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Effect of C/N ratio on extracellular polymeric substances (EPS) and physicochemical properties of activated sludge flocs

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ABSTRACT

The influences of C/N ratio on the extracellular polymeric substances (EPS) and physicochemical properties of the activated sludge flocs were investigated using laboratory-scale sequencing batch reactors (SBRs). Flocs sizes decreased when C/N ratio increased from 20 to 100 and decreased from 20 to 4. The amount of total EPS, TB-EPS, and the carbohydrate and protein contents in TB-EPS were independent of the C/N ratio. In LB-EPS, the protein content increased and the carbohydrate content decreased at decreased C/N ratio, whereas the protein content decreased and the carbohydrate content increased at increased C/N ratio. Effluent suspended solids (ESS) content, turbidity, sludge volume index (SVI), capillary suction time (CST), and specific resistance to filtration (SRF) increased when the C/N ratio decreased, indicating poor flocculation, settleability and dewaterability of the flocs. However, when the C/N ratio increased, only ESS content, SVI and CST value increased. These properties of the flocs were deteriorated greatly under decreased C/N ratio as compared to increased C/N ratio. The characteristics of the flocs could be recovered when C/N ratio returned to the original value. Only the content of protein in LB-EPS was positively correlated with the flocculation, settleability and dewaterability of the flocs.

1. Introduction

The activated sludge process is one of the major biological wastewater treatment techniques. The efficiency of the process depends, first, upon the growth of metabolically capable microorganisms and, second, upon the efficient separation of these organisms from the treated effluent. The bioflocculated microbial aggregates, known as activated sludge flocs, are the essential components of the process. It is, therefore, an undisputed fact that the physicochemical characteristics of activated sludge flocs play an important role in the performance of the process. Indeed, almost all the performances of the activated sludge process are related to or dependent on the physicochemical properties of the flocs, including flocculation, sludge settling and sludge dewatering.

Extracellular polymeric substances (EPS) which are the representative components of the flocs, originate from metabolism or lysis of microorganisms and the wastewater itself, and are made up of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) [1,2]. EPS are considered to determine the physicochemical properties of the flocs and give the floc its structural and functional integrity. It is reasonable to assume that EPS play a leading role in flocculation, settling and dewatering properties of the flocs. The exact role in the floc formation is not well understood, but previous studies indicated that they are important for flocculation to occur [3]. In addition to, the precise role of EPS in biosolid–liquid separation is not well understood. EPS are highly hydrated and may contain a high level of water in the sludge. There does appear to be a relationship between the quantity of EPS in the sludge and the sludge dewaterability, and an increase in the level of EPS present causes the sludge become more difficult to be dewatered [4].

The nature and content of EPS are sensitive to the operational and environmental conditions. If certain culture condition is induced, then the nature of the polymers changes accordingly. Nutritional parameters, such as C/N ratio, affect the microbial physiology, thereby affect the nature and content of EPS of the sludge. Therefore, the C/N ratio of the influent in the activated sludge systems can be important determinant of how microorganisms flocculate, settle and dewater. However, the dynamics of EPS production under different C/N ratio has not been well characterized [5]. Furthermore, there is limited information available on the effect of operating condition, especially C/N ratio of the feed to activated sludge system, on the physic-

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ochemical properties of activated sludge flocs. Thus, the effects of C/N ratio on the sludge handling property in relation to bioflocculation, settleability and dewaterability also need to be investigated.

Even though there are many studies on these physicochemical properties in the literature, they are not comparable due to different types of reactors and other operating conditions. Considering that the physiology is determined by many nutritional parameters such as C/N ratio of the influent, and that there is not a consensus on the correlations between EPS and bioflocculation, settleability and dewaterability of the sludge, this study aims to characterize the chemical constituents (LB-EPS, TB-EPS, protein and carbohydrate contents in EPS) in activated sludge flocs from laboratory-scale sequencing batch reactors (SBR) and sludge physicochemical properties under different C/N ratio, and to evaluate the effect of C/N ratio on the flocculating ability, settleability and dewaterability of the activated sludge flocs. It will be important to elucidate the roles that EPS content and components have in bioflocculation, settling and dewatering phenomena in activated sludge system to deal with biosolid–liquid separation and sludge disposal problems.

2. Materials and methods

2.1. Activated sludge systems

Nine reactors (inner diameter 25 cm, height 50 cm) with an effective volume of 8-l used as activated sludge reactors, were placed in parallel and operated under three different C/N ratios of 4, 20 and 100. The reactors were seeded with the sludge collected from an aeration basin in Ningbo municipal wastewater plant, Ningbo, China. The sludge in the reactors was well suspended by continuous aeration from the bottom through stone air diffusers. Three reactors were fed once a day with a synthetic wastewater with different C/N ratio. The C/N ratio is calculated as the ratio of chemical oxygen demand (COD) to total nitrogen (TN) measured in mg/l. The reactors with a C/N ratio of 20 were used to represent the condition of conventional activated sludge treating municipal wastewater, whereas the reactors with a C/N ratio of 4 represented a carbon-limited case. The reactors with a C/N ratio of 100 were used to represent a nitrogen-limited case. Synthetic wastewater with a C/N ratio of 20, is composed of starch (2 g/l), urea (0.21 g/l), KH_2PO_4 (18 mg/l), and K_2HPO_4 (25 mg/l). For the other C/N ratios, the nitrogen content was adjusted by modifying the urea amount in the feed, keeping the starch content the same. The organic loading rates for all reactors were kept at 0.6 g COD/gSS/d. The composition and concentrations of micronutrients in the feed were: $MgSO_4 \cdot 7H_2O$, 5.07 mg/l; $FeSO_4 \cdot 7H_2O$, 2.49 mg/l; $Na_2MoO_4 \cdot 2H_2O$, 1.26 mg/l; $MnSO_4 \cdot 4H_2O$, 0.31 mg/l; $CuSO_4$, 0.25 mg/l; $ZnSO_4 \cdot 7H_2O$, 0.44 mg/l; NaCl, 0.25 mg/l; $CaSO_4 \cdot 2H_2O$, 0.43 mg/l; $CoCl_2 \cdot 6H_2O$, 0.41 mg/l.

The sludge retention time (SRT) in all the reactors was controlled at 5 days. All reactors were operated in sequence: filling for 15 min; aeration for 17.5 h; idling for 6 h; withdrawing for 15 min at a room temperature of $25 \pm 1\,^{\circ}C$.

The experimental procedure was conducted in three stages. Initially, the reactors were operated at the C/N ratio of 20 for 15 days to achieve the steady state. Secondly, the C/N ratio of the feed in three reactors was shifted from 20 to 4, and in other three reactors was shifted from 20 to 100, while in another three reactors was unchanged. The different C/N ratios were operated from day 16 to day 36, when the performance of the reactors fed with the wastewater of the C/N ratio of 4 and 100 deteriorated seriously and could not assessed as a normal state. Finally, the C/N ratios in all the reactors were changed back to 20.

2.2. Sludge flocculation and settleability

Sludge suspension was slowly mixed at 50 rpm for 3 min in a beaker. The flocs size was measured at this time. The sludge suspension was settled for 30 min, and the supernatant fraction was collected as the effluent. The turbidity of the effluent and effluent suspended solids (ESS) were measured to indicate the performance of the sludge flocculation. The SVI was used to evaluate the settleability of the flocs. A turbidimeter (HACH, Model 2100 AN) measured the turbidity. The ESS content and SVI (mixed liquor was diluted to about 2 g/l before the SVI test) were determined according to the Standard Methods [6].

2.3. Sludge dewaterability

The capillary suction time (CST) and specific resistance of filtration (SRF) were used to evaluate the dewaterability of the flocs in this study. CST measures the time when the filtrate requires to travel a fixed distance on a specific filter paper, reported in seconds. A high value of CST usually implies a poor filterability and dewaterability. The CST was measured by a CST instrument (Triton, Model 304 M, UK) as detailed in Standard Methods [6] with a CST paper purchased from Triton company. The CST for distilled water was stable at 11 s. It was stated that for a specific sludge, the CST was related to and dependent on the suspended solids concentration. In this study, the CST was measured at a MLSS concentration approximately 4000 mg/l, and should, therefore, be comparable with each other. The test was made in triplicate with a standard deviation of 5%. The SRF was conducted in a 250 ml stirred cell using a filter with 0.45 μm filter paper. The stirred cell was filled with 100 ml of the sludge suspension, and a constant pressure was applied by an air pump. The production of filtrate under pressure was continuously recorded. The SRF (s^2/g) of the sludge was calculated by

$$SRF = \frac{2000A^2\,\Delta pb}{\mu C}$$

where Δp (35 kPa) is the pressure applied, A (0.00502 m^2) is the filter area, μ (1.0 mPa s) is the viscosity of the permeate, C is the sludge concentration in MLSS (kg/m^3) and b (s/m^6) is the time-to-filtration ratio, which is the slope of the curve that is obtained by plotting the ratio of the time of filtration to the volume of filtrate (t/V) versus the filtrate volume (V).

2.4. Floc size

The floc size was determined by a Malvern Mastersizer/E instrument with a 300 mm lens which enables the measurement of particles in the range of 0.9–546 μm. The samples were diluted in distilled water to avoid multiple scattering. The activated sludge suspension was then continuously recycled through the sample cell of the Malvern with a peristaltic pump to be exposed to a 2 mW He–Ne laser (wavelength 633 nm). Each sample was measured three times with a standard deviation 0.1–4.5%. The scattered light is detected by means of a detector that converts the signal to a size based on volume. The average size of the flocs was given as the mean based on the volume equivalent diameter.

2.5. Extraction of EPS and determination of EPS components

The sludge was fully mixed prior to commencing the extraction procedure. EPS extraction was carried out using a sonication/thermal extraction process. The activated sludge was harvested by centrifugation (6000 × g, 10 min) and washed with distilled water prior to extraction to remove the loose slime polymers found in the sludge. The dewatered sludge pellet was resuspended in a 0.05% (w/w) NaCl solution with several glass beads, sonicated at 20 kHz for 2 min, shaken horizontally at 150 rpm for 10 min, sonicated again for an additional 2 min. The liquor was centrifuged at 8000 × *g* for 10 min to separate solids and supernatant. The collected supernatant was regarded as the LB-EPS of the sludge sample. The residual sludge pellet left in the centrifuge tube was resuspended in a 0.05% (w/w) NaCl solution, sonicated for 2 min, then heated at 70 °C for 30 min, finally centrifuged at 11,000 × *g* for 30 min to collect supernatant. The collected supernatant was regarded as the TB-EPS in the sludge sample. The quantity of polymers extracted was related to the SS content of that sludge. The sum of the amount of LB-EPS and TB-EPS was considered to represent the total amount of EPS.

2.6. Chemical analysis

The COD, TN, and suspended solid (SS) content were determined according to the Standard Methods [6]. Both the LB-EPS and TB-EPS extractions were analyzed for total organic carbon (TOC), protein and carbohydrate. TOC was measured by a TOC analyzer (Elementar, Liqui TOC/TNb, Germany), and expressed as mg TOC/gSS. Proteins were determined by an adaptation of the Lowry method [7] using casein (Shanghai Sangon Biotechnology Co., Ltd., China) as the standard. Carbohydrate was determined using the anthrone method with a glucose standard. All samples were made in triplicate.

2.7. Statistical analysis

All statistical analysis was carried out with the software SPSS version 11.0 for Windows (SPSS, Chicago, IL, USA). The Pearson's correlation coefficient (R_p) was used to estimate linear correlation between two parameters. The Pearson's R_p coefficient is always between −1 and +1, where −1 means a perfect negative correlation, +1 a perfect positive correlation and 0 the absence of a relationship. Correlations were considered statistically significance at a confidence interval ($p < 0.05$).

3. Results and discussion

3.1. Effect of C/N ratio on EPS production and components

According to previous studies [8,9], protein was the principal component, and carbohydrate was the second component of the EPS matrix in the activated sludge system. Therefore, in this investigation, only protein and carbohydrate of EPS were analyzed.

As shown in Fig. 1, the change of C/N ratio resulted in considerable changes in sludge's EPS production and the chemical constituents. The LB-EPS contents of all sludge samples were low (<10 mg/g SS), but the TB-EPS contents appeared to be much higher (>100 mg/g SS). The findings were consistent with the results of the previous studies [2,10]. Due to the dominant member of TB in the total EPS and its unchanged under different C/N ratios, the total amount of EPS did not change significantly, although the amount of LB-EPS increased by 66% at the C/N ratio of 4. Increasing in C/N ratio from 20 to 100 did not cause obvious change in LB-EPS, but reduction of C/N ratio from 20 to 4 caused remarkable increase in LB-EPS from 5.6 to 9.3 mg TOC/g SS in 5 days. Thereafter, LB-EPS decreased gradually and stabilized at about 4.8 mg TOC/g SS when the C/N ratio was shifted back to 20, the original value before C/N ratio changed.

Regardless of LB-EPS and TB-EPS, the proteins were more dominant than carbohydrates as expected at different C/N ratios. As shown in Fig. 2, when the C/N ratio decreased from 20 to 4, the carbohydrate content in LB-EPS decreased by 200%, but the protein content in LB-EPS increased by 300%, resulting in increased content

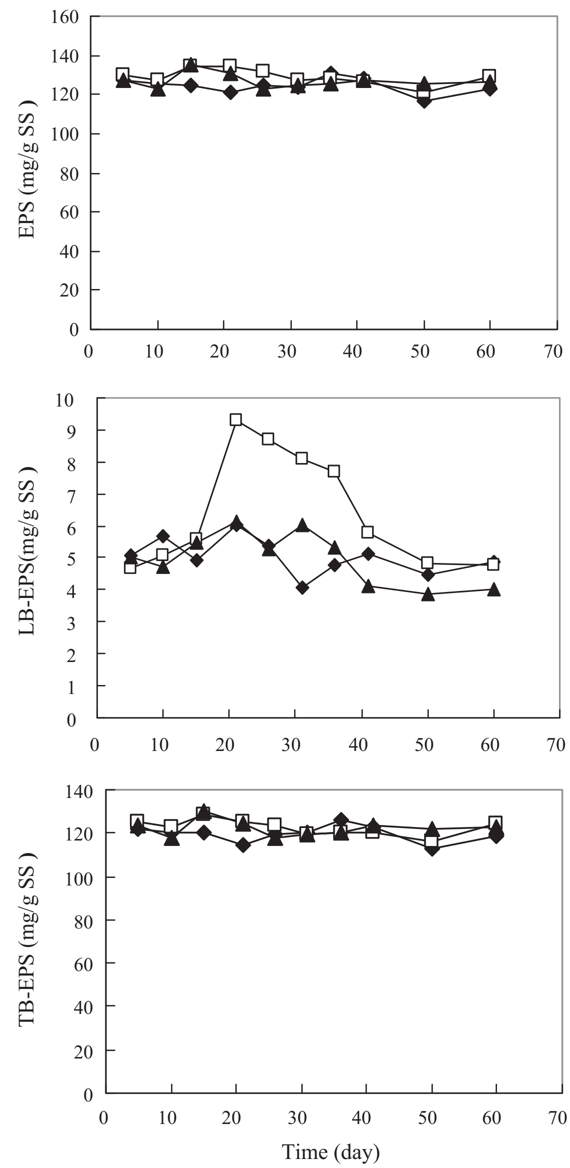

Fig. 1. Effect of C/N ratio of the influent on the total amount of EPS, LB-EPS and TB-EPS. C/N ratio: (♦) 20; (□) 4; (▲) 100.

of LB-EPS. At the low C/N ratio, the amount of nitrogen in the feed is high as compared to carbon. Microorganisms in the sludge flocs utilize this nitrogen in the synthesis of proteins and nucleic acids. However, almost all carbon is utilized by the microorganisms in the biomass synthesis, rather than in extracellular polysaccharide production. With the return of the C/N ratio to the original level (C/N = 20), the carbohydrate contents in LB-EPS decreased gradually to a level of around 4.0 mg TOC/g SS, and the protein contents in LB-EPS increased gradually to a level of around 14.0 mg TOC/g SS. On the other hand, as the C/N ratio increased from 20 to 100, the carbohydrate contents in LB-EPS increased by about 50%, but the protein contents in LB-EPS decreased by approximately 50%, resulting in unchanged content of LB-EPS. At the high C/N ratio, microorganisms can use the excess carbon in the synthesis of cells and also in extracellular polysaccharide production. After the C/N ratio was shifted back to 20, the carbohydrate and protein contents in LB-EPS were all stabilized gradually at the original values. Similar results were reported by other studies [9,11,12]. However, Sponza [13] found that nitrogen deficiency increased the protein level in the EPS and was associated with good settling (low SVI).

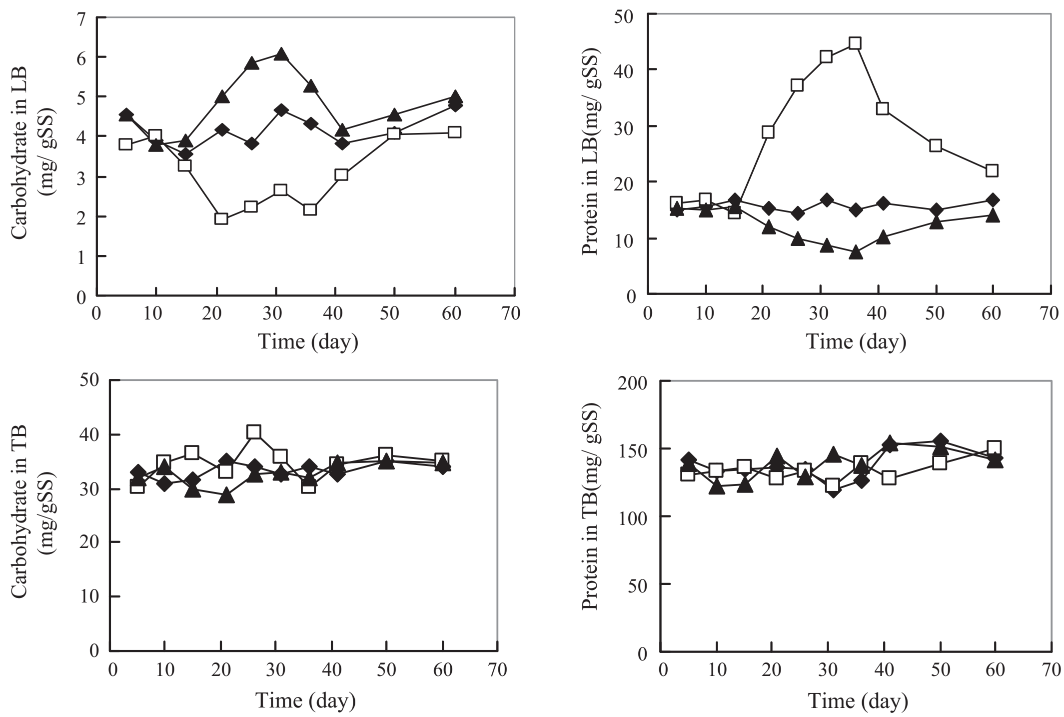

Fig. 2. Effect of C/N ratio of the influent on the carbohydrate and protein amount in LB-EPS and TB-EPS. C/N ratio: (♦) 20; (□) 4; (▲) 100.

Compared to the variations in LB-EPS with the change in C/N ratio, the change extent in TB-EPS at different C/N ratio was less significant. The carbohydrate and protein contents in TB-EPS also did not vary obviously as the C/N ratios changed. The rather consistent level of the TB-EPS content and its chemical constituents under different C/N ratio were in agreement with the other observations [2,10]. The EPS production is a microbial response to external environmental conditions. The results indicated that the more EPS in terms of LB-EPS will be produced at unfavorable environmental conditions, such as increased or decreased C/N ratio from optimum 20.

3.2. Effect of C/N ratio on sludge flocculation, settleability and dewaterability

The change in C/N ratio resulted in marked variations in the sludge-water separation properties. When the reactors were feed with the wastewater of the same C/N ratio of 20, the size of the flocs ranged from 180 to 210 μm, which is in agreement with previous experimental observations [8,14]. The flocs size decreased to 60 μm and 85 μm at the C/N ratio of 4, and 100, respectively (Fig. 3). The effect of decreased C/N ratio on the flocs size was more significant than increased C/N ratio. Cetin and Erdincler [11] found that the activated sludge flocs become larger and stronger with the increase of C/N ratio. However, in their study, as compared to the optimum C/N ratio of 20, the highest C/N ratio (C/N = 30) was not high enough to induce nitrogen-deficient condition. Therefore, the effect of increased C/N ratio on the physicochemical properties was not comparable with our present results. When the C/N ratios were changed to the original level (C/N = 20), the sizes of the flocs increased gradually. However, the reactors with the C/N ratio of 100 recovered faster than the reactors with the C/N ratio of 4. At the end of the investigation, the size of the activated sludge flocs in the reactors with the C/N ratio of 4 was still lower than those in the reactors with the C/N ratio of 20 and 100.

Generally, large size flocs, low turbidity and ESS content imply good flocculating ability of the sludge. The sludge with large size and compact structure has good flocculating ability, resulting in low ESS and super settleability. Therefore, bioflocculation does not only affect the settleability of the sludge, but it also has a significant impact on the dewaterability of the sludge flocs.

After the C/N ratio change was imposed, the sludge became worse in bioflocculation and settleability. As presented in Fig. 4, when the C/N ratio increased from 20 to 100, the turbidity was not different significantly. However, ESS content increased from 0.3 g/l to about 1 g/l, and SVI increased from 85 to 170. The results also implied that the turbidity may be not a good indication for flocculation and settlerability of the sludge. On the other hand, when the C/N ratio decreased from 20 to 4, the turbidity, ESS content and SVI increased greatly, indicating seriously deteriorated flocculation and settleability of the sludge. As soon as the C/N ratios changed to the original level (C/N = 20), the turbidity, ESS content and SVI values all restored to the original levels.

The strongly flocculated flocs have higher degree of compressibility of the activated sludge determined as SVI. Sponza [8] found that very small and big flocs show high SVI values indicating

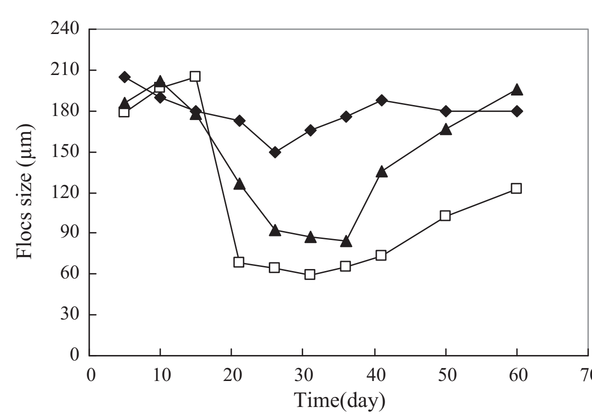

Fig. 3. Effect of C/N ratio of the influent on the flocs size. C/N ratio: (♦) 20; (□) 4; (▲) 100.

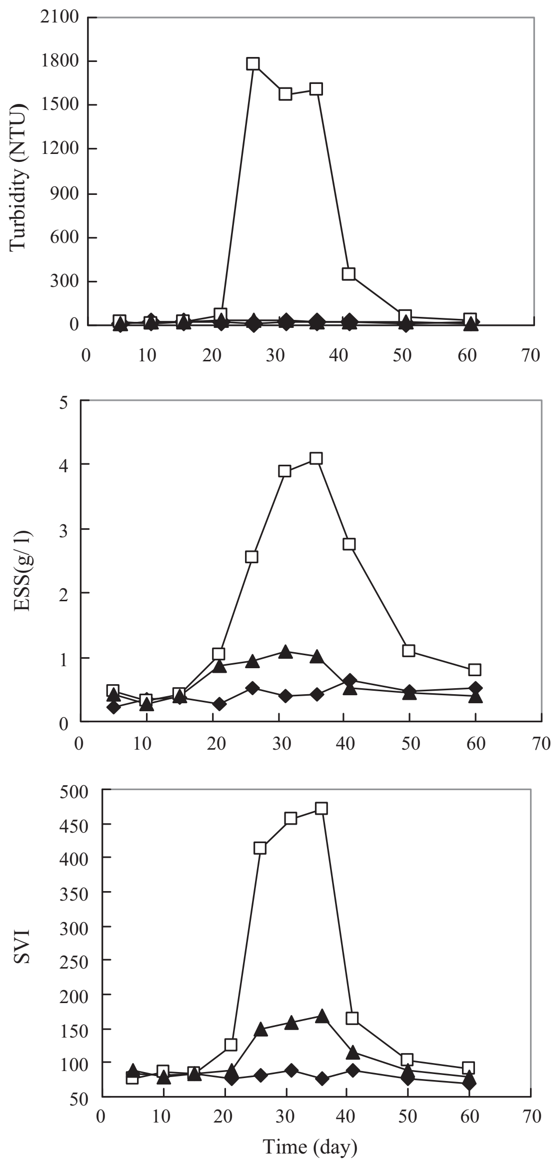

Fig. 4. Effect of C/N ratio of the influent on sludge flocculation and settleability as measured by the turbidity, ESS content and SVI. C/N ratio: (♦) 20; (□) 4; (▲) 100.

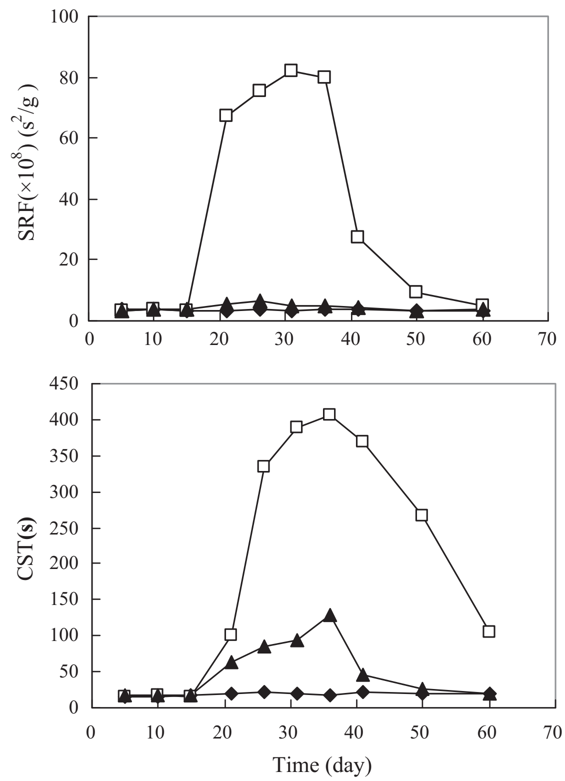

Fig. 5. Effect of C/N ratio of the influent on sludge dewaterability as measured by the CST and SRF. C/N ratio: (♦) 20; (□) 4; (▲) 100.

poor settling properties. The sludge flocs with a high number of filamentous microorganisms were also large, but had relatively poor settleability. Microscope examination of the sludge samples showed that filamentous microorganisms were not noticeable in any of the sludge flocs. Therefore, in this study, the decrease of settleability was due to the small flocs as shown in Fig. 3.

Changing the C/N ratio from 20 to 4 or 100 caused deterioration in sludge dewaterability. As shown in Fig. 5, the CST values increased from 17 s to 400 s and 130 s, respectively, while the SRF values were almost unchanged as the C/N ratio increased from 20 to 100. However, the CST and SRF all increased remarkably when the C/N ratio decreased from 20 to 4. The results suggested that SRF may not be a sensitive indication of the sludge dewaterability. The results were inconsistent with the other reports. Sanin et al. [12] stated that SRF decrease slightly and sludge filterability do not change significantly when C/N ratio increased from 9 to 21. But when C/N ratio increased further to 43, SRF increased about hundred times. Once the C/N ratio shifted back to the original level (C/N = 20), the SRF and CST values recovered gradually, whereas CST value of the sludge flocs in the reactors with the C/N ratio of 4 was still higher than those in other reactors.

Higgins and Novak [15] stated that the "supracolloidal" particles in the range of 1–100 μm have the greatest influence on the dewaterability of the activated sludge, and the dewaterability decline with the increase of the concentration of the particle in this size range. The poor dewaterability of the sludge flocs after the C/N ratio changed from the optimum value of 20, may be also due to the small flocs. The poorer dewaterability of the sludge flocs might be attributed to the smaller size of the flocs or more percent of the flocs under 100 μm in the reactors with the C/N ratio of 4, as compared to the C/N ratio of 100.

The lack of linear correlation between CST and SRF in this study is not surprising as we found similar phenomenon in our previous study. Therefore, the measurements of both CST and SRF correspond to a similar indication in term of dewatering rate of activated sludge, but the efficiency of dewatering of the activated sludge depends on both the dewatering rate and the extent of dewatering. It is necessary to find a suitable parameter to indicate the dewatering degree. It has been widely accepted that short CST is associated with good dewaterability of the sludge [16]. However, the difficulty is that CST test does not quantify a particular, fundamentally based physical parameter of the sludge.

The results indicated that change in C/N ratio of the influent has an adverse impact on the performance of sludge-water separation.

3.3. Corrections between EPS and sludge flocculation, settleability and dewaterability

Together with the varied contents of carbohydrate and protein in LB-EPS and the total amount of LB-EPS, we found that there is no correlation between the carbohydrate or LB-EPS content and flocculation, settleability and dewaterability of the activated sludge.

However, the protein contents in LB-EPS positively correlated with the turbidity ($R_p = 0.71$, $p < 0.05$), ESS content ($R_p = 0.76$, $p < 0.05$), SVI value ($R_p = 0.73$, $p < 0.05$), SRF ($R_p = 0.79$, $p < 0.05$), and CST value ($R_p = 0.76$, $p < 0.05$). The flocculation, settleability and dewaterability of the activated sludge were reduced significantly at decreased C/N ratio than increased C/N ratio. Therefore, we suggested that protein is more important than carbohydrate in flocculating, settling and dewatering of the sludge flocs, which was in agreed with that reported by Higgins and Novak [15]. They found that the degradation of protein by proteolytic enzymes result in the disintegration of the sludge and deteriorated dewaterability, whereas the degradation of carbohydrate by cellulose have no noticeable effect. Wu et al. [17] compared nitrogen-deficient and nitrogen-rich activated sludge. They found that the nitrogen-deficient sludge have higher carbohydrate content, lower protein content, and poorer dewaterability. From Cetin and Erdincler's [11] results, we found that the changed degree is nearly same (the C/N ratio decreased from 19 to 8, and increased from 19 to 30), filterability and compactibility of the sludge at the C/N ratio of 8 is worst. This was consistent with our results that flocculation, settleability and dewaterability of the sludge at the C/N ratio of 4 were much deteriorated than those at the increased C/N ratio, even if at very high C/N ratio of 100.

There was no correlation between the TB-EPS content and the flocculation, settleability and dewaterability (data not shown). Although the quantity of TB-EPS was greater than that of LB-EPS, it was LB-EPS, function as the primary surface for cell interaction and attachment. The results were in agreement with other studies [2,10]. Li and Yang [2] demonstrated that the LB-EPS have a negative effect on bioflocculation and sludge-water separation. The parameters for the performance of sludge-water separation were much more closely correlated with the amount of LB-EPS than with the amount of TB-EPS. Wilén et al. [18] found that the increase of the protein content of the EPS coincide with higher ESS concentrations. They also found that there is poor correlation between EPS contents and stirred sludge volume index (SSVI) at a full scale plant over a 2-year period. Another study found that SVI increase with the proteins of EPS, but have no correlation with carbohydrates of EPS [19].

EPS are expected to have an influence on sludge dewaterability through the high level of hydration of the polymer surrounding the bacteria cell and the role in flocculation. Indeed, the amount of EPS extracted from the activated sludge using a sodium hydroxide solution was positively correlated with the SRF [20]. The SRF value was closely correlated with the LB-EPS content [2]. Jin et al. [14] found that concentrations of the individual polymers and total EPS measured in the extracted EPS have negative correlations with CST, and the amount of protein measured in the total sludge and the extracted EPS correspond oppositely to the CST, indicating that high amount of the total extracted EPS was associated with low CST. In another study, Cetin and Erdincler [11] stated that dewaterability of sludge is improved considerably with the increasing carbohydrate part and the decreasing protein part of the sludge EPS. Furthermore, Yu et al. [21] suggested that the proteins and proteins/carbohydrates in the supernatant and slime layers, which are usually decanted, markedly impact sludge dewaterability.

4. Conclusions

This paper has presented a comparative study of EPS and its chemical constituents, as well as flocculation, settleability and dewaterability of the activated sludge flocs at different C/N ratio of the influent. The following conclusions may be drawn from this study:

(1) The different C/N ratio in the feed produced the activated sludge flocs with similar total amount of EPS and TB-EPS, but different contents of LB-EPS, carbohydrate and protein in LB-EPS.
(2) When the C/N ratio increased from 20 to 100, the turbidity of the supernatant and SRF were nearly unchanged, but ESS content SVI and CST all increased, whereas the flocs size reduced.
(3) When the C/N ratio decreased from 20 to 4, the turbidity, ESS content, SRF, SVI and CST all increased greatly, whereas the flocs size reduced.
(4) The results indicated that flocculation, settleability and dewaterability of the sludge flocs are all adversely influenced when the C/N ratio increased to 100 or decreased to 4 from the optimum value of 20. However, the extent of influence was much significant at decreased C/N ratio of 4.
(5) The carbohydrate in LB-EPS decreased, and the content of protein in LB-EPS increased as the C/N ratio decreased. The carbohydrate in LB-EPS increased, and the content of protein in LB-EPS decreased as the C/N ratio increased. However, the carbohydrate and protein contents in TB-EPS were not influenced by the C/N ratio. There was no strong relationship between total amount of EPS and the flocculation, settleability and dewaterability of the activated sludge. However, the contents of protein in LB-EPS correlated with the flocculation, settleability and dewaterability of the activated sludge.

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