

Effects of Fe(III) on floc characteristics of activated sludge

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Abstract: The effects of Fe(III) on floc characteristics of activated sludge were investigated in nine parallel sequencing batch reactors (SBRs). The results showed that Fe(III) improved the quality of organic matters in the effluent of reactors. Concentrations of Fe(III) up to 23.8 mg dm^{-3} decreased suspended solids and turbidity in effluent but overdosage resulted in deterioration of these parameters. Activated sludge floc size measurements indicated that Fe(III) led to a shift in the size distribution from large to small flocs. Concentrations of Fe(III) less than 23.8 mg dm^{-3} did not significantly change the proportion of larger flocs, but overdosage of Fe(III) markedly decreased the fraction of larger flocs and produced a large number of smaller flocs, which may be responsible for the deterioration of effluent suspended solids and turbidity. Scanning electronic microscopic (SEM) observation suggested high Fe(III) concentrations lead to significant changes in floc morphology and reduction of filamentous microorganisms available for the formation of large aggregates.

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1 INTRODUCTION

A key aspect in the operation of activated sludge systems is the separation of the biological solids from the treated wastewater. The effectiveness of settling of activated sludge from mixed liquor is related to the physical properties of activated sludge flocs, such as floc size, structure and density.^{1–3} These properties can vary rapidly with varying conditions in the wastewater treatment plant. The settling capacity of the floc is of current interest in wastewater treatment. Various process parameters have been demonstrated to affect the properties of activated sludge flocs: dissolved oxygen concentration (DO),² organic loading rate,³ solids retention time (SRT),^{4–6} and cations^{7–9} in the wastewater.

Activated sludge floc formation is the result of physical and chemical interactions between microorganisms, inorganic particles, extracellular polymeric substances (EPS) and cations.^{10–12} Since the overall floc and other components, including bacterial cells and EPS, are negatively charged, the possible role of cations in activated sludge has been considered important and investigated by many researchers. Cousin and Ganczarzyk reported that the addition of sodium to a biological suspension increased floc size and improved floc porosity.¹³ Zita and Hermansson observed that floc stability was affected by solution ionic strength and K^+ and Ca^{2+} produced similar effects on particle stability.¹⁴ In contrast, Novak

and coworkers have demonstrated that an increase in calcium and magnesium ions improves settling properties, whereas an increase in sodium results in the deterioration of settling and dewatering properties of activated sludge.^{8,15–18} Models suggested that divalent cations participate in bridging of negatively charged sites on the EPS. The charge bridging between the EPS promotes an increase in floc size, density and floc resistance to shear.⁸

Although the possible effects of monovalent and divalent cations have been investigated, the role of trivalent cations in activated sludge solid–liquid separation is not well understood. However, municipal and some industrial wastewater contain high concentrations of ferric ions. Moreover, ferric salts have gained wide applications in the coagulation process as a common method of wastewater pretreatment. The addition of ferric iron in the aerated tank of activated sludge systems is common practice for phosphorus removal.

There is limited information available on the effect of Fe(III) on activated sludge floc characteristics. Wuhrmann reported that ferric chloride dosing caused the virtual disappearance of protozoa from the activated sludge culture and an increase of the turbidity in the effluent.¹⁹ It was not clear whether the turbid effluents were due to poorly flocculated ferric-hydroxyphosphate particles or as a result of dispersed activated

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sludge particles due to the absence of protozoa. Nielsen and Keiding have reported that reduction of Fe(III) to FeS led to a weakening of floc strength and disintegration of activated sludge flocs.²⁰ Novak *et al* showed that iron contributed to floc strength, and deterioration in sludge dewatering was noticed when the iron was reduced.¹⁵

In this research, the effects of Fe(III) on activated sludge floc properties was studied within a wide range of ferric ion concentration. The influence of Fe(III) on treatment performance, sludge settleability, floc structure and size distribution was investigated in laboratory-scale sequencing batch reactors (SBR).

2 MATERIALS AND METHODS

2.1 Reactors and wastewater

The experimental system consisted of nine parallel SBRs, and temperature was controlled at 25 ± 0.5 °C by a water bath. Each SBR was constructed from glass and had an operating volume of 2 dm^3 . Each reactor was operated and controlled by four timers through four stages in sequence: filling, aeration, settling and withdrawal. The reactors were operated continuously in cycles of 12 h, with a filling period of 10 min, an aeration period of 11 h, a settling period of 40 min and a withdrawal period of 10 min. At the end of the cycle 1.4 dm^3 of effluent was pumped out of the reactors, resulting in a hydraulic retention time (HRT) of 17.1 h. The solids retention time (SRT) was maintained at 10 days by withdrawing 200 cm^3 mixed liquor every day. The sludge suspension was continuously mixed with a magnetic stirrer ($\phi 0.6 \text{ cm} \times 5 \text{ cm}$) placed at the bottom of each reactor. Air was introduced into the reactors through a stone air diffuser. The dissolved oxygen concentration in each reactor was monitored frequently and kept in the range of $3\text{--}5 \text{ mg dm}^{-3}$.

Throughout the experiment, the wastewater was authentic domestic wastewater taken from the sewage outlet in our university campus. This wastewater was transported daily to the laboratory and was stored at 4 °C in a refrigerator until used. The characteristics of the wastewater used in these experiments varied over wide ranges of pH 7.4–8.1, chemical oxygen demand (COD) $452 \pm 65 \text{ mg dm}^{-3}$, suspended solids (SS) $200 \pm 43 \text{ mg dm}^{-3}$, total phosphorus (TP) $7.2 \pm 1.9 \text{ mg dm}^{-3}$, dissolved organic carbon (DOC) $120 \pm 32 \text{ mg dm}^{-3}$, and total nitrogen (TN) $47 \pm 11 \text{ mg dm}^{-3}$.

Initially, the reactor system was inoculated with activated sludge (TS, 2050 mg dm^{-3} ; VS, 1460 mg dm^{-3})

from the outlet of aeration tank of a municipal wastewater treatment plant (Gaobeidian wastewater treatment plant, Beijing, China). Following a three-SRT stabilization period, different dosages of FeCl_3 were added into the SBRs at the filling period of each cycle. Since the Fe(III) concentration range adopted in the experiment was comparatively wide and the number of reactors was limited, the experiment was divided into two stages. In each stage, an SBR was operated as control reactor without any FeCl_3 addition, and the Fe(III) concentrations in different reactors are shown in Table 1. Operation at steady state was determined by monitoring several parameters, including mixed liquor suspended solids (MLSS), sludge volume index (SVI), effluent COD, effluent TP, and effluent DOC. Steady state operation is defined as being reached when these parameters remained stable for at least 2 weeks.

2.2 Analytical methods

COD, DOC, effluent suspended solid (ESS), MLSS, TP, mixed liquor volatile suspended solid (MLVSS) and DO were measured in accordance with standard methods.²¹

The concentrations of protein and polysaccharide in the effluent were measured using the methods of Lowry *et al*²² and of Dubois *et al*,²³ respectively. Protein standards were prepared with bovine serum albumin, and polysaccharide standards with glucose.

2.3 Floc size and zeta potential measurements

Floc size distributions were measured on-line with a Malvern Mastersizer (Mastersizer 2000, UK) according to the technique reported by Chaignon *et al*.²⁴ Results are volume based and represented as suspended particle volume versus sphere diameter of equivalent volume. At least three measurements were made on each sample.

The zeta potential of sludge flocs in each SBR was determined using a Malvern zeta analyzer (Zetasizer 2000, UK).

2.4 Electron microscope observations

Floc samples were preserved with 2.5% (v/v) glutaraldehyde and 1% (v/v) osmium tetroxide. Both reagents were diluted in sodium phosphate, 0.1 mol dm^{-3} , buffer solution. The samples were dehydrated in a graded series of acetone solutions, and subsequently stained with uranyl acetate and lead citrate to enhance the electron contrast of biological material. The floc morphology was observed by

Table 1. The dosages of Fe(III) in SBR reactors during the experiment

	Reactor								
	Stage I				Stage II				
	SBR-1	SBR-2	SBR-3	SBR-4	SBR-5	SBR-6	SBR-7	SBR-8	SBR-9
Fe^{3+} concentration (mg dm^{-3})	0	3.0	6.0	10.0	0	23.8	35.7	71.4	142.8

scanning electron microscopy (SEM) (Model S-570, Hitachi, Japan).

2.5 EPS extraction and elemental analysis

The thermal extraction/solvent precipitation techniques described by Forster and Lewin,²⁵ Brown and Lester²⁶ and Forster²⁷ were used to extract polymeric material from the different sludges of the second stage experiment. The EPS extraction procedure followed the method of Morgan *et al.*²⁸ with some modification. One hundred cm³ sludge solids was concentrated by centrifugation (3000 rpm, 3 min) and resuspended in 50 cm³ sterile double distilled water. To reduce bacterial lysis and consequent release of intracellular products, thermal treatment at 80 °C was used.²⁹ The extracted polymers were harvested by removal of the sludge solids by centrifugation, first at 3000 rpm for 3 min and then at 10 000 rpm for 30 min. The supernatant was subsequently filtered through 0.22 µm pore size Millipore filters to remove cells. The EPS in the filtrate was precipitated using a mixture of acetone and ethanol (3:1 v/v), and left overnight at room temperature. The precipitate was subsequently recovered by centrifugation (3000 rpm, 3 min) and then resuspended in sterile double distilled water. Elemental analysis of the EPSs was carried out according to the method described by Cloete and Oosthuizen.³⁰

3 RESULTS AND DISCUSSION

3.1 Overall performance of reactors

The quality of effluents of the reactors under steady state conditions is listed in Table 2. It can be seen from Table 2 that the increases of ferric salt dosage improved the quality of organic matter, as well as dissolved organics, in the effluent. Figure 1 shows the changes in effluent COD, polysaccharide and protein as a function of iron dosage. Ferric chloride dosing removed most of the soluble protein and some polysaccharide, which may account for the noted decreases in effluent COD. These results together with those of Murthy and Novak¹⁷ demonstrate that iron may retain most of the protein within the floc while a smaller amount of polysaccharide is retained.

Table 2 presents different trends of effluent SS and turbidity with iron dosage increases in the two stages of

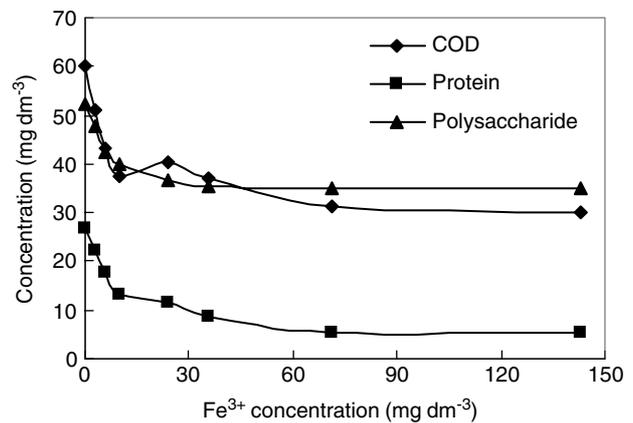


Figure 1. Profiles of COD, protein and polysaccharide in reactor effluent as a function of Fe³⁺ concentration.

the experiment, suggesting that iron addition may alter the ability of activated sludge to flocculate particulate and colloidal matter. At stage I, the concentration of suspended solids and the turbidity in effluent diminished as the dosage of Fe(III) increased. In contrast, the quality of SS and the turbidity first improved and then deteriorated when the Fe(III) dosages were increased at stage II. These results suggested that appropriate concentration of Fe(III) may promote the formation of activated sludge flocs and enhance the ability of activated sludge to retain particulate and colloidal matter within flocs. However, overdosages of Fe(III) weakened the process of activated sludge aggregation and resulted in dispersed flocs.

The characteristics of activated sludge from the SBRs under steady state conditions are shown in Table 3. The MLSS concentrations of SBRs increased with an increase in iron chloride dosage, while the MLVSS concentrations first increased and then stayed at a relatively constant level in all of these reactors. As a result, the MLVSS/MLSS ratio decreased from 0.83 to 0.61, which implied that the fraction of inorganic matter in the sludge increased with iron addition. Owing to iron addition, the amount of inorganic matter, and hence the density of the sludge, can be increased if these ferric salts are retained in the sludge. As expected, the settleability of the sludge as measured by SVI improved since Fe(III) addition increased the concentration of MLSS.

Table 2. The quality of reactor effluents under steady state conditions

Fe ³⁺ concentration (mg dm ⁻³)	COD (mg dm ⁻³)	DOC (mg dm ⁻³)	SS (mg dm ⁻³)	Turbidity (NTU)
Stage I				
0	57.4 ± 15.3	12.5 ± 2.7	35 ± 11	10.4 ± 2.4
3.0	51.2 ± 10.7	11.4 ± 1.1	27 ± 8	9.1 ± 3.0
6.0	41.0 ± 7.6	10.7 ± 0.9	18 ± 5	8.1 ± 2.4
10.0	37.6 ± 3.5	9.3 ± 1.1	8 ± 3	4.2 ± 0.7
Stage II				
0	66.1 ± 12.4	14.3 ± 2.8	33 ± 15	10.7 ± 3.1
23.8	43.5 ± 4.7	10.9 ± 2.1	14 ± 5	7.6 ± 1.4
35.7	37.0 ± 4.9	9.2 ± 1.9	36 ± 17	11.9 ± 4.2
71.4	31.5 ± 6.6	9.7 ± 2.1	44 ± 12	13.8 ± 5.2
142.8	30.2 ± 3.2	10.1 ± 1.6	56 ± 20	19.5 ± 5.5

Table 3. Characteristics of activated sludge in the SBRs under steady state conditions

Fe ³⁺ concentration (mg dm ⁻³)		MLSS (g dm ⁻³)	MLVSS (g dm ⁻³)	SVI (ml ³ g ⁻¹)	Zeta potential (mV)
Stage I	0	2.56 ± 0.31	2.14 ± 0.18	81 ± 7	-20.6 ± 0.4
	3.0	2.86 ± 0.23	1.97 ± 0.32	72 ± 10	-20.2 ± 0.6
	6.0	3.48 ± 0.26	2.31 ± 0.15	71 ± 7	-18.7 ± 0.3
	10.0	3.84 ± 0.29	2.36 ± 0.26	75 ± 8	-18.2 ± 0.5
Stage II	0	2.55 ± 0.15	1.98 ± 0.12	88 ± 10	-19.8 ± 0.8
	23.8	3.77 ± 0.13	2.32 ± 0.20	85 ± 6	-19.6 ± 0.5
	35.7	4.30 ± 0.11	2.84 ± 0.14	82 ± 8	-19.6 ± 1.1
	71.4	4.61 ± 0.18	2.77 ± 0.90	78 ± 17	-19.4 ± 0.9
	142.8	4.85 ± 0.21	2.98 ± 0.22	70 ± 6	-19.5 ± 0.3

As shown in Table 3, there was no significant difference in the zeta potential between the flocs in the SBRs at the steady state. These results suggested that charge neutralization does not play an important role in the flocculation of activated sludge. Pavoni *et al*³¹ also showed that surface charge reduction may not be the prime mechanism responsible for bioflocculation.

3.2 The size distribution of activated sludge flocs

As shown in Fig 2, the addition of ferric chloride caused a shift of the size distribution from large to small flocs. In stage I, Fe(III) addition caused visible changes in the size distribution in comparison with the control reactor. As Fe(III) concentration increased up to 10 mg dm⁻³, the highest frequency peak decreased from 120 to 83 μm, and *d*₅₀ (the size of particles below which 50% volume of the sample lies) decreased from 97.7 to 74.2 μm. In stage II, further increases of Fe(III) concentration led to significant decreases in the floc size of the activated sludge. When Fe(III) concentration increased to 23.8 mg dm⁻³, the *d*₅₀ of activated sludge decreased from 94.7 to 71.8 μm. For the reactor with 142.8 mg dm⁻³ Fe(III), the value of *d*₅₀ was less than 30 μm.

Table 4 shows the changes in the sizes of the activated sludge flocs in four size categories. Even though Fe(III) decreased the size of flocs at low Fe(III) concentrations, ie less than 23.8 mg dm⁻³, Fe(III) did not greatly increase the amount of small flocs, which did not settle rapidly, and those flocs greater than 50 μm comprised 70–80% of the total volume of activated sludge. Moreover, Fe(III) addition improved settleability, expressed as SVI, of activated sludge and decreased the particulate and colloidal matter in the effluent of reactors, which indicated that appropriate concentrations of Fe(III) could promote bioflocculation. Novak *et al* reported that iron contributed to floc strength and reduction of iron led to deterioration in sludge dewatering.¹⁵ In contrast, we observed that maintaining a high Fe(III) concentration in the solution resulted in substantial production of small activated sludge flocs, which may be present in the effluent and contribute to SS and turbidity. Meanwhile, high concentrations of Fe(III) greatly decreased the proportion of flocs larger than

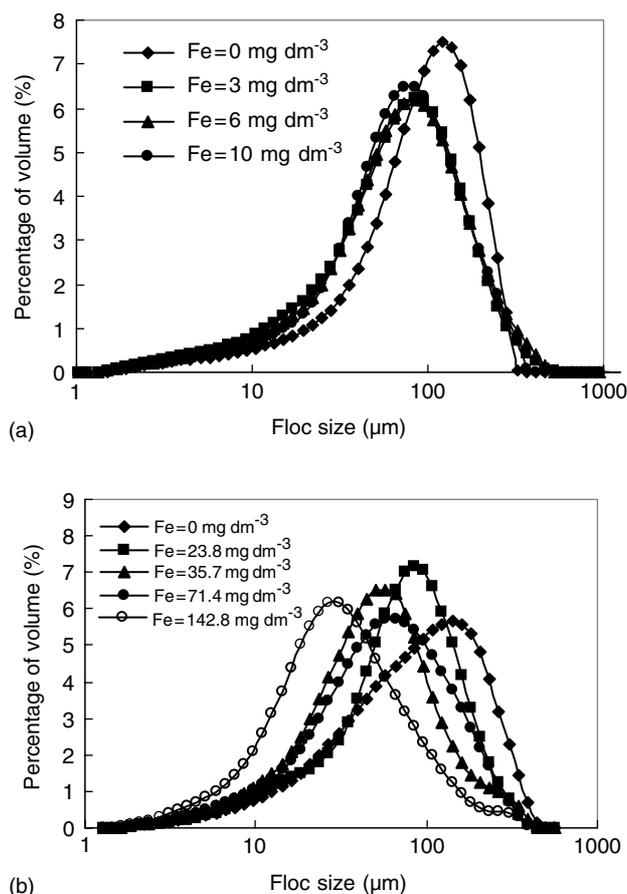


Figure 2. The influence of Fe³⁺ concentration on the floc size distribution of activated sludge: (a) stage I, (b) stage II.

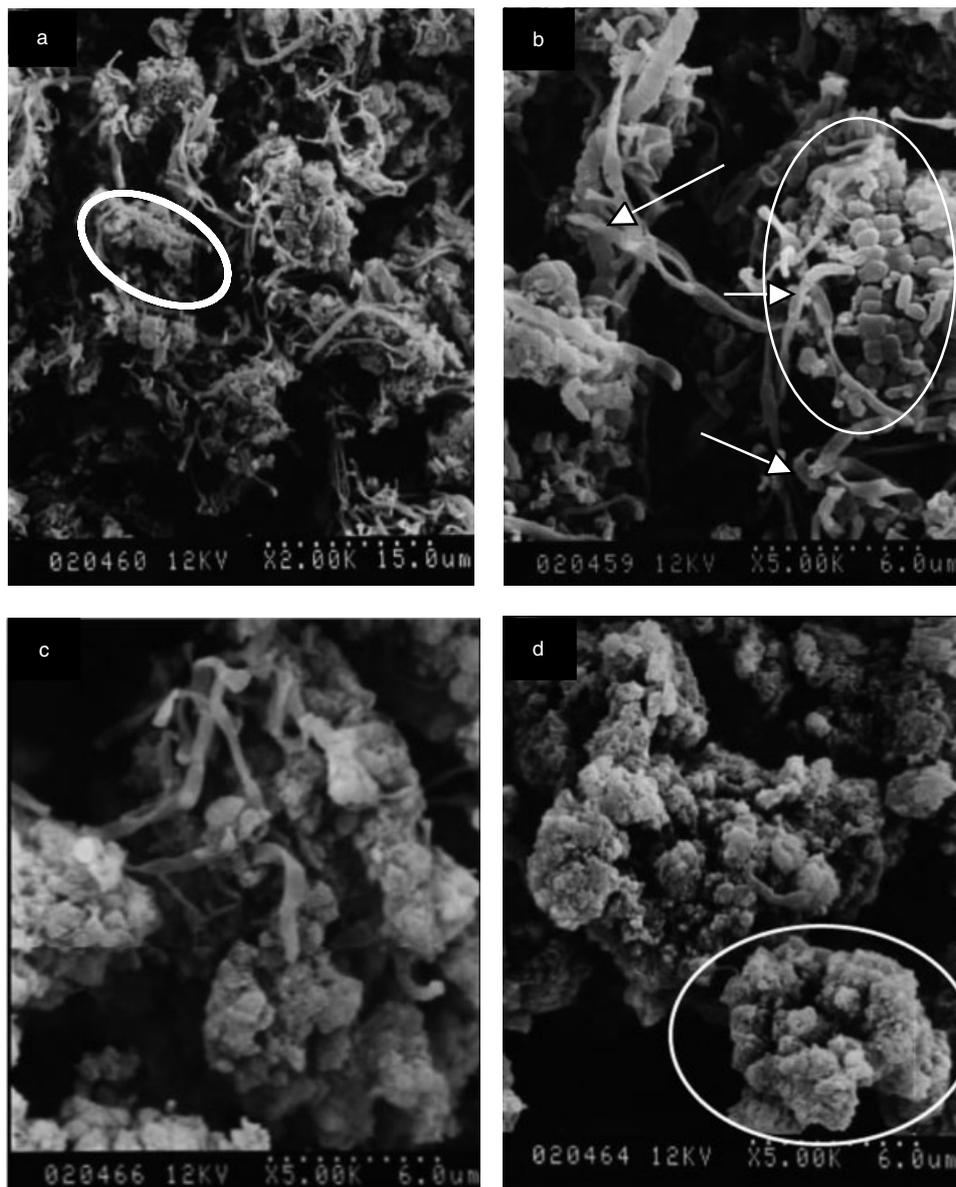
50 μm. Therefore, overdosage of Fe(III) reduced the fraction of the flocs with settleable sizes and impaired the bioflocculation of activated sludge.

3.3 Morphology of activated sludge flocs

Since high concentrations of Fe(III) significantly changed floc size distribution and deterioration of performance of activated sludge reactors, morphology of activated sludge flocs was studied at stage II using scanning electron microscopy. SEM of activated sludge flocs revealed marked alteration in their morphology when FeCl₃ was added to the mixed liquor (Fig 3). Figure 3(a and b) shows a typical activated sludge floc sampled from SBR-5. The flocs

Table 4. Distribution of activated sludge flocs under steady state conditions

Fe ³⁺ concentration (mg dm ⁻³)	Percentage of volume (%)				
	<10 μm	10–50 μm	50–80 μm	>80 μm	
Stage I	0	4.03	14.00	26.80	55.17
	3.0	5.69	23.29	32.34	38.68
	6.0	5.36	22.31	32.99	39.30
	10.0	4.71	21.78	35.04	38.48
Stage II	0	4.53	20.49	25.61	49.35
	23.8	4.69	20.55	35.85	38.9
	35.7	6.25	36.64	36.79	21.34
	71.4	6.23	30.48	33.33	29.99
	142.8	11.23	54.25	23.48	11.03

**Figure 3.** SEM micrographs of activated sludge particles from different SBRs. (a) SBR-1, ×2000; (b) SBR-5, ×5000; (c) SBR-6, ×5000; (d) SBR-9, ×5000. Circles in micrographs represent activated sludge particles, and arrows represent filamentous microorganisms.

had a comparatively loose structure: the boundaries of individual primary particles were generally ill-defined, and the arrangement of bacteria could be easily distinguished. It could be clearly seen that bacterial

cells adhered to EPS to form microbial clusters and filamentous microorganisms acted as a ‘backbone’ of these microbial clusters and intertwined with each other to form large biological aggregates.

On the other hand, flocs exposed to FeCl_3 addition (Fig 3(c and d)) exhibited a significantly different type of morphology. Boundaries of individual particles were very clear, and the connections between individual particles were much lower than the sludge without Fe(III) addition. Bacterial cells cannot be clearly distinguished and little filamentous microorganisms could extend out from the sludge particles. These filamentous microorganisms extending out of individual floc particles were considered to be very important in the aggregation of primary particles. Namely, Fe(III) seemed to reduce the amount of filamentous microorganisms available for formation of large aggregates. Furthermore, some inorganic matter could also be observed on the surfaces of bacterial cells, which made their shapes indistinct. Experimental results indicated that the more iron addition, the greater change in floc morphology. For example, some filamentous microorganisms were seen at the surface sludge flocs with $23.8 \text{ mg dm}^{-3} \text{ Fe(III)}$ (Fig 3(c)), while almost no filamentous microorganisms could be observed in the reactor with $142.8 \text{ mg dm}^{-3} \text{ Fe(III)}$ (Fig 3(d)).

3.4 Elemental analysis of extracellular polymeric substances

Because of the importance of extracellular biopolymers in bioflocculation of activated sludge, elemental analysis was conducted at stage II of experiment in order to investigate the role of Fe(III) in bioflocculation of activated sludge. Elemental analysis of EPS samples is listed in Table 5, and the amount of each element is expressed as weight percentage. All EPS samples contained considerable amounts of phosphorus (24–34%), while sulfur, silicon and chloride were also found in some samples. These results are similar to those observed by Cloete and Oosthuizen.³⁰ As counter-ions to these anions, magnesium, potassium and calcium were dominant in the EPS of the control reactor. Since microbial cells and EPS are the negatively charged components in activated sludge flocs, cations were considered to

Table 5. Typical data set for elemental analysis of EPS in the reactors

Element ^a	Weight range (%)				
	SBR-5	SBR-6	SBR-7	SBR-8	SBR-9
Na-K	8.03	4.52	ND ^b	4.91	3.15
Mg-K	13.39	10.07	8.86	5.07	4.21
Al-K	ND ^b	ND ^b	ND ^b	ND ^b	2.10
Si-K	3.12	ND ^b	ND ^b	1.76	ND ^b
P-K	27.68	30.10	34.18	27.53	24.21
S-K	7.58	8.25	11.39	6.40	8.42
Cl-K	9.38	4.33	ND ^b	10.00	11.57
K-K	17.86	14.67	11.39	10.44	10.52
Ca-K	15.18	15.99	17.72	12.88	11.58
Fe-K	6.70	12.07	16.46	20.79	24.21

^a The K following every element indicates the K-shell of the specific atom.

^b ND – not detected.

play an important role in bioflocculation of activated sludge. With increasing concentration of Fe(III) , the proportions of iron in the EPS samples increased while the percentages of monovalent and divalent cations decreased. The results showed that the reduction in the amounts of the various cations differed, with $\text{Mg}^{2+} > \text{Ca}^{2+}$, $\text{Na}^+ > \text{K}^+$. These results suggest that Fe(III) in solution participates in bridging between the negatively charged groups in EPS and, also, replaces the monovalent and divalent cations from within the flocs through ion-exchange effects, as suggested by previous studies.^{8,16,17} Nielsen and Keiding²⁰ have shown that reduction of activated sludge grown in the presence of Fe(III) resulted in floc disintegration, and that the released matter was mainly EPS and some bacteria. Because of its higher valence, Fe(III) may physiochemically interact with more negatively charged functional groups than divalent cations in EPS, thus making the flocs more compact and denser. Meanwhile, high dosing of Fe(III) decreased the amount of filamentous microorganisms and weakened the ability of primary sludge particles to aggregate, thus deteriorating bioflocculation of activated sludge.

4 CONCLUSIONS

- (1) Addition of ferric salts to the influent of SBR system contributed to the improvements in the organic matter in reactor effluent, such as COD, DOC, protein and polysaccharide. Appropriate concentrations of Fe(III) enhanced the ability of activated sludge to retain particulate and colloidal matter within flocs, but overdosages of Fe(III) weakened the process of activated sludge aggregation and resulted in dispersed flocs.
- (2) Fe(III) improved the settleability of activated sludge and the zeta potential of flocs was little affected by Fe(III) addition.
- (3) The addition of ferric chloride caused a shift of the size distribution from large to small flocs. Overdosages of Fe(III) markedly decreased the fraction of larger flocs and produced a large number of smaller flocs. Because these smaller flocs could not be effectively removed in settlers, overdosages of Fe(III) resulted in the deterioration of effluent suspended solids and turbidity.
- (4) Element analysis of extracellular polymeric substances of activated sludge indicated that Fe(III) interacted with some components of these biopolymers. With the increasing dosage of FeCl_3 , the percentages of iron increased in the EPS samples while the proportions of monovalent and divalent cations declined.
- (5) Ferric ions led to a marked alteration in the morphology of activated sludge flocs. High Fe(III) concentration seemed to weaken the connections between activated sludge particles and reduce the amount of filamentous microorganisms available for formation of large aggregates.

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