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Review

The interactions between nanoscale zero-valent iron and microbes in the subsurface environment: A review

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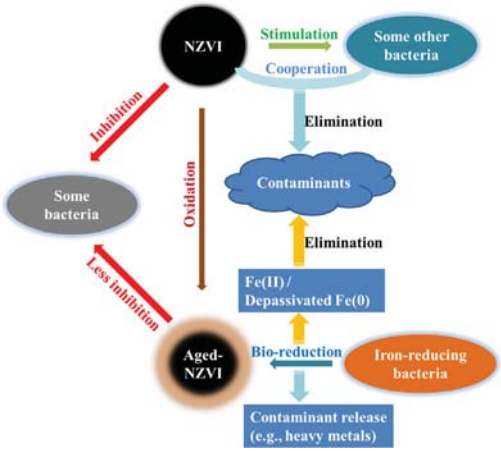
HIGHLIGHTS

- The interactions between various microbes and NZVI were summarized.
- The adverse and positive effects of NZVI on the growth of microbes were reviewed.
- The synergistic effects of NZVI and bacteria on pollutant removal were reviewed.
- The effects of iron-reducing bacteria on the aged NZVI were reviewed.
- Future challenges to study the interactions between NZVI and microbes are suggested.

GRAPHICAL ABSTRACT



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ABSTRACT

Nanoscale zero-valent iron (NZVI) particles, applied for in-situ subsurface remediation, are inevitable to interact with various microbes in the remediation sites directly or indirectly. This review summarizes their interactions, including the effects of NZVI on microbial activity and growth, the synergistic effect of NZVI and microbes on the contaminant removal, and the effects of microbes on the aging of NZVI. NZVI could exert either inhibitive or stimulative effects on the growth of microbes. The mechanisms of NZVI cytotoxicity (i.e., the inhibitive effect) include physical damage and biochemical destruction. The stimulative effects of NZVI on certain bacteria are associated with the creation of appropriate living environment, either through providing electron donor (e.g., H_2) or carbon sources (e.g., the engineered organic surface modifiers), or through eliminating the noxious substances that can cause bactericidal consequence. As a result of the positive

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interaction, the combination of NZVI and some microbes shows synergistic effect on contaminant removal. Additionally, the aged NZVI can be utilized by some iron-reducing bacteria, resulting in the transformation of Fe(III) to Fe(II), which can further contribute to the contaminant reduction. However, the Fe(III)-reduction process can probably induce environmental risks, such as environmental methylation and remobilization of the previously entrapped heavy metals.

Contents

1. Introduction

Over the last two decades, nanoscale zero-valent iron (NZVI) has been increasingly used to tackle polluted waters, soils and sediments [1–6]. Given the large specific surface area and high reactivity, NZVI particles could be directly injected into the pollution zone for the in-situ remediation, which was recognized as a highly effective method to remove/degrade a wide range of pollutants [7–11]. However, similar to other engineered nanomaterials (e.g., nano-silver, nano-titanium oxide, carbon nanotubes), the engineered NZVI confronted skepticism from those who doubt about its net benefits according to its uncertainties and potential risks to human health and ecological security [12].

Since anthropogenic perturbation and/or environmental restriction could trigger a response of the microbial community, numerous studies have demonstrated that NZVI could have potential harmful impacts on many microbes, especially for the gram-negative bacteria [13–15]. Interestingly, some studies reported that NZVI particles appear to exert selective pressure on the microbial community, inhibiting some microbial groups but promoting the dominance of others [16–18]. Moreover, the effect of NZVI on the microbes could be influenced by the surface chemistry of NZVI. For instance, the engineered surface modifiers (e.g., carboxymethyl cellulose), employed to enhance the stability and dispersion of NZVI, were found to be able to diminish NZVI toxicity to the susceptible bacteria, and even provide the available nutrient substances (i.e., carbon source) for the microbial community [15,19–21].

In contrast to the studies focusing on the toxic effects of NZVI on the microbes, some other studies reported the synergistic effects of NZVI and microbes on the contaminant removal [4,20,22,23]. Even though NZVI can function well in in-situ remediation, most contaminated sites still depend on microbes as polishing agents for the concurrent or terminal bioremediation process. It is well known that microbial communities can carry out crucial functions for the degradation and immobilization of organic compounds and inorganic salts in soil, water and sediments [16,24,25]. Nevertheless, the bioremediation process may entail a relatively long time and is unable to degrade some refractory contaminants. Therefore, some studies have combined NZVI with various bacteria to remediate contamination, by taking the advantages of each other [4,20,22,23]. Taking the dissimilatory iron reducing bacteria (DIRB) as an example, they can play an important role in the biogeochemical cycling of iron in the environment, which can reconvert oxidized Fe(III) to Fe(II) by their respiration [26–28]. Accordingly, employing DIRB in the NZVI remediation process may promote the abiotic remediation of pollutant in a distinctive way [29]. However, the interaction between DIRB and the aged NZVI may also cause the remobilization of the previously immobilized contaminants in the aged NZVI, posing potential environment risk [30–32].

In the different stages of its life cycle from fresh Fe^0 to the aged nano-colloid, NZVI applied to the environment could inevitably interact with various microbes. As illustrated above, the applied NZVI could possibly exert either positive or negative impacts on the indigenous microbes of the treated sites, and the microbes could also possibly influence the fate of the applied NZVI. It is thus essential to comprehensively summarize the interactions between NZVI and the microbes in the subsurface environment, as this is helpful to gain a fundamental understanding of the microbial fate of NZVI and ensure the effective remediation and safe application of NZVI. Although some review papers on the relationships between NZVI and the microbes have been published in recent years, most of them mainly focused on the NZVI toxicity to bacteria, because of the broad concern on the potential ecological risks that NZVI

might create when released into the natural environment. Very few review papers concentrated on the positive interrelationship between NZVI and the microbes, and none of them extensively summarize the interactions between various microbes and NZVI in different stages of life cycle. Therefore, the objectives of this study are to (1) summarize the effects of NZVI on the microbial activity and growth, (2) summarize the interactions between NZVI and microbes in the pollutant removal process, (3) summarize the influences of microbes on the behavior of the aged NZVI particles, and (4) propose the future research requirements to tackle the pertinent engineering challenges.

2. Effects of NZVI on the microbial activity and growth

2.1. Toxic effect of NZVI

NZVI particles have been prevalently used to remediate the pollution zone contaminated by inorganic salt (e.g., NO_3^-), chlorinated organic pollutants (e.g., TCE) and heavy metals (e.g., Cr(VI)) [7–9]. Then, there are a large quantity of NZVI-related nanoparticles (NPs) intentionally being released to soil and aquatic environments, which has raised concerns of environmental safety and human health. These engineered iron NPs probably enter subsurface biosphere and increase subsurface organisms' exposure to iron NPs. It is amazing that a remarkably high proportion of microbes can be essential contributors to the remediation process, such as the remediation of halogenated contaminants [33], nitrate [34], U(VI) [35] etc. However, NZVI may alter soil bacterial community structure, adversely affect microbial populations in the subsurface and insistently accumulate in the ecosystem [36].

2.1.1. Adverse effects of NZVI on the microbial growth

Previous studies have demonstrated that NZVI could easily attach to the bacterial cell surface due to its particular physicochemical property [12,15,37,38], no matter by Brownian movement, electrostatic attraction, coulombic attraction, hydrogen bonds or even dipole–dipole interactions [39,40]. During their contact, some interactions could occur between NZVI and the bacteria, which may be physical, chemical, biological reaction or any cooperation of them. It has been reported that NZVI particles exert some negative influences on cells, including inhibition and toxicity [12,14,41]. Specific patterns of action for the cytotoxicity of NZVI have been postulated, including damage to the cell membrane integrity [42], reductive decomposition of protein functional groups in the cell membrane as a result of the strong reducing conditions at the interface of cell-NZVI system [43], interference with respiration [44], and oxidative damage of DNA or enzymatic proteins from intracellular reactive oxygen species (ROS) produced from Fenton's chemistry [45]. In addition, toxicokinetics may differ among various kinds of microbes, the intact organism or the diverse life stages of the tested organism [46].

For the gram-positive/-negative bacteria and the fungus, all of them present so distinctive cell structures and characters that their sensitivities to NZVI are also diverse appreciably [37,47–49]. Under the identical test conditions, gram-positive bacteria such as *Bacillus* spp. and *Streptomyces* spp. show a greater resistance to NZVI compared to gram-negative bacteria like *Escherichia coli* (*E. coli*), *Pseudomonas fluorescens* etc. [13,14]. The reason why gram-positive bacteria are less sensitive to NZVI may be due to the presence of the thick (20–80 nm) peptidoglycan layer in the cell walls [37]. Definitely, peptidoglycans that contain mucopeptides, glycopeptides and mureins are the structural elements of almost all bacterial cell walls. Their domination in the cell wall of some gram-positive bacteria is substantial, but seems to be less in gram-negative bacteria. It was speculated that this discrepancy in membrane structure may result in their different absorption ability onto the NZVI-related NPs [48]. Moreover, fungal cell walls are thicker than either type of bacterial cell walls, which comprise chitin instead of peptidoglycan [50]. This may explain the phenomenon that fungal cells are more tolerant to the NPs than the positive bacteria. Hence, it was demonstrated that there is an association between cell wall architectures and sensitivity to NZVI, which indicates that either membrane disruption or differential membrane permeability play a part in cytotoxicity of NZVI.

As for the intact organisms, some previous studies have investigated the toxicity of NZVI with various concentrations to several different species [3]. For example, the high doses of NZVI could cause acute toxic effects on collembola and ostracods [3]. The common field doses of NZVI ($\geq$500 mg kg^{-1} soil) has acute adverse impacts on the earthworms [51]. Also aquatic organism like medaka (especially for the larvae) are significantly affected by NZVI under environmentally relevant exposure [52].

There is no lack of studies that elucidating the diverse NZVI toxicity at the different life stages of organisms [41,46,52]. The exposure of *Mytilus galloprovincialis* sperm to NZVI has caused negative effects on sperm itself and on its subsequent vitro fertilization [41]. Severe disruptions of the development consist of 30% mortality among sperm cells followed by 20% decline in fertilization success and development. In addition, the medaka at the embryonic stage is relatively vulnerable in its development and also especially sensitive to NZVI toxicity [46,52]. Interestingly, the histopathological damage in medaka larvae gills with NZVI appears inapparent in contrast with medaka at mature stage [53]. It was speculated that at the early life stage, larval gills are still rudimentary in development with small unbranched knobs, so the histopathological changes of gill structures could not be exactly illustrated by the staining techniques[54].

As mentioned above, NZVI dispersed into aqueous phase and soil can attach to relevant organisms inevitably. Then either NZVI permeating into cell or organisms englobing the NPs by themselves can trigger a series of toxic effects on the organisms. It can be surmised that the NZVI-related NPs carriers could endanger other organisms and even human through food chains.

2.1.2. Mechanisms of NZVI toxicity to the microbes

It is well known that there are multiple kinds of nanomaterials which have been demonstrated to display antimicrobial properties, such as silver [55], copper [56], gold [57], fullerenes [58], carbon nanotubes [59], titanium oxide [60], magnesium oxide [61], cerium oxide [62] and NZVI. Given a recent summary of the biophysicochemical interactions in the NPs-cell system, the mechanisms for toxicity may include two aspects: one is physical damage: disruption of the cell membrane architectures, enhancement of membrane permeability [15,42]; the other is biochemical destruction: interference in energy transduction and exchange [44], gene and protein damage [63]. The aforementioned modes of the action of NZVI cytotoxicity are in line with the mechanisms illustrated herein. However, the microbial cells have several countermeasures to resist those adverse influences, and the fate of cells is decided by the final confrontation between the NZVI damage and bacterial defense, which is illustrated in Fig. 1.

Plenty of in vitro studies have shown that the soluble portions of NZVI suspension aroused the equivalent toxic effects to the particles, which indicates that the consequence is caused by the metals released into solution rather than the particles themselves [12,38]. In other words, the cytotoxicity exposed to NZVI is comparable to the response observed when cells are exposed to the same concentration of dissolved Fe(II). Some researchers even reported that Fe(II) was more toxic to ostracods (Heterocypris incongruens) than NZVI in soil [3]. Although iron is an essential element for almost all organisms, the excess uptake of Fe(II) could cause oxidative dam-

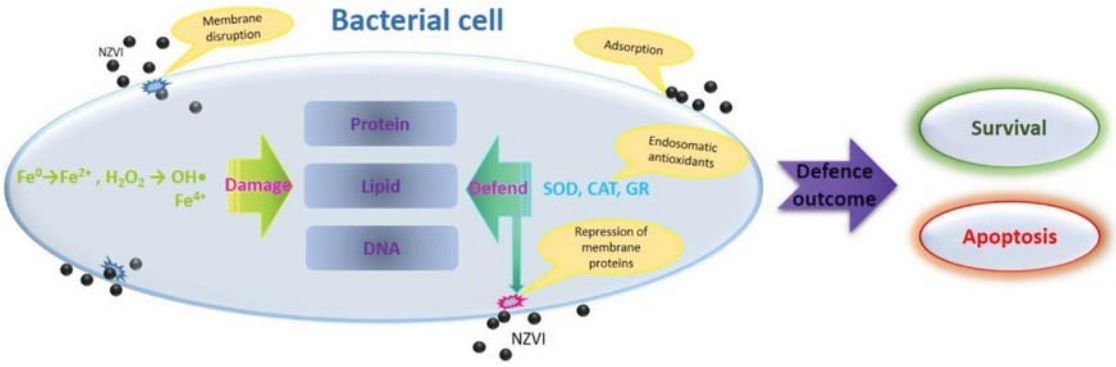

Fig. 1. Illustration of the confrontation between the NZVI damage and bacterial defense.

ages in the body via redox cycling of iron and the internal ROS generation, thus leading to cell death and acute toxic effects.

For appraisal of the toxicological effect of NZVI on microorganisms, it should be noted that before the acute toxic effects occur at the cellular level (typically growth and survival), the initial changes have arisen at the molecular level [64]. It is believed that NZVI could induce oxidative stress via redox cycling of iron and the ROS generation, resulting in lipid peroxidation and DNA damage for human bronchial epithelial cells, *E. coli* etc [12,65]. The greater inactivation of *E. coli* mutants which lacked superoxide dismutase (SOD) proved that ROS might play an important role in NZVI toxicity from another perspective [45]. The generation of ROS such as hydroxyl radicals (•OH) can stem from the interplay of oxygen with the reducing iron species (Fe^0 and/or Fe^{2+}) or from the interruption of the electronic and/or ionic transport chains due to the great affinity of the cell membrane for the nanoparticles [45]. Commonly, oxygenation of the reducing Fe species (Fe^{2+} and/or Fe^0) is the main pathway for ROS fabrication (Eqs. (1)–(3)) [15]. Although the hydroxyl radical is often reported to be the main product of the Fenton reaction, an alternative oxidant (e.g., ferryl ion) can be formed under some conditions [66]. For instance, Fe(IV) appeared to be the primary oxidant produced by NZVI in the absence of ligands at circumneutral pH values [67]. or just by Fe(II) in phosphate-buffered solutions [68].

$$Fe^0 + O_2 + 2H^+ \rightarrow Fe^{2+} + H_2O_2 \quad (1)$$

$$Fe^0 + H_2O_2 \rightarrow Fe^{2+} + 2OH^- \quad (2)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\bullet} + OH^- \quad (3)$$

Notably, under the conditions of the bioassay, Fe(II) was oxidized rapidly (Eq. 3) and it was improbable that significant quantities of Fe(II) were taken up by the cells in short order [12]. Therefore, the internal ROS production is most probably triggered by the external oxidative stress that sets off redox-response signaling pathways in cells, including the expression of genes which play a role in responses to inflammation and cause intracellular ROS production [69]. With the internal ROS levels increasing, the activity of endosomatic antioxidants such as SOD (converting internal O_2^- to H_2O_2), CAT (a cellular H_2O_2 scavenger) and GR (a ROS scavenger) alter in varying levels [46]. Whereas the concentration levels of NZVI will decide whether the cells undergo oxidative stress or oxidative damage, NZVI at low concentrations can initiate the homeostasis mechanism by antioxidant enzymes to eliminate the iron-induced ROS inside cells. Conversely, superfluous internal ROS deactivates the balance of the antioxidant defense system. Then, the accumulated ROS can further interact with macrobiomolecules (e.g., lipids, proteins or DNA), thus resulting in irreversible sublethal or lethal toxic effects [46,52,69].

With regard to maintaining cellular homeostasis against NZVI, the proteomic approach is adopted to provide new insights. Changes in the protein expression signal a molecular response caused by cells to counteract the impact of NZVI, which includes two principal defense mechanisms: the repression of membrane proteins to restrain iron uptake and the overproduction of proteins to scavenge ROS, thereby diminishing intracellular oxidative stress and inhibiting abnormally folded proteins (chaperonins, heat shock proteins) [14]. For example, *Klebsiella oxytoca* could establish an adaptive stress response involving tryptophanase and indole, which might be regarded as signal molecules. And the overexpression of tryptophanase enabled to minimize and even counteract the adverse impact of NZVI [64]. However, for *Bacillus cereus*, a ubiquitous soil gram-positive bacterium, a distinctive defense strategy has been employed [13]. Once *B. cereus* encounter with NZVI, the cell would rapidly respond by entering in an early sporulation stage so that it could survive under the stressful conditions and adjust the expression ratio of specific proteins.

Except for the protection and defense performed by organisms themselves, the natural water and soil may also possess the ability to attenuate NZVI toxicity when NZVI particles are applied to these sites. It is well known that both the natural water and soil environments are complicated, containing various mineral substance, ions, natural organic matter (NOM) and so on. There are so many factors that can influence the toxic effects of NZVI on the organisms [15,21,63]. For example, the metallic ions (e.g. Ca^{2+}) at proper concentration could weaken the toxicity of NZVI particles to bacteria, which might be attributed to the charge neutralization on the surface of NZVI and then an enhancement of NZVI aggregation. Besides, NZVI particles added to soil perhaps have an impact on both the compositional structure and functional capacity of the soil microbial community, but clay and organic matter may attenuate such effects [70]. In the subsurface environment where soil organic matter is high, the bactericidal effects of NZVI are usually diminished.

2.1.3. Effects of surface modification and aging on the NZVI toxicity to the microbes

It has been reported that bare NZVI particles aggregated rapidly in the aqueous phase, making them difficult to migrate in the subsurface [71,72]. For the purpose of decreasing aggregation and enhancing the mobility of NZVI, surface stabilizers (e.g., surfactant, macromolecule organic matters, polyelectrolyte) can be typically coated onto the surface of the particles to afford electrostatic repulsion, and steric or even electrosteric stabilization [15,47,73–75], as illustrated in Fig. 2. Electrosteric repulsion, a combination of the electrostatic and steric repulsion provided by large molecular weight polyelectrolytes [19], is most effective in NZVI stabilization, and its repulsive force is less sensitive to the alteration in pH or ionic

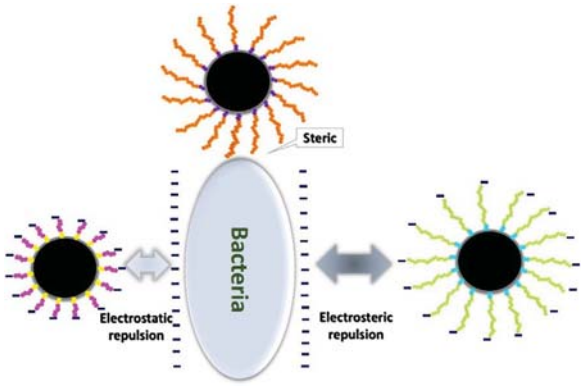

Fig. 2. The inhibited physical contact of bacteria with NZVI modified with different surface stabilizers.

strength than electrostatic repulsions. Electrosteric repulsions provided by the negatively charged polymer coatings or adsorbed NOM may significantly decrease physical interaction between NZVI and the bacteria cell wall and therefore its toxicity [15,63].

Carboxymethyl cellulose (CMC), one type of representative polyelectrolyte, is usually used as a NZVI stabilizer and found to be able to diminish the disruption of cell membrane integrity and minimize oxidative stress response, resulting in mitigated cytotoxicity towards bacteria (e.g., *E. coli*, *Agrobacterium* sp. PH-08) compared with the uncoated counterpart [15,21]. Interestingly, transmission electron microscope (TEM) shows a discernible enhancement in physical contact between CMC-NZVI (CNZVI) and cells rather than attenuation, which may be owing to the better dispersion and distribution of CNZVI in bacterial culture, but the enormous CNZVI on the cell surface seems not to destroy membrane integrity severely relative to the uncoated form. Even the CNZVI-treated cells somewhat preserve intact structure and exert a minor antibacterial activity. In addition, some people surmised CMC could act as one of the highly reactive and unselective species, and the hydroxyl radical caused by the released Fe^{2+} might well attack on CMC. The most likely explanation was that CMC served as a radical scavenger by several studies ($^{\bullet}OH + CMC \rightarrow H_2O + CMC^{\bullet}$) [21]. Therefore, CMC can compete with bacteria for oxidants, decreasing hydroxyl radical generation and then reducing the NZVI toxicity to the bacteria.

Similarly, NOM adsorbed to the particles could decrease NZVI toxicity comparably to the polyelectrolytes tested under the same conditions. It is supposed that NOM is probably served as a polyelectrolyte to provide electrosteric repulsions between NZVI and the cells [63]. Although the surface modification increases NZVI's dispersion and decreases its toxicity, the degradation rate of halogenated organic compounds (e.g., TCE) also diminishes in a certain extent in the presence of coatings [47]. This is probably due to occlusion or occupation of reactive sites on the surface of NZVI [76]. Hence, it is proposed that the presence of surface coatings offers a tradeoff for NZVI-based remediation, with higher potential for concurrent or sequential bioremediation at the cost of partially inhibiting abiotic reactivity with the target contaminant.

The surface modification of NZVI by organic stabilizers is considered as an efficient approach to diminish NZVI toxicity to microbe, however, the modification of NZVI by noble metals (the so-called bimetallic NPs) is generally believed to increase the toxicity of NZVI. In general, a partial coverage of NZVI by metals like Ni and Ag can increase NZVI reactivity in various applications, such as removal of stubborn contamination [77,78]. As known, the loaded metals are often used as catalysis for Fe^0 reaction. Although the Fe/Ni bimetallic nanoparticles enhance particle stability in the air, these NPs may have a more inhibitory effect on the cells than bare NZVI. It is believed that Ni may be more toxic to free cells and cause cell growth to decrease. As to Fe–Ag NPs, each of them is renowned for its antimicrobial activity at least to some extent. In addition to cost-effective activity for contamination removal, Fe–Ag NPs have presented antimicrobial action against a wide variety of bacteria and yeasts [55].

The adverse effects of NZVI on tested organisms appeared to be temporary and weakened after oxidation [3] and the increase of incubation time or reaction period also alleviated NZVI toxicity. NZVI was less toxic to bacteria (e.g., *E. coli*) exposed under aerobic conditions compared to exposure under anaerobic conditions [43,63]. In general, NZVI exposed to environmental conditions would be oxidized under either oxygen-rich or deficient conditions [63,79]. Oxidation of NZVI in groundwater could generate different iron oxide layers, such as magnetite, hematite, and lepidocrocite [79]. Then it can be presumed that partially oxidized (aged) NZVI particles are less toxic to bacteria than the fresh NZVI particles.

From the foregoing, NZVI can readily produce Fe^{2+} in aqueous phase and then lead to large amount of ROS generation (Eqs. (1)–(3)). Moreover, cytotoxicity and internal ROS production in cells exposed to dissolved Fe(II) is equivalent to the response observed when cells are exposed to the same concentration of NZVI [12]. The iron precipitates from oxidation of NZVI may deposit on the reactive surface of NZVI and diminish its biocidal activity [43]. As the end products of this oxidation process, Fe_3O_4 and γ-FeOOH respectively accounts for different proportion under dissimilar circumstances. Specifically, γ-FeOOH particles are the main product of NZVI oxidation in the abiotic suspension, whereas Fe_3O_4 particles predominate in the presence of bacteria [45]. Two assumptions can explain the alteration in the Fe_3O_4/γ-FeOOH ratio: firstly, the range of pH suited for γ-FeOOH crystallization is quite narrow (between 5 and 7) [80]; secondly, bacterial respiration may consume a considerable portion of the oxygen present thereby decreasing the O_2/Fe^{2+} ratio and favoring the crystallization of Fe_3O_4 particles. Besides, it is known that Fe^{2+} ions exhibit a greater toxicity than Fe^{3+} ions toward cells [81]. Therefore, it is suggested that the redox changes between $Fe^0/Fe^{2+}/Fe^{3+}$ may be directly related to the increase of cytotoxicity observed with γ-FeOOH < Fe_3O_4 < NZVI[15,52].

2.2. Stimulative effect of NZVI

Although NZVI has exhibited its toxic activity to a plenty of microbes, it is not universally bactericidal in aquifer systems [16,17]. NZVI exerts selective pressure on the microbial community, inhibiting some microbial groups (e.g., β- and *γ-Proteobacteria subclasses*) but promoting the dominance of others (e.g., *Archaea*, LGC gram-positive bacteria and α-*Proteobacteria*) [18]. Although there are still no definite explanations for the growth of these species promoted by NZVI, it is generally believed that the hydrogen gas generated from NZVI corrosion could be one factor that stimulates the growth of those bacteria which can use hydrogen as electron donor for respiration [82–84]. As expounded previously, surface modifiers like polyelectrolytes are commonly coated onto the surface of the particles to enhance NZVI stabilization and mitigate its toxicity to cells, but it does not end there. Some coatings (e.g., CMC, polyaspartate) could consolidate the stimulative effect on bacteria by NZVI [4,16]. In addition, some contaminants contained in the pollution zone can inhibit the growth of autochthonous microflora or even kill them. However, injection of NZVI results in a rapid decrease in contaminant concentration and thus benefits the microflora to a great extent [16,85–87]. In general, the microbial communities are able to flourish following NZVI or SM-NZVI injection and it is probably attributed to the modified environmental conditions associated with the injection, rather than the interaction with NZVI itself [4]. The mechanisms of the stimu-

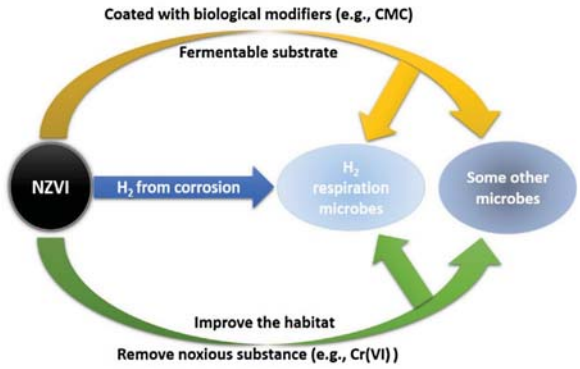

Fig. 3. Illustration of the mechanisms of the stimulative effects of NZVI on microbial growth.

lative effects on microbes by NZVI are schematically illustrated in Fig. 3.

2.2.1. Effect of H_2 generated from NZVI corrosion

With slight toxicity to the strain, NZVI reacted with water to generate cathodic hydrogen [88], a preferred electron donor for many metabolic groups of bacteria including reductive dehalogenators, methanogens, denitrifying bacteria, sulfate reducers, and etc [82–84]. Previous studies have demonstrated that NZVI stimulated methanogenic activity but inhibited biological dechlorination in a mixed culture containing *Dehalococcoides* spp. [16,89]. Methanogens can impede dechlorination by competing for H_2 with dechlorinating bacteria. In fact, the relatively high amount of cathodic H_2 available resulted in a preferential biostimulation of methanogens [90]. While compared to methanogens, *Dehalococcoides* spp. was more sensitive to NZVI's interventions. Their dechlorination activity could be initially inhibited by NZVI and pull through after a lag period. It was suggested that H_2 evolved from NZVI via anaerobic iron oxidation could be utilized as electron donor by *Dehalococcoides* spp.; therefore, they could recuperate after the partial oxidation and passivation of NZVI. As for the denitrifying bacteria, although NZVI could inhibit its microbial activity [91], NZVI might still facilitate microbial removal of nitrate by the cathodic H_2 produced through iron corrosion. Nevertheless, with the increase of NZVI concentration, the removal efficiency gradually decreased [92], which was perhaps due to the toxic effect of NZVI gradually exceeded the stimulation effect of H_2 on the denitrifying bacteria. Although H_2 is one of the most thermodynamically favorable anaerobic substrates for those microbes, its low solubility and concentration perhaps cannot totally satisfy their metabolic demand [93].

2.2.2. Effect of organic surface coatings

Some laboratory studies suggested that NZVI inhibited microbial activity in mixed cultures containing *Dehalococcoides* spp. [17,90,93], while the inhibitive effects seemed not to arise within days when using the NZVI modified with polymer coating. Thus, the polymer coating on the surface of NZVI may play an important role in the microbe-NZVI interaction. It was reported that CMC-NZVI injection can stimulate the native populations of organohalide-respiring microorganisms by 1 order of magnitude of increment throughout the CMC-NZVI affected area relative to preinjection abundance [4]. As known, polymers may act as a fermentable substrate in the NZVI suspension and the fermentation production of hydrogen can be used by organohalide-respiring bacteria [94]. As a biological substrate, CMC may be metabolized to cellulose rapidly and appear to promote microbial reductive dechlorination in situ. This advantage is very analogous with polyaspartate coatings, which may also be combined with NZVI to exhibit stimulatory effect on bacteria. Apparently, polyaspartate is a biodegradable polymer that could be able to hydrolyze to aspartate [95], increasing the available carbon and nitrogen for the microbial community [16]. However, the olefin maleic acid copolymer can not only overcome the significant inhibitory effect on organohalide-respiring microorganisms by NZVI, but also up-regulate both *tceA* and *vcrA* (two model genes coding for reductive dehalogenases) considerably after two days of exposure. It is hypothesized that NZVI coating may enhance the expression of dechlorinating genes and the metabolic activity of *Dehalococcoides* spp. in the culture [17].

Not just the polymer coated NZVI, the produced long-lasting emulsified colloidal substrate (LECS), which contained NZVI, vegetable oil, surfactants, lactate, molasses, minerals, and vitamins, could also make some microbial consortia thriving, especially for some anaerobic bacteria [5]. It was suggested that LECS could form a stable oil-in-water emulsion with uniformly tiny droplets (0.7 μm). The addition of LECS to the aqueous phase could lead to an increase in total organic carbon (TOC) concentrations and provide biodegradable substrates to indigenous microbial community. Thus, LECS promoted the growth of microbes in the related circumstance and engendered a significant increase in the microbial population. Interestingly, the added LECS caused the decrease in dissolved oxygen (DO) concentrations and thus shifted the oxidation–reduction stage of the subsurface environment from aerobic to anaerobic condition, which could also indirectly activate organohalide-respiring bacteria.

2.2.3. Impacts of noxious substance

Some contaminates such as halogenated organic matters and heavy metals can cause serious bactericidal consequence on indigenous microbial consortia [3,85,96]. The application of NZVI in these pollution zones cannot just remove these hazardous materials to mitigate their toxic effect on bacteria, but also activate the growth of some bacteria [85,86]. For instance, 2,4,6-Trichlorophenol (2,4,6-TCP) has a significant inhibitory effect on methanogens' activity, which influences the biological dechlorination of 2,4,6-TCP and the final mineralization by anaerobic microorganism. Yet the surface-modified nanoparticles (NZVI@SiO_2-NH_2) in anaerobic granule sludge system promoted the anaerobic biodechlorination system [86]. The addition of appropriate concentration of NZVI@SiO_2-NH_2 in the treatment system might attenuate the adverse influence of 2,4,6-TCP on the anaerobic microorganisms and improve the electron transport system, stimulating the methanogenesis markedly.

Because of its solubility, mobility and high oxidizing potential, Cr(VI) could have high toxicity [97] to the environmental microbes. The injection of NZVI could lead to a quick decrease in the Cr(VI) and total Cr concentrations in the subsurface water. Apparently, because of the toxic hexavalent form of Cr, the total amount of the bacteria presented a negative correlation with the concentration of Cr(VI). In contrast, the application of NZVI stimulates the growth of gram-positive bacteria and thus results in a positive correlation between the bacteria population and the concentration of NZVI injected [98]. As well, there are numerous other organic compounds (e.g., toluene, phenol) and inorganic materials (e.g., Cd^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}) that could probably exert adverse impacts on microbes [98], NZVI can remove them and create a relatively decent environment for the growth of the microbes. It can be revealed that removing the hazardous materials by NZVI has a positive effect on the autochthonous microflora in spite of the concern about NZVI ecotoxicity. Moreover, it can be speculated that the initial alterations in microbial communities are caused principally by the NZVI induced changes in geochemical conditions, such as hydrogen evolution, nutriment complement and hazardous composition degradation, but not by direct microbial interactions with nanoparticles.

Table 1
Review of the effects of NZVI-based particles on the microbes in the subsurface environment.

Reference	NPs	NPs concentrations	Microorganisms	Experimental conditons	Major results
[63]	NZVI; NOM-modified NZVI; Aged-NZVI	100 mg/L	*Escherichia coli*	Bicarbonate solution, 1 h	Exposure to 100 mg/L of bare NZVI under anaerobic conditions caused a 5.2-log inactivation after 60 min. Adsorbed NOM on the same NZVI significantly decreased its toxicity, resulting in less than 0.2-log inactivation after 60 min. Complete oxidation of Fe0 in NZVI (Aged-NZVI) under aerobic conditions was relatively benign to bacteria
[55]	NZVI; Bimetallic Fe–Ag nanoparticles	0-3.5 g/L	*Enterococcus faecalis* CCM 4224, *Pseudomonas aeruginosa* CCM3955, *Staphylococcus epidermidis* etc.	Deionized water, 24 h	NZVI did show any antibacterial activity against all microorganisms. Fe−Ag NPs could significantly inhibit the growth of the tested bacteria
[15]	NZVI; CMC-modified NZVI	500 mg/L	*Escherichia coli*	Normal saline, 1h	The CMC-coating could significantly weaken the cytotoxicity of NZVI towards *Echerichia coli*
[74]	NZVI	14–18 g kg^{-1} soil	Soil microbial communities	Soil from an untreated agricultural field, 10 days	The results showed that the bacterial molecular response of Gram-negative bacteria to the addition of nZVI exceeds that of the Gram-positive bacteria
[36]	Polyacrylic acid (PAA)-coated NZVI	10 g kg^{-1} soil	Soil bacterial community containing chloroaromatic mineralizing microorganisms	Soil from an arable field, 0, 1, 4, 7 and 28 days	NZVI addition caused altered microbial composition, and reduced the activity of chloroaromatic mineralizing microorganisms
[89]	NZVI	17 mg NZVI g^{-1} soil; 0–10 mg/L	Soil microorganisms, *C. elegans*	Bulk soil samples, 7 days; K-medium, 4 days	The toxic impact of NZVI on soil organisms was shown to be generally higher in in vitro than in soil assays. The complexity of the soil matrix could probably diminish nZVI bioavailability and thus its potential toxic effect on soil organisms
[90]	NZVI	1 g/L	Methanogens, *Dehalococcoides spp.*	HEPES and mineral salts solution, 190 h	NZVI could significantly biostimulate the growth of Methanogens; whereas dechlorinating organisms were initially inhibited by NZVI, the dechlorination activity and ethane production could recovered after a lag period. The water-derived cathodic H_2 production evolved from NZVI via during its anaerobic corrosion was utilized as electron donor by these two types of microbes
[16]	NZVI; Polyaspartate-modified NZVI	1.5 g/L	Complex bacterial communities	Wet aquifer material (i.e. clayey sand, glacial and coastal sandy soil), 68-250 days	NZVI may only be noxious to certain groups of microbes, and select for others. In addition, toxicity may be reduced because wet aquifer material commonly contains surfaces, clays, and other components to which NZVI tends to adsorb, limiting contact with microbial surfaces. Polyaspartate coatings can be hydrolyzed by certain bacterial, which can cause an increase of the available carbon and nitrogen for the microbial community, stimulating microbial growth
[85]	NZVI	2 kg NZVI/ton of soil	Indigenous bacterial communities	A pilot-scale in-situ remediation test in the saturated zone of a historically Cr(VI)-contaminated site, 0,1,21,154.217 days	The results showed a negative correlation between the microflora and the Cr concentrations before the application of NZVI and an obvious positive correlation between the concentration of Fe and bacteria after the application of nZVI. NZVI could stimulate the growth of Gram-positive bacteria considerably

Representative researches on the effects (either positive or negative effects) of NZVI-based particles on the microbes in the subsurface environment are summarized in Table 1. The various experimental conditions (i.e., particle concentration, contact time, soil/water system, and the corresponding microbes) and the main results are illustrated.

3. Synergistic effect of NZVI and microbes on the pollutant removal

Given the larger surface area and higher reactivity relative to the micro-scale ZVI, NZVI has been developed as an highly effective method to remove/degrade a wide range of chemical pollutants

[7,10]. However, no matter how well NZVI functions in remediating pollutants in situ, most contaminated sites still rely on bioremediation as a concurrent or terminal process to achieve remediation goals. Microbial communities are paramount not just to nutrient cycling (C, N, S, Fe, etc.) in ecosystems, but also to contaminant and heavy metal degradation and immobilization [16,24]. As mentioned in the previous part, NZVI may generate selective pressure on the microbial community, promoting the dominance of some microbial groups and the diminution of others [90]. Recent studies have combined NZVI with various bacteria to remediate contamination, and the results suggested that an appropriate quantity of NZVI could stimulate the bio-iron system for effective remediation [4,20,23]. In general, surface modifiers (e.g. anionic polymer, NOM) can be employed to decorate NZVI in the field scale application, which provide better colloidal stability and mobility during NZVI injection and delivery [99] and/or facilitate their interaction with non-aqueous phase liquids (NAPL) [100]. Besides, some biodegradable coatings, such as CMC and NOM, can not only act as a buffer between microbial cell walls and the highly reductive NZVI surface to mitigate the adverse effect of NZVI on biological reduction activity [15,17], but also serve as nutrient source to supply available carbon and nitrogen for the microbial community [4]. Overall, these results indicate that the appropriate NZVI coatings may make the synergetic system (NZVI-microbes) more favorable in the remediation of contaminated sites.

3.1. Bio-NZVI system for dehalogenation

Reductive dehalogenation by microorganisms under anaerobic conditions (or oxic in a few cases [101]) is believed to be the main biotransformation process, which exerts enormous potency for pollutant removal [102–104]. Anaerobic dehalogenation may increase the biodegradability of dense non-aqueous phase liquids (DNAPL, such as TCE) in the environment and promote the natural attenuation. However, this process entails a relatively long time for effective dechlorination and the bio-dechlorination rate may decrease with the increasing halogenation degree, which possibly causes the accumulation of more toxic intermediates [101,105]. Although some dehalogenating bacteria (e.g. *Dehalococcoides* sp. strain CBDB1, strain BAV1) have broad dehalogenation activity on halogenated aromatic compounds, they could only utilize lower brominated polybrominated diphenyl ethers (PBDEs) (<7 bromines) as electron acceptor [106,107]; no debromination activity could be detected for *Dehalococcoides* sp. after exposed to high-brominated congeners (e.g. deca-BDE or octa-BDE) even for a rather long period. As known, the genus *Dehalococcoides*, the only organisms known to completely dechlorinate TCE to vinyl chloride (VC) and ethene, is able to dechlorinate TCE sequentially to dichloroethene (DCE) isomers (*cis*-DCE, *trans*-DCE, and 1,1-DCE), VC, and ultimately to the innocuous product ethane [104,108,109]. However, as a significant intermediate product of reductive dehalogenation, VC may exhibit toxicity and carcinogenicity to organisms with its (temporary) accumulation [110]. Besides, some chemicals like 2,4,6-TCP have an intrinsic inhibition on anaerobic microorganism and the toxic chlorophenols probably lead to the accumulation of organic acids and pH decrease, which finally affect their biological dechlorination by anaerobic microorganism [86].

NZVI has been extensively studied for its potential application in the remediation of DNAPL-contaminated sites [1,101] and has been shown to effectively degrade a great amount of halogenated organic contaminants (e.g., PCB, TCE) [90,101,103,105]. As known, NZVI could reduce TCE directly into acetylene, ethene, and ethane, with only trace production of *cis*-DCE and toxic VC [111]. The toxic 2,4,6-TCP could be directly chemically reduced and/or be adsorbed onto the surface layer of NZVI [86]. Given the high surface area and strong reducibility, NZVI could readily dehalogenate lots of higher-halogenated aromatic ring compounds (e.g. deca-BDE or nona-BDE) and sequentially generate low halogenated congeners such as mono-BDEs to tri-BDEs as products. Nevertheless, NZVI particles are quickly oxidized and difficult to reduce the low-brominated congeners completely [101]. Moreover, some certain halogenated organic pollutants like 1,2-DCA could resist to the abiotic reduction by NZVI [112]. Even in the case of bimetallic NPs, which were formed via modifying NZVI with second catalytic metals such as nickel (Ni) or palladium (Pd), the degradation of the above refractory pollutants was still relatively sluggish [20,113]. This undoubtedly indicates that further biological bioremediation after NZVI application is necessary in order to completely remove the pollutants.

Some studies have attempted to combine the NZVI with *Dehalococcoides* spp. for the remediation of halogenated solvent contaminated plumes [17]. In the bio-NZVI system, decabromodiphenyl ether (DBDE) was first reduced to lower brominated congeners by NZVI and then dehalogenated by debrominating bacteria to diphenyl ether (DE). This method combined the advantages of both NZVI and Dehalococcoides to completely reduce DBDE to DE in the bio-NZVI system [101,105], as presented in Fig. 4. Additionally, the injection of NZVI could generate cathodic H_2 as it reduce water-derived protons [33] and cause strongly reducing conditions [114] that were highly beneficial to biotic reductive dechlorination. Accordingly, two main mechanisms are involved in the enhanced contaminant remediation by the bio-iron synergetic system: firstly, both NZVI and *Dehalococcoides* spp. can degrade/remove the contaminants simultaneously or sequentially; secondly, *Dehalococcoides* may be stimulated by the corrosion H_2, which is a preferable electron donor for their halorespiration [115].

However, the effectiveness of the bio-NZVI system could be influenced by several factors. It was reported that the dechlorination rate of TCE by the combined bio-NZVI system decreased to less than one-half compared to the dechlorinating bacteria alone

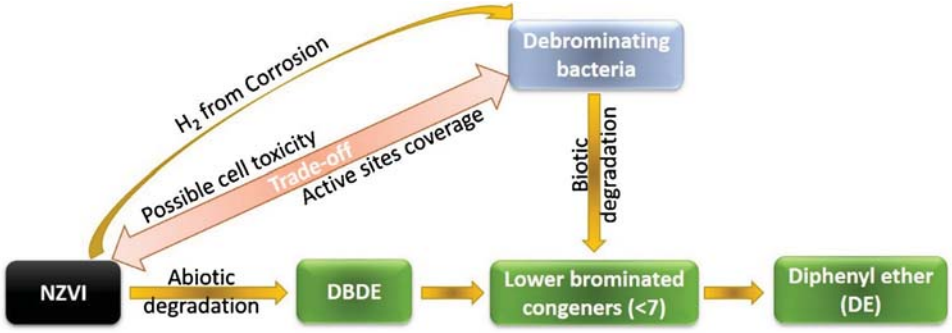

Fig. 4. The process of decabromodiphenyl ether (DBDE) debromination by the synergistic system containing NZVI and debrominating bacteria.

[90].The attachment of NZVI on the surface of cells could possibly affect the bacterial membrane functions and even result in cell apoptosis. Meanwhile, the effective area of NZVI for reductive dehalogenation (i.e., numerous active sites) can be sheltered to a great extent. Besides, it is noteworthy that during the combined treatment, biotic degradation appeared to become the dominant degradation process as the available Fe^0 had been oxidized, exhausting reactivity of NZVI particles and constraining the long-term extent of abiotic degradation [4]. Moreover, the existence of other oxidants in the bio-iron system might compete with the targeted contaminant for the consumption of Fe^0. Therefore, greater amount of NZVI will be needed to reduce a quantity of TCE. However, the concentration of NZVI should be regulated to avoid the adverse impacts on *Dehalococcoides* spp.

Considering the possible inhibition of biodechlorination in the system, besides downregulating the NZVI concentration appropriately, some modifiers have been employed to overcome this problem [4,17,20,116–118]. As illustrated in previous section (2.1.3 and 2.2.2), the organic coatings (e.g., CMC polymer, starch and soy protein) on the surface of NZVI can also act as a fermentable bio-substrate that produce hydrogen, benefitting organohalide-respiring microorganisms remarkably [4,116,117]. Besides, the additional biodegradable natural modifiers would provide sufficient carbon sources as well as electron donor to stimulate microbial growth [20]. It was evident that both biotic and abiotic processes exhibited substantial effects on the degradation of 1,2-DCA using Fe-Pd NPs stabilized with the biodegradable surfactant [20]. The results reveal that the appropriate NZVI coatings may alter environmental conditions and enable the concurrent or sequential participation of dechlorinating bacteria in the remediation process [17]. Nevertheless, it should be noted that the application of surface modifiers proposes a tradeoff between the NZVI-bacteria synergistic remediation and the partial inhibition of NZVI reactivity with the target contaminant [47].

All in all, it can be concluded that combining the NZVI and dehalogenation microbes together may overcome the shortcomings of each technology, and reach a comparatively higher effectiveness in DNAPL treatment. In the optimizing combination system with appropriate doses of NZVI and dehalogenation microbes for DNAPL treatment, NZVI can (i) abiotically degrade most of the DNAPL in the source-plume directly followed by biotic dechlorination to remediate residual halogenated organic compound as a “polishing” step; (ii) offer hydrogen for dechlorinating bacteria to diminish the requirement for additional electron donor (e.g., lactate, pyruvate); (iii) dramatically flourish the microbes by its biodegradable modifiers (e.g., CMC); (iv) abate the toxic effect of some DNAPL on dechlorinating bacteria by decreasing their aqueous concentration in the source zone; and (v) shorten the remediation time by effectively dechlorinating the DNAPL mass [90]. However, considering the uncertainty and complexity of bio-iron system, more laboratory and pilot studies should be conducted to enable this technique to become more effective and feasible in site restoration, including the exploration of bio-iron interaction mechanisms, the reactivity of both NZVI and *Dehalococcoides spp*., the behavior with the latent inhibitory chemicals and the need for pH control [105].

3.2. Bio-NZVI system for denitrification

In recent years, NZVI has been investigated as an novel denitrification technique due to its large specific surface area and high surface reactivity area [119]. Complete denitrification of nitrate has been accomplished quickly just by employing NZVI in the aqueous solutions under anaerobic conditions at near neutral pH [120]. The reaction for nitrate reduction by Fe^0 could be described by Eq. (4).

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

As indicated above, the abiotic reduction of nitrate by NZVI generated abundant ammonium and then raised the ambient pH, which could probably intensify the formation of iron hydroxide precipitates [120]. Both of the two problems could hinder the extensive application of NZVI in the denitrification of nitrate. Besides, it was reported that the high nitrate concentration may also present inhibition on the rates of nitrate reduction and iron corrosion [121].

Biological denitrification is considered to be an alternative approach for the denitrification of nitrate, where denitrifying bacteria use nitrates as terminal electron acceptors and reduce nitrate to nitrogen [25]. Although this approach is cost-effect, environmental friendly, the elongated cycle times and lower denitrification rate are always inevitable just like other bioremediation process [122,123]. Moreover, biological denitrification can generate redundant biomass and soluble microbial products, which needs secondary treatment, especially when heterotrophic bacteria participate in the process [124]. Alternatively, autotrophic denitrification can use H_2 as an electron donor and denitrify nitrate more cleanly with fewer residual organics [125]. Nevertheless, on account of the high cost and technical difficulties in handling or storing, the engineered application of H_2 to denitrification systems seems to be localized. Therefore, the combination of autotrophic denitrifier and NZVI may provide an alternative for the denitrification of nitrate, since the hydrogen generated from anaerobic iron corrosion may stimulate the denitrifying populations [126].

Using NZVI for the biologic denitrification can preclude the drawbacks of traditional biological denitrification, which depends on the use of explosive hydrogen gas or even organic substrates, and maintain the advantages that fully reduce nitrate in a few minutes offered by NZVI [127]. Furthermore, adding the autotrophic bacteria can apparently decline ammonium generation in the nitrate treatment using NZVI. Ultimately, nitrates are removed completely by the bio-NZVI synergetic system with a smaller proportion of ammonium [128]. It was proposed that nitrate removal in the synergetic system could be divided into two stages: an abiotic reduction period and a biological denitrification period [22]. This process was also in line with the dynamic of ammonium generation, which was a biphasic process including an increasing period and a stable period. To be specific, NZVI could reduce nitrate merely in the first period and produce ammonium as soon as it came into contact with the nitrate. During this period, autotrophic microbes were in an adaptive phase with little activity to utilize nitrate. After that, NZVI turned into hydrogen donors for the bacteria, and the autotrophic bacteria denitrified the residual nitrate, while the ammonium was in a stable period. Accordingly, it is suggested that the key mechanism of nitrate removal converts gradually from chemical reduction into biological denitrification as the biomass reaches a certain concentration [128], which is schematically described in Fig. 5.

However, in the integrated denitrification system, NZVI and the autotrophic bacteria (e.g., *Alcaligenes eutrophus*) may exhibit a subtle competition in the denitrification, which is probably due to that the bacteria modify the system pH and NZVI surfaces, and even expedite the oxidation of the NZVI. As pH value attained to a high medium pH (> 8.0), the NZVI got devitalized by formation of an oxide iron layer and then bacteria would dominate the nitrate reduction rather than NZVI [22]. Furthermore, plenty of bacteria might cause serious cell agglomeration and a small exposure of NZVI surface area, as well intensify the competition for electrons with abiotic denitrification [128]. Consequently, the first order rate constant rose with the decrease of the initial bacteria concentration

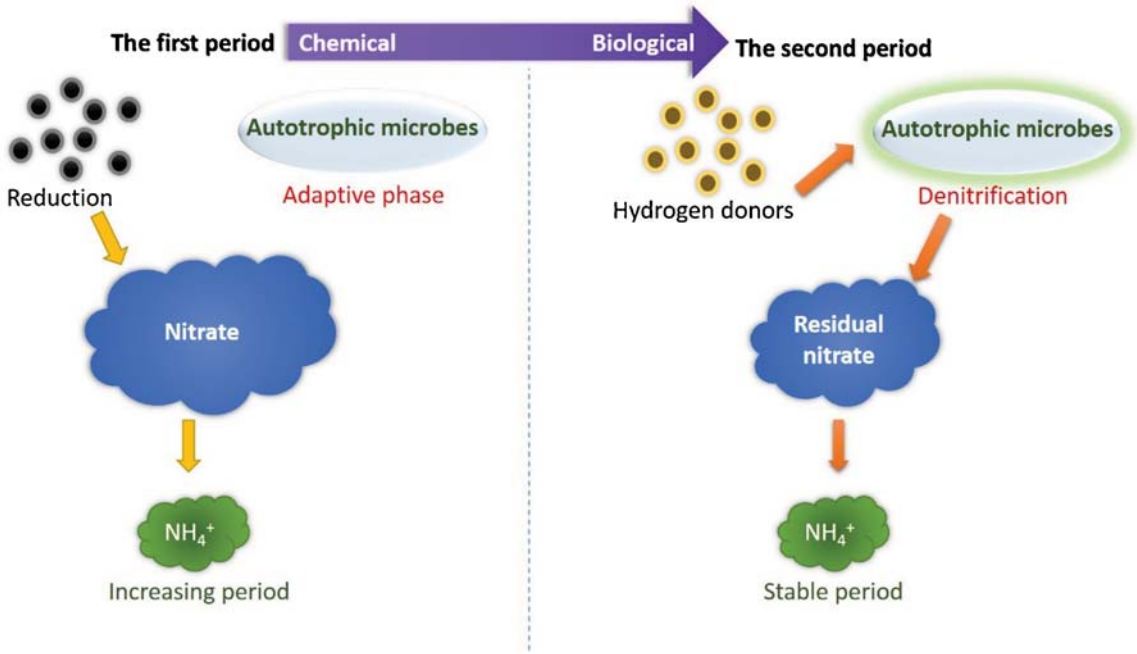

Fig. 5. The removal process of nitrate by the bio-NZVI synergetic system.

and the increase of the initial iron nanoparticle content. However, when the addition of NZVI reached to a high degree, the microbial activities would be inhibited obviously. A high amount of NZVI mass may exhibit obvious toxicity towards autotrophic bacteria by excess uptake of Fe (II) and ROS, which eventually lead to oxidative damage to the cell [91]. Therefore, the concentration of ammonium increases again and would inhibit the reductive activity of NZVI anew.

In spite of some studies indicating the slight toxicity of NZVI even at low concentrations, there were still some other studies showing the addition of 50–1000 mg L^{-1} of NZVI particles under aerobic conditions, as an electron donor source, could accelerate nitrate reduction by autotrophic microbes (e.g., *Paracoccus* sp.) [129,130]. Definitely, the toxic impact of NZVI on bacteria is dose- and species-dependent, and mostly influenced by environmental conditions. It is indicated that the optimized proportion of NZVI and initial biomass concentrations can minimize the ammonium generation and promote the denitrification efficiency of the composed system.

While applying NZVI particles to water treatment under continuous aerobic/anaerobic conditions, various bacterial communities just like reductive dehalogenators, autotrophic denitrifiers, methanogens [131], and sulfate reducers [132] that existed in the environment are all inevitable to contact with NZVI directly or indirectly. Regardless of the possible toxicity of NZVI, the hydrogen gas from the corrosion of NZVI can be the most striking benefit for many metabolic groups of these bacteria as a preferred electron donor. Considering the extensive applications of NZVI and distributions of functional bacteria, more studies about their combination should be carried out for the efficient remediation of on the recalcitrant contaminants.

3.3. DIRB assisted contaminant reduction by NZVI

Commonly, NZVI can be oxidized to ferrous or ferric iron, leading to decreased reactivity with time. Several lab-/field-scale studies involving the long-term performance of Fe^0 indicated that the contaminant degradation rates would decline with time [133,134]. The temporal effect on contaminant degradation rates is ascribed to the accumulation of thick layers of amorphous corrosion products that develops on the metal surface [135], which may probably act as a physical barrier between the underlying reactive sites on the Fe^0 core and the dissolved contaminants. Even though some studies proposed that the corrosion products accumulated on Fe^0 surfaces could also be highly redox-reactive and serve as new sites for contaminant catalysis and adsorption, the inhibition of contaminant access to the Fe^0 surface seems to be more worrisome [29,136].

An emerging technology for the in situ remediation of contaminated sites is the stimulation of dissimilatory metal reducing bacteria (DMRB) [137]. Various organic contaminants (e.g., nitroaromatic compound and chlorinated solvents) and reducible inorganic (e.g., Cr(VI), Tc(VII), Co(III) and U(VI)) could be either directly reduced by DMRB or indirectly by the biogenic Fe(II) [138,139]. The dissimilatory iron reducing bacteria (DIRB) are distributed extensively in both pristine and polluted terrestrial, aquatic, and subsurface environments [133]. DIRB may play an important role in the biogeochemical cycling of iron in anoxic sediments and groundwater. For instance, respiration by DIRB can reconvert oxidized Fe(III) to Fe(II), which would contribute to further contaminant reduction [133].

The reduction of Fe(III) to Fe(II) can generate surface reactive Fe(II) species or lead to the removal of passivating ferric precipitates on the ZVI surface [133]. Reductive dissolution of ferric iron precipitates decreases the thickness of corrosion layer, which might improve the mass transport of contaminants to surface reactive sites or benefit to the electron transport from the central Fe^0 to reaction sites on the corrosion product-covered surface [29]. Because of these advantages, it is proposed that the application of DIRB (e.g., *Shewanella alga BrY, S. putrefaciens* CN32) to the abiotic remediation of contamination by NZVI would prolong the reactivity of NZVI by reducing the outer ferric iron to its ferrous form. Then the management cost of NZVI remediation is expected to shrink due to IRB metabolism, which is a biological and ecological means, restoring reaction potential with no harmful effect on the ecosystem of remediation sites [140].

For instance, the presence of *S. alga BrY* in Fe^0 reactive barriers could affect their longevity by reducing the oxidized iron particles to Fe^{2+}, which became available to reduce the additional contam-

inants, such as TCE [141]. It was reported that DIRB could reduce structural Fe(III) oxides, which in turn potentially dechlorinated carbon tetrachloride to chloroform following the reaction below (Eq. (5)). Apparently, DIRB have a positive influence on the performance of ZVI by restoring the reactivity of passivated iron [133].

$$2Fe^{2+} + CCl_4 + H^+ \rightarrow 2Fe^{3+} + CHCl_3 + Cl^- \quad (5)$$

As known, some laboratory studies have extensively investigated the microbial reduction of Cr(VI) by DIRB [142,143]. Potentially, the coupling of microbial and chemical reduction by DIRB could be a promising approach for the reduction of Cr(VI) to Cr(III). DIRB exploited the organic carbon or H_2 as the electron donor to reduce metals Cr (VI) and Fe (III) in aqueous phase to Cr (III) and Fe (II), respectively. Meanwhile, the yielded Fe (II) by DIRB could act as a strong reductant to chemically reduce Cr(VI) in the tested system [23]. Additionally, some studies have demonstrated that NZVI could function as an effective tool for managing sites contaminated with Cr(VI) by reducing Cr(VI) to stable Cr(III) significantly [144–146]. The combination of NZVI and DIRB for remediation of Cr(VI) may be worthy to be taken into consideration, excluding the possible toxicity of both NZVI and Cr(VI) for the DIRB.

However, a flourishing DIRB population in Fe^0 remediation sites may have an adverse impact on the reactivity of Fe^0. The mass transport of dissolved contaminant to iron surface could be hindered by the development of thick biofilms [147], thus diminishing the overall reactivity of NZVI. On the other hand, the reduced Fe^{2+} could particularly attach to gram-negative bacteria (e.g., *S. alga BrY* and *S. putrefaciens* CN32) [148,149] or complex with HCO_3^- or PO_4^{3-} to form siderite ($FeCO_3$) or vivianite ($Fe_3(PO_4)_2 \cdot (H_2O)_8$) [149], which might cover the reactive surfaces of NZVI and inhibit the abiotic reduction of contaminants (e.g., TCE) by NZVI [141].

The reduction of oxide layers of Fe^0 by *Shewanella putrefaciens* occurred principally by electron transfer at the cell membrane/mineral interface [150], and required adherence to the oxyhydroxide surface directly. However, several studies have indicated that indirect electron shuttling mechanisms of dissimilatory metal reduction for DIRB to reduce crystalline Fe(III) (e.g., hematite) were more efficient than that employing direct contact between the bacteria and Fe(III)-oxide [151,152]. NOM (e.g., humic substances) is known to transfer electrons for DIRB, and can also shuttle electrons by themselves in abiotic reactions [47]. This process could employ electron bridges like the quinone functional groups [153], or polyphenolic and aromatic groups [32], which would be temporarily reduced and then permit the electron transfer to Fe(III) eventually. This mechanism leads to a substantial increase in the Fe(III) reduction rate, and the degree of reduction per cell, by avoiding the need of direct contact between the cell membrane and Fe(III), especially for the aggregated iron particles where tiny pore spaces are inaccessible to DIRB [154].

It was suggested that postponing the sorption/precipitation of aqueous biogenic Fe (II) could enhance the Fe (III) oxide bioreduction and then contribute to restore the reactivity of Fe^0 obviously [155]. Previous studies have derived several ways to control their accumulation onto reactive sites of the iron oxides:

(i) Addition of the organic ligands such as humic acid, citrate, NTA and EDTA, which could either complexe with Fe (III) followed by its solubilization/generation at the NZVI surface or complexe with the biogenic Fe (II), might delay the iron precipitation on DIRB cells/NZVI oxide surface [156];
(ii) Addition of fresh media and inoculum was also found to revive on the retarded reduction activity [157];
(iii) Other solid phase compounds (e.g., kaolin, alumina, layered silicates, quartz) have also been found to act as Fe (II) sinks, which might draw away the biogenic Fe (II) [158], and thus decelerate the surface deposition of Fe (II) complex on the NZVI surface and promote the reactivity of NZVI for contamination remediation.

Furthermore, several studies have indicated that DIRB (e.g., *S. alga BrY*) could preferentially adhere to Fe(III) coated surfaces of the corroded iron [159], and they would be more possible to catalyze reactions of NZVI with chlorinated aliphatics on the solid iron oxide surface than on the surface of untreated Fe^0 [133]. The ferrous ions produced by *S. alga BrY* entailed the existence of the solid iron oxide surface to degrade TCE efficiently [141]. Over all, it can be presumed that inoculation of the DIRB along with the medium to the aged or partially aged NZVI could be one practical and efficient way to sustain the NZVI system for the remediation of contaminated sites.

4. Influences of microbes on the behavior of the aged NZVI particles

The high reactivity of NZVI is associated with its core–shell structure closely, which comprises a metallic iron (Fe^0) core wrapped by a thin oxide shell [160]. Once NZVI was introduced into the environment, the Fe^0 core in the NZVI would be oxidized upon reactions with contaminant, water or oxygen. Then NZVI could entirely or partially transform from Fe^0 to various iron oxides/hydroxides [161,162] and other forms of minerals (e.g., siderite and vivianite) [163]. Furthermore, a considerable fraction of iron oxide minerals in nature was believed to exist in the form of nanosized colloids [39] and play an essential role in numerous biogeochemical processes [164]. Such nanoparticles can be developed in environments with great geochemical gradients that facilitate supersaturation and rapid nucleation, like those occurring in the process of the rapid enzymatic oxidation of Fe(II) by bacteria [165]. These nanoparticles may appear in stable colloidal suspension, albeit aggregating as a stable cluster of multiple particles that may be spatially more accessible for microbes than those large aggregates via flocculation [166].

The aged NZVI exhibited little toxicity to organisms mainly due to the lack of oxidative stress, simultaneously, maintained the cellular metabolism and promoted the cellular respiration significantly via acting as electron conduits particularly for several soil microbial populations (e.g. *Geobacter* species) [167,168]. It was reported that crystalline iron oxides such as magnetite and goethite were typically abundant (about 2- to > 10-fold more abundant) relative to amorphous forms (e.g., ferrihydrite) in various subsurface sediments [169]. Although previous studies have proposed that DIRB could only reduce amorphous and poorly crystalline iron oxides and that crystalline phases were relatively recalcitrant [26], some DIRB belonging to the genus *Shewanella* were able to reduce more crystalline forms of ferric iron oxide [27,28] and low-grade (semitaconite) ferric ore [170]. Roden and Zachara [27] examined the initial rate and long-term extent of reduction of a range of crystalline iron-(III) oxide using a dissimilatory iron(III) oxide-reducing bacterium (*Shewanella alga* strain BrY). It was found that the Fe(III) reduction rates were in order of several $\mu mol\ L^{-1}\ min^{-1}$, depending on the oxide surface area and cell numbers. Oxide reduction rates reached an asymptote at cell concentrations around $10^9/m^2$ of oxide surface. Kostka and Nealson [28] reported that marine and freshwater strains of the bacterium *S. putrefaciens* are capable of the rapid dissolution and reduction of magnetite, converting millimolar amounts to soluble Fe(II) in a few days at room temperature. Accordingly, it is believed that the DIRB in the remediation sites would influence the long-term fate of the aged NZVI [140].

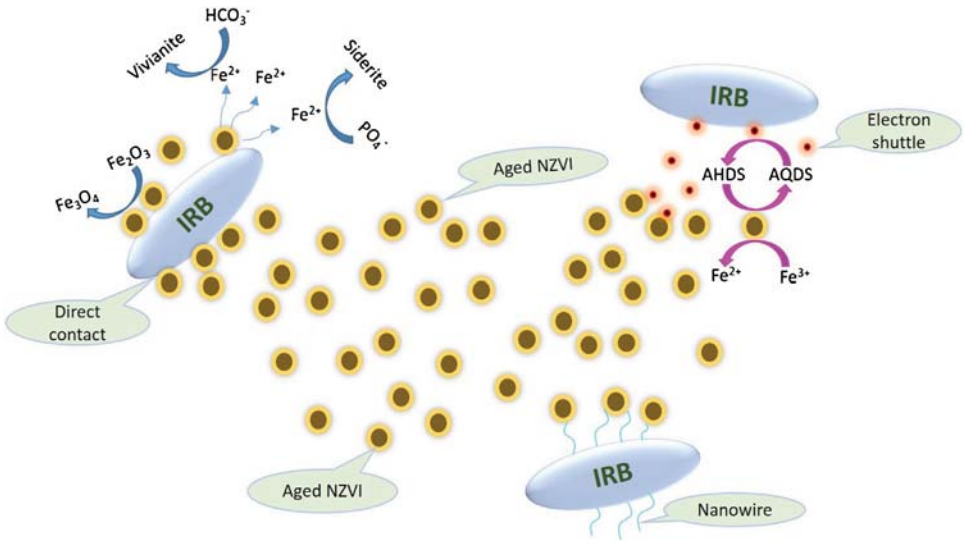

Fig. 6. Illustration of the three major approaches (i.e., direct contact, electron shuttle and nanowire) for the bio-reduction of the aged NZVI and the formation of secondary phases.

4.1. Approaches for the electron transfer between DIRB and the aged NZVI particles

DIRB have substantially developed three strategies to conquer the crucial problem of association of the cell's electron transport system (ETS) with the indissolvable aged NZVI particles, including (i) transferring electrons to Fe(III)-bearing minerals directly via cell surface-localized cytochromes [171] or (ii) electrically conductive pili or "nanowires" [172], or (iii) "shuttling" electrons indirectly from terminal points of the ETS to the Fe(III) oxides surfaces by using humic substances [173] or bacteria metabolites [174]. All of the three approaches for iron reduction were depicted schematically in Fig. 6.

Under conditions of low acidity and ionic strength, both the electrostatic repulsion among nano-iron oxide particles and the coulombic attraction between nanoparticles and bacteria can promote the contact between the Fe(III) colloids and the cells. Even the shortage, non-coulombic interactions like dipole–dipole interactions and hydrogen bonds may also play an important role in their contact [40]. Correspondingly, large quantities of aged NZVI colloidal particles, such as ferrihydrite, goethite (α-FeOOH), and hematite (α-Fe_2O_3) are tightly attached to the cell surface [175]. Contrary to bulk ferric iron phases, colloidal particles may fully cover the bacteria surfaces with no spatial limitation, which probably follows a Langmuir isotherm [176]. Furthermore, colloids undergo Brownian movement constantly, which could increase their chances to capture electrons from the cell membrane by higher frequency of contact with reactive sites on the DIRB surface [176].

It has been recognized that the direct contact between the DIRB and the oxide surface might be a major mode for the reduction because of iron oxide's low solubility and the other properties mentioned above [177]. To be specific, direct electron transfer from outer membrane reductases to the ferric minerals entails close contact below 14 Å between the terminal ferric reductase on the bacteria surface and iron oxide molecule at the mineral surface [178]. Therefore, the distance may be the key factor for limiting the rates of electron transfer from cell to mineral. In addition to the contact between outer-membrane cytochromes and solid-phase iron oxide particles, bacterial nanowires, a kind of electrically conductive pilus-like appendages produced by some bacteria (e.g., *Shewanella oneidensis* MR-1), could also transfer electrons to iron oxide particles efficiently at sites distal from bacteria surface [179]. However, some researchers claimed that the formation of nanowires in appropriate culture environment within hours appeared to be rather unlikely [39].

Even though several studies have shown that direct contact between cell and iron oxide was essential to the occurrence of electron transfer, while others have suggested that the electron shuttles (e.g., quinones, riboflavin) synthesized by cell could mediate the terminal reduction of ferric compounds [154]. It was reported that the indirect electron shuttling was more efficient for DIRB to reduce crystalline Fe(III) compared to the direct contact [152,180]. As a result of the tiny and complex interstices in the aggregated ferric particles, DIRB could hardly access them and unlikely reduce them to a large extent. The acceleration in bacterial reduction of crystalline Fe(III)-oxides by electron shuttles might be due to their better accesses to those ferric compounds that were not easily reduced. This is in accordance with the finding that the aggregation of aged NZVI particles exhibited negligible impact on its bacterial reduction by employing electron shuttles in the bacterial-nanoparticle system [154].

As known, the *Shewanella* species could create their own endogenic organic ligands that activated Fe(III) before reduction efficiently [181]. Besides, the natural organic ligands could also play the same role in the bio-reduction of iron oxides. It was reported that the humic substances themselves could supply the electrons required for the reduction of Fe(III) oxides, meanwhile, promoted their availability as electron acceptors for respiring bacteria [182]. For example, the obvious enhancement of reducibility of Fe(III) oxides by AQDS, a common humic analog that were employed to IRB-Fe(III) oxides system frequently [183], might be attributed to the production of hydroquinone form of AQDS (i.e., AHDS) by *Shewanella* CN32. The AHDS exhibited thermodynamic power to reduce the Fe(III) oxides [24]. Namely, AQDS could serve as an electron acceptor/repository for cell respiration by the following reaction:

$$\mathrm{AQDS} + 2\mathrm{H}^+ + 2\mathrm{e}^- = \mathrm{AHDSE}^0 = 0.23\,\mathrm{V} \quad (6)$$

Specifically, it can be postulated that AQDS may act as an electron shuttle between the bacterial electron transport chain and the Fe^{3+} oxide surface, and abolish the necessity for direct bacterial-nanoparticle contact. The bacteria provided electrons for AQDS at the terminal of the electron transport chain located in the outer

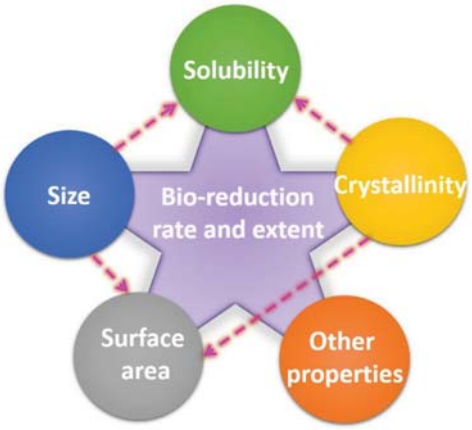

Fig. 7. The major factors influencing the rate and extent of bio-reduction of Fe(III) oxide nanoparticles by DIRB.

membrane or periplasmic space, and then reduced it to AHDS [184]. Subsequently, the generated AHDS developed a surface complex on the Fe^{3+} oxide surface, delivering electrons to the Fe^{3+} [24]. AHDS reoxidation to AQDS was coupled with Fe^{3+} reduction to Fe^{2+}, and AQDS could participate in the bioreduction of Fe(III) oxides once again [185]. Furthermore, the increase of bioreduction rate in the presence of humic acid was not just due to its role of electron shuttle, but also by its effect on the controlling of the size, shape and density of the oxyhydroxides indirectly via complexation with iron species [182].

Additionally, it is interesting that some nanosized iron oxides (e.g., ferrihydrite, hematite) themselves could act as electron shuttles between bacterial cell surface and the bulk iron oxide [39,168]. The copious adsorption of colloidal oxides onto the bulk mineral could probably form an easily accessible surface layer for microbial utilization, which would activate the surface by conducting electrons to the interior of the bulk iron oxide. Particularly when those iron oxide nanoparticles were with high mobility due to the colloidal suspension, they might serve as feasible agents joining the iron-reducing and oxidizing zones in this concept [39]. Moreover, some *Shewanella* spp. (e.g., *oneidensis* MR-1 and *loihica* PV-4) could establish electrically conductive networks with those nanocrystalline (semi)conductive iron oxides, which acted as electron conduits between the cell surface and distant Fe(III) mineral [186]. Given nanocrystalline iron oxides are plentiful in soils and aquatic sediments, the electrically conductive networks may be widely distributed in the surroundings.

4.2. Influences of major characteristics of the aged NZVI particles on their bio-reduction

The rate and degree of NZVI oxidation and the types of mineral phases generated subsequently would depend on the geochemical conditions under which they were exposed [79]. Furthermore, the differences in particle size, solubility, crystallinity and surface area can all potentially influence the rate and extent of Fe (III) oxide bioreduction by DIRB [27,140,150]. Meanwhile, the relations among these factors are also complicated (Fig. 7).

It was suggested that the reactive surface sites for interfacial reaction between iron and IRB influenced the bio-reduction rate of ferric oxides significantly [181]. Understandably, the smaller the particle size was, the higher the respiratory rate of IRB [140] and the more complete reduction extent could be attained. For example, less than 10% of the Fe(III) was reduced for 50 nm particles by *Geobacter sulfurreducens*, but 50% could be reduced for 10–30 nm nanoparticles under the same reaction conditions [187]. Likewise, the reduction rate of colloidal iron oxides could be up to 2 orders of magnitude more rapid than the bulk mineral of identical iron phase [39]. And the opinion that particle aggregation was a main factor for relatively low reduction rates is consistent with that as mentioned above. In addition, size effects appear to bring about nanoparticles to be less soluble [188] or more soluble [189] than the coarser particles. Thus solubility may be another rate-controlling factor for Fe(III) oxyhydroxides reduction [150]. For instance, the soluble ferric citrate was reduced 9 times faster than the ferrihydrite nano-colloids [39].

Generally, low solubility corresponds to high crystallinity, which can lower the reaction rates [150]. Thus, the crystalline bulk iron oxides like hematite or goethite are poorly reducible by IRB, compared with the amorphous ferrihydrite [27]. Given the crystallinity differences (or microheterogeneities), it was reported that the synthetic Fe(III) oxides seem to be less reducible than their geologic counterparts. The synthetic oxides can be reduced in a qualitative trend according to their surface area and free energy: hydrous Fe(III) oxide > goethite > hematite [24]. However, when the geologic Fe(III) oxide particles reached a sufficiently small size, their crystal structure might become negligible in determining the reduction rates of Fe(III) nanoparticles, in contrast to the bulk aggregates [39]. Obviously, the well crystalline minerals possess smaller surface areas and the effects of solubility and surface area cannot be distinguished severely [190]. The smaller particle size with increased surface area can also arise higher Fe(III) oxide bio-reduction activity [155]. Moreover, it was reported that the bioreduction rate of Fe oxides is strongly correlated with their specific surface areas, not responding intensively to the mineral thermodynamic properties or reactivity [191]. Specifically, because the Fe(III) reduction occurred at the outer cell membrane, the reduction rates should be associated with the available reactive surface area rather than the total particle surface area [39]. In other words, bioreduction rate of the aged NZVI particles reveal a strong linear correlation with the relative coverage of the IRB surface by nanoparticles [192].

Besides the four major characteristics of nanosized Fe(III) particles, there are other properties, such as their surface bonding environments [193], thermodynamic stability effects [194] or the internal and surface atomic structures [194], that probably influence the rate and extent of their bio-reduction by IRB. Additionally, the external environmental factors, e.g., solute ions [154] and electron shuttles as elaborated before, may exert influences on their bio-reduction. More specifically, the solute ions in the context may be adsorbed onto the surface of nanoparticles, leading to the mitigation of surface charges, increase of nanoparticle aggregation and finally weakening the rate and extent of Fe(III) nanoparticles [195]. Moreover, some ions like NO_3^- could be the coexisting electron acceptors that compete with ferric nanoparticles for IRB reduction [196].

4.3. Adverse impacts of the bio-reduction of iron oxides

Although the IRB can couple the reduction of iron oxide minerals with the oxidation of contaminants effectively, they may also exert some adverse impacts towards iron environment, such as engendering steel corrosion [133], environmental methylation [31] and contaminates desorption from the iron oxide nanoparticles [197,198]. Evidently, either the inhibitory or corrosive behavior of microbes was aroused at biofilmed metal surfaces where complex biofilm interacted with the shielding films [199].

Several studies have speculated DIRB probably played a part in environmental methylation [30,31]. It was reported that Fe(III) bioreduction by DIRB could stimulate the formation of MeHg, whose toxicity was stronger than any other Hg species like Hg^0 and Hg^{2+}. Beyond question, the occurrence of the stimulative reaction was

hazardous to the health of organisms. Besides, mercury methylation and Fe(III) reduction might compete to occur, leading to the decline of Fe(III) reduction rate. However, on account of the mercury complexation and low availability, the mercury methylation rate could be restrained with high Fe(III) additions. Yet, the risk of mercury methylation should still be noticed in the process of Fe(III) bio-reduction by DIRB.

The redox transformations of Fe(III) oxide nanoparticles, which were considered to have high sorption capacities for numerous metals and metalloids such as As(V), Cu(II), Hg(II), and Zn(II), could significantly influence the transport of contaminants and their environmental fate [32]. Previous studies have paid massive attention to the arsenic desorption from the bio-reduction of the available Fe(III) oxide nanoparticles by DIRB [194,197,198]. As reported, As(V)-treated NZVI was gradually turned into magnetite/maghemite corrosion products over 90 days and a larger fraction of As(V) was immobilized in the crystalline phases after aging, involving surface complexation reactions in which the oxygen moiety of arsenate replaced an hydroxyl group on the iron oxide surface to form an inner-sphere complex [7,134]. However, DIRB such as *Shewanella alga strain BrY* could drive arsenic mobilization from a crystalline ferric arsenate (i.e., scorodite) as well as from the sorption sites within sediments via dissimilatory reduction of Fe(III) to Fe(II) [197]. Additionally, microbial cells and the extracellular polymeric substances (EPS) secreted could also mobilize arsenic from Fe(III)-(Hydr)oxides because of the competitive interactions between the adsorbed arsenic and the functional groups of cell surface molecules and EPS coordinated at Fe(III) mineral surfaces [198]. Moreover, both the surface-bound As(V) and the released As(V) might be further reduced directly by arsenic-reducing bacteria or indirectly through chemical reduction (e.g., by H_2S) to As(III) [200]. The product As(III), which is even more toxic than As(V), has a lower affinity for ferrihydrite, goethite, and hematite at the near-neutral pH [134,198]. Therefore, during the interaction between the As(V)-treated iron oxide nanoparticles and DIRB, both As(V) and As(III) could potentially be released into the environment; especially for As(III), which has the higher desorption potential and toxicity, can cause a more serious threat to environmental health.

5. Conclusions and future challenges

In our opinion, the impact of NZVI on bacterial activity and growth is dose- and species-dependent, meanwhile, it can be largely influenced by the environmental conditions. Besides the commonly known toxic effect of NZVI on the bacterial growth, the engineered NZVI particles could also possibly stimulate the growth of some bacteria by creating suitable environmental conditions for the bacterial activity. Given lots of microbial communities in the ecological system can contribute to the contamination remediation, matching the relevant microflora with appropriate dose of NZVI can eliminate the contaminant more efficiently and quickly, which would also achieve more substantial cost-effect. While the engineered NZVI particles are gradually aged in the field application, the produced Fe(III) oxides can be utilized by DIRB and reconverted to Fe(II). This could possibly lead to two different results: i) the bio-generated Fe(II) would act as a reductant to reduce more contaminants; and ii) the bio-induced dissolution of Fe(III) oxides could result in the remobilization of the previously entrapped contaminants.

Although NZVI technology has been widely used and developed in the past 15 years, it is still being suspected of its reliability as well as eco-safety. A lot of studies have probed the impacts of NZVI on the microbial activity, yet a fundamental understanding of the interactions between NZVI and microorganisms is still not obtained. Research and development in this field are essential to conquer or diminish these suspicions, and to expand the field application of NZVI technology. Based on this review, the crucial aspects for future research are proposed as follows:

(1) Assessing the impact of NZVI in real subsurface environment (i.e., groundwater and soil system with different physicochemical properties). Most experiments were conducted in pure bacterial culture, deionized water or a single model soil, which does not reflect the real situation;
(2) Development of new approaches to probe the underlying mechanism for the cytotoxicity of NZVI and the cellular defense mechanism of those microbes susceptible to NZVI (e.g., isotopic tracer method);
(3) Further development of multifunctional surface modifiers that enable to preserve the unique physicochemical property of NZVI, and that are nuisance-free, readily biodegradable to bacteria;
(4) Development of new bio-assessment tools in both laboratory and field scales to elucidate the influence of NZVI on microbial community structure, as well as specific bacterial populations;
(5) The NZVI-microbe synergetic technologies developed are still in as stage of bench- or pilot-scale experiments. It is necessary to verify their practicability in field-scale, meanwhile, to ensure they are safe, effective, sustainable and low-cost;
(6) Design, classification and cost-benefit test of various NZVI-microbe synergetic technologies for different application fields in appropriate conditions and expand the utilization of NZVI-based technologies;
(7) Tailored reaction pathway of pollutants (e.g., chlorinated organic contaminants, nitrate) degraded by NZVI-microbe synergetic system to avoid the formation of noxious byproducts;
(8) Gain insight into the bio-transformation process of NZVI during its aging, and clarify its converted products and secondary phase, then explore potential utilization of these NZVI-related particles for contamination abatement;
(9) Better understanding of the interactions between DIRB and iron-containing sludge generated from the process of pollutants sequestration by NZVI, and avoiding the release of contaminants into the environment, ultimately reaching safe disposal.

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