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Effect of nickel on the flocculability, settleability, and dewaterability of activated sludge

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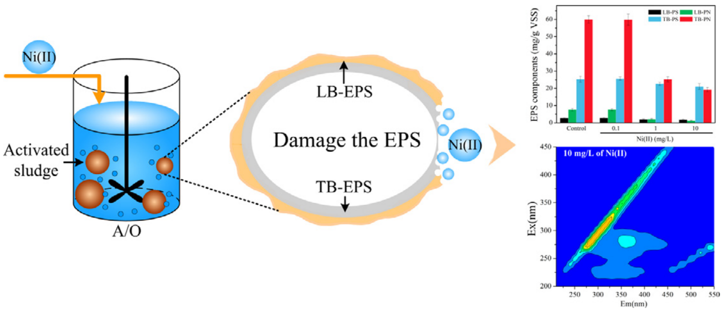
HIGHLIGHTS

- Long-term exposure to Ni(II) exhibited adverse effects on the sludge flocculability.
- The content of PN in EPS decreased after long-term exposure to Ni(II).
- EEM and FTIR showed the change of structures and functional groups in EPS.
- The hydrophobicity of sludge decreased with the increasing Ni(II) level.
- The deleterious effect of Ni(II) could be mitigated by adding the EDTA or citrate.

GRAPHICAL ABSTRACT



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ABSTRACT

Short-term and long-term effects of nickel (Ni) (0.1–10 mg/L) on the physicochemical properties of activated sludge, including the flocculability, settleability, and dewaterability, were investigated. It was found that these properties were unaffected after short-term exposure (1 day) to Ni(II) even at the level of 10 mg/L. After long-term exposure (60 days) to 1 and 10 mg/L of Ni(II), however, the sludge flocculability has seriously deteriorated, while the settleability, and dewaterability became gradually better than the control. The mechanism studies revealed that long-term exposure to Ni(II) resulted in the decrease of protein content in extracellular polymeric substances (EPS) and the damage to EPS structures. Although Ni(II) did not bring any adverse effect on the cell membrane, the relative hydrophobicity of activated sludge was significantly decreased. The negative effects on the flocculability and phosphorus removal performance of activated sludge could be completely eliminated by adding the chelator such as EDTA and citrate.

1. Introduction

Nickel (Ni) has widely been used in many consumer products such as batteries, high quality iron-based alloys, catalysts, and paints due to its unique physical and chemical properties (Gikas, 2007; You et al., 2009). The extensive applications of Ni inevitably cause its release into municipal wastewater treatment plants (WWTPs) (Gikas, 2008; Wang et al., 2013). Previous studies documented that Ni(II) concentration in the municipal wastewaters is greater than 0.1 mg/L in India, Greece, and Turkey, even greater than 0.5 mg/L (Karvelas et al., 2003; Singh et al., 2004; Üstün, 2009). In the municipal WWTPs that receive some specific

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industrial wastewaters, this level is even higher. It is reported that it is generally greater than 10 mg/L in the Ni-relevant industrial wastewaters (Akbal and Camci, 2011; Malakootian et al., 2015). Therefore, the potential effect of Ni(II) on WWTPs has attracted increasing attention.

Recently, the effect of Ni on the biological treatment process has concentrated on the removal of organic pollutants and nitrogen (Gikas, 2008; Hu et al., 2002, 2003, 2004; Ong et al., 2004; You et al., 2009). These removal performances are relevant to or dependent upon the physicochemical properties of activated sludge, including the flocculability, settleability, and dewaterability (Ye et al., 2011a,b). Therefore, the variations of these properties are also used to predict the effect of toxic pollutants such as heavy metal ions on activated sludge systems (Chen et al., 2014). Some researchers have investigated the effect of Ni(II) on the settleability and dewaterability of sludge (Li et al., 2011; Ong et al., 2004; Wang et al., 2010). However, these investigated Ni (II) concentrations were usually in the range of 60–240 mg/L, which were much higher than that in real municipal wastewater. Thus, the potential effect of Ni(II) in relatively low concentration should also be studied. Moreover, to date, little is known about the short-term and long-term effect of Ni(II) on the flocculability of activated sludge.

Extracellular polymeric substances (EPS) are thought to be the glue that binds microorganisms cells together to form activated sludge flocs and play a leading role in the physicochemical properties of the flocs (Li and Yang, 2007; Ye et al., 2011b). EPS have a dynamic double-layered structure, including the loosely bound EPS (LB-EPS) and the tightly bound EPS (TB-EPS), and mainly consist of protein (PN), polysaccharide (PS), and small amounts of humic acids, lipids and nucleic acids (Luo et al., 2013; Yang et al., 2010). The structures and compositions of EPS are closely correlated with the flocculability, settleability, and dewaterability of sludge, and can be affected by heavy metal (Liao et al., 2001; Sun et al., 2009). In addition, the physicochemical properties are relevant to the surface properties of activated sludge such as the hydrophobicity, the surface charge, and the integrity of cell membrane (Jin et al., 2003). These surface properties can also be affected by heavy metal cations such as Cu(II) (Chen et al., 2014). Therefore, Ni(II), as a highly soluble heavy metal cation, may change the surface properties and the structures and compositions of EPS, further affecting the physicochemical properties of sludge. For the purpose of giving deep insight into the effect of Ni(II), these possibilities should be clarified by experiments.

When Ni(II) enters the WWTPs, another important job is to explore an effective method to reduce its deleterious effect on activated sludge. Some publications point out that the heavy metal toxicity to activated sludge is strongly correlated with the activities of free metal ions. Their activities can significantly be altered by adding the strong complexing agent (e.g., EDTA) (Hu et al., 2002). Accordingly, the effect of Ni(II) on the physicochemical properties and removal performance may be mitigated by adding the chelator, and yet this assumption needs to be confirmed.

The objectives of this work are therefore to (i) investigate the short-term and long-term effects of Ni(II) ranging from 0.1 to 10 mg/L on the flocculability, settleability, and dewaterability of activated sludge in enhanced biological phosphorus removal (EBPR) system; (ii) explore the details of how Ni(II) affects these properties from the aspects of the compositions and structures of EPS by using excitation-emission matrix (EEM) fluorescence spectroscopy and Fourier-transform infrared (FTIR) spectroscopy, and the surface properties of activated sludge; (iii) find a valid strategy to mitigate the adverse effect of Ni(II).

2. Materials and methods

2.1. Start-up of parent sequencing batch reactor (SBR)

A parent SBR with 80 L working volume was operated under alternate anaerobic-aerobic model to culture the EBPR activated sludge. The seed sludge was collected from the return sludge in Guozhen Wastewater Treatment Plant, Changsha, China. The reactor was operated with three cycles daily at controlled room temperature (21 ± 1 °C). Each cycle (8 h) comprised 20 min of feeding, 2 h of anaerobic and 3 h of aerobic stage, followed by 80 min of settling, 20 min of decanting and 60 min of idle stage. After settling period, 50 L supernatant was discharged from the reactor, and replaced with the same volume of fresh feeding medium (component detailed as below) at the feeding stage. The influent pH value was maintained at 7.5 ± 0.2 by using 2 M HCl or 2 M NaOH. At the anaerobic stage, the SBR was continuously mixed with a mechanical stirrer, and then air was provided to keep the dissolved oxygen (DO) level between 2 and 3 mg/L at the aerobic stage. In order to control the sludge retention time (SRT) at about 10 days, 2.66 L sludge mixture was wasted at the end of aerobic stage per cycle but before settling. After 60 days of cultivation, the relatively stable effluent suspended solids (ESS) and P removal efficiency (>98%) were achieved in the parent SBR.

The feeding medium was prepared daily, and its components were as follows (mg/L): 255.4 CH_3COONa, 49.03 $K_2HPO_4{\cdot}3H_2O$ and 14.62 KH_2PO_4, leading to a theoretical ratio of chemical oxygen demand (COD) to P (=20 mg COD/mg P), which was thought to be favorable for biological P removal (Wang et al., 2012). The other nutrients in the medium consisted of (per liter): 57.2 mg NH_4Cl, 10 mg $MgSO_4{\cdot}7H_2O$, 5 mg $CaCl_2$, and 0.5 mL of trace metals solution. This trace-element solution comprised (g/L): 0.03 $CuSO_4{\cdot}5H_2O$, 0.06 $Na_2MoO_4{\cdot}2H_2O$, 0.12 $MnCl_2{\cdot}4H_2O$, 0.12 $ZnSO_4{\cdot}7H_2O$, 0.15 $CoCl_2{\cdot}6H_2O$, 0.15 H_3BO_3, 0.18 KI, 1.50 $FeCl_3{\cdot}6H_2O$, and 10 ethylenediamine tetra-acetic acid. Allylthiourea (10 mg/L) was provided in this medium to inhibit nitrification.

2.2. Batch Ni(II) toxicity experiments

Three Ni(II) levels (i.e., 0.1, 1, and 10 mg/L, from environmentally relevant level to high level) were set to assess the short-term and long-term effect of Ni(II) on the physicochemical properties. The 12 L biomass obtained from the parent SBR, was firstly washed for 3 times with distilled water, and then divided evenly to four identical SBRs with 3 L working volume. All reactors operated according to the procedure as same as the parent SBR. After settling period, 2 L supernatant was taken out from each SBR, and replaced with 2 L fresh feeding mediums containing 0 (i.e., the control reactor), 0.1, 1, and 10 mg/L of Ni(II) at the feeding stage, respectively. The feeding mediums were prepared by diluting 0, 2, 20, and 200 mL nickel sulfate stock solution (100 mg/L), respectively. Except for Ni(II) concentrations, the other components were identical to the parent reactor. After the addition of Ni(II), the effluent P concentrations, ESS, sludge volume index (SVI) and capillary suction time (CST) values in all SBRs were measured every day to indicate the effect of Ni (II). In this study, the short-term exposure was defined as the exposure of activated sludge to Ni(II) only 1 day. After 60-days exposure, these determined data reached relatively stable. So the 60-days exposure to Ni(II) was defined as the long-term exposure. At the day 60, the compositions and structures of EPS, the surface hydrophobicity, the cell surface charge, and the integrity of cell membrane were determined to reveal the long-term effects of Ni(II).

2.3. Reduction Ni(II) toxicity experiments

The complexing agents including EDTA and citrate were chosen to explore the strategy for reducing Ni(II) toxicity. Six identical SBRs with 3 L working volume each were continuously worked for 60 days. Four identical SBRs were fed with the mediums containing 10 mg/L of Ni(II) (about 0.17 mM), and 0.2 or 1 mM of EDTA and citrate, respectively. Other two reactors were fed with the mediums without the Ni(II) and containing 1 mM of EDTA or citrate as the control. Six SBRs were continuously worked for 60 days.

2.4. Analytical methods

2.4.1. Ni(II) analysis

To determine the total Ni(II) concentrations in activated sludge, 0.2 g of each powdered sample was wet-digested by adding 9 mL of concentrated nitric acid-perchloric acid (HNO_3-$HClO_4$; 2:1) mixture in a 25 mL of Teflon perfluoroalkoxy vial. After 2 mL of hydrofluoric acid (HF) acid was added, the mixtures were then heated until a clear solution was formed. This process was continued to near dryness. The cooled residue was dissolved in 5 mL of HNO_3 (2%), and the solution was diluted to 25 mL with deionized water for measuring the total Ni(II) content in the sludge. The different fractions of Ni(II) in activated sludge were extracted according to the BCR European Communities Bureau of Reference's (BCR) sequential extraction procedure as described in our previous study (Chen et al., 2008). The concentration of Ni(II) was detected by using a flame atomic absorption spectroscopy (PEAA700, Switzerland).

2.4.2. EPS analysis

A heat extraction method was used to extract the LB-EPS and TB-EPS of activated sludge, and the detailed extraction procedure was reported in our previous study (Liu et al., 2016). The extractions of LB-EPS and TB-EPS were analyzed for the PN, PS, and total organic carbon (TOC). TOC was determined by a TOC analyser (TOC-$V_{CPH/CPN}$, Shimadzu) using the combustion-infrared method. The PN were detected according to the modified Lowry method with bovine serum albumin as the standard, and the PS content was measured using the phenol-sulphuric acid method (Liu et al., 2016).

In order to characterize the structure of EPS, the EEM fluorescence spectra of LB-EPS and TB-EPS extractions were determined by using a luminescence spectrometry (F-4600 FL Spectrophotometer, Hitachi, Japan). To obtain the EEM spectra of EPS, the emission (Em) and excitation (Ex) wavelengths were increased at 5 nm increments from 210 to 550 nm and from 200 to 450 nm, respectively. The Em and Ex slits were set at 5 nm, and the scanning speed was kept at 4800 nm/min for all samples.

The extractions of LB-EPS and TB-EPS were freeze-dried to powder prior to FTIR spectroscopy analysis. Then the samples and dried potassium bromide (KBr, IR grade) were mixed at a ratio of 1:100. The mixture was ground and homogenized in an agate grinder, and then compressed between two polished and clean iron anvils at 10 kPa to form a KBr window. FTIR spectra were obtained with an FTIR spectrometer (IR Prestige-21, Shimadzu, Japan).

2.4.3. Other analysis

The measurements of P, ESS, SVI, SV30, MLSS and MLVSS were performed following the standard methods (APHA, 2005). The CST value was determined with a CST instrument (model 304B, Triton, UK) equipped with Whatman No.17 chromatography-grade and paper 18 mm diameter funnel. The relative hydrophobicity of sludge was measured by microbial adhesion to hydrocarbons assay (Chen et al., 2014). The lactate dehydrogenase (LDH) release assay was conducted to investigate the cell membrane integrity of activated sludge (Chen et al., 2012). The net surface charge of activated sludge was detected by colloidal titration (Wilen et al., 2003).

2.5. Statistical analysis

All measurements were conducted in triplicate and results were expressed as mean ± standard deviation. An analysis of variance was used to evaluate the significance of results, and $p < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Effect of Ni(II) on the flocculability, settleability, and dewaterability of activated sludge

Table 1 presents the flocculability, settleability and dewaterability variations of activated sludge after short-term exposure to 0 (control), 0.1, 1, and 10 mg/L Ni(II). It can be found that the ESS, SVI, and CST values in all Ni(II) exposure reactors were almost same as those in the control test. The result indicated that short-term exposure to Ni(II) even at the level of 10 mg/L had little effect on these physicochemical properties of sludge. It is known that WWTPs are long-term operated, and short-term exposure experiments cannot represent the long-term effect of Ni(II). Thus, the long-term exposure tests were also conducted in this study.

As shown in Fig. 1, the physicochemical properties of sludge in the reactor exposed to 0.1 mg/L of Ni(II) almost kept constant with the increase of exposure time, suggesting that the environmentally relevant level of Ni(II) even in long-term experiments cannot cause significant effect on the flocculability, settleability and dewaterability of sludge. However, Fig. 1a shows that the ESS concentrations in the 1 and 10 mg/L of Ni(II) exposure reactors were remarkably increased with the exposure time, which were much higher than that in the control test. This result indicated that long-term exposure to the higher concentration of Ni(II) would result in the deterioration of sludge flocculability and effluent clarification. The data in Fig. 1b and c indicated that the SVI and CST values at the Ni(II) exposure concentration of 1 and 10 mg/L obviously decreased as the exposure time increased. The result implied that the settleability and dewaterability of sludge became better after long-term exposure to higher concentration of Ni(II), which was consistent with previous investigations (Li et al., 2011; Wang et al., 2010).

It was worth noting that the long-term effect of Ni(II) on the physicochemical properties of sludge was quite different from its short-term effect. This result might be attributed to the fact that the total Ni(II) concentration in activated sludge after the long-term exposure was much higher than that after short-term exposure. As shown in Table 2, the total Ni(II) content was increased with the increase of the Ni(II) feeding concentration, and yet this content was relatively small even after the short-term exposure to 10 mg/L of Ni(II) (2.19 mg/g MLSS). After long-term exposure to 1 and 10 mg/L of Ni(II), however, the total concentrations of

Table 1
Characteristics of the sludge and effluent in EBPR system after short-term to Ni(II).[a]

Ni(II) concentration (mg/L)	ESS (mg/L)	SVI (mL/g)	CST (s)
Control	18.9 ± 2.3	109.1 ± 2.9	50.4 ± 1.5
0.1	20.2 ± 2.2	111.5 ± 2.4	47.8 ± 1.9
1	22.6 ± 2.1	109.9 ± 3.5	48.9 ± 2.0
10	21.5 ± 1.9	111.2 ± 3.9	49.5 ± 2.6

[a] Results are the averages and their standard deviations of triplicate determinations.

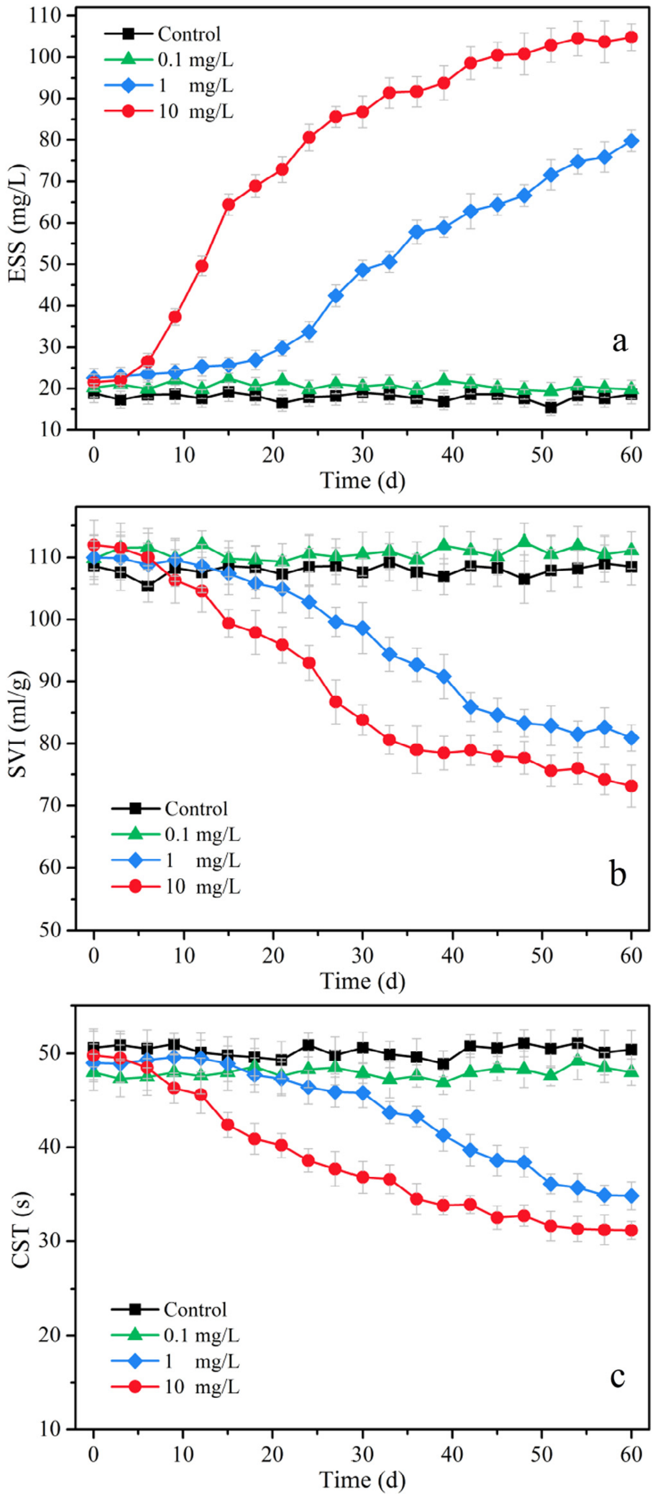

Fig. 1. Variations of ESS (a), SVI (b), and CST (c) values during the long-term exposure to different Ni(II) concentrations. Error bars represent standard deviations of triplicate determinations.

Ni(II) in sludge went up 8.71 and 96.64 mg/g MLSS respectively. Apart from relying on the total concentration, the eco-toxicity and bioavailability of heavy metals also depend on their fractions in sludge (Chen et al., 2008). Based on the BCR's sequential extraction program, the total Ni(II) in activated sludge is divided into four fractions: exchangeable/acid soluble fraction, reducible fraction, oxidizable fraction, and residual fraction. In general, the exchangeable/acid soluble fraction and the reducible fraction are identified as the direct effect fractions (Chen et al., 2008). In this study, the predominant fractions of Ni(II) in the activated sludge were these two fractions, and their contents in the long-term test were also much higher than that in the short-term test (Table 2). This result once again explained why the long-term effect of Ni(II) is more obvious than the short-term effect.

3.2. Effect of Ni(II) on the compositions and structures of EPS

Fig. 2a shows that no significant variations of the LB-EPS and TB-EPS content were found after long-term exposure to 0.1 mg/L of Ni(II) compared with the control. However, their content significantly decreased when the exposure concentration increased to 1 or 10 mg/L (Fig. 2a). It is widely reported that the increased EPS content can reduce the sludge dewaterability since EPS are highly hydrated (Mikkelsen and Keiding, 2002). Therefore, the decreased EPS content induced by Ni(II) might be responsible for the improved dewaterability. As PN and PS are thought to be the major components of EPS, the content of PN and PS in EPS is also determined in this study. As shown in Fig. 2b, the levels of PS in LB-EPS and TB-EPS among the investigated reactors had little change, while the corresponding PN content was remarkably decreased under exposure to higher Ni(II) concentrations. These results indicated that the decreased content of LB-EPS and TB-EPS in the higher Ni(II) reactors was mainly due to the decrease of PN content, which was coincident with the fact that Ni(II) can damage protein structure and function. Moreover, PN of EPS is reported to be more important than PS in the flocculability, settleability and dewaterability of activated sludge (Ye et al., 2011a). Higgins and Novak (1997) pointed out that the degradation of PN could lead to the disintegration of the sludge. Some publications reported that the sludge dewaterability and settleability were significantly improved with the decreasing PN level of EPS (Cetin and Erdincler, 2004; Martinez et al., 2000). As a consequence, the decreased PN content of EPS caused by Ni(II) might be a reason for the good settleability and dewaterability as well as the deteriorated flocculability.

Previous studies confirmed that PN comprises large quantities of aromatic structures, which have fluorescence characteristics. In this study, the EEM was used to investigate the effect of Ni(II) on the EPS structures. Fig. 3 presents the EEM fluorescence spectra of LB-EPS and TB-EPS after long-term exposure to different Ni(II) concentrations. Peak A in LB-EPS and TB-EPS is identified at excitation/emission (Ex/Em) of 220–225/305 nm, which is relevant to simple aromatic proteins such as tyrosine. Peak B is located at Ex/Em of 220–225/335–345 nm, and peak C is situated at 275–280/335–350 nm. The two peaks are related to aromatic protein-like substances (peak B) and tryptophan protein-like substances (peak C). Peak D is observed at Ex/Em of 335–350/420–440 nm, which is assigned to humic acid-like substances (Wang et al., 2014).

It is well-known that the variations of fluorescence peak intensities and locations are relevant to the changes of EPS structures (Luo et al., 2013). Compared with the control, the locations and fluorescence intensities of peaks A-D in LB-EPS and TB-EPS exhibited no significant variations after long-term exposure to 0.1 mg/L of Ni(II) (Fig. 3). This result suggested that environmentally relevant level of Ni(II) was not enough to change the EPS structures even in the long-term exposure, which was similar to its negligible effect on the physicochemical properties. However, the fluorescence intensities of these four peaks in LB-EPS and TB-EPS were significantly quenched at the Ni(II) concentration range of 1–10 mg/L (Fig. 3). It is reported that the fluorescence quenching caused by metal ions is through changing the electronic polarization of both the binding site and the metal, and the binding capac-

Table 2
The constituents of Ni(II) in activated sludge after short-term and long-term exposure to Ni(II).

Ni(II) concentration (mg/L)	Short-term experiment				Long-term experiment			
	0[c]	0.1	1	10	0[c]	0.1	1	10
Exchangeable/acid soluble fraction [a]	0	0.014	0.21	1.49	0	0.78	5.80	62.06
Reducible fraction [a]	0	0.0059	0.083	0.54	0	0.31	1.73	22.92
Oxidizable fraction [a]	0	0.0021	0.027	0.20	0	0.09	0.51	4.77
Residual fraction [a]	0	0	0	0	0	0	0.61	5.73
Sum of four fractions [a]	0	0.022	0.32	2.23	0	1.18	8.65	95.48
Total Ni(II) [a]	0	0.023	0.33	2.19	0	1.14	8.71	96.64
Recovery rate (%) [b]	–	95.65	96.97	101.83	–	103.51	99.31	98.80

[a] The unit is mg/g MLSS.
[b] The recovery rate was calculated as the summation of four fractions in the total contents.
[c] The control test.

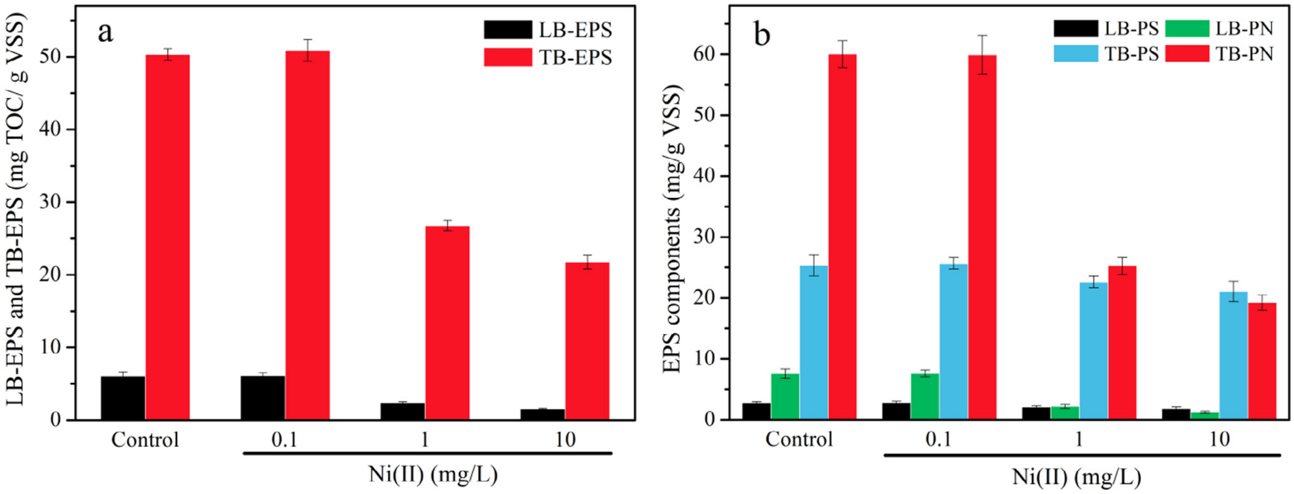

Fig. 2. Variations of the quantities and components of EPS after long-term exposure to different Ni(II) concentrations; (a) quantities of LB-EPS and TB-EPS in terms of TOC, (b) PN and PS quantities of LB-EPS and TB-EPS. Error bars represent standard deviations of triplicate determinations.

ity can be indicated by quenching degree (Cabaniss, 1992). Thus, the phenomenon of fluorescence quenching in LB-EPS and TB-EPS confirmed that Ni(II) had strong binding capacity on EPS.

Particularly, disappearance of peaks A, B and D in LB-EPS were found in the 1 and 10 mg/L Ni(II) exposure reactors, suggesting that the structures of aromatic proteins and humic acid-like substances in LB-EPS were destroyed by the higher levels of Ni(II). In addition, the location of peak C in TB-EPS had no shift in these two reactors, but there were 10 nm of emission wavelength red shift (from 350 nm to 360 nm) in LB-EPS. This shift once again confirmed that the higher levels of Ni(II) could change the chemical structure of LB-EPS. The above results also demonstrated that exposure of Ni(II) could bring more severe effect on the LB-EPS than TB-EPS. Previous studies showed that LB-EPS played a more important role in the flocculability of activated sludge than TB-EPS, since LB-EPS in sludge may function as the primary surface for cell flocculation and attachment (Li and Yang, 2007). Accordingly, the deteriorated flocculability caused by Ni(II) might be closely correlated with the destroyed LB-EPS structures.

It is reported that EPS contain many chemical functional groups such as hydroxyl and carboxylic groups, which can effectively bind metal ions (Mu et al., 2012). Thus, the effect of Ni(II) on the possible functional groups dominated in LB-EPS and TB-EPS was investigated by FTIR. According to previous studies, the broad absorption band near 1630 cm^{-1} is related to the stretching C=O of β-sheets in secondary PN structures, namely amide I (Wang et al., 2008). The band at about 1400 cm^{-1} is associated with the stretching COO- and C-O of carboxylic groups from PN, which is assigned to the amine III (Eboigbodin and Biggs, 2008).

There were some changes (decrease and shift) in the bands of 1630 and 1400 cm^{-1} after long-term exposure to 1–10 mg/L of Ni (II) (Fig. S1). The intensities of these two bands in LB-EPS and TB-EPS decreased with the increase of Ni(II) concentration, suggesting that the changes are closely correlated with the decreased PN of EPS. Moreover, the band near 1630 cm^{-1} in LB-EPS and TB-EPS exhibited significant red-shifts at Ni(II) concentrations of 1 and 10 mg/L, implying that β-sheets of PN was significantly affected. It is reported that the bands between 1630 and 1640 cm^{-1} are associated with β-sheets of PN, and the locations between 1648 and 1657 cm^{-1} are relevant to α-helix of PN. Therefore, this red-shift might indicate that the higher Ni(II) concentration resulted in the decrease of β-sheets and the increase of α-helix in PN. Qin and Buehler (2010) found that the increase of β-sheets structures and the decrease of α-helix content could significantly improve protein's strength, stiffness and energy dissipation capacity. As a result, Ni(II) may reduce the protein's strength, stiffness and energy dissipation capacity, thereby damaging the structure of PN.

The band near 1100 cm^{-1} in LB-EPS and TB-EPS, representing the O-C-O group of PS (Sun et al., 2009), showed a light red-shift from 1078 to 1082 cm^{-1} and the change of intensity after long-term exposure to different Ni(II) concentrations was negligible. This observation was consistent with the above result that the PS were not affected by Ni(II) (Fig. 1b). Some bands belonging to the fingerprint region (<1000 cm^{-1}) might be related to the phosphate

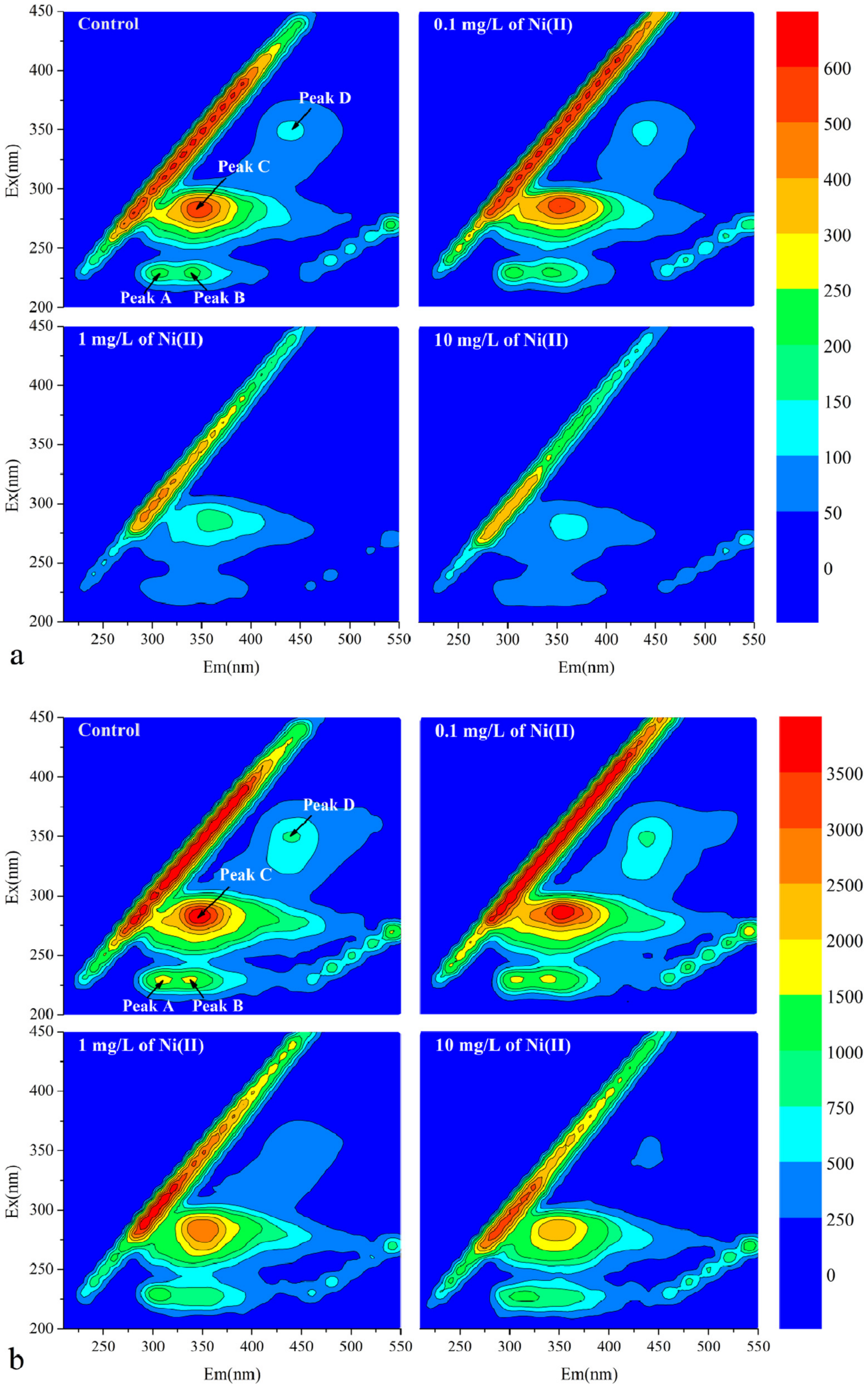

Fig. 3. The EEM fluorescence spectra of LB-EPS (a) and TB-EPS (b) after long-term exposure to different Ni(II) concentrations.

group, which is regarded as the functional groups of nucleic acids, and yet it is hardly to be predicted (Mu et al., 2012). Clearly, when activated sludge was chronically exposed to higher Ni(II) concentrations, the changes of the functional groups in EPS mainly occurred in PN.

3.3. Effect of Ni(II) on the surface properties and P removal performance of activated sludge

It is reported that the surface hydrophobicity of activated sludge plays a vital role in the flocculability (Jin et al., 2003). In general, as the hydrophobic molecules are non-polar, they are difficult to mix in water compared with the polar molecules, and beneficial for the biding together of sludge (Urbain et al., 1993). Indeed, the higher hydrophobicity is in favor of flocculation of sludge. Table 3 presents the variations of sludge surface hydrophobicity after long-term exposure to different Ni(II) concentrations. It could be found that long-term exposure to higher levels of Ni(II) would make the relative hydrophobicity of sludge become poor, which might be an alternative reason for the deteriorated flocculability.

In recent literature, the hydrophobicity of bacteria was reported to be relevant to cell surface charge. Generally, cells with a large number of negative surface charges appear to be more hydrophobic (Urbain et al., 1993). Therefore, the variations of cell surface charge after long-term exposure to Ni(II) were also investigated, and shown in Table 3. Compared with the control, the surface charge of sludge after long-term exposure to 1 and 10 mg/L of Ni(II) increased from −0.77 ± 0.031 to −0.43 ± 0.023 and −0.32 ± 0.022 meq/g MLSS, respectively, which was consistent with the decreased hydrophobicity (Table 3). The increased surface charge might be due to the binding of Ni(II) with the negative functional groups of EPS. This assumption can be supported by the results of FTIR spectra (Fig. S1).

Chen et al. (2014) pointed out that the integrity of cell membrane plays an important role in the flocculability since it occurs between microbial surfaces. Thus, the long-term effect of Ni(II) on the integrity of cell membrane was also investigated by the LDH release test. As seen in Fig. S2, little variations were found in this LDH release test, indicating that long-term exposure to Ni(II) even at the leave of 10 mg/L was not enough to destroy the cell membrane of microorganisms. These results suggested that the changes of EPS compositions and structures, and the lower hydrophobicity rather than the damage of cell membrane were the major reason for the deteriorated flocculability induced by Ni(II).

As the physicochemical properties changed, especially the declined flocculability, activated sludge had a tendency to exist as dispersive small particles. These small particles will provide a larger specific surface area to interact with Ni(II), which might improve its toxicity to activated sludge (Chen et al., 2014). Thus, when the exposure of Ni(II) showed detriment to the flocculability, the P removal efficiency of activated sludge might be affected. As shown in Fig. S3, the P removal efficiencies significantly decreased with the increase of exposure time in the reactors exposed to 1 and 10 mg/L of Ni(II). After long-term exposure, the corresponding P removal efficiencies completely collapsed (Table 4). These results are consistent with the declined flocculability reported in Fig. 1a, and confirm the above assumption. However, contrary results were found in the 0.1 mg/L of Ni(II) exposure reactor. The ESS values were almost unaffected during long-term operation in this reactor (Fig. 1a), but the P removal performance completely deteriorated after 30-day exposure (Fig. S3 and Table 3). The results suggested that the P removal was sensitive to Ni(II) than the flocculability, and thus using the variation of this property as an indicator to assess the risk of Ni(II) might underestimate its effect.

3.4. Reduction of Ni(II) toxicity by complexing agents

In this study, EDTA and citrate were selected as the chelating agent to explore the strategy for reducing the inhibitory effect of Ni(II) on the physicochemical properties and removal performance in EBPR system. As shown in Table 4, long-term exposure to 10 mg/L Ni(II) had little effect on the ESS, SVI, and CST values in the presence of EDTA or citrate. The result demonstrated that these chelating agents could eliminate the negative effect of Ni(II) on the physicochemical properties of activated sludge. It was likely due to the fact that the free Ni(II) could be chelated by EDTA and citrate to mainly form $NiEDTA^{2-}$ and $NiCitrate^{-}$, respectively, and these two resultants were essentially inert and nonbioavailable (Hu et al., 2002).

As exposure time increased, no significant variations of P removal efficiency were also found in these reactors simultaneously exposed to Ni(II) and EDTA (0.2 or 1 mM) (Fig. 4a), suggesting that EDTA could reduce the toxicity of Ni(II) to the P removal performance in EBPR system. However, the P removal efficiency is gradually decreased with the increase of time at the 0.2 mM citrate reactor, while Ni(II) toxicity effect was completely obviated with the addition of 1 mM citrate (Fig. 4b). Clearly, the reduced extent of toxicity effect was associated with the concentration of these chelators. These results also confirmed that EDTA was a better complexing agent than citrate to reduce the toxicity of Ni(II), which should attribute that EDTA could form stronger complexes with Ni(II) than citrate (formation constants of $NiEDTA^{2-}$ and $NiCitrate^{-}$ are $10^{20.4}$ and $10^{6.6}$, respectively) (Hu et al., 2002).

Table 3
Variations of relative hydrophobicity, surface charge, and P removal efficiency after long-term exposure to different Ni(II) concentrations.[a]

	Ni(II) concentrations (mg/L)			
	Control	0.1	1	10
Relative hydrophobicity (%)	85.2 ± 2.5	84.7 ± 3.4	64.4 ± 2.1	55.6 ± 2.6
Surface charge (meq/g MLSS)	−0.77 ± 0.031	−0.75 ± 0.035	−0.43 ± 0.023	−0.32 ± 0.022
P removal efficiency (%)	98.8 ± 1.1	0	0	0

[a] Results are the averages and their standard deviations of triplicate determinations.

Table 4
Characteristics of the sludge and effluent in EBPR system after long-term simultaneous exposure to Ni(II) and the chelating agent.[a]

	Ni(II) + EDTA			Ni(II) + Citrate		
	Control	10 mg/L +0.2 mM	10 mg/L +1 mM	Control	10 mg/L +0.2 mM	10 mg/L +1 mM
ESS (mg/L)	21.5 ± 3.1	20.5 ± 2.8	20.8 ± 3.2	21.1 ± 2.9	21.2 ± 2.7	22.8 ± 3.2
SVI (mL/g)	107.4 ± 4.2	108.2 ± 3.4	110.1 ± 3.8	108.5 ± 4.4	110.2 ± 3.9	109.8 ± 4.2
CST (s)	49.4 ± 1.9	48.9 ± 2.3	50.1 ± 2.4	49.1 ± 2.9	50.4 ± 3.1	49.8 ± 2.5

[a] Results are the averages and their standard deviations of triplicate determinations.

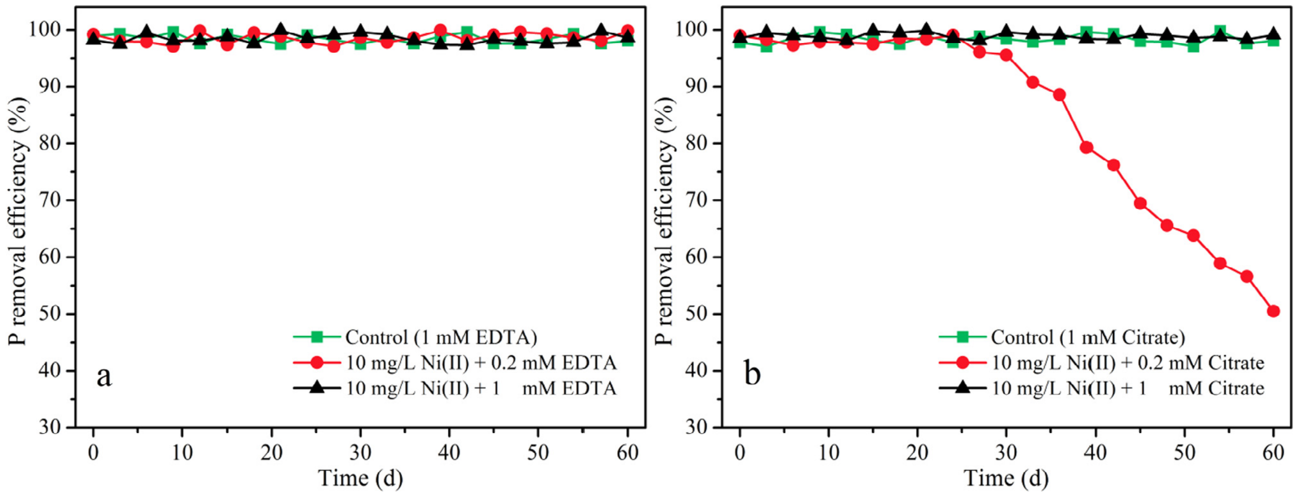

Fig. 4. Variations of P removal efficiency in EBPR system during long-term simultaneous exposure to Ni(II) and EDTA (a), or Ni(II) and citrate (b).

4. Conclusions

The sludge flocculability has seriously deteriorated after long-term exposure to 1 and 10 mg/L of Ni(II). The mechanism studies revealed that the changes of EPS structures, and the decrease of the protein content and hydrophobicity were the major reason for the deteriorated flocculability. Moreover, the worsened flocculability made the sludge flocs more dispersed, and thereby might increase the toxicity of Ni(II) to the P removal microorganism in activated sludge. These adverse effects of Ni(II) could be completely eliminated by adding the EDTA or citrate, but the extent was associated with the concentration of these chelators.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.11.018.

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