

Effect of Metal Nanoparticles on Biological Denitrification Process: A Review

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Abstract

Globally, increasing nitrate concentrations have become a serious environmental problem and a potential risk to public health. The World Health Organization (WHO) has set a limit of (10 mg/ (L)) NO₃⁻ for human consumption and (100 mg/ (L)) NO₃⁻ for animals. Water above these limits requires denitrification. Nitrate causes cancer, blue-baby syndrome, hypertension and thyroid hypertrophy. Several treatment processes can remove nitrates from water with varying degrees of efficiency, cost, and ease of operation. Among these processes chemical denitrification and biological denitrification are useful processes considering efficiency cost and maintenance. Denitrifying microorganisms are able to reduce nitrate to innocuous nitrogen gas using suitable source of electron. Oxides of metal nanoparticles, hydrogen gas, and sulfur reduced compounds; pyrite and arsenate have been used as electron donors for biological nitrate reduction. Among these electron donors H₂ and oxides of metal nanoparticles have better performance in the removal of nitrate. Bio-denitrification coupled to metal nanoparticle oxidation is an inexpensive and advantageous process for nitrate removal from source of water. In this review article the biological denitrification efficiency was studied between free cell and cell amended metal nanoparticle such as nano scale zero valent iron (nZVI). This study investigated the potential applicability of employing metal nanoparticle as source of electron for biological nitrate reduction. Temperature, pH, nitrate concentration and dissolve oxygen concentration are the major factors affecting nitrate removal, with or without the presence of metal nanoparticles that studied in this article.

Keywords: Water, Biological Denitrification, Metal Nanoparticles, Nano Scale Zero Valent Iron

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Introduction

Nitrate is a serious water contaminant in many areas in the world. Consumption of nitrate contamination water causes methemoglobinemia and other disorder such as hypertension [1], goiter [2], stomach cancer [3], thyroid disorder and birth defects [4]. In animals, it may lead to muscular weakness and abdominal pains [5]. Because of its risk to environmental and human health, nitrate should be removed from water supplies. In recent years biological denitrification has gained increasing interest for nitrate removal. This method is considered to be a most economical technique among other conventional techniques [6]. There is considerable ongoing attempt focused on autotrophic denitrification using metal nanoparticle, since it is a clean method with high nitrate removal efficiency. The main advantage of autotrophic denitrification using metal nanoparticle is the use of hydrogen and oxides of metal nano particles as an electron donors, which are harmless to human [7]. In this study investigated autotrophic denitrification which was combined with metal nanoparticle for treating nitrate contamination water. This combined technology could achieve both high nitrate removal and low by- product.

denitrification of nitrate has been accomplished using n- ZVI in aqueous solutions, indicating its potential

applications for nitrate removal from nitrate pollutant waters [8]. Few studies have focused on this combined technology for water treatment [9- 11]. Therefore, the main objective of this review article is to investigate the autotrophic denitrification using n-ZVI and other metal nanoparticles.

Source of nitrate

Generally major sources of nitrate pollution of water supplies divided to several group, including: accumulation of waste, overuse of animal and chemical fertilizers, explosive industries, municipal waste, industrial effluents and septic tank leakage [7, 12].

Nitrate removal methods

Nitrate is highly soluble anion which is colorless and odorless. Because of these features it is difficult to remove nitrate using conventional water treatment technology such as filtration [13].

There are different methods for removing nitrate from water supplies like ion exchange [14], reverse osmosis [15], catalysis and electro- dialysis [16]. Each of techniques has its advantages and disadvantages. Physical and chemical technology are very expensive for pilot scale operation [17]. However, use of these techniques is limited to energy costs and post- treatment of large volume of wastewater [18]. Biological treatment process is widely used for



nitrate removal from water source, compared with physio-chemical denitrification methods, this method removes nitrate without need of post-treatment. Despite its lower denitrification rate, biological nitrate removal method is a cost-effective and friendly to the environment [7].

Biological denitrification

The biological nitrate removal from drinking water supply first became operation in 1981, at the Chateau-Landon in France [19]. Biological denitrification removes nitrate from water supply using microorganisms. The microbial reduction of nitrate to innocuous nitrogen gas is termed biological denitrification or nitrate respiration. [20].

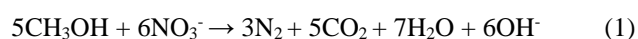
Usually, biological denitrification is anoxic process, that occurring in the absence of molecular oxygen and presence of nitrate as a final electron acceptor. This process involves four steps, from nitrate to nitrogen gas ($\text{NO}_3^- \rightarrow \text{NO}_2^- \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2$). Each step in this process is catalyzed by separate enzyme system [21]. Microorganisms known to denitrify are facultative organisms, but most of them are not obligate anaerobes. This microorganisms under anoxic conditions use nitrate as a final electron acceptor. The most recognized denitrify microorganisms including: *Achromobacter*, *Alcaligenes*, *Bacillus*, *Chromobacter*, *Corynebacterium*, *Halobacterium*, *Methanomonas*, *Moraxella*, *Paracoccus*, *Propionibacterium*, *Pseudomonas*, *Spirillum*, *Thiobacillus*, and *Xanthomonas* [22, 23]. According to organic carbon source, biological denitrification divided to autotrophic and heterotrophic denitrification [24-25]. The main difference between autotrophic and heterotrophic denitrification is type of electron donor [26].

Heterotrophic denitrification

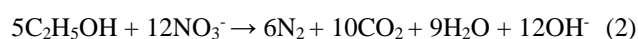
Heterotrophic denitrifying microorganisms need a reduced organic substrate for metabolisms. In heterotrophic denitrification, different organic carbon substrate used as an electron donor. Generally, the most prominent carbon sources are glucose, methanol, ethanol and acetic acid. A number of studies utilized natural compounds, such as wheat straw as organic carbon source for use in heterotrophic denitrification [27].

The following stoichiometric relationships for methanol, ethanol and acetic acid have been formulated in equations Eq(s) (1) - (3):

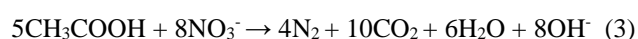
Methanol [27].



Ethanol [27].



Acetic acid [27].



The major harm of using methanol is production of toxic residual methanol in water supply. Due to the toxic effect of residual methanol, ethanol has been used as safe organic carbon source instead methanol.

Heterotrophic biological denitrification is not only cost-effective but also high nitrate removal rate, observed in this method.

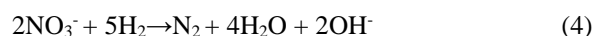
Despite these advantages, pretreatment process in this method is complex and lengthy. Moreover, heterotrophic biological denitrification is efficient in nitrate removal from supply water by sufficient organic carbon source but in situations such as lake by insufficient organic carbon source, application of this method is inefficient, unless organic substrate added to situation [27].

Autotrophic denitrification

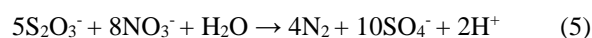
In autotrophic denitrification process, nitrate converted to innocuous nitrogen gas by autotrophic denitrifying microorganisms such as *Thiobacillus denitrificans*, *Thiomicrospira denitrificans*, *Paracoccus denitrificans*. In autotrophic denitrification an inorganic energy source such as sulfur, hydrogen or reduced sulfur species (thiosulfate, sulfite) is needed. Despite Heterotrophic denitrification, in autotrophic denitrification the carbon required for bacterial growth achieved from inorganic carbon compounds including bicarbonate or carbon dioxide that these compounds used for cell synthesis. Beijerinck, Lieske and Baalsruud [28] studied physiological and biochemical characteristics of sulfur-oxidizing microorganisms. Claus and Kutzner [29] investigated kinetics of *Thiobacillus denitrificans* using thiosulfate as an electron donor.

The following stoichiometric relationships for hydrogen and thiosulfate have been reported in Eq(s)(4)-(5):

Hydrogen [23].



Thiosulfate [29].



Autotrophic denitrification has advantages including no requirement for an external organic carbon compounds such as methanol. Furthermore autotrophic denitrification is cost-effective and less sludge production, which decreases post-treatment process. Despite these advantages autotrophic microorganisms have slow growth [6].

Removal of nitrate by metal nanoparticles

Most of the metal nanoparticles due to their electron-donating tendency can removed different pollutant chemical pollutants including the anions through reduction. This property of the metals has attracted considerable attention of environmental researchers. Recently, some types of nanoparticles have been found in sewage and wastewater treatment, and these nanoparticles were observed to be adsorbed on to activated sludge [30]. Some of the metal nanoparticles should not be used because they are either toxic or expensive. Most of zero-valent metal nanoparticles can be used for chemicals removal including: Fe, Mg, Cu, Ti, Al, Ni, Pd, Pt, Cr and Ce [30].

Different metal nanoparticles for nitrate removal

Alumina nanoparticles (Al_2O_3) have been used in many fields. Chen et al., investigated both short-term and long-term effects of Al_2O_3 nanoparticles on wastewater nitrate removal efficiency [31]. Scanning electron microscope analysis showed that most of Al_2O_3 nanoparticles were adsorbed onto activated sludge. Results of their study was found that short-term exposure to (1 and 50 mg/L) Al_2O_3

nanoparticles induced marginal influences nitrate and phosphorus removal.

Nevertheless, high Al₂O₃ nanoparticles concentration (50 mg/L) was decreased nitrate removal efficiency from 80.4% to 62.5% due to the suppressed denitrification process. Their study indicated that compared with the control, (50 mg/L) Al₂O₃ nanoparticles decreased the abundance of denitrifying bacteria in activated sludge. Enzyme activity tests showed that the activities of key denitrifying enzymes (nitrate reductase and nitrite reductase) were inhibited, which due to the negative effects of (50 mg/L) Al₂O₃ nanoparticles on wastewater nitrate removal.

Hou et al., [32] evaluated the effects of CeO₂ nanoparticles on biological nitrate removal in a sequencing batch biofilm reactor (SBBR). In their study at low concentration of CeO₂ nanoparticles (1mg/L), no significant effect was observed on nitrate removal, but, at high concentrations (10 and 50 mg/L), the nitrate removal efficiency reduced from 74.09% to 64.26% and 55.17% respectively. Scanning electron microscope imaging showed large amounts of CeO₂ nanoparticles attached on the biofilm, which increased the production of reactive oxygen species. The high concentration of CeO₂ nanoparticles (50 mg/L) significantly affected the lactate dehydrogenase release. Confocal laser scanning microscopy showed that high concentrations of CeO₂ nanoparticles measurably reduced bacterial number [32].

Cai et al., [33] studied bimetallic Fe-Ni nanoparticles supported by kaolinite (K-Fe-Ni) to simultaneously remove Cu (II) and nitrate. Results of their study showed that the removal of either Cu (II) or nitrate using K-Fe-Ni was affected. Specifically, 42.5% of nitrate was reduced in the presence of (200 mg/L) Cu (II), while only 26.9% of nitrate was reduced when Cu (II) was absent. Similar results were also obtained for the removal of Cu (II) in the absence or presence of nitrate. However, the effect of nitrate concentrations on the removal of Cu (II) was less than that for Cu (II) concentrations regarding the reduction of nitrate [33]. Shi et al., investigated supported bimetallic Fe-Pd nanoparticles that were prepared by loading Fe and Pd on chelating resin by two different methods. Their results showed a remarkable nitrate removal (more than 95%) [34].

Wang et al., investigated Polycrystalline Pt nanoparticles with controlled ratio of Pt(100) and Pt(110) were adopted to study the surface sensitivity to nitrate reduction. Pt nanoparticles with more Pt(100) oriented surface exhibit higher reactivity to electro-reduction of nitrate. The mechanism studied by a cyclic voltammogram in low nitrate concentration at high scan rate indicates that the initial reduction of nitrate ion happens on the short-range order Pt(100) sites, leading to the formation of NO that acts as the intermediate of the nitrate reduction to ammonia, while Pt(110) is hardly involved in nitrate reduction in this condition [35].

Lubpho et al., evaluated nanocomposite tree metals, (Cu-Pd-Fe⁰) for enhancing both nitrogen gas selectivity and nitrate removal. The results of their study showed under the conditions of temperature of 50°C, pH7 and initial

nitrate concentration (100 mg/L), the highest N₂ gas selectivity of 60.5% and nitrate removal of 90% were achieved [36].

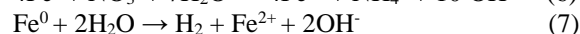
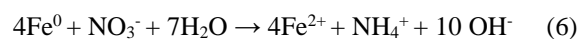
In another study that occurred with An et al., bimetallic nanoparticles (nano Fe-Ni, nano Fe-Cu) and coated iron nanoparticles (chitosan-Fe⁰, sodiumoleate-Fe⁰) were utilized to support autotrophic denitrification. In comparison to nano scale zero-valent iron (nZVI) particles, Fe-Ni nanoparticles resulted in faster nitrate removal, but generated 17% more ammonium. The nano Fe-Cu integrated system, required two days less than the unmodified nZVI integrated system to remove the entire nitrate and decrease ammonium by 13%, but a large amount of nitrite remained in the system. Compared to uncoated nZVI particles, chitosan-coated nanoparticles allowed the same nitrate removal time but 23% more ammonium production. The sodiumoleate-Fe⁰ nanoparticles decreased the toxicity of the nanoparticles to bacteria. Coating nZVI particles with sodium oleate have better performance in the denitrification since less ammonium and nitrite was accumulated with these particles than uncoated particles [37].

Nitrate removal by Zero Valent Iron nanoparticles (nZVI)

Many studies have been carried out in the denitrification techniques using iron nanoparticles, with or without other metals such as nickel or palladium in water supplies. nZVI has been extensively studied for environmental remediation of a variety of contaminants including halogenated organic substance, heavy metallic ions, arsenate, perchlorate and nitrate [38].

Oxidation of nano zero valent iron produces Fe²⁺ ion and H₂ which not only may be inducing the growth of microorganisms, but also can be used as an electron donor.

The advantages of nZVI include having a larger specific surface area and higher surface reactivity. Recently some studies reported that to accelerate the denitrification rate, nZVI has been used as an electron source for biological nitrate removal. Nitrate was reduced using a nZVI-cell reactor since the H₂ was generated from nZVI corrosion as an electron donor for nitrate reduction [38- 40]. Studies have shown compared with micro-scale ZVI, nano scale zero-valent iron has a higher surface area and faster reaction rate [41]. Hence, in recent years, nZVI has been investigated as an alternative denitrification technique [42]. As an electron donor for nitrate, nZVI could promote microbial removal of nitrate through the reactions indicated in following Eq (s)(6)- (7): [43].



Combination biological denitrification with nanoparticles

Liu et al investigated efficiency of denitrification of aqueous solutions in the presence of nanoscale zero-valent iron and *Paracoccus sp.* strain YF1. In their study, various influencing factors were studied, such as oxygen, pH, temperature, and anaerobic corrosion products (Fe²⁺, Fe³⁺ and Fe₃O₄). The results of their study indicated with slight toxicity to the bacteria, nZVI enhanced denitrification efficiency by providing additional electron sources under aer-

obic conditions. For example, (50 mg) nZVI increased the nitrate removal efficiency from 66.9% to 85.2%. Also their results showed, as for anaerobic corrosion products, compared with Fe^{2+} and Fe^{3+} , Fe_3O_4 increased nitrate removal by serving as an electron donor [10].

Jiang et al., studied effects of nanoscale zero-valent iron (nZVI) on the growth of *Paracoccus sp.* strain and biodenitrification under aerobic conditions, specific factors were evaluated in their study like pH, concentration of nitrate, Fe (II) and carbon dioxide. The results of this study indicated that low concentration of nZVI (50 mg) promoted both cell growth and biodegradation from 69.91% to 76.16% while nitrate removal fell to 67.10% in the presence of high nZVI concentration (1000 mg). This is due to the high concentration of ions produced in nZVI corrosion being used as an electron source for the biodegradation of nitrate.

However, the excess uptake of Fe (II) causes oxidative damage to the cells. To confirm this, nitrate was completely removed after 20 hour when (100 mg) Fe (II) was added to the solution, which is much faster than the control (86.05%, without adding Fe (II)). Also, nitrate removal reached only 45.64% after 20 hour with low cell density in the presence of 300 mg Fe (II) [11].

In other study that occurred with Shin and Cha [38] microbial reduction of nitrate in the presence of nZVI evaluated. Results of their study showed that nitrate was completely reduced after 72 hour using Fe^0 -cell reactor, while only 50% of the nitrate reduced after 168 hour using Fe^0 .

The effects of different factors on nitrate removal efficiency

The effect of pH

pH control can be effective on the efficiency of nitrate reduction. Chen et al., [44] evaluated nitrate removal efficiency at pH ranged from 6 to 9. The results of their study showed the greatest accumulated nitrite level was increased at pH 9. Zhu et al., proved abiotic nitrate reduction by nZVI on optimal pH range of 4- 7. They proved half of nitrate was converted to gaseous nitrogen at pH 4 [45]. Di Capura et al., demonstrated that low pH stimulates a biotic nitrate reduction by increasing proton accessibility [46]. In a study conducted by Westerhoff and James [47] decrease in initial pH from 8.5 to 7.1 enhanced nitrate removal up to 95%. Chung et al., proved complete denitrification occurred after 27 hour at pH 8 [48]. Xia et al., reported that maximum efficiency for autotrophic denitrification was at pH 7 [49].

Since pH influences the enzyme activity of bacteria, it plays a major role in nitrite accumulation in the denitrification reactors. Accumulation of nitrite was detected at alkaline pH, and the nitrite level was increased with pH increased from 8 to 9, the reason for this decline was that high pH value could inhibit the nitrite reductase activity of bacteria [50] and consequently the denitrification reaction was not completed under this conditions.

The effect of temperature

The temperature has a prominent effect on nitrate removal efficiency. In a study occurred with Komori and Sakakibara [51] demonstrated that denitrification process can be

occur in the range 2- 50°C. Vasilidou et al., proved higher temperature enhanced growth and denitrification performance of microorganisms [52]. Another study reported by Kurt et al., [21] showed that denitrification rate in a fluidized-bed reactor were doubled for every 10°C increase in temperature. Rezanian et al., suggested that the denitrification rate increased as temperature increased from 12 to 25°C [50]. Zhou et al., demonstrated that the suitable temperature range was 30- 35°C and by increasing temperature from 25 to 35°C, maximum nitrate removal efficiency observed at 25°C, however in this temperature nitrite accumulation was observed [53]. Chen et al., evaluated the effect of temperature on nitrate reduction in the range 25- 40°C in airtight flasks. The result of their study showed that the nitrate reduction rate increased as temperature increased from 20 to 35°C. A slight decline in nitrate removal rate and nitrite accumulation observed at 40°C [54]. Liu et al., investigated nitrate removal and cell growth rates in free cells and nZVI-amended cells in the range 25- 35°C. The results of their study showed a high temperature at 35°C has a positive effect on bacteria activity and cell growth and finally increased nitrate removal in free cells and nZVI-amended cells [10]. The results of all studies demonstrated that the rate of denitrification continuously increased as the temperature increased and the activity of the microorganisms influenced by temperature. Investigation of above studies showed that maximum nitrate removal efficiency observed at high temperature since all metabolic activity of microorganism and participation of enzymes enhanced in high temperature.

The effect of nitrate concentration

Nitrate concentration plays an important role in denitrification efficiency. In the study occurred with Chen et al., [55] defined that increase of initial nitrate concentration from 30–130 mg/L enhanced hydrogenotrophic efficiency but at higher concentration above (130 mg/L) denitrification performance was inhibited. In other study Changet et al., [56] reported that denitrification performance at high nitrate concentration was not inhibited and the bacteria were able to tolerate high nitrate concentration. Zhou et al., [53] showed that when initial nitrate concentration was (10 mg/L), complete nitrate removal was observed while of higher concentrations above 30 mg/L denitrification was inhibited and nitrite accumulation was observed. More initial nitrate concentration will need more hydraulic retention time (HRT) for complete nitrate removal. Also, high initial nitrate concentration can affect denitrification process by inhibiting the production of N_2 gas from N_2O [57].

The effect of dissolved oxygen

Denitrifying bacteria prefer oxygen over nitrate under aerobic conditions. Under anaerobic conditions denitrifying bacteria use NO as terminal electron acceptor. hence dissolved oxygen (DO) has significant effect on nitrate removal efficiency. High DO has negative effect on denitrification, since in these conditions microorganisms use oxygen as the electron acceptor over nitrate. Also aerobic conditions repress enzymes involved in nitrate removal [58]. Generally denitrification occurs at dissolved oxygen concentration less than 2 mg/L, but Unexpectedly in the study occurred with Hansen et al increase in

dissolved oxygen up to 8 mg/L enhanced nitrate removal, probably due to the formation of ferric iron that could be used as an electron donor [59].

Conclusion

Nitrate contamination has been a major environmental challenge because of adverse effects on environment and human health. Nitrate removal from water resources has been investigated in numerous studies. Among the several techniques described for nitrate removal, biological nitrate removal is a widely applicable method with high efficiency. The evaluation of combined biological denitrification methods and metal nanoparticles in treatment of contaminated waters indicated that this method has potential advantages over the other nitrate removal methods. The results of different studies showed that conventional nitrate removal methods are not adequate due to many reasons that discussed in this paper. Denitrification through use of zero valent metal nanoparticles as a developed the treatment technique that attracted considerable attention of the researchers. Recently, in many studies nZVI used as an electron source in the autotrophic denitrification process where nitrate is reduced to innocuous products like nitrous oxide and nitrogen gas. Furthermore many factors influenced nitrate removal that discussed in review article. Results of studies confirmed that increasing temperature and decreasing initial nitrate concentration and dissolved oxygen concentration at neutral pH was affected the activity of the microorganism and consequently the bio-denitrification increased with or without the presence of metal nanoparticles.

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