



Escherichia Coli Removal from Water Using Electrophotocatalytic Method

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ABSTRACT: Electrochemical has the suitable method of drinking water disinfection. This method leads to production of hydroxyl radicals which are known powerful oxidant agent. In recent years, water disinfection using electrophotocatalytic method is spreading. The aim of this experimental applied study is to evaluate the removal of *Escherichia Coli*, as the microbial contamination indicator of water, from drinking water using electrophotocatalytic method. The contaminated water in an electrophotocatalytic reactor were prepared by adding 10^2 - 10^3 cell of *E. coli* bacteria to drinking water. The studied variables were pH (6-8), the number of bacterial suspensions (10^2 - 10^3 cells / ml), the UV-A lamps (2-4 W), times (5-40 min), the distances between electrodes (2-3.5 cm), layering of zinc oxide nanoparticles (1-3), and voltages (10-40). The findings showed the correlation between removal of cells and UV-A lamps, voltage, and time of electrolysis. Optimal removal (MPN: 0) was obtained at pH 8, time of electrolysis: 5 minutes, 2 layer of nano ZnO, and voltage of 10 V. This result offers that this method is an efficient method for water disinfection. @JASEM

Keywords: *Escherichia Coli*, Water disinfection, Electrophotocatalytic, UV- A.

According to WHO report, at least 1.1 billion people does not have access to safe water (Li et al., 2008). Many of methods have been used for inactivation of microbial pollutants, including; chlorination, ozone, ultra violet, and photocatalytic process (Chong et al., 2010). Small-scale or at point of use water treatment systems, based on nanoparticles, can be used for inactivation of bacterial microorganisms in areas with low population which are not connected to central drinking water network. Chlorination is the most common method of drinking water chemical disinfection.

This method is not effective against some pathogenic agents as *Cryptosporidium* Oocyst and also leads to formation of trihalomethanes (THMs) which are known carcinogen for bladder (Rahmani et al., 2005; Bisneto et al., 2003). Therefore, it is necessary to employ more advanced methods for water disinfection. There is a need to apply a high technology to succeed chlorination (Chong et al., 2010). Electrophotocatalytic method has been considered as a promising method for water disinfection (Li et al., 2008; Liu et al., 2003). This process is an advanced oxidation processes (AOPs) in water treatment (Sobczynski et al., 2001; Banerjee et al., 2006). This process is a combination of external electric field and the heterogeneous photocatalytic, so as to avoid of recombination hole / electron (Benedix et al., 2000; Devilliers, 2006). The advantages of thin layer electrophotocatalyst stabilized on metal surface are: not requiring stir for homogeneous

mixing, and more homogeneous radiation of UV to catalyst (Benedix et al., 2000; Khanna, 2008).

Effective factors on the optimal performance of thin layer electrophotocatalyst stabilized on metal surface are: catalyst characteristics such as gap bond (higher photocatalytic activity is observed in catalysts with more extensive indirect gap bond), improvement of photocatalytic efficiency because of the smaller size of particles and higher special surface area of the catalyst for more active adsorption of light and water molecules, layer thickness, light source, wavelength of radiated light, intensity of light (Brunet et al., 2009; Wunderlich et al., 2004; Jin et al., 2009). Different studies have shown bactericidal effects for photocatalytic method using titanium oxide nanoparticles against bacteria such as *Streptococcus mutans*, *Escherichia coli*, and *Saccharomyces cerevisiae*, as well as *Poliovirus*, and inactivation of spores of *Cholestridium perfrogenus* (up to 98% after 152 seconds) (Nguyen et al., 2008).

Kerr studied inactivation of *E. coli* and demonstrated a linear relationship between production of hydroxyl radicals and inactivation of *E. coli*. Kerr suggested that electrophotocatalytic method is useful in disinfection of water contaminated with fecal indicators such as *E. coli* and *Cholestridium perfrogenesis* (Kerr, 2004). Liu et al. reported rapid death of *E. coli* cells in complete flow reactor using titanium dioxide as photocatalyst. The role of zinc oxide in absorption of optically excited oxygen in its suspension was reported.

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They found that Zinc oxide nanoparticles had greater UV photocatalytic effect, compared with titanium dioxide. Zinc oxide photocatalyst nanoparticles killed 10^8 /ml *E. coli* in 40 minutes (Liu et al., 2003). In this study the coupling of light emitted by UV-A lamp and immobilized ZnO semiconductor on zinc electrode have introduced a new method to meeting a more efficient kill of *E. coli* cells. The aim of this study is removal of *E. coli*, a Gram-negative bacterium considered as the fecal indicator of drinking water, from drinking water using a thin layer of electrophotocatalytic ZnO nanoparticles stabilized on zinc. As safe drinking water should not contain *E. coli*, this organism was studied as the model organism and an indicator in this study.

MATERIALS AND METHODS

The ZnO nanoparticles with special area $50 \text{ m}^2 \text{ g}^{-1}$ and particle size 20 nm were purchased from Amohr Co. (Germany). Nutrient agar culture media, brain heart infusion, sodium chloride, sodium hydroxide, and nitric acid were purchased from Merck Co. (Germany). Nitric acid and sodium hydroxide (1 N) were used for pH adjustment. 5 grams of zinc oxide nanoparticles was poured into 100 ml of distilled water. The suspension was mixed with a magnetic stirrer for 30 min and then sonicated in ultrasonic bath (MATR. N.B., Italy) for 22 min in order to improve the dispersion of ZnO in water. The weight of zinc electrode was measured after hydroxylating, and washing with distilled water. Zinc electrode was used as the substrate for immobilization of ZnO nanoparticles. The dip-coating method was performed for thin film fabrication. The Zinc electrode was pre-treated with detergent and methanol for increasing the number of OH groups. After the pre-treatment, zinc electrode was weighted, immersed in the colloidal solution, and dried in an oven for 30 min at 35°C . The coated was then calcined in a muffle furnace at temperature 105 and 320°C for 60 min. For 2- and 3-layer coatings, the process was repeated twice and three times. They were washed with distilled water for the removal of free ZnO nanoparticles. Figure 1 showed a batch reactor made of a 360ml glass vessel. The characteristics of electrodes were as follows: two electrodes of thin layer zinc oxide nanoparticles immobilised on zinc and copper electrode. The area of each electrode was 36 cm^2 . The distance between the UV-A lamp and Zn-ZnO electrode was adjusted 2-3.5cm. The AC electrical source had the power of electrical energy production

equal to 1-5A. The LED UV-A lamp had the radiation intensity 120 mW cm^{-2} , and wavelength 395 nm. To evaluate the effect of electrolysis, catalyst, and UV light on the disinfection process, samples underwent with UV-A lamp with (240, 360, and 480 mW cm^{-2}), the electrode of thin layer zinc oxide nanoparticles immobilized on zinc (5%, 10%, and 15%), different voltages (10, 20, 30, and 40 V), and different times (5, 10, 20, and 40min). Magnetic stirrer was used for homogeneous mixing of contaminated water samples. percentage cell reduction was calculated according to the following equation:

$$\text{Removal (\%)} = (1 - A/B) * 100$$

Where R was the percentage of cell reduction; B and A were the average of number of live cell per milliliter before and after treatment. *E. coli* (ATCC 25922) was reactivated from frozen stock (15% glycerinated brain heart infusion broth) in a 100 ml Erlenmeyer having 50 ml of BHI broth (Merck). In order to obtain the bacterium inculca, the surface of BHI was rinsed with sterile water and scraped with a spatula. Three rising procedures were carried out in 50 ml tubes: The three rinse were made with phosphate buffer saline at 12000 rpm for 10 min. The sample was incubated at 37°C for 12 h. Optical density of the cell suspension was measured with a spectrophotometer (UNICO) at a 610 nm wavelength. The described procedure resulted in suspensions with a cell concentration of 10^2 and 10^3 CFU/ml. After each round of the study, 1ml of reactor water was picked and cultured on nutrient agar plates to evaluate the efficiency of the removal process. After incubation at 37°C for 18h, the number of cells formed on the agar plate was counted and the results were expressed as mean cell per milliliter. Electrophotocatalytic reactor without microbe and electrophoto was used as the test control.

RESULTS AND DISCUSSION

The studies related to the removal of cells are obtained using water disinfection with electrophotocatalytic method. Electrophotocatalytic removal of *E. coli* cells was observed using ZnO nanoparticles immobilized on Zn plate. Electrophotocatalytic experiments were carried out an initial cell concentration in the range of 10^2 to 10^3 cells in ml.

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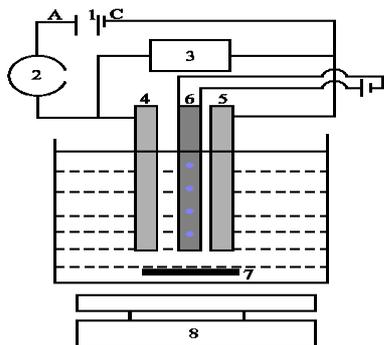


Fig 1. The batch electrophotocatalytic reactor of thin layer zinc oxide nanoparticles immobilized on zinc (1.Power supply, 2.Amper, 3.Voltage, 4.Copper electrode/negative pole, 5.Zinc/ Zinc oxide electrode/positive pole, 6.UV-A lamp, 7.Magnetic stirring bar, 8.Magnetic stirring)

Figure 2 showed the effect of the initial cell loading on removal efficiency. The efficiency of *E. coli* cells removal was reduced by an increase in the cell number from 10^2 to 10^3 CFU/ml. The electrophotocatalytic system showed percentage removal of 97%, 97%, and 100% with the initial cell count (10^2 cell in ml) removed with 5 min irradiation in pH 6, 7, and 8, respectively. The electrophotocatalytic system showed percentage removal with 82%, 96%, and 100% of the initial cell count (10^3 cell in ml) removed with 5 min irradiation in pH 6, 7, and 8, respectively. As expected, for the number of *E. coli* cells was increased, accordingly the number of photocatalytic sites and fixed UV light also were fixed. This phenomenon was the same as *Pseudomonas aeruginosa* bacterium (Daneshvar et al., 2007). They were investigated the effect of photocatalytic disinfection on *Pseudomonas aeruginosa*. These experiments were done in TiO_2 concentration of 325 ppm and microorganism MPN / 100 ml of 50 to 1600 and that increasing the initial concentration of microorganism, increased its removal efficiency (Daneshvar et al., 2007).

The electrophotocatalytic system showed percentage removal of 100% with the initial cell count (10^2 and 10^3 cell in ml) removed with 5 min irradiation in pH 8. Photocatalytic treatment time required for complete cell inactivation (10^2 and 10^3 cell in ml) were 5 min. This finding was the same as photocatalytic experiments were carried out using a Degussa- TiO_2 alloy electrode and an initial spore concentration in the range of 1×10^4 to 2×10^5 spores ml (Dunlop et al., 2008). They found the photocatalytic treatment efficiency required for complete spore inactivation increased

with higher initial spore loadings. Rapid death of *E. coli* cells using titanium dioxide was reported (Liu et al., 2003). They found that Zinc oxide photocatalyst nanoparticles killed 10^8 /ml *E. coli* in 40 minutes.

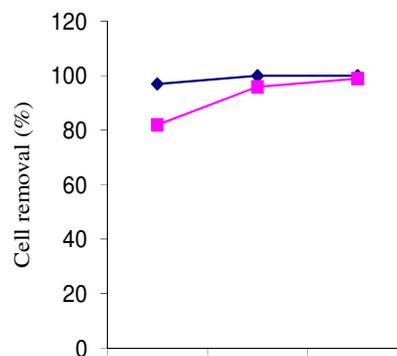


Figure 2. Percentage of removal efficiency of electrophotocatalytic in *E. coli* removal from contaminated water (10^2 , and 10^3 CFU/ ml) at pH, electrolysis time 5 minute, distance between the UV-A lamp and Zn/ZnO electrode 2 cm, voltage 10 v, zinc oxide nanoparticles 5%, and LED UV-A lamp power 240 mw cm^{-2}

Figures 2, and 3 showed the effect of the pH on removal efficiency. Electrophotocatalytic analysis of efficiency of *E. coli* removal at pH 6, 7, and 8 demonstrated that at pH 8, lower voltage and electrical current is needed, compared with the two other voltages. It is found that optimum pH for reaching to microbial standard (MPN 0) was pH 8. It was expected that negative surface charge of *E. coli* logarithmic growth phase might affect the solution pH during photocatalytic oxidation. The bactericidal effect of the method was highly dependent on pH, and was increased by an increase in pH.

This finding was the same as photocatalytic experiments were carried out using TiO_2 activated with UV light (Liu et al., 2003). Although, many of researcher reported that photochemical removal of coliform bacteria was unaffected by the pH of the sample in the range of 6-8 pH units (Cho et al., 2004). This enhancing effect could be attributed in part to amore efficient formation of hydroxyl radical from OH^- than from water (Khodja et. al, 2002).

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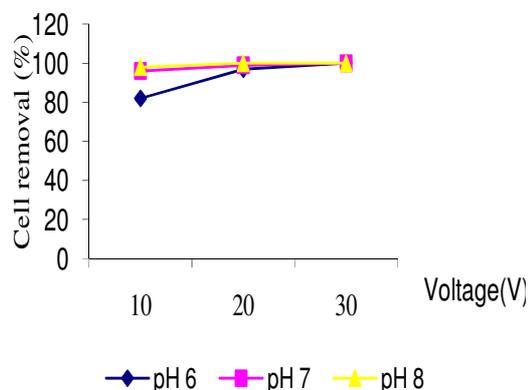


Figure 3. Electrophotocatalytic effect of zinc oxide nanoparticles 5% on removal percentage of *E. coli* from contaminated water (10^3 CFU/ml) at pH, electrolysis 5 min, distance between the UV-A lamp and Zn/ZnO electrodes 2 cm, voltage, and LED UV-A lamp power 240 mw cm^{-2}

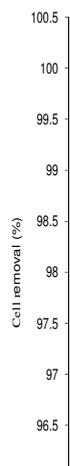


Figure 4. Electrophotocatalytic effect of zinc oxide nanoparticles 5% on removal percentage of *E. coli* from contaminated water (10^2 per ml) at pH, electrolysis 5 min, distance between the UV-A lamp and Zn/ZnO electrodes 2 cm, voltage, and LED UV-A lamp power 240 mw cm^{-2}

Figure 5 showed the effect of the UV-A radiation on removal efficiency. The efficiency of *E. coli* removal increased by an increase in the power of the lamp (Melemeni et al., 2009; Nguyen et al., 2008). Figure 6 showed the ineffectiveness of using only UV-A in photoelectrolysis in *E. coli* removal. Higher power of the lamp decreased electrolysis time, and voltage. Optimum power of the lamp for reaching to microbial standard (MPN 0) was 4 watt. The above increased optical activity was justified by more efficient production of electron donor hydroxyl radical from hydroxide anion of water and higher production of superoxide. This finding was the same as Photocatalytic experiments were carried out using a TiO_2 nanoparticles (Brunet et al., 2009; Khodja REZAAE, A; KASHI JONIDI-JAFARI, A; KHATAEE, A R; NILI-AHMADABADI, A

et al., 2002; Melemeni et al., 2009; and Nguyen et al., 2008).

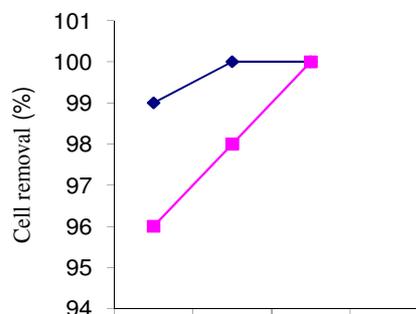


Figure 5. Electrophotocatalytic effect of zinc oxide nanoparticles 5% on removal percentage of *E. coli* from contaminated water (10^3 , and 10^2 CFU/ml) at pH 7, electrolysis 5 min, distance between the UV-A lamp and Zn/ZnO electrode 2 cm, voltage 10 v, and LED UV-A lamp power

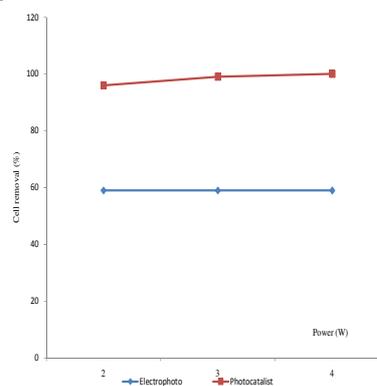


Figure 6. Electrophotocatalytic effect of zinc oxide nanoparticles 5% on removal percentage of *E. coli* from contaminated water (10^3 CFU/ml) at pH, electrolysis 5 time, distance between the UV-A lamp and Zn/ZnO electrode 2 cm, voltage 10 v, and LED UV-A lamp power

Figure 7 showed the effect of the amounts of zinc oxide and UV-A on removal efficiency. The efficiency of *E. coli* removal was greatly increased in the presence of zinc oxide photocatalyst nanoparticles and UV-A. Higher power of the lamp along with higher amount of zinc oxide catalyst up to solution 10% wt decreased electrolysis time, and voltage. At a fixed lamp power it was expected that an optimum catalyst amount would exist where the photocatalyst would generate a maximum concentration of reactive oxygen species (ROS) which could take part in reaction at the outer film surface. Optimum amount of zinc oxide catalyst solution, and optimum power of the lamp for

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reaching to microbial standard (MPN 0) were 10% wt. and 3 watt, respectively. The efficiency of *E. coli* removal increased as the number of ZnO nanoparticle layers increased to two, which may be attributed to an increase the surface area for inactivation of bacteria. This finding was the same as photocatalytic experiments were carried out using a TiO₂ thin films (Habibi et al., 2007).

They showed that decomposition rate constants of red sulphonyl 3BL depended on the film thickness. The rate constants increased with increasing film thickness. However, a limiting value can be observed at thick films due to increase in opacity and light scattering leading to a decrease in the passage of irradiation through the film (Habbibi et al., 2007).

The decrease in reduce rate of *E. coli* at higher catalyst loadings (i.e. above two layers) could be attributed to an decrease in UV penetration to the outer layers of the film, and an decrease in protection effect of clusters blocking UV from reach catalyst surface.

The optimal catalyst loading of 0.5 mg cm⁻² was used (Yu, and Zhao, 2001).It was also explained that the efficiency of *E. coli* removal was increased in the presence of zinc oxide photocatalyst nanoparticles and UV-A , was due to the production of hydroxyl radicals.

This finding was the same as Photocatalytic experiments were carried out using TiO₂ (Melemeni, et al., 2009; Liu, et al., 2003, and Daneshvar, et al., 2007). Hydroxyl radicals led to fat peroxidation of cellular membrane and degradation of the different compounds of the cell (Banerjee et al., 2006; Brunet et al., 2009).

Super oxide radical anion, hydro peroxy radical and hydrogen peroxide, formed by the reduction of dissolved oxygen in anode, can also feed into the photocatalytic disinfection mechanism. These species which could contribute to the cell inactivation. The photocatalytic inactivation of gram-positive and negative bacteria in water had been reported (Blake et al., 1999).

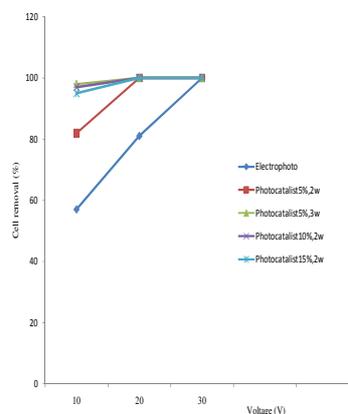


Figure 7. Electrophotocatalytic effect of zinc oxide nanoparticles on removal percentage of *E. coli* from contaminated water (10³ CFU/ml) at pH 6, electrolysis 5 min, distance between the UV-A lamp and Zn/ZnO electrode 2 cm, voltage, and LED UV-A lamp power

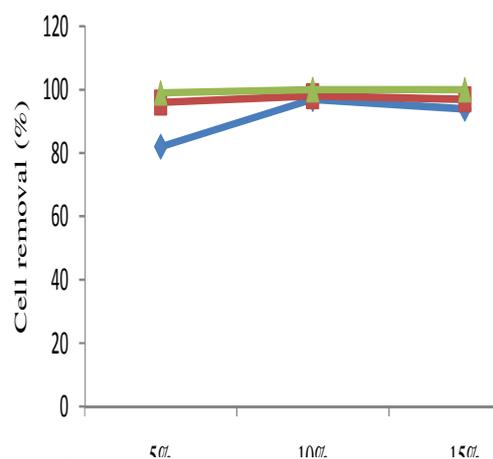


Figure 8. Electrophotocatalytic effect of zinc oxide nanoparticles on removal percentage of *E. coli* from contaminated water (10³ CFU/ ml) at pH 6, electrolysis 5 min, distance between the UV-A lamp and Zn/ZnO electrodes 2 cm, voltage 10v, and LED UV-A lamp power 2 w

Figure 9 showed the effect of the electrical current on removal efficiency. The efficiency of *E. coli* removal increased as the electrolysis voltage and time increased. Higher electrical current increased the efficiency of *E. coli* removal and could shorten electrolysis time.

Optimum electrical current for reaching to microbial standard (MPN 0) was 20 volt. Figure 2 showed that the photocatalytic treatment time required for complete cell inactivation increased with higher initial cell loadings. Regarding the effect of level voltage on the

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photoelectrocatalytic inactivation rate, experimental results showed that the voltage electrode increased, the resulting gradient separated electron-hole, decreasing its recombination rate, increasing the photocurrent rate, and eventually accelerating the cell inactivation as shown in Fig. 2.

Moreover under higher applied voltages and times the external electric field could also improve the direct and/ indirect electro-oxidation reactions at anode. The biocidal efficiency must be proportional to the specific surface area of photocatalysts and the quantum yield of photocatalytic system because the number of OH• was proportional to the specific surface area and inversely proportional to the electron-hole recombination rate. This finding was the same as photocatalytic experiments were carried out (Dheaya, et al., 2009). Additionally photoelectrocatalytic increased mass transfer by electro-migration of negatively charged bacteria towards the electrode. This finding was the same as photocatalytic experiments were carried out using graphite-supported TiO₂ (Palmisano, et al., 2009).

Regarding the effect of the irradiation level on the photoelectrocatalytic inactivation rate, experimental results showed that the more power the radiation reaching the photocatalytic electrode was, the faster the cell inactivation progresses. As expected, for the electrolysis voltage and time was increased, accordingly the efficiency of *E. coli* removal also were increased as shown in Fig. 9. This finding was the same as photocatalytic experiments were carried out using TiO₂ reactor (Melemani, et al., 2009; and Nguyen, et al., 2008).

It was also explained that increase in electrolysis voltage and time led to faster production of electrolysis products such as OH⁻ and Cl⁻ ions in cathode and anode electrodes, respectively. These products were responsible for water disinfection. Increased voltage caused an increased drift force on electrode surface, which was the main factor in electrochemical processes.

This finding was the same as experiments were carried out using electrode (Rahmani, et al., 2005). The oxygen produced in anode electrode led to higher bactericidal effect against *E. coli*, because oxygen molecule played a important role in photocatalysis stage, and transformed to

superoxide anion radical ([•]O₂⁻) in capacity bond of zinc oxide photocatalyst nanoparticles. This finding was the same as photocatalytic experiments were carried out using TiO₂ (Liu, et al., 2003). The efficiency of *E. coli* absorption by zinc electrode layered by zinc oxide nanoparticles as positive pole (anode) was directly related to an increase in electrolysis voltage and time. It was also explained that the Gram-negative bacterium *E. coli* had a complex structure of cell wall, a peptidoglycan layer between the outer membrane and cytoplasmic membrane. The negative charge of lipopolysaccharide molecules of outer membrane the Gram-negative bacterium *E. coli* led to its absorption by the zinc electrode. This finding was the same as experiments were carried out using several nanoparticles (Tam, et al., 2007; Yoon, et al., 2007; and 2008).

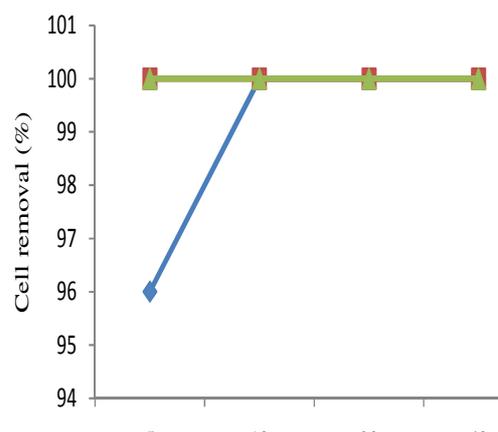


Figure 9. Electrophotocatalytic effect of zinc oxide nanoparticles 5% on removal percentage of *E. coli* from contaminated water (10³ CFU/ml) at pH 7, electrolysis 5 min, distance between the UV-A lamp and Zn/ZnO electrode 2 cm, voltage, and LED UV-A lamp power 2w

Figure 10 showed the effect of the distance between the UV-A lamp and Zn/ZnO electrode on removal efficiency. The efficiency of *E. coli* removal on zinc electrode layered by zinc oxide catalyst nanoparticles, as the positive pole (anode), was decreased as it distance with the UV-A source increased. Optimum zinc electrode layered by zinc oxide catalyst nanoparticles distance with the UV-A source for reaching to microbial standard (MPN 0) was 2 centimeter.

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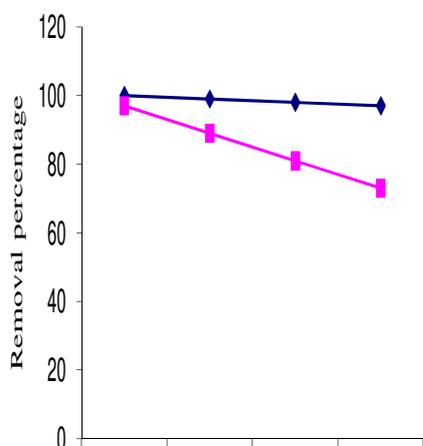


Figure 10. Electrophotocatalytic effect of zinc oxide nanoparticles 5% on removal percentage of *E. coli* from contaminated water (10^3 CFU/ml) at pH 7, electrolysis 5 min, distance between the lamp UV-A and Zn/ZnO electrode, voltage 10 v, and LED UV-A lamp power 2 w

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