



Effect of proteins, polysaccharides, and particle sizes on sludge dewaterability

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Abstract

Four batch experiments of hydrolysis and acidification were carried out to investigate the distributions of proteins (PN) and polysaccharides (PS) in the sludge, the PN/PS ratio, the particle sizes, and their relationship with sludge dewaterability (as determined by capillary suction time, CST). The sludge flocs were stratified through centrifugation- and ultrasound-based method into four fractions: (1) slime, (2) loosely bound extracellular polymeric substances (LB-EPS), (3) tightly bound EPS (TB-EPS), and (4) pellet. The results showed that PN was mainly partitioned in the pellet (80.7%) and TB-EPS (9.6%) fractions, while PS distributed evenly in the four fractions. During hydrolysis and acidification, PN was transferred from the pellet and TB-EPS fractions to the slime fraction, but PS had no significant transfer trends. The mean particle sizes of the sludge flocs decreased with hydrolysis and acidification. The pH had a more significant influence on the dewaterability of sludge flocs than temperature. Sludge dewaterability during hydrolysis and acidification processes greatly deteriorated from 9.7 s at raw sludge to 340–450 s under alkaline conditions. However, it was just slightly increased under acidic conditions. Further investigation suggested that CST was affected by soluble PN, soluble PN/PS, and particle sizes of sludge flocs, but was affected slightly by total PN, PS, or PN/PS in the whole sludge flocs and other fractions (except slime).

Key words: dewaterability; extracellular polymeric substances; particle size; polysaccharides; proteins; sewage sludge

Introduction

Improper disposal of the large amounts of sludge generated a significant threat to ecological systems during wastewater treatment poses (Rai *et al.*, 2004). Sludge dewatering has been proven to be an effective method to reduce the volume of sludge. The dewaterability of sludge was dependent on several factors, such as particle size, proteins (PN) content, polysaccharides (PS) content, and the PN/PS ratio in extracellular polymeric substances (EPS) and a quantity of EPS (Veeken and Hamelers, 1999; Houghton *et al.*, 2001; Houghton and Stephenson, 2002; Rosenberger and Kraume, 2002; Liu and Fang, 2003). However, controversy remains concerning the extracellular organic characteristics that influence sludge dewaterability, owing to the complex dynamics of the sludge matrix.

To better understand the factors influencing sludge dewaterability, the structure of sludge flocs should be further well-defined. EPS and cells form bioaggregates, such as biofilms, sludge flocs, and river snow (Nielsen and Jahn, 1999). EPS in sludge flocs is composed of soluble EPS (slime) and bound EPS. The latter exhibits a dynamic double-fractioned structure and is made up of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS)

(Poxon and Darby, 1997; Ramesh *et al.*, 2006; Li and Yang, 2007). After the EPS is extracted, the cells in the residues form a pellet (Yu *et al.*, 2007, 2008). Hence, from the outer surfaces to the cores of the granules, the sludge flocs possess a multi-fractioned structure consisting of slime, LB-EPS, TB-EPS, and pellet. To the best of our knowledge, the chemical compositions of these fractions and of sludge as a whole have not been studied with respect to the dewaterability of sludge flocs.

Hydrolysis and acidification can convert the complex organic substances in sludge flocs into volatile fatty acids (VFA), which can further be used as an energy and carbon source for biological nutrient removal (Barnard, 1983; Elefsiniotis and Oldham, 1994). Meanwhile, the EPS content of sludge flocs during hydrolysis and acidification process changes with time. It is believed that the sludge flocs are broken first and then EPS of the sludge flocs is released into the bulk solution in the hydrolysis and acidification process. The released EPS will affect the sludge dewaterability. To date, however, a few investigators studied the influence of hydrolysis and acidification process on dewaterability. The objectives of this article are to investigate the changes of PN, PS, and particle sizes in different fractions of sludge flocs and to explore their influences on the sludge dewaterability.

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1 Materials and methods

1.1 Sludge sample

The activated sludge sample was collected from the aerated basin of a municipal wastewater treatment plant (WWTP) in Shanghai, China. The plant treats 75000 m³/d of wastewater (93% from domestic and 7% from industrial origins) using anaerobic-anoxic-oxic process. The collected samples were transported to a laboratory within 30 min after sampling. The sludge was first settled for 1.5 h at 4°C with the supernatant being collected as bulk solution. The sludge sediments were also collected and subsequently screened through a 1.2-mm screen with the characteristics listed in Table 1.

1.2 Operation of reactors

Four batch experiments of hydrolysis and acidification were carried out using airtight vessels with a working volume of 1.5 L. For each vessel, 1 L of the sludge sediment (Table 1) pH was adjusted to 5.5 or 10.0 by 2 mol/L HCl or 2 mol/L NaOH. Oxygen in the vessels was removed from the headspace by nitrogen gas (N₂) sparging for 2 min. The vessels were then capped with rubber stoppers and placed in an air-bath shaker (100 r/min) for 20 d. For vessels the pH was adjusted to 5.5 or 10.0 every day, and the temperature was maintained at 37°C for two of them and at 55°C for another two. During the operation, no gas production was detected. In the first 5 days of operation, the variations of pH every day were less than 2. After 5-day operation, the pH every day was almost unchangeable. The sludge samples 100 mL were collected every five days, and used for the stratification extraction and other analysis.

1.3 Stratification protocol

Sludge stratification protocol was modified according to the previously described method (Yu *et al.*, 2007, 2008). In brief, the sludge sample was centrifuged at 2000 ×g for 15 min. The solution was collected as the slime, i.e., the fraction that could be removed by soft centrifugation. The bottom sediments were collected and re-suspended to their original volumes using a buffer solution (pH 7) consisting of Na₃PO₄, NaH₂PO₄, NaCl, and KCl at the molar ratio of 2:4:9:1. The conductivities of the buffer were then adjusted using a distilled water to about 331 μS/cm as of the sludge sediment samples. The suspensions were centrifuged again at 5000 ×g for 15 min and the bulk solution and solid phase were collected separately. The organic matter in the bulk solution was the LB-EPS. The collected sediments were re-suspended with the aforementioned buffer solution to the original volumes and then extracted by subjecting

the solution to ultrasound at 20 kHz and 480 W for 10 min. The extracted solutions were centrifuged at 20000 ×g for 20 min. The organic matter in the bulk solution was the TB-EPS, and the residues (solid phase) were again re-suspended with the buffer solution to the original volumes. This fraction comprised the pellet.

1.4 Sludge characterization assay

All chemical analyses were carried out in duplicate using chemicals of analytical grade. PN was determined by the modified Lowry method (Frølund *et al.*, 1995), using casein (Shanghai Sangon Biotechnology Co., Ltd., China) as the standard. PS was measured by the Anthrone method (Gaudy, 1962), with glucose as the standard. The COD and SCOD were analyzed using a HACH DR/2000 Spectrometer (Hach company, USA). Conductivity was determined by a conductivity meter (DDSJ 308A, Leici Co., Ltd., Shanghai, China). Other parameters, including TSS and VSS, were analyzed following the standard methods (APHA *et al.*, 1998).

The dewaterability of the sludge flocs and four fractions was measured with a CST instrument (Model 319, Triton, UK) equipped with an 18-mm diameter funnel and Whatman No. 17 chromatography-grade paper.

The mean particle size of the sludge flocs was determined by an EyeTech instrument (Ankersmid, USA) with a 300-mm lens, which enables the measurement of particles in the range 0.1–1000 μm. The sample was diluted in filtrated effluent (0.45-μm PTFE membrane) to avoid multiple scattering and measured three times.

Statistical analysis was carried out using the software SPSS version 11.0 for Windows (SPSS, Chicago, IL, USA). Pearson's correlation coefficient (*R*) was used to evaluate the linear correlation between two parameters. The Pearson's coefficient is always between −1 and +1, where, −1 denotes a perfect negative correlation, +1 denotes a perfect positive correlation, and 0 denotes the absence of a relationship. The correlations were considered statistically significant at a 95% confidence interval (*p* < 0.05).

2 Results

2.1 PN and PS during hydrolysis and acidification processes

Figures 1 and 2 present the variations of PN and PS with time during the four hydrolysis and acidification processes. In the raw sludge flocs, the PN and PS contents were 667 ± 99.7 and 95.6 ± 14.3 mg/g VSS, respectively. The results showed that PN was the major component in the sludge flocs, accounting for about 66.6% of the organic matters in

Table 1 Characteristics of the activated sludge after settlement

pH	TSS (mg/L)	VSS (mg/L)	COD (mg/L)	SCOD (mg/L)	CST (s)	PN/VSS (mg/g)	PS/VSS (mg/g)	Conductivity (μS/cm)
6.4 ± 0.1	7.1 ± 0.4	6.7 ± 0.0	9100 ± 380	94 ± 5	9.7 ± 0.6	670 ± 99	96 ± 14	331 ± 14

TSS: total suspended solids; VSS: volatile suspended solids; COD: chemical oxygen demand; SCOD: soluble COD; CST: capillary suction time; PN: proteins; PS: polysaccharides.

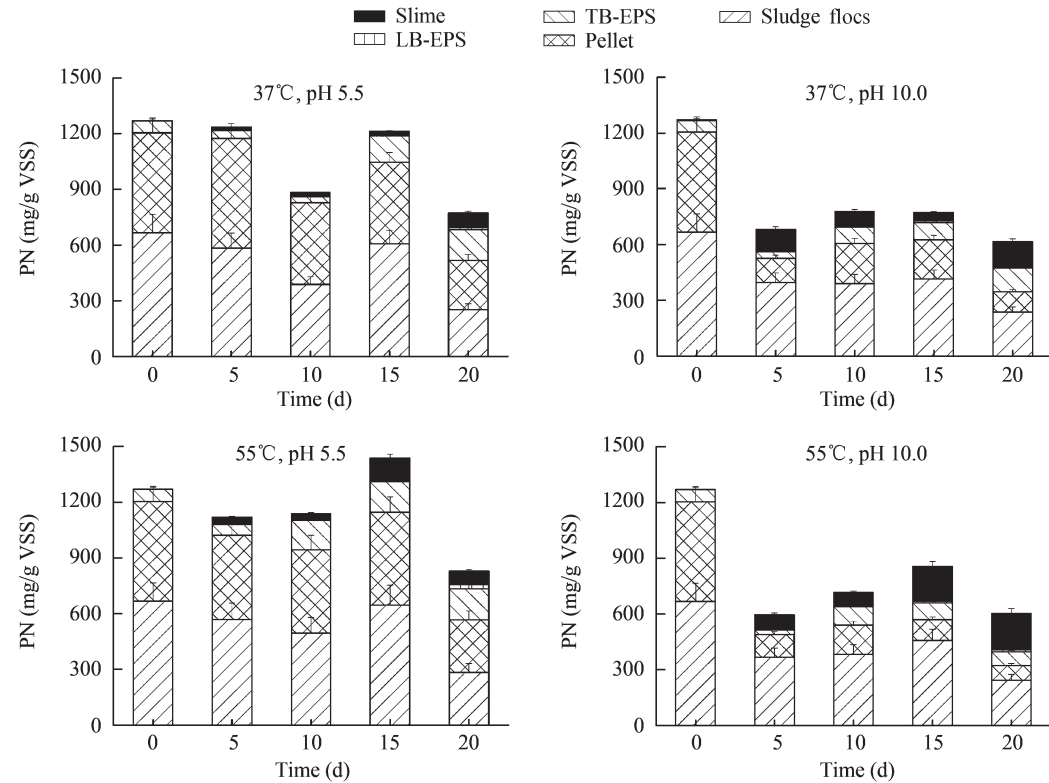


Fig. 1 Variations of proteins (PN) with time during the hydrolysis and acidification processes. Error bars represent the standard deviation of duplicates.

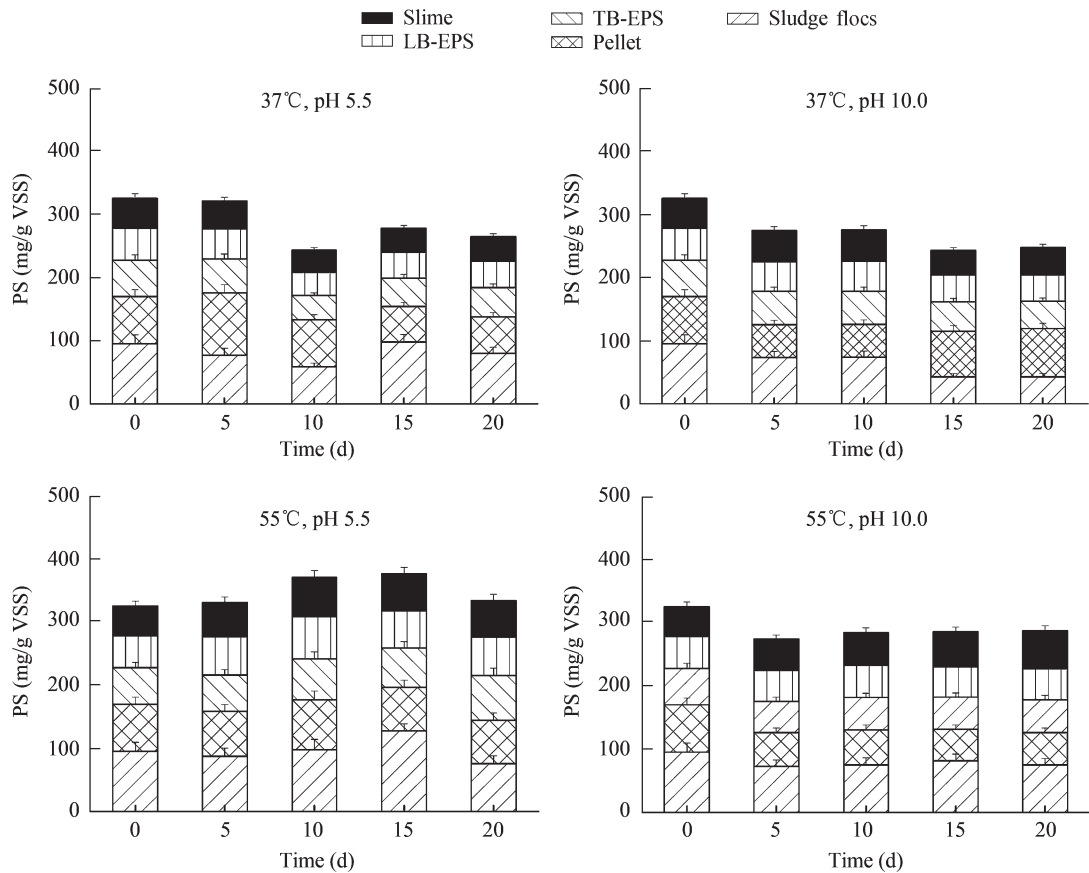


Fig. 2 Variations of polysaccharides (PS) with time during the hydrolysis and acidification processes. Error bars represent the standard deviation of duplicates.

the sludge flocs. PN was mainly (80.7%) distributed in the pellet, less (9.6%) in the TB-EPS, and virtually not in the LB-EPS and slime. As for PS, it distributed evenly in the different fractions of the sludge flocs.

It was observed that more PN in the sludge flocs degraded than PS during the four hydrolysis and acidification processes. At 55°C, considerably more PN was degraded than 37°C under the same pH condition. Meanwhile, at pH 10.0, the degradation rate of PN was faster than pH 5.5 under the same temperature. As for PN in different fractions of sludge flocs, it decreased in the pellet while it increased in the TB-EPS and slime fractions, suggesting that PN was transferred from the inner fraction (pellet) to the outer fractions (TB-EPS and slime) after hydrolysis and acidification. In other words, PN was originally embedded in the core of sludge flocs by the EPS matrix, while during hydrolysis and acidification processes, it was released to the brim of the broken sludge flocs. In contrast, there was no clear trend for PS transferring in the different fractions of the sludge flocs. The phenomenon may be owing to that the production of PS was a dynamic balance during the growth of bacteria (Chen *et al.*, 2007).

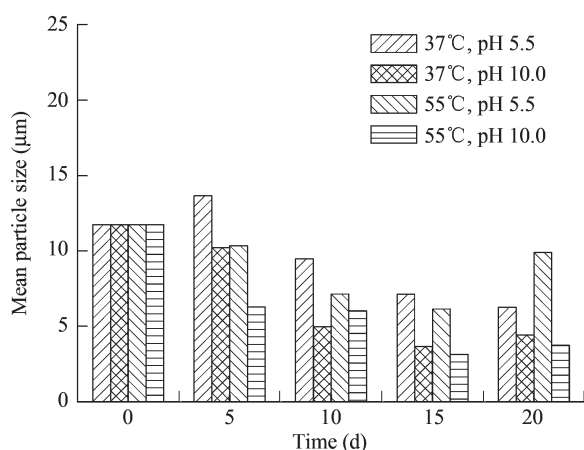
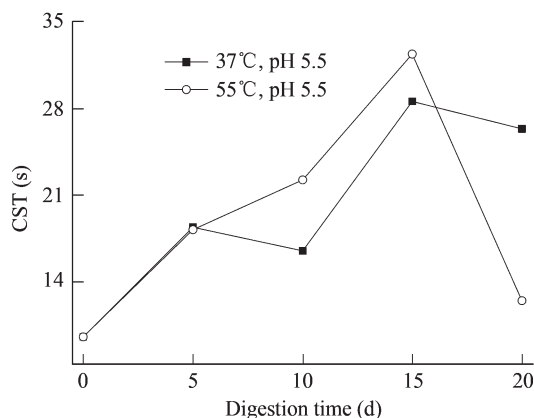


Fig. 3 Mean particle sizes of sludge flocs with time during the hydrolysis and acidification processes.



2.2 Particle sizes during the hydrolysis and acidification processes

Figure 3 shows that the particle sizes of the sludge flocs all decreased with time. It was noted that pH had a more significant influence on the mean particle sizes of the sludge flocs than temperature. At pH 10.0, the mean particle sizes were smaller than pH 5.5. Comparing the variations of PN and PS (Figs. 1 and 2), it can be inferred that at pH 10.0, the solubilization of sludge flocs may be better than at pH 5.5.

2.3 Sludge dewaterability during the hydrolysis and acidification processes

Figure 4 depicts the variations of CST during the four hydrolysis and acidification processes. All four processes led to the CST increment to some extents, suggesting that the sludge dewaterability was deteriorated during the hydrolysis and acidification processes. It was observed that pH played a more important role on the CST of the sludge flocs than temperature. At pH 10.0, the CST climbed rapidly from 9.7 s for the raw sludge to about 340–450 s after 5 days of hydrolysis and acidification. Then, it reached a plateau value until 20 days. However, at pH 5.5, the CST value increased slightly during the hydrolysis and acidification processes. The difference can be owing to that under alkaline condition, the release of organic matters (mainly PN and PS) in the sludge flocs was higher than under acidic condition (Figs. 1 and 2). Therefore, the variations of PN may play an important role in the dewaterability of the sludge flocs.

Table 2 Coefficients of Pearson correlation between PN, PS, PN/PS, and CST ($n = 17$)

Sludge fraction	CST		
	PN	PS	PN/PS
Slime	0.63**	0.07	0.67**
LB-EPS	0.14	-0.29	0.25
TB-EPS	-0.29	-0.37	-0.17
Pellet	-0.86**	-0.54*	-0.86**
Sludge flocs	-0.46	-0.53*	0.03

** $p < 0.01$ (2-tailed); * $p < 0.05$ (2-tailed).

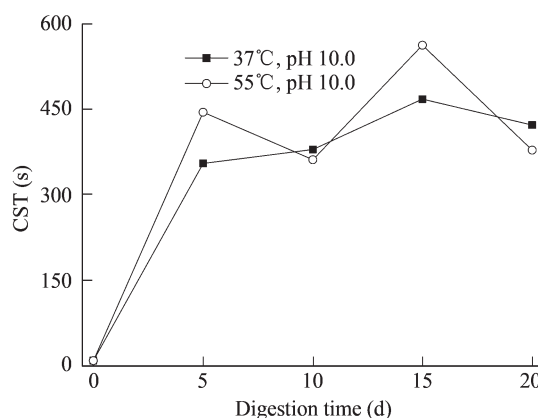


Fig. 4 Variations of capillary suction time (CST) during the hydrolysis and acidification processes.

3 Discussion

3.1 Effects of PN, PS, and PN/PS on CST

Table 2 shows the Pearson correlations between PN, PS, PN/PS of the different fractions in the sludge flocs and the CST, respectively. It was noted that CST had a significant positive correlation ($R > 0.63$, $p < 0.01$) with PN and PN/PS in the slime fraction. In contrast, it correlated poorly ($R < -0.46$, $p > 0.05$) with PS in the slime fraction, with PN, PS, and PN/PS in the LB-EPS and TB-EPS fractions, and with PN and PN/PS in the sludge flocs. Additionally, negative correlation ($R > -0.53$, $p < 0.05$) was found between sludge dewaterability and PN, PS, PN/PS in the pellet, and PS in sludge flocs. These statistical results suggested that the increase of soluble organic matters (mainly PN in the slime) could result in the deterioration of sludge dewaterability, while the increase of organic matters in the pellet could improve the dewaterability. In other words, the sludge dewaterability will not deteriorate if PN is embedded in the inner fraction of the sludge flocs.

As seen in Figs. 1 and 4, at pH 10.0, more organic matters in the sludge flocs were degraded than at pH 5.5, but led to poorer dewaterability. Under the same pH, the hydrolysis and acidification processes at 55°C could degrade more organic matters in the sludge flocs, while led to poorer dewaterability than that at 37°C. Therefore, if the aim was the degradation of sludge flocs, it was better to select the thermophilic process at pH 10.0. Additionally, the sludge dewaterability in the mesophilic process at pH 5.5 was just slightly deteriorated.

Li and Yang (2007) reported that the amount of LB-EPS had a more significant influence on the performance of sludge dewatering (as determined by specific resistance to filtration, SRF) than that of TB-EPS. In their study, however, the decanted part before EPS extraction was corresponded to the slime in this study. Murthy and Novak (1999) found that the release of PN and PS in the flocs to the bulk solution under lower divalent cations associated with the worsening of dewatering property. Our results revealed that PN in the slime fraction rather than in the LB-EPS or TB-EPS fraction was a predominant factor that influenced sludge dewaterability. The different results could in part be owing to the difference of the EPS extraction method applied. It was worthy to note that the decanted part before EPS extraction (i.e., slime) in all the previous studies had an important impact on sludge dewaterability. During the hydrolysis and acidification processes, PN in the inner fraction of the sludge flocs could become soluble and release to slime owing to the effect of alkaline pretreatment, thus worsening the sludge dewaterability. The deterioration reasons may be that PN in slime fraction had a more strong water binding capability and more contribution to the sludge dewaterability than in the other fractions.

3.2 Effects of particle size on CST

The particle size of sludge flocs was another parameter affecting the sludge dewaterability (Karr and Keinath,

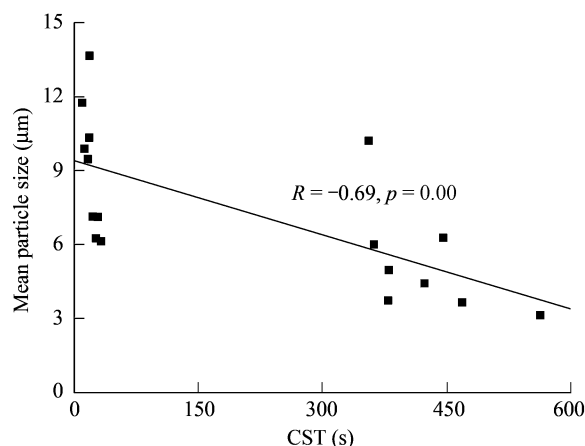


Fig. 5 Pearson correlation between particle sizes of sludge flocs and CST.

1978). Figure 5 shows the Pearson correlation between the mean particle sizes of the sludge flocs and the CST, which expressed the filterability of sludge flocs. Significant correlation between the mean particle size and the CST was found, suggesting that the particle sizes of sludge flocs had an important effect on the sludge dewaterability. The result was in accordance with the earlier conclusion (Karr and Keinath, 1978; Novak *et al.*, 1988). The sludge dewaterability in their studies was determined by specific resistance filterability (SRF), which expressed the compactibility of sludge flocs. Higgins and Novak (1997) indicated that the “supracolloidal” particles in the range 1–100 μm had the greatest effect on the dewaterability of sludge flocs. In this study, all the particle sizes belonged to the “supracolloidal” particles, and they had a marked influence on the dewaterability. Compared with the variations of PN (Fig. 1), it could be seen that the relationship between sludge dewaterability and its particle size consisted with that of PN. It could infer that the decrease of particle size during the hydrolysis and acidification processes could mainly attribute to the degradation of PN.

4 Conclusions

PN was the main component of the sludge flocs, which did not distribute uniformly in the four different fractions of the sludge flocs. PN was mainly distributed in the pellet, less in the TB-EPS, and virtually not in the LB-EPS and slime. However, the distribution of PS was relatively uniform during the four processes and no significant transfer trend existed.

During the hydrolysis and acidification processes in the sludge, the pH had a more significant influence on the degradation of the sludge flocs than temperature. Under alkaline condition, more PN was transferred from the pellet and TB-EPS fractions to the slime fraction during hydrolysis and acidification, and subsequently led to a significant increment of the CST value, thus deteriorating the dewaterability of the sludge flocs.

The pH had considerably more significant influence on the dewaterability of the sludge flocs than temperature.

Sludge dewaterability during hydrolysis and acidification processes was greatly deteriorated under alkaline conditions. However, it was just slightly increased under acidic conditions. Sludge dewaterability was affected by soluble PN, soluble PN/PS, and particle size, but virtually not affected by total PN, PS, or PN/PS in the sludge flocs and other fractions (except slime). Increase of organic matters in the slime fraction could result in the deterioration of sludge dewaterability, while the increase of organic matters in the pellet fraction could make the dewaterability good.

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