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Research Article

Investigation of Zinc Oxide Nanoparticles Effects on Removal of Total Coliform Bacteria in Activated Sludge Process Effluent of Municipal Wastewater

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Abstract

Background: One of the problems of rapid population growth is contamination of water resources by wastewater. Wastewater may contain microbial contaminants such as total coliform bacteria that can be a threat to the public health. Zinc oxide nanoparticles effects on removal of total coliform bacteria from the activated sludge process effluent of municipal wastewater was investigated. **Materials and Methods:** The samples were taken from the effluent of the secondary sedimentation tank of wastewater treatment plant. After cultivation of the samples by multiple tube fermentation method, the total coliform rate was evaluated by MPN table. Different concentrations of ZnO nanoparticles were applied and in different contact times, the efficiency of total coliform removal rate was calculated. **Results:** Findings showed that ZnO nanoparticles could remove 100% of total coliform in municipal activated sludge effluent in concentration of 1.1 g L^{-1} in 90 min contact time. Also, total coliform removal percentage showed a significant difference in 20 min with that of 40, 60 and 90 min ($p<0.001$). No significant relation was seen in the concentration of ZnO nanoparticles with the removal efficiency ($p>0.05$). **Conclusion:** The results show that ZnO nanoparticles are a proper antibacterial for removing total coliform bacteria from municipal wastewater effluent and application of ZnO nanoparticles as a disinfectant for removing total coliform bacteria can be studied further.

Key words: Wastewater treatment, effluent disinfection, activated sludge, nanoparticles, total coliform

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Rapid world population growth has caused lots of problems for human beings. One of them is the environmental pollution specially the water resources by wastewater. In addition to different environmental hazards, this problem threatens the human health[1]. Municipal wastewater is one of the major environmental pollution sources which imposes high cost to the economy of countries each year[2]. Wastewater contains pathogen and non-pathogen microorganisms and organic and non-organic materials. When these compounds are released into the environment untreated, they pose immense threat to creatures and environment. Therefore, wastewater treatment before discharge is seriously necessary[3]. Existence of diseases due to pathogens in the discharged effluent of wastewater treatment plants which affects the microbial quality of water resources, causes significant threats to the public health[4]. Microbes are transferred to humans through infiltration of human wastewater to drinking water and consequently different diseases are transferred to humans by water. Some of these diseases are cholera, typhoid, microbial diarrhea and dysentery, hepatitis, tuberculosis and blood and liver parasites. Near 1 billion humans suffer from the diseases related to contaminated water which causes the death of 10 million people[5]. Death of 2.2 million of under 5 years old children has been reported because of unhealthy water[6,7]. The most common disinfection methods of bacterial contamination in water are chemical disinfection, ozonation, UV radiation and membrane processes[5]. Right now, chlorination is the most common method for disinfection of sewage around the world before discharge into receiving streams, rivers and oceans[4]. Application of some of these processes are limited because of the expensive treatment cost, additional treatment requirement, sludge production and other byproducts formation, increase of dissolved ions and low performance[8]. For example, halogens such as chlorine and bromine as antibacterial agents are well known and widely used but the direct use of halogens as a bactericide has a lot of problems because of their high toxicity and pure vapor pressure[9]. So, there is a challenge for finding a suitable disinfection method without formation of harmful byproducts. Also, increasing need to decentralized systems or point-of-use water treatment and recycling systems requires a new technology for disinfection and microbial control[6]. Numerous studies have shown that nanoparticles formulations can be used as an efficient antimicrobial agent[10]. Because of the catalytic and antimicrobial characteristics of nanoparticles, they are considered as efficient alternatives for disinfection of water and wastewater systems[11]. Antibacterial features of metal nanoparticles are because of their small size and their high rate of surface to volume which lets them react closely to the microbial membrane and destroy them[12]. The operating mechanism of the effect of nanoparticles on bacteria is by damaging the protein, DNA and destroying the cell wall[13]. Unlike common chemical disinfectants, antimicrobial nano- materials are not strong oxidants and are partly neutral in water. So, they are not expected to produce harmful Disinfection By-Products (DBP_s). If nanomaterial is correctly entered to the process, it can enhance the common methods of disinfection or be replaced with them[6].

Other studies clearly indicate that ZnO nanoparticles, in comparison with five other nano-metal oxides have significant growth inhibitory effects in natural light. They specially have wide antibacterial effect on some microorganisms[10]. The study of Malakootian and Toolabi[13] showed that the effect of ZnO nanoparticles on gram positive and negative bacteria was more than TiO_2 and CuO nanoparticles. Jones *et al.*[10] study in 2008 indicated that among six examined nanoparticles, ZnO nanoparticles with their relatively small particle size showed a wide range of antibacterial effect in visible light on different strains of *Staphylococcus aureus*. The study of Brayner *et al.*[14] with the title of toxicological impact studies based on *Escherichia coli* bacteria in ultrafine ZnO nanoparticles colloidal medium showed that *E. coli* cells were damaged after contacting with ZnO and caused destruction of Gram-negative triple membranes. Hrenovic *et al.*[15] demonstrated that application of metal oxide nanoparticles was a novel method in disinfection of secondary effluent and removing pathogen microorganisms. In the previous studies, antibacterial effect of ZnO nanoparticles have been proved but its efficiency in removing total coliform bacteria has not been investigated yet. Therefore, considering the great significance of this subject and lack of similar studies, the effect of effective parameters like concentration of ZnO nanoparticles and reaction time on the removal process rate of total coliform bacteria of wastewater treatment plant effluent was investigated.

MATERIALS AND METHODS

This study was an experimental study and was done in a laboratory scale. Effluent samples were taken from the secondary sedimentation tank effluent of municipal wastewater treatment plant in the summer of 2015. In this study, ZnO concentrations of 0.3, 0.5, 0.7, 0.9 and 1.1 g L^{-1} were used in four contact time of 20, 40, 60 and 90 min and in pH = 7.2-7.4 (the natural pH of the samples) and the removal total coliform efficiency was calculated.

Materials: The ZnO nanoparticles used in this study were bought from Iranian Nanomaterial Pioneers Company which had been produced by an American company (US Research Nanomaterials). Lactose broth medium and brilliant green bill broth medium which were used in this study were from Merk company made in Germany and bought from Poiesh Shimi company in Iran.

ZnO nanoparticles structure: The ZnO nanoparticles with the size of 10-30 nm and 99% purity and SSA>15 were used (Table 1). The seller had done the x-ray powder diffraction (XRD), Scanning Electron Microscope (SEM), Transmission Electron Microscopy (TEM) analysis in the nano laboratory unit which is approved by Iran Nanotechnology Initiative Council (Fig. 1).

Table 1: Zinc oxide nanopowder (ZnO) characterization

Purity (%)	APS (nm)	SSA (m^2 g^{-1})	Color	Crystal phase	Crystal morphology	True density (g cm^{-3})
99+	10-30	20-60	Milky white	Single	Nearly spherical	5.606

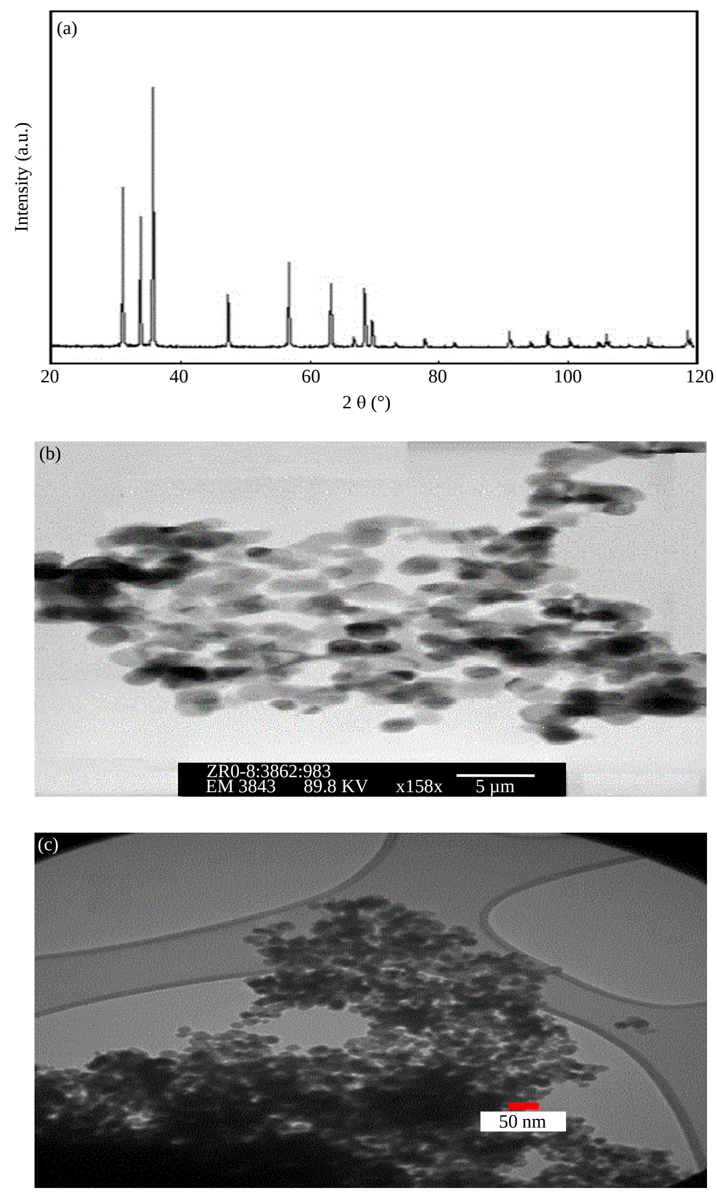

Fig. 1(a-c): ZnO nanoparticles (a) XRD, (b) SEM and (c) TEM

Experiment: First, the effluent was diluted up to 1/100 (in order to make 1/100 concentration, 10 mL of the effluent was mixed with 1000 mL saline). Then, after cultivation, total rate of total coliform in the sample was calculated. Total coliform bacteria cultivation was done by multiple tube fermentation method in lactose broth and brilliant green bill broth medium in concentrations of 0.1, 1 and 10 mL according to 9221 B. experiment of standard method book published in 2012. Total coliform bacteria counting was done using Most Probable Number (MPN) chart. Lactose broth medium was used in concentrations of 0.1, 1 and 10 mL to evaluate the probability of the presence of total coliform. The cultivated samples were incubated in 35±0.5°C for 48 h. If the samples were positive (gas aggregation in Durham tubes), they were transferred to brilliant green bill broth medium to confirm the presence of total coliform. They were cultivated in concentrations of 0.1, 1 and 10 mL. The cultivated samples in brilliant green bill broth medium were incubated in 35±0.5°C for 24 h. When the mentioned time was passed, the number of positive tubes were counted by MPN chart. In the next step, different concentrations of ZnO nanoparticles were added to one liter of diluted wastewater in a glass reactor in different contact times (to accomplish this process, the reactor was set on a magnetic stirrer). Before adding the contacted sample to nanoparticles in the medium, in order to prevent further contact, the mixture was centrifuged (to have identical conditions, the raw effluent sample was centrifuged too and then it was cultivated). The negative tubes which had no gas indicated that no total coliform bacteria existed in them[16]. Cultivation of bacteria was done in lactose broth and brilliant green bill broth medium as was mentioned before. The experiments were done in natural light. In order to make sure about the correctness of results and avoid any possible mistakes, each experiment was repeated three times. To investigate the effect of the process and comparison of different concentrations and times, repeated measures variance analysis was used. Then, data were analyzed with SPSS ver.16 software.

RESULTS

In this study, the effect of different concentrations of ZnO nanoparticles (0.3, 0.5, 0.7, 0.9 and 1.1 g L^{-1}) in different reaction times (20, 40, 60 and 90 min) on removing efficiency of total coliform bacteria from municipal wastewater's effluent of activated sludge process was investigated. Table 2 shows that in concentration of 0.3 g L^{-1}, during 20-90 min of the experiment, total coliform removal efficiency reached 96% from 66%. The removal rate of ZnO nanoparticles in concentration of 0.5 g L^{-1} from 20-90 min reaction time increased from 75-98.66%. In concentration of 0.7 g L^{-1}, in 20 min 65% of total coliform reduced and in 90 min removal efficiency reached 99.4%. In concentration of 0.9 g L^{-1} of nanoparticles, removal efficiency changed from 69-99.7% during 90 min. Finally, in the concentration of 1.1 g L^{-1} of ZnO in 90 min, 100% of the municipal effluent's total coliform was removed. On the whole, by increase of time, removal efficiency was increased (Table 2).

According to the results of Repeated Measures variance analysis (Table 3), since the Mauchly's test was significant

Table 2: Mean and standard deviation of total coliform removal in different times

	Time (min)			
Concentration (g L^{-1})	20	40	60	90
0.3	66.03±21.14	91.40±1.35	94.43±2.05	96.40±2.51
0.5	74.90±14.6	89.56±3.09	95.33±5.03	98.66±1.15
0.7	65.56±22.06	90.00±5.4	91.73±4.35	99.40±0.79
0.9	69.46±18.51	87.50±11.24	94.53±4.93	99.76±0.4
1.1	78.72±17.08	90.36±7.14	98.43±1.6	100.00
Total	70.93±16.81	89.76±5.73	94.89±3.96	98.84±1.74

Table 3: Tests of within-subjects effects

Source	Type III sum of squares	df	Mean square	F	Significance	Partial Eta squared
Time						
Sphericity assumed	6869.541	3	2289.847	27.252	0.000	0.732
Greenhouse-Geisser	6869.541	1.162	5912.440	27.252	0.000	0.732
Time concentration						
Sphericity assumed	291.904	12	24.325	0.290	0.987	0.104
Greenhouse-Geisser	291.904	4.648	62.809	0.290	0.900	0.104
Error (time)						
Sphericity assumed	2520.745	30	84.025			
Greenhouse-Geisser	2520.745	11.619	216.954			

and the sphericity assumption was not established, Greenhouse-Geisser statistics was used. According to this test, time was statistically significant ($p<0.001$). Interaction of concentration and time was not statistically significant (Table 3).

Figure 2 shows the changes of ZnO nanoparticles concentration and time in removing total coliform. As the results indicate, removal percentage is significantly increased by the increase of time. Removal percentage of total coliform in 20 min has a significant difference with three other times ($p<0.001$). Comparing the removal percentage in 40 min with 20 and 90 min, there is a great difference ($p<0.001$ and $p = 0.019$, respectively) but with 60 min it has no considerable difference ($p = 0.177$). Also between the removal percentage of 60 min with 90 min, there is no significant difference ($p = 0.295$).

The results of the removal efficiency in different concentrations of ZnO nanoparticles can be seen in Fig. 3. As shown in the graph, concentration of 1.1 g L^{-1} has the most

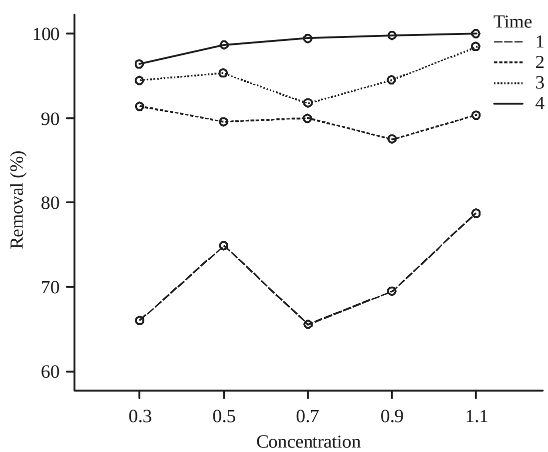

Fig. 2: Efficiency of total coliform removal by ZnO nanoparticles in terms of time

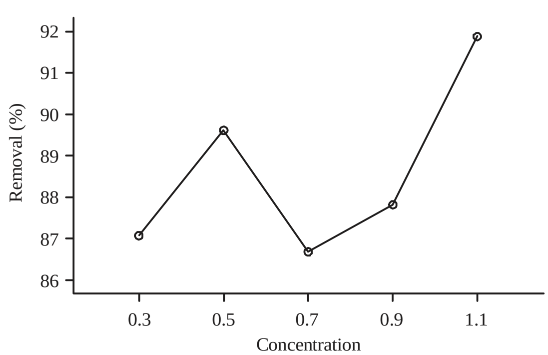

Fig. 3: Average efficiency of total coliform removal by ZnO nanoparticles in terms of concentrations

removal percentage of total coliform with 91.88%. Then, the concentration of 0.5 g L^{-1} with the removal of 89.61% and the concentrations of 0.9, 0.3, 0.7 g L^{-1} with the removal percentage of 87.81, 87.06 and 86.67%, respectively. The observed difference in removal efficiency in different concentrations of ZnO nanoparticles are not statistically significant ($p>0.05$).

DISCUSSION

The development of science and nano-technology in the recent decades has provided the opportunity for discovering the antibacterial characteristics of metal nanoparticles. Nanoparticles are an efficient option for disinfection of water and wastewater systems because of their catalytic and antimicrobial characteristics[11]. This study was done to investigate the effect of ZnO nanoparticles on removal rate of total coliform of municipal wastewater treatment plant effluent.

This study shows that as contact time is increased, the removal percentage of total coliforms is also increased. This result is in accordance with some other researches[5,7,17]. It can be concluded that when the reaction time is increased, ZnO nanoparticles have more time for penetrating into the cells and destructing the membrane cells of bacteria and killing more percentage of total coliform bacteria. Aggregation of ZnO nanoparticles in the membrane and cytoplasm of bacteria can inactive them and cause bacteria's growth inhibition. In this regard, due to very small size of particles, membrane piercing is easer[18]. Also, by increase of time, more H_2O_2 is released from bacteria cells which increases toxic effects[19]. During all the experiment process in concentration of 0.3-1.1 g L^{-1}, ZnO nanoparticles remove 65-100% of wastewater's total coliform. This result is very similar to Noroozi and Mehdinezhad[5] study which in concentration of 0.2-1 g L^{-1} removal rate is 70-100%.

By increasing the ZnO concentration from 0.3-0.5 to 0.7-1.1 g L^{-1}, the removal of total coliform increases. With the increase of ZnO nanoparticle's concentration, antibacterial activity increases which can be because of the increase of hydrogen peroxide's rate. ZnO is a semiconductor with a large band gap. It means that when a photon energy greater than the band gap is applied, holes (h^+) and free electrons are formed. The holes react with water to form •OH and electrons in cb react with oxygen to form $•O_2^-$ and •OH. All these reactions will ultimately end in forming[20] H_2O_2. Different studies indicate that by increase of nanoparticles' concentration, antibacterial effect is increased[13,17,20,21]. In the concentration of 1.1 g L^{-1}, ZnO nanoparticles removed 100% of the municipal effluent's total coliform in 90 min. In

Alikhani *et al*.[18] study, optimum doze of ZnO for photocatalytic removal of *Escherichia* is considered as 1 g L^{-1}, which is compatible with the results of this study. Study of Malakootian and Toolabi[13] determined that 1000 mg L^{-1} of ZnO nanoparticles can remove 60-100% of Gram-negative and Gram-positive bacteria which is similar to this study. Zhang *et al*.[22] concluded that during 1 day and with the concentration of 2 g L^{-1} of ZnO nanoparticles, with or without light, 99.99% of the *E. coli* bacteria are removed. This difference may be because of different size of nanoparticles which in this study is 10-30 nm and Zhang *et al*.[22] study is approximately 198.4 nm. This difference caused the necessity of more time for removal. According to the study of Franklin *et al*.[23] smaller particles are more toxic.

Concentration of 1.1 g L^{-1} has the most percentage of total coliform removal with 91.88% while in the concentrations of 0.5, 0.9, 0.3 and 0.7 g L^{-1}, the removal percentage is 89.61, 87.81, 87.06 and 86.67%, respectively. The results show that between concentrations of 0.5-0.7 g L^{-1}, despite the increase of ZnO concentration, reduction of bacteria has not occurred. This may be due to bacteria metabolism because they can use zinc ion as a factor for cellular processes and as a result it is not toxic for *E. coli* bacteria. High concentrations of ZnO can inactive bacteria; while, in low concentrations it can be a growth factor[24]. Roslli *et al*.[25] study shows that concentration of 10^{-3} to 10^{-4} M of ZnO nanoparticles in 31 h of treatment have no effect on cell permeability. Study of Brayner *et al*.[14] show results similar to this experiment occurs and it means that with the increase of ZnO nanoparticles in concentration of 1.5×10^{-3} to 10^{-3} M, besides increase of bacteria numbers, removal percentage is reduced. Low dose of concentrations in those studies, in comparison with this experiment, may be because of nanoparticle's size which is larger in this study and also different cultivation conditions.

CONCLUSION

According to the results, ZnO nanoparticles are a proper probable antibacterials for removing total coliform bacteria from municipal wastewater effluent. So, application of ZnO nanoparticles as a proper replacement for municipal wastewater effluent disinfection for removing total coliform bacteria can be studied further.

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