Characteristics of Biochemical and Fractal Structure of Activated Sludge with Thermochemical Lysis

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Abstract In this study, we investigated the structural characteristic and biochemical properties of waste-activated sludge after thermochemical pretreatment. The results show that with the increase dosage of hydrochloric acid or sodium hydroxide, the concentration of suspended solid (SS) and volatile suspended solids (VSS) declined, especially at pH 12 + H (“H” means heating). At the same time, soluble chemical oxygen demand (SCOD) all increased as well, especially at pH 12, the greatest lysis effect appeared. Protein and polysaccharide presented a similar law with SCOD. Furthermore, the specific surface area (SSA), two-dimensional fractal dimension (D_2), and three-dimensional fractal dimension (D_3) all increased to a certain degree with acid/alkali pretreatment whether or not heating. Otherwise, the median particle size ($d_{0.5}$) and zeta potential decreased leading to more compact and stable floc structure and reduction effect compared with the original sludge. In Pearson correlation analysis, SSA and SS, SSA and VSS, zeta potential and SCOD, and zeta potential and protein have significant negative

correlations; D_3 and SSA have a significant correlation with SS, VSS, SCOD, and protein. Consequently, measuring the structural parameters D_3 and SSA online can reflect the effects of sludge lysis indirectly, which will be helpful to guide the practical application.

Keywords Sludge · Lysis pretreatment · Structural characteristics · Biochemical parameters · Correlation

1 Introduction

Activated sludge process was the most common and reliable process for wastewater treatment. However, growing amount of waste-activated sludge (WAS) generated from the process has become a pressing problem in recent years, environmentally and economically (Nguyen et al. 2014; Zhou et al. 2014b). Lysis-cryptic growth is a process to minimize the generation of waste-activated sludge (Eastman and Ferguson 1981). Enhancing lysis means that cells of microorganism are destroyed and intracellular matter is released (Zhang et al. 2016). Numerous chemical and physical methods for enhancing sludge lysis have been studied including thermal hydrolysis (Wilson and Novak, 2009), mechanical disintegration (Kampasa et al. 2007), ultrasonic process (Guo et al. 2011), ozonation (Braguglia et al. 2012), acid/alkali (Xie et al. 2014), and hybrid pretreatment (Takashima and Tanaka, 2010).

In the above methods, thermal hydrolysis and acid/alkali treatment are most conventional and economical method for sludge pretreatment as they are easy to

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control, stable in performance, and flexible during operation. Bougrier et al. (2008) reported that 40–60% sludge solubilization could be obtained from thermal treatment of WAS at 170–190 °C for 30 min. Similarly, Wang et al. (2010) suggested that by thermal treatment, the release of biomass materials increased with treatment time (up to 3.0 h) and temperature (at 50, 70, and 90 °C), while the temperature had the more significant influence than the time. pH range from 3.0 to 10.0 was unable to cause substantial releases of organic and nutrient materials; even at a higher pH of 12.0, no apparent reduction of total solid was observed. Besides, Sheng et al. (2008) concluded that alkali pretreatment could reduce sludge quantity well and accelerate the hydroxylation of carbohydrates. Furthermore, Neyens et al. (2003) found the increase of soluble chemical oxygen demand (SCOD) concentration from 510 to 11,000 mg/L in the sludge supernatant after thermal and acid pretreatment, and the biodegradability of sludge has been enhanced greatly. They concluded that the temperature and pH affected the solubilization degree of sludge and the sludge reduction ratio. Xiao and Liu (2008) discussed the impacts of three pretreatments (acid, alkaline, and thermal treatments) on the characteristics of sludge and found that thermal and alkaline treatments could disintegrate the sludge, decrease the particle size ($d_{0.5}$), and increase the specific surface area (SSA) and the consistency of sludge as well.

Since the changes in the morphology of sludge flocs are very significant for lysis performance, researchers have attempted to employ fractal theory (Mandelbrot and Wheeler 1982) for the study of sludge structural characteristics. Fractal dimension (D_f) is used to depict the irregular degree of sludge structure, representing microstructure change of sludge during lysis process. A high D_f value (range from 1 to 3) indicates compact aggregates, whereas a low value corresponds to “looser” aggregates (Britt-Marie et al. 2003). Gao et al. (2010) revealed that D_f provides an approach for quantification of dense and regular level of sludge; furthermore, it could be used to characterize the status of sludge, such as balking. In this study, we use D_f to describe the sludge floc's degree of irregularity to analyze its micromechanism changes, furthermore to estimate the efficiency of lysis process in order to provide a new way for the study of sludge reduction and its mechanism.

Previous studies mainly focused on the disintegration of sludge, and little is known on the changes of

biochemical characteristics and structural characteristics and the relationships between the structural characteristic and biochemical properties of sludge during cell lysis pretreatment, while it is vital to study and understand its impact on sludge reduction performance. Therefore, the effects of structural characteristics and biochemical properties on sludge with thermochemical pretreatment were investigated in this study. At the same time, fractal theory is employed here for the study of sludge fractal structural characteristics in lysis process, incorporating cell biological analysis from a microscopic perspective. Moreover, characterization approach and structure assessment for sludge minimization will be proposed; hence, in-depth theory and technical guidance about sludge reduction can be finally obtained.

2 Materials and Methods

2.1 Sewage Sludge

Sewage sludge sample used in this study was collected from the secondary sedimentation tank of a local sewage treatment plant (in Beijing, China), screened to remove big debris, and precipitated for 0.5 h and then stored at 4 °C. Air aeration activated sludge process is adopted in the sewage treatment plant, with a daily treatment capacity of 1 million cubic meters. The characteristics of raw sludge are presented in Table 1.

2.2 Experimental Equipment and Methods

The raw sludge was treated in a high-pressure (1.5 atm) reactor at 121 °C for 30 min and then cooled. Hydrochloric acid (6 M) or sodium hydroxide (10 M) was added to adjust the pH of the sludge to 2, 4, 6, 7, 8, 10, 11, and 12, with stirring to stabilize the pH for 5 min.

2.3 Biochemical and Structural Characteristic Parameters

Biochemical parameters include suspended solid (SS), volatile suspended solids (VSS), SCOD, protein, and polysaccharide concentration in sludge supernatant. The structural characteristic parameters including $d_{0.5}$, SSA, zeta potential, two-dimensional fractal dimension (D_2), and three-dimensional fractal dimension (D_3) were tested as well.

Table 1 Basic properties of original sludge

Parameter	Water content (%)	pH	SS (g/L)	VSS (g/L)	SCOD (mg/L)	$d_{0.5}$ (μm)
Value	99.25 ± 0.25	7 ± 0.10	4.90 ± 0.05	3.35 ± 0.05	260 ± 9.50	84.49 ± 1.20

SS suspended solid, VSS volatile suspended solids, SCOD soluble chemical oxygen demand, $d_{0.5}$ median particle size

2.4 Measurements of Biochemical and Structural Characteristic Parameters

SS, VSS, and SCOD in this study were measured following standard methods (APHA, 1995). The sludge supernatant was obtained by centrifuging the mixed liquor at 4500 rpm for 15 min; afterwards, it was screened through the 0.45- μm cellulose acetate membrane. The particle size analyzer (Mastersizer 2000, Malvern, UK) was used to inspect particle size and SSA of sludge samples (Yu et al. 2009). The protein content was determined by modified Lowry method (Frølund et al. 1995; Lowry et al. 1951). The polysaccharide content was determined by the phenol-sulfuric method (Dubois et al. 1956). The zeta potential analyzer (Zetasizer 2000, Malvern, UK) was used to inspect zeta potential of sludge samples. D_2 were calculated by image analysis with the software HisArea, based on the box count method. D_3 were calculated from the raw light scattering data from the Malvern Mastersizer/E instrument according to the method by Spicer et al. (1998) and Guan et al. (1998). Each experiment was performed in triplicate, and average values were then obtained.

2.5 Methods of Statistical Analysis

Statistical analysis was conducted using SPSS version 18.0 for Windows. Two-tailed T detection was used to evaluate the correlation between the structural characteristic and biochemical parameters.

3 Results and Discussion

3.1 Effect of pH on Biochemical Properties

3.1.1 Effect of pH on SS and VSS

Total solids (TS) can be divided into soluble solids (DS) and SS. Usually, SS accounted for more than 90% of TS (Wang and Wang 2005). VSS solubilization depicts the trend of sludge becoming an inorganic product, and it is

also used as a sludge reduction indicator (Yu et al. 2008). Therefore, the change of SS and VSS plays a decisive role in the change of sludge characteristics.

The acid treatment method is efficient to dissolve the amphoteric substances in EPS and destroy the sludge floc structure (Du 1992; Zhou 1993). While the thermal treatment is observed to disrupt the chemical bonds of the cell wall and membrane, denatured biologic macromolecules such as protein and nucleic acid thus solubilize the cell components to achieve sludge reduction. As shown in Fig. 1, during acid/alkali treatment, the concentration of SS and VSS declined with the increase dosage of hydrochloric acid or sodium hydroxide, whether or not heating, especially at pH 12 + H ("H" means heating). Since thermal-alkali combined pretreatment can generate the synergistic effect to accelerate hydrolysis process of protein and nucleic acids and destroy the enzymatic and structure of cell, resulting in the hydrolysis of cell wall and membrane, hence, the solubility of organic matter contained in the microbial cells is increased, so the amount of sludge decreased. The results were reflective of a fact that proper amounts of acid and alkali are both capable of dissolving microbial cells in sludge, and the stronger is acid or alkali, the faster is the rate and more thorough is cell lysis. This result was in agreement with another work (Do et al. 2009), which observed that thermochemical sludge treatment is

Fig. 1 Effect of pH on suspended solid (SS) and volatile suspended solid (VSS)

Fig. 2 Effect of pH on soluble chemical oxygen demand (SCOD)

capable of disrupting the sludge floc and cell, releasing the organic matter into soluble phase and accelerating sludge degradation.

3.1.2 Effect of pH on SCOD

The SCOD value was used to evaluate the efficiency of sludge disintegration. Organic matter hydrolysis, protein, and carbohydrate in solid organic matter gradually released into liquid phase during thermal pretreatment. Furthermore, organic matter of sludge cells dissolves and transfers into soluble small molecule organic matter in liquid phase. Thermal treatment has an obvious promotion on the release of SCOD in sludge. As shown in Fig. 2, whether acid, alkali, or thermal treatment, the concentration of SCOD increased, especially at pH 12 the most effective of sludge lysis appeared. These results are in agreement with Xiao and Liu (2006), who

Fig. 3 Effect of pH on protein

Fig. 4 Effect of pH on polysaccharide

observed that the pH is a marked factor of affecting the release of SCOD and sludge reduction.

3.1.3 Effect of pH on Protein and Polysaccharide

Proteins and polysaccharides are two of the most predominant organic components of sludge. Therefore, the measurements of proteins and polysaccharides after cell lysis pretreatment can provide a more complete understanding of the influences of cell lysis pretreatment on sludge disintegration. When sludge disintegration methods are applied, microbial cells undergo lysis or death during which cell contents (protein and polysaccharide) are released. Besides, the granular materials of sludge were transformed into soluble polysaccharide and protein. Protein and SCOD presented similar law. Similarly, protein was increased in different degree after acid, alkali, and thermal pretreatment. As the pH increases, protein of the supernatant increased rapidly

Fig. 5 Effect of pH on D_3 ("H" means heating)

Fig. 6 Effect of pH on D_2

and reached the maximum at pH 12 (Fig. 3), meaning that alkali pretreatment may be more effective to destroy microbial cell walls and dissolve sludge. Meanwhile, a great quantity of polysaccharides is released after acid/alkali pretreatment and released further when heating (Fig. 4), indicating that heating conditions were more conducive for the dissolution of polysaccharides compared to acid/alkali pretreatment.

3.2 Effect of pH on Structural Characteristics

3.2.1 Effect of pH on Fractal Dimension

As shown in Fig. 5, during acid/alkali treatment, whether or not heating, D_3 increased with the increasing of pH value, and the structure of sludge floc becomes more compact, further with thermal treatment. Acid treatment with lower pH (pH 4, pH 6) can increase the density of sludge, while D_3 decreased significantly at pH 2. This result reflected a fact that acid and thermal treatment

Fig. 7 Effect of pH on $d_{0.5}$ and SSA

Fig. 8 Effect of pH on zeta potential

(except for pH 2 and pH 4 + H) can also increase D_3 . The highest value of D_3 is achieved at pH 6 and pH 6 + H, at which can enhance the degree of sludge density remarkably. Besides, D_3 decreased remarkably at pH 12 + H, indicating that thermal-alkali pretreatment affect the structure of floc by restructuring the floc morphology.

Figure 6 showed that D_2 increased to a certain extent with acid/alkali pretreatment compared with the original sludge whether or not heating. It worth noting that contrary to D_3 , D_2 increased at pH 12 + H instead of decrease. This result suggested that there is no comparability between D_2 and D_3 due to the different expression of meanings. In fact, D_3 reflects the level of irregularity of sludge floc in space while D_2 is measured on the plane. This suggests that only one fractal dimension is insufficient to describe the floc structure (Britt-Marie et al. 2003).

3.2.2 Effect of pH on $d_{0.5}$ and SSA

Activated sludge is a typical microbial flocculation. The size distribution of the particles was formed with the increasing of the size of the particles during the formation of sludge. Figure 7 presents that as the pH increase, $d_{0.5}$ of sample by alkali treatment alone showed downward trend and decreased further with heat treatment. $d_{0.5}$ reached the minimum as 52.696 μm at pH 12 + H. Besides, the $d_{0.5}$ value dramatically increased at pH 2 and pH 4 + H, while it significantly decreased to 63.040 μm on the condition of pH 2 + H. This is because of the disruption of the sludge's floc structure and the complete breaking of the sludge cell under the condition of heating and strong acid/alkali.

Table 2 Pearson's correlation coefficient among the biochemical parameters of sludge

	SS	VSS	SCOD	Protein	Polysaccharide
SS	—	—	—	—	—
VSS	0.973 ^a	—	—	—	—
SCOD	-0.432	-0.470	—	—	—
Protein	-0.545	-0.558	0.970 ^a	—	—
Polysaccharide	-0.540	-0.465	0.266	0.392	—

^aCorrelation is significant at the 0.01 level (two-tailed)^bCorrelation is significant at the 0.05 level (two-tailed)

Figure 7 also indicated that alkali treatment alone has little effect on SSA of sludge, while SSA showed a downward trend with the rising of pH values especially on the condition of pH 12 + H because of the obvious decrease of $d_{0.5}$. However, acid treatment has little effect on SSA alone, except a slight decrease at pH 2. SSA increased significantly at pH 2 + H. All of the above revealed that there is a strong positive correlation between SSA and $d_{0.5}$.

3.2.3 Effect of pH on Zeta Potential

The presence of ionizable groups such as carboxyl, phosphate, and amino groups, in the EPS and cell surfaces is responsible for the density of surface charge. As shown in Fig. 8, with the increase of pH, zeta potential decreased and dropped further at pH 12, at which the sludge becomes more stable. This is probably because of variations in the degree of dissociation of weakly ionizable groups on sludge surfaces with respect to the pH (Liao, 2000). Furthermore, the influence of amino groups on the surface charge is more complicated. At a low pH value, amino groups (R-NH_2) tend to make the surface more positively charged by absorbing H^+ to form R-NH_3^+ , and this explains why zeta

potential increased with the decrease of pH (Zhu et al. 2008). Zeta potential closes to 0 at pH 2 and pH 4 + H, and the sludge particles are very unstable and easy to agglomerate into large particles, which verified the previous measurement results of $d_{0.5}$ (see in Fig. 7).

3.3 Correlation Analysis

3.3.1 Correlations Between the Biochemical Parameters

Correlation analysis between the biochemical parameters of sludge was showed in Table 2. The result suggested that there were significant positive correlations between SS and VSS, protein, and SCOD as well.

3.3.2 Correlations Between the Structural Characteristic Parameters

Correlation analysis between the structural characteristic parameters of sludge was showed in Table 3. It revealed that SSA and $d_{0.5}$, D_3 , and SSA have a significant negative correlation, respectively, while Table 4 indicated no obvious correlation between D_2 and other structural characteristic parameters.

Table 3 Pearson's correlation coefficient between D_3 and other structural characteristic parameters

	$d_{0.5}$	SSA	Zeta	D_3
$d_{0.5}$	—	—	—	—
SSA	-0.787 ^a	—	—	—
Zeta	0.268	-0.287	—	—
D_3	0.346	-0.815 ^a	0.099	—

^aCorrelation is significant at the 0.01 level (two-tailed)^bCorrelation is significant at the 0.05 level (two-tailed)**Table 4** Pearson's correlation coefficient between D_2 and other structural characteristic parameters

	$d_{0.5}$	SSA	Zeta	D_2
$d_{0.5}$	—	—	—	—
SSA	-0.815 ^a	—	—	—
Zeta	0.158	-0.263	—	—
D_2	-0.267	0.427	-0.492	—

^aCorrelation is significant at the 0.01 level (two-tailed)^bCorrelation is significant at the 0.05 level (two-tailed)

Table 5 Pearson's correlation coefficient between D_3 and biochemical parameters

		SS	VSS	SCOD	Protein	Polysaccharide	D_3
Non-heating	D_3	0.810 ^a	0.255	0.217	0.155	-0.271	–
Heating	D_3	0.974	0.972	-0.888	-0.885	-0.441	–

^a Correlation is significant at the 0.01 level (two-tailed)

^b Correlation is significant at the 0.05 level (two-tailed)

3.3.3 Correlations Between D_f and the Biochemical Parameters

Correlation analysis between D_3 and the biochemical parameters of sludge in Table 5 showed that D_3 and SS have significant positive correlation and no obvious correlation between D_3 and other biochemical parameters under the condition of non-heating. Besides, D_3 and other biochemical parameters have a significant correlation except the polysaccharide when heating, while the result of correlation analysis between D_2 and biochemical parameters of sludge in Table 6 showed that there is no obvious correlation between them.

3.4 Classification of Correlation Analysis

According to the results of correlation analysis above, among structural characteristic parameters, SSA and D_3 and SSA and $d_{0.5}$ have significant negative correlations. Among biochemical parameters, VSS and SS and protein and SCOD have significant positive correlations. SSA and SS, SSA and VSS, zeta and SCOD, and zeta and protein have significant negative correlations, while D_3 and SS, D_3 , and VSS have significant positive correlations among structural characteristics and biochemical parameters of sludge.

In addition, classifying the results of correlation analysis according to positive and negative correlation of the parameters revealed that VSS and SS, protein and SCOD, D_3 and SS, and D_3 and VSS have significant positive correlations, while SSA and $d_{0.5}$, SSA and SS, SSA and VSS, SSA and D_3 , zeta and SCOD, zeta and protein, D_3 and SCOD, and D_3 and protein have

Table 6 Pearson's correlation coefficient between D_2 and biochemical parameters

	SS	VSS	SCOD	Protein	Polysaccharide	D_2
D_2	-0.335	-0.47	0.257	0.199	-0.088	–

significant negative correlations. It is worth noting that structural characteristics D_3 and SSA have obvious correlations with SS, VSS, SCOD, and protein. The cell lysis effect is usually related to the decrease of SS and VSS and the increase of SCOD and protein as well. Therefore, D_3 and SSA can be used to reflect the effect of sludge lysis indirectly, which can be measured online to guide practical application.

4 Conclusions

1. With the increase of acid and alkali dosage, SS and VSS showed downward trend and significant decrease at pH 12 + H. SCOD increased regardless of acid, alkali, or thermal treatment. Alkali treatment induced the highest release of SCOD at pH 12, which is beneficial in sludge lysis. Protein showed similar changes in the law with SCOD. Meanwhile, polysaccharide of sludge dissolved extensively after alkali treatment and increased further with thermal treatment. Acid/alkali and thermal treatments are observed to accelerate disintegration of the cell wall and enhance the release of intracellular substance.
2. Whether or not heating, both D_2 and D_3 of sludge increased to a certain degree in acid and alkali treatment. The $d_{0.5}$ of sample by alkali treatment alone showed downward trend and decreased further with heat treatment. The particle size of sludge usually increased in the acid treatment, except an obvious decrease at pH 2 + H. While as the pH increased, SSA showed increasing tendency and the most significant value appeared at pH 12 + H in thermal/alkali treatment. SSA by acid treatment alone performed little impact except a slight decrease at pH 2. Besides, SSA showed a correlation with particle sizes. Normally, the smaller is the particle size, the larger is the SSA. With the increase of pH, zeta potential increased and the sludge became more stable.

3. Among the correlation analysis of structural characteristic and biochemical parameters of sludge, SSA and SS, SSA and VSS, zeta and SCOD, as well as zeta and protein have significant negative correlations, while structural characteristic parameters (D_3 and SSA) of sludge have obvious correlations with SS, VSS, SCOD, and protein. Consequently, measuring D_3 and SSA online can be used as parameters reflecting the effects of sludge lysis in order to guide the practical production.

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