Effect of Hydrogen Nutrition Denitrifying Bacteria on Nanoscale Zero-Valent Iron Settlement

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Effect of Hydrogen Nutrition Denitrifying Bacteria on Nanoscale Zero-Valent Iron Settlement

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Abstract. Nanoscale zero-valent iron (nZVI) particles has an extremely small size and can be suspended in groundwater for long periods of time to remove contaminants. However, more and more experimental results show that the migration performance of nZVI particles in groundwater is far lower than expected, and it is difficult to meet the actual needs of in-situ remediation. In order to study the effect of hydrogenotrophic denitrifying bacteria on Nano-iron, the sedimentation test was used as the main research method to discuss the demonstration. The experimental results showed that within the pure water system and in the culture solution system, the sedimentation rate of Nano-iron was decreased after adding bacteria. As the amount of bacteria in the system increases, the sedimentation rate of Nano-iron tends to decrease. When the amount of bacteria reaches a certain amount, it will have a saturating effect on the sedimentation effect of Nano-iron. XRD spectra showed that Fe₃O₄ and Fe₂O₃ were the main iron oxides in the initial oxidation, while Fe₂O₃, Fe₃O₄ and Fe₂O₃ were obtained in the final time of sedimentation. Amorphous δ-FeOOH (corrosion product of zero-valent iron in pure water) replaces Fe₂O₃ and Fe₃O₄ as major corrosion products after the addition of bacterial reaction.

1. Introduction

The need for safe water has forced developing countries to develop a water treatment method that is innovative and economically valuable, including improved Nano-iron drug delivery and in situ remediation of groundwater [1]. Therefore, the huge potential of Nano-iron in the treatment of groundwater pollution has aroused widespread concern. In order to effectively carry out in-situ groundwater remediation, Nano-iron needs to have a low sedimentation rate and agglomeration level, otherwise the migration ability of Nano-iron in the ground will be greatly limited [2].

The ability of Nano-iron to migrate and settle in a groundwater environment depended on many factors [3], such as particle size, pH, ionic strength, soil composition, groundwater flow rate, etc. Agglomeration was a major factor causing a decrease in the migration properties of Nano-iron. Due to magnetic attraction and van der Waals force, Nano-iron was easily agglomerated, and the particle size and surface potential of the agglomerate made it easy to settle in the water environment, thus seriously affecting its migration ability [4]. In addition, the oxidation products formed by anaerobic corrosion of Nano-iron in water will also greatly reduce its migration properties through adsorption, sedimentation [5].

The existing research focused on the effects of hydrological parameters and water quality conditions on the mobility of Nano-iron, but the influence of indigenous microorganisms in groundwater cannot be ignored. It is of great significance to study the influence of microorganisms on the sedimentation performance of Nano-iron and its mechanism in groundwater [6]. Therefore, this study focused on the sedimentation of Nano-iron under the action of hydrogen autotrophic denitrifying
bacteria and attempted to explore the mechanism.

2. Materials and Methods

2.1. Cultivation of Microorganisms

Alcaligenes eutrophus was purchased from China Center of Industrial Culture Collection (Beijing, China). Medium for the bacterial growth was prepared with 52 mg·L$^{-1}$ of ZnCl$_2$, 190 mg·L$^{-1}$ of CoCl$_2$·6H$_2$O, 100 mg·L$^{-1}$ of MnSO$_4$·7H$_2$O, 24 mg·L$^{-1}$ of NiCl$_2$·6H$_2$O, 29 mg·L$^{-1}$ of CuCl$_2$·2H$_2$O, 36 mg·L$^{-1}$ of Na$_2$MoO$_4$·2H$_2$O and 30 mg·L$^{-1}$ of H$_3$BO$_3$, respectively, as described by Shin and Cha\cite{7}.

The culture tank contained 5 L concentrated medium and was seeded with 50 ml cell suspension which contained 20 mg·L$^{-1}$ of A. eutrophus. The culture tank was purged with argon gas for 20 min following inoculation to remove dissolved oxygen, and incubated in an illumination incubator (250-D, Jiangsu) at 30 °C. The gas tank was filled with 5.0 g commercial iron powder (300 meshes) and 50 ml HCl (1M) to produce H$_2$ gas, and then the top cap was screwed in position. According to the consumption of nitrate, the culture solution is updated in time until the absorbance of the bacterial solution reaches 6005 or higher.

2.2. Preparation of NZVI particles

The preparation of NZVI used in this experiment was carried out under a flow of Ar gas. In addition, all solvents were also saturated with Ar gas. Based on previous work\cite{8}, NZVI particles in this study were synthesized by reduction of ferrous iron solution by KBH$_4$ in ethanol–water solution. After the reaction, the iron nanoparticles were repeatedly washed by deionized water and anhydrous ethanol and then dried under the protection of Ar gas. The average particle size of NZVI was approximately 30 nm.

2.3. Batch Experiments

2.3.1 Feasibility test

Dilute 8 mL of the bacterial solution into the colorimetric tube, and introduced 10 ml of Nano-iron suspension (unmodified, average particle size 30 nm) by vacuum line, and dilute to 25 mL with deionized water. After adjusting the pH of the reaction system to 7.0 with HCl, it was sealed and stored, and the whole process was protected with nitrogen. Based on the preparation method of the above-mentioned iron bacteria synergy system, and appropriately adjusted according to the test requirements (7 ml culture solution was added in the batch test containing the culture solution). Four kinds of reaction systems (Table 1) such as "Nano-iron + bacteria + culture solution", "Nano-iron + bacteria + water", "Nano-iron + culture solution", and "Nano-iron + water" were prepared. Three batches of each batch were repeated to reduce the test error and ensure the accuracy of the test.

| Number | NZVI content (mL) | Bacteria (mL) | Culture medium (mL) | H$_2$O (mL) |
|--------|------------------|--------------|---------------------|-------------|
| A1     | 10               | 8            | 15                  | 0           |
| A2     | 10               | 8            | 0                   | 15          |
| A3     | 10               | 0            | 25                  | 0           |
| A4     | 10               | 0            | 0                   | 25          |

2.3.2 Effect of iron bacteria content ratio

In the case of the coexistence of Nano-iron and hydrogen autotrophic bacteria, the experiment in the previous section pointed out those hydrogen autotrophic bacteria will interfere with the sedimentation rate of Nano-iron, and the effect of bacteria on Nano-iron may be multi-faceted. We focused on whether the effect of bacteria on Nano-iron in different proportions of iron bacteria (Table 2) will have different results, so as to observe the sedimentation law of Nano-iron under different bacterial concentrations.
### Table 2. Batch test on the effect of hydrogen autotrophic denitrifying bacteria under different dosages

| Number | NZVI content (mL) | bacteria (mL) | H₂O (mL) |
|--------|-------------------|---------------|----------|
| B1     | 10                | 0             | 15       |
| B2     | 10                | 4             | 11       |
| B3     | 10                | 8             | 7        |
| B4     | 10                | 12            | 2        |

3. Results and Discussion

3.1. Feasibility Test

As shown in Fig. 1, in the pure water system and the culture solution system, the sedimentation rate of Nano-iron decreased after the addition of bacteria. In the pure water system, when sterilized, the concentration of Nano-iron was reduced by 70% within 40 minutes, and after the addition of bacteria, the concentration of Nano-iron was only 60% in the same time; In the culture system, when sterilized, the concentration of Nano-iron decreased by 65% within 40 minutes, and after the addition of bacteria, the concentration of Nano-iron decreased by only 58% in the same time. At the same time, the results of the t-test showed that there was a significant difference between the sedimentation data of the sterile group and the additive group (p<0.05). This indicated that the presence of bacteria can significantly inhibit the sedimentation rate of Nano-iron in water (pure water system or culture system).

![Nano iron concentration curve under different treatments](image)

**Figure 1.** Nano iron concentration curve under different treatments

On the other hand, the survival of bacteria depended on the supply of nutrients. Therefore, we have also conducted corresponding research on the interference effect of the culture solution. The results showed that the presence of the culture solution had a significant effect on the sedimentation of the Nano-iron during sterility, and the presence of the culture solution significantly inhibited the sedimentation of the Nano-iron in 40 minutes. However, after the addition of bacteria, the interference effect of the culture solution was reduced. In the bacteria system, the difference of sedimentation rate of Nano-iron in the pure water system and the culture solution system was reduced from 0.057 min⁻¹ to 0.039 min⁻¹.

In addition, in the 40 minutes of the test of each group of Nano-iron sedimentation rate showed a slow decline trend. However, there is a phenomenon here that is worth noting. In the water group, the sedimentation rate of Nano-iron was greatly different in the first 40 minutes between the pure water system and the culture solution system, but the difference between the two groups were almost completely eliminated by 70 minutes. It showed that the culture solution influence the sedimentation
3.2. Effect of Iron Bacteria Content Ratio

As shown in Fig. 2, as the amount of bacteria in the system increased, the sedimentation rate of Nano-iron tended to decrease. In a pure water system, the concentration of Nano-iron was reduced by 70% within 40 minutes when sterile. In the case of 4 ml of bacteria, the concentration of Nano-iron decreased by 66% in 40 minutes. The results of t-test showed that there was no significant difference between the sedimentation data of the none bactericidal and the 4 mL addition group (p>0.05). This means that when the amount of bacteria in the system was small, the concentration change of Nano-iron with time was not much different from the blank test, that is, it has no effect on the sedimentation rate of Nano-iron. In the presence of a small amount of bacteria, the effect on the settlement of Nano-iron was not significant. The reason may be that Nano-iron has a toxic effect on bacteria to a certain extent. When the amount of bacteria was too small, the activity of bacteria in the system was affected by Nano-iron. In the past decade, some scholars [9-11] have studied the effects of Nano-iron particles on organisms and the environment, and there are some indications of the toxic effects of Nano-iron.

However, after adding 8 mL of bacteria, the concentration of Nano-iron decreased by 58% within the same time. At the same time, the results of the t-test showed that there was a significant difference (p<0.05) between the nanoscale iron deposition data of the sterile group and the 8 mL and 12 mL addition groups. It showed that the presence of bacteria can significantly inhibit the sedimentation rate of the Nano-iron in the water system (pure water system).

Under the condition of 8ml and 12ml of bacteria, the concentration ratio of Nano-iron at the same time tends to be consistent. According to SPSS data analysis, the data of the two groups of batches test results is p>0.05, and the difference between the two groups is not significant. It means that when the amount of bacteria reaches a certain amount, it will have a saturating effect on the sedimentation effect of Nano-iron. Even if the amount of bacteria is increased, the sedimentation rate of Nano-iron cannot be continuously increased.

3.3. Analysis of Agglomeration Behavior of Nano-Iron

In the pure water system, the state of presence of Nano-iron in water at the initial time is a full spherical shape, and the Nano-iron particles are arranged in clusters, and the overall surface morphology is uniform and densely arranged (Figure 3a). Over time, Nano-iron will agglomerate,
which was quite different from the initial morphology of Nano-iron (Fig. 3b). After the addition of bacteria, the Nano-iron dispersed in the pure water in a disperse state. From the TEM image, it can be seen that the Nano-iron is full spherical; the Nano-iron particles have no adhesion clusters, and the sedimentation rate of the Nano-iron changes. Compared with the aseptic state, the degree of oxidation of the surface of the Nano-iron was reduced, which was characterized by relatively less gray-white oxide on the surface of the Nano-iron in the transmission electron micrograph (Fig. 3d), and the degree of agglomeration was lighter.

We used XRD technology to analyze the oxidation products of Nano-iron. The results showed that in the pure water system, when it was aseptic (Fig. 4a), the absorption peak at 36° characterized the existence of Fe0, and the absorption peak at 43.5°, characterized the existence of Fe2O3. The absorption peak at 43° and 62° characterized the presence of Fe3O4. After adding bacteria (Fig. 4b), the absorption peak at 43° and 62° characterized the existence of Fe3O4, the absorption peak at 36°, characterized the existence of Fe0, the absorption peak at 43.5° characterized the existence of Fe2O3 and FeOOH.

Figure 3. Transmission electron micrograph of Nano-iron

Figure 4. XRD characterization of Nano-iron
4. Conclusions

Based on the results obtained, the following conclusions can be drawn:

1. Within the pure water system and in the culture solution system, the sedimentation rate of Nano-iron was decreased after adding bacteria. As the amount of bacteria in the system increases, the sedimentation rate of Nano-iron tends to decrease. At the same time, the results of the t-test showed that there was a significant difference (p<0.05) between the nanoscale iron deposition data of the sterile group and the 8 mL and 12 mL addition groups. It showed that the presence of bacteria can significantly inhibit the sedimentation rate of the Nano-iron in the water system (pure water system).

2. When the amount of bacteria reaches a certain amount, it will have a saturating effect on the sedimentation effect of Nano-iron. Even if the amount of bacteria is increased, the sedimentation rate of Nano-iron cannot be continuously increased.

3. XRD spectra showed that Fe$_3$O$_4$ and Fe$_2$O$_3$ were the main iron oxides in the initial oxidation, while Fe$_2$O$_3$, Fe$_3$O$_4$ and Fe$_2$O$_3$ were obtained in the final time of sedimentation. Amorphous δ-FeOOH (corrosion product of zero-valent iron in pure water) replaces Fe$_2$O$_3$ and Fe$_3$O$_4$ as major corrosion products after the addition of bacterial reaction.

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