

Effects of Ni²⁺ on the characteristics of bulking activated sludge

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ABSTRACT

This study investigated effects of the continuous addition of a high-concentration of Ni²⁺ on the characteristics of bulking activated sludge as well as on the variety and recovery of their ability removing organic pollutants and ammonia in a sequencing batch reactor (SBR). To the knowledge of the authors, this is the first study to examine the changes in quantity and composition of the extracellular polymeric substances (EPS) in bulking activated sludge before and after the addition of a high-concentration of Ni²⁺. The correlation between these changes and the sludge characteristics was also investigated. The results showed that after a 40-day acclimation to the addition of 60 mg/L Ni²⁺, the ability to remove organic pollutants was recovered; but the ability to remove ammonia was not. The addition of Ni²⁺ made a great change in the quantity and composition of the EPS in bulking sludge, including both exterior soluble EPS (EPS_S) and interior bound EPS (EPS_b), showing a slight increase followed by a large decrease before reaching a steady state. The difference between the change in the amounts of EPS_S and EPS_b was not significant. However, among the component fractions, the change in protein content was obvious than that of polysaccharide and humic acid.

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1. Introduction

Activated sludge process or its modifications have been widely applied in wastewater treatment due to its cost-effective and operation-easy. Heavy metals are biologically toxic at high concentrations. They strongly disrupt the normal metabolism of microorganisms and have severe negative effects on activated sludge process [1–5]. Ni²⁺ is one of the common heavy metals found in electroplating wastewater. However, the majority of the studies to date [1,4–9] have only investigated the effect of Ni²⁺ on the organic pollutants and ammonia removal by activated sludge in a batch reactor or a continuous-flow reactor with shock addition of Ni²⁺. Only a few researchers [10–12] have examined the effect of continuous addition of Ni²⁺ on the ability of activated sludge to remove the organic pollutants in a continuous-flow reactor. In addition, their tested Ni²⁺ concentrations were relatively low, with a maximum range of 10–30 mg/L [10–12]. Therefore, it is needed to study the effects of continuous addition of high-concentration Ni²⁺ on both organic pollutants and ammonia removal abilities of activated sludge in a continuous-flow reactor.

The bulking of activated sludge is a frequently encountered problem in the operation of biological wastewater treatment plants. There have been studies on the effects of metal ions such as calcium, magnesium and iron on the inhibition of bulking in acti-

ated sludge [13,14]. However, little is known about the effects of heavy metals such as Ni²⁺ on bulking control in activated sludge. Only a few researchers [10,12] reported that the settleability and condensation of the sludge in a Ni²⁺-loaded reactor were better than those of the sludge in the control group without the addition of Ni²⁺. Ong et al. [12] observed that the addition of 5 or 10 mg/L of Ni²⁺ could reduce the number of filamentous organisms, as reflected by the decrease of sludge volume index (SVI) from 100 to 85 mL/g and from 100 to 70 mL/g, respectively. Lombraña et al. [10] also reported that the sludge SVI value in an SBR reactor with the addition of 30 mg/L of Ni²⁺ was 29–52 mL/g, which was 30–57% lower than that of the control group without Ni²⁺.

Extracellular polymeric substances (EPS) are products of activate secretion, cell surface material shedding, cell lysis, and sorption from the environment; EPS are composed of a variety of organic substances including polysaccharide, protein, humic-like substances, uronic acids, and deoxyribonucleic acids (DNA) [15]. EPS are the major components of biological flocculation, and can account for up to 50–90% [16,17] of the total organic substances in activated sludge. EPS have a great effect on the settleability, condensation and dewatering of the activated sludge [18–21]. To date, there has been no report on bulking in an activated sludge system under the inhibition of a high-concentration of Ni²⁺, or documented data showing the changes in EPS content and composition in the activated sludge before and after the addition of Ni²⁺. In addition, there has been no report on the correlation between EPS (including its components) and the settleability and dewatering of bulking sludge under such inhibition.

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This study investigated the settleability and dewatering of bulk-activated sludge in a SBR reactor, as well as the changes in, and recovery of, the ability of the sludge to remove organic wastes and ammonia–nitrogen before and after the continuous addition of high-concentration Ni^{2+} (60 mg/L). The study also examined the content and composition changes of the total EPS (EPS_t), the exterior soluble EPS (EPS_s) and the interior bound EPS (EPS_b) of the activated sludge before and after the addition of 60 mg/L of Ni^{2+} , and discussed the correlation between these changes and the sludge settleability and dewatering properties.

2. Experiment

2.1. Experimental methods

The experiment used artificial wastewater with sodium benzoate solution as the organic substrate, having a chemical oxygen demand (COD) of around 800 mg/L. Other components were 80 mg/L NH_4Cl , 8 mg/L K_2HPO_4 , 8 mg/L KH_2PO_4 , 15 mg/L MgSO_4 and 15 mg/L CaCl_2 . The heavy metal salt $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ was added at a later stage, and the influent concentration of Ni^{2+} was 60 mg/L.

The experiment was performed at room temperature using a sequencing batch reactor (SBR). The SBR was run twice per day with 12 h per cycle. Each cycle included 0.5 h of decant, 0.5 h of filling, 6 h of aeration, 2 h of settling and 3 h of idling. The working volume not including the headspace was 4 L, and the total volume was 6 L; 2 L of the clear supernatant water was decanted during the decanting stage in each cycle, then the SBR was refilled with 2 L of fresh wastewater. Within 30 min before the SBR completed the aeration period, the proper level of the mixed liquor was decanted manually on a daily basis in order to keep the sludge residence time at around 16 days. The “seed” sludge was the returned sludge from the settling tank at the Shanghai Quyang wastewater plant. While the SBR was operating, dissolved oxygen during the aeration period was kept at 2.5–3.5 mg/L, mixed liquor suspended solids (MLSS) was kept at 2500–3500 mg/L and pH was 6.5–8.0. The COD and the concentration of ammonia–nitrogen in the influent and effluent water, the sludge volume after 30 min of sedimentation (SV_{30}), SVI, sludge specific resistance to filtration (SRF) and EPS of activated sludge in the SBR were measured periodically.

2.2. Analysis methods

During the experimental period, COD was analyzed with method 5220B, ammonia–nitrogen with method 4500– NH_3 Nitrogen, MLSS with method 2540B, MLVSS with method 2540E, SVI with method 2710D, SV_{30} with method 2710C. All these methods were listed in Standard Methods for the Examination of Water and Wastewater of APHA [22].

The SRF test was performed according to the method of Viadero et al. [23] and Djedidia et al. [24]. The equation for SRF calculation was as following: $\text{SRF} = 2bPA^2/(\mu C)$, where P is the vacuum, b is the slope of the plot, A is the filter area, μ is the viscosity, and C is the weight of solids/unit volume of filtrate, $C = 1/[C_i/(100 - C_i) - C_f/(100 - C_f)]$, where C_i is the initial moisture content, and C_f is the final moisture content.

The analysis of EPS included extraction and measurement. Extraction of EPS was carried out using a heat extraction process similar to that of Morgan et al. [25] and Houghton and Stephenson [26]. The extraction of EPS was done by the following procedure with a TGL-16C high-speed centrifuge: 5 mL of the sludge mixture was spun at 4000 rpm for 10 min in a 7-mL centrifuge tube; the supernatant was collected and filtered through a 0.22- μm filter membrane to remove microbial cells and low molecular-weight metabolites. Filtered samples were washed and resuspended in

5 mL of distilled H_2O , and centrifuged again at 12,000 rpm for 20 min. The supernatant was collected and filtered through a 0.22- μm membrane to remove extractant residues in the solution. The composition of EPS_s in the filtered solution was measured. The retentate after the EPS_s extraction was resuspended in 5 mL of distilled H_2O , mixed well, heated in an 80 °C water bath for 30 min, and then centrifuged at 12,000 rpm for 20 min. The supernatant was filtered through a 0.22 μm membrane to remove extractant residues, and the composition of EPS_b in the filtered solution was measured. The sum of EPS_s and EPS_b was named EPS_t .

The contents of DNA and other small amount of substances such as uronic acids were very few and could hardly influence the content of EPS. This study focused on the variation of EPS content before and after the addition of Ni^{2+} , and the EPS_s , EPS_b , respectively, in this study were composed of three major component contents including polysaccharide, protein, humic acid. Protein and humic acid were measured with the modified Lowry method [27], and the polysaccharide was measured with the anthrone colorimetric assay [28].

3. Results and discussion

3.1. The effect of Ni^{2+} on the settleability and dewatering of activated sludge

Before the addition of 60 mg/L Ni^{2+} , the sludge was severely bulking. As shown in Fig. 1a, SV_{30} was maintained at about 950 mL/L and SVI at about 416–653 mL/g, with an average of 545 mL/g. At day 48, when 60 mg/L Ni^{2+} was added, SV_{30} started to decrease, reaching the value of 898 mL/L at day 60 and 600 mL/L at day 72, then stabilising at about 350 mL/L thereafter. However, unlike SV_{30} , SVI did not decrease until day 60. This is because, during the period between day 48 and 60, the bacteria degrading organic wastes were significantly inhibited, with cell synthesis and proliferation almost stopped, which resulted in the decreases in VSS and suspended solids (SS) in the reactor. SV_{30} continued decreasing until day 60, then discharging sludge was stopped and VSS and SS were maintained in the reactor until MLSS was higher than 3000 mg/L, such that the SVI significantly decreased, to 399 mL/g at day 72 and 155 mL/g at day 84, before stabilising at 104–121 mL/g. The settleability of the sludge was significantly improved, and sludge was no longer in the bulking condition. Similar results were observed by other investigators [10,12], although the SVI values of sludge in their reactors were all lower than 150 mL/g before the addition of Ni^{2+} , and not in the bulking condition [29].

SRF represents the relative resistance offered to drainage of its liquid component [30], and SRF is extensively used to characterize the dewaterability of sludges. A smaller value of SRF indicates better dewaterability [31,32]. The SRF value of activated sludge with relatively good dewaterability is less than 1×10^{12} cm/g, while that with relative bad dewaterability is high than 1×10^{13} cm/g [33]. In this study, SRF at day 36 in bulking state was 2.55×10^{12} cm/g. At day 48, 60 mg/L Ni^{2+} was added in the influent. The SRF decreased to 1.30×10^{12} cm/g at day 52, to 9.50×10^{11} cm/g at day 80, and the dewatering was significantly improved. Therefore, 60 mg/L Ni^{2+} significantly inhibits the bulking of sludge, and improves its settleability and dewatering.

3.2. The effect of Ni^{2+} on bulking sludge waste-removal ability

3.2.1. The effect of Ni^{2+} on bulking sludge organic waste-removal ability

As shown in Fig. 2, the average effluent soluble COD in the SBR was 40 mg/L, with a removal rate of 95.1% before the addition of Ni^{2+} , which indicated that severely bulking sludge still had a strong

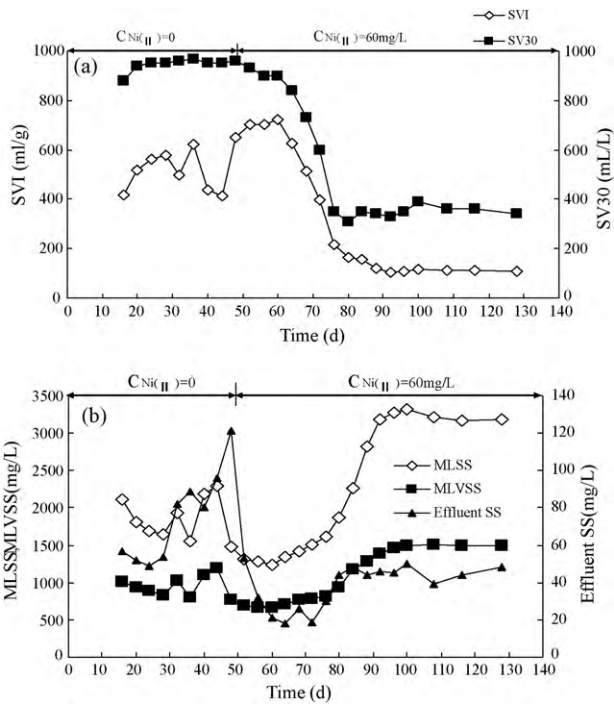


Fig. 1. (a) The effect of Ni^{2+} on sedimentation performance of bulking sludge. (b) The effect of Ni^{2+} on MLSS, MLVSS, and effluent SS of the SBR system.

SVI (mL/g)	SV30 (mL/L)	Time (d)
416.2	880	16
519.1	940	20
561.8	950	24
580.3	950	28
498.7	960	32
622.3	967	36
435.8	950	40
415.6	950	44
652.6	960	48
704.5	930	52
701.5	900	56
721.9	898	60
625	840	64
515.5	730	68
398.9	600	72
216.6	350	76
165.2	310	80
154.9	350	84
120.7	340	88
103.5	330	92
106.7	350	96
117.3	390	100
112.1	360	108
113.9	360	116
117.2	340	128

Time (d)	MLSS (mg/L)	MLVSS (mg/L)	Effluent SS (mg/L)
16	2114	1012	57
20	1811	936	52
24	1691	891	49
28	1637	826	54
32	1925	1020	82
36	1554	796	89
40	2180	1105	80
44	2286	1196	96
48	1471	773	121
52	1320	700	53
56	1283	669	32
60	1244	659	21
64	1344	706	18
68	1416	773	26
72	1504	792	19
76	1616	816	30

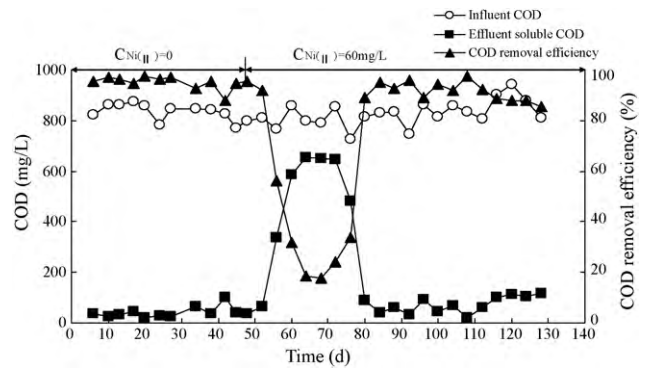


Fig. 2. The effect of Ni^{2+} on COD removal in bulking sludge system.

Influent COD (mg/L)	Effluent soluble COD (mg/L)	COD removal efficiency (%)	Time (d)
824	36	95.6	6
863	23	97.3	10
862	33	96.2	13
877	46	94.8	17
860	20	97.7	20
782	28	96.4	24
846	24	97.2	27
847	63	92.6	34
842	36	95.7	38
827	100	87.9	42
771	40	94.8	45
798	36	95.5	48
810	64	92.1	52
767	336	56.2	56
861	587	31.8	60
800	653	18.4	64
790	650	17.7	68
854	647	24.2	72
726	482	33.6	76
817	88	89.2	80
830	39	95.3	84
835	62	92.6	88
745	31	95.8	92
864	94	89.1	96
815	45	94.5	100
860	70	91.9	104
835	20	97.6	108
807	61	92.4	112
904	100	88.9	116
944	113	88.0	120
880	106	88.0	124
810	118	85.4	128

ability to remove organic wastes. At day 48 of the SBR operation, 60 mg/L Ni^{2+} was added to the influent. The soluble COD concentration in the effluent gradually increased from 36 mg/L at day 48 to 653 mg/L at day 64, with a removal rate of only 18.3%. Such a deteriorated removal efficacy indicated a strong inhibition of bacteria that degrade organic wastes in the reactor. Later, the concentration of effluent soluble COD slowly decreased. Starting from day 76, it rapidly decreased from 482 to 62 mg/L at day 88, and eventually stabilised at about this level, and the average removal rate

Fig. 1 (Continued)

80	1877	934	44
84	2260	1182	48
88	2817	1282	44
92	3188	1384	46
96	3280	1469	45
100	3325	1486	50
108	3210	1502	39
116	3162	1487	44
128	3189	1496	48

was 91.3%, indicating the activity of bacteria that degraded organic wastes had significantly recovered from the previous inhibition. Therefore, 60 mg/L Ni^{2+} significantly inhibits bacteria that degrade organic wastes in the reactor. After a certain period, the bacteria can adapt to 60 mg/L Ni^{2+} , and can therefore efficiently degrade organic wastes and lower the effluent soluble COD close to the level prior to the addition of Ni^{2+} . The bacteria in the reactor can efficiently degrade organic wastes in the presence of 60 mg/L Ni^{2+} . This concentration is far beyond the previously reported concentrations of 10 mg/L [12], 25 mg/L [11] or 30 mg/L [10]. Ong et al. [12] reported that 10 mg/L Ni^{2+} significantly inhibited the bacteria that degraded organic wastes in the SBR, and the almost full recovery of the bacterial activity that was shown by the SBR treatment performance required the discontinuation of 10 mg/L Ni^{2+} addition into the influent, which was not agreeable with the result of this study.

At the early stage after the addition of 60 mg/L Ni^{2+} , the processing efficiency of the activated sludge on soluble COD significantly worsened due to the severe inhibition of bacterial metabolism by the excessive amount of heavy metal. However, the bacteria in the activated sludge were capable of adapting to the changed environment, and the intrinsic metabolism of the bacteria could gradually recover. Therefore, the level of effluent soluble COD in the SBR system gradually decreased with an increasing removal rate, and the quality of the water was improved.

3.2.2. The effect of Ni^{2+} on bulking sludge ammonia–nitrogen removal ability

As shown in Fig. 3, during the first 34 days of reactor operation, the removal rate of ammonia–nitrogen was maintained at about 52.5%, and the effluent ammonia–nitrogen was kept under 40 mg/L. With the reactor running, its ability to remove ammonia–nitrogen gradually increased, reaching the highest removal rate of 87.5% and the effluent ammonia–nitrogen reduced to 10 mg/L. After the addition of Ni^{2+} at day 48, the efficacy of ammonia–nitrogen removal decreased immediately. The concentration of effluent ammonia–nitrogen increased to 35 mg/L at day 52, to 69 mg/L at day 56 and to 77 mg/L at day 60. The removal rate was zero at this point, with almost no removal of influent ammonia–nitrogen. After that, the concentration of effluent ammonia–nitrogen decreased slowly, although it was still maintained at about 61 mg/L at day 128.

Compared with the COD removal efficiency, the inhibition of 60 mg/L Ni^{2+} on nitrobacteria and nitrosobacteria was stronger than that on bacteria that degraded organic wastes, and it was more difficult for nitrobacteria and nitrosobacteria to adapt to the inhibition and recover in the SBR system. So that the effluent soluble COD could be recovered to the level prior to the addition of Ni^{2+} after certain period, whereas the level of effluent ammonia–nitrogen could not.

You et al. [5] reported that, in batch experiments, the specific ammonia utilisation rate (SAUR) inhibitions were about 90% and 70%, respectively, at 10 mg/L influent Ni^{2+} for the activated sludges taken from an anaerobic-anoxic-oxic (A_2O) process and from a SBR. Hu et al. [34] found that 11.7 mg/L Ni^{2+} caused 30% inhibition of ammonia oxidation when a mixed nitrifying microbial culture was growing in a batch system, while inhibition over 95% occurred when the same culture was growing in a continuous system with a 24-h shock addition. In this experiment, with a continuous loading of 60 mg/L Ni^{2+} into the SBR under operation, the results were consistent with those previously reported, in that the removal of ammonia–nitrogen was strongly inhibited.

3.3. The effect of Ni^{2+} on EPS of bulking sludge

3.3.1. The effect of Ni^{2+} on the EPS content of bulking sludge

The EPS contents of bulking sludge when Ni^{2+} was added and the changes after the addition of Ni^{2+} are shown in Fig. 4. In bulk-

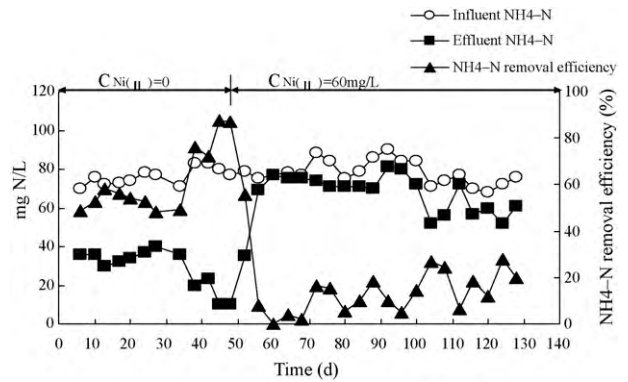


Fig. 3. The effect of Ni^{2+} on $\text{NH}_4\text{-N}$ removal in bulking sludge system.

Influent $\text{NH}_4\text{-N}$ (mg/L)	Effluent $\text{NH}_4\text{-N}$ (mg/L)	$\text{NH}_4\text{-N}$ removal efficiency (%)	Time (d)
70	36	48.6	6
76	36	52.6	10
72	30	58.3	13
73	32	56.2	17
74	34	54.1	20
78	37	52.6	24
77	40	48.1	27
71	36	49.3	34
83	20	75.9	38
83	23	72.3	42
80	10	87.5	45
77	10	87.0	48
79	35	55.7	52
75	69	8.0	56
77	77	0.0	60
78	75	3.8	64
77	75	2.0	68
89	74	16.5	72
84	71	15.5	76
75	71	5.3	80
79	71	9.7	84
86	70	18.6	88
90	81	10.0	92
84	80	4.8	96
84	72	14.3	100
71	52	26.8	104
74	56	24.3	108
77	72	6.5	112
70	57	18.6	116
68	60	11.8	120
72	52	27.8	124
76	61	19.7	128

ing sludge, the contents of EPS_t and its EPS_s and EPS_b components were relatively high, with values of 294.9 mg/g-VSS, 128.4 mg/g-VSS and 166.4 mg/g-VSS, respectively. Starting from day 48 after the addition of 60 mg/L Ni^{2+} , eight days later, i.e., at day 56, the contents of EPS_t , EPS_s and EPS_b all slightly increased. They then decreased slightly, and the decrease became obvious at day 72. At day 88, the decrease gradually levelled off, and the contents of EPS_t , EPS_s and EPS_b were only 18.1%, 16.6% and 19.3% of the corresponding contents of the bulking sludge at day 48 before the addition of 60 mg/L Ni^{2+} . By comparison, the content of EPS_s was higher than that of EPS_b in bulking state, while in steady state after the addition of 60 mg/L Ni^{2+} , the content of EPS_s was close to that of EPS_b .

Some researchers have investigated the variation of EPS production affected by heavy metals. Aquino and Stuckey [35] suggested that after the spike with chromium at 50%, 75%, and 100% of the threshold value that was 150 mg/L, respectively, the biomass had produced more EPS material. Aquino and Stuckey investigated the anaerobic bacteria with batch experiment, and the exposure to chromium lasted comparatively short. Sheng et al. [36] reported

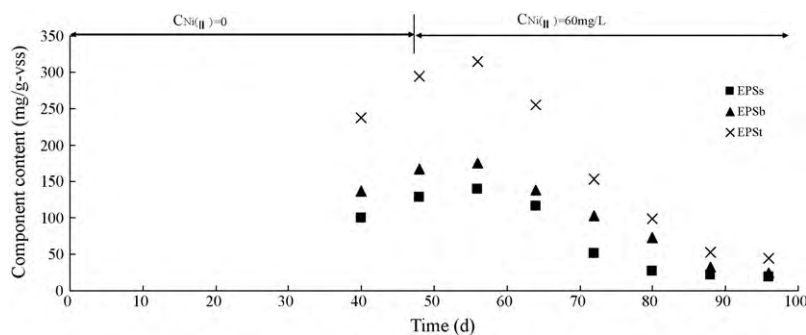


Fig. 4. The effect of Ni^{2+} on EPS component contents of bulking sludge.

EPSs (mg/g-vss)	EPSb (mg/g-vss)	EPSt (mg/g-vss)	Time (d)
			0
			8
			16
			24
			32
101.05	136.88	237.92	40
128.42	166.43	294.85	48
139.52	175.56	315.08	56
116.64	138.2	254.84	64
51.13	102.44	153.57	72
26.68	72.89	99.57	80
21.32	32.12	53.44	88
19.6	24.9	44.5	96

that at concentrations of 30 mg/L Cu(II), 40 mg/L Cr(VI), 5 mg/L Cd(II), the EPS content secreted by *R. acidophila* increased by 5.5, 2.5, 4.0 and 1.4 times, respectively, than the control without the dosage of toxicant. In Sheng's study, the *R. acidophila* taken from anaerobic sludge was incubated in a vial for only 70 h, and was exposed to the heavy metal for a comparatively short-term. Both Aquino and Stuckey and Sheng et al. based their studies on anaerobic bacteria with batch experiments, and both of their studies focused on the effects of shock addition of heavy metal on comparatively short-term cultured bacteria. They reported that the EPS production increased after the dosage of heavy metal than the control group. However, this experiment investigated the EPS variation of aerobic bacteria affected continuously by heavy metal during a long period, and got the disagreeable result with Both Aquino and Stuckey and Sheng et al., which suggested that effects of heavy metal on EPS production of aerobic bacteria and that of anaerobic bacteria were different, and that the variation of EPS production under continuous exposure to heavy metal was not consistent with the variation of EPS production influenced by shock dosage of heavy metal. Therefore, it was necessary for further investigation in the difference of EPS variation secreted by different class of bacteria affected by toxicant.

In the early stage after the addition of 60 mg/L Ni^{2+} , the normal metabolism of bacteria was inhibited, and the secretion of EPS was dramatically decreased. A large number of bacteria with weak tolerance apparently died, as evidenced by the substances released from these lysed cells being absorbed by living bacteria and transformed into EPS. A small population of bacteria with a stronger tolerance may secrete EPS at a higher than normal level, alleviating the negative effect of Ni^{2+} . Taken together, the contents of EPS_t , EPS_s and EPS_b did not change significantly at the early stage after the addition of Ni^{2+} , showing a slight increase followed by a slow decrease. At the later stage, it was likely that fewer bacterial cells with a weak tolerance lysed. Although bacteria adapted to a high-concentration of Ni^{2+} proliferated, the newly-generated bacteria secreted less EPS, thereby resulting in the dramatic decrease of EPS_t , EPS_s and EPS_b . Previous studies have shown that bacteria can secrete a large amount of EPS to protect themselves against

the toxic effect of metal ions [15,21]. This experiment, however, demonstrated that the EPS content in bacteria pre-adapted to the high-concentration of Ni^{2+} dramatically decreased compared to that without Ni^{2+} , and that the compositions of the EPS also changed significantly.

The percentages of EPS_s and EPS_b in EPS_t at day 48 in bulking state were 43.6% and 56.4%, respectively. After the addition of Ni^{2+} , the percentages of EPS_s and EPS_b in EPS_t at day 88 were 39.9% and 60.1%, respectively. From day 48 to day 88, the percentage of EPS_s in EPS_t decreased 3.7% while that of EPS_b in EPS_t increased 3.7%. EPS_s plays a role as a microbial protective barrier and it is the most active EPS towards the metallic cations such as Ni^{2+} [37], which suggests EPS_s is more liable to be affected by Ni^{2+} compared with EPS_b . Furthermore, EPS_b only attaches to the cell surface as peripheral capsules and can be shed into the surrounding environment as the less organized EPS_s [38]. After the addition of 60 mg/L Ni^{2+} , ion intensity increased, and this led to less EPS_b shed into the surrounding environment. Therefore, the percentage of EPS_s in EPS_t decreased while that of EPS_b increased.

3.3.2. The effect of Ni^{2+} on compositions of bulking sludge EPS

During the first 48 days of reactor operation, the activated sludge was in the bulking condition. The sludge at day 48 before the addition of 60 mg/L Ni^{2+} was taken as a representative example of bulking sludge and the sludge at day 88 was taken as a representative example of sludge adapted to 60 mg/L Ni^{2+} . When analyzing, two samples were taken from the reactor after slightly churning up the sludge-water mixture 5 min before stopping aeration, and each sample was analyzed for two times. The compositions of sludge EPS at day 48 and 88 are compared in Fig. 5. Fig. 5a shows that before the addition of Ni^{2+} , the contents of bulking sludge EPS_t were 40.8 mg polysaccharide/g-VSS, 116.8 mg protein/g-VSS and 137.3 mg humic acid/g-VSS, in the order of predominance: humic acid > protein > polysaccharide. After the addition of Ni^{2+} , the content of EPS_t was stable until day 88, when the contents of the different components were 15.6 mg polysaccharide/g-VSS, 70.4 mg protein/g-VSS and 37.4 mg humic acid/g-VSS. It noted

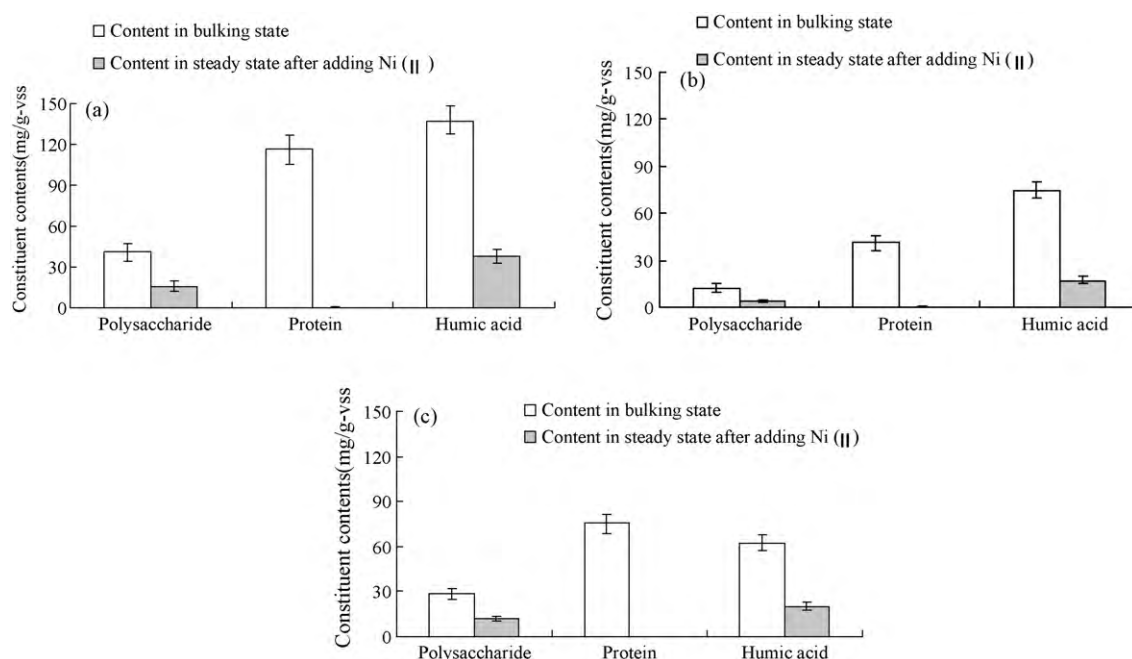


Fig. 5. The effect of Ni^{2+} on EPS composition. (a) The effect of Ni^{2+} on EPSt component contents of bulking sludge, (b) the effect of Ni^{2+} on EPSs component contents of bulking sludge and (c) the effect of Ni^{2+} on EPS_b component contents of bulking sludge.

Total EPS component contents	Polysaccharide	Protein	Humic acid
In bulking state (mg/g-vss)	40.8	116.8	137.3
After adding Ni(II) (mg/g-vss)	15.6	0.4	37.4
Soluble EPS component contents	Polysaccharide	Protein	Humic acid
In bulking state (mg/g-vss)	12.4	41.3	74.7
State after adding Ni(II) (mg/g-vss)	3.7	0.4	17.2
Bound EPS components	Polysaccharide	Protein	Humic acid
In bulking state (mg/g-vss)	28.4	75.4	62.6
After adding Ni(II) (mg/g-vss)	11.9	0.0	20.2

that the content of every component decreased dramatically. The protein content decreased the most, a 99.7% decrease compared to that in the bulking condition. The polysaccharide content decreased by 61.7% and the humic acid content decreased by 72.7%. The order of the fractional compositions changed to humic acid > polysaccharide > protein. Among these, the protein content was too low to be detected, and could be ignored, such that the sludge EPS_t was mainly composed of humic acid (70.0%) and polysaccharide (29.2%). Similar results were obtained in the analysis of EPSs. The contents of total EPSs and components thereof all dramatically decreased in the stabilised sludge after Ni^{2+} addition. The extent to which the protein content decreased was 99.0%, which is the largest change, compared to 70.3% for polysaccharide and 76.9% for humic acid. The order of predominance in the fractional compositions changed from humic acid > protein > polysaccharide to humic acid > polysaccharide > protein, with the major component being humic acid (80.9%), plus some polysaccharide (17.3%) and very little protein (1.9%).

The order of EPS_b compositions in the bulking sludge was slightly different from that of EPS_t or EPSs. As shown in Fig. 5c, the order was protein (75.4 mg protein/g-VSS) > humic acid (62.6 mg humic acid/g-VSS) > polysaccharide (28.4 mg polysaccharide/g-VSS). In the stabilised sludge after Ni^{2+} addition, the pattern of EPS_b changes was similar to that of EPS_t and EPSs, namely the order humic acid > polysaccharide > protein, with the main component again being humic acid (62.9%), plus a little more polysaccharide (37.1%) and very little protein (close to 0).

Though many researchers had investigated the biosorption of heavy metals by EPS [39–42] or the variation of EPS content under the effect of heavy metals [43,44]. Few reported clearly about the variation of EPS component contents under the effect of heavy metals. Jang et al. [45] only reported the changes of EPSs component contents including carbohydrate and protein when the biofilm was exposed to heavy metals, however, neither the change of EPS_b components nor the component of humic acid in EPSs and EPS_b was described. This experiment studied the EPS of activated sludge, and reported the variation of component contents of EPSt, EPSs, and EPS_b. Sheng et al. [36] investigated EPS secreted by *R. acidophila* taken from anaerobic sludge, and reported that the proteins/carbohydrates ratios in the presence of 30 mg/L Cu(II), 40 mg/L Cr(VI), 5 mg/L Cd(II), respectively, were always higher than that of control. However, this experiment studied EPS secreted by aerobic bacteria, and observed that the proteins/carbohydrates ratio decreased after the dosage of 60 mg/L Ni^{2+} . Therefore, it seemed that effects of heavy metal on EPS components of aerobic bacteria and that of anaerobic bacteria were different, and it was necessary to carry out further investigation.

By comparing the results in Figs. 1, 4 and 5, with the bulking sludge, the high contents of EPS_t, EPSs and EPS_b are associated with high SV₃₀ and SVI values. From day 48, 60 mg/L Ni^{2+} was dosed in the reactor, and the toxicity of Ni^{2+} could inhibit the growth of filamentous bacteria [46], which stopped the deterioration of activated sludge bulking. Meanwhile, EPS in sludge was the most important parameter with respect to sludge structure

[47], and the presence of EPS formed a negatively charged polymer network [15,47,48]. Due to the strong bonding of cations with negatively charged EPS molecules [15], the dosage of 60 mg/L Ni^{2+} resulted in the cations' bridging between negatively charged EPS molecules, which also improved the characteristics of sludge settling and thickening. Between days 48 and 60, the SVI remained high even though the SV_{30} decreased. This was due to the decrease in the amount of bacteria and the slight increase in EPS content. The sludge EPS_s and EPS_b did not significantly decrease until day 72 (when there was also a significant decrease of EPS_t), after which the settleability of the sludge improved significantly. Therefore, it appears that if there is too much EPS outside the bacterial cells with a high protein content, condensed sludge flocculation is difficult, compromising the settleability.

Before the addition of Ni^{2+} , the bulking sludge had a high level of EPS. The high hydratability of EPS could keep water in the sludge matrix, thus negatively affecting sludge dewatering. The high hydratability of EPS resulted from the large number of hydrophilic groups found in its protein and polysaccharide components. Upon reaching steady state after the addition of Ni^{2+} , the content of EPS and EPS components decreased dramatically, particularly that of polysaccharide and protein, which directly resulted in the decrease of hydrophilic groups in EPS, therefore significantly improving the dewatering of sludge.

4. Conclusions

- (1) With the continuous dosage of high-concentration (60 mg/L) Ni^{2+} into the influent of the SBR, sludge bulking was dramatically inhibited. SV_{30} , SVI and SRF of the sludge gradually decreased and its settleability and dewatering were significantly improved.
- (2) Bulking activated sludge had a stronger capability of removing pollutants such as organic substances and ammonia–nitrogen. The addition of 60 mg/L of Ni^{2+} greatly inhibited the ability of the bulking sludge to remove the organic wastes and ammonia–nitrogen. However, after a 40-day acclimation, the existing and newly-generated bacteria degrading organic wastes adapted to 60 mg/L of Ni^{2+} . Thereafter, the soluble COD in the effluent decreased from 653 to 62 mg/L during the inhibition period. However, nitrobacteria and nitrosobacteria could not adapt to 60 mg/L of Ni^{2+} , even after an 80-day acclimation, nor could the nitrification capability be significantly recovered.
- (3) The addition of 60 mg/L of Ni^{2+} altered the EPS in bulking activated sludge. The contents of the EPS_t as well as that of EPS_s and EPS_b changed, first showing a slight increase, followed by a dramatic decrease and finally reaching a steady state.
- (4) The EPS_s in bulking activated sludge contained more humic acid than protein and polysaccharide (humic acid > protein > polysaccharide), while EPS_b contained more protein than humic acid and polysaccharide (protein > humic acid > polysaccharide), and EPS_t contained more humic acid than protein and polysaccharide (humic acid > protein > polysaccharide).
- (5) In non-bulking activated sludge which had adapted the dosage of 60 mg/L Ni^{2+} , the contents of the EPS_s and EPS_b decreased dramatically to 19.3% and 18.1%, respectively, of the values from bulking activated sludge before the addition of Ni^{2+} , as did the contents of their major components including polysaccharide, protein and humic acid, to only 38.3%, 0.3%, 27.3% of their initial values. The effects of Ni^{2+} on EPS_s and EPS_b in bulking sludge were similar. In addition, the predominance orders of the EPS_s and EPS_b component contents both changed to humic acid > polysaccharide > protein. Protein content was the lowest with the largest decrease. After this adaptation, the settleability and dewatering of the sludge were improved.

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