[Downloaded free from http://www.ijehe.org on Thursday, January 26, 2023, IP: 5.238.149.81]

Original Article

Bactericidal effect of starch-stabilized zero-valent iron nanoparticles on *Escherichia coli*

Mohammad Mosaferi, Roya Zarei[1], Mohammad Hosein Soroush Barhagi[2], Mohammad Asghari Jafar-abadi[3], Alireza Khataee[4]

Department of Environmental Health, Tabriz Health Services Management Research Center, Tabriz University of Medical Sciences, Tabriz, Iran and Department of Environmental Health, Tehran University of Medical Sciences, Tehran, Iran, [1]Department of Environmental Health, Center of Student Researches, Faculty of Health, Tabriz University of Medical Sciences, Tabriz, Iran, [2]Department of Microbiology, Faculty of Medicine, University of Tabriz, Tabriz, Iran, [3]Department of Statistics and Epidemiology, Medical Education Research Center, Faculty of Health, Tabriz University of Medical Sciences, Tabriz, Iran, [4]Department of Applied Chemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran

Address for correspondence:
Eng. Roya Zarei,
Center of Student Researches, Faculty of Health,
Tabriz University of Medical Sciences, Tabriz, Iran.
E-mail: rzarei29@yahoo.com

ABSTRACT

Aims: The present study reports the antibacterial efficiency of starch-stabilized nano scale zero-valent iron (S-NZVI) particles on *Escherichia coli*.

Materials and Methods: NZVI was synthesized using $NaBH_4$ and $FeSO_4.7H_2O$, and characterized by scanning electron microscopy, as well as X-ray diffraction. The effects of concentration, contact time, dissolved oxygen, and stabilization were tested. *E. coli* was determined by the pour plate method.

Results: The results revealed that the complete inactivation (100%) of *E. coli* was occurred at using 100 mg/l of NZVI after 30 min under anaerobic condition. The inactivation efficiency was decreased in an aerobic condition. When NZVI concentration increased to 500 and 1000 mg/L, complete inactivation was achieved under both anaerobic and aerobic condition. In general, *E. coli* inactivation efficiency using NZVI was strongly dependent on the contact time and the concentration of NZVI particles with its maximum efficiency at 500 mg/L within 120 min. Stabilization-NZVI by starch did not improve its bactericidal activity and the inactivation of *E. coli* by stabilized nanoparticles required higher concentration compared to that by nonstabilized nanoparticles.

Conclusion: The present study showed that nonstabilized Fe^0 nanoparticles have higher bactericidal efficiency than that of S-NZVI. This investigation also suggests that NZVI can be used as an effective and strong agent for antimicrobial applications.

Key words: *Escherichia coli*, inactivation, stabilized nanoparticles, starch, zero-valent iron

Access this article online

Quick Response Code:

Website: www.ijehe.org

DOI: 10.4103/2277-9183.179195

INTRODUCTION

Microbial contamination of water is a significant health threat which is an important issue for drinking water supplies,[1] so that disinfecting drinking water supplies is considered to be an important advance in public health.[2] Recently, there has been an increasing interest in the potential use of engineered nanomaterials for treatment of polluted

Copyright: © 2015 Mosaferi M. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

This article may be cited as:
Mosaferi M, Zarei R, Barhagi MH, Jafar-abadi MA, Khataee A. Bactericidal effect of starch-stabilized zero-valent iron nanoparticles on *Escherichia coli*. Int J Env Health Eng 2016;5:2.

[Downloaded free from http://www.ijehe.org on Thursday, January 26, 2023, IP: 5.238.149.81]

waters.[3] Antimicrobial nanoparticles could overcome critical challenges associated with traditional chemical disinfectants such as harmful disinfection by-products (DBPs) (e.g., carcinogenic trihalomethanes, BrO_3)[2] and they could enhance performance of the existing technologies such as ultraviolet inactivation of viruses, solar disinfection of bacteria, biofouling-prone membrane filtration, and advanced oxidation processes.[4] It can also abate concerns of water quality degradation within distribution networks and in large centralized water treatment systems.[5] A variety of nonmaterial including nano-Ag, nano-ZnO, nano-TiO_2, nano-Ce_2O_4, carbon nanotubes, and fullerenes have been proven as an effective microbicide with the lowest rate of DBPs formation.[6]

Nano scale zero-valent iron (NZVI) is one of the first generations of nano scale technologies which has been used in the environmental remediation[7] and water treatment.[2] NZVI is able to remove a wide range of contaminants such as arsenic (As),[8,9] chromium (Cr^{6+}),[10] lead (Pb^{2+}),[11] chlorinated solvents including perchloroethylene and trichloroethylene and polychlorinated biphenyls.[12]

Several studies have also shown the potential applicability of NZVI in inactivating and removing bacteria[13-16] and viruses.[17,18] Previously, Diao and Yao have shown that Fe^0 nanoparticles inactivated *Bacillus subtilis* var. niger and *Pseudomonas fluorescens* bacteria.[14] Kim *et al*. have demonstrated the inactivation of MS2 coliphage (MS2) by nano zero-valent iron (ZVI) particles in an aqueous solution.[18] Auffan also found that Fe^0 nanoparticles induce intracellular oxidative stress toward *Escherichia coli* via generating reactive oxygen species (ROS), which causes membrane damages and cell death.[19] It was indicated in another study that the release of Fe^{2+} ion and disturbance of the enzymatic function is responsible for the antimicrobial activity of nano Fe particles.[15]

Since magnetic nanoparticles (e.g., NZVI) can be separated from water by a relatively low magnetic field,[6] they could be used as a platform to develop multifunction nano composite materials.[2] This would enable both chemical disinfection and photocatalytic destruction of waterborne pathogens while enhancing the retention of nano materials.[2] It has been shown that NZVI particles (NZVIPs) have more antimicrobial activity than other iron-based nanoparticles.[16] Rapid degradation of more contaminations is feasible using NZVI because it's large surface areas and higher surface reactivity, which would provide the best performance for contamination removal.[20]

Despite these benefits, NZVI has a strong tendency to agglomeration, because of van der Waals and magnetic attraction among magnetic nanoparticles, which is an unfavorable property for remediation.[4,21] In addition, uncoated nanoparticles are very susceptible to some environmental agents such as pH, temperature, electrolytes, and solvent.[22] It has been suggested that surface modification of ZVI nanoparticles by stabilizers helps to produce smaller particles with a controlled shape which are more mono-dispersed.[21,23,24]

Although several studies have shown the antimicrobial properties of NZVI,[14-16,25] little is done on comparison between bactericidal effect of starch-SNZVIPs and non-S-NZVIPs. In the present work, the bactericidal efficiency of starch-S-NZVI was studied in comparison with bare NZVI (b-NZVI) (none stabilized nanoparticles). Starch was used as a cost effective and environment-friendly stabilizer. The Gram-negative *E. coli* (ATCC strain 8739) was used as the bacteria in this study.

MATERIALS AND METHODS

Synthesizing starch-stabilized nano scale zero-valent iron particles

The starch-S-NZVIPs and b-NZVIPs were synthesized according to the procedure used by Keenan *et al*.,[26] via aqueous phase reduction of ferrous sulfate ($FeSO_4.7H_2O$) by sodium borohydride ($NaBH_4$) at room temperature.

Briefly, stabilized Fe^0 nanoparticles were synthesized by adding 100 mL of 0.5 M $NaBH_4$ (MERKCO/Germany) in drop-wise fashion to a three neck flask containing 0.14 M solution of $FeSO_4.7H_2O$ (MERK KGaA/Germany) (1:1 volume) and 0.2% (w/w) starch as a stabilizer with continuous mixing. To ensure efficient use of the reducing agent and prevent NZVI particles from being oxidized, the reactions had to be performed in anaerobic conditions. Accordingly, N_2 gas was bubbled to remove the dissolved oxygen (DO) during the synthesis. Once all of $NaBH_4$ solution was added, the sample was continuously stirred for 30 min to complete the reaction and ensure uniform growth of iron nanoparticles. The NZVI was synthesized via the following reaction:

$$2Fe^{2-} + BH^-_4 + 3H_2O \rightarrow 2Fe^0\downarrow + H_2BO^-_3 + 4H^+ + 2H_2$$

The prepared solution was centrifuged (jouan-b_3.11) at 2500 rpm for 5 min then the supernatant was decanted. After the nanoparticles had been washed with ethanol for 3 times, they were centrifuged, and the supernatant was decanted. Finally, the samples stored and dried in vacuum (iemierson-c55jxhrl-420-5) overnight. b-NZVIPs (nonstabilized nano particles) were prepared through the same procedure as were S-NZVIPs without using starch.[27]

Analytical method

In order to specify structure and composition of freshly synthesized NZVIPs, X-ray diffraction (XRD) analysis was carried out on a Siemens D5000 (Germany) diffractometer using Cu-Kα radiation (40 kV, 30 mA, λ = 0.15418 nm). The Samples were scanned for a 2 θ range of 20-85°. Crystalline size of the nanoparticles was measured using Sherrer formula from the line broadening of XRD peak.

[Downloaded free from http://www.ijehe.org on Thursday, January 26, 2023, IP: 5.238.149.81]

Surface morphology and size of the NZVIPs were characterized by scanning electron microscopy (SEM) on a Hitachi S 4160 model (Japan) instrument.

Microbial agent's preparation

Escherichia coli (ATCC strain 8739) as the more precise indicator of microbial pollution was employed in this study. Bacteria suspension was obtained following the same procedures described by Lee *et al*.[15] *E. coli* was inoculated on petri dishes with tryptic soy broth agar (Merck KGaA/Germany) and grown at 37°C for 18 h. The bacteria were obtained by centrifugation at 1000 *g* for 10 min; which were then washed twice with 50 mL of 150 mM phosphate buffer saline (PBS, pH = 7), and re-suspended in 50 mL of PBS to prepare the bacteria stock solution.[15] The resultant bacterial suspension had an *E. coli* concentration of approximately 2-3 × 10^5 CFU/mL.

Inactivation experiments

The microbial suspensions were diluted to about 10^3 CFU/mL. NZVIP concentration of 5, 10, 50, 100, 500, and 1000 mg/L were tested in this study. The mixture samples were shaken at 250 rpm under room temperature for 5, 15, 30, 60, 120 min.

Escherichia coli was determined via the pour plate method. The number of colony forming units (CFUs) was counted on nutrient agar plates after they were incubated at 37°C for 18-24 h. Samples were plated in triplicate to ensure fidelity of the results. Colony counts were reported as the average value over three samples.

All the experiments were conducted under both anaerobic conditions (N_2 gas bubbled prior to initiation of the experiments to remove DO, and the test tubes were capped with parafilm) and aerobic conditions (exposed to the atmosphere).[15]

The following equation was used to evaluate bactericidal activity of the NZVIPs:

$$\text{Inactivation rate (\%)} = \frac{CFU_{\text{control}} - CFU_{\text{exposed}}}{CFU_{\text{control}}} \times 100$$

where $CFU_{control}$ represents the number of CFUs in the absence of NZVIPs, and $CFU_{exposed}$ indicates of the CFUs after doing the experiment with NZVIPs on nutrient agar plates.

The effect of parameters including contact time, NZVI concentration, DO, and stabilization on the bacteria removal efficiency was examined using starch-stabilized and bare Fe^0 nanoparticles. The experiments were performed by applying 360 samples in triplicates.

Statistical analysis of the data

Data were summarized in mean (±standard deviation). Data distribution was checked for normality by one-sample Kolmogorov–Smirnov test. Since the normality was supported by this test, a parametric statistical test was used to assess the mean effect of concentration and DO. In addition, a three-way analysis of variance was performed to find their interactions. In the case of significant effects, *post-hoc* was applied to confidence intervals by implementing Bonferoni correction. All the analyses were performed using Minitab 16.

$P < 0.05$ at a confidence level of 95% were considered statistically significant.

RESULT

Characterization results

X-ray diffraction and SEM results of Fe^0 nanoparticles in this study are shown in Figures 1 and 2. According to XRD analysis, the synthesized crystals were ZVI. As shown in the figure, characteristic main diffraction peak at 2θ = 44.7° confirmed crystallization of Fe^0 nanoparticles. The calculated crystalline size for Fe^0 nanoparticles by Sheerer formula was found 10 nm.

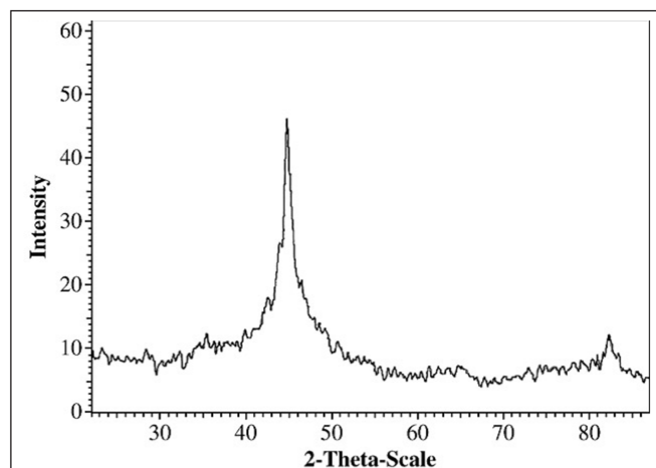

Figure 1: X-ray diffraction pattern of synthesized starch-stabilized nano scale zero-valent iron

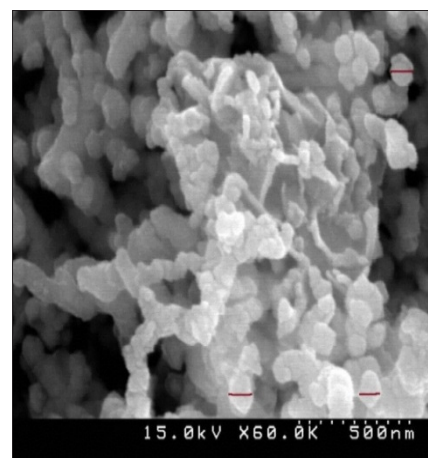

Figure 2: Scanning electron microscopy image of synthesized starch-stabilized nano scale zero-valent iron

[Downloaded free from http://www.ijehe.org on Thursday, January 26, 2023, IP: 5.238.149.81]

SEM image for nanoparticles of Fe^0 is presented in Figure 2 which indicates that the stabilized synthesized NZVIPs were spherical in shape with an average diameter of about 50-100 nm.

Escherichia coli inactivation

Statistical analysis indicated that effects of the nanoparticles concentration ($P < 0.001$), contact time ($P < 0.001$) and oxidation status ($P < 0.001$) on the bacterial inactivation were significant.

Effect of nano scale zero-valent iron concentration on the inactivation efficiency

Figures 3a and b and 4a and b illustrate inactivation efficiency of *E. coli* after being treated with different concentration of S-NZVI and b-NZVI within different contact times. It is apparent from these figures that there was a direct relationship between inactivation efficiency of *E. coli* and nanoparticles concentration under both aerobic and anaerobic conditions, and increasing concentration of NZVI resulted in higher inactivation efficiency. Figure 3a illustrates that the inactivation efficiency increased from 50% to 100% as the S-NZVI concentration increased from 5 to 500 mg/L. According to Figure 3b, the removal efficiency of *E. coli* was about 26% using S-NZVI at 5 mg/L for 30 min; however, it reaches 100% when the S-NZVI concentration exceeded 100 mg/L.

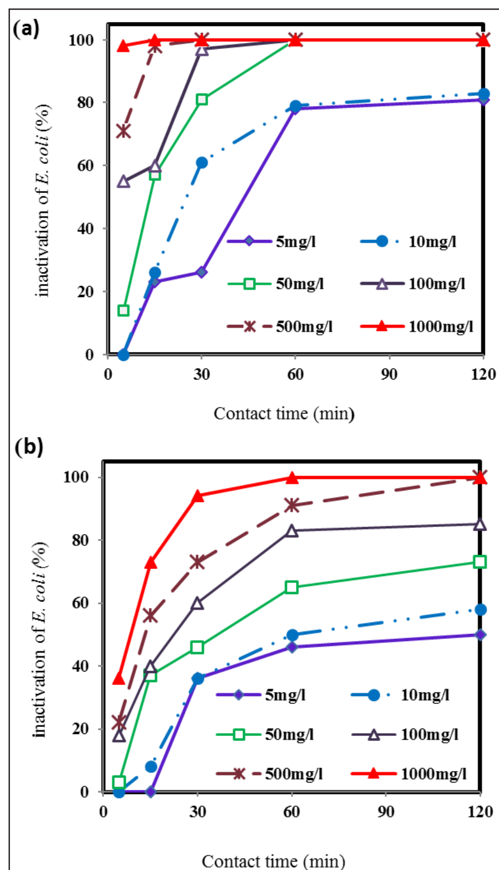

Figure 3: Effect of stabilized nano scale zero-valent iron concentration-contact time (a) at anaerobic (b) and aerobic conditions

At the highest concentrations of NZVI (500, 1000 mg/L), a strong bactericidal effect (100%) was observed; even during a short contact time (5 min) [Figures 3 and 4]. Statistical analysis indicated that there was no significant difference between 1000 mg/L of NZVI within 5 min of exposure time versus 5 mg/L of that after 60 min of exposure ($P = 0.163$).

Effect of contact time

Bactericidal activity of NZVI was tested by culturing samples after 5, 15, 30, 60, and 120 min of treatment. In general, there was a positive correlation between inactivation rate and contact time. Figures 3a and b and Figure 4a and b show that there has been an abrupt increase in *E. coli* inactivation, after 30 min of exposure. At higher contact times, a small dosage of nanoparticles was able to effectively inactivate *E. coli*. For example, required concentration of NZVI to complete removal of bacteria population was 1000 mg/L after 5 min, whereas it was reduced to 50 mg/L when contact time increased to 120 min [Figure 4a].

From the data in Figures 3a and 4a, we can see that in anaerobic condition, stabilized and bare Fe^0-NPs with the treatment time period of 120 min were capable to considerably reducing bacterial growth (80-90%) at their lowest concentrations (5 and 10 mg/L), and eventually increase it to 100% at higher concentrations (100, 500

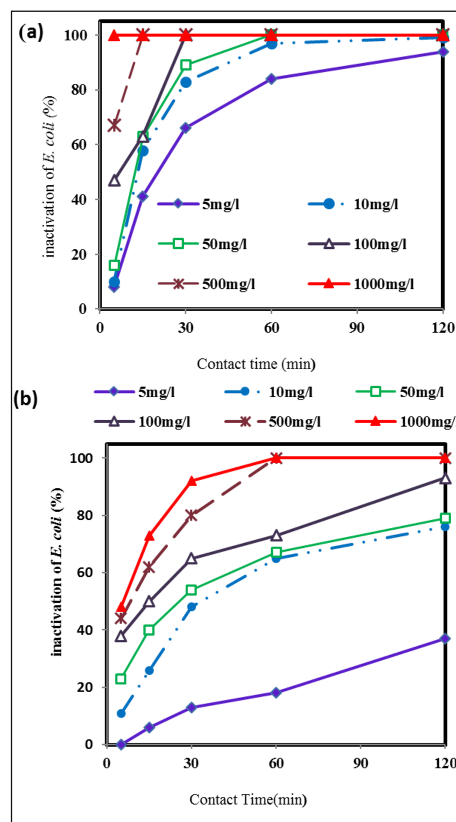

Figure 4: Effect of bare nano scale zero-valent iron concentration-contact time (a) at anaerobic (b) and aerobic conditions

[Downloaded free from http://www.ijehe.org on Thursday, January 26, 2023, IP: 5.238.149.81]

and 1000 mg/L). According to the statistical analysis, no significant difference was found between exposure time of 30 and 60 min ($P = 0.26$), also 60 and 120 min ($P = 0.21$).

Effect of dissolved oxygen

Based on statistical analysis, there was a significant difference between aerobic and anaerobic conditions ($P < 0.05$).

Figure 5a and b depict a comparison between inactivation efficiency of nanoparticles at two conditions: Aerobic and anaerobic. As shown in Figure 6, inactivation efficiency of b-NZVIPs and S-NZVIPs was decreased in comparison with those of anaerobic condition.

In aerobic condition, the required concentration of nanoparticles for complete inactivation (100%) was about 10 times higher than that of the anaerobic condition. According to statistical analysis, there was no significant difference between the obtained efficiency with 100 mg/L in the anaerobic condition or 1000 mg/L in aerobic condition ($P = 1$).

In anaerobic conditions, concentration of 50 mg/L was sufficient to inhibit 100% bacterial growth after 1 h of treatment; whereas it was decreased to 65% under aerobic conditions [Figure 5b]. Figure 5a and b show that the presence of oxygen hindered inactivation of *E. coli* so that a negligible bactericidal activity (%) was resulted after a short mixing time (5 and 15 min). In the presence of oxygen, highly efficient inactivation of bacteria (90-100%) was only achieved with concentrations exceeding 500 mg/L after 60 min of shaking.

Effect of stabilization

Table 1 shows a statistic comparison between NZVI and S-NZVI at the equilibrium levels of concentrations. This table shows that there was no significant difference between NZVI and S-NZVI with exceptions at 100 and 10 mg/L.

Figure 6a compares the inactivation efficiency by stabilized and b-NZVI under aerobic condition. As can be seen, stabilization of NZVI reduced its toxicity toward *E. coli*, when exposed to the same concentration of nanoparticles within the same exposure time.

Table 1: Statistic comparison between NZVI and S-NZVI

NZVI concentration (mg/L)	S-NZVI concentration (mg/L)	*P*	*t*
5	5	1	0.6
10	10	0.00	5.59
50	50	0.79	1.84
100	100	0.009	3.77
500	500	0.9	1.6
1000	1000	1	0.54

NZVI: Nano scale zero-valent iron, S-NZVI: Stabilized nano scale zero-valent iron

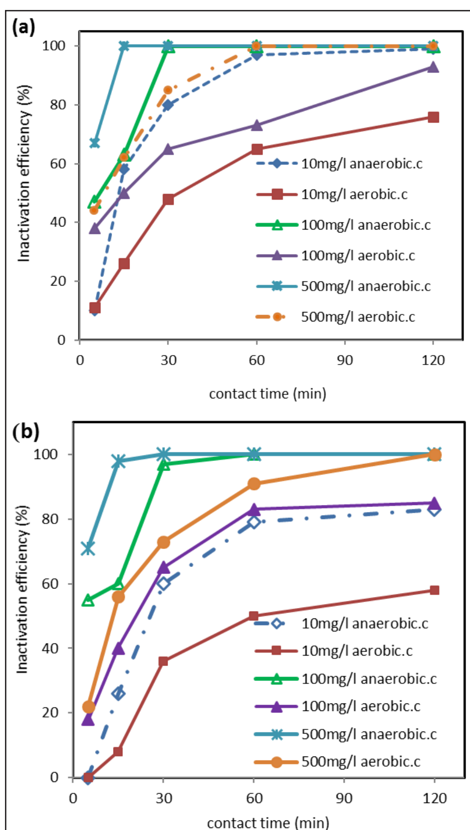

Figure 5: Comparison of inactivation efficiency at aerobic and anaerobic condition using (a) bare nano scale zero-valent iron (NZVI) (b) stabilized NZVI

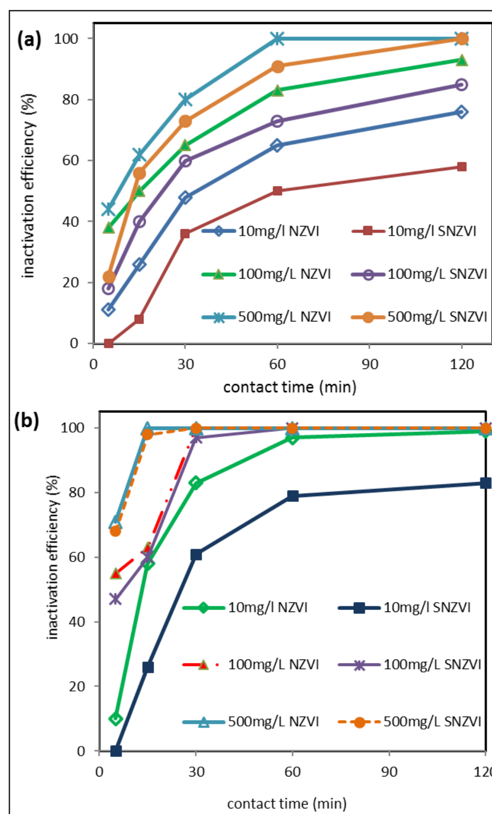

Figure 6: Comparison of inactivation efficiency of bare and stabilized-nano scale zero-valent iron under (a) aerobic and (b) anaerobic condition

[Downloaded free from http://www.ijehe.org on Thursday, January 26, 2023, IP: 5.238.149.81]

Figure 6b compares the results obtained from S-NZVI and b-NZVI under anaerobic condition. It is apparent from this figure that at low concentration (10 mg/L), b-NZVI shows higher inactivation efficiency than stabilized nanoparticles. However, similar efficiency was resulted using both nanoparticles as NZVI concentration increased to 100 and 500 mg/L.

DISCUSSION

The results suggested that the cytotoxicity of nanoparticles was strongly depended on NZVIPs concentration which was consistent with the previous reports.[14,15] This result may be due to increasing number of nanoparticle attachments to the membrane and deepening intracellular penetrability of nanoparticles at higher concentrations.[28] In anaerobic conditions, as nanoparticles concentration increased, a considerable reduction was observed in the bacterial growth. This result could be attributed to the residual ratio of nonoxidized NZVI. Once initial oxygen was consumed in the oxidation reaction, the residual NZVI plays a role in the bactericidal activity.[15]

Our results indicated that there was an increase in *E. coli* inactivation within increasing contact time under aerobic condition. In contrast, Lee *et al*. found that inactivation rate of *E. coli* decreased over time under air saturation.[15] Increasing exposure time could enhance the chances of particle-bacterial cell interaction; it can also increase accessibility of the Fe^0 surface for bacteria through keeping oxidation products suspended and preventing from oxide film formation on Fe^0.[29,30] This operation may explain the relatively good correlation between shaking time and inactivation rate of *E. coli* under aerobic conditions.

The results of this study showed that inactivation efficiencies of both b-NZVIPs and S-NZVIPs were lower at the presence oxygen when compared to anaerobic conditions. This finding was in agreement with those of Lee's indicating that the presence of DO significantly reduces the antimicrobial activity of nano-Fe^0.[15] This result might be associated with rapid oxidation of Fe^0 into Fe^{+3} by DO resulting NZVI surface to be coated by an iron oxide layer.[17] Considering the fact that physical contact of cells with the ZVI nanoparticles plays an important role in disruption of cell membranes and generation of ROS,[19,31] one may deduce that NZVI surface coating with an iron oxide layer might contribute to decrease its antimicrobial activities. There are, however, other possible explanations. A possible explanation might be that under aerobic conditions, *E. coli* secretes enzymes like superoxide dismutase (SOD). It has been reported in the literature that SOD as a superoxide radical scavenger, prevents ROS including superoxide, peroxide hydrogen, and hydroxyl radicals from being formed[32] by suppressing the subsequent Fenton reaction.[33] Chang *et al*. suggested that adding SOD to silver nanoparticle suspensions, reduced toxicity of these particles toward *E. coli* membranes.[34] Another possible explanation is that in the presence of oxygen, various types of Fe-oxide such as magnetite and maghemite can be formed at the particle surface that these compounds are less toxic than Fe^0.[32] Even recent evidences have suggested that oxidation of iron can affect its biological impacts on bacteria by controlling ion release.[35]

Results obtained from the stabilization of nanoparticles indicated that inactivation efficiency of bare particles was higher than that of stabilized particles. They also showed that stabilization of nanoparticles with 0.2% (w/w) of starch reduced the rate of *E. coli* inactivation. This finding was consistent with that of Voladker's who found that starched copper nanoparticles were less toxic than cupric ions.[36] In addition, Zhou *et al*. studies suggested that carboxymethyl cellulose-coating decreases toxicity and oxidizing capacity of NZVI toward bacteria *Agrobacterium*.[37] Li *et al*. also found that NZVI modification by polymer decreased its inactivation efficiency from 5.2 to 0.2 logs.[32] Considering the fact that generation of ROS is one of the hypothesized mechanisms for inactivation of bacteria with NZVI,[19,38] generating intracellular ROS and subsequently decomposing protein functional groups would require close proximity of nanoparticles to the cells.[32,39] Starch might have protected *E. coli* cells in coated nanoparticles, reducing interactions of ROS or nanoparticles with cells.[40] This causes a reduction in the physical disruption of cell structures; especially at low concentration and short contact time.

One of the most probable antimicrobial mechanisms in metal nanoparticles is a physical disruption of cell structures.[41] Therefore, coating nanoparticles by stabilizers may contribute to weaken nanoparticles attachments to the bacteria surface via reducing contact area between nanoparticles and bacteria which may lead to attenuate cell membrane degradation.[42]

Pan *et al*. showed that encapsulation of magnetite nanoparticles changes surface reactivity through a diffusion barrier toward contaminants. It has also been shown that humic acid and synthetic polymers decreased NZVI toxicity against *E. coli* by surrounding the nanoparticles surface and limiting their adhesion to the bacteria.[32,43] Although stabilization may cause a reduction in the interactions between nanoparticles as well as alteration of aggregation pattern, its toxicity is alleviated by keeping nanoparticles from interaction with bacteria. In addition, a previous study has suggested that free ions could lead damages to DNA, proteins,[44] and cell wall while they enhance penetrability of nanoparticles inside the cell[45] in which slower release of iron ions from the capped nanoparticles occurred.[36] While NZVI toxicity may be mediated through a release of Fe^{2+}, it is reasonable to assume that the close proximity of NZVI to the bacteria would increase its toxicity potential.

[Downloaded free from http://www.ijehe.org on Thursday, January 26, 2023, IP: 5.238.149.81]

CONCLUSION

This study set out to determine the effect of starch-S-NZVIPs on *E. coli*. However, application of starch as the stabilization agent did not enhance inactivation efficiency of NZVIPs. The results of this investigation have revealed that generally the starch-SNZVIPs and b-NZVIPs have strong bactericidal activity against *E. coli*.

According to the statistical results, the concentration of NZVIPs and contact time were the most effective parameters in the bactericidal activity of NZVI. In addition, the results showed that bacteria removal using bare nanoparticles under anaerobic condition was more effective than that of S-NZVI under aerobic conditions. Nanoparticles surface coating with such compounds as iron oxide might reduce the potential toxicity of nano-Fe^0 while limiting its antimicrobial activities. This is one of the NZVI limitations deals with in disinfection application. Thus, additional research is required to overcome this drawback.

ACKNOWLEDGMENTS

This research was partially supported by Tabriz University of Medical Sciences, Iran.

Financial support and sponsorship

Tabriz University of Medical Sciences.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. You Y, Han J, Chiu PC, Jin Y. Removal and inactivation of waterborne viruses using zerovalent iron. Environ Sci Technol 2005;39:9263-9.
2. Diallo M, Street A, Sustich R, Duncan J, Savage N. Nanotechnology Applications for Clean Water: Solutions for Improving Water Quality. Norwich: William Andrew; 2009.
3. Crane RA, Scott TB. Nanoscale zero-valent iron: Future prospects for an emerging water treatment technology. J Hazard Mater 2012;211-212:112-25.
4. Li Q, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, *et al*. Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. Water Research 2008;42:4591-602.
5. Brame J, Li Q, Alvarez PJ. Nanotechnology-enabled water treatment and reuse: Emerging opportunities and challenges for developing countries. Trends Food Sci Technol 2011;22:618-24.
6. Qu X, Alvarez PJ, Li Q. Applications of nanotechnology in water and wastewater treatment. Water Res 2013;47:3931-46.
7. Comba S, Di Molfetta A, Sethi R. A comparison between field applications of nano-, micro-, and millimetric zero-valent iron for the remediation of contaminated aquifers. Water Air Soil Pollut 2011;215:595-607.
8. Ramos MA, Yan W, Li XQ, Koel BE, Zhang WX. Simultaneous oxidation and reduction of arsenic by zero-valent iron nanoparticles: Understanding the significance of the coreâˆ' shell structure. J Phys Chem C 2009;113:14591-4.
9. Kim SA, Kamala-Kannan S, Lee KJ, Park YJ, Shea PJ, Lee WH, *et al*. Removal of Pb (II) from aqueous solution by a zeolite "nanoscale zero-valent iron composite. Chem Eng J 2013;217:54-60.
10. Shi C, Wei J, Jin Y, Kniel KE, Chiu PC. Removal of viruses and bacteriophages from drinking water using zero-valent iron. Sep Purif Technol 2010;84:72-8.
11. Tehrani MR, Shamsai A, Vossughi M. *In-situ* Pb2+ remediation using nano iron particles. J Environ Health Sci Eng 2015;13:1.
12. He F, Zhao D, Paul C. Field assessment of carboxymethyl cellulose stabilized iron nanoparticles for *in situ* destruction of chlorinated solvents in source zones. Water Res 2010;44:2360-70.
13. Barnes RJ, van der Gast CJ, Riba O, Lehtovirta LE, Prosser JI, Dobson PJ, *et al*. The impact of zero-valent iron nanoparticles on a river water bacterial community. J Hazard Mater 2010;184:73-80.
14. Diao M, Yao M. Use of zero-valent iron nanoparticles in inactivating microbes. Water Res 2009;43:5243-51.
15. Lee C, Kim JY, Lee WI, Nelson KL, Yoon J, Sedlak DL. Bactericidal effect of zero-valent iron nanoparticles on *Escherichia coli*. Environ Sci Technol 2008;42:4927-33.
16. Mahdy SA, Raheed QJ, Kalaichelvan PT. Antimicrobial activity of zero-valent iron nanoparticles. Int J Mod Eng Res 2012;2:578-81.
17. Kim JY, Lee C, Sedlak DL, Yoon J, Nelson KL. Inactivation of MS2 coliphage by Fenton's reagent. Water Res 2010;44:2647-53.
18. Kim JY, Lee C, Love DC, Sedlak DL, Yoon J, Nelson KL. Inactivation of MS2 coliphage by ferrous ion and zero-valent iron nanoparticles. Environ Sci Technol 2011;45:6978-84.
19. Auffan M, Achouak W, Rose J, Roncato MA, Chanéac C, Waite DT, *et al*. Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward *Escherichia coli*. Environ Sci Technol 2008;42:6730-5.
20. Phenrat T, Long TC, Lowry GV, Veronesi B. Partial oxidation ("aging") and surface modification decrease the toxicity of nanosized zerovalent iron. Environ Sci Technol 2009;43:195-200.
21. Gupta AK, Gupta M. Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 2005;26:3995-4021.
22. Wei D, Sun W, Qian W, Ye Y, Ma X. The synthesis of chitosan-based silver nanoparticles and their antibacterial activity. Carbohydr Res 2009;344:2375-82.
23. Mohanty S, Mishra S, Jena P, Jacob B, Sarkar B, Sonawane A. An investigation on the antibacterial, cytotoxic, and antibiofilm efficacy of starch-stabilized silver nanoparticles. Nanomedicine 2012;8:916-24.
24. He F, Zhao D, Liu J, Roberts CB. Stabilization of Fe-Pd nanoparticles with sodium carboxymethyl cellulose for enhanced transport and dechlorination of trichloroethylene in soil and groundwater. Ind Eng Chem Res 2007;46:29-34.
25. Auffan M, Achouak W, Rose J, Roncato MA, Chanéac C, Waite DT, *et al*. Relation between the redox state of iron-based nanoparticles and their cytotoxicity toward *Escherichia coli*. Environ Sci Technol 2008;42:6730-5.
26. Keenan CR, Goth-Goldstein R, Lucas D, Sedlak DL. Oxidative stress induced by zero-valent iron nanoparticles and Fe (II) in human bronchial epithelial cells. Environ Sci Technol 2009;43:4555-60.
27. Alidokht L, Khataee AR, Reyhanitabar A, Oustan S. Reductive removal of Cr (VI) by starch-stabilized Fe0 nanoparticles in aqueous solution. Desalination 2011;270:105-10.
28. Tiwari DK, Behari J, Sen P. Time and dose-dependent antimicrobial potential of Ag nanoparticles synthesized by top-down approach. Curr Sci 2008;95:647-55.
29. Polasek P. Differentiation between different kinds of mixing in water purificationâ€"Back to basics. Water SA 2007;33:249-52.
30. Noubactep C. The suitability of metallic iron for environmental remediation. Environ Prog Sustain Energy 2010;29:286-91.
31. Tran N, Mir A, Mallik D, Sinha A, Nayar S, Webster TJ. Bactericidal effect of iron oxide nanoparticles on Staphylococcus aureus. Int J Nanomedicine 2010;5:277-83.
32. Li Z, Greden K, Alvarez PJ, Gregory KB, Lowry GV. Adsorbed polymer and NOM limits adhesion and toxicity of nano scale zerovalent iron to *E. coli*. Environ Sci Technol 2010;44:3462-7.
33. Kim JY, Lee C, Love DC, Sedlak DL, Yoon J, Nelson KL. Inactivation of MS2 coliphage by ferrous ion and zero-valent iron nanoparticles. Environ Sci Technol 2010;45:6978-84.

[Downloaded free from http://www.ijehe.org on Thursday, January 26, 2023, IP: 5.238.149.81]

34. Chang Q, Yan L, Chen M, He H, Qu J. Bactericidal mechanism of Ag/Al2O3 against *Escherichia coli*. Langmuir 2007;23:11197-9.
35. Auffan M, Rose J, Bottero JY, Lowry GV, Jolivet JP, Wiesner MR. Towards a definition of inorganic nanoparticles from an environmental, health and safety perspective. Nat Nanotechnol 2009;4:634-41.
36. Valodkar M, Rathore PS, Jadeja RN, Thounaojam M, Devkar RV, Thakore S. Cytotoxicity evaluation and antimicrobial studies of starch capped water soluble copper nanoparticles. J Hazard Mater 2012;201-202:244-9.
37. Zhou L, Thanh TL, Gong J, Kim JH, Kim EJ, Chang YS. Carboxymethyl cellulose coating decreases toxicity and oxidizing capacity of nanoscale zerovalent iron. Chemosphere 2014;104:155-61.
38. Biswas P, Wu CY. Nanoparticles and the environment. J Air Waste Manag Assoc 2005;55:708-46.
39. Auffan M, Rose J, Wiesner MR, Bottero JY. Chemical stability of metallic nanoparticles: A parameter controlling their potential cellular toxicity *in vitro*. Environ Pollut 2009;157:1127-33.
40. Pan G, Li L, Zhao D, Chen H. Immobilization of non-point phosphorus using stabilized magnetite nanoparticles with enhanced transportability and reactivity in soils. Environ Pollut 2010;158:35-40.
41. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. Langmuir 2002;18:6679-86.
42. Starr KF. Microbial implication of iron oxide nanoparticles. Alabama, USA: Auburn University; 2010.
43. Chen J, Xiu Z, Lowry GV, Alvarez PJ. Effect of natural organic matter on toxicity and reactivity of nano-scale zero-valent iron. Water Res 2011;45:1995-2001.
44. Panacek A, Kvítek L, Prucek R, Kolar M, Vecerova R, Pizúrova N, *et al.* Silver colloid nanoparticles: Synthesis, characterization, and their antibacterial activity. J Phys Chem B 2006;110:16248-53.
45. Neal AL. What can be inferred from bacterium-nanoparticle interactions about the potential consequences of environmental exposure to nanoparticles? Ecotoxicology 2008;17:362-71.