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Inactivation of Heterotrophic Bacteria in Well Water Using ZVI, TiO_2 and ZnO Nanoparticles

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Background & Aims of the Study: The heterotrophic bacteria are widely used as a water microbial pollution index for drinking water. The aim of this study was to investigate the effect of metallic nanoparticles such as Zero Valent Iron (ZVI), Titanium dioxide (TiO_2) and Zinc oxide (ZnO) on Heterotrophic Bacteria inactivation in well water.

Materials & Methods: We performed an experimental-laboratory study that the effect of nanoparticles type Zero valent iron (ZVI), Titanium Dioxide (TiO_2) and Zinc oxide (ZnO)) in constant contact time (30 min) and nanoparticles dose (1 g/L) was investigated on heterotrophic bacteria inactivation.

Results: The results showed that TiO_2 was detected more effective than ZnO and ZVI. The HPC inactivation after 30 min of retention time by TiO_2, ZnO and ZVI nanoparticles were 71.5, 50 and 36.4 as percent, respectively. The maximum bacteria inactivation was 98.82% in the presence of TiO_2 nanoparticles.

Conclusions: It is concluded that nanoparticles used in this study could be effectively used to increase the efficiency of removing heterotrophic bacteria from water and can be considered for microorganisms' inactivation.

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Background

There are several microbial indicators for assessment of the microbiological safety of drinking water. One of the standard methods for controlling of microbiological water quality in the water treatment systems is heterotrophic plate counts that described as colony-forming units (CFU) (1). High HPC is threatening to human health and may cause gastrointestinal diseases (2). In recent years, a lot of discussion has been raised about the effects of the common disinfectant in water, such as: carcinogenic effects, forming harmful disinfection byproducts (DBPs) (3,4) and microorganisms resistant that the need for research to find alternatives disinfection has increased (5). The rapid development of nanotechnology and its wide use in the environmental field due to high reactivity and the large surface area has attracted the attention of many studies (4,6-9). Nanotechnology has introduced different types of nanomaterial including Titanium dioxide (TiO_2), silver nanoparticles, etc to water industry that can have antimicrobial properties (10-12). The several studies have been carried out on the use of TiO_2 in the removal of contaminants from aquatic environments (4,10,13,14). The research showed that titanium dioxide can be effective in removal of different types of pollutants including microorganisms (bacteria, viruses, cysts, fungi, algae and protozoa), due to effect of OH radicals, hydrogen peroxide, superoxide anions and metal ions (15-17). In recent years, some studies have been performed on the microbial quality of water, for instance Maness et al. (18) and Cho et al. (13) investigated that inactivation of E.coli in the presence of the TiO_2 photocatalytic disinfection. Nowadays nZVI due to high reduction capacity, high efficiency, abundance, cheapness and also their unique atomic, molecular and chemical properties was used in the treatment of contaminated water (19,20). In addition, one of the important features nZVI is high reduction capacities that have been considered by many scientists in various fields (21). Diao et al. (22) reported that complete inactivation was achieved both for B. Subtilis var. Niger and P. fluorescens when treated with NZVI particles. Also, antibacterial properties of ZnO have been much attention. Zhang et al. (23) reported that photocatalytic degradation and inactivation of Escherichia coli can be obtained by $ZnO/ZnAl_2O_4$.

According to literature review there is no research about the effect of nanoparticles on heterotrophic bacteria inactivation.

Aims of the study:

The aim of this study was to evaluate the ability of nanoparticles to heterotrophic bacteria inactivation in water.

Materials & Methods

Materials:

Zero-valent iron powder and Zinc oxide nanoparticles were purchased from Research Institute of Petroleum Industry of Iran (RIPI). Titanium dioxide was purchased from Iranian nanomaterials Pioneers Company. All nanoparticles were used without any more treatment. Table 1 shows the properties of nanoparticles, according to manufactures.

Table1) characteristics of nanoparticles

Nanoparticles	Particle sizes (nm)	Purity (%)	Specific surface areas (m^2/g)	Color
ZVI	8-18	65	59-79	black
TiO_2	10-25	>99	200-240	white
ZnO	10-30	>99	20-60	White

Nanoparticles:

The morphology of ZnO and ZVI nanoparticles were determined in the RIPI by X ray diffraction (XRD) pattern model PW1840 Philips, Transmission Electron Microscopy (TEM) model Bruker AT-210 and Scanning Electron Microscopy (SEM) model S360 Cambridge instrument UK. The TEM image of TiO_2 was determined by Transmission Electron Microscopy model Hu-12A.

Sampling of water:

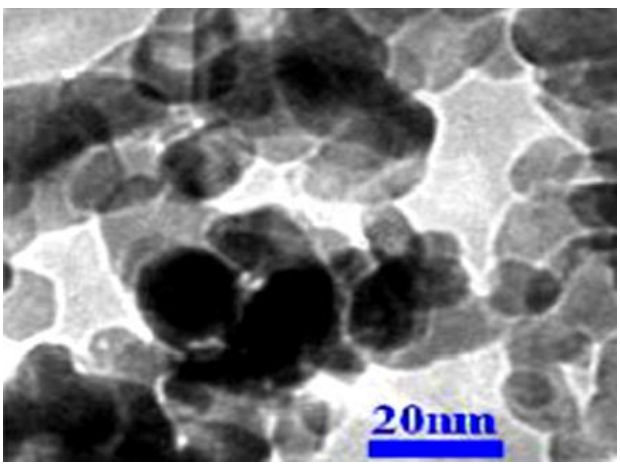
A total of 32 samples were collected from eight sites from several deep wells supplying water to the Mazandaran University of Medical Sciences located in Khazar road, Sari, Iran. Sampling was conducted between the hours of 9 am to 1 pm. Water treated with a plain chlorination. Water sampling was done according to the Standard Methods for the Examination of Water and Wastewater, method 9060 A (24).

Heterotrophic bacteria inactivation experiments:

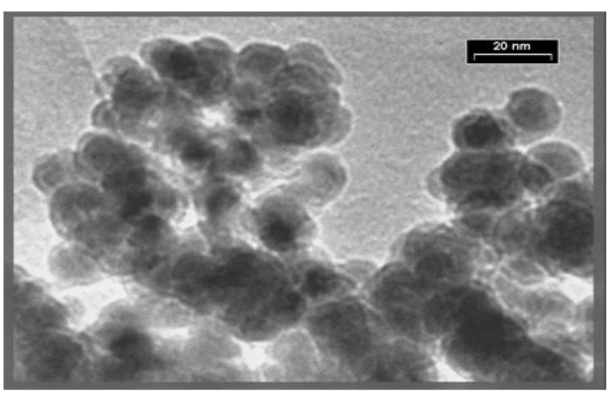
All equipment used in the experiments to be sterilized in an autoclave before the tests. In the laboratory; every eight collected samples were divided into four parts. One sample was examined without any treatment from each siteas the control. Based on the literature review (10,22,25) 1g/L certain nanoparticles (ZVI, TiO_2 and ZnO) were added into the water and shaken with a rate of 200 rpm using a horizontal shaker (Sibata) for 30 min. After the required time, the suspension was centrifuged for 30 minute at 4000 rpm. After the centrifugation, the samples were filtered using membrane filter with 0.45 µm pore size (24). To avoid the filtration effect on the test results, the samples were not filtered before to test. The Standard Methods for the Examination of Water and Wastewater, method 9215c (Spread Plate Method) was used for analysis of HPC (24). R_2A culture medium (Merck) was prepared and then sterilized in an autoclave at 121 ^oC and then 0.1-0.5 mL of sample volume was spread onto the surface of R_2 agar medium and incubated for 48 h at 35^oC. The results were analyzed at the end.

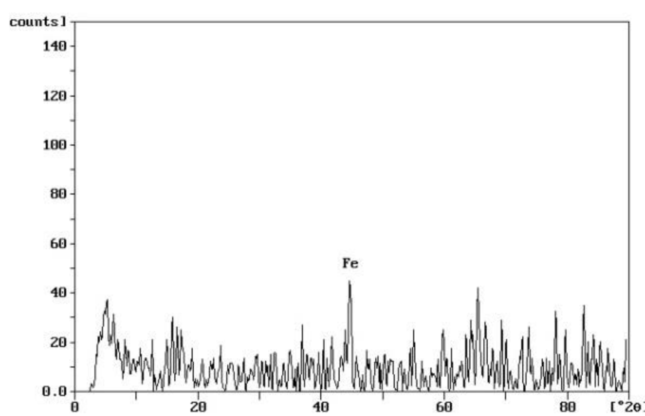
Results

Characterization of nanoparticles:
The images of XRD, TEM and SEM of used nanoparticles are shown in Figures 1 to 3. In the Figure 2 (B), the main peaks are characteristic peaks of iron and it should be noted that the samples were amorphous because they were prepared through liquid method. Therefore, only iron index peaks are evident in the 44.7 area.



(A) (B)
Figure 1) (A) TEM images and (B) SEM image of ZnO





(A) (B)
Figure 2) (A) TEM images and (B) XRD pattern of nZVI

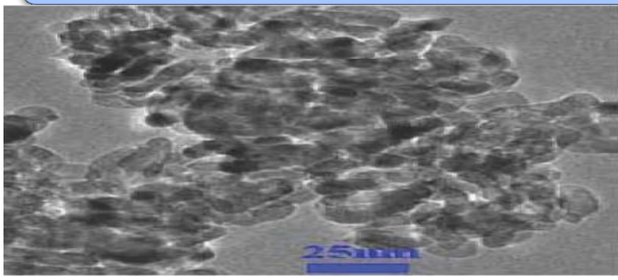


Figure 3) TEM images of TiO_2

Effect of TiO_2:

TiO_2 have a significant effect on heterotrophic bacteria inactivation. Maximum efficiency is observed in the experiment No.7 that the number of bacteria is reduced from eighty-five to one after treatment with TiO_2 nanoparticle. The heterotrophic bacterial inactivation efficiency by different nanoparticles was listed in table 2 based on this table remarkable amounts of bacteria (98.8 %) are destroyed by TiO_2. It is noteworthy that this high efficiency has been created no UV radiation and activation of nano. An antibacterial property of the TiO_2 has been attributed to two mechanisms: generation reactive oxygen species (ROS), damage to various parts of the cell (4).

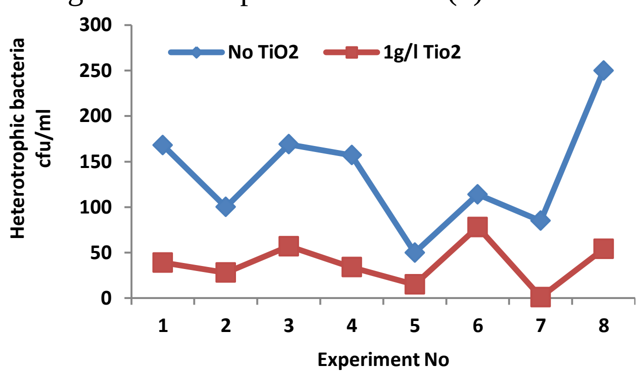


Figure 4) Effect of TiO_2 on the removal of heterotrophic bacteria at 30 min

Table 2) Heterotrophic bacterial inactivation

nanoparticles	Heterotrophic bacterial inactivation (%)		
	Min	Mean	Max
ZVI	6.5	36.4	67.1
ZnO	13.6	50	91.8
TiO_2	31.6	71.5	98.8

Effect of ZnO:

The highest efficiency of ZnO nanoparticles in the removal of heterotrophic bacteria was 91.8% that close to TiO_2 efficiency. In addition to the above-mentioned mechanisms for titanium dioxide, production of Zn ions and H_2O_2 are effective on toxicity of ZnO (4,26).

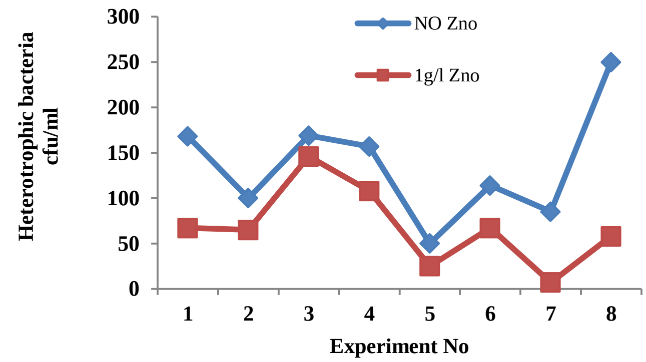


Figure 5) Effect of ZnO on the removal of heterotrophic bacteria at 30 min

Effect of nZVI:

A number of studies have shown that iron nanoparticles have antibacterial properties (22,27,28). The average removal of heterotrophic bacteria was obtained 36.4% at 8 experiments by nZVI. Average removal of ZnO and TiO_2 nanoparticles was estimated 5 and 71.5 percent, respectively. Mechanisms of nZVI in destroying bacteria are: physical impaired of the cells, production fenton conditions, generation oxidative stress (29).

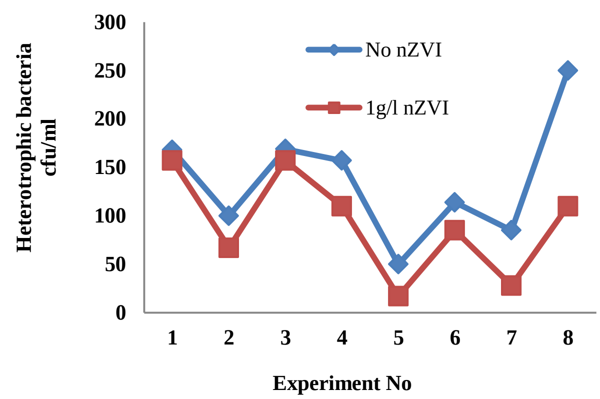


Figure 6) Effect of nZVI on the removal of heterotrophic bacteria at 30 min

Discussion

The results of this study are different with results reported by Adams et al. who demonstrated antibacterial activity of ZnO nanoparticlesis higher than of TiO_2 and SiO_2 in the removal of Gram-positive Bacillus subtilis and Gram-negative Escherichia coli (25). Similar results were reported by Rezaei-Zarchi et al. (30) they found that nano-TiO_2 has proven to be more efficient antibacterial agent as compared to nano-CdO. Moreover, Kasemets et al. (31) have used of ZnO, CuO and TiO_2 to evaluate the toxic effect of nanoparticles to Saccharomyces cerevisiae. Their results showed that nano and bulk TiO_2 were not toxic and nano CuO was about 60-fold more toxic than bulk CuO to Saccharomyces cerevisiae that was notsimilar to this research. Aruojaet al. (14) showed that ZnO nanoparticles compared with CuO and TiO_2 are most toxic to microalgae Pseudokirchneriella sub capitata. Their research results are not close to data obtained by present study. In another study conducted by Sawaiet al. for the study of antibacterial metallic oxide powders (32). The results indicated that ZnO powder was the least effective against E. coliamong the, MgO and CaO powder. Additionally, Dasari et al. (33) stated that the LC_{50} values was in the order of $ZnO<CuO<TiO_2<Co_3O_4$ under light condition, their results showed that ZnO were the most toxic among the tested nanoparticles, which is not in accordance with present findings. The toxicity of nanoparticulate and bulk ZnO, Al_2O_3 and TiO_2 to the nematode Caenorhabditiselegans was studied by Wang et al. (34). They reported that ZnO has minimum LC_{50} among the tested nanoparticles that are different with this research results. Various studies demonstrated the mechanism of ZnO on a wide range of bacteria (35-38). In a study was done by Leeet al. on the effect of nZVI on the Escherichia coli (28). It was reported that Escherichia coli are inactivated in the presence of nZVI rapidly (28). Also Diao et al. realized that 95%, 80% inactivation of B. subtilis var. nigerat nZVI concentrations of 1, 0.1 mg/ml (22) which was much higher than the values obtained in this study. The effects of ZVI nanoparticles on a natural river water bacterial community were studied by Barnes et al. (27). They reported that the addition of 100 mg/L ZVI nanoparticles to aerobic river water was not toxic to the indigenous river water bacterial community.

Conclusion

This is the first study on the effects of various nanoparticles on heterotrophic bacteria that is an important indicator in drinking water. The results showed that the nanoparticles can be used in of heterotrophic bacteria inactivation in drinking water with further investigation of their fate in the distribution network and its impact on human healthand the environment and may be a perfect alternative to traditional methods of water disinfection. The HPC inactivation after 30 min of retention time by TiO_2, ZnO and ZVI nanopaticles were 71.5, 50 and 36.4 as percent, respectively. Table 2 shows the heterotrophic bacterial inactivation efficiency by different nanoparticles. TiO_2 was more effective than nano ZnO and nano ZVI.

Footnotes

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Conflict of Interest:

The authors declared no conflict of interest.

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