Korean J. Chem. Eng., 36(7), 1140-1147 (2019)
DOI: 10.1007/s11814-019-0298-7

pISSN: 0256-1115
eISSN: 1975-7220

Mechanism of the simultaneous removal of nitrate and Ni(II) by *Enterobacter* sp. CC76 through mixotrophic denitrification processes

Jun Feng Su*,,†, Shu Yang*,**, Ting Lin Huang*,**, Xue Chen Bai***, Jin Suo Lu*,**, Lei He***, and Min Li*,****

*Key Laboratory of Northwest Water Resource, Environment and Ecology, MOE,
Xi'an University of Architecture and Technology, Xi'an 710055, P. R. China
**Shaanxi Key Laboratory of Environmental Engineering, Xi'an University of Architecture and Technology,
Xi'an 710055, P. R. China
***China United Northwest Institute for Engineering Design and Research Co., Ltd. (CUCED), Xi'an 710077, P. R. China
(*Received 13 February 2019 • accepted 15 May 2019*)

Abstract−We studied the mechanism for the simultaneous removal of nitrate and Ni(II) by *Enterobacter* sp. CC76. Response surface methodology results showed that the maximum removal ratios of nitrate and Ni(II) were 95.02% and 75.99% under the following conditions: pH 7.37, 54.31 $mg \cdot L^{-1}$ Fe(II), and 10.00 $mg \cdot L^{-1}$ Ni(II). The mechanism of Ni(II) removal involved Fe-oxide adsorption and the increase of pH. In addition, meteorological chromatography analysis indicated that Ni(II) affected gas composition during denitrification. Scanning electron microscopy and X-ray photoelectron spectroscopy confirmed that Fe-oxide adsorption was the main contributor to Ni(II) removal. This study shows that *Enterobacter* sp. CC76 can enhance the adsorption of Ni(II) onto Fe-oxides while removing nitrate.

Keywords: Mixotrophic Denitrification, Ni(II) Removal, Nitrate Removal, Response Surface Methodology (RSM), Adsorption

INTRODUCTION

In recent years, the problem of nitrate contamination in groundwater has become more serious [1]. When nitrate enters the body, it is reduced to nitrite, causing methemoglobinemia and has a certain carcinogenic effect [2,3]. Therefore, the issue of controlling nitrate pollution is urgent. At present, the nitrate removal method mainly includes three aspects: biological, physical and chemical [4,5]. The biological method is to remove nitrate from water by using microorganisms to convert nitrate into nitrogen, which has the advantage of less investment, and thus is widely used [6]. However, when excess organic are provided, biological denitrification, including autotrophic denitrification, also produces some secondary pollution [7].

The content of nickel in the earth's crust is second only to silicon, oxygen, iron and magnesium. It is widely found in various compounds in soil and water [8]. Metal nickel and nickel compounds have great commercial and industrial uses, such as coin making, mobile communications [9]. Nickel enters the human body through ingestion, respiratory inhalation and epidermal absorption [10], which can cause harm to human health. Previous studies have demonstrated the harmful effects of nickel on the human body, including affecting protein activity, causing dermatitis and carcinogenesis [11, 12]. The widespread use of nickel-containing products causes nickel and its by-products to pollute the environment [13]. For example, wastewater discharge from the electroplating industry [14] and the mineral processing industry [15] causes nickel to enter the aqueous environment [16]. Ni(II) is also widely found in many nitrogen-rich wastewaters [17]. Di Capua et al. and Zou et al. discussed the effects of nickel on autotrophic and heterotrophic denitrification [18,19], which helped to carry out the research on the simultaneous removal of nitrate and nickel from water.

The treatment methods for nickel-containing wastewater mainly include chemical precipitation, physical adsorption and ion exchange [20]. Pandey et al. used calotropis procera to make an adsorbent that removed 85% of nickel from industrial wastewater [21]. Moosavirad et al. used ion exchange to remove 77% of Ni(II) from wastewater of ceramics plants [22]. In terms of biological methods, Al-Gheethi et al. used a drug-resistant strain to remove Ni(II) from pharmaceutical wastewater, with a removal ratio of 58.32% [23]. Quintelas et al. used an *Escherichia coli* biofilm supported on zeolite NaY to treat low metal concentration wastewater with an 82% removal ratio for Ni(II) [24]. The Ni(II) removal ratio in this study was 75.99%, which is basically at the same level as the above methods. Note that this study still has a high removal ratio (95.02%) for nitrate while removing Ni(II). The simultaneous removal of such multiple pollutants is our highlight.

We used the previously isolated *Enterobacter* sp. CC76 to determine the mechanism of the simultaneous removal of nitrate and Ni(II) [25]. According to the previous reports, CC76 can use sodium acetate and Fe(II) as electron donors to combine Fe cycle and denitrification to enable efficient nitrate removal, the maximum nitrate removal rate is 0.24 $mg \cdot L^{-1} \cdot h^{-1}$, and the product is N_2 [25,26]. On the basis of response surface methodology (RSM), the effects of pH, initial Ni(II) and Fe(II) concentrations on the process were investigated. The removal mechanism of Ni(II) was investigated by X-ray photoelectron spectroscopy (XPS) and scanning electron micros-

†To whom correspondence should be addressed.
E-mail: sjf1977518@sina.com

copy (SEM). Gas chromatography was used to detect the difference in gas production during denitrification under different conditions. The feasibility of the simultaneous removal of nitrate and Ni(II) was demonstrated.

MATERIALS AND METHODS

1. Media

Mixotrophic medium was used for cultivation of *Enterobacter* sp. CC76. The medium contained the following components (per liter of distilled water): 0.10 g of $NaNO_3$, 0.25 g of $NaHCO_3$, 0.125 g of CH_3COONa, 0.10 g of K_2HPO_4, 0.05 g of $MgSO_4{\cdot}7H_2O$ and 2.00 mL of trace elements solution. The final pH of medium was adjusted to 7.00 by using 1 M HCl or NaOH. The trace element solution used in this study was comprised of the following reagents: 0.50 $g{\cdot}L^{-1}$ $MgSO_4{\cdot}7H_2O$, 1.00 $g{\cdot}L^{-1}$ EDTA, 1.00 $g{\cdot}L^{-1}$ $ZnSO_4$, 0.10 $g{\cdot}L^{-1}$ $MnCl_2{\cdot}4H_2O$, 0.50 $g{\cdot}L^{-1}$ $FeSO_4{\cdot}7H_2O$, 0.50 $g{\cdot}L^{-1}$ $CuSO_4{\cdot}5H_2O$, 0.20 $g{\cdot}L^{-1}$ $CoCl_2{\cdot}6H_2O$. The medium was then sterilized by autoclaving at 0.11 MPa, 121 °C for 30 min. Concentrated soluble sterile Fe(II) and Ni(II) stock solutions were made by adding $FeSO_4{\cdot}7H_2O$ and $NiCl_2{\cdot}6H_2O$ to distilled water.

2. Optimization of Cultivation Conditions for Maximum Removal of Nitrate and Ni(II) by Using RSM

The nitrate and Ni(II) removal ratios were optimized by Box-Behnken Design (BBD) of RSM with the help of Design Expert 8 software. Ranges and levels of the three variables were designed: Fe(II) concentration (20.00, 40.00, 60.00 $mg{\cdot}L^{-1}{\cdot}h^{-1}$); Ni(II) concentration (10.00, 20.00, 30.00 $mg{\cdot}L^{-1}$) and pH (6.00, 7.00, 8.00). All experimental setups were performed as previously described, i.e., the medium contained 0.10 $g{\cdot}L^{-1}$ of $NaNO_3$.

3. Study on the Mechanisms of Ni(II) Removal

To demonstrate that the nickel removal in this study was indeed related to the biological effects of strain CC76 and not just the adsorption of heavy metals onto Fe-oxides, three sets of experiments were designed as shown in Table 1. The mixotrophic medium was poured into a 100 mL bottle for sterilization, and a given volume of 10 $mg{\cdot}mL^{-1}$ Ni(II) stock solution was added to the bottle. The headspace of each bottle was purged with argon gas at the beginning of the experiment. The bottles were sealed using a rubber stopper under an anoxic atmosphere. The adsorption experiment was in a constant temperature incubator. Samples were taken every day to observe the removal of Ni(II). By comparing the difference between the control group and the experimental group, the biological effect of strain CC76 on Ni(II) removal was demonstrated.

To demonstrate that the increase in nickel removal may be related to the changes in pH, a comparative experiment was designed to test this hypothesis. The medium was treated as before, and 1 M HCl or NaOH was added to the experimental group to keep the pH constant at 6.60. The initial Ni(II) concentration was set to about 8.00 $mg{\cdot}L^{-1}$, and 5% of the *Enterobacter* sp. CC76 was inoculated.

4. Detection and Analysis of Gas Emission During Denitrification

As previously described, the mixotrophic medium was poured into a 1,000 mL bottle for sterilization, then inoculated with 5% *Enterobacter* sp. CC76 and sealed with a rubber stopper to ensure an anaerobic environment. Two sets of experiments were designed for gas detection. A certain amount of Ni(II) solution was added to the experimental group to make the initial Ni(II) concentration 10.00 $mg{\cdot}L^{-1}$, and the control group was not treated. The initial pH was adjusted to 7.00. Gas was withdrawn from the top of the bottle daily to detect changes in N_2 and N_2O.

5. Analytical Methods and Statistical 1Analysis

NO_3^--N, NO_2^--N, Fe(II) concentration and optical density (OD_{600}) in the aqueous solution were confirmed by ultraviolet spectrophotometer (DR 5000, HACH, the USA) according to standard methods. The concentration of Ni(II) in the solution was measured with an atomic absorption spectrophotometer (ICE 3500, USA). The pH was measured with a pH instrument (HQ11d, HACH, USA). Scanning electron microscopy (SEM, JSM-5800, Japan JEOL) was used for the analysis of the microstructure of Fe-oxides. The composition of the Fe-oxides was analyzed by X-ray photoelectron spectroscopy (XPS). The gas were analyzed by a gas chromatography instrument (PerkinElmer Clarus 600, American).

RESULTS AND DISCUSSION

1. The Growth of *Enterobacter* sp. CC76 with Nitrate and Ni(II) Removal in Mixotrophic Condition

Fig. 1 shows the growth experiment of strain *Enterobacter* sp. CC76. The initial Ni(II) concentration was set to 9.50 $mg{\cdot}L^{-1}$, sodium bicarbonate was used as inorganic carbon source, Fe(II) and sodium acetate were used as electron donors, nitrate as an electron acceptor. The C/N ratio used in this study was approximately 2.60. Qambrani et al. used acetate as electron donor to repair nitrate-contaminated groundwater by combined autotrophic and heterotrophic denitrification and, using a C/N ratio of 2.68 [27], which was similar to this research.

For the experimental group, the strain *Enterobacter* sp. CC76 grew slowly and the nitrate removal rate was low (0.11 $mg{\cdot}L^{-1}{\cdot}h^{-1}$) in the first 16 hours. This may be because the toxicity of Ni(II) inhibited the growth of the strain. Subsequently, the nitrate content decreased rapidly from 15.31 $mg{\cdot}L^{-1}$ at 16 h to 0 $mg{\cdot}L^{-1}$ at 48 h with a removal

Table 1. Conditions of the adsorption experiments

Group	Content	Explain
Control group 1	Medium+Fe(II) solution	
Control group 2	Medium	Blank control
Experimental group	Medium+biological Fe-oxides composites*	

*The composites were from the experimental product of previous experiment (filtering and air drying the biological Fe-oxides)
*The composites were added in an amount of 0.10 g

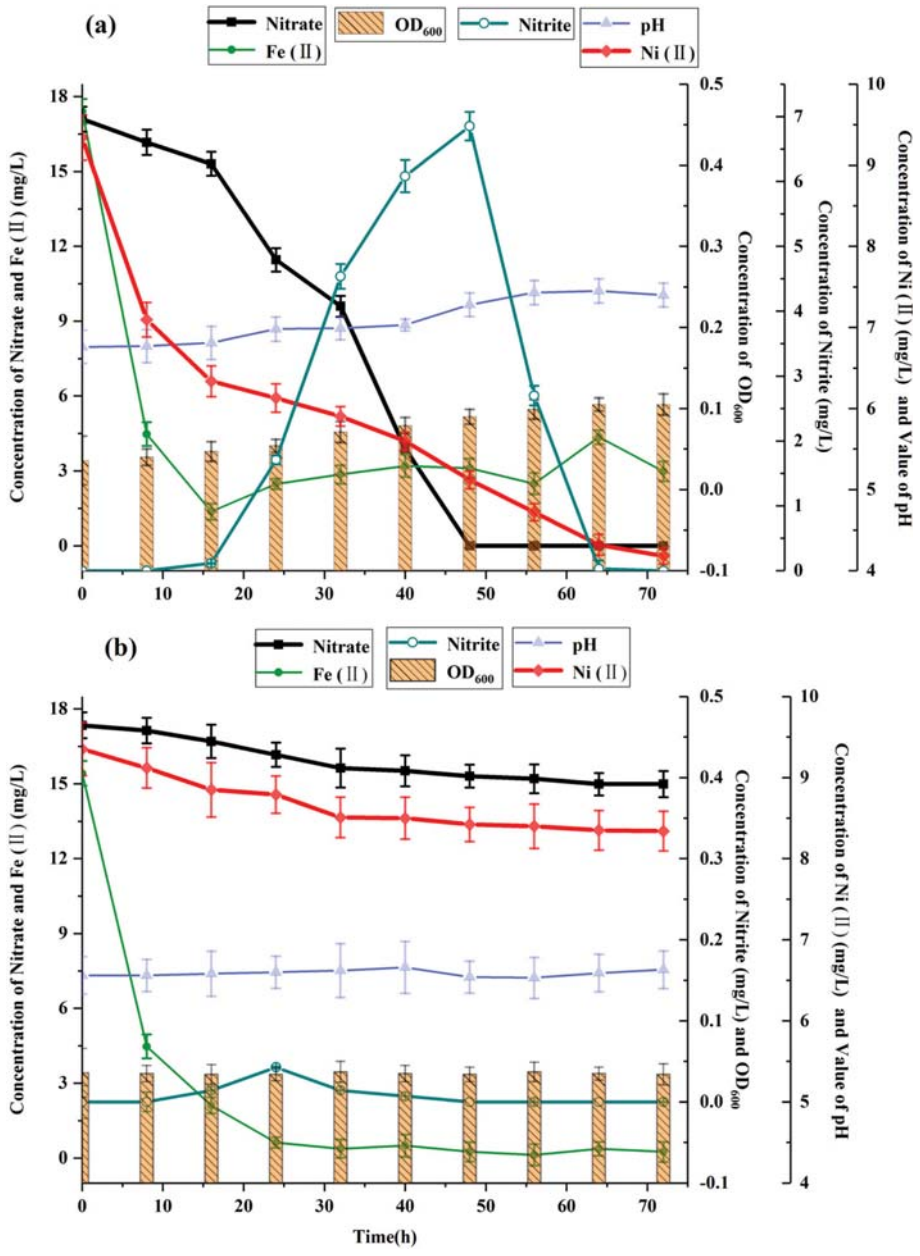

Fig. 1. The growth experiment of strain CC76 and its blank control (a) Experimental group (b) Blank control group.

rate of 0.47 mg·L^{-1}·h^{-1}. The OD_{600} value increased significantly from 0.036 to 0.090 from 0 to 48 h. These results indicate that the bacteria adapted to the new growth environment and successfully removed the nitrate.

On the other hand, the concentration of Ni(II) decreased from the initial 9.50 mg·L^{-1} to 4.18 mg·L^{-1}, and the pH slowly changed from 6.75 to 7.41 after 72 h. In addition, nitrite accumulation up to 6.85 mg·L^{-1} was observed at 48 h, and then nitrite gradually decreased to zero within the next 24 h.

Within the first 16 h, the Fe(II) content decreased rapidly, which may be due to natural oxidation. Subsequently, due to the Fe reduction effect of strain CC76, part of the Fe(III) was reduced to Fe(II) (the Fe(II) content increased), which provided more electron donors for the reaction. During the entire reaction, when the Fe(II) became Fe(III), it lost electrons (the Fe(II) content decreased), and the nitrate accepted electrons to become nitrite (the nitrate content decreased and the nitrite content increased). However, when the strength of Fe reduction was higher than the strength of denitrification, the content of Fe(II) increased and the nitrate content decreased, that is, the case shown in Fig. 1(a) from 16 h to 48 h. The ability of strain CC76 to lead to autotrophic and heterotrophic denitrification, Fe(II) oxidation, and Fe(III) reduction at the same time was confirmed in previous studies [25,26].

Compared with the experimental group, the blank control group did not contain the strain, the nitrate content did not change significantly, and the OD_{600} remained at around 0.030. In addition, no accumulation of nitrite was observed and pH did not change. The concentration of Ni(II) decreased from 9.50 mg·L^{-1} to 8.34 mg·L^{-1}.

This may be because the natural oxidation of Fe(II) produces Fe-oxides precipitation and, then the adsorption of heavy metals onto Fe-oxides leads to a reduction in Ni(II) [28]. The content of Fe(II) continued to decrease due to self-oxidation, and no increase in Fe(II) content was observed. These results indicate that Fe(II) had little influence on the removal of Ni(II) when strain *Enterobacter* sp. CC76 was not present.

2. Optimization of Nitrate and Ni(II) Removal by Using RSM

RSM is used to analyze three factors that affect the removal ratio of nitrate and Ni(II): Fe(II) concentration; Ni(II) concentration and pH. Using software to derive the relationships between the three and removal ratios are as follows:

$$Y_1=55.22+15.05X_1+15.52X_2-11.04X_3+0.76X_1X_2-3.44X_1X_3 -2.66X_2X_3-4.44X_1^2-10.29X_2^2-4.43X_3^2$$

$$Y_2=90.71-5.37X_1+6.01X_2-6.90X_3+4.83X_1X_2+1.57X_1X_3 +2.05X_2X_3-5.17X_1^2-5.24X_2^2-0.74X_3^2$$

where Y_1=Ni(II) removal ratio (%), Y_2=nitrate removal ratio (%), X_1=pH, X_2=Fe(II) concentration (mg·L^{-1}) and X_3=Ni(II) concentration (mg·L^{-1}).

The F-value, p-value and correlation coefficient of the response are shown in Table 2.1 and 2.2. The p-value represents the probability of data error, and the F-value represents the ratio of the estimated parameter effect to the estimated parameter standard deviation [29]. The results in Table 2.1 indicate that pH, Fe(II) concentration and Ni(II) concentration (P<0.001) have a significant effect on the removal of Ni(II). The interaction between pH and Fe(II) concentration (P=0.5202) had no significant effect on the removal of Ni(II). The data in Table 2.2 show that the most influential factor for nitrate removal is Ni(II) concentration (P=0.0010), followed by Fe(II) concentration (P=0.0022).

The optimized conditions as predicted by the software for the maximum removal ratio of nitrate and Ni(II) were, pH 7.37, 54.31 mg·L^{-1} Fe(II), and 10.00 mg·L^{-1} Ni(II). Under these conditions, the maximum ratios of nitrate and Ni(II) removal were 95.02% and 75.99%, respectively.

It can be observed from Fig. 2(a) that as the Fe(II) concentration increased from 20.00 mg·L^{-1} to 60.00 mg·L^{-1}, the removal ratio of Ni(II) significantly improved. It is presumed that the presence of Fe-reducing bacteria can effectively increase the adsorption of heavy metals onto Fe-oxides [30]. Previous studies pointed out that Fe-oxides are one of the most important substances for the adsorption of heavy metals in water sediments [28], and the addition of

Table 2.1. Analysis of variance table of Y_1 (Ni(II) removal ratio)

Source	Coefficient estimate	F-value	p-Value Prob>F
Model	55.22	118.74	<0.0001***
A-pH	15.05	354.86	<0.0001***
B-Fe(II) concentration	15.52	377.01	<0.0001***
C-Ni(II) concentration	−11.04	190.89	<0.0001***
AB	0.77	0.46	0.5202
AC	−3.44	9.24	0.0189*
BC	−2.66	5.54	0.0508
A^2	−4.44	16.27	0.0050**
B^2	−0.29	87.24	<0.0001***
C^2	−4.43	16.20	0.0050**

Table 2.2. Analysis of variance table of Y_2 (nitrate removal ratio)

Source	Coefficient estimate	F-value	p-Value Prob>F
Model	90.71	10.84	0.0024**
A-pH	−5.37	17.76	0.0040**
B-Fe(II) concentration	6.01	22.20	0.0022**
C-Ni(II) concentration	−6.90	29.28	0.0010**
AB	4.83	7.18	0.0316*
AC	1.57	0.76	0.4119
BC	2.05	1.29	0.2939
A^2	−5.17	8.66	0.0216*
B^2	−5.24	8.89	0.0205*
C^2	−0.74	0.18	0.6857

***Vitally significant (p-value<0.001)
**Very significant (0.001<p-value<0.01)
*Significant (0.01<p-value<0.05)

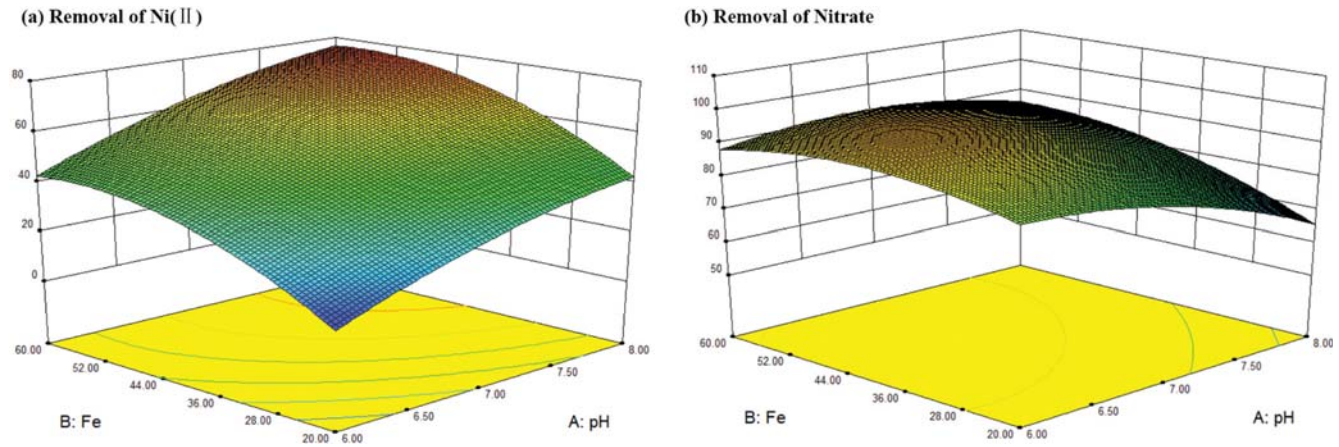

Fig. 2. 3-D surface plots of nitrate and Ni(II) removal ratio as the function of (a) Fe(II) and pH for Ni(II), (b) Fe(II) and pH for nitrate.

microorganisms can observably increase its adsorption capacity [31]. Small et al. reported this mechanism: bacteria can alter the electrochemical surface properties of Fe oxide itself, thereby increasing the adsorption capacity to heavy metals [30]. Lee et al. pointed out that Fe-reducing bacteria can improve arsenic mobility in Fe oxide minerals [32]. Daughney et al. reported the immobilization of cadmium by bacteria-Fe oxide complexes [33]. Hohmann et al. also reported that Fe bacteria have the ability to co-precipitate or adsorb arsenic during the formation of Fe(III) minerals [34]. Meanwhile, Fig. 2(a) shows the Ni(II) removal ratio gradually increased with increasing pH from 6.00 to 8.00. Coup and Swedlund found that the adsorption capacity of Fe-oxides was enhanced when the pH value increased from 5.00 to 10.00, which was similar to this study [35].

Fig. 2(b) shows the response surface and contours of the nitrate removal ratio as a function of Fe(II) concentration and pH. The responses remained horizontal when the pH increased from 6.00 to 7.00 and, then declined in the next phase. Previous research has shown that pH plays an important role in microbial activities during the denitrification process, because, in acidic conditions, the denitrifying bacteria can better utilize Fe(II) as an electron donor to enhance the removal of nitrate [36]. A maximum nitrate removal ratio of 95.02% could be observed at 54.30 $mg \cdot L^{-1}$ Fe(II), and this may be due to the increased content of electron donors, which is conducive to the synthesis of cellular materials during denitrification.

3. Study on the Mechanism of Ni(II) Removal

3-1. Adsorption Experiment

Fig. 3 shows the results of the adsorption of Ni(II) in different groups: (a) experimental group (0.1 g of biological Fe-oxides composites was added to); (b) control group 1 (only added with Fe(II) solution); (c) control group 2 (blank).

In the control group 1, the concentration of Ni(II) dropped slightly (from 13.84 to 12.12 $mg \cdot L^{-1}$), and the concentration of Ni(II) did not change in control group 2. Conversely, the concentration of Ni(II) decreased from 12.79 to 4.88 $mg \cdot L^{-1}$ in experimental group and,

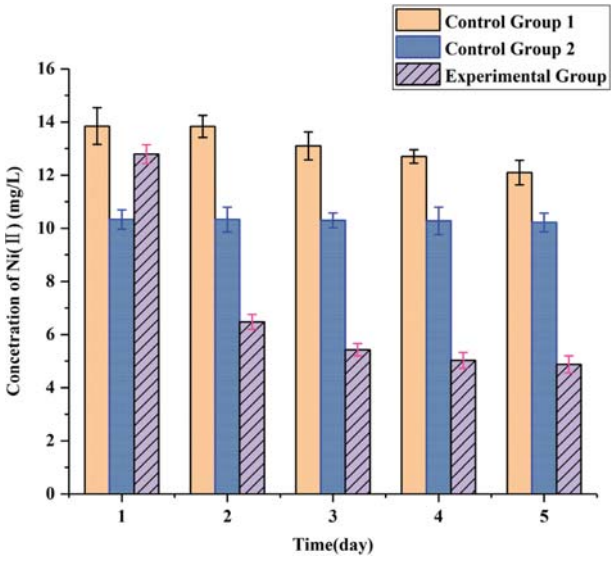

Fig. 3. Ni(II) adsorption results in different groups.

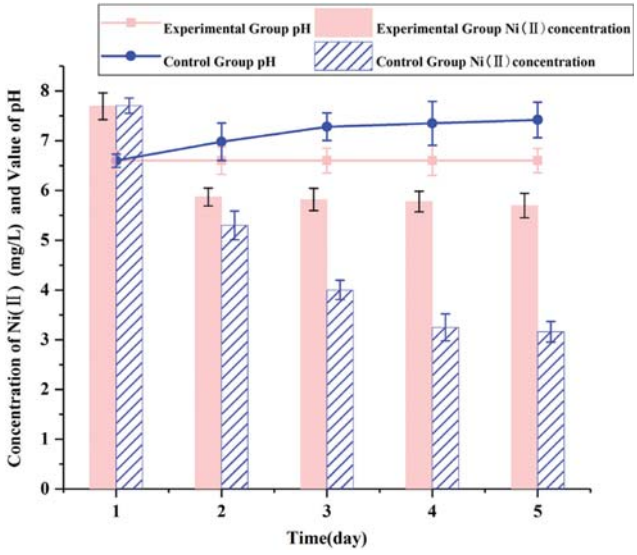

Fig. 4. Influences of pH on Ni(II) removal processes.

the removal ratio of Ni(II) was 61.84%. The phenomenon in control group 1 can be explained by the adsorption of heavy metals onto Fe-oxides [37]. In the experimental group, the Fe-oxides were transformed to the bacteria-Fe-oxides due to the strain CC76, which enhanced the ability of adsorption on heavy metals [31]. The above results indicate the following: Ni(II) was not removed under natural conditions; the adsorption of heavy metals onto Fe-oxides is the cause of the decrease in Ni(II) content; the bacteria further strengthen this effect.

3-2. Influences of pH on the Ni(II) Removal Processes

The influences of pH on Ni(II) removal were investigated. Two sets of experiments were designed: (a) control group (untreated); (b) experimental group (pH constant at 6.60).

Fig. 4 shows the differences between the two sets of experiments. In the control group, the pH increased from 6.60 to 7.42, and the concentration of Ni(II) decreased from 7.70 to 3.16 $mg \cdot L^{-1}$. In contrast, the concentration of Ni(II) only dropped slightly on the first day in the experimental group (from 7.70 to 5.87 $mg \cdot L^{-1}$), and there was almost no change after that. This may be due to the pH remaining constant at 6.60.

The above results indicate that pH has a great influence on the removal of Ni(II), and the reasons may be as follows:

1. The increase in the pH caused deprotonation of the Fe-oxides, which made the surface of Fe-oxides negatively charged, and thus could more easily adsorb metal cations in solution. Therefore, the higher the pH of the solution, the stronger the adsorption performance to cations [38,39].

2. The increase in the pH led to a decrease in the hydrogen ion content in the system, which reduced the competitive adsorption with cations, thereby promoting the adsorption of heavy metals onto Fe-oxides [40,41].

3. The pH of 6.60 was probably not the optimal condition for adsorption of Ni(II) onto the Fe-oxides. Previous studies have shown that the conditions for different types of Fe-oxides to achieve maximum adsorption capacity are different. For example, Yang et al.

found that a change of pH from 5.00 to 9.00 had no influence on Sb(V) adsorption onto Fe-Mn binary oxides [42]. However, in Li et al., when the pH was less than 9, the iron-manganese oxide had almost no adsorption effect to the thallium, and when the pH was more than 11, the adsorption was remarkably enhanced [43].

4. Detection and Analysis of the Gas Composition During Denitrification

Two sets of gas analysis experiments were designed to investigate the effects of different Ni(II) concentrations on the denitrification process. Fig. 5 shows the gas changes in the control group (initial Ni(II) concentration 0 $mg \cdot L^{-1}$ and initial nitrate concentration 33 $mg \cdot L^{-1}$) and the experimental group (initial Ni(II) concentration 10.00 $mg \cdot L^{-1}$ and initial nitrate concentration 33 $mg \cdot L^{-1}$). From day 1 to day 3, the N_2 concentration of the two groups increased significantly. Meanwhile, the N_2 concentration of the control group was higher than that of the experimental group. These data indicate that the combined heterotrophic and autotrophic denitrification with organic matter and Fe(II) as electron donors occurred, a large amount of nitrate was transformed to N_2, and there may be a more efficient denitrification process in the control group. The nitrate was removed by the following conversion pathway [44]:

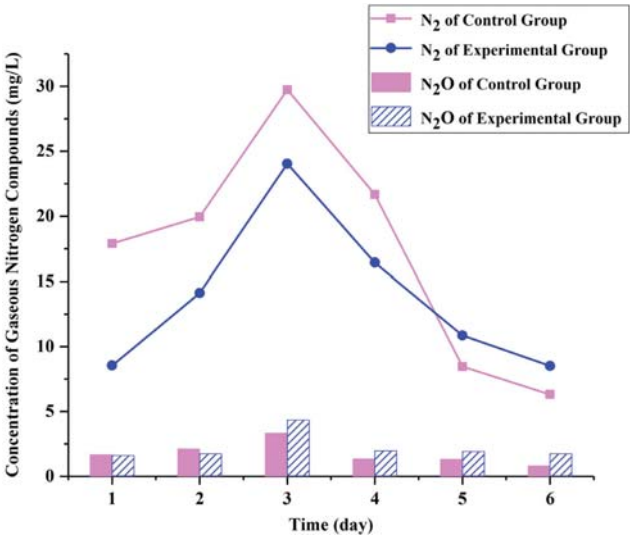

Fig. 5. Gas changes in different groups during denitrification.

$$NO_3^- + 2Fe^{2+} + H_2O = NO_2^- + 2Fe^{3+} + 2OH^-$$
$$2NO_2^- + 6Fe^{2+} + 4H_2O = N_2 + 6Fe^{3+} + 8OH^-$$
$$NO_3^- + 0.25CH_3COO^- = NO_2^- + 0.5CO_2 + 0.25H_2O + 0.25OH^-$$
$$NO_2^- + 0.375CH_3COO^- + 0.125H_2O = 0.5N_2 + 0.75CO_2 + 1.375OH^-$$

After the third day, the N_2 concentrations in both groups gradually decreased. In addition, a low degree of accumulation of N_2O was observed in both groups throughout the experiment. The above results show that: (1) denitrification occurred in the system and, NO_3^--N was reduced to N_2O and N_2; (2) Ni(II) has an inhibitory effect on nitrate removal.

5. Adsorption Mechanisms of Fe-oxides

The surface morphology of the two sets of bacteria-Fe-oxides (blank and Ni(II) added) was characterized using SEM, as shown in Fig. 6. After the addition of Ni(II), the surface morphology of the oxides changed greatly, and some nubby precipitates were observed, presumably because Ni(II) was adsorbed onto the surface of the Fe-oxides. The composition and structure of the Fe-oxides were further examined by XPS analysis. According to the XPS spectrum of Fig. 7, a peak of nickel was observed at 856.03 eV [45], which indicates that Ni(II) adsorbed onto the surface of the Fe-oxides. At the

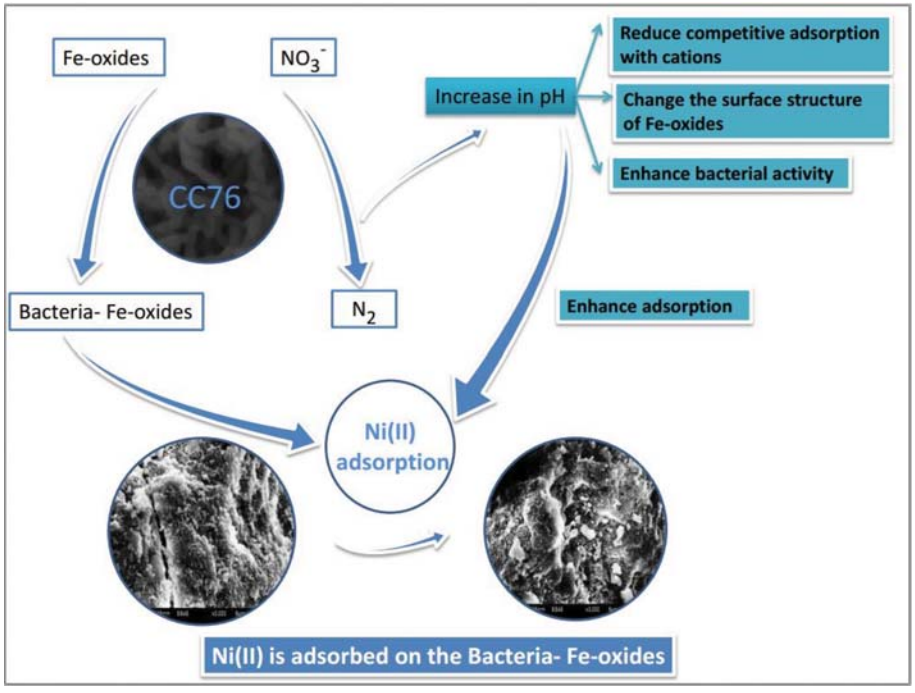

Fig. 6. Potential pathway of nitrate and Ni(II) removal of strain CC76.

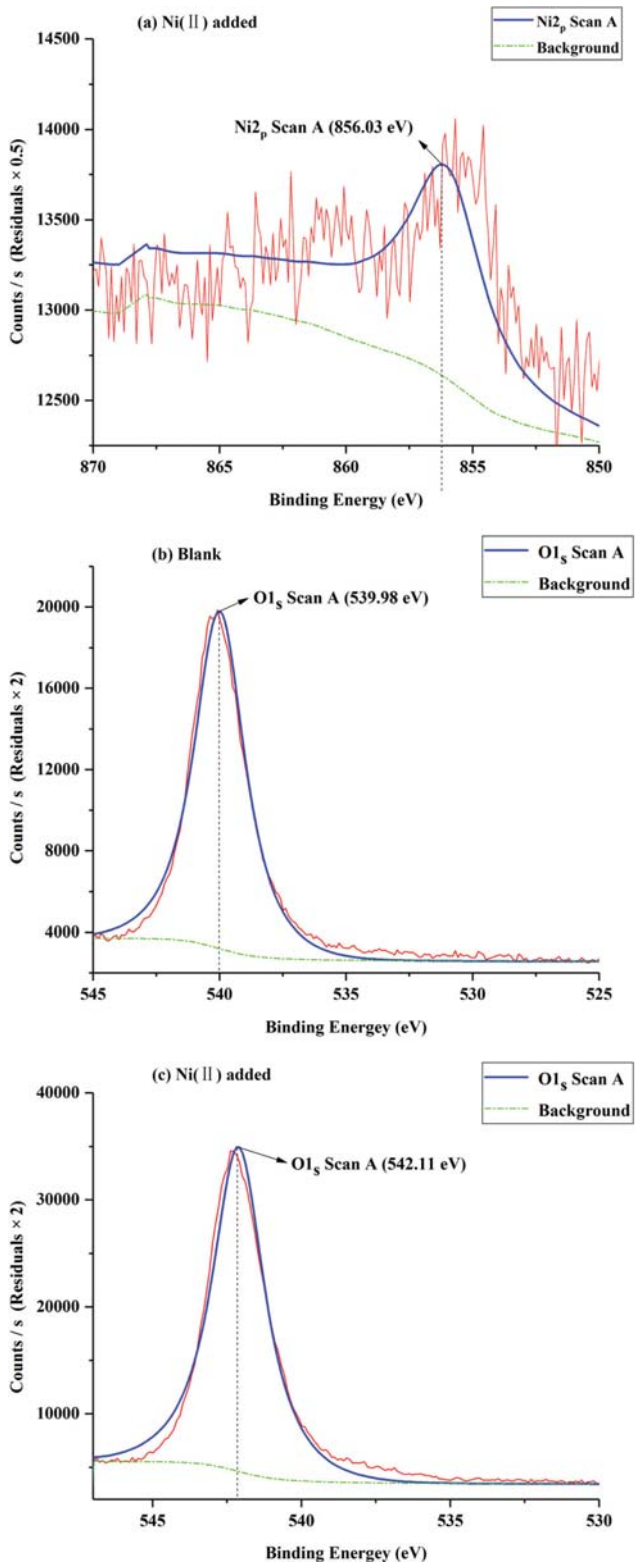

Fig. 7. XPS spectrum of different oxides.

same time, after the addition of Ni(II), the position of the oxygen peak was shifted from 539.98 eV to 542.11 eV, indicating that Ni(II) destroyed the Fe-O bond, further demonstrating the adsorption of Ni(II) [46].

CONCLUSIONS

This study demonstrates that *Enterobacter* sp. CC76 can use organics and Fe(II) as electron donors for combined heterotrophic and autotrophic denitrification and can simultaneously remove Ni(II). Using RSM analysis, the maximum removal ratios of nitrate and Ni(II) were 95.02% and 75.99% at pH 7.37, 54.31 mg·L^{-1} Fe(II), and 10.00 mg·L^{-1} Ni(II), respectively. XPS and SEM were used to observe bacteria-Fe-oxides. and the results demonstrated that the removal of Ni(II) was via Fe-oxides adsorption. The presence of *Enterobacter* sp. CC76 raised the pH of the environment, which further enhanced this adsorption.

ACKNOWLEDGEMENTS

This research work was partly supported by the National Natural Science Foundation of China (NSFC) (No. 51678471, 51778523). The National Key Research and Development Program of China (2016YFC0400706). Key Scientific Technological Innovation Team Plan of Shaanxi Province (No. 2017KCT-19-02). Key Research and Development Program in Shaanxi Province (2018ZDXM-SF-029).

REFERENCES

1. Ahmad, Badaruddin, H., Abbas, Yasir, Hussain, Mazhar, Akhtar, Naeem, Ansari and T. Mahmood, *Korean J. Chem. Eng.*, **31**, 284 (2014).
2. G. Huang, H. Fallowfield, H. Guan and F. Liu, *Water, Air, & Soil Pollution*, **223**, 4029 (2012).
3. T. Huang, G. Lin, H. Zhang, J. Su, W. Gang and Z. Kai, *Bioresour. Technol.*, **196**, 209 (2015).
4. Z. F. Song, J. An, G. H. Fu and X. L. Yang, *Aquaculture*, **319**, 188 (2011).
5. Z. Zhou, T. Huang, B. Yuan and X. Liao, *J. Soil Contamination*, **25**, 89 (2016).
6. S. Xia, F. Zhong, Y. Zhang, H. Li and X. Yang, *J. Environ. Sci.*, **22**, 257 (2010).
7. F. Di Capua, F. Pirozzi, P. N. L. Lens and G. Esposito, *Chem. Eng. J.*, **362**, 922 (2019).
8. I. Bertini, A. Sigel and H. Sigel, *J. Org. Chem.*, **659**, 203 (2001).
9. M. Schmidt and M. Goebeler, *J. Mol. Medicine*, **89**, 961 (2011).
10. E. Denkhaus and K. Salnikow, *Crit. Rev. Oncol. Hematol.*, **42**, 35 (2002).
11. B. Zambelli, V. N. Uversky and S. Ciurli, *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, **1864**, 1714 (2016).
12. B. Zambelli and S. Ciurli, *Metal Ions in Life Sci.*, **13**, 321 (2013).
13. V. Coman, B. Robotin and P. Ilea, *Resour. Conserv. Recycl.*, **73**, 229 (2013).
14. G. S. Hoseinian, B. Rezai, E. Kowsari and M. Safari, *Miner. Eng.*, **119**, 212 (2018).
15. F. Fu and Q. Wang, *J. Environ. Manage.*, **92**, 407 (2011).
16. N. P. Raval, P. U. Shah and N. K. Shah, *J. Environ. Manage.*, **179**, 1 (2016).
17. T. He, D. Xie, J. Ni and Z. Li, *J. Hazard. Mater.*, **368**, 594 (2019).
18. G. Zou, A. Ylinen, F. D. Capua, S. Papirio, A. M. Lakaniemi and J. Puhakka, *Adv. Mater. Res.*, **825**, 500 (2013).

19. F. D. Capua, I. Milone, A. M. Lakaniemi, E. D. V. Hullebusch, P. N. L. Lens and G. Esposito, *Bioresour. Technol.*, **238**, 534 (2017).
20. R. Idhayachander and K. Palanivelu, *J. Chem.*, **7**, 1412 (2012).
21. P. Pandey, S. Choubey, Y. Verma, M. Pandey, S. Kamal and K. Chandrashekhar, *Int. J. Environ. Res. Public Health*, **4**, 332 (2007).
22. S. M. Moosavirad, R. Sarikhani, E. Shahsavani and S. Z. Mohammadi, *J. Water Chem. Technol.*, **37**, 191 (2015).
23. A. A. S. Al-Gheethi, I. Norli, J. Lalung, A. M. Azlan, Z. A. N. Farehah and M. O. A. Kadir, *Clean Technologies Environ. Policy*, **16**, 137 (2014).
24. C. Quintelas, Z. Rocha, B. Silva, B. Fonseca, H. Figueiredo and T. Tavares, *Chem. Eng. J.*, **152**, 110 (2009).
25. J. f. Su, C. Cheng, T. l. Huang, F. Ma, J. s. Lu and S. c. Shao, *J. Taiwan Institute Chem. Engineers*, **66**, 106 (2016).
26. J. f. Su, D. x. Guo, T. l. Huang, J. s. Lu, X. c. Bai and X. f. Hu, *Environ. Eng. Sci.*, **35**, 1228 (2018).
27. N. A. Qambrani, S. H. Jung, S. O. Yong, S. K. Yong and S. E. Oh, *Environ. Sci. Pollut Res. Int.*, **20**, 9084 (2013).
28. M. Komárek, J. Antelo, M. Králová, V. Veselská, S. Číhalová, V. Chrastný, V. Ettler, J. Filip, Q. Yu, J. B. Fein and C. M. Koretsky, *Chem. Geol.*, **493**, 189 (2018).
29. M. Abigail, M. S. Samuel and R. Chidambaram, *J. Taiwan Inst. Chem. Engineers*, **49**, 156 (2015).
30. T. D. Small, L. A. Warren and F. G. Ferris, *Appl. Geochem.*, **16**, 939 (2001).
31. J. H. Lee and H. G. Hur, *J. Korean Soc. Appl. Biol. Chem.*, **57**, 123 (2014).
32. S. W. Lee, *J. Mater. Cycles Waste Manage.*, **15**, 362 (2013).
33. C. J. Daughney, M. Fakih and X. Chatellier, *Geomicrobiol. J.*, **28**, 11 (2011).
34. C. Hohmann, E. Winkler, G. Morin and A. Kappler, *Environ. Sci. Technol.*, **44**, 94 (2010).
35. K. M. Coup and P. J. Swedlund, *Chem. Geol.*, **398**, 97 (2015).
36. K. Kiskira, S. Papirio, E. D. van Hullebusch and G. Esposito, *Environ. Sci. Pollut. Res.*, **24**, 21323 (2017).
37. N. J. Barrow, J. Gerth and G. W. Brümmer, *Eur. J. Soil Sci.*, **40**, 437 (2010).
38. E. J. Kim, C. S. Lee, Y. Y. Chang and Y. S. Chang, *Appl. Mater. Interfaces*, **5**, 9628 (2013).
39. Q. Qin, Q. Wang, D. Fu and J. Ma, *Chem. Eng. J.*, **172**, 68 (2011).
40. L. Lei, G. Zhang, J. Lin, X. Wang, S. Wang and Y. Jia, *Appl. Geochem.*, **98**, 418 (2018).
41. A. M. Yusof and N. A. N. N. Malek, *J. Hazard. Mater.*, **162**, 1019 (2009).
42. K. Yang, J. Zhou, Z. Lou, X. Zhou, Y. Liu, Y. Li, S. Ali Baig and X. Xu, *Chem. Eng. J.*, **354**, 577 (2018).
43. H. Li, Y. Chen, J. Long, X. Li, D. Jiang, P. Zhang, J. Qi, X. Huang, J. Liu and R. Xu, *J. Hazard. Mater.*, **338**, 296 (2017).
44. J. Su, C. Gao, T. Huang, Y. Gao, X. Bai and L. He, *Chemosphere*, **222**, 970 (2019).
45. H. W. Nesbitt, D. Legrand and G. M. Bancroft, *Phys. Chem. Miner.*, **27**, 357 (2000).
46. A. Tamilselvan, S. Balakumar, M. Sakar, C. Nayek, P. Murugavel and K. K. Saravana, *Dalton Trans.*, **43**, 5731 (2014).