Synthesis of nZVI/PVP nanoparticles for bioremediation applications

Anatoli Sidorenko, Tatiana Gutula, Dmitri Dvornikov, Mine Gül Şeker, Tuğçe Arı, Evgenii Gutul, Anatoli Dimoglod, and Ashok Vaseashta

Abstract

The objective of this investigation is to synthesize and investigate zero-valent iron (ZVI) nanoparticles (NPs) for bioremediation applications. The ZVI-NPs were fabricated by chemical reduction using a ferrous salt solution with poly(N-vinylpyrrolidone) (PVP), used as a stabilizer. The synthesis was conducted with and without ultrasonic treatment. The ZVI NPs were fabricated and characterized using scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray powder diffraction (XRD) analysis, and Fourier Transform Infrared Spectroscopy (FTIR). Experimental observations demonstrate that depending on synthesis conditions and coordination of stabilizers, NPs with different morphologies are formed. Colloidal solutions of the synthesized NPs were used in antimicrobial activity tests and biofilm formation assays for nine different control microorganisms: Escherichia coli (ATCC 25922), Pseudomonas aeruginosa (ATCC 15692), Enterococcus faecalis (ATCC 29122), Klebsiella pneumoniae (ATCC 29212), Staphylococcus aureus (ATCC 29213), Bacillus cereus (DSMZ 4312), Bacillus subtilis (ATCC 6633), and Candida albicans (ATCC 10231). All control strains did not show antibacterial effect against PVP-stabilized ZVI NPs synthesized without ultrasonic treatment. However, biofilm results show that the highest absorbance values of the micro-organisms were tested in control wells. Although B. subtilis, E. coli, and K. pneumoniae were observed during biofilm formation, B. cereus, S. aureus, and P. aeruginosa biofilm formation reduced noticeably by Fe0/PVP-US (A1) NPs. For control strains, such as E. faecalis and C. albicans, no biofilm formation was observed. For Fe0/PVP (A2) NPs, biofilm formation of B. subtilis, E. faecalis, E. coli, K. pneumoniae, P. vulgaris, and C. albicans demonstrated positive effect, and B. cereus, S. aureus, P. aeruginosa showed negative effect. A strategic utilization of nZVI-PVP nanoparticles showed a great potential for effective, efficient, and sustainable bioremediation applications.

Introduction

The environmental pollution due to anthropogenic contaminants is increasing and remains as one of the top global challenges of the 21st century to be addressed by scientists and policymakers. Anthropogenic changes and their impact on the natural environment are often not well understood due to the evolution cycle, and challenges and complexities related to differentiating natural variation from anthropogenic changes. With recent advances in nanomaterials, the pace of disruptive technological innovation has advanced from linear to parabolic. The material aspect of several innovations is based on novel and unique characteristics of nanomaterials due to its reduced dimensions and large surface area per unit mass (Vaseashta 2005), which can be useful in hazardous waste site remediation and contaminant reduction. Nanoscale zero-valent iron (nZVI) particles demonstrate the potential to rapidly transform many environmental contaminants to benign products and serve as a promising in situ remediation agent. Due to toxic impacts associated with pesticides, many recent
studies have focused on the decomposition of pesticides in aqueous media and soils using nanoparticles (NPs), since they can be injected where contamination remediation and reclamation of soils and lands is of utmost necessity.

A strategic approach to remediation and reclamation of soil investigation is based on the optimal use of natural processes that involve self-purification and self-regeneration, such as bioremediation and other similar techniques using new technologies, such as iron-containing NPs [magnetite (Fe3O4), goethite FeO(OH), hematite α-Fe2O3, and zero-valent iron (ZVI) Fe0] exhibiting pronounced redox properties (Asadishad et al. 2017; Basnet et al. 2016; Yin et al. 2019). It has been proposed that these NPs should be used for the remediation of both organochlorine pesticides and nitroaromatic pesticides. There are only a few studies on nitro pesticides that include trifluralin and are mostly focused on the degradation of the pesticides in water (Xie et al. 2017).

Here, a special experimental niche is applied using ZVI NPs, which has demonstrated an extremely high efficiency in the treatment to remove heavy metals and persistent organic pollutants (POPs) in wastewater. In particular, the use of iron NPs and nanocomposites is based on its remediation of residual pesticides [organochlorine dichlorodiphenyltrichloroethane (DDT), dichloro-diphenyldichloroethylene (DDE), dichlorodiphenyldichloroethane (DDD), and hexachloro-cyclohexane (HCH)] having potential toxic effects on soil microorganisms (Rostek et al. 2018).

Several researchers have concluded that the microbial community in soils mostly exists in biofilms. It was shown (Chaithawiwat et al. 2016; Fang et al. 2012; Rodrigues and Elimelech 2010) that 99% of bacteria in the natural environment are localized in biofilm matrix. It is further known that biofilms are complex communities of one or several types of interconnected microorganisms, which are held together by an extracellular polymeric substance (EPS) (Basnet et al. 2016). Along with the fact that bacterial communities are considered to have a pathogenicity factor, recent studies have shown that they play other roles as well. Biofilms can be involved in various catalytic processes as living catalyst systems (Yin et al. 2019) and soil biofilms play an important role in biogeochemical processes in soils (Kocur et al. 2016; Jamei, Khosravi-Nikou and Anvaripour 2013). A small number of studies are focused on the effect of silver, gold, palladium, and iron NPs on the formation of biofilms. The number of studies on the effect of ZVI NPs on the formation of biofilms is even smaller. Since the composition of a soil biofilm can include various types of bacteria, protozoa, fungi, and algae, which effectively interact with each other, the following control microorganisms were selected for our studies: *Escherichia coli* (ATCC 25922) representing Gram negative bacteria, *Pseudomonas aeruginosa* (ATCC 15692), *Enterococcus fæcalis* (ATCC 29122), *Proteus vulgaris* (laboratory isolates), *Staphylococcus aureus* (ATCC 29213) representing Gram positive bacteria, *Bacillus cereus* (DSMZ 4312), *Bacillus subtillis* (ATCC 6633) used as a soil microorganism, and *Candida albicans* (ATCC 10231) selected as a representative of fungal microorganisms. This investigation is focused on studying the effect of ZVI NPs of different morphologies on biofilm formation during their growth of selected control microorganisms.

**Materials and methods**

**Chemicals**

Laboratory-grade high-purity chemicals, such as iron(II) sulfate, FeSO₄ (purity ≥99.7%), saturated iron(III) chloride, FeCl₃ solution (purity ≥99%), poly(N-vinylpyrrolidone) (C₆H₉NO)n (PVP, MW: 40,000 avg.), and sodium borohydride, NABH₄ were purchased from Sigma-Aldrich, USA. Methanol (purity ≥99.9%) and acetone (purity ≥99.9%) used in conjunction with synthesis processes were also purchased from Sigma-Aldrich, USA. Deionized water (>18 MΩ) was used for the entire experimentation. All chemicals were used as received and without any additional purification.

**NP synthesis**

The method to prepare ZVI NPs using FeSO₄ and FeCl₃ is reported by Han et al. (2016), but was modified to include a chemical reduction
procedure. Low-molecular-mass PVP was used as the stabilizer. Synthesis was conducted at 15°C in an argon atmosphere under constant stirring for 4 h. NPs were synthesized with an ultrasonic treatment [FeO/PVP-US NPs (A1)] and without using ultrasonic treatment [FeO/PVP NPs (A2)]. The black powder of nZVI, prepared as described earlier, was separated from the host solution, washed with acetone and ethanol, and dried at 100°C.

**Characterization**

The ZVI NPs synthesized as described earlier were studied by Fourier-transform infrared (FTIR) spectroscopy using a Perkin Elmer spectrometer model Spectrum 100 – an optical system with data collection and having spectral range of 370–7800 cm⁻¹ using KBr pellets in sample compartment. A PANalytical Empyrean diffractometer, using CuKα radiation (λ = 1.936 Å) was used at an accelerating voltage of 45 kV and ~40 mA current for X-ray diffraction analysis in a range of 2θ = 10°–80° (with 2θ linearity better than ±0.01), at room temperature. Scanning electron microscopy (SEM) images were observed using a Quanta 200 electronic microscope operating at 30 kV with secondary and backscattering electrons in a high-vacuum mode, and transmission electron microscopy (TEM) studies were performed using a JEOL JEM-2100F instrument. The ultrasonic treatment unit used for processing ZVI-NP had the following parameter specification: an ultrasonic bath (ISOLAB Laborgeräte GmbH), an ultrasonic power of 120 W, a heating power of 180 W, and a frequency of 40 kHz. Colloidal solutions of FeO/PVP-S1 US NPs (sample 1) and FeO/PVP NPs (sample 2) in concentrations of 20 mg/mL were dissolved in dimethylsulfoxide (DMSO). The resulting colloidal solutions of iron-containing NPs and microorganisms were mixed and incubated in accordance with a procedure described below.

**Cell biology – measurement of antimicrobial activity**

The antimicrobial activity test was performed on nine control microorganisms: E. coli (ATCC 25922), P. aeruginosa (ATCC 15692), E. faecalis (ATCC 29122), K. pneumonia (laboratory isolates), P. vulgaris (laboratory isolates) representing Gram negative bacteria, S. aureus (ATCC 29213), B. cereus (DSMZ 4312), B. subtilis (ATCC 6633) representing Gram positive bacteria, and C. albicans (ATCC 10231), selected as representatives of fungal microorganisms. The tests were conducted by using the agar well method (Valgas et al. 2007; Clinical and Laboratory Standards Institute 2010) on Mueller Hinton Agar (MHA) for bacterial control strains and on Sabouraud dextrose agar (SDA) for fungal strain.

Fresh cultures of strains were set to 0.5 McFarland [about 10⁸ cfu (colony forming unit)/mL for E. coli] by using a BD CrystalSpec™ nephelometer (Bacton Dickinson, United States) in 0.85% (wt/vol) sterile physiologic serum (SPS) for the initial cell suspension of control microorganism strains. One hundred microliters of each bacterial suspension were inoculated on MHA with a sterile swab. FeO, FeO/PVP-US (sample A1), and FeO/PVP NPs (sample A2) synthesized by three different methods (A1, A2) were dissolved in DMSO to 20 mg/mL as a stock solution. After that, 100 μL of the stock solution were poured into wells on Petri dishes. The inoculated Petri dishes were incubated at 37°C for bacterial strains and 30°C for fungal strain for 24 h. After the incubation period, the inhibition zones around the agar wells (if any) were measured and recorded in millimeters (mm). In all experiments, chloramphenicol (C30) and nystatin were used as a positive control, while DMSO was used as a negative control.

**Biofilm formation assay**

All of the control microbial strains were grown in 5 mL of a LB medium at 30°C overnight. Biofilm production assays were performed using a tryptic soy broth (TSB) medium with 1% glucose. After a 24-h incubation, fresh broth cultures of each bacteria were adjusted to 0.5 McFarland in 0.85% (wt/vol) sterile physiologic serum using a nephelometer. Stock solutions of all substances were prepared in DMSO to a concentration of 20 mg/mL. Fresh micro-organism cultures (0.5
McFarland) were directly mixed with 100 µL of Fe⁰ NPs (A1, A2 10 mg/mL) and TSB (1% glucose) in 96-well plates, which were incubated for 72 h at 37°C. Bacteria cultures in a TSB medium with 1% glucose and in a DMSO/blank medium were used as positive and negative controls, respectively. All plates were washed with tap water and air dried after 72 h. All wells were filled with 30% acetic acid solutions (200 µL) and incubated at room temperature. Finally, the resulting biofilms were measured by using a microtiter plate reader at an optical density of 600 nm (OD600). Results were estimated with respect to control samples.

**Results and discussion**

Using X-ray powder diffraction (XRD), SEM, and TEM, it has been shown that the synthesis with an ultrasonic treatment leads to the formation of iron NPs with a magnetic core size of 12.4 Å; in this case, the iron oxide layer and the polymer layer have a thickness of 1.5–2 and 18–20 nm, respectively; as shown in Figure 1a,b that the NPs are distributed discretely.

The NPs synthesized with the use of an ultrasonic treatment are discrete spherical agglomerates distributed in the polymer matrix. Without ultrasonic treatment, iron NPs with a core size of 1.24 Å are agglomerated into aggregates of 20–200 nm; in this case, the iron oxide layer and the polymer layer have a thickness of 2–4 and 10–12 nm, respectively. Figure 2 shows the morphology of the Fe⁰/PVP-US NP sample synthesized in the presence of PVP with clearly visible globules composed of NPs surrounded by PVP.

The NPs synthesized without ultrasonic treatment are characterized by the formation of ferromagnetic chain structures with a fragment size of 200 nm, which are shown in Figure 3, as a skeleton architecture consisting of chips.

Figure 4 shows diffraction patterns of the nanopowder of Fe⁰/PVP-US and Fe⁰/PVP NPs. The main diffraction peaks correspond to the crystallographic planes of cubic inverted spinel (space group Fd3m, a = 8.3952 Å). Size d of the Fe⁰ crystallites is d = 12 ± 1 Å. The results are in good agreement with the spectra from the diffractionograms database (Costerton et al. 1987), thereby confirming crystallinity of the synthesized iron nanopowder and NPs. Size d of the Fe⁰ crystallites was calculated using the half-width of the diffraction peaks by the Scherer formula (Patterson 1939):

\[ d = \frac{k \cdot \lambda}{(\beta \cos \theta)} \]  

where \( k (=1) \) is a dimensionless shape factor, \( \lambda = 1.93604 \) Å is the X-ray wavelength, \( \beta \) is the line broadening at half the maximum intensity (FWHM), and \( \theta \) is the Bragg diffraction angle. According to Eq. (1), the sizes of these crystallites of Fe⁰/PVP-US and Fe⁰/PVP NPs were calculated to be 12.0 and 12.4 Å, respectively, as per X-ray diffraction line width.
Results of FTIR spectroscopic studies of the samples (FTIR spectra of the Fe\textsuperscript{0}/PVP-US and Fe\textsuperscript{0}/PVP NPs) are shown in Figure 5. The absorption band around 2054–2302 cm\textsuperscript{-1} is attributed to the presence of CO\textsubscript{2} molecules in the air (Kafayati et al. 2013). FTIR spectroscopy was used to investigate the interaction of PVP with iron NPs. Analysis of the FTIR spectra of the Fe\textsuperscript{0}/PVP-US and Fe\textsuperscript{0}/PVP samples, as shown in Figure 5, suggests that the polymers under investigation are coordinated for dimensionality using chelation by binding via oxygen atoms of the PVP ring and iron atoms, and also by binding via nitrogen atoms, as appears to be the case for gold NPs, according to Kiran et al. (2014). The shift of the absorption band of the C=O bond from 1694 cm\textsuperscript{-1} for the pure polymer (Figure 5) to 1735 cm\textsuperscript{-1} is usually representative of the bond and the formation of a C=O–Me (metal) bond (Wu et al. 2019). In this case, a larger shift to the blue region is observed for the samples synthesized under an ultrasonic treatment, which is associated with a more highly ordered packing and is consistent with the microscopy data, as shown in Figure 2. At 500–600 cm\textsuperscript{-1}, the observed peak results from the Fe-O bond, which for this investigation, is evident by a small peak in the region of 580 cm\textsuperscript{-1} and is attributed to Fe\textsubscript{2}O\textsubscript{3} present in Figure 5a: Fe\textsuperscript{0}/PVP-US NPs, and in the region of 564 cm\textsuperscript{-1} to Figure 5b: Fe\textsuperscript{0}/PVP NPs, formed during the synthesis.

During these experiments, PVP was used as a polymeric stabilizer for the formation of iron NPs. The introduction of the polymer during synthesis provides the formation of a negatively charged carbonyl group on the surface of the iron NPs, which contribute to the formation of a stable colloidal solution; the presence of large PVP molecules provides the occurrence of a

**Figure 2.** SEM image of the morphology of Fe\textsuperscript{0}/PVP-US NPs. PVP stabilizing ZVI NPs forms layers with a thickness of 3–5 nm for (a): Fe\textsuperscript{0}/PVP-US-A1 and (b): 20–25 nm for Fe\textsuperscript{0}/PVP-A2.

**Figure 3.** SEM image of the morphology of Fe\textsuperscript{0}/PVP NPs under different magnifications and recording parameters: (a): Mag = 14.63k×, 1 μm, (b), Mag = 148.16k×, 100 nm.
steric effect and hinders their aggregation. Colloidal solutions based on the synthesized NPs were used in biotests.

According to the antimicrobial test conducted in this investigation, the results of two types of NPs showed no antibacterial effects. Furthermore, effects of Fe$^0$ NPs on the biofilm formation ability of ATCC control strains have been studied. According to results of the assay, three Fe$^0$ NPs synthesized by different methods do not show any antimicrobial activity of control strains compared with their positive controls. However, biofilm results show that the highest absorbance values of the tested micro-organisms in control wells are found for S. aureus, B. cereus, P. aeruginosa, and C. albicans. Other microorganisms tested, such as E. coli, B. subtilis, E. faecalis, K. pneumoniae, and P. vulgaris, have lower biofilm absorbance values. In addition, the estimation of the results concerning specific microorganisms has shown that A2 is the most effective in the formation of biofilms of E. coli, K pneumoniae, and C. albicans of all the tested NPs. The C. albicans formed biofilm has the best characteristics. The Fe$^0$/PVP-A2 NPs have an inhibitory effect on biofilm formation of all the test microorganisms (Figure 6). Considering the fact that biofilm formation is a preferred architecture in nature, in this study, the synthesized Fe$^0$/PVP-US-A1 NPs are not antibacterial and do not promote biofilm formation.

Apparently, different results of the bioassay on biofilm formation can primarily be attributed to the difference in the ZVI NP synthesis methods: with and without an ultrasonic treatment of the synthetic solution; the different methods lead to the formation of NPs with different morphologies, which are shown in micrographs in Figures 2 and 3. In tests with Fe$^0$/PVP-US-A1 NPs having a more highly ordered structure, a core of 12.0 Å, smaller aggregates of 10–15 nm, and a higher surface area, which is characteristic of NPs prepared using an ultrasonic treatment, the growth of the biofilm is inhibited to a greater extent. According to Koczkur et al. (2015), Postolachi et al. (2019), and Sidorenko et al. (2020), ZVI NPs exhibit a high reactivity; accordingly, ZVI NPs can be involved in the Fenton reaction to form active oxygen species. Active oxygen species have a detrimental effect on cell membranes; this fact was noted in our earlier studies for soil micro-organisms (Fang et al. 2012) and in studies of Simonin and Richaume (2015). As a consequence, A1 NPs have a smaller effect on the formation of biofilms of the tested micro-organisms, as shown in Figures 6a,b.

It is known that the formation of biofilms occurs in several stages, and one such stage is adhesion, by the fixation of microorganisms. During this stage, a biofilm is formed at the surface of skeleton magnetic aggregates of NPs, which were prepared without exposure to ultrasound, which is preferable considering its properties, viz. charge state and dimension of each of the micro-organisms. In soil environment, biofilm formation impacts microbial community profile and microbial processes (Wu et al. 2019; Costerton et al. 1987). Therefore, biofilm conformation provides suitable environment to locate...
colony residents, and also it plays an important role in shaping of soil. At the same time, this structure is highly preferred in terms of balancing the redox potential in the formation of soil architecture, in order to establish and maintain the plant–microorganism interaction (Mhlongo et al. 2018). Thus, given the fact that biofilm formation is the preferred architecture in nature, the A2 NPs synthesized in this study are not antibacterial, as they promote the formation of biofilms. The control microorganisms used in this study are generally soil-living bacteria and it shows that the NPs synthesized in this study can be used in sustainable agriculture in terms of promoting both biological degradation and biofilm formation.

It is also very important to use a stabilizer forming ZVI NPs. The stabilizer generally is water-soluble polymer PVP and is commonly used in the synthesis of NPs owing to the unique properties. This offers an interesting possibility of preparing stable aqueous colloids, as evidenced by several studies by Ikuma, Decho and Lau (2015) and Vaseashta (2015). N-Vinylpyrrolidone stabilizing ZVI NPs exhibits high-adhesion properties; therefore, it can contribute to an increase

Figure 6. Effect of Fe⁰/PVP-US-A1 (a) and Fe⁰/PVP-A2 Nps (b) synthesized by different methods on the formation of a biofilm of control strains.
in the aggregation of micro-organisms into a biofilm. In addition, a larger polymer layer is formed on NPs prepared without ultrasonic treatment, although according to IR spectroscopy, the polymer layer formed with the use of an ultrasonic treatment is more coordinated.

**Conclusions**

In this investigation, ZVI NPs were synthesized by the reduction of iron salts and stabilized with a low-molecular-mass hydrophilic polymer (PVP) with and without exposure to an ultrasonic treatment. The morphology of ZVI NPs was determined using the SEM and TEM methods. The magnetic core size is almost identical for Fe/PVP-US Fe/PVP and Fe/PVP; it is 12.0 and 12.4 Å, respectively. The NPs synthesized with the use of an ultrasonic treatment are discrete spherical agglomerates distributed in the polymer matrix. The NPs synthesized without ultrasonic treatment are characterized by the formation of ferromagnetic chain structures with a fragment size of 200 nm. FTIR spectroscopy has revealed the formation of a C–O–Fe bond for iron NPs synthesized using an ultrasonic treatment.

Colloidal solutions based on the synthesized Fe⁰/PVP-US and Fe⁰/PVP NPs have been used to study the formation of biofilms during their growth and determine the antimicrobial activity. It has been shown that the formation of biofilms of various microorganisms is obviously affected by the methods used to synthesize the NPs and, accordingly, the morphology of the resulting NPs, dimension, and coordination of the stabilizer. The results have shown that Fe⁰ NPs, which have a stimulating effect on biofilm formation in control microorganisms, should be studied with other agriculture-friendly Plant Growth-Promoting Rhizobacteria (PGPRs) to determine the possibilities of their use in agriculture for future prospects, especially due to their bioremediation nature. Hence, it is concluded that the use of ZVI NPs for bioremediation is an emerging and essential field, playing an increasingly important role in addressing innovative and effective solutions to a vast range of environmental challenges.

**Disclosure statement**

The authors declare no conflict of interest.

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**ORCID**

Anatoli Sidorenko [http://orcid.org/0000-0001-7433-4140](http://orcid.org/0000-0001-7433-4140)
Anatoli Dimoglo [http://orcid.org/0000-0001-8638-3679](http://orcid.org/0000-0001-8638-3679)
Ashok Vaseashta [http://orcid.org/0000-0002-5649-0067](http://orcid.org/0000-0002-5649-0067)

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