# Synergetic Interactions of Nanoscale Zero-Valent Iron (nZVI) and Anaerobic Bacteria in Groundwater Remediation: A Review

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**Abstract: During the last century, large scale production of halogenated organic compounds and heavy metals, specifically by industrial processes, and the inappropriate management of those products caused a wide spreading of a variety of hazardous contaminants into the environment including a massive contamination of the groundwater. Their presence and persistence have significantly influenced human health and the environment. Recently, many technologies have been employed in order to reduce their impacts. However, the majority of those technologies did not achieve the target, because of their high cost and low efficacy in the reduction of contaminants. Nevertheless, a new technology of synergetic interactions of (nZVI) zero-valent iron nanoparticles with two types of anaerobic bacteria; the organohalide respiring bacteria (OHRB) and sulfate-reducing bacteria (SRB), have been investigated as a promising technology for *in-situ* groundwater remediation. This powerful technique was successfully utilized for the reduction of pollutants and converted to environmentally benign forms. This article reviews and emphasizes the coupling effect of (nZVI-OHRB) and (nZVI-SRB) on the remediation process of contaminated sites, in addition to a detailed illustration of the mechanism of the integration of (nZVI-OHRB) and (nZVI-SRBs), and discussion of the influencing factors on the integrated system. Actually, the technology presented here, though proven successfully, needs more case studies to better understanding of the interactions between microorganisms and nZVI, as well as with the surrounding environment for a better efficacy and finding the best solutions.**

**Keywords: Chlorinated (Halogenated) Solvents, Groundwater Remediation, Heavy Metals, Microbial Reductive Dehalogenation, Nanoscale Zero Valent Iron (nZVI), Organohalide Respiring Bacteria (OHRB), Sulfate-Reducing Bacteria (SRB)**

## 1. Introduction

During the last century, short-chain chlorinated aliphatic hydrocarbons ($C_1$-$C_3$) had been manufactured in large amounts and widely utilized in industrial, agricultural, domestic, and military applications. The extensive use of chlorinated aliphatic hydrocarbons is primarily based on their proper characteristics consisting of low cost, easily accessible, a distinction as solvents, chemically stable, and safe toward the fire. Many chlorinated solvents are non-flammable and do not produce explosions

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when introduced to the air. The huge use, careless application and storage, lack of awareness of health effects and environmental risks, and the lack of regulations over many years of considerable use caused extensive-ranging groundwater pollution (Löffler, Ritalahti, & Zinder, 2013).

Chlorinated aliphatic hydrocarbons, including chlorinated ethanes and ethenes, had been excessively used as metallic degreasers and cleaning agents, solvents and adhesives, and reagents in numerous industries. These compounds, 1,1,1-trichloroethane (1,1,1-TCA) was recognized as an ozone-reducing compound under the Montreal Convention of 1987, and its utilized was weed out beginning in 1989 (Yang, Previdsa, Edwards, & Sleep, 2020). With the extensive industrialization and urbanization which have come about over the previous century, pollution of the aquatic environment with organic compounds and heavy metals contamination has become a solemn universal problem. Heavy metals and metalloids are frequently present in water as a result of anthropogenic activities (Wu et al., 2020). Heavy metallic contamination has constantly been an extreme environmental problem due to its characteristic properties such as extreme toxicity, non-degradability, and bioaccumulation with the food chain (Zhao, Li, Xiao, Peng, & Fan, 2020). Sediment, as an essential component of aquatic ecosystems, is a basin of heavy metals from the water via deposition, adsorption, and biological activities, therefore the content of heavy metals in sediment maybe hundreds of thousands of times that in water, despite sediment performs crucial roles in the transport, speciation transformation and food chain processes of heavy metals in aquatic ecosystems. The heavy metals collected in sediment can also tend to be released into the aquatic environment once more when the sediment is disturbed through geogenic or anthropogenic activities, leading to extreme threatens to the environment and human health (Zhao et al., 2020).

In reality, the use of anaerobic bacteria in bioremediation for chlorinated ethenes such as tetrachloroethene (PCE) and trichloroethene (TCE) via reductive de-chlorination to ethene was restricted to the handling of dissolved-phase plumes rather than source areas., which are associated with the release of dense non-aqueous phase liquids (DNAPLs).(Hood et al., 2008). However, the realized limitation of bioremediation for the remediation of source areas is the belief that microbial activity is inhibited by the high concentrations of PCE and TCE within source areas and the prediction that biodegradation rates were so slow to significantly influence the rate of pollutant mass removal. Nevertheless, various studies have conducted that the activity of dechlorinating microorganisms is unchanged at the chloroethene concentrations that are much higher than those commonly noticed in source areas (Hood et al., 2008).

Bioremediation is affordable and would not yield secondary contamination during the remediation process which is considered as a potential process for in-situ groundwater treatment (Lacina et al., 2015; Wei et al., 2012). In the subsurface climate, the anaerobic bacteria could oxidize carbon as a source of energy and minimize pollutants through co-metabolism (Lacina et al., 2015; Nemecek et al., 2016). Because of the longevity of biological remediation and low reduction rates to persistent organic pollutants and the inadequate energy source this process is also in need of improvement (Kocur et al., 2016; F. Liu et al., 2018). Stimulation of the growth of indigenous microorganisms (bio-stimulation) or inoculation of non-native halogenated hydrocarbons degrading bacteria (bio-augmentation) has been recognized as successful steps to accelerate the detoxification of the contaminated site with minimal impact on the ecological system (Tanzadeh, Ghasemi, Anvari, & Issazadeh, 2020). While bioremediation offers an excellent and versatile recovery method for the various contaminants, it is inefficient to deal with high pollutant concentrations, xenobiotics, or refractory compounds, resulting in unsustainable treatment and recovery times (Mapelli et al., 2017). One of the most promising alternatives in groundwater, soil and wastewater remediation for heavy metal immobilization is expected to be sulfate-reducing bacteria SRB (Zhao et al., 2020).

As an important reducing agent, nanoscale zero-valent iron (nZVI) can reduce inorganic and organic contaminants as well as heavy metals (Diao et al., 2018; Dong et al., 2018; Phenrat, Saleh, Sirk, Tilton, & Lowry, 2007; Shi, Zhang, Wang, & Fan, 2018; T. Wang, Lin, Chen, Megharaj, & Naidu, 2014; Zhang, 2003). Nanoscale zero-valent iron nZVI particles have a diameter less than (100 nm), nZVI particles have a core-shell structure produced by reaction with water and oxygen. The inner core is made of non-oxide ($Fe^0$) and the outer shell is made of (hydr)oxide layer, the thin and distorted layer of oxide allows the electron to pass (1) directly from the metal through defects like pits or pinholes (2) via the oxide conduction band, impurity, or localized band indirectly (3) from sorbed or ferrous structural, thereby preserving the particle's potential for contaminant reduction (Li, Elliott, & Zhang, 2006). The outer layer of (hydr)oxide can also serve as an effective adsorbent for different pollutants, including heavy metals (O'Carroll, Sleep, Krol, Boparai, & Kocur, 2013). Last years the technique of coupling nZVI with functional anaerobic bacteria has been considered one of the most promising techniques for in-situ groundwater remediation (Chen et al., 2012; Kocur et al., 2015; Kocur et al., 2016; Nemecek et al., 2016).

The purpose of this review is to present the recent developments of synergetic interactions of nanoscale zero-valent iron (nZVI) and anaerobic bacteria (OHRB-SRB) in-situ remediation of chlorinated solvents and heavy metals and demonstrates the principles of integration of bio-nano remediation technology and the cooperation effect and mechanisms of (nZVI) and (OHRB and SRB) in subsurface remediation. Also, the influencing factors on the synergetic of (nZVI) and (OHRB and SRB) in the remediation process is discussed.

## 2. Principles of Bio-Nano Remediation Technology

Approximately 10 million tons of poisonous chemical compounds are released by industry annually (Avio, Gorbi, & Regoli, 2017; Thompson & Darwish, 2019). These compounds further react after release and form different chemicals, such as polychlorinated dibenzo-p-dioxins or polychlorinated dibenzofurans, which are by-products produced of certain chemical processes containing chlorine atoms (Vázquez-Núñez, Molina-Guerrero, Peña-Castro, Fernández-Luqueño, & de la Rosa-Álvarez, 2020). Despite their cytotoxicity, the significant variations in physicochemical properties of these chemical compounds and multiple interactions with biotic and abiotic environmental factors, such as inorganics, microorganisms, plants, animals, water, minerals, organic matter, air, etc. The successful application of remediation technologies has been complicated (Hurtado, Montano-Chávez, Domínguez, & Bayona, 2017; Jeon, Murugesan, Baldrian, Schmidt, & Chang, 2016). The combined use of nanotechnology with biotechnologies could provide a step-change in remediation capability, prevent intermediate processes and increase the degradation rate (Fulekar & Pathak, 2017; Kang, 2014).

### 2.1 The Concept of (nZVI) Mediated Reductive Dehalogenation and Heavy Metals Removal

The core-shell organized nZVI involves inner nZVI and outer (hydr)oxide shell surface, which offers bulk-reducing mediators for de-chlorination of organohalide contaminants (Martin et al., 2008; Matheson & Tratnyek, 1994; Nurmi et al., 2005). The electrons transferred to most organohalides from nZVI can catalyze the reductive dehalogenation process directly (Novak, 1998; S. Wang et al., 2016; Weathers, Parkin, & Alvarez, 1997). Based on the molecular orbital analysis, with an increased number of halogen substituents (electron-withdrawing groups), both the (HOMO) and (LUMO) of polybrominated diphenyl ethers (PBDEs) are increasing. and highly halogenated Organohalides exhibiting an increased affinity to the electron respond to nucleophiles or electron donors, an event confirmed by experimental proof (Hu et al., 2005; Liu, Wang, & Zhou, 1994; Luo, Hu, Zhuang, Wei, & Huang, 2013; Wang et al., 2016; Zhao, Tao, & Zeng, 2008). nZVI catalyzes abiotic halogen elimination, from a broad variety of organohalides, involving chloroethenes, chloroethanes with the exclusion of 1,2-dichloroethane, pentachlorophenol, chlorobenzenes, polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers and polychlorinated dibenzodioxins (PCDDs) (Cao, Xu, Tang, Tang, & Cao, 2011; Chen, Al-Abed, Ryan, & Li, 2001; Kim, Tratnyek, & Chang, 2008; Li et al., 2007; Lowry & Johnson, 2004; Song & Carraway, 2005; Tso & Shih, 2015; Wang et al., 2016). As a powerful reducing agent, the reduction potential ($E^o$) = - 0.440 V, nZVI reduces chlorinated compounds by reducing dehalogenation reaction at the same time itself is oxidized according to the following reactions (Arnold & Roberts, 2000; Galus, Bard, Parsons, & Jordan, 1985; Matheson & Tratnyek, 1994; O’Carroll et al., 2013; Orth & Gillham, 1995; Roberts, Totten, Arnold, Burris, & Campbell, 1996).

$$Fe^0 \longrightarrow Fe^{+2} + 2e^- \quad E^o = -0.440V \qquad (1)\ Oxidation\ of\ nZVI$$

$$ClCH = CCl_2 + H^+ + 2e^- \longrightarrow ClCH = CHCl + Cl^- \qquad (2)\ Hydrogenolysis$$

$$ClCH = CCl_2 + 2e^- \longrightarrow CH \equiv CCl + 2Cl^- \qquad (3)\ \beta - Elimination\ reaction$$

$$CH_2 = CCl_2 + 2e^- \longrightarrow CH_2 = C : + 2Cl^- \qquad (4)\ \alpha - Elimination\ reaction$$

$$Fe^0 + 2H_2O \longrightarrow Fe^{+2} + 2OH^- + H_{2\,(gas)} \uparrow \qquad (5)\ Oxidation\ of\ nZVI\ by\ water$$

Despite the above reaction, hydrogenation reaction could happen especially after α-Elimination and β-Elimination reactions, eventually led to the increasing degree of saturation compounds. The hydrogen gas and two electrons produced from reducing water and oxidizing nZVI, both electrons and hydrogen have participated in complete dehalogenation of halogenated organic compounds (O’Carroll et al., 2013). The redox potential E0 of the metal contaminants has a significant impact on nZVI mechanisms to remove of heavy metals contaminants, in such a way, metals with E0 greater than Fe0 like Cr, As, Cu, U and Se are favorably eliminated by reduction and precipitation. On the other hand, metals with redox potential E0 of less or similar to Fe0 like Cd and Zn are removed by adsorption mechanism, while metals with somewhat more positive E0 than Fe0 (for example, Pb and Ni) can be eliminated by both reduction and adsorption. However, oxidation and complexation by iron (hydr)oxides are the other probable reaction depend on the environmental situations e.g., pH, Eh, etc. (Arnold & Roberts, 2000; Matheson & Tratnyek, 1994; O’Carroll et al., 2013; Orth & Gillham, 1995; Roberts et al., 1996).

### 2.2 High Efficiency Removal of Halogens and Heavy Metals

ZVI's efficacy depends greatly on the characteristics surface area of the particle on which the reduction reaction mainly happens (Wang et al., 2016; Weber, 1996). In contrast, mZVI, nZVI (less than 100 nm in diameter) has a larger surface area and higher surface reactivity. For instance, nZVI with a 20-40m2/g surface area provided 10-1000 times more reactive than micro-scale mZVI with a surface area of less than 1m2/g (Wang & Zhang, 1997). Furthermore, the longevity and reactivity of the nZVI surface are further improved by the addition of secondary metal to form a bimetallic nanoparticle, for example, doping Fe0 with palladium or nickel as a metallic catalyst enhanced the rate of heavy metal reduction as well as increased the rate of dehalogenation reactions of a wide range of halogenated aliphatic and aromatic hydrocarbons, such as chloroethenes, polychlorinated dibenzodioxins (PCDDs), polybrominated diphenyl ethers (PBDEs), chlorophenols, polychlorinated biphenyls (PCBs) (Doong & Lai, 2005; Fang, Qiu, Chen, & Qiu, 2011; Kim et al., 2008; Kim & Carraway, 2000; Kim, Shin, & Ko, 2004; Wang et al., 2016; Zhuang, Jin, & Luthy, 2012).

The following five points describe the removal mechanisms by nZVI of different metals and heavy metals

| | |
|---|---|
| 1. Precipitation | Cu, Pb, Cd, Co, Zn. |
| 2. Co-precipitation | Cr, As, Ni, Se. |
| 3. Adsorption | Cr, As, U, Pb, Ni, Se, Co, Cd, Zn, Ba. |
| 4. Reduction | Cr, As, Cu, U, Pb, Ni, Se, Co, Pd, Pt, Hg, Ag. |
| 5. Oxidation/reoxidation | As, U, Se, Pb. |

Certain metals react with nZVI through several mechanisms, for example, Se and As, were removed by Co-precipitation, Adsorption, Reduction and Oxidation/reoxidation (Li, Cao, & Zhang, 2008; Manning, Kiser, Kwon, & Kanel, 2007; O'Carroll et al., 2013; Ponder, Darab, & Mallouk, 2000).

### 2.3 nZVI as an Environmentally Friendly and Cost-Effective Agent

The production source of nZVI for environmental remediation applications is essential, for instance, nZVI green synthesized from pomegranate juice extraction, green tea and oak leaves, have been developed that are environmentally friendly and economically favorable. The nZVI produced using these green techniques shows beneficial characteristics, including inexpensive, decreased cytotoxicity and significantly reduced effects on the community of microbes. A cost comparison case study in New Jersey on the remediation of trichloroethene and tetrachloroethene in contaminated sites assessed that using pump-and-treat, PRB, and nZVI tactics would charge nearly $4.16 million, $2.2 million and $0.45 million individually, the cost comparison study showed that nZVI would have cost savings of over 80% on both methods (Hoag et al., 2009; Karn, Kuiken, & Otto, 2009; Kharissova, Dias, Kharisov, Perez, & Perez, 2013; Machado, Pacheco, Nouws, Albergaria, & Delerue-Matos, 2015; Nadagouda, Castle, Murdock, Hussain, & Varma, 2010; Wang et al., 2016)

### 2.4 nZVI Limitations

#### 2.4.1 Long-Distance Transportation Deficiency of nZVI

Due to large surface area and high surface reactivity, nZVI nanoparticles lead to naturally aggregated, producing large flocs, and bulky micro-scale structures. The nZVI size, shape, concentration, composition of the solution, magnetism, and surface chemistry have significantly influenced the aggregation of nZVI to reach a steady state. nZVI injected to groundwater at high concentration may interact with groundwater constituents such as organic and inorganic constituents, thus restraining the transportable distance of nZVI in groundwater without reduction of target contaminants like heavy metals and halogenated organic compounds. In order to solve this issue, coating of nZVI by organic polymer or nZVI bimetallic nanoparticles will lead to increasing the longevity, stability, electrostatic and electrosteric stabilization of nZVI and increase the adsorption ability and selectivity towards target contaminants. For example, polymers include chitosan, polyelectrolyte-olefin maleic acid, poly aspartate, and carboxymethyl cellulose (CMC) which are biodegradable. It may even be a slow-releasing source of Carbon for fermentation bacteria around the environment and benefit (OHRB)organohalide respiring bacteria (Elliott & Zhang, 2001; He, Zhang, Qian, & Zhao, 2009; He & Zhao, 2005, 2007; Johnson, Johnson, Nurmi, & Tratnyek, 2009; Kim, Phenrat, Tilton, & Lowry, 2012; Kocur et al., 2015; Li, Greden, Alvarez, Gregory, & Lowry, 2010; Petosa, Jaisi, Quevedo, Elimelech, & Tufenkji, 2010; Phenrat et al., 2010; Phenrat, Kim, et al., 2009; Phenrat, Liu, Tilton, & Lowry, 2009; Phenrat et al., 2007; Saleh et al., 2008; Tang & Lo, 2013; Xiu, Gregory, Lowry, & Alvarez, 2010).

### 2.4.2 Low Electron Transfer Efficiency from nZVI to OHRB and SRB

It has been illustrated under extremely redox conditions, nZVI is vulnerable to erosion in aqueous media. Hydrogen ions (H+) dissociated from water act as electron acceptors and bid for the reduction equivalents of nZVI with organohalides and sulfate-reducing bacteria (SRB) (Dong et al., 2019; Naresh Kumar, Chaurand, Rose, Diels, & Bastiaens, 2015; S. Wang et al., 2016; Xiao, Sheng, Mu, & Yu, 2013). As demonstrated in the below equation:

$$Fe^{0} + 2H_2O \longrightarrow Fe^{+2} + 2OH^{-} + H_{2\,(gas)}\uparrow \qquad (5)\ Oxidation\ of\ nZVI\ by\ water$$

A significant source of hydrogen is the corrosion of nZVI in an anaerobic aqueous medium. Over 80% of the total electrons from iron corrosion transferred in the form of H2 to the surrounding environment, that is determined by Farrell et al. (2000)(Farrell, Melitas, Kason, & Li, 2000; S. Wang et al., 2016). Nevertheless, H2 is not an efficient reducing agent in the absence of an effective catalyst and will not specifically lead to the reductive dehalogenation of organohalides (L. J. Matheson & P. G. Tratnyek, 1994). In addition, iron corrosion and abiotic reductive dehalogenation processes are slowed by extreme H2 buildup at the Fe0 surface (J. L. Chen et al., 2001). The formed H2 will finally be used as an electron donor to reduce the dehalogenation of halogenated organohalides by means the processes of (OHRB) and (SRB), which increases the overall efficiency in electron transfer from nZVI to organohalides and sulfate-reducing bacteria (Dong et al., 2019; Naresh Kumar et al., 2015; Rysavy, Yan, & Novak, 2005; Wang et al., 2016; Xiao et al., 2013).

### 2.4.3 Incompletes Remove of Heavy Metals and Halogenation Process

The process of dehalogenation of highly halogenated aromatic hydrocarbons to slightly halogenated species is a multistage process, which progresses on the surface of nZVI, the reductive dehalogenation activities are controlled by molecular properties of the organohalides, involving heat formation (Hf), Gibbs free energy formation and LUMU energies (ELUMU). In general, extremely halogenated compounds with high (Hf) and low (ELUMO) are more susceptible than their less halogenated analogous to reductive dehalogenation. For instance, it has been ascertained the rate constant values (Kobs) of debromination (PBDE) analogues reduces from ($1.41 \times 10^{-1}\ d^{-1}$) to ($1.80 \times 10^{-3}\ d^{-1}$) as the number of bromines reduces from (10) to (1)(Keum & Li, 2005). This may lead to the accumulation of more toxic byproducts as tri- to hepta-BDEs, by the reduction of nZVI-mediated BDE 209 debromination (Keum & Li, 2005; Liang et al., 2014). The elimination of halogens from organohalides depends on the number of halogens as well as on the positions of the halogen atoms. As stated by Keum and Li (2005), the debromination of PBDEs by nZVI with bromine elimination from each position follows a general trend: ortho- > meta- > para-bromines. Less-brominated PBDEs represent more danger to the environment, and subsequently, further treatments are expected to accomplish total debromination (Keum & Li, 2005; Wang et al., 2016).

## 2.5 (nZVI) - (OHRB and SRB) Interactions and Their Effects on Indigenous Microbial Communities

nZVI cytotoxicity is the major reciprocal influence between nZVI and microorganisms. nZVI reactivity has a significant impact on anaerobic micro-organisms activities and their microbial products, so the generation of selective growth conditions for hydrogenotrophic anaerobes by nZVI depends on the nZVI concentration and reactivity (He, Zhao, & Paul, 2010; Kirschling, Gregory, Minkley, Lowry, & Tilton, 2010; M. Zhang, He, Zhao, & Hao, 2011). nZVI with high reactivity may cause microorganism cell toxicity (Chaithawiwat et al., 2016; Z. Li et al., 2010; Xiu, Jin, et al., 2010). The main mechanisms contributing to nZVI cytotoxicity are cell membrane disruption and oxidative stress derived from the generation of Fe2+ and reactive oxygen species by nZVI under aerobic or aerobic/anaerobic alternative conditions. Also, the inhibitive impacts are strain-explicit and partly rely upon cell structures and development stage (Auffan et al., 2008; Chaithawiwat et al., 2016; Wang et al., 2016). Direct contact among nZVI and microorganisms is required for nZVI to be toxic in anaerobic conditions (Z. Li et al., 2010). As a result, surface treatments with functional polymers reduce the toxicity of nZVI to surrounding microorganisms (He et al., 2010; Xiu, Gregory, et al., 2010). H2 produced during nZVI corrosion and the fermentation of organic substrates, provides a good source of energy for many hydrogenotrophs in subsurface environments, including organohalide-respiring bacteria, homoacetogens, sulphate reducers and hydrogenotrophic methanogens (Gu et al., 1999; Kirschling et al., 2010; Lampron, Chiu, & Cha, 2001; Novak, 1998; Wang et al., 2016; Weathers et al., 1997). The injection of carboxymethyl cellulose CMC/nZVI, a polymer-coated nZVI, enhances the development of a microbial community capable of actively dehalogenation of organohalide contaminants. In this case, carboxymethyl cellulose (CMC) and other organic substrates act as excellent sources of carbon (He et al., 2010).

## 2.6 Principle of (OHRB) Reductive Dehalogenation

Microbial reductive dehalogenation is usually mediated by organohalide respiring bacteria. using multiple sets of functional enzymes to transfer electron pairs from H2 and other organic compounds (electron donors) to organohalide halogen removal electron accepters (Mohn & Tiedje, 1992; Seshadri et al., 2005). By metabolic dehalogenation through organohalide respiration, organohalide-respiring bacteria obtain energy for growth, which is favoured over co-metabolic dehalogenation due to easier implementation, maintenance and control of the metabolic process (Loffler & Edwards, 2006; Rittmann et al., 2006). Reductive dehalogenase (RDase) is the key enzyme catalyzing halogen elimination in organohalide-respiring bacteria. which uses RDase-bound cobalamin to attack the halogen atom by electron transfer from Cob(I) alamin reduced by a [4Fe-4S] cluster to organohalides. However, different mechanisms may be used by dehalogenations of different bacterial ancestries and remain indefinable in binding organohalide-respiratory bacteria (Bommer et al., 2014; Goris et al., 2015; Kublik et al., 2016; Louie & Mohn, 1999; Parthasarathy et al., 2015; Payne et al., 2015).

## 3. The Cooperative Effect of nZVI and OHRB - SRB

nZVI, as a powerful reducing agent, has been commonly used for the direct reduction of halogenated hydrocarbons. nZVI is oxidized into Fe+2 during this chemical process and the halogenated organic compounds are reduced to alkenes and alkanes (Velimirovic, Simons, & Bastiaens, 2015; Xu, Wang, & Lu, 2014). Though highly halogenated organic compounds can be degraded rapidly and efficiently with nZVI. nZVI does not normally reduce the lower halogenated organic compounds (Xie et al., 2017; Xu et al., 2014). This deficiency can be compensated by Dehalococcoides because they are more effective for halogen elimination from the lesser halogenated substrate and thus can thoroughly reduce highly halogenated organic solvents by connecting nZVI. Furthermore, the existence of nZVI could create appropriate reduction conditions for OHRB development, as described above. In addition, the hydrogen formed by the erosion of nZVI might be an electron donor to the breakdown of hydrogenotrophic bacteria or dehalo-respiring bacteria (Wang et al., 2016; Xiu, Jin, et al., 2010; Xu et al., 2014).

The ZVI analysis could decrease the higher brominated polybrominated diphenyl ethers (PBDEs) to lower brominated PBDEs, which could be further degraded by Dehalococcoides sp. strain CBDB1. The complete degradation of PBDEs was then accomplished by integrating ZVI with Dehalococcoides sp. strain CBDB1. The degradation of PCE by combined nZVI and Dehalococcoides strains was investigated and the consequences revealed that Fe0 might be an electron donor in the dehalogenation of PCE by Dehalococcoides strains, and the PCE could be entirely reduced to ethene in this integrated method (Dong et al., 2019; Rosenthal, Adrian, & Steiof, 2004). Regarding SRB, the nZVI might have a positive influence on SRB growth and activity (Xiao et al., 2013). The highly reducing and alkalescent state produced by (Fe0) corrosion would therefore be appropriate for SRB growing, hydrogen(H2) and (Fe+2) iron can act as an electron donor for sulfate reduction by sulfate reduction bacteria (SRB) (Naresh Kumar et al., 2015).

### 3.1 Mechanism of Reduction of Contaminants by nZVI-OHRB System

In the combined nZVI and Dehalococcoides strain treatment system, there are primarily three routes to supply electrons to organic halogens. First of all, the nZVI could supply electrons for organic halogen reduction. β-elimination is the key mechanism of this chemical reduction by nZVI, and chloroethylene and ethyne are the degradation intermediates (Arnold & Roberts, 2000; Liu, Majetich, Tilton, Sholl, & Lowry, 2005; Liu, Phenrat, & Lowry, 2007; Velimirovic et al., 2015). Secondly, electrons could also be generated by the hydrogen created by the corrosion of nZVI. Besides, the lactate could be used as a carbon source by certain Dehalococcoides strains, and the lactate could provide hydrogen in the bacteria's metabolism (Carr & Hughes, 1998; Gerritse et al., 1999). Nevertheless, the water solubility of hydrogen (H2) is very limited, which would be a barrier to the reduction of aimed pollutants in groundwater and may thus have slight effect on the process of degradation (Kumar et al., 2014). Thirdly, by oxidizing organic carbon to carbon dioxide and water, the Dehalococcoides strains may supply electrons to eliminate the organic halogens via microbe co-metabolism, the target contaminants could be completely removed (Arnold & Roberts, 2000; Velimirovic et al., 2015).

Typically, the microorganisms would have a lag phase at the early stages of this combined chemical and microbial degradation treatment process and the microorganisms could never quickly degrade the target contaminants (Shanbhogue, Bezbaruah, Simsek, & Khan, 2017; Wang et al., 2016). Consequently, the nZVI chemical reduction may be the key mechanism to degrade organic halogens throughout that whole lag phase of bacteria. Following the lag phase, microorganisms’ function would be recovered and the products produced during nZVI chemically reduced could be degraded entirely (Dong et al., 2019). The successful debromination of decabromodiphenyl ether using the incorporation of Dehalococcoides sp. strain CBDB1 and nZVI was recorded by Xu et al. (2014). In this step, the bacteria were able to convert the lower halogen compounds formed by ZVI from the chemical reduction phase to ethene and ethane, and then the absolute degradation is accomplished (H. Dong et al., 2019). Figure (1) explains the synergetic degradation mechanism of halogenated organic compounds by (nZVI-OHRB) system (Dong et al., 2019).

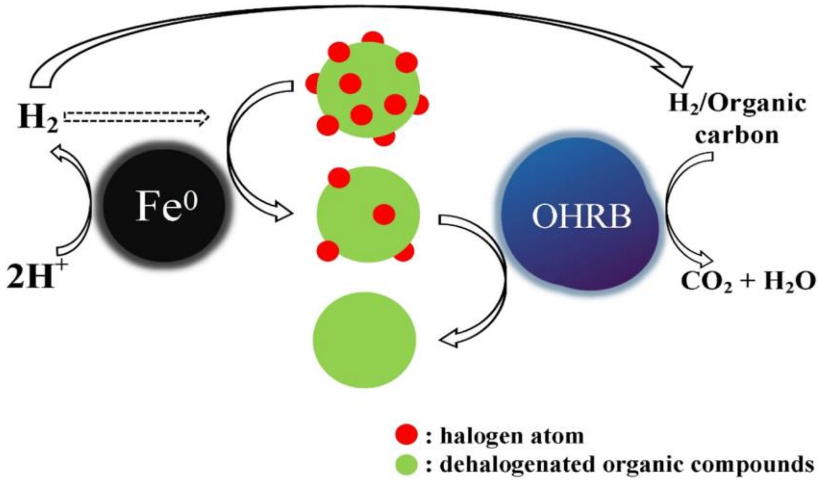

Figure 1: Degradation mechanism of halogenated organic compounds by (nZVI-OHRB) system (Dong et al., 2019).

### 3.2 Influencing Factors on nZVI-OHRB System

Many considerations may have an effect on contaminants' degradation efficacy in the integrated treatment system (nZVI- OHRB) (Tan, Fontes, & Byrne, 2014; Velimirovic et al., 2015; S. Wang et al., 2016). These considerations include nZVI species and concentrations, inorganic ions, organic matter, pH and type of contaminants.

### 3.2.1 nZVI Physicochemical Properties and Their Concentration

The nZVI may have a negative impact on the bacteria by disturbing the cell membrane integrity and triggering the oxidative stress reaction. Therefore, nZVI is commonly considered to have a twofold effect, upon the degrading efficacy and activity of bacteria, which is determined by the concentration, particle size, and kind of nZVI (Tilston, Collins, Mitchell, Princivalle, & Shaw, 2013). Earlier experiments showed that nZVI toxicity of OHRB activity could be detected when nZVI levels were above 0.1g/L. The highest concentration of nZVI that the bacterial community could tolerate was 0.05 g/L, analyzed by Velimirovic et al. (2015). Likewise, Xiu et al. (2010b) observed that the expression of the tceA and vcrA reduction dehalogenase genes and further degradation efficiency of TCE could be inhibited by nZVI. Obviously, the high concentration of nZVI could hinder the combined (nZVI-OHRB) treatment systems. In addition, the size of nZVI could also have an impact on the activity of nZVI since the smaller size of nZVI could have a larger surface area and a higher surface reactivity and degradation activity. nZVI has been modified to enhance particle mobility in groundwater, and surface coating has been developed (Haoran Dong et al., 2017). While bare nZVI can inhibit microbial activity, by using surface-coated modified nZVI, the inhibitive effects could be alleviated (Dong et al., 2019; Xiu, Gregory, et al., 2010).

### 3.2.2 Organic and Inorganic Constituents

Organic compounds could have a double impact on degradation efficacy in the combined OHRB and nZVI treatment systems. Former research has shown that organic compounds could serve as an electron transfer mediator or adsorbent to facilitate the transfer of electrons or to reduce the reaction sites in the nZVI degradation process (Tan et al., 2014; Tratnyek, Scherer, Deng, & Hu, 2001). Humic acid (HA) is an essential source of natural organic compounds in groundwater, it is essential to investigate the impacts of HA on the degradation of halogenated organic compounds by (nZVI - OHRB) or nZVI and OHRB individually. The function of HA has two aspects to the degradation of the pollutants by nZVI and/or OHRB. On the one side, the adsorbed HA on the nZVI surface could prevent the reaction sites and inhibit the transmission of electrons in reducing organic halides (Tratnyek et al., 2001). For instance, the high concentration (500 µmol/L) of HA could have a negative effect on the degradation of TCE by Dehalococcoides strains (Zhang et al., 2011). In groundwater, some types of inorganic materials coexist with organohalides, and these inorganic materials may contest with organohalides to reduce equivalents or have passivating effects on nZVI. The effect of inorganic anions on the degradation rate of TCE was studied by (Liu et al., 2007; Wang et al., 2016) and the findings suggested that nitrate could contest for electrons with organic halides and thus reduce the degradation rates of TCE. Nitrate could be reduced by nZVI to nitrite and ammonium, and the ammonium formed by nitrate reduction is also toxic to bacteria (Hu, Wang, Tao, Wang, & Ding, 2011). The chloride, sulfate, bicarbonate and hydrogen phosphate could also form a complex with iron (hydr)oxides, and the passivation layer formed on the surface of the particles could reduce the reaction sites and thereby prevent the degradation rate of TCE (Liu et al., 2007). Thus, the affinity of these inorganic anions to iron (hydr)oxides determines this inhibiting effect. The inhibiting influence of these anions on TCE reduction dehalogenation, in turn, is hydrogen phosphate ions > bicarbonate ions > sulfate > chloride ions (Dong et al., 2019).

#### 3.2.3 pH and Organohalide Compounds

The pH value is very crucial for microbial activity and the optimal pH value for the growing of Dehalococcoides sp. strains is almost neutral. Mostly during nZVI reaction with water and halogenated organic compounds, the pH value would increase. The pH value might decline throughout the microbe co-metabolism process in the anaerobic degradation remediation system. In a combined nZVI and Dehalococcoides system, the pH value can be adjusted suitably to create an ideal condition for the growth of bacteria (Velimirovic et al., 2015). The degradation efficacy of PBDEs decreased with a decrease in bromines in the treatment system (nZVI-OHRB), and degradation of the ortho or meta position is likely to occur instead of para position due to the length of C-Br bonds.

### 3.3 The Synergetic Effect of Integrated nZVI-SRB System

The nZVI may have a positive impact on SRB development and activity. Normally, because of the undesirable environmental conditions and absence of carbon source or electron doner, SRBs may have a long interim phase in the site remediation. But, the addition of nZVI, could reduce the SRB delayed period and improve the remediation efficacy (Guo, Kang, & Feng, 2017). The integrated (nZVI-SRB) led to increasing the efficacy of heavy metal removal and precipitation stability of products (Yi, Bin, Yang, & Zou, 2009). In the integrated (mZVI-SRB) system, the precipitate formed was more stable than that in the SRB and mZVI individually systems. The more stable metal precipitates are, the harder it is to go back to groundwater (X. Li et al., 2016). Organic carbon could provide electrons for the reduction of sulfate by SRB in the combined (nZVI-SRB) remediation process, and hydrogen and ferrous ions derived from nZVI corrosion could also be used as the electron donor for the reduction of sulfate.

#### 3.3.1 Mechanism of Reduction of Contaminants by nZVI- SRB System

The four major pathways of removing heavy metals are involved in the combined (nZVI-SRB) remediation process. The following removal processes occur concurrently, but the key elimination method in the nZVI treatment system is reductive precipitation, and adsorption is the key elimination strategy in the SRB treatment system.

1. Reductive precipitation: (nZVI) could reduce certain heavy metals (e.g., $Ni^{+2}$, $Cu^{+2}$, $Pb^{+2}$, $Zn^{+2}$ and $Cr^{+6}$) and further produce insoluble metal precipitates to detoxify heavy metals and reduce ecological impacts.
2. Co-precipitation: ferrous ions could react with sulfide to produce iron sulfides, which are good adsorbents for heavy metals and halogenated organic compounds. Consequently, the other heavy metals could be reduced by the coprecipitation with iron sulfides.
3. Sulfide precipitation: With the SRB metabolites the heavy metals could be precipitated. The sulfate could be reduced to the sulfide ($H_2S$, $HS^{-1}$, $S^{-2}$) during the sulfate reduction by the metabolism of SRB which can then react with metal ions to produce the sulfide precipitates
4. Biosorption: The SRB cell membrane and extracellular polysaccharides could be in interaction with heavy metals directly and adsorbed by physical or chemical adsorption through the bacterial surface.

Figure (2) illustrates the cooperative degradation mechanism of halogenated organic compounds by nZVI-SRB system.

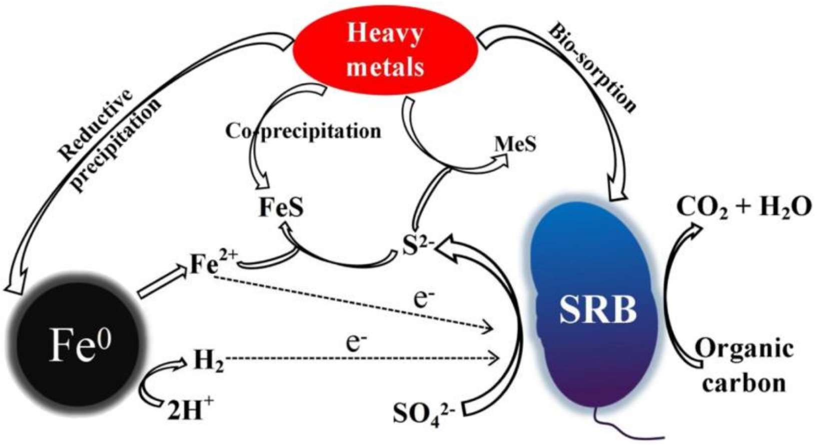

Figure 2: Summarized degradation mechanism of heavy metals by (nZVI-SRB) system (Dong et al., 2019)

## 4. Conclusions

The extremely efficient potential has been illustrated for the application of synergetic interactions of nZVI- (OHRB and SRB) for remediation of a broad spectrum of preference contaminants in bench, pilot, and case studies. However, this technology is a complicated application process, because it is influenced by a range of factors: (1) nZVI cytotoxicity, crystal phase, coating, size and shape; (2) the average pH and temperature; (3) the type of contaminants, stability and toxicity; (4) microorganism's biological nature, tolerance toward nZVI, pollutants, pH, temperature and energy source for metabolisms. So appropriate conditions should be considered to get an optimal efficiency for environmental remediation by integration of nZVI- (OHRB and SRB) system. Finally, the integration of nZVI- (OHR and SRB) could produce environmental benefits, due to low cost and environmentally friendly in comparison to the other techniques that have been used in groundwater remediation. Further research is required especially in a field study to better preceptive and thoughtful of the potential of synergetic interactions of nZVI- (OHR and SRB) for the subsurface remediation process.

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