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ORIGINAL ARTICLE

Inactivation of *Escherichia coli* in water by silver-coated $Ni_{0.5}Zn_{0.5}Fe_2O_4$ magnetic nanocomposite: a Box–Behnken design optimization

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Abstract

The present research studied the antibacterial effect of silver-coated $Ni_{0.5}Zn_{0.5}Fe_2O_4$ magnetic nanoparticles on Gram-negative bacteria *Escherichia coli* (*E. coli*) from water. The effects of pH (6, 7 and 9), disinfectant dose (2, 5 and 10 g/L) and contact time (10, 20 and 30 min) have been also investigated. To obtain important factors, the interactions between factors and optimal experimental design in surface response method were used based on Box–Behnken design. According to the research findings, the system is efficient in eliminating *E. coli*. The results showed that *E. coli* elimination efficiency intensified through increasing the amount of nanoparticles from 2 to 10 g/L. The results also demonstrated no significant change in *E. coli* elimination through pH increasing of 6 to 9. Expanding contact time from 10 to 30 min also heightened *E. coli* elimination rate. R^2 for *E. coli* elimination is 0.9994 indicating a good agreement between model experimental data and forecasting data.

Keywords Magnetic nanoparticles · *Escherichia coli* · Silver nanoparticles · Box–Behnken design

Introduction

Different countries throughout the world are concerned for supplying drinking water requirements due to population growth and depletion of drinking water sources. Water scarcity seriously resulted from increasing environmental pollution has turned water supply and sanitary requirements into one of the main issues of the present world (Thatai et al. 2019). Diseases caused by water contamination have led to the death of tens of thousands people around the world. However, the possibility of refining water provides access to resources for several purposes, and may, in some cases, compensate for water resources scarcity (Plessis 2019). Increasing the production and consumption in various industries, natural and artificial pollutants have challenged access to traditional water treatment practices to achieve the standard as the pollutants approaching to surface and groundwater resources. Sewage discharge to water resources is of water pollution sources to be identified, controlled and deterred. The first step is to monitor water pollution. Particular organisms including coliform family must be applied rather than monitoring all parameters to assess pathogenic microbial contamination. Coliform family is not pathogenic and remains in the environment for a relatively long time in large numbers; further, as they are exclusively intestinal, their presence in the environment is an indication of fecal contamination (Ashbolt 2004). Coinciding with the start of drinking water disinfection with chlorine in 1904, outbreak of epidemics associated with contaminated water consumption was severely reduced (Wolf et al. 2018). Then, different methods of drinking water disinfection like ozone and UV light for infection prevention were applied. However, because of numerous advantages of chlorine and its derivatives, drinking water disinfection using these compounds is the most global common method of disinfection (Alicia and Alvarez 2000; El-Shafy and Grünwald 2000). Using new methods is inevitable on account of population growth, the need for water supply and sanitary requirements due to widespread pollution of water sources. Today, nanotechnology is proposed to solve the issues of water quality and quantity (Vikesland 2018). The effect of metal ions on water disinfection has been studied by many scientists (Jain and Pradeep 2005; Deng et al. 2017; Fan et al. 2018; Mnatsakanyan and Trchounian 2018; Motshekga et al. 2018; Park et al. 2018). Silver, copper and zinc ions have been long known for their

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antimicrobial properties. Some studies have shown that the metal ions react with proteins through binding to sulfhydryl groups (–SH) in enzymes, and finally disable proteins (Yoon et al. 2007). If the metals are tiny, they would show better antimicrobial properties as a result of increased surface to volume ratio (Zhang et al. 2008). The metal nanoparticles can be used for coating some parts for antimicrobial properties and filters in medical equipment. Using the materials endures some challenges including microbial resistance against chemical antimicrobial agents as well as producing disinfection using conventional disinfectants. Innovation in new technologies development of disinfection and water treatment is a new achievement in the field of nanotechnology. *Escherichia coli* bacteria are of a streptococcus genus, Gram-positive organism, catalase-negative, oval and non-sporulation, facultative (lactic acid production from lactose) with complex nutritional requirements that often exist in most vegetables, herbs and foods, especially foods of animal origin such as dairy products. Also, it is part of normal intestinal flora of some mammals and humans (Rezaei-Zarchi et al. 2010). Berendjchi et al. (2011) performed a study to evaluate the activity of copper nanoparticles prepared in the form of a coating on cotton layer through sol–gel method. The results revealed that nanoparticles are effective in both *E. coli* (Gram −) and *S. aureus* (Gram +) (Berendjchi et al. 2011). Dong et al. (2011) analyzed antibacterial activity of magnetic nanoparticles (Fe_3O_4). According to the research results, modified nanoparticle is more effective against *E. coli* bacteria. Nanoparticles in a magnetic field can be recovered from sample (Dong et al. 2011). Cortés et al. (2006) conducted a study to investigate magnetic properties and antibacterial activity of a quad-core copper complex. The results displayed that the 4-phenylamidazole complex is effective for cereus bacteria and other Gram-positive bacteria, whereas complex pyridine *N*-oxide and 2-methylamidazole are only effective against Gram-negative bacteria (Cortés et al. 2006). Sanpo et al. (2013) examined spinel ferrite nanoparticle antibacterial activity using citric acid as a chelating agent through sol–gel method. According to the research findings, zinc and copper substitution in nanocobalt ferrite particles significantly increases *E. coli* and *Staphylococcus aureus* antibacterial activity (Sanpo et al. 2013). Tian et al. (2014) explored a nanocomposite antibacterial activity consisting of iron oxide, silver oxide and graphene oxide nanoparticles as the core, and only compared silver nanoparticles antibacterial activity. According to the research findings, obtained nanocomposites (GO-IONP-Ag) showed more powerful antimicrobial activity than silver nanoparticles; moreover, antibacterial effect was also observed on Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*) (Tian et al. 2014). Nickel–zinc ferrites have drawn noticeable consideration from researchers because of their remarkable magnetic properties, large permeability and very high electrical resistivity (Sharma et al. 2010). They have an extensive list of potential applications in such areas as high–density information storage devices, microwave devices, transformer cores, magnetic fluids (Liu et al. 2018), etc. We thus hypothesize that silver-coated $Ni_{0.5}Zn_{0.5}Fe_2O_4$ magnetic nanoparticles are viable filtration–sorption media to consider for practical application of bacteria removal. These nanocomposites are considered as one of the most suitable alternatives for magnetic and biochemical applications, considering the economic aspects and the properties of biodegradability and non-toxicity. Silver-coated $Ni_{0.5}Zn_{0.5}Fe_2O_4$ magnetic nanoparticles can have a very favorable outlook for the water and wastewater industry, taking into account advantages such as desirable health, economic and environmental aspects, as well as high efficiencies in the removal of microbial contaminants from water and wastewater, which may lower human dependence on chlorine. The present study aimed to evaluate the effect of silver-coated red soil on *E. coli* removal as a water microbial pollution index and effects of some parameters on its efficiency.

Materials and methods

General

This is an applied research carried out at a laboratory experimenting lyophilized strains of *E. coli*: ATCC 25922 prepared by a center of fungi collection and industrial bacteria in Iran. The materials used in this study include zinc chloride, nickel chloride, iron chloride, hydrochloric acid, sodium chloride, silver nitrate, sodium borohydride and culture media of lauryl sulfate broth, brilliant green bile broth, EC broth, eosin methylene blue, azide dextrose broth, bile esculin azide agar, Brain heart infusion, R_2A agar, nutrient broth and triple soy broth produced by Merck and Chem Lab company. B150 Nabertherm model furnace, SSIMS ONOS SW3H ultrasonic bath machine, Shaker incubator machine 8480 and also Rigaku RAD-IIA spectrometer with radiation of (1.5418 Å), 40 kV and 30 mA were also applied. The experiments were carried out according to the standard methods contained in the standard methods for water and sewage testing (Apha 2012). The magnetic characteristic was investigated by vibrating sample magnetometer (VSM, LDJ9600).

Synthesis of $Ni_{0.5}Zn_{0.5}Fe_2O_4$

Sixty ml of egg white was poured into a container and diluted with 100 ml distilled water; next, it was poured into a beaker for uniformity, then placed on a heater stirrer so that a homogeneous mixture is obtained. In the next step, 1.48, 1.45 and 8 g $FeCl_3$, $NiCl_2$ and $ZnCl_2$ salts were weighed and

added to a small amount of distilled water, then mixed and finally added to the homogeneous egg whites. The mixture was quickly stirred for 30 min. Following, it was heated at 80 °C for drying and turning into the powder. The obtained powder at this stage was then placed in the furnace at 500 °C for 2 h, and magnetic nanoparticles were prepared (Gabal et al. 2012).

Synthesis of $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag nanocomposite

In this procedure, 3 g of $Ni_{0.5}Zn_{0.5}Fe_2O_4$ was dispersed in 30 mL of sodium borohydride ($NaBH_4$) solution with constant stirring. To this mixture, 60 mL of a 0.1 M silver nitrate solution was added drop by drop. After all the silver nitrate solution is added, the mixture was further stirred for 30 min. The prepared $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag nanocomposite was then separated from solution by magnetic decantation and washed three times with distilled water, dried at 60 °C overnight and milled to achieve smooth powder. Finally, it was stored in a dark container. The inductively coupled plasma atomic emission spectroscopy (ICP-AES) was applied for determining the amount of the Ag nanoparticle loading on magnetic nanoparticles. After coating silver nanoparticles on $Ni_{0.5}Zn_{0.5}Fe_2O_4$, its effect on disinfection and removal of Gram-negative bacterium of *E. coli* was investigated.

Culture medium

Escherichia coli species were cultured according to the manufacturer guidelines. Briefly, a single colony of *E. coli* was taken from a refrigerated stock and pre-cultured in 20 mL tryptic soy broth (TSB) by incubation at 37 °C for 24 h. Then, it was transferred into tryptic soy agar (TSA) and incubated for 24 h at 37 °C. The top of each colony was touched with a sterile loop, and the growth was transferred into a tube containing 4 to 5 mL of distilled water (IROST 2016). A McFarland standard 0.5 was used to determine the cell concentrations. The cell density was compared to that standard using a UV/VIS spectrophotometer, an equivalent optical density of 0.1 at 625 nm with regard to the calibrated standard cell suspensions in distilled water (Dhara and Tripathi 2013). A barium sulfate turbidity standard was used to standardize the inoculums density for a susceptibility test; its turbidity was equivalent to that of a 0.5 McFarland standard the latter made according to Garcia (2010). To obtain the required cell suspensions, the stock was serially diluted in distilled water. This resulted in a suspension containing approximately 10^2, 10^4 and 10^6 CFU/mL. The standard plating method was applied to confirm the bacterial concentrations. This test was done in triplicates on tryptic soy agar. Samples were plated in triplicates. The colonies were visually identified and counted after incubation at 37 °C overnight.

Response level method

The response level method is a collection of useful statistical and mathematical methods for modeling and analyzing problems, in which the desired response level is affected by multiple variables (Ahmadi et al. 2005). In recent years, the method has been well considered in the field of water treatment, as it is very easy with a quick and accurate design. The method provides a second-order polynomial model to fit the test responses as follows (Myers et al. 2004):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 \quad (1)$$

where Y is the response, (X_1, X_2 and X_3) are the encoded variable factors, b_0, b_i and b_{ij} ($i, j = 1, 2, 3$) are the model estimated coefficients.

In the response level method, the Box–Behnken design (BBD) has been used to optimize the responses. The parameters significance level was 95%. To eliminate *E. coli*, from contaminated water, the effect of nanoparticle factors, pH and contact time on removing *E. coli* has been investigated. The factors' levels are presented in Table 1. Regarding the number and levels of selected factors using response level and Box–Behnken design method, through Design-Expert software 8.0.1, 15 tests have been introduced in the proposed range. The results were analyzed using ANOVA table and 3D charts.

To calculate the removed amount of *E. coli* by nanoparticle, the following equation is used:

$$R(\%) = \left[1 - \left(C_0/C_t\right)\right] \times 100 \quad (2)$$

where R represents the percentage of bacterial elimination, C_0 is the number of primary bacteria, and C_t is the number of bacteria remaining after disinfection time t.

Table 1 Selected factors and levels for the removal of *E. coli*

Factors	Actual and code values of factors		
	1 +	0	−1
$Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag (g/L)	10	5	2
pH	9	7	6
Contact time (min)	30	20	10

Results and discussion

Characterization of $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles

$Ni_{0.5}Zn_{0.5}Fe_2O_4$ magnetic nanoparticles were synthesized according to the reported procedure by Gabal et al. (2012) and fully characterized based on our previous reports (Beigzadeh and Moeinpour 2016; Omidvar-Hosseini and Moeinpour 2016). The inductively coupled plasma atomic emission spectroscopy (ICP-AES) was applied for determining the amount of the Ag nanoparticle loading on $Ni_{0.5}Zn_{0.5}Fe_2O_4$. It showed 0.071 mol of silver nanoparticle per gram of the prepared disinfectant.

In order to verify the formation of silver layer on $Ni_{0.5}Zn_{0.5}Fe_2O_4$ magnetic nanoparticles, UV spectra were taken from both samples, as shown in Fig. 1. The peak formed in a wavelength of about 350 nm in the UV spectrum of silver nanoparticles (b) reveals the presence of a silver layer on magnetic nanoparticles (Wang et al. 2011).

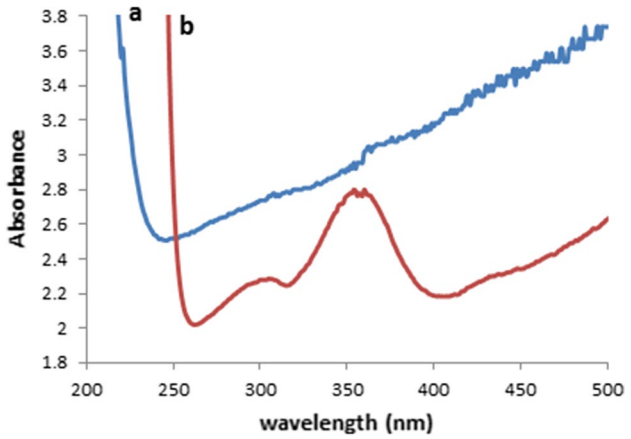

Fig. 1 UV spectra of (a) $Ni_{0.5}Zn_{0.5}Fe_2O_4$ magnetic nanoparticles; (b) $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag

The magnetic characteristics of the $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles were identified using vibrating sample magnetometer (VSM). As can be seen in Fig. 2, $M(H)$ hysteresis loop was completely reversible for the sample, indicating that the $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles show super paramagnetic characteristics. The hysteresis loops of them reached saturation up to the ultimate applied magnetic field. At room temperature, the magnetic saturation value of the $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles is about 25 emu g^{-1}. The high permeability of particles in the magnetization showed that they could be separated by a typical magnetometer.

Development and evolution of prediction model

Table 2 shows experimental results of eliminating *E. coli* according to the design table with the response level and BBD with $Ni_{0.5}Zn_{0.5}Fe_2O_4$ magnetic nanoparticles.

The results of analysis of variance (ANOVA) for the independent variables are presented in Table 3. The confidence level was considered 95%. For this reason, *P* ratio must be less than 0.05 so that the model or the factors effect is significant. This means that there is a 5% probability of error that a non-important factor is considered important. One of the most important factors in the statistical analysis of *F* ratio is the model statistical significance. If the *F* ratio of an agent is higher for, it indicates that this factor is significant and its effect on the response rate is more important. The analysis of variance for the removal of *E. coli* is shown in Table 3.

Considering *P* value and *F* ratios of eliminating *E. coli*, the effect of pH factors, disinfectant dosage and contact time on *E. coli* elimination is significant. Disinfectant dosage

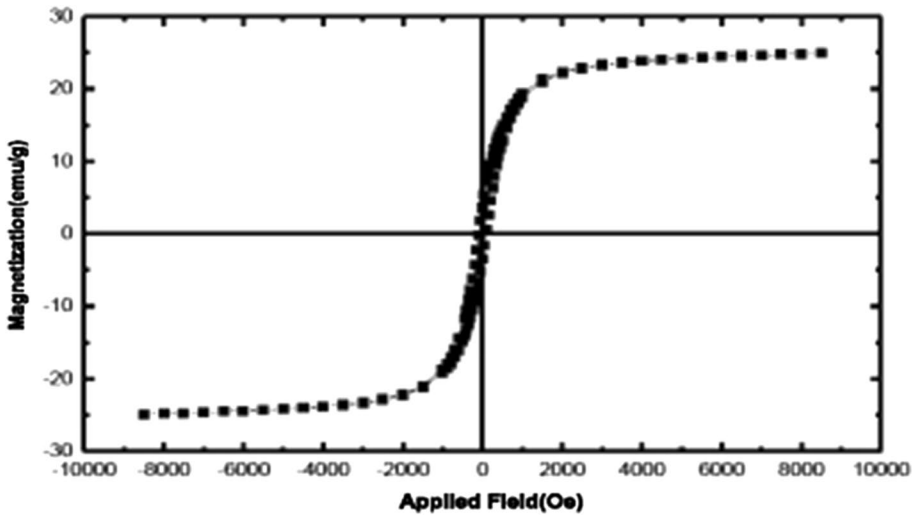

Fig. 2 Magnetization curve of the $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles at room temperature

Table 2 The Box–Behnken design matrix with three independent variables (*A*, *B* and *C*)

Experiment no.	*A* Disinfectant dosage; g (code)	*B* pH (code)	*C* Contact time; min. (code)	*R* % *E. coli* removal
1	2 (− 1)	7 (− 1)	20 (0)	94.7
2	5 (0)	9 (1)	30 (1)	99.2
3	10 (1)	9 (1)	20 (0)	99.1
4	10 (1)	8 (0)	30 (1)	100
5	10 (1)	8 (0)	10 (− 1)	96.4
6	2 (− 1)	9 (1)	20 (0)	94.1
7	2 (− 1)	8 (0)	30 (1)	95.3
8	5 (0)	8 (0)	20 (0)	100
9	5 (0)	7 (− 1)	10 (− 1)	96
10	5 (0)	8 (0)	20 (0)	99.9
11	5 (0)	8 (0)	20 (0)	100
12	5 (0)	7 (− 1)	30 (1)	99.9
13	10 (1)	7 (− 1)	20 (0)	99.9
14	5 (0)	9 (1)	10 (− 1)	94.9
15	2 (− 1)	8 (0)	10 (− 1)	91.2

factor has the greatest effect on *E. coli* removal. Other factors showed no significant relationships.

Mathematical model

The value of R^2 for *E. coli* elimination is 0.9994 indicating a good agreement between experimental data and the model predicted data. The comparison of the experimental model and predicted results of *E. coli* removal rate is presented in Fig. 3.

Also, insignificance of the term not fitted in Table 3 implies that the proposed statistical model provided is well fitted to the experimental data. The second-order statistical model, offering the design of *E. coli* removal rate in terms of agents actual values (not coded values), is presented as follows:

$$R_1(\%) = 99.97 + 2.51A - 0.4B + 1.99C - 0.05AB - 0.13AC + 0.1BC - 2.4A^2 - 0.62B^2 - 1.85C^2 \quad (3)$$

where disinfectant dosages (A) are in g/L, contact time (C) in minutes, and elimination of *E. coli* (R_1) is in percentage.

The effect of parameters

The effect of $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles dosage

The $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles dosage is one of the most important factors, as shown in Fig. 4a, b (3D diagrams); bacteria elimination rate has been raised by intensifying nanoparticles dosage in the process of bacterial elimination, for example for an increased amount from 2 to 5 g for *E. coli*, the elimination percentage has increased from 95.7 to 100%, which can be attributed to an escalation in the contact surface of the nanoparticles with bacteria; as well as an increase in hydrogen peroxide concentration produced from nanoparticles intensifying bacteria elimination percentage (Sawai et al. 1996). The results of the present study are consistent with Zhang et al. (2007). It should be noted that in these experiments, the control test was performed at following conditions:

Contact time = 20 min.; disinfectant concentration (uncoated $Ni_{0.5}Zn_{0.5}Fe_2O_4$) = 0.05 g/mL; *E. coli* no. = 10^4 CFU/mL. After culture on the medium (R_2A agar), the bacteria grew and formed a colony. The bacterial inactivation percent was zero.

Table 3 Analysis of variance for the response rate of *Escherichia coli* elimination

Source	*df*	Sum of squares	Square mean	*F* value	*p* value prob > *F*	Remark
Model	9	115.32	12.28	926.27	< 0.0001	Significant
A (disinfectant dosage)	1	50.5	50.5	3650.69	< 0.0001	Significant
B (pH)	1	1.28	1.28	92.53	0.0002	Significant
C (time)	1	31.6	31.6	2284.43	< 0.0001	Significant
$A \times B$	1	0.01	0.01	0.72	0.434	
$A \times C$	1	0.062	0.062	4.52	0.0869	
$B \times C$	1	0.04	0.04	2.89	0.1498	
A^2	1	21.19	21.19	1532.09	< 0.0001	Significant
B^2	1	1.42	1.42	102.88	0.0002	Significant
C^2	1	12.58	12.58	909.4	< 0.0001	Significant
Lack of fit	3	0.062	0.021	6.25	0.141	
Pure error	2	0.006	0.003			

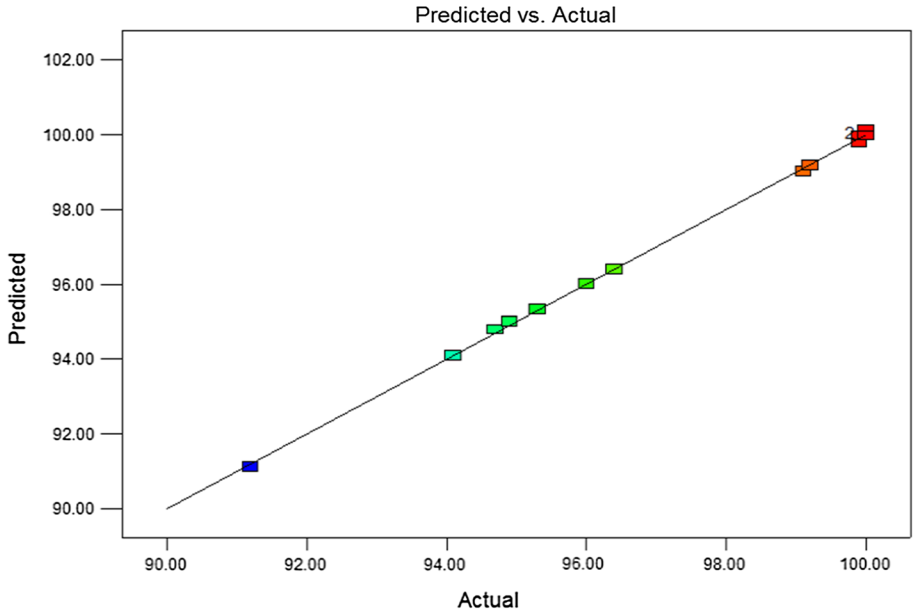

Fig. 3 The experimental model (squares) and predicted results (dash) for *Escherichia coli* removal

The effect of contact time

Contact time is the second most important factor. As shown in Fig. 4a, b for *E. coli*, the increase in contact time has led to an increase in bacterial elimination percentage. For example, an increase from 10 to 20 min heightened *E. coli* removal percentage from 95.1 to 100%, which results in expanded process time.

The effect of pH

The effect of pH factor on removing understudied bacteria has been lower than other investigated factors. In spite of the removal research in the field of chemistry, it is associated with living organisms that the organisms' reactions may cause pH changes. As shown in Fig. 4a, b (diagrams of *E. coli* removal), the results indicated no significant change by a pH change of 6 to 9 in *E. coli* elimination percent. It is interpreted that the intestinal bacteria are not pH-tolerant. The results of the study are consistent with Alikhani et al. (2012) and Haavik (1974). The control test was performed at the conditions described above.

Optimization

Respecting economic dimension, the software has the optimum point with a 97.2% elimination rate for *E. coli* and a concentration of 3.2 g/L magnetic nanoparticles, a pH of 6.35 and a contact time of 15.3 min. Moreover, if the optimal point is determined by economic dimension, the maximum removal at these points equals 79.6%, at a point where the concentration of nanoparticles = 2 g/L, pH = 6.16 and contact time = 11.4 min. If maximum removal percentage is the objective, regardless of economic dimension, the highest percentage of removal is 100% where nanoparticle concentration is 10 g/L, pH = 6.19, and contact time is 30 min.

The effect of the number of bacteria

Figure 5 illustrates that bacterial removal rate decreases by augmenting the number of bacteria. For instance, in an *E. coli* sample with an increase of 10^3 to 10^5 CFU/mL in the number of bacteria, disinfection efficiency decreases from 100 to 99.92% as nanoparticles contact surface with bacteria is declined by increased number of bacteria, and the turbidity is intensified, due to diminishing nanoparticles' disinfection properties. The control test was performed at the conditions described above.

According to our results, the mechanism of bacterial inactivation by red soil coated Ag is ionic. The mechanism operates based on the transformation of microorganisms by converting—SH bonds to—SAg bonds. Silver nanoparticles disable the enzyme by releasing Ag^+ ions and absorbing the—SH bonds which are the basis of the protein enzymes at the surface of bacteria. Therefore due to the lack of absorption of phosphate by the cell, the bacteria are inactivated. This mechanism does not terminate with the destruction of the bacteria and is a permanent process (Davies and Etris 1997).

Conclusion

The results of the present study demonstrated that Gram-negative *E. coli* bacterium is sensitive to $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles. Intensifying nanoparticles

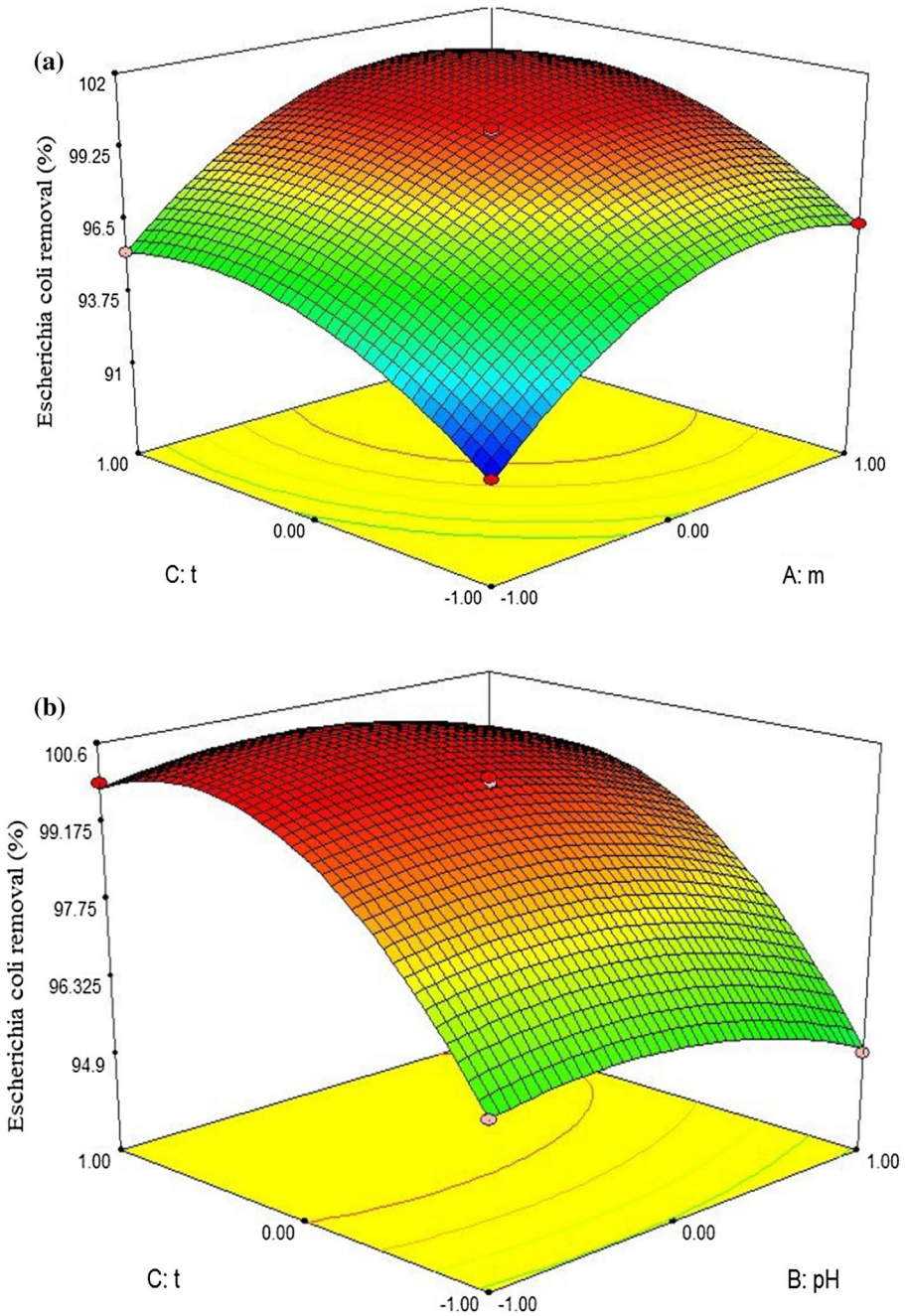

Fig. 4 3D diagram of changes in *Escherichia coli* removal rate in terms of code and actual values; **a** nanoparticles dosage and contact time; **b** pH and contact time

concentration increases bacterial removal percentage; furthermore, raising the number of bacteria reduces nanoparticles disinfection properties. Extended contact time of the bacteria with magnetic nanoparticles may increase bacterial elimination percentage. The results also revealed no significant changes in *E. coli* elimination by increasing pH from 6 to 9. R^2 value for *E. coli* elimination is 0.9994, implying a good agreement between the experimental model and predicted data. Of major advantages of disinfection is separating nanoparticles from solution by a magnetic force, which not only reuses disinfection and is economically feasible, but also prevents the entrance of nanoparticles into the environment. Scientific nano-advances, it is possible to use $Ni_{0.5}Zn_{0.5}Fe_2O_4$/Ag magnetic nanoparticles in water and wastewater industry. Respecting silver nanoparticles, one of the limitations considered as a disadvantage of using disinfectant is the high price. However, silver reusing and recycling may, to some extent, moderate the constraint of this strong disinfectant.

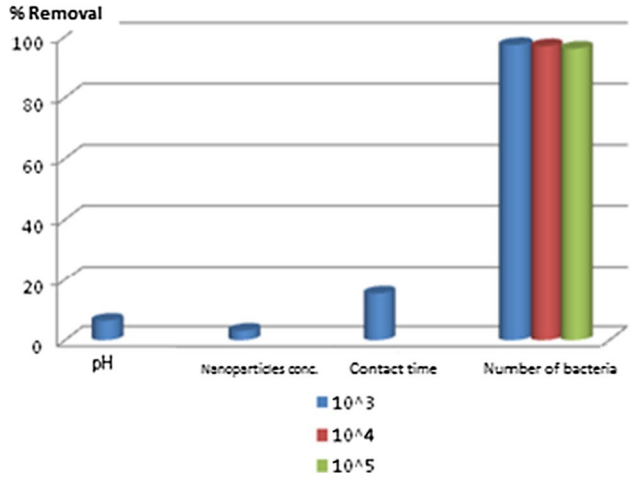

Fig. 5 The effect of the number of bacteria on magnetic nanoparticles disinfection efficiency at optimum point for *Escherichia coli*

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