Chemical Engineering Journal 313 (2017) 415–423

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Nickel toxicity to the performance and microbial community of enhanced biological phosphorus removal system

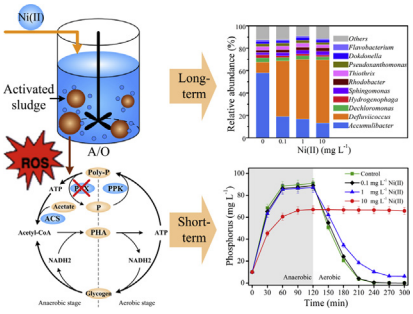
Jian Sun, Qi Yang *, Dongbo Wang *, Shana Wang, Fei Chen, Yu Zhong, Kaixin Yi, Fubing Yao, Chen Jiang, Sibei Li, Xiaoming Li, Guangming Zeng

College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China
Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, PR China

HIGHLIGHTS

- High concentration of Ni(II) has an adverse effect on the EBPR system.
- The transformations of PHAs were suppressed by short-term exposure of Ni(II).
- Impact of Ni(II) on the surface integrity of activated sludge was negligible.
- Inhibition of PPX activity was likely attributed to high ROS production.
- Long-term exposure to Ni(II) changed the microbial community of EBPR system.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 8 October 2016
Received in revised form 28 November 2016
Accepted 16 December 2016
Available online 19 December 2016

Keywords:
Nickel toxicity
Phosphorus removal
Polyphosphate accumulating organisms
Glycogen accumulating organisms

ABSTRACT

Nickel (Ni(II)) is commonly present in municipal and industrial wastewaters, and thus its potential toxicity to activated sludge in wastewater treatment plants attracts increasing concerns. Although considerable efforts have been paid to this topic, the potential effect of Ni(II) on biological phosphorus removal has not been reported. In this work, short-term and long-term effects of Ni(II) in the range of 0.1–10 mg·L^{-1} on enhanced biological phosphorus removal (EBPR) were therefore investigated. Compared with the control, short-term exposure to 1 and 10 mg·L^{-1} of Ni(II) resulted in the decrease of phosphorus removal efficiency from 99.7% to 38.3% and 0, respectively. The phosphorus removal was unaffected after short-term exposure to 0.1 mg·L^{-1} of Ni(II), but it was completely collapsed after 30-day exposure. The mechanism studies revealed that the cell membrane of microorganisms in activated sludge was not damaged, though the production of reactive oxygen species increased with the increase of Ni(II) exposure concentration. However, the presence of Ni(II) inhibited the anaerobic release of polyphosphate and the activity of exopolyphosphatase but enhanced the transformations of poly-3-hydroxyvalerate and glycogen. Microbial community investigation with high-throughput sequencing analysis showed that *alphaproteobacterial* glycogen accumulating organisms instead of polyphosphate accumulating organisms became the predominant microorganisms in EBPR systems after long-term exposure to Ni(II).

1. Introduction

In recent years, nickel (Ni) has been extensively applied in many consumer products such as high quality iron-based alloys, catalysts, paints and batteries due to its unique physicochemical

* Corresponding authors at: College of Environmental Science and Engineering, Hunan University, Changsha 410082, PR China.
E-mail addresses: sunjian0915@hnu.edu.cn (J. Sun), yangqi@hnu.edu.cn (Q. Yang), w.dongbo@yahoo.com (D. Wang).

http://dx.doi.org/10.1016/j.cej.2016.12.078
1385-8947/

property [1,2]. Meanwhile, it is identified as potential carcinogen to humans by the International Agency for Research on Cancer [3,4]. The production and utilization of Ni inevitably leads to its release into wastewater treatment plants (WWTPs) [5–7]. It is reported that the Ni(II) concentration in the municipal wastewaters of India, Greece, and Turkey is greater than 0.1 $mg{\cdot}L^{-1}$, even greater than 0.5 $mg{\cdot}L^{-1}$ [8–10]. With the increasing requirement of Ni-based products, this level should be increased year by year. Additionally, Ni(II) is highly soluble and can enter into cells, and once inside the cell, it can damage protein structure and function [11,12]. Therefore, the potential toxicity of Ni(II) to activated sludge in WWTPs has attracted increasing concerns.

The main function of activated sludge is to remove organic pollutants, nitrogen (N), and phosphorus (P) from wastewater via a series of microorganisms such as heterotrophic bacteria, ammonia/nitrite oxidizing bacteria, denitrifying bacteria, and polyphosphate accumulating organisms (PAOs) [13,14]. However, most of previous studies with regard to Ni(II) toxicity conducted so far have concentrated on the two former functions [2,5,11,15–17]. For example, the specific oxygen uptake rate and total organic carbon removal rate reduced by 57% and 51%, respectively, after the addition of 10 $mg{\cdot}L^{-1}$ Ni(II) into sequencing batch reactor (SBR) [5,17]. Hu et al. [16] pointed out that 11.7 $mg{\cdot}L^{-1}$ of Ni(II) caused 30% inhibition on ammonia oxidation in a mixed nitrifying culture. Also, denitrifying bacteria were found to be more vulnerable to Ni (II) than nitrifying bacteria [18]. It was observed that 0.5 $mg{\cdot}L^{-1}$ Ni (II) caused a significant suppression on the denitrification process, but no obvious impact was detected on the nitrification.

Although a large number of endeavors have been dedicated to evaluating the effects of Ni(II) on the biological removal of organic matter and N, little is known about the toxicity of Ni(II) to biological P removal so far. Moreover, the toxic investigations of heavy metals to N removal cannot represent their effects on P removal. Chen et al. [19] confirmed that total N removal was more sensitive to Cd(II) than P removal at the same concentration. Thus, there is a need to explore the potential effect of Ni(II) on the biological P removal.

Enhanced biological phosphorus removal (EBPR) is widely applied to P removal in WWTPs, which can be achieved by circulating PAOs through anaerobic and aerobic conditions [20]. It is well-known that intracellular polyhydroxyalkanoates (PHAs) and glycogen are the key intermediates during metabolic process of PAOs. Furthermore, the metabolic processes are directly related to some key enzymes, such as exopolyphosphatase (PPX), polyphosphate kinase (PPK) and acetyl-CoA synthases (ACS) [21,22]. It is reported that Ni(II) can induce reactive oxygen species (ROS) production on the human bronchial epithelial cells [4]. The produced ROS may inhibit the activities of key enzymes involved in biological P removal [23]. In addition, the produced ROS and Ni(II) may also affect the transformations of metabolic intermediates, thereby affecting the metabolic process of PAOs. To date, however, all these possibilities have not yet been clarified.

Another important issue that usually upsets and deteriorates the EBPR process is the excessive proliferation of glycogen accumulating organisms (GAOs). GAOs compete with PAOs for the limited carbon source such as volatile fatty acids at the anaerobic stage, without performing P removal. As a result, the proliferation of GAOs leads to the deterioration of EBPR performance [24,25]. Recently, several studies have been devoted to investigating the effects of some heavy metals on the PAOs-GAOs competition. It was found that Cr(VI) and Ag(I) provided a competitive advantage to GAOs over PAOs [23,26]. Therefore, it is necessary to confirm whether GAOs become predominant after exposure to Ni(II).

The aim of this work is to systematically evaluate the potential toxicity of Ni(II) to biological P removal. Firstly, short-term and long-term effects of Ni(II) ranging from 0.1 to 10 $mg{\cdot}L^{-1}$ on biological P removal were investigated. Then, the details of how Ni(II) affects P removal were explored from the aspects of the surface integrity of activated sludge assayed by lactate dehydrogenase (LDH) release, the transformations of metabolic intermediates, and the activities of key enzymes. Finally, the effects of Ni(II) on PAOs-GAOs competition were also analyzed by microbial community analysis with high-throughput sequencing.

2. Materials and methods

2.1. Activated sludge cultures

An anaerobic-aerobic parent SBR with a working volume of 40 L was operated for the enrichment of PAOs. The seed sludge was obtained from the recycling sludge in Guozhen WWTP, Changsha, China. The reactor was worked in a temperature controlled room (21 ± 2 °C) and operated with four cycles daily. Each cycle (6 h) included 2 h anaerobic and 3 h aerobic phase, followed by 40 min settling, 10 min decanting and 10 min idle phase. After settling phase, 25 L of supernatant was discharged from the SBR and replaced with the same volume of synthetic feeding medium (composition detailed as below). The influent pH value was adjusted to 7.5 ± 0.2 by using 2 M NaOH or 2 M HCl. The SBR was constantly mixed with a mechanical stirrer at the anaerobic stage. At the aerobic stage, air was provided to ensure the dissolved oxygen (DO) concentrations between 2 and 3 $mg{\cdot}L^{-1}$. The solids retention time (SRT) was maintained at about 10 days by wasting 1 L of the sludge mixture (once per cycle) at the end of aerobic stage but before settling. After acclimation for 60 days, the relatively stable P removal efficiency (>98%) was achieved in the SBR. The mixed liquid suspended solids (MLSS) and mixed liquid volatile suspended solids (MLVSS) were 3020 ± 186 and 2130 ± 151 $mg{\cdot}L^{-1}$ in the parent SBR. The total P content of the sludge at the end of aerobic stage was 61.3 ± 3.5 $mg{\cdot}g^{-1}$ MLSS. The sludge volume index (SVI) was 109.1 ± 5.9 $mL{\cdot}g^{-1}$, indicating that the settling ability of activated sludge was good.

The feeding medium was prepared daily and comprised ($mg{\cdot}L^{-1}$): 255.4 sodium acetate, 14.62 KH_2PO_4 and 49.03 $K_2HPO_4{\cdot}3H_2O$, yielding a theoretically influent chemical oxygen demand to P ratio (COD/P) of 20 mg COD/mg P, which was supposed to be favorable for the growth of PAOs [27]. The concentrations of the other nutrients in the medium were as follows (per liter): 57.2 mg NH_4Cl, 5 mg $CaCl_2$, 10 mg $MgSO_4{\cdot}7H_2O$ and 0.5 mL of trace-element solution. The trace metals solution consisted of: 1.50 $g{\cdot}L^{-1}$ $FeCl_3{\cdot}6H_2O$, 0.06 $g{\cdot}L^{-1}$ $Na_2MoO_4{\cdot}2H_2O$, 0.03 $g{\cdot}L^{-1}$ $CuSO_4{\cdot}5H_2O$, 0.12 $g{\cdot}L^{-1}$ $MnCl_2{\cdot}4H_2O$, 0.15 $g{\cdot}L^{-1}$ $CoCl_2{\cdot}6H_2O$, 0.12 $g{\cdot}L^{-1}$ $ZnSO_4{\cdot}7H_2O$, 0.15 $g{\cdot}L^{-1}$ H_3BO_3, 0.18 $g{\cdot}L^{-1}$ KI and 10 $g{\cdot}L^{-1}$ ethylenediamine tetra-acetic acid. Allylthiourea was provided in the feeding medium (10 $mg{\cdot}L^{-1}$) to inhibit nitrification.

2.2. Short-term exposure experiments

According to previous studies, the environmentally relevant concentration of Ni(II) in WWTPs was about 0.1 $mg{\cdot}L^{-1}$ [1,5]. With the wide application of Ni-based products, the Ni(II) released into environmental might further increase. In general, Ni(II) concentration is greater than 10 $mg{\cdot}L^{-1}$ in the Ni production relevant industrial wastewaters [28]. When municipal WWTPs receive these specific industrial wastewaters, Ni(II) level in influent would be even higher. Thus, the potential effects of higher Ni(II) concentrations (1 and 10 $mg{\cdot}L^{-1}$) were also considered in this study.

To carry out the short-term experiments, four identical SBRs with a working volume of 3 L each were operated. All experiments were performed in triplicate to ensure reliable results. The biomass for these reactors was taken out from the parent SBR at the end of