Effect of Cobalt, Cadmium and Manganese on Nitrogen Removal Capacity of *Arthrobacter arilaitensis* Y-10

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Received: 11 May 2020; Accepted: 10 June 2020; Published: 14 June 2020

**Abstract:** The aim of this study was to investigate the possibility of a simultaneous nitrification–denitrification hypothermic bacterium for applying in Cd(II), Co(II), and Mn(II)-contaminated wastewater. The influence of Cd(II), Co(II), and Mn(II) on the inorganic nitrogen removal capacity of the hypothermia bacterium *Arthrobacter arilaitensis* Y-10 was determined. The experimental results demonstrated that low concentration of Cd(II) (2.5 mg/L) exhibited no significant impact on bioremediation of ammonium. The nitrate and nitrite removal activities of strain Y-10 were enhanced by 0.1 and 0.25 mg/L of Cd(II), but hindered by more than 0.25 and 0.5 mg/L of Cd(II), respectively. However, the cell growth and denitrification activity ceased immediately once Co(II) was supplemented. In terms of Mn(II), no conspicuous inhibitory impact on ammonium bioremediation was observed even if Mn(II) concentration reached as high as 30 mg/L. The bioremediation of nitrates and nitrites was significantly improved by 0.5 mg/L of Mn(II), and then dropped sharply along with the increase of Mn(II). The order of the degree of inhibitory influence of the three heavy metal ions on the nitrogen bioremediation ability of strain Y-10 was Co(II) > Cd(II) > Mn(II). All the results highlighted that the heterotrophic nitrification was less sensitive to the inhibitory effects of Cd(II), Co(II), and Mn(II) relative to aerobic denitrification.

**Keywords:** *Arthrobacter arilaitensis*; heterotrophic nitrification; heavy metal ions; aerobic denitrification

1. Introduction

An aerobic bacterium which could conduct nitrification and denitrification simultaneously has been considered as one of the most innovative developments in biological wastewater treatment for inorganic nitrogen removal in recent years. The strain of *Arthrobacter arilaitensis* Y-10 possesses the capacity of simultaneous nitrification–denitrification at low temperature [1]. The simultaneous nitrification–denitrification strategy could convert ammonium, nitrates, and nitrites to gaseous and biomass nitrogen using less energy, which is more environmentally friendly than physical, chemical, and other biological approaches. In such biological nitrogen removal process, various inorganic nitrogen compounds could be removed simultaneously using an identical wastewater treatment system. Meanwhile, the accumulated nitrite nitrogen in the wastewater treatment system could be further removed by aerobic denitrifying microorganisms.
Although the inorganic nitrogen removal ability of *Arthrobacter arilaitensis* Y-10 has been extensively investigated, the ability of strain Y-10 to remove inorganic nitrogen compounds from heavy metals-contaminated wastewater is unclear. Nitrogen biodegradation bacteria could be sensitively influenced by many factors present in wastewater, such as temperature, substrate concentration, dissolved oxygen, pH, carbon sources, and C/N [1–5]. Heavy metal ions, another influencing factor, seriously threaten the ecosystem due to their acute or persistent toxicity to many aquatic organisms [6,7]. Nevertheless, it is unclear how heavy metal ions affect the aerobic denitrifying bacterium of strain Y-10.

Cd(II), one of the top five heavy metals (Cr, Hg, As, Pb, and Cd), is commonly present in metal coatings, phosphorus fertilizer products, and batteries [8–10]. The existing Cd(II) could be transported to and accumulated in cells due to its high solubility. Cd(II) could be toxic to many organisms due to carcinogenicity, teratogenicity, endocrine and reproductive toxicity, and thus it is considered one of the most severe pollutants [11–13]. Additionally, a large number of enzymes could be suppressed, the normal metabolism could be disrupted, and even the cell membrane could be disrupted if Cd(II) is present in a toxic concentration [7]. Therefore, Cd(II) is usually undesirable when discharged into the environment. Based on the literature quoted above, Cd(II) generally affects the activities of microorganisms adversely. However, there is little information on the effect of Cd(II) on the nitrogen transformation process of the simultaneous nitrification–denitrification bacteria. In addition to Cd(II) pollution, Co(II) is generally discovered in porcelain enameling, iron, paint formulating, and steel plant wastewater [14]. Co(II) has been proved as the least toxic heavy metal ion among Cu(II), Co(II), Zn(II), and Ni(II) in terms of nitrification [15]. Gikas [16] summarized the majority of the literature data about the effects of Co(II) on activated sludge and found that Co(II) is more likely to act as a cell growth stimulant rather than an inhibitor. Low concentration of Co(II) (less than 2 mg/L) has never been found toxic to microorganisms. Similarly, Mn(II) is also frequently found in the nitrogen-rich wastewater, including landfill leachates, steel manufacture, and swine wastewater [17]. When Mn(II) exceeded 0.05 mg/L in water, it could result in the water becoming brown–black in color, acquiring metallic taste, precipitating in the distribution system, and eventually clogging the pipe [18]. In particular, the central nervous system could be affected by Mn(II) once it is ingested by organisms [19]. Dissimilarly, the nitrogen removal efficiency could be improved by addition of Mn(II) in long-term nitritation–anammox [17]. A nearly double nitrogen removal efficiency could be observed by adding 0.05 mmol/L (equal to 2.8 mg/L) Mn(II) to activated sludge [20]. However, there are few studies of the effect of Mn(II) on nitrification–denitrification hypothermic aerobic bacteria. In conclusion, Cd(II) is the strongest toxicity ion for cell growth and metabolism of microorganisms, and Co(II) is the most beneficial metal ion among such heavy metals as Cd(II), Co(II), and Mn(II), according to the previously reported literature.

Although several researchers have reported that some heavy metals impacted the ammonium oxidation process in activated sludge, few studies have investigated their influence on aerobic denitrification. Heavy metals Cd(II), Co(II), and Mn(II) are ubiquitous in wastewater environments, but few studies have examined their effects on the nitrogen removal process, especially in the aerobic denitrification process at low temperature. Previous reports manifested that the toxicity degree of heavy metal ions depends on the type of microorganisms, the concentration of the biomass, the nature and concentration of the heavy metals, the interaction with other metal ions or non-metal ions in a complex activated sludge system [21]. Additionally, the absence of sludge may lower the tolerance of the pure bacteria to heavy metal ion inhibition. It was difficult to reveal the effect mechanism of a certain heavy metal ion on the nitrogen removal process in a sludge system. Therefore, this study focused on the influence of Cd(II), Co(II), and Mn(II) on the nitrogen removal capacity of the pure strain of *Arthrobacter arilaitensis* Y-10. It is expected that the results of this research will be instructional for application of *Arthrobacter arilaitensis* Y-10 to the inorganic nitrogen, Cd(II), Co(II), and Mn(II)-containing wastewater.
2. Materials and Methods

2.1. Strain and Medium

*Arthrobacter arilaitensis* Y-10 (KP410739) was selected as a pure bacterial source, which was previously deposited in China (China General Microbiological Culture Collection Center (CGMCC) No. 10536) by He and Li [22]. The strain Y-10 could tolerate low temperature (15 °C) and effectively perform simultaneous nitrification–denitrification in the mixed inorganic nitrogen sources of ammonium and nitrate/nitrite-stimulated wastewater [1,22].

The basal medium composition (synthetic wastewater) in this research was as follows (per liter): 0.1 g MgSO$_4$$\cdot$7H$_2$O, 2.56 g CH$_3$COONa, 0.236 g (NH$_4$)$_2$SO$_4$, 1.5 g KH$_2$PO$_4$, 3.5 g K$_2$HPO$_4$, (0.362 g KNO$_3$ or 0.247 g NaNO$_2$), pH = 7.2. About 50 mg/L of NH$_4^+$$\cdot$N, NO$_2^-$·N, and NO$_3^-$·N were used as the initial concentrations of nitrogen sources, respectively. To investigate the effects of Cd(II), Co(II), and Mn(II) on the nitrogen bioremediation ability of strain Y-10, the metal ions of Cd(II), Co(II), and Mn(II) in the basal medium were derived from the chemical compounds of CdSO$_4$$\cdot$8H$_2$O, CoSO$_4$$\cdot$7H$_2$O, and MnSO$_4$$\cdot$H$_2$O. The composition of the Luria–Bertani (LB) liquid medium was as follows (per liter): 5.0 g yeast extract, 10 g NaCl, 10.0 g tryptone, pH = 7.0, which was used to enrich bacteria. To make solid plates, 2.0% agar was added to the LB liquid medium. The basal and LB media were sterilized at 121 °C and 0.11 MPa for 30 min.

2.2. Assessment of the Influence of Cd(II), Co(II), and Mn(II) on the Nitrogen Bioremediation Performance of Strain Y-10

LB medium solid plates and the liquid medium were used to activate and enrich the strain Y-10. After cultivation of strain Y-10 at 150 rpm and 15 °C for 36 h in the LB liquid medium, we took out 6 mL of the pre-culture, continued with centrifugation at 4000× rpm for 8 min, discarded the supernatant liquid, washed twice under sterile conditions, inoculated into the basal medium (100 mL), which was supplemented with different concentrations of Cd(II), Co(II), and Mn(II), and then cultivated for 48 h at 150 rpm and 15 °C. Cell density, ammonium, nitrite, nitrate, and total nitrogen concentrations were examined before and after cultivation. All the experiments described above were conducted in three replicates. The nitrogen removal efficiencies were calculated using Equation (1).

\[
R_m = \frac{(C_1 - C_2)}{C_1} \times 100\%
\]  

where $R_m$, $C_1$, and $C_2$, respectively, represent the removal efficiency of different types of nitrogen, initial, and final nitrogen concentrations.

2.3. Analytical Methods

The spectrophotometer (DU800, Beckman coulter, USA) was used to determine cell growth and concentrations of various inorganic nitrogen compounds. The optical density (OD$_{600}$) was measured at 600 nm without centrifugation for evaluating the bacterial growth. The pH and concentrations of various inorganic nitrogen compounds were measured with supernatants after centrifuging for 5 min at 8000 rpm. The indophenol blue method was used to determine ammonium nitrogen. Nitrates were calculated by the absorbance value at 220 nm subtracting two times the background absorbance value at 275 nm. Nitrite nitrogen was determined by ultraviolet spectrophotometry at the wavelength of 540 nm after adding 1 mL of a chromogenic reagent including 0.1 mL of phosphoric acid, 0.002 g of N-(1-naphthyl)-1,2-diaminoethane dihydrochloride, and 0.04 g of sulfanilamide. Total nitrogen was calculated using the absorbance value at 220 nm subtracting two times the background absorbance value at 275 nm using alkaline potassium persulfate digestion chromatography [23].

2.4. Statistical Analysis

SPSS Statistics 22 and Origin 8.6 were used to conduct data analysis and create graphs.
2.5. Kinetic Model

The inhibitory impacts of each heavy metal ion on the nitrogen bioremediation efficiency of strain Y-10 were evaluated using a modified non-competitive inhibition model [24].

\[ I(\%) = 100 \times \left(1 - \frac{1}{1 + (\frac{[Y]}{a})^b}\right) \]  
(2)

where $I(\%)$ is the nitrogen removal efficiency inhibition response, $[Y]$ is the concentration of a heavy metal ion. The 50% inhibitory concentrations of Cd(II), Co(II), and Mn(II) and the fitting parameters were represented by the letters $a$ and $b$, respectively.

3. Results and Discussion

3.1. Impacts of Cadmium on the Nitrogen Bioremediation Characteristics of Strain Y-10

The nitrogen removal capacity of strain Y-10 was investigated with different concentrations of Cd(II), as shown in Figure 1A. The initial Cd(II) dosages were fixed at 0.1, 0.25, 0.5, 2.5, and 5.0 mg/L. Low concentration of Cd(II) (≤2.5 mg/L) caused no conspicuous impacts on ammonium and total nitrogen removal compared with the control treatments (without supplementation of Cd(II)). When the concentrations of Cd(II) changed from 0 to 2.5 mg/L, the ammonium and total nitrogen bioremediation efficiency reached about 100% and 88%; this contradicted the report that 2–2.5 mg/L of Cd(II) could cause 50% inhibition of the specific ammonium utilization in activated sludge systems [25]. With the further increase in the concentration of Cd(II) to 5.0 mg/L the ammonium and total nitrogen bioremediation efficiency prominently dropped to 90.2% and 73.2%; this contradicted the report that the total nitrogen removal efficiency could still reach up to 88% with the addition of 10 mg/L of Cd(II) to activated sludge [26]. This phenomenon might be explained by possibly lower bioavailability of Cd(II) when used in the concentration of 5 mg/L instead of 10 mg/L in an activated sludge wastewater treatment system, because a large amount of Cd(II) is precipitated and adsorbed by the sludge. Semerci and Çeçen [25] summarized several publications and found that the toxicity of dissolved heavy metal ions is strongly related to the free metal ion dosages rather than to the total concentrations. During the nitrification process, neither nitrates nor nitrates were detected. The modified non-competitive inhibition model was used to describe the impact of Cd(II) on heterotrophic nitrification. Although Cd(II) did exert prominent toxicity for nitrification with its concentration ranging from 2.5 to 5.0 mg/L, the ammonium and total nitrogen removal efficiency did not drop below 50%. Hence, the IC₅₀ value of Cd(II) for heterotrophic nitrification could not be calculated. Meanwhile, without the Cd(II) addition, the value of OD₆₀₀ was only 1.21. Low concentration of Cd(II) (less than 2.5 mg/L) significantly enhanced the bacterial reproduction compared with that in the control group. The value of OD₆₀₀ was about 1.4 with the Cd(II) concentration ranging from 0.1 to 2.5 mg/L. The pH was positively correlated with the change of cell growth, which might be due to the fact that the denitrification process occurred simultaneously along with nitrification and the cell growth of strain Y-10.

Despite the well-documented low toxicity of Cd(II) in terms of the ammonium removal ability of strain Y-10 in the above experiments, the impact of Cd(II) on aerobic denitrification was still unclear. Thus, the nitrate was used as the sole nitrogen source to investigate the influence of Cd(II) on the aerobic denitrification process. Figure 1B depicted the cell growth and nitrate reduction in the presence of different Cd(II) concentrations. The denitrification capacity of strain Y-10 was positively correlated with the Cd(II) dosage. In the absence of Cd(II), the removal efficiencies of nitrate and total nitrogen were 60.2% and 31.3%, respectively. Meanwhile, 11.21 mg/L of nitrates were detectable (the nitrite accumulation data are not shown in the Figures). When 0.1 mg/L of Cd(II) was added to the denitrification system, the nitrate and total nitrogen bioremediation efficiency markedly increased to 65.4% and 48.7%.
The total nitrogen removal efficiency (48.7%) was higher than that (31.3%) in control treatment. This result may be due to that the enzymes of nitrite oxidoreductase and nitrate reductase were enhanced by 0.1 mg/L Cd(II) and thus resulted in only 6.96 mg/L of nitrite accumulation was detected at the end cultivation.

Besides, the final OD$_{600}$ and pH values increased by 0.32 and 0.18 with the addition of 0.1 mg/L Cd(II) compared with the control treatments. These results demonstrated that 0.1 mg/L Cd(II) could not only enhance the denitrification capacity of strain Y-10, but also stimulate the cell growth along with lower nitrite accumulation. However, as the Cd(II) concentrations continually climbed from 0.25 to 5.0 mg/L, the detrimental impacts on denitrification gradually became more serious, where the nitrate and total nitrogen bioremediation efficiency dropped from 55.7% and 35.9% to 2.2% and 0.9%, respectively. Certainly, the accumulation concentration of nitrites also decreased with the increase of Cd(II), and only 0.07 mg/L of nitrites were detected at 5.0 mg/L of Cd(II), which might be due to strong inhibition of the enzymes nitrate reductase and nitrite reductase by the high concentration of Cd(II) [27].

Additionally, the modified non-competitive inhibition model was used to evaluate the inhibitory effects of Cd(II) on the nitrogen removal activity. The nitrate and total nitrogen bioremediation efficiency was shown using regression equations: Equation (3) and Equation (4). According to the regressions, the 50% inhibitory Cd(II) concentration (IC$_{50}$) for the nitrate and total nitrogen bioremediation efficiency was calculated to be 0.3268 and 0.1024 mg/L, respectively, indicating that the nitrate removal process was less sensitive to the Cd(II) inhibition effect than the total nitrogen removal process by strain Y-10. Compared to the effect of Cd(II) on ammonium bioremediation, the nitrate bioremediation was more susceptible to Cd(II) supplementation.

\[
I(\%) = 100 \times \left(1 - \frac{1}{1 + ([Cd]/0.3268)^{-0.7822}}\right), \quad R^2 = 0.9200 \quad (3)
\]

\[
I(\%) = 100 \times \left(1 - \frac{1}{1 + ([Cd]/0.1024)^{-0.6762}}\right), \quad R^2 = 0.9567 \quad (4)
\]

where the Cd(II) concentration (mg/L) is represented by [Cd].

Similarly, the nitrite was added to the basal medium instead of the nitrate. With the concentration of Cd(II) increased from 0 to 0.25 mg/L, the nitrite and total nitrogen bioremediation efficiency conspicuously increased by 28.6% and 33.1% (Figure 1C). Nevertheless, as the concentration of Cd(II) climbed continuously to 5.0 mg/L, the nitrite bioremediation efficiency sharply decreased to 1.3% without any total nitrogen reduction. The cell growth was also enhanced by lower than 0.25 mg/L Cd(II) compared with the concentration in the control treatment. The change of the pH value was positively related to the nitrogen removal trend. The maximum nitrogen removal efficiency and high pH values occurred at 0.25 mg/L of Cd(II), which were higher than those in the nitrate removal process (0.1 mg/L). Based on the regression equations (Equations (5) and (6)), the IC$_{50}$ for the nitrite and total nitrogen removal were calculated to be 0.7565 and 0.5312 mg/L, respectively. The IC$_{50}$ values of Cd(II) for nitrite and the corresponding total nitrogen removal efficiency (0.7565 and 0.5312 mg/L) were apparently higher than that in the nitrate bioremediation system (0.32677 and 0.10237 mg/L), but lower than that in the ammonium removal system. This was that the reason why the ammonium and total nitrogen bioremediation efficiency could reach up to 90.2% and 73.2%, respectively, even at the Cd(II) concentration of 5.0 mg/L.

\[
I(\%) = 100 \times \left(1 - \frac{1}{1 + ([Cd]/0.7565)^{-1.3169}}\right), \quad R^2 = 0.7732 \quad (5)
\]

\[
I(\%) = 100 \times \left(1 - \frac{1}{1 + ([Cd]/0.5312)^{-1.4888}}\right), \quad R^2 = 0.8702 \quad (6)
\]
Previous studies suggested that Cd(II) is one of the most severe heavy metal pollutants for microorganisms [11–13]. In this study, Cd(II) is thought to have a positive rather than negative influence on aerobic denitrification when the concentration of Cd(II) is approximately 0.1 mg/L in the nitrate removal system and 0.25 mg/L in the nitrite-contaminated wastewater. The reason why Cd(II) could facilitate aerobic denitrification needs to be studied further.

However, when the concentration of Cd(II) was up to 5.0 mg/L, the ammonium, nitrate, and nitrite bioremediation efficiency decreased by 9.8%, 63.2%, and 96.5%, respectively, as compared with 2.5 mg/L of Cd(II) for ammonium, 0.1 mg/L of Cd(II) for nitrates, and 0.25 mg/L of Cd(II) for nitrite nitrogen. These results indicated that high concentration of Cd(II) (>5.0 mg/L) could conspicuously inhibit nitrogen biodegradation, especially nitrate and nitrite removal. The results of the impact of Cd(II) on the nitrogen removal ability of Arthrobacter arilaitensis Y-10 demonstrated that the order of the degree of toxic effect on heterotrophic nitrification and aerobic denitrification was as follows: nitrates > nitrites > ammonium. The inhibition phenomenon may be ascribed to the fact that the transcription of nirK and norB could be regulated by Cd(II) [28]. Gui et al. [29] reported that only 2.5 mg/L of Cd(II) could totally inhibit the expression concentration of the nirS gene. Besides, Cd(II) could affect activity of oxidoreductase, which could transform intracellular polyhydroxyalkanoates and glycogen, and then results in the decrease of the nitrate oxidizing bacteria abundance [26]. It was probably another reason contributing to the adverse effect of high Cd(II) concentrations on biological nitrogen removal.

![Figure 1. Effects of Cd(II) on ammonium, nitrate, and nitrite removal abilities of strain Y-10. (A) ammonium; (B) nitrates; (C) nitrites. The values shown are the means ± the SD (Standard Deviation) of the results of three replicates (error bars). Different letters indicate a significant difference between treatments at p < 0.05. OD<sub>600</sub>: the optical density at 600 nm.](image)

3.2. Impacts of Cobalt on the Nitrogen Bioremediation Characteristics of Strain Y-10

The previous study demonstrated that Co(II) is the least inhibitory heavy metal ion for nitrification among the following four heavy metal ions: Cu(II), Zn(II), Co(II), and Ni(II) [15]. The biodegradation in the municipal wastewater treatment system could be stimulated by low concentration of Co(II) [29]. Trace amounts of Co(II) may act as activated sludge growth stimulant and the COD (chemical oxygen demand) removal efficiency could be improved by 30% in a batch activated sludge system by addition of 5 mg/L Co(II) [30]. However, the influence of Co(II) on the nitrification and denitrification process in the pure culture and even in the activated sludge has not been widely evaluated by international researchers. In this experiment, a pure simultaneous nitrification–denitrification bacterium was employed as the research object, which could help more clearly clarify the mechanism of impact of Co(II) on the biological nitrogen removal process compared to that in activated sludge. Figure 2 depicted the ammonium, nitrate, and nitrite removal characteristics at different Co(II) concentrations.
The ammonium removal efficiency decreased by 71.1% with the addition of 0.5 mg/L of Co(II) compared with that in the control treatment (Figure 2A).

This result contradicted the report that the anammox activity could not be affected by 2 mg/L of Co(II), and the activity only decreased by 15%, 51%, and 64% when the Co(II) concentration increased to 5, 10, and 12 mg/L, respectively (Kimura and Isaka, 2014). Furthermore, 29.47 mg/L of Co(II) did not exert any effect on either the growth of Bacillus subtilis PK15 or the removal of ammonia [31]. A noteworthy observation was that microbial growth could be sustained even with 50 mg/L of Mn(II), which was inconsistent with the literature stating that addition of Mn(II) could not be a beneficial biochemical heavy metal ion for cell growth. This result was exactly opposite to that in the control treatment (Figure 2A).

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The value of pH exhibited a similar trend with cell growth. The reason why low concentration of Co(II) exerted high toxicity to the nitrogen removal capacity of strain Y-10 needs to be studied further.

The above results concern the inhibitory influence of Co(II) on the heterotrophic nitrification by strain Y-10, but this influence on the aerobic denitrification process has not been examined. According to Figure 2B,C, unfortunately, aerobic denitrification activities were totally inhibited once Co(II) was added to the wastewater treatment system regardless of whether nitrates or nitrites were selected as the sole nitrogen source. All results indicated that the nitrogen transformation ability of strain Y-10 was negatively correlated with the Co(II) concentration and Co(II) is not crucial for strain Y-10 to perform biological nitrogen removal and cell growth. Conversely, Bruins et al. [32] demonstrated that Co(II) could stimulate microbial growth, and thus be considered one of the “essential” heavy metal ions, whereas Cd(II) was considered one of the “non-essential” heavy metal ions, because it was not a beneficial biochemical heavy metal ion for cell growth. This result was exactly opposite to the effect of Co(II) and Cd(II) on the nitrogen conversion characteristics of strain Y-10. This findings offer new insights into the interaction between heavy metals (Co(II), Cd(II)) and biological nitrogen removal behaviors and thus improve our understanding of the potential roles of Co(II) and Cd(II) in the nitrogen cycle.

![Figure 2](image_url)

Figure 2. Effects of Co(II) on ammonium, nitrate, and nitrite removal abilities of strain Y-10. (A) ammonium; (B) nitrate; (C) nitrite. The values shown are the means ± the SD of the results of three replicates (error bars). Different letters indicate a significant difference between treatments at \( p < 0.05 \).

### 3.3. Impacts of Manganese on the Nitrogen Bioremediation Characteristics of Strain Y-10

Previous reports demonstrated that Mn(II) generally exists in natural groundwater bodies and acts as an electron donor [33,34]. The abundance of anaerobic ammonium-oxidizing bacteria and the nitrogen removal rate in the long-term nitritation–anammox could be enhanced by addition of Mn(II) [17]. To estimate the impact of Mn(II) on the nitrogen transformation capacity of strain Y-10, the concentration of Mn(II) was set as 0, 0.5, 5, 10, 20, and 30 mg/L. As shown in Figure 3A, no significant differences were observed in the ammonium removal efficiency regardless of whether Mn(II) was
exposed to the nitrogen transformation system or not. The ammonium removal efficiency remained about 100% even if the Mn(II) concentration reached up to 30 mg/L. Nevertheless, the total nitrogen removal efficiency conspicuously dropped by 6.7% when exposed to 0.5 mg/L of Mn(II) compared to the control treatments.

After that, the total nitrogen removal efficiency was close almost to 78% when exposed to 0.5–30 mg/L of Mn(II), which was inconsistent with the literature stating that addition of Mn(II) could promote the ammonium removal ability of Acinetobacter harbinensis HITLi7\textsuperscript{[35]}. These results revealed that the presence of Mn(II) exerted no damage on the capacity of strain Y-10 to remove ammonium, although the total nitrogen bioremediation capacity of strain Y-10 was adversely affected by Mn(II) supplementation. During this nitrification process, Mn(II) supplementation also caused no effect on cell growth compared to the control treatment.

In the Huang’s work, the activities of anaerobic ammonium-oxidizing bacteria were improved by 0.05 mmol/L (equal to 2.8 mg/L) of Mn(II) and a nearly double improvement of the nitrogen removal efficiency was observed \textsuperscript{[20]}. Moreover, 27.47 mg/L of Mn(II) did significantly influence the cell growth of PK15 \textsuperscript{[36]}. Similar results were obtained by Li et al. \textsuperscript{[17]} who revealed that the nitrogen removal rate dramatically improved with addition of 2.0 mg/L Mn(II) in the nitration–anammox process. These results indicated that Mn(II) is beneficial for nitrogen bioremediation. The value of pH was closely bound with the cell growth at the Mn(II) concentration from 0.5 to 30 mg/L, further demonstrating that the strain Y-10 could continuously conduct heterotrophic nitrification at high concentration of Mn(II).

Next, the aerobic denitrification capacity of strain Y-10 was carried out when ammonium was substituted by nitrates. The nitrate and total nitrogen bioremediation efficiency increased dramatically from 61.5% and 32.2% to the peak values of 66.1% and 44.9%, respectively, accompanying the Mn(II) increase from 0 to 0.5 mg/L, and then sharply decreased to 9.1% and 7.8% at 5 mg/L of Mn(II). With further addition of Mn(II), the aerobic denitrification ability of strain Y-10 continuously decreased and even ceased (Figure 3B). The maximum nitrate accumulation (11.59 mg/L) occurred in the control treatments. These experimental results revealed that 0.5 mg/L of Mn(II) could not only improve the nitrogen removal efficiency, but also lower the nitrite accumulation during the aerobic denitrification process. Meanwhile, 0.5 mg/L of Mn(II) could stimulate cell growth, but the value of OD\textsubscript{600} sharply dropped from 0.93 to 0.58 as the Mn(II) concentration increased from 0.5 to 30 mg/L. The pH was comparatively constant at low concentrations of Mn(II) (less than 0.5 mg/L) and well-correlated with the rate of cell growth at other concentrations. Therefore, all the results showed that 0.5 mg/L of Mn(II)
was the optimum concentration for enhancing aerobic denitrification and the growth of strain Y-10 with nitrates. The abilities of strain Y-10 to remove nitrates and total nitrogen were suppressed by high Mn(II) concentration, and the simulation results from the modified non-competitive inhibition model are presented below (Equation (7) for nitrate and Equation (8) for total nitrogen removal inhibition). The equations suggested that IC$_{50}$ for nitrate and total nitrogen removal was 0.8773 and 0.4008 mg/L, respectively. This result implied that low concentration of Mn(II) (less than 0.4 mg/L) may be more beneficial for nitrogen removal.

\[
I(\%) = 100 \times \left(1 - \frac{1}{1 + ([Mn]/0.8773)^{-1.1635}}\right) \quad R^2 = 0.9911
\]  
\( (7) \)

\[
I(\%) = 100 \times \left(1 - \frac{1}{1 + ([Mn]/0.4008)^{-0.9393}}\right) \quad R^2 = 0.9937
\]  
\( (8) \)

Nitrites were used to further investigate the effect of different Mn(II) concentrations on the aerobic denitrification capacity of strain Y-10. Figure 3C showed that 0.5 and 5 mg/L of Mn(II) caused no inhibition effect on the nitrite removal efficiency compared with the control treatment, corresponding to the removal efficiency of about 69.5%. Additionally, 5 mg/L of Mn(II) could markedly improve cell growth and the peak value of OD$_{600}$ was 1.08. At the same time, the total nitrogen removal ability exhibited a similar trend with nitrites as depicted in Figure 3C. For instance, the total nitrogen removal efficiency obviously increased from 50.3% to the maximum value of 64.1% with the concentration of Mn(II) increasing from 0 to 0.5 mg/L and then evidently decreased to 23.3% at 30 mg/L of Mn(II). The aerobic denitrification with nitrites could be persistently conducted by strain Y-10 even at a high concentration of Mn(II), which was inconsistent with the results that the nitrate and total nitrogen removal almost stopped with the addition of 30 mg/L of Mn(II).

The OD$_{600}$ and pH values were higher than 0.83 and 8.08, respectively, even if the Mn(II) concentration reached 30 mg/L. These results demonstrated that the strain Y-10 could tolerate a high concentration of Mn(II) when wastewater contained nitrite nitrogen. According to the regression below (Equation (9) for nitrite and Equation (10) for total nitrogen removal inhibition), the 50% inhibitory concentrations of Mn(II) for the nitrite and total nitrogen removal efficiency were determined to be 5.1547 and 1.7766 mg/L, respectively, which is significantly higher than those (0.8773 and 0.4008 mg/L) in the nitrate experiment.

\[
I(\%) = 100 \times \left(1 - \frac{1}{1 + ([Mn]/5.1547)^{-0.5296}}\right) \quad R^2 = 0.6337
\]  
\( (9) \)

\[
I(\%) = 100 \times \left(1 - \frac{1}{1 + ([Mn]/1.7766)^{-0.4179}}\right) \quad R^2 = 0.9675
\]  
\( (10) \)

Due to the extensive use of Cd(II), Co(II), and Mn(II), it appears that a considerable amount of the abovementioned heavy metal ions will inevitably discharge into the aquatic environment or biological wastewater treatment system. Indeed, several kinds of nitrogen-contaminated wastewater, such as mining, landfill leachate, pigments, hospital wastewater, and metal refinery wastewater generally contain high concentrations of various heavy metals [9,37,38]. In particular, heavy metal ions could not be biodegraded typically and could be accumulated by the living organisms, which makes the situation even worse. Nitrogen removal from wastewater is usually achieved by heterotrophic nitrification and aerobic denitrification bacteria. Nitrifiers and denitrifiers are sensitive to several heavy metal ions as well as to other substances. Up to now, researchers mainly focused on the effects of heavy metals on nitrification by using the activated sludge as the research object [26,39,40]. Keep in mind that the
bioavailable concentration of heavy metal ions should be less than the total concentration of added heavy metal ions, because these parts of heavy metal ions could be adsorbed by the activated sludge. Additionally, the aerobic denitrification with nitrites or nitrates has always been neglected.

This study revealed that heterotrophic nitrification with ammonium and aerobic denitrification with nitrates/nitrites of strain Y-10 could be affected by heavy metal ions of Cd(II), Co(II), and Mn(II). Cd(II) is listed as one of the top five toxic heavy metals, which is the most toxic to the environment, nitrifying bacteria, and even human health [41]. However, in current studies, low concentration of Cd(II) (2.5 mg/L) exhibited no obvious effects on ammonium transformation. Furthermore, the nitrate and total nitrogen bioremediation efficiency could significantly be enhanced by 0.1 mg/L Cd(II). Likewise, the nitrite and total nitrogen bioremediation efficiency was also improved by 0.25 mg/L of Cd(II).

By contrast, the previous work proved that Co(II) is the least inhibitory metal ion of Cu(II), Zn(II), Ni(II), and Co(II) in the nitrifying sludge, and the effect of Co(II) on bacteria is not crucial if the contact time with the biomass is short [42,43]. Nevertheless, Co(II) strongly inhibits the heterotrophic nitrification ability of strain Y-10, and even worse, it lost its denitrification ability once Co(II) was added to the wastewater. In terms of Mn(II), the 50% inhibitory concentration for the anammox activity was calculated to be 7.33 mg/L [44], while 30 mg/L of Mn(II) in this research showed no conspicuous deterioration for ammonium removal. The order of the inhibitory effect was Co(II) > Cd(II) > Mn(II) when both nitrogen removal efficiency and the 50% inhibitory concentration of a heavy metal were taken into account. Additionally, the results of this study mainly revealed effect of a single heavy metal ion on heterotrophic nitrification with ammonium and aerobic denitrification with nitrates/nitrites alone. It highlighted that the aerobic denitrification with nitrates and/or nitrites was more sensitive to the inhibitory effects of Cd(II), Co(II), and Mn(II) relative to the heterotrophic nitrification with ammonium. Therefore, the effect of heavy metal ions on nitrification alone does not have an explanatory power for such inhibition of the whole biological nitrogen removal process unless denitrification is also considered.

4. Conclusions

The order of the inhibitory degree for the nitrogen bioremediation capacity of Arthrobacter arilaitensis Y-10 was Co(II) > Cd(II) > Mn(II). The heterotrophic nitrification was less sensitive to the inhibitory effects of Cd(II), Co(II), and Mn(II) relative to aerobic denitrification. This is the first report to state that the most inhibitory heavy metal ion is Co(II), and that the denitrification activity is completely suppressed once Co(II) is added. Therefore, when using strain Y-10 to remove nitrogen, attention should be paid to the Co(II) concentration in the wastewater treatment system.

Author Contributions: T.H. was the executor and writer of this study; D.X. and J.N. participated in the revision of the paper. D.X. and J.N. participated in some experiments and data analysis; Z.L. (Zhu Li) and T.H. provided financial support; Z.L. (Zhenlun Li) provided the experimental ideas; All authors have read and agreed to the published version of the manuscript.

Funding: This research was found by the Gui Da Zhuan Ji He Zi, grant number (2019) 04 and the Construction Program of the Biology First-Class Discipline in Guizhou, grant number (GNYL (2017) 009).

Conflicts of Interest: The authors declare no conflict of interest.

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