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Removal of waterborne phage and NO_3^- in the nZVI/phage/NO_3^- system: competition effect†

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Waterborne pathogenic viruses are a threat to public health. Nanoscale zero-valent iron (nZVI) has increasingly been applied to the removal of viruses. However, current studies are usually based on single component systems, which are not consistent with reclaimed water containing various pollutants in complex mixtures. In this study, a coexisting system containing microorganisms and chemical substances was constructed. Phage f2 and NO_3^- were selected as the model virus and nutrient substance in water to investigate the removal of waterborne phage and a chemical substance in an nZVI/phage/NO_3^- system. The results showed that phage f2 and NO_3^- could coexist without interference in a phage/NO_3^- system, while there was competition between phage f2 and NO_3^- for nZVI when nZVI was added. The removal efficiency of phage f2 decreased with an increase in NO_3^- concentration (0–100 mg L^{-1}). When the initial concentration of virus was 8×10^5 PFU mL^{-1}, the virus removal efficiency was not altered by NO_3^-; however, it was significantly reduced by NO_3^- when the initial concentration of the virus was increased (8×10^6 to 8×10^7 PFU mL^{-1}). In addition, the virus (8×10^6 PFU mL^{-1}) reduced the NO_3^- (20 mg L^{-1}) removal by nZVI (60 mg L^{-1}). With an increase in nZVI dosage, the virus removal efficiency first increased and then decreased irrespective of NO_3^- being present. Nevertheless, the turning point of virus removal efficiency was retard in the presence of NO_3^-. The removal efficiency of NO_3^- increased with an increase in the nZVI dosage (20–120 mg L^{-1}) irrespective of whether the virus was present, but the effect of virus on NO_3^- removal was weakened. Under acidic conditions, phage f2 was superior to NO_3^- in reacting with nZVI, and NO_3^- was superior to phage f2 under alkaline conditions.

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1. Introduction

Reusing wastewater is an effective way to solve the problem of water shortage worldwide.[1] However, the sources of reclaimed water are usually the secondary effluents from municipal wastewater treatment plants, which commonly contain toxic trace organics, heavy metals, and different types of pathogenic microorganisms including bacteria, viruses and parasites.[2] Viruses, with small sizes of approximately 0.01–0.1 μm and strong resistance to traditional water treatment,[3] pose serious health threats. It was reported that approximately 600 000 children all over the world die from rotavirus infection every year.[4] Therefore, it is essential and urgent to remove waterborne pathogenic viruses from reclaimed water.

Chlorine and UV disinfection are the two main technologies applied in water and wastewater disinfection. For chlorine disinfection, the formation of disinfection byproducts, including trihalomethanes, haloacetic acids and nitrosamines, has been a great challenge for more than a century.[5] Another concern with chlorination is that some viruses such as Cryptosporidium and Giardia tend to develop resistance to chlorine. As a result, higher doses of chlorine are needed for complete virus inactivation.[6] UV disinfection has received much attention since no disinfection byproducts are produced.[7] However, UV disinfection has some disadvantages including high energy consumption and high water treatment cost.[8] Moreover, the phenomenon of photoreactivation can sometimes occur.[9]

Nanoscale zero-valent iron (nZVI), with sizes of approximately 1–100 nm, have been used for a wide variety of applications including the removal of groundwater pollutants and the harvesting of oleaginous micro alga.[10–12] Recently, nZVI has increasingly been applied in removing and inactivating viruses, such as f2, MS2 and φX174,[13–15] due to its small size, large specific surface area and high reactivity.[16] In previous studies, virus removal with nZVI under different conditions and the inactivation mechanism were studied.[13,14] However, current studies are usually based on the systems with a single

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component, which are not consistent with real reclaimed water where various pollutants are present as complex mixtures.[17] When nZVI is injected into the reclaimed water for removing viruses, it also interacts with other pollutants.

As a nutrient element, nitrogen is essential for microorganisms. However, an excessive release of nitrogen can cause eutrophication. In addition, the release of nitrogen species is a threat to public health. In particular, nitrate has been identified as a potential health hazard to humans, particularly to pregnant women and infants.[18,19] In recent years, the reduction of nitrate by nZVI was reported in several studies, considering factors influencing nitrate reduction and possible products of nitrate reduction.[20–24]

In this study, NO_3^- was selected as a pollutant, and the pathogenic virus phage f2, which has similar properties to some pathogenic viruses such as Norwalk, poliovirus and hepatitis A virus, was chosen as the model virus. In addition, the effect of NO_3^- on virus removal by nZVI and the effect of the virus on NO_3^- removal by nZVI were studied. We believe that the interaction between nZVI, virus and NO_3^- was important for the removal of virus and NO_3^-, and it is necessary to study the removal of the virus and NO_3^- in an nZVI/virus/NO_3^- system, which is rather limited to date. In addition, effects of nZVI concentration and pH value on the interactions between phage f2 and NO_3^- were also investigated.

2. Materials and methods

2.1 Materials

Chemicals used in the experiments were of reagent grade. All chemicals including agar, nutrient broth, nutrient agar medium, ferrous sulfate, sodium borohydride, sodium hydroxide, hydrochloric acid and sodium nitrate were purchased from the Sinopharm Chemical Reagent Co., Ltd (Beijing, China). All solutions were prepared using ultrapure water before use (Milli-Q, Millipore, US).

2.2 Synthesis and characterization of nZVI

The synthesis of nZVI was conducted in a four-open neck flask (Fig. 1), and nZVI was prepared by a chemical reduction method in aqueous solutions. Argon gas was used to remove oxygen from the flask, and anaerobic conditions were maintained throughout the process. A mechanical stirrer was used to blend the solution and prevent the reunion of nZVI. A lifting table was used to adjust the height of the flask. A 100 mL aliquot of an aqueous solution of 1.00 M $NaBH_4$, in a bottle, was added dropwise to the four-open neck flask with 100 mL of an aqueous solution of 0.20 M $FeSO_4$, and nZVI was obtained through the following chemical reaction [eqn (1)]. The flow rate regulator was used to adjust the dropping speed. The ultrapure water in the flask could be deoxygenated during the reaction process and then used for washing the synthesized particles. The as-prepared particles were washed 3 times with degassed ultrapure water, dried in a vacuum dryer, and then characterized with a scanning electron microscopy (SEM) and X-ray diffraction (XRD). The SEM image and XRD spectrum of nZVI are shown in the ESI (Fig. S1 and S2†).

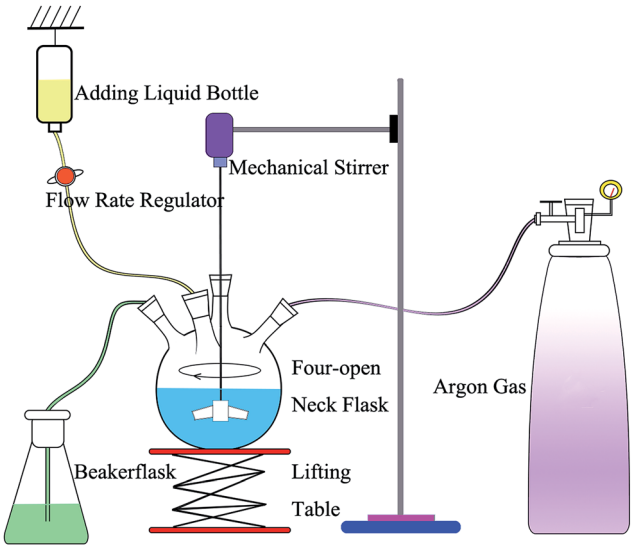

Fig. 1 Diagram of the device for preparing nZVI.

$$2Fe^{2+} + BH_4^- + 3H_2O \rightarrow 2Fe^0 + H_3BO_3 + 3H^+ + 2H_2 \quad (1)$$

2.3 Preparation of phage f2

Phage f2 was prepared using *E. coli* 285 as a host. Phage f2 and *E. coli* 285 were purchased from the Institute of Hygiene and Environmental Medicine, Academy of Military Medical Sciences (Beijing, China). The culture medium of *E. coli* 285 was as follows: 10 g of peptone, 5 g of sodium chloride and 3 g of beef extract in 1 L of ultrapure water.

Phage f2 concentrate was prepared as described with the following procedures. *E. coli* 285 was incubated at 37 °C for 12 h, and a single colony was added into a flask containing 10 mL of liquid medium and incubated at 37 °C for 6–8 h. Then, 1 mL of liquid culture was added into a flask containing 100 mL of liquid medium to prolong the incubation at 37 °C for 6–8 h. After that, 1 mL of phage f2 was added and incubated at 37 °C for 24 h. The mixture was collected, centrifuged (4000 rpm, 10 min) and filtered with a 0.22 μm microporous membrane. The filtrate was the phage f2 concentrate.

2.4 Experimental procedure

Experiments were conducted in a flask with solution volume of 500 mL. A certain amount of nitrate solution and phage f2 were added to the flask containing a certain amount of nZVI. Then, the flask was placed on a shaker with constant temperature (30 °C) with a required rotation rate (120 rpm). The experiments were performed with exposure to air. The initial pH value of reactant solution was adjusted by sodium hydroxide and hydrochloric acid. A certain amount of sample was withdrawn from different test groups at regular intervals. The nitrate solution and phage f2 solution were taken as controls. Each experiment was performed in triplicate.

For the experiments referring to the effects of NO_3^- on the phage f2 removal, the nZVI dosage was 60 mg L^{-1} and the initial

pH value was 7.0. When testing the effects of the NO_3^- concentration, the initial concentration of phage f2 was 8×10^6 PFU mL^{-1}, and the initial concentrations of NO_3^- were 10, 50, 100 mg L^{-1}. When testing the effects of virus concentration, NO_3^- added was 20 mg L^{-1}, and the initial concentrations of phage f2 were 8×10^5, 8×10^6, 8×10^7 PFU mL^{-1}.

For the experiments referring to the effects of phage f2 on the NO_3^- removal, the nZVI dosage, initial pH value, virus concentration, and the NO_3^- added were 60 mg L^{-1}, 7.0, 8×10^6 PFU mL^{-1}, 20 mg L^{-1}, respectively.

For the experiments referring to the effects of nZVI dosage, the initial pH value, virus concentration, and the NO_3^- added were 7.0, 8×10^6 PFU mL^{-1}, 20 mg L^{-1}, respectively. The nZVI dosages were set to be 20, 40, 60, 80, 100 mg L^{-1}.

For the experiments referring to the effects of pH, the nZVI dosage, virus concentration, and the NO_3^- added were 60 mg L^{-1}, 8×10^6 PFU mL^{-1}, 20 mg L^{-1}, respectively. The initial pH values were set to 5.0, 7.0, and 9.0.

2.5 Analytical methods

The concentration of phage f2 was determined by the double layer agar method.[25] The sample was diluted with phosphate buffered saline (PBS), incubated at 37 °C, and then the plaque forming units of each dish were counted. The phage f2 concentration was reported as plaque forming unites per milliliter (PFU mL^{-1}). The concentrations of total nitrogen, NO_3^-–N, NH_4^+–N and NO_2^-–N in the solution were analyzed with spectrophotometric determination method using a UV-Vis spectrophotometer (DR 6000, HACH, US).[24,26,27] The pH value of the solution was measured with a pH meter (STARTER 3100, OHAUS, US).

3. Results and discussion

3.1 Effects of NO_3^- on the phage f2 removal by nZVI

3.1.1 Effects of NO_3^- concentration on the phage f2 removal. First, a series of test experiments were conducted to test the effects of NO_3^- on the virus survival in the phage/NO_3^- system. When NO_3^- concentrations were 0, 10, 50 and 100 mg L^{-1}, the phage f2 concentrations were 6.9, 6.9, 7.0 and 6.9 log after 2 h with NO_3^-, respectively. Apparently, NO_3^- had no impact on the virus survival during the experimental time.

Fig. 2 illustrated the effects of NO_3^- concentration on the phage f2 removal by nZVI. After a 120 min reaction, the removal efficiencies of phage f2 were 6.9, 4.1, 2.6 and 0.9 log by nZVI in the presence of 0, 10, 50 and 100 mg L^{-1} NO_3^-, respectively. Clearly, the virus removal efficiency by nZVI was significantly reduced with the addition of NO_3^-. As more NO_3^- was added, a lower virus removal efficiency was obtained. On one hand, parts of the reactive sites on the surface of nZVI were occupied by NO_3^-. Therefore, the chance for phage f2 to contact with nZVI was decreased. On the other hand, the reactive oxygen species including ·OH, H_2O_2 and $\cdot O_2^-$ would be produced by the reaction between nZVI and oxygen in the nZVI/O_2/H_2O system [eqn (2)–(6)],[14] which should be an important factor for virus inactivation. However, in the presence of NO_3^-, nZVI

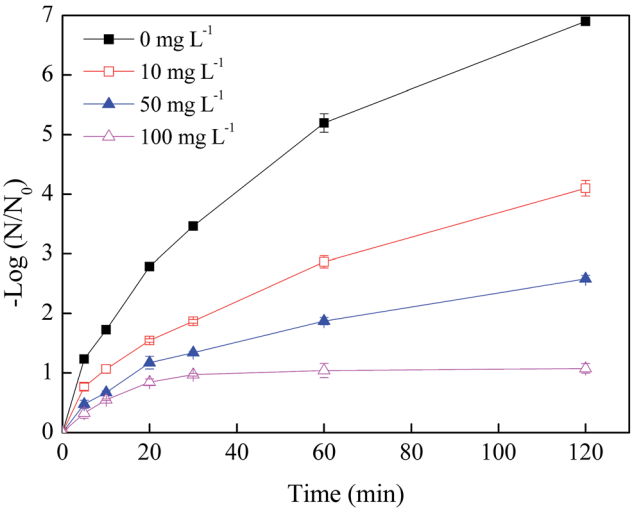

Fig. 2 Effects of NO_3^- concentration on the virus removal by nZVI (nZVI: 60 mg L^{-1}, phage f2 initial concentration: 8×10^6 PFU mL^{-1}, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm).

could be consumed by the reaction between nZVI and NO_3^- directly, since NO_3^- was an electron acceptor and nZVI was a reducing nanomaterial. Then, reactive oxygen species generated by nZVI decreased.[28–30] In addition, nZVI would be rapidly corroded and deactivated.

$$Fe^0 + O_2 + 2H^+ \rightarrow Fe(II) + H_2O_2 \quad (2)$$

$$Fe^0 + H_2O_2 + 2H^+ \rightarrow Fe(II) + 2H_2O \quad (3)$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + \cdot OH + OH^- \quad (4a)$$

$$Fe(II) + H_2O_2 \rightarrow Fe(IV) + H_2O \quad (4b)$$

$$Fe(II) + O_2 \rightarrow Fe(III) + \cdot O_2^- \quad (5)$$

$$Fe(II) + \cdot O_2^- + 2H^+ \rightarrow Fe(III) + H_2O_2 \quad (6)$$

The effects of products of NO_3^- on virus removal were considered. As noted in Section 3.2, NH_4^+ and NO_2^- were the products of NO_3^- reduction by nZVI. Then, the effects of NH_4^+ and NO_2^- on phage f2 were studied. The results showed that the removal efficiencies of phage f2 by NH_4^+ after a 120 min reaction were 0.04 log and 0.05 log when the concentrations of NH_4^+ were 0.5 mg L^{-1} and 1 mg L^{-1}, respectively. Moreover, the removal efficiency of phage f2 by NO_2^- was 0.02 log after a 120 min reaction when the concentration of NO_2^- was 0.02 mg L^{-1}. Clearly, NH_4^+ and NO_2^- had no significant impact on the virus survival under the experimental conditions.

3.1.2 Effects of phage f2 initial concentration on the virus removal. As shown in Fig. 3, when the initial concentration of phage f2 was 8×10^5 PFU mL^{-1}, the removal efficiency of phage f2 in the initial 30 min in the presence of NO_3^- was lower than that of the system without NO_3^-. However, all of the phage f2 was removed within 120 min irrespective of whether NO_3^- was present. As explained in Section 3.1.1, NO_3^- would compete

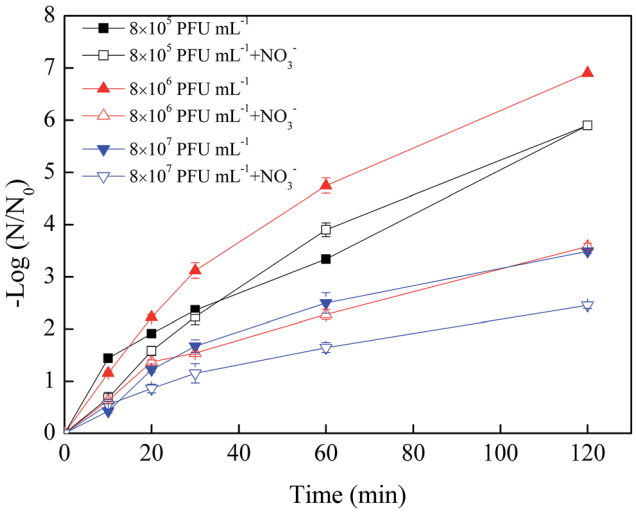

Fig. 3 Effects of virus initial concentration on the virus removal by nZVI with NO_3^- (nZVI: 60 mg L^{-1}, NO_3^-: 20 mg L^{-1}, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm).

with the virus for nZVI. However, when nZVI was sufficient enough, the virus removal would be affected very little. The results indicated that the nZVI in the experiment (60 mg L^{-1}) was relatively sufficient for the virus with lower concentration (8 × 10^5 PFU mL^{-1}) even in the presence of NO_3^- (20 mg L^{-1}). In other words, though NO_3^- took up and consumed some of the nZVI, the virus could be completely removed within 2 h by the remaining nZVI. When the initial concentrations of phage f2 were 8 × 10^6 and 8 × 10^7 PFU mL^{-1}, the removal efficiencies of phage f2 were considerably reduced in the presence of NO_3^-. This indicated that the nZVI might be insufficient for the virus with a higher concentration in the presence of NO_3^-. Particularly, when the initial concentration of phage f2 was 8 × 10^6 PFU mL^{-1}, the virus could be completely removed within 120 min in the absence of NO_3^-, but the removal efficiency was 3.6 log in the presence of NO_3^-. This result revealed the significant competition for nZVI posed by NO_3^-. Part of the nZVI was consumed by the reaction between nZVI and NO_3^-. As a result, the virus removal efficiency decreased.

In the absence of NO_3^-, the removal efficiencies of phage f2 by nZVI were 5.9, 6.9 and 3.5 log after a 120 min reaction when the virus initial concentrations were 8 × 10^5, 8 × 10^6 and 8 × 10^7 PFU mL^{-1}, respectively. Clearly, the nZVI was sufficient for virus removal when the virus initial concentration was lower than 8 × 10^6 PFU mL^{-1}, but it may have been insufficient when the virus initial concentration increased to 8 × 10^7 PFU mL^{-1}. Similar results were obtained in our previous studies.[13] In addition, phage f2 with high concentration would be an aggregate, which protected the inner phage from being inactivated by nZVI.

3.2 Effects of phage f2 on NO_3^- removal by nZVI

In the nZVI/NO_3^- system, NO_3^- can be reduced to NH_4^+, NO_2^- and N_2 by nZVI *via* a series of reactions. The possible reaction pathways are proposed for the NO_3^- reduction by nZVI as eqn (7)–(14).[18,20,31–34]

$$4Fe^0 + NO_3^- + 10H^+ \rightarrow NH_4^+ + 3H_2O + 4Fe^{2+} \quad (7)$$

$$Fe^0 + NO_3^- + 2H^+ \rightarrow Fe^{2+} + NO_2^- + H_2O \quad (8)$$

$$2.82Fe^0 + 0.75Fe^{2+} + NO_3^- + 2.25H_2O \rightarrow 1.19Fe_3O_4 + NH_4^+ + 0.5OH^- \quad (9)$$

$$3Fe^0 + NO_3^- + 3H_2O \rightarrow Fe_3O_4 + NH_4^+ + 2OH^- \quad (10)$$

$$3Fe^0 + NO_2^- + 8H^+ \rightarrow 3Fe^{2+} + NH_4^+ + 2H_2O \quad (11)$$

$$5Fe^0 + 2NO_3^- + 6H_2O \rightarrow 5Fe^{2+} + N_2 + 12OH^- \quad (12)$$

$$3Fe^0 + 2NO_2^- + 8H^+ \rightarrow 3Fe^{2+} + N_2 + 4H_2O \quad (13)$$

$$15Fe^0 + 8NO_3^- + 4H_2O \rightarrow 5Fe_3O_4 + 4N_2 + 8OH^- \quad (14)$$

Fig. 4 illustrates the total nitrogen mass and the evolution processes of three nitrogen species including NO_3^-, NH_4^+ and NO_2^- in the nZVI/phage/NO_3^- system. Along with an increase in reaction time, the total nitrogen in the solution decreased in the absence of phage f2. This indicated that part of NO_3^- was reduced to N_2 by nZVI based on the nitrogen balance [eqn (12)–(14)].[20,32] Compared with that in the system without phage f2, the total nitrogen increased in the presence of the virus. To determine the source of nitrogen, some control experiments were conducted, and the different nitrogen species were determined. The results showed that NO_3^-–N, NH_4^+–N and total nitrogen in the f2 solution was 0.23 mg L^{-1}, 0.10 mg L^{-1} and 0.71 mg L^{-1}, respectively. In addition, NO_2^-–N was not detected in the f2 solution. This confirmed that parts of the nitrogen in the system with virus came from the original f2 solution. In addition, in the nZVI/phage system, the total nitrogen mass increased from 0.71 mg L^{-1} to 0.87 mg L^{-1} after a 120 min reaction. This indicated that the virus could be decomposed after being inactivated by nZVI. Then, the nitrogen in the protein and RNA from the virus were eventually released into the solution, which resulted in an increase in the total nitrogen mass of the solution. Considering the varying process, there was no significant change in the total nitrogen in the nZVI/phage/NO_3^- system over time, though there was a slight fluctuation during the reaction. This indicated that very little N_2 was produced in the system with the virus, which was different from the system without the virus analyzed earlier.

With regards to NO_3^-, the changes in the processes over time were similar in the two systems. In the absence of phage f2, approximately 19.1% NO_3^- was removed by nZVI after a 120 min reaction. In the presence of phage f2, 17.0% NO_3^- was removed after a 120 min reaction. The NO_3^- removed by nZVI was a little less when the virus was present. This indicated that virus might inhibit the NO_3^- reduction by nZVI, but the effect was not so prominent.

Among the products of NO_3^-, NH_4^+ was the main product of NO_3^- reduction by nZVI. This indicated that NO_3^- was reduced by nZVI to NH_4^+ [eqn (7)–(11)].[18] The concentration of NH_4^+–N in the presence of virus (1.10 mg L^{-1}) was higher than that in the system without the virus (0.60 mg L^{-1}). As mentioned above, there was 0.10 mg L^{-1} NH_4^+–N in the original f2 solution.

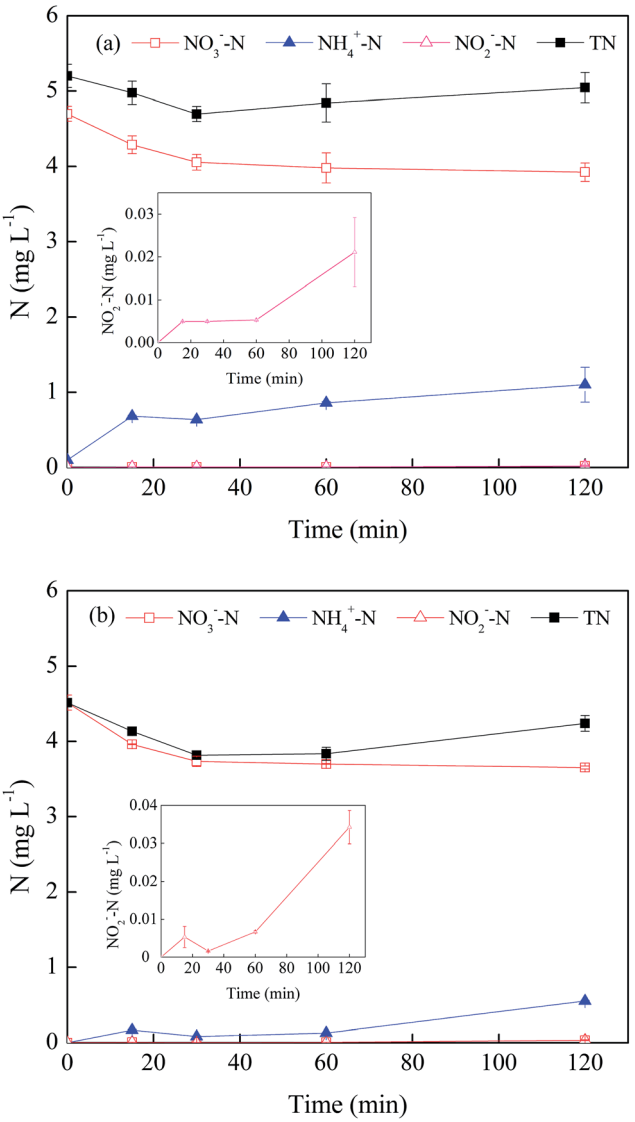

Fig. 4 The evolution processes of nitrogen species in the system: (a) nZVI/phage/NO_3^-; (b) nZVI/NO_3^- (nZVI: 60 mg L^{-1}, phage f2 initial concentration: 8 × 10^6 PFU mL^{-1}, NO_3^- added: 20 mg L^{-1}, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm).

However, the amount could not compensate the difference of NH_4^+–N between the two systems. This meant that there was less NO_3^- removed but more NH_4^+ produced when the virus was present. There were two possibilities to explain these observations. Organic nitrogen and NO_3^- from the f2 solution could be transformed into NH_4^+ through the effect of nZVI. In addition, the nitrogen in the protein and RNA of the virus could be transformed into NH_4^+ *via* a series of reactions. NO_2^-–N was also detected in the product of NO_3^-, but the mass was very low (<0.03 mg L^{-1}) [eqn (8)]. Similar results were obtained in the NO_3^- reduction by nZVI.[18,33] Moreover, there was some fluctuation in NO_2^-–N concentration, and it indicated that NO_2^- was the intermediate product in the NO_3^- reduction process by nZVI.[33] Then, NO_2^- was continuously transformed to other substances such as NH_4^+ [eqn (12)].[34]

In general, in the presence of phage f2, the reduction process of NO_3^- was affected since parts of the nZVI were responsible for phage f2 inactivation. In addition, the pathway for the transformation of NO_3^- to N_2 might be inhibited.

3.3 Effects of nZVI dosage on the interactions

3.3.1 Phage f2 removal by nZVI with different dosages. As shown in Fig. 5, the removal efficiency of phage f2 increased as the nZVI dosage increased when the nZVI dosage was lower. Similar results were obtained in previous studies.[13,14] Along with the increase in nZVI dosage, the number of reactive oxygen species increased. As a result, more phage f2 was inactivated. However, the phage f2 removal efficiency began to drop as the nZVI dosage became higher. The probability for agglomeration increased with an increase in the nZVI dose,[35] and the particle size became larger after agglomeration. Then, the reactivity of nZVI rapidly reduced. As a result, the phage f2 removal efficiency began to drop when the dosage of nZVI was in excess.

In general, the removal efficiency of phage f2 by nZVI in the absence of NO_3^- (2.4–4.8 log) was higher than that of the system with NO_3^- (1.5–2.6 log). Clearly, NO_3^- could inhibit the virus removal by nZVI. The existence of NO_3^- would decrease the chance for the virus to make contact with nZVI and reactive oxygen species generated by nZVI. Moreover, the corrosion and deactivation of nZVI would be accelerated. As a result, the virus removal efficiency decreased. In the absence of NO_3^-, the virus removal efficiency began to drop when the nZVI dosage was greater than 60 mg L^{-1}. However, the virus removal efficiency began to drop when the nZVI dosage was greater than 80 mg L^{-1} in the presence of NO_3^-. Clearly, the required dosage of nZVI to achieve the maximal virus removal efficiency by nZVI was different in the absence of NO_3^- (60 mg L^{-1}) and in the presence of NO_3^- (80 mg L^{-1}). It indicated the competition between phage f2 and NO_3^- for nZVI in the nZVI/phage/NO_3^- system. In the presence of NO_3^-, nZVI could be consumed by NO_3^-, and

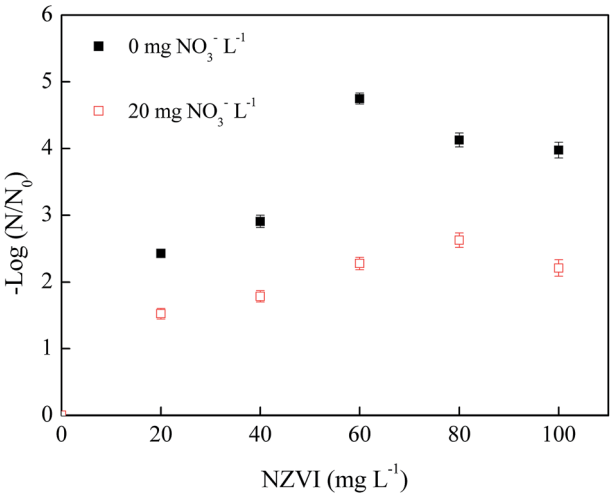

Fig. 5 Effects of nZVI dosage on phage f2 removal with/without NO_3^- (phage f2 initial concentration: 8 × 10^6 PFU mL^{-1}, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm, time: 60 min).

the agglomeration of nZVI would be relieved to some extent. This means that NO_3^- inhibited the virus removal by nZVI, and the required dosage of nZVI to achieve the maximal virus removal efficiency increased.

3.3.2 NO_3^- removal by nZVI with different dosages. Fig. 6 illustrates the three nitrogen species including NO_3^-, NH_4^+ and NO_2^- in the phage/NO_3^- system, nZVI/NO_3^- system and nZVI/phage/NO_3^- system. In the phage/NO_3^- system, NO_2^-–N was not detected in the solution, and there were no changes in the concentrations of NH_4^+–N and NO_3^-–N. Clearly, phage f2 had little impact on the existence of NO_3^-. Moreover, it showed that NO_3^- (0–100 mg L^{-1}) had no impact on the virus survival in Section 3.3.1. This indicated that phage f2 and NO_3^- survived independently in the phage/NO_3^- system under experimental conditions.

In the absence of phage f2, the removal efficiency of NO_3^- by nZVI was increased by 5.5% when the nZVI concentration increased from 20 to 120 mg L^{-1}. NO_3^- could be removed by the direct reduction effect of nZVI. As a result, the NO_3^- removal efficiency was increased when the nZVI dosage increased. Moreover, some products of nZVI including Fe^{2+} and Fe_3O_4 could be produced during the experiment. Moreover, Fe^{2+} and Fe_3O_4 could promote the removal of NO_3^-.[36] Similar results were obtained in the presence of phage f2. Compared with the system without phage f2, the removal efficiencies of NO_3^- by nZVI were decreased by 2.8% and 0.9% in the presence of phage f2 when the nZVI dosages were 20 and 120 mg L^{-1}, respectively. Although the difference was not so obvious between the system with phage f2 and without phage f2, the difference had fallen along with the increase in the nZVI dosage. This meant that the effect of virus on the NO_3^- removal decreased with the increase in nZVI dosage, which indicated a competition between the virus and NO_3^-.

When the nZVI dosages were 20 and 120 mg L^{-1}, the concentrations of NH_4^+–N in solution were 0.3 and 0.6 mg L^{-1} in the absence of phage f2, and were 0.8 and 1.1 mg L^{-1} in the presence of phage f2, respectively. On one hand, the concentration of NH_4^+–N increased with an increase in the nZVI dosage. Similar results were obtained by previous studies.[37,38] On the other hand, the concentration of NH_4^+–N in the presence of the virus was higher than that of the system without the virus. As explained in Section 3.2, part of the extra NH_4^+–N in the system with the virus might come from the f2 solution. Moreover, organic nitrogen from decomposed phage f2 could also transform into NH_4^+–N. In addition, NO_2^-–N was also detected throughout the reduction process, but the mass was very low (<0.02 mg L^{-1}). Similar results were reported in previous studies.[18,33]

3.4 Effect of pH value on the interactions

3.4.1 Phage f2 removal by nZVI at different pH values. First, control experiments with the nitrate solution and phage f2 solution at different pH values were conducted. The initial concentration of phage f2 was 6.4 log. When the initial pH values were 5.0, 7.0 and 9.0, the concentrations of phage f2 were 6.4, 6.4 and 6.4 log after a 120 min reaction in the phage/NO_3^- system, respectively. The concentrations of nitrate were 4.69, 4.71 and 4.70 mg L^{-1} after a 120 min reaction, respectively, which was consistent with those before the reaction. In addition, NO_2^-–N was not detected in the solution, and the concentration of NH_4^+–N had not changed. This showed that phage f2 and NO_3^- did not affect each other in the phage/NO_3^- system at different pH values under the experimental condition.

As shown in Fig. 7, when the initial pH value was 5.0, the removal efficiency of phage f2 was reduced in the initial 60 min in the presence of NO_3^-. However, all of the phage f2 was removed within 120 min whether NO_3^- was present. This meant that the reaction rate was hindered by NO_3^-, but the virus could be completely and finally removed within 120 min. Products of nZVI generated from the reaction between nZVI and NO_3^- could contribute to the virus removal. For example, Fe(II) was more

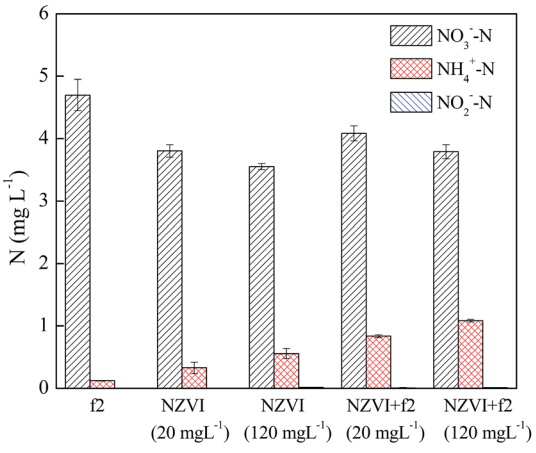

Fig. 6 The three nitrogen species in the phage/NO_3^- system, nZVI/NO_3^- system and nZVI/phage/NO_3^- system with different nZVI dosages (phage f2 initial concentration: 8×10^6 PFU mL^{-1}, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm).

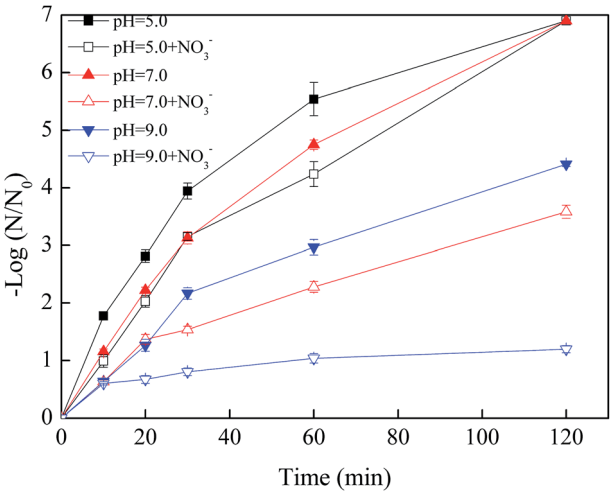

Fig. 7 Effects of pH value on the virus removal by nZVI with/without NO_3^- (nZVI: 60 mg L^{-1}, phage f2 initial concentration: 8×10^6 PFU mL^{-1}, NO_3^- added: 20 mg L^{-1}, *T*: 30 °C, shaking rate: 120 rpm).

stable under acidic conditions. As a result, more viruses were inactivated.[39] In addition, some substances, such as Fe(II), Fe(III), Fe(IV), ·OH and H_2O_2, would be produced when nZVI reacted with oxygen in water.[14] In addition, different radical species dominated the mixture at different pH values. Under acidic conditions, ·OH was expected to be the dominant radical[35] and made a significant contribution to the virus removal due to its high reactivity.

When the pH values were 7.0 and 9.0, the virus removal efficiencies were reduced within 120 min in the presence of NO_3^-, and the reduction increased along with the reaction time (Fig. 7). On one hand, the reaction between NO_3^- and nZVI was the acidic-driven reaction process. Products under a natural or alkaline condition, such as Fe^{2+}, would be less than that of the acidic condition.[40] On the other hand, Fe(IV) and $\cdot O_2^-$ were expected to be the dominant radicals under a natural or alkaline condition, which had less impact on the virus removal due to their low reactivity.[14]

3.4.2 NO_3^- removal by nZVI at different pH values. As discussed in Section 3.4.1, phage f2 had little impact on the existence of NO_3^- in the NO_3^-/phage system at experimental pH values. Fig. 8 illustrates the three nitrogen species including NO_3^-, NH_4^+ and NO_2^- in the phage/NO_3^- system, nZVI/NO_3^- system and nZVI/phage/NO_3^- system at different pH values.

In the absence of phage f2, approximate 25.5%, 19.1% and 10.0% NO_3^- were reduced by nZVI after a 120 min reaction when the pH values were 5.0, 7.0 and 9.0, respectively. Clearly, the NO_3^- removal by nZVI decreased with an increase in the pH value, which was similar to the results of previous studies.[28,41,42] The NO_3^- removal by nZVI proceeded on the nZVI surface. At low pH values, the formation of iron oxides, which would reduce the activity of nZVI, could be retarded.[28] In addition, the passive layer on the nZVI surface could be dissolved under acid conditions, and then the regenerated Fe^0 could effectively reduce NO_3^-.[37] In the presence of phage f2, approximately 10.2%, 16.5% and 10.0% NO_3^- were reduced by nZVI after a 120 min reaction when the pH values were 5.0, 7.0 and 9.0, respectively. What is interesting is that the removal efficiency of NO_3^- under neutral conditions was the highest when phage f2 was present. This is much different from that without phage f2. As mentioned above, a higher solution pH value was not favorable for NO_3^- reduction by nZVI according to eqn (7) and (8). As a result, the removal efficiency of NO_3^- was relatively lower under alkaline conditions irrespective of whether phage f2 was present. However, the removal efficiency of NO_3^- was also relatively lower under acidic conditions when phage f2 was present. As mentioned, an acidic condition was favorable for both NO_3^- reduction and phage f2 inactivation by nZVI. Moreover, the virus removal efficiency was still higher under an acidic condition than that under neutral conditions when NO_3^- was present as stated in Section 3.4.1. Therefore, the results indicated that phage f2 was superior to NO_3^- in reacting with nZVI under acidic conditions.

Compared with the system without phage f2, the removal efficiencies of NO_3^- by nZVI decreased by 15.3%, 2.6% and 0 in the presence of phage f2 when the pH values were 5.0, 7.0 and 9.0, respectively. Clearly, the effect of the virus on NO_3^- removal

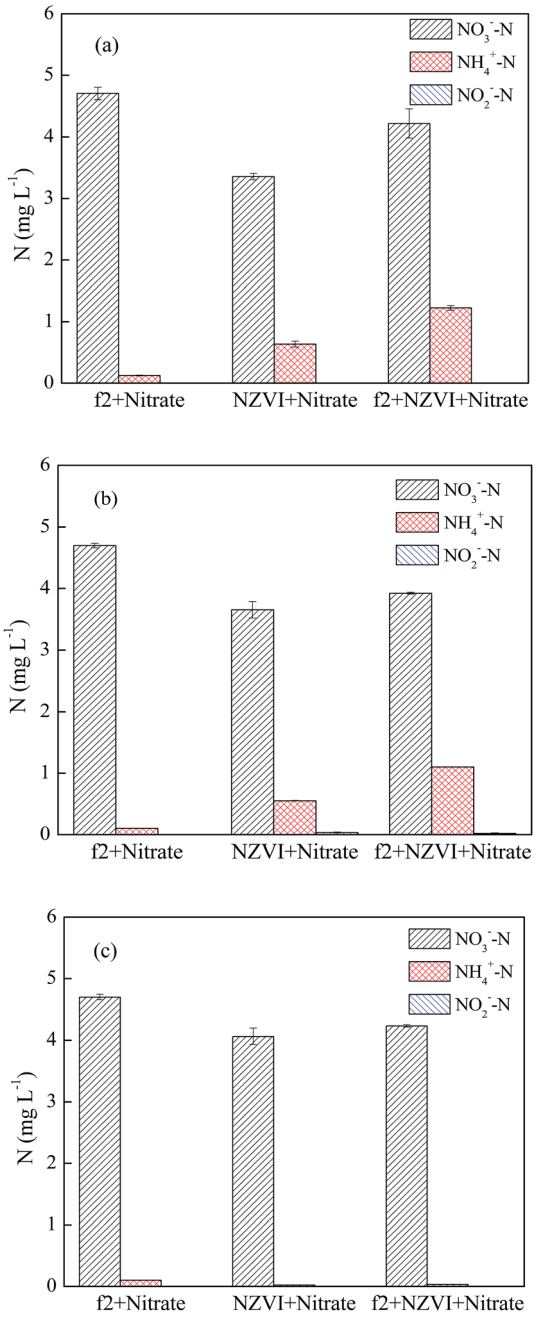

Fig. 8 The three nitrogen species in the phage/NO_3^- system, nZVI/NO_3^- system and nZVI/phage/NO_3^- system at different pH values: (a) pH = 5.0; (b) pH = 7.0; (c) pH = 9.0 (nZVI: 60 mg L^{-1}, phage f2: 8×10^6 PFU mL^{-1}, NO_3^- added: 20 mg L^{-1}, *T*: 30 °C, shaking rate: 120 rpm).

by nZVI rapidly decreased when the pH value increased from 5.0 to 7.0. Particularly, when the pH value was 9.0, the removal efficiency of NO_3^- by nZVI was almost the same irrespective of whether phage f2 was present. As mentioned, the activity of nZVI was relatively lower under alkaline conditions. Hence, the alkaline condition was not favorable for both NO_3^- reduction and phage f2 inactivation by nZVI. However, the removal efficiency of NO_3^- was not affected under alkaline conditions when phage f2 was present, while the virus removal efficiency was greatly reduced when NO_3^- was present. This meant that NO_3^-

was superior to phage f2 in reacting with nZVI under alkaline conditions.

In the absence of phage f2, the concentrations of NH_4^+–N in solution were 0.60, 0.50 and 0.02 mg L^{-1} when the pH values were 5.0, 7.0 and 9.0, respectively. Clearly, the NH_4^+–N concentration decreased with an increase in the pH value. As explained in Section 3.4.1, NO_3^- removal by nZVI was the acidic-driven reaction process. As a result, more NO_3^- was transformed to NH_4^+ at lower pH values. In the presence of virus, the concentrations of NH_4^+–N were 1.2, 1.1 and 0.03 mg L^{-1} when the pH values were 5.0, 7.0 and 9.0, respectively. Clearly, the concentration of NH_4^+–N in the presence of virus was higher than that of the system without the virus. As explained in Section 3.2, organic nitrogen and NO_3^- from the f2 solution, and nitrogen in the protein and RNA of the virus could be transformed into NH_4^+ *via* a series of reactions. The virus removal efficiency by nZVI under acidic conditions was higher than that of an alkaline condition.[13,14] Therefore, the concentration of NH_4^+–N under acidic conditions was higher than that of alkaline conditions. In addition, NO_2^-–N was also detected throughout the reduction process, but the mass was very low (<0.02 mg L^{-1}). Similar results were obtained in the NO_3^- reduction by nZVI.[18,33]

4. Conclusions

In a phage/NO_3^- system, phage f2 and NO_3^- could coexist without interferences, while there was competition between phage f2 and NO_3^- for nZVI when nZVI was added into the system. NO_3^- reduced phage f2 removal, and phage f2 also reduced NO_3^- removal.

The removal efficiency of phage f2 by nZVI decreased with an increase in the NO_3^- concentration (0–100 mg L^{-1}). When the initial concentration of virus was 8×10^5 PFU mL^{-1}, the removal efficiency of phage f2 was not severely altered by NO_3^-, and all of the phage f2 could be removed within 120 min by nZVI (60 mg L^{-1}) in the presence of NO_3^- (20 mg L^{-1}). However, the virus removal efficiency was obviously reduced in the presence of NO_3^- when the virus initial concentration was increased (8×10^6 to 8×10^7 PFU mL^{-1}). Also, virus (8×10^6 PFU mL^{-1}) reduced the NO_3^- (20 mg L^{-1}) reduction by nZVI (60 mg L^{-1}), and the pathway for the transformation of NO_3^- to N_2 might be inhibited. NH_4^+ was the main product, and NO_2^- (<0.03 mg L^{-1}) was the intermediate product of NO_3^- reduction by nZVI.

With an increase in nZVI dosage, the virus removal efficiency was firstly increased and then decreased whether NO_3^- was present. Nevertheless, the turning point of virus removal efficiency was retard in the presence of NO_3^-. In the absence of NO_3^-, the virus removal efficiency began to decrease when the nZVI dosage was greater than 60 mg L^{-1}. However, the virus removal efficiency began to decrease when the nZVI dosage was greater than 80 mg L^{-1} in the presence of NO_3^-. With regards to NO_3^-, the removal efficiency of NO_3^- increased with an increase in the NZVI dosage (20–120 mg L^{-1}) whether the virus was present, but the effect of the virus on the NO_3^- removal by nZVI was weakened.

The virus removal efficiency decreased with an increase in the initial pH value whether NO_3^- was present. Also, the effect of NO_3^- on virus removal by nZVI was much stronger under neutral and alkaline conditions. The removal efficiency of NO_3^- also decreased with an increase in the initial pH value when phage f2 was absent. However, when phage f2 was present, the removal efficiency of NO_3^- under a neutral condition was the highest. When the pH value was 9.0, the removal efficiency of NO_3^- by nZVI was not affected by phage f2. This meant that phage f2 was superior to NO_3^- under acidic conditions and NO_3^- was superior to phage f2 under alkaline conditions in reacting with nZVI.

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