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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 $8 \times 10^5$ 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 ($8 \times 10^6$ to $8 \times 10^7$ PFU $mL^{-1}$). In addition, the virus ($8 \times 10^6$ 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†).

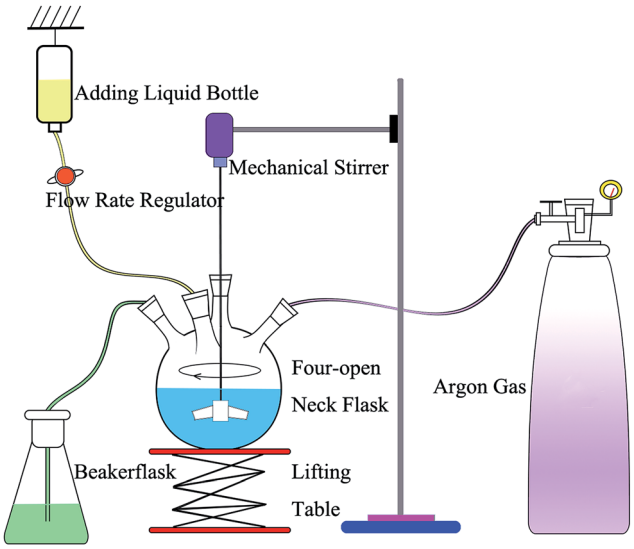


Fig. 1 Diagram of the device for preparing nZVI.

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

### 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 $8 \times 10^6$ 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 $8 \times 10^5$, $8 \times 10^6$, $8 \times 10^7$ 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, $8 \times 10^6$ 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, $8 \times 10^6$ 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 $L^{-1}$, $8 \times 10^6$ 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

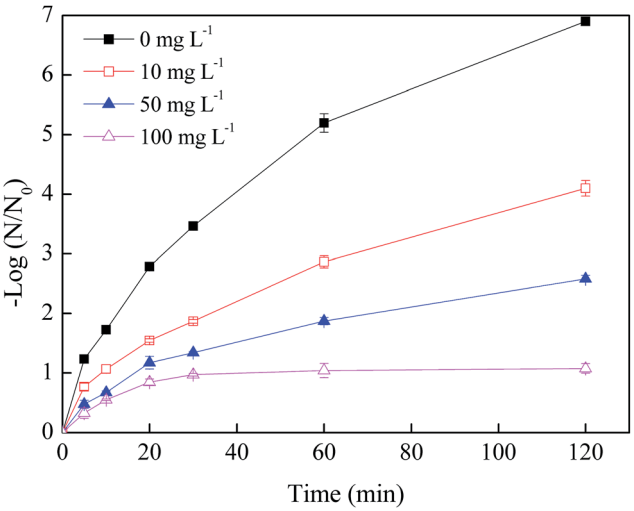


Fig. 2 Effects of $NO_3^-$ concentration on the virus removal by nZVI (nZVI: 60 mg $L^{-1}$, phage f2 initial concentration: $8 \times 10^6$ PFU $mL^{-1}$, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm).

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 $8 \times 10^5$ 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

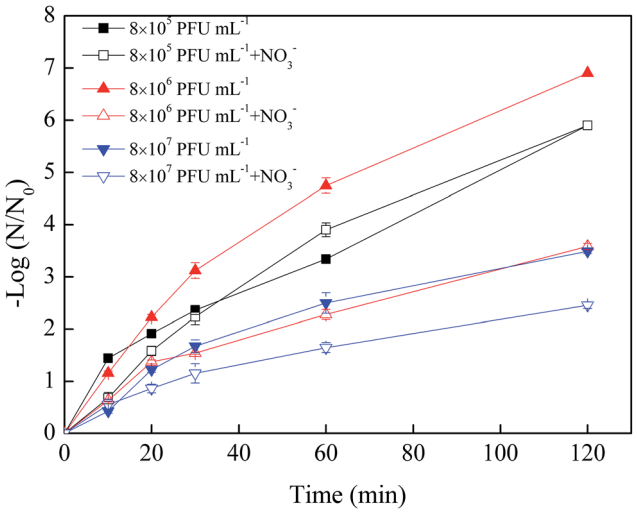


Fig. 3 Effects of virus initial concentration on the virus removal by nZVI with $NO_3^-$ (nZVI: 60 mg $L^{-1}$, $NO_3^-$: 20 mg $L^{-1}$, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm).

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.

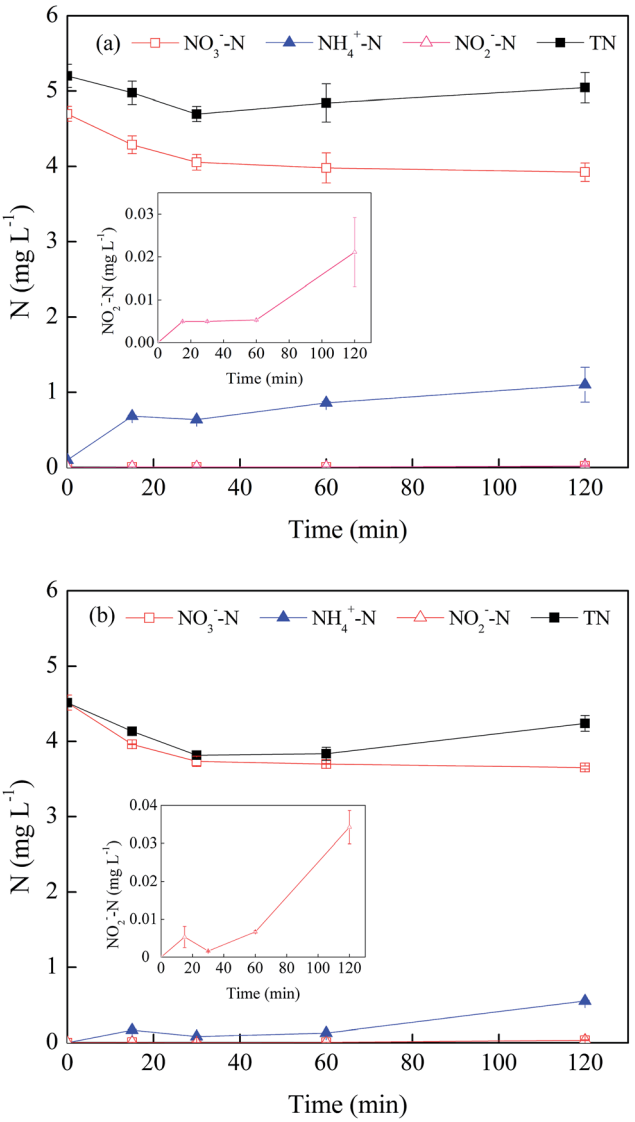


Fig. 4 The evolution processes of nitrogen species in the system: (a) nZVI/phage/$NO_3^-$; (b) nZVI/$NO_3^-$ (nZVI: 60 mg $L^{-1}$, phage f2 initial concentration: 8 × $10^6$ PFU $mL^{-1}$, $NO_3^-$ added: 20 mg $L^{-1}$, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm).

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

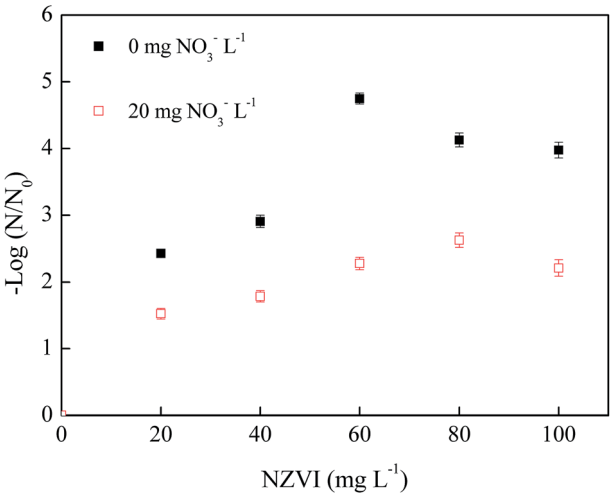


Fig. 5 Effects of nZVI dosage on phage f2 removal with/without $NO_3^-$ (phage f2 initial concentration: 8 × $10^6$ PFU $mL^{-1}$, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm, time: 60 min).

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

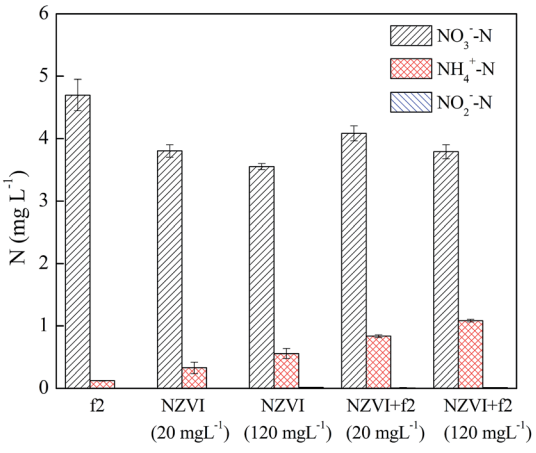


Fig. 6 The three nitrogen species in the phage/$NO_3^-$ system, nZVI/$NO_3^-$ system and nZVI/phage/$NO_3^-$ system with different nZVI dosages (phage f2 initial concentration: $8 \times 10^6$ PFU $mL^{-1}$, pH: 7.0, *T*: 30 °C, shaking rate: 120 rpm).

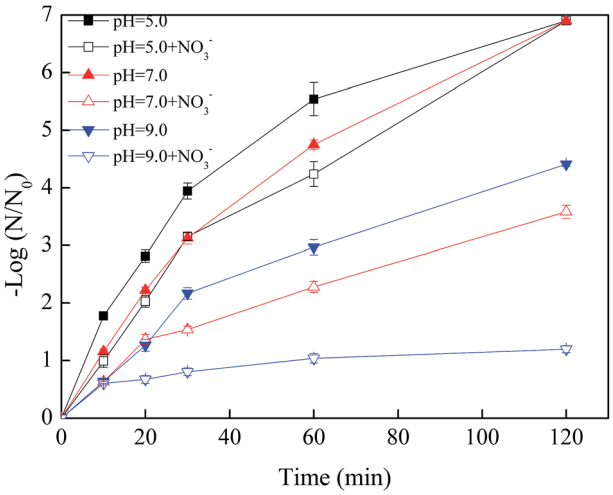


Fig. 7 Effects of pH value on the virus removal by nZVI with/without $NO_3^-$ (nZVI: 60 mg $L^{-1}$, phage f2 initial concentration: $8 \times 10^6$ PFU $mL^{-1}$, $NO_3^-$ added: 20 mg $L^{-1}$, *T*: 30 °C, shaking rate: 120 rpm).

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

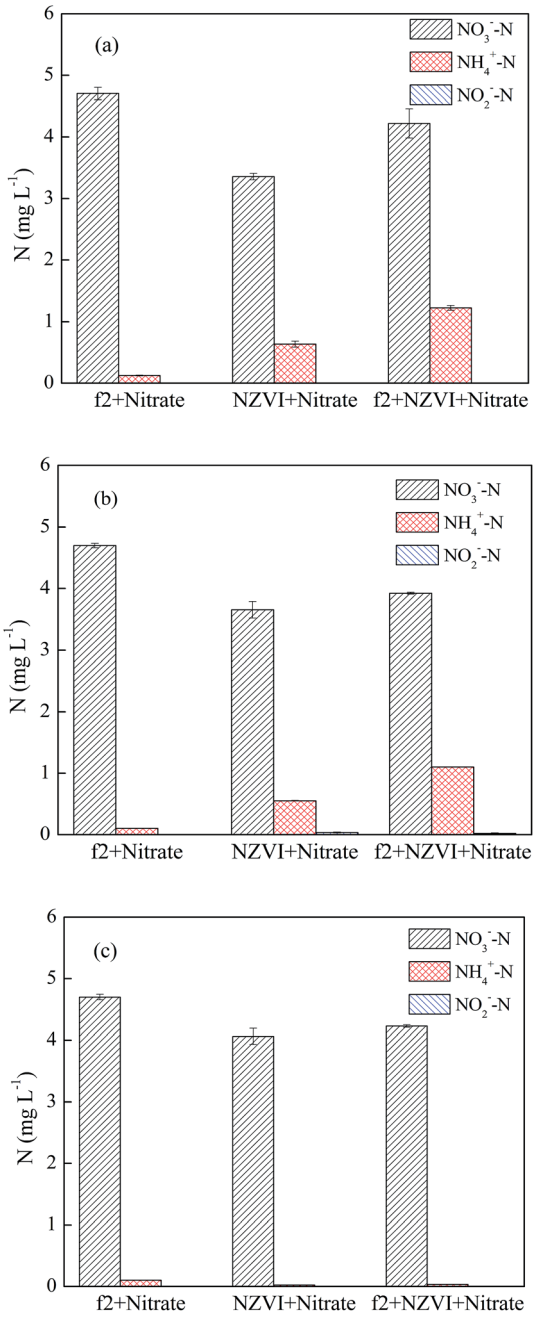


Fig. 8 The three nitrogen species in the phage/$NO_3^-$ system, nZVI/$NO_3^-$ system and nZVI/phage/$NO_3^-$ system at different pH values: (a) pH = 5.0; (b) pH = 7.0; (c) pH = 9.0 (nZVI: 60 mg L$^{-1}$, phage f2: $8 \times 10^6$ PFU mL$^{-1}$, $NO_3^-$ added: 20 mg L$^{-1}$, *T*: 30 °C, shaking rate: 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 $8 \times 10^5$ 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 ($8 \times 10^6$ to $8 \times 10^7$ PFU $mL^{-1}$). Also, virus ($8 \times 10^6$ 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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