewaterability and the presence of microbial extracellular polymer, Water Sci. Technol. 44 (2–3) (2001) 373–379.
[8] F.D. Sanin, P.A. Vesilinel, Effect of centrifugation on the removal of extracellular polymers and physical properties of activated sludge, Water Sci. Technol. 30 (8) (1994) 117–127.
[9] L.H. Mikkelsen, K. Keiding, Physio-chemical characteristics of full scale sewage sludges with implications to dewatering, Water Res. 36 (10) (2002) 2451–2462.
[10] L.H. Mikkelsen, K. Keiding, The shear sensitivity of activated sludge: an evaluation of the possibility for a standardised floc strength test, Water Res. 36 (12) (2002) 2931–2940.
[11] M.F. Dignac, V. Urbain, D. Rybacki, A. Bruchet, D. Snidaro, P. Scribe, Chemical description of extracellular polymers: implication on activated sludge floc structure, Water Sci. Technol. 38 (8–9) (1998) 45–53.
[12] Standard Methods for the Examination of Water and Wastewater, 21st ed., [M], American Public Health Association, Washington, DC, 2005, 2710G.
[13] S. Comte, M. Cuibaud, M. Baudu, Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and EPS complexation properties: part I. Comparison of the efficiency of eight EPS extraction methods, Enzyme Microb. Tech. 38 (1–2) (2006) 237–245.
[14] S. Comte, M. Cuibaud, M. Baudu, Relations between extraction protocols for activated sludge extracellular polymeric substances (EPS) and complexation properties of Pb and Cd with EPS: part II. Consequences of EPS extraction methods on Pb^{2+} and Cd^{2+} complexation, Enzyme Microb. Tech. 38 (1–2) (2006) 246–252.
[15] C. Park, J.T. Novak, Characterization of activated sludge exocellular polymers using several cation-associated extraction methods, Water Res. 41 (8) (2007) 1679–1688.
[16] J. Wingender, T.R. Neu, H.C. Flemming, What are bacterial extracellular polymeric substances? in: J. Wingender, T.R. Neu, H.C. Flemming (Eds.), Microbial extracellular polymeric substances: characterization, structure, and function, Springer, Berlin, 1999, pp. 1–19.
[17] H.Y. Pei, W.R. Hu, J. Li, Dewaterability and particle size distribution of activated and digestion sludge Environ. Sci. 28 (10) (2007) 2236–2242.
[18] P.A. Vesilind, Capillary suction time as a fundamental measure of sludge dewaterability, J. Water Pollut. Control Fed. 60 (2) (1988) 215–220.
[19] S. Miklas, Review of recent trends in capillary suction time (CST) dewaterability testing research, Ind. Eng. Chem. Res. 44 (2005) 8157–8163.
[20] D.E. Smiles, Water flows in filter paper and capillary suction time, Chem. Eng. Sci. 53 (12) (1998) 2211–2218.
[21] V. Chaignon, B.S. Lartiges, A.E. Samrani, C. Mustin, Evolution of size distribution and transfer of mineral particles between flocs in activated sludges: an insight into floc exchange dynamics, Water Res. 36 (3) (2002) 676–684.
[22] C.P. Chu, D.J. Lee, B.V. Chang, C.S. You, J.H. Tay, "Weak" ultrasonic pre-treatment on anaerobic digestion of flocculated activated biosolids, Water Res. 36 (11) (2002) 2681–2688.
[23] P.R. Karr, T.M. Keinath, Influence of particle size on sludge dewaterability, J. Water Pollut. Control Fed. 50 (1978) 1911–1930.
[24] S. Na, Y.U. Kim, J. Khim, Physiochemical properties of digested sewage sludge with ultrasonic treatment, Ultrason. Sonochem. 14 (2007) 281–285.
[25] X. Feng, J.C. Deng, H.Y. Lei, T. Bai, Q.J. Fan, Z.X. Li, Dewaterability of waste activated sludge with ultrsound conditioning, Bioresource Technol. 100 (3) (2009) 1074–1081.
[26] B.S. Lartiges, S. Deneux-Mustin, G. Villemin, C. Mustin, O. Borres, M. Chamerois, B. Gerard, M. Babut, Composition, structure and size distribution of suspended particulates from the Rhine river, Water Res. 35 (3) (2001) 808–816.
[27] M. Mecozzi, R. Acquistucci, V. Di Noto, E. Pietrantonio, M. Amici, D. Cardarilli, Characterization of mucilage aggregates in Adriatic and Tyrrhenian Sea: structure similarities between mucilage samples and the insoluble fractions of marine humic substance, Chemosphere 44 (4) (2001) 709–720.

[28] G. Guibaud, N. Tixier, A. Bouju, M. Baudu, Relation between extracellular polymers' composition and its ability to complex Cd, Cu and Pb, Chemosphere 52 (10) (2003) 1701–1710.
[29] C. Garnier, T. Görner, B.S. Lartiges, S. Abdelouhab, P. Donato, Characterization of activated sludge exopolymers from various origins: a combined size-exclusion chromatography and infrared microscopy study, Water Res. 39 (13) (2005) 3044–3054.
[30] H.C. Flemming, J. Wingender, Relevance of microbial extracellular polymeric substances (EPSs)—part I: structural and ecological aspects, Water Sci. Technol. 43 (6) (2001) 1–8.
[31] V. Korstgens, H.C. Flemming, J. Wingender, W. Borchard, Influence of calcium ions on the mechanical properties of a model biofilm of mucoid *Pseudomonas aeruginosa*, Water Sci. Technol. 43 (6) (2001) 49–57.
[32] Y.C. Wu, E.D. Smith, R. Novak, Filterability of activated sludge in response to growth conditions, J. WPCF 54 (5) (1982) 444–456.
[33] P.H. Nielsen, B. Frψlund, K. Keiding, Changes in the composition of extracellular polymeric substances in activated sludge during anaerobic storage, Appl. Microbiol. Biotechnol. 44 (6) (1996) 823–830.
[34] R. Bura, M. Cheung, B. Liao, J. Finlayson, B.C. Lee, I.G. Droppo, G.G. Leppard, S.N. Liss, Composition of extracellular polymeric substances in the activated sludge floc matrix, Water Sci. Technol. 37 (4–5) (1998) 325–333.