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Full Length Research Paper

Pseudomonas aeruginosa removal from water using electrophotocatalytic method

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Application of electrophotocatalytic (EPC) methods for drinking water disinfection was broadly used in the recent years. These methods led to production of strong oxidant agents such ashydroxyl ($OH^{\cdot}$) radical. The goal of this applied-analytical research was to investigate *Pseudomonas aeruginosa* as a source of nosocomial infection caused by waterborne bacteria, removal from urban drinking water by batch EPC reactor with usage of zinc oxide (ZnO) nanoparticles immobilized on zinc (Zn) sheet-copper electrode, and lamp emitting dynode (LED) ultraviolet-A (UV-A) lamp. The contaminated water sample was prepared by adding 10^2-10^3 cells of *P. aeruginosa* bacteria per ml of drinking water. The studied variables were pH (6-8), the number of bacteria (10^2-10^3 cells/ml), the lamp intensity (360-600 mW/cm^2), radiation time (7.5-30 min), the distance between lamp and electrode (1.5 cm), layering of zinc oxide nanoparticles (1-3), and current density (3-9 mW/cm^2). The results showed the correlation between removal of cells and UV-A lamp intensity, current density, and radiation time. Optimal removal (0) was obtained at pH 8, radiation time: 7.5 min, 2- layer of ZnO nanoparticles, and current density of 6 mW/cm^2. The findings indicated that removal efficiency was increased with increasing current density, radiation time, and lamp intensity.

Key words: Bacterium, lamp emitting dynode, electrophotocatalytic, nosocomial infection, *Pseudomonas aeruginosa*, urban drinking water.

ABBREVIATIONS USED: AC - Alternative Current; **AOPs** - Advanced Oxidation Processes; **BHI** - Brain Heart Infusion; **DCP** - Dichlorophenol**; EPC** - Electrophotocatalytic; **LED** - Light Emitted Dynode; **MPN** - Most Probable Number; **OD** - Optical Density; **$OH^{\cdot}$** - Hydroxyl; **$O_2^{\cdot}$** - Superoxide Radical Anion; **PCP** - Pentachlorophenol; **PECFC** - Photoelectrolytic Fuel Cell; **ROS** - Reactive Oxygen Species; **THMs** - Ttrihalomethanes; **TiO_2** - Titanium oxide; **UV** - Ultraviolet; **Zn** - Zinc; **ZnO** - Zinc oxide.

INTRODUCTION

The drinking water quality must obey strict regulations about microbial and chemical pollutants (Belhacova et al., 1999). *Pseudomonas aeruginosa*, opportunistic bacterium, remained in hospital water source for long periods, and resulted in nosocomial outbreak. Nosocomial waterborne infections such as *P. aeruginosa* were caused by bacteria tolerant to at least 2 classes of antibacterial agents (Elias et al., 2002). *P. aeruginosa*, member of the Gamma Proteobacteria class of bacteria, was responsible for 10-20% of nosocomial infections, particularly in patients with severe burns, and in cancer and AIDS patients (Trautmann et al., 2001; Bitton, 2005). These bacteria might cause minor problems associated with color, taste, odor, and turbidity of the water. The main concern was related to the biological slime they form. These slimes, which protected *P. aeruginosa*, also had the ability to harbor other disease-causing bacteria such as *coliforms*. Chlorination was the most usual method of drinking water chemical disinfection. This method led to formation of trihalomethanes (THMs) which

were known as carcinogen for bladder (Kiriluk et al., 2012). The surrogate methods had been applied for inactivation of microbial pollutants, including ozone and ultraviolet (UV). The high treatment expenses and dosage were attributed to these methods (Johnson et al., 2010). Therefore, it was necessary to apply more efficient methods for water disinfection (Chong et al., 2010). Small-scale or at point of use water treatment systems, based on nanoparticles, could be used for inactivation of bacterial microorganisms in areas with low population which were not connected to central drinking water network. Electrophotocatalytic (EPC) method had been considered as a promising and innovative method for water disinfection (Mahamunia and Adewuyi, 2010). This process was a combination of external electric field and the heterogeneous photocatalytic, so as to avoid recombination hole/electron (Devilliers, 2006). This process was a part of advanced oxidation processes (AOPs) in water treatment (Banerjee et al., 2006). The advantages of thin layer electrophotocatalyst stabilized on metal surface are: it does not require stirring for homogeneous mixing, and more homogeneous radiation of UV to catalyst (Khanna, 2008). Effective factors on the optimal performance of thin layer electrophotocatalyst stabilized on metal surface were: catalyst characteristics such as gap bond, improvement of photocatalytic efficiency, layer thickness, light source, light intensity, and water quality such as the presence of particle associated microorganisms (Wunderlicha et al., 2004). Recent research had shown that EPC technologies could propose a good opportunity to remove microbial and chemical pollutants. The application of photoelectrolytic fuel cell (PECFC) technology by using tungsten trioxide/visible light for the treatment of organic pollutants [pentachlorophenol (PCP), dichlorophenol (DCP)], and pathogenic strains of *Esherichia coli* were reported (Macphee et al., 2010).

Different studies had shown bactericidal effects for photocatalytic method using titanium oxide (TiO_2) nanoparticles against bacteria (complete degradation of *Legionnela pneumophila* after 20 h), polio virus, and inactivation of spores (up to 98% after 152 seconds) (Hayakawa et al., 2007). Inactivation of *E. coli* was reported and it demonstrated a linear relationship between production of hydroxyl ($OH^{\cdot}$) radicals and inactivation of *E. coli* (Kerr, 2004). In this study, the coupling of light emitted dynode (LED) UV-A lamp and immobilized zinc oxide (ZnO) semiconductor on zinc (Zn) electrode had introduced a new method to meeting a more efficient kill of *P. aeruginosa* cells. The aim of this study was to investigate the removal of *P. aeruginosa*, a Gram-negative bacterium which was considered a bacterium tolerant to antibacterial agents, and nosocomial waterborne infections, from drinking water using a thin layer of photocatalytic ZnO nanoparticles stabilized on Zn. The studied variables were pH, the number of bacteria, the lamp intensity, the radiation time, layering of ZnO nanoparticles, and current density. As safe drinking water should not contain *P. aeruginosa*, this organism was studied as the model organism and an indicator in this study.

MATERIALS AND METHODS

Materials

The ZnO nanoparticles with special area 50 m^2/g and particle size of 20 nm were supplied from Amohr Co. (Germany). Asparagine broth, set amid broth, brain heart infusion (BHI), nutrient agar, sodium chloride, sodium hydroxide, and nitric acid were purchased from Merck Co. (Germany). Nitric acid and sodium hydroxide (1 N) were applied for pH adjustment.

Preparation of ZnO nanoparticles

About 5 g of ZnO nanoparticles were placed in 100 ml of distilled water. The suspension was mixed with a magnetic stirrer for 30 min and then sonicated in an ultrasonic bath (MATR. N.B., Italy) at a frequency of 50 kHz for 22 min to improve the dispersion of ZnO in distilled water. The weight of zinc electrode was measured after hydroxylation and washing with distilled water.

Preparation of electrodes

The Zn electrode was used as the substrate for the immobilization of ZnO nanoparticles. The zinc electrode was pre-treated by detergent and sodium hydroxide solution at 0.01 N to increase the number of OH groups.

Immobilization of ZnO nanoparticles

To prepare the ZnO films, dry methods were used (Malato et al., 2009; Zuolian et al., 2010). In this study, a Zn plate was used for immobilization. After the pre-treatment, the Zn electrode was weighted, immersed in the colloidal solution, and dried in an oven at 35°C for 30 min. The coated particles were then calcined in a muffle furnace at 105 and 320°C for 60 min. The thermal treatment of immobilized ZnO films led to developing good mechanical stability of the films. For 2- and 3-layer coatings, the process was repeated two to three times, after which they were washed with distilled water to remove any free ZnO nanoparticles.

Batch EPC reactor

The experimental setup is shown in Figure 1. The batch reactor was a 360-ml glass vessel (10×6×6 cm). The characteristics of electrodes were as follows: two

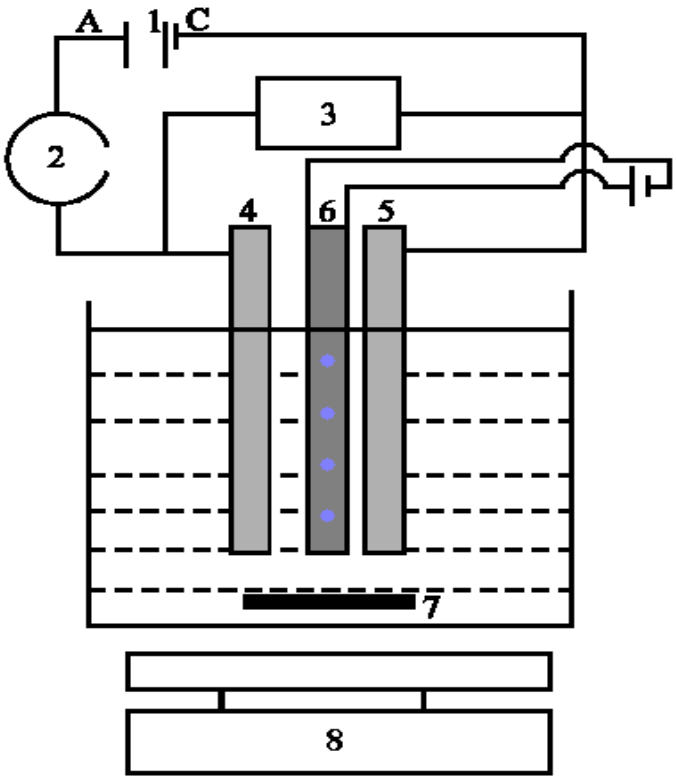

Figure 1. The batch EPC reactor of thin layer ZnO nanoparticles immobilized on Zn (1) power supply, (2) current volume, (3) voltage volume, (4) copper electrode, (5) zinc/zinc oxide electrode, (6) light emitted dynode ultraviolet-A lamp, (7) magnetic stirrer bar, and (8) magnetic stirrer.

electrodes of thin layer ZnO nanoparticles immobilized on Zn (anode), and copper electrode (cathode). The area of each electrode was 36 cm^2 (9×4×0.1 cm). The distance between the bottom of the reactor and the electrodes was 1 cm, and the distance between the LED UV-A lamp and the Zn/ZnO electrode was adjusted to 1.5 cm. The alternative current (AC) electrical source had an electrical energy production equal to 1-5 A, and a maximum electrical power of 60 W. The LED UV-A lamp had an electrical power of 1 W, radiation intensity of 120 mW/cm^2, a wave length of 395 nm, and a voltage of 3.4 V. To evaluate the effect of the current densities, catalyst, and UV light on the disinfection process, samples underwent LED UV-A lamp treatments (at 360, 480, and 600 mW/cm^2), with an electrode of thin layer ZnO nanoparticles immobilized on Zn (at 5, 10 and 15%), different current densities (at 3, 6 and 9 mW cm^{-2}), different pHs (at 6, 7 and 8), and different radiation times (at 7.5, 15 and 30 min). A magnetic stirrer was used for homogeneous mixing of the contaminated water samples. The percentage cell reduction was calculated according to the following equation (Hua et al., 2007):

$$R\ (\%) = (1 - B_t/B_{t0}) \times 100 \quad (1)$$

where R was the percentage of cell reduction, B_{t0} and B_t are the average of number of live cells per milliliter before and after treatment.

Preparation of *P. aeruginosa*

Suspension of *P. aeruginosa* (ATCC 27853) in water was obtained following the technique proposed by other researchers (Kisko and Szabo-Szabo, 2011). *P. aeruginosa* was reactivated from frozen stock (15% glycerinated BHI broth) in a 100-ml Erlenmeyer flask containing 50 ml of BHI broth (Merck). The sample was incubated at 35°C for 24 h. The bacterial cell was isolated at 5000 rpm for 15 min after inoculating strain in BHI broth at 35°C. Strain was stored on nutrient agar at 4°C. The strain was grown on BHI agar at 35°C for 24-48 h. The optical density (OD) of the cell suspension was measured with a spectrophotometer at 600 nm. The described procedure resulted in suspensions with a cell concentration of 10^2 and 10^3 CFU/ml. The *P. aeruginosa*

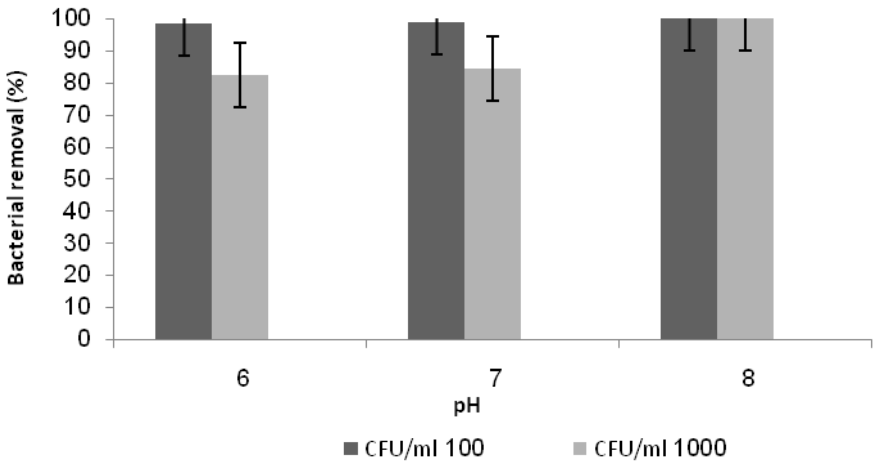

Figure 2. Effect of the initial number of *P. aeruginosa* and pH on efficiency of bacterial removal (pH, 6-8; temperature, 25°C; radiation time, 7.5 min; UV-A lamp intensity, 480 mw/cm^2; initial bacterial number, 10^2-10^3; current density, 3 mA/cm^2; zinc oxide concentration, 10% wt).

was measured by standard method 9213F (APHA, 2005). In this method, the bacteria could be detected by production of fluorescent pigments, which were detectable by UV irradiation. This method reported the number of microorganisms as most probable number (MPN). After each round of the study, reactor water was picked and cultured on asparagine, and set amid broth tubes to evaluate the efficiency of the removal process. After incubation at 37°C for 48 h, the number of cells was counted, and the results were expressed as MPN. EPC reactor without microbe and electrophoto were used as the test control. EPC experiments were at least duplicated and all samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Effect of initial number of *P. aeruginosa*

The effect of the initial number of *P. aeruginosa* on the removal efficiency of the EPC process is shown in Figure 2. The removal efficiency was decreased by an increase in the cell number from 10^2 to 10^3 CFU/ml. The EPC reactor showed that the removal percentage for *P. aeruginosa* cells (10^2 in ml) increased from 98.5 to 100% as the pH increased from 6 to 8, with 7.5 min irradiation, whereas the removal percentage for *P. aeruginosa* cells (10^3 in ml) increased from 82.5 to 97.5% as the pH increased from 6 to 8, with 7.5 min irradiation. This effect was attributed to the fact that the increasing number of *P. aeruginosa* cells accordingly matched the number of photocatalytic sites and UV-A light. This phenomenon was the same for *E. coli* bacterium. The effect of photocatalytic disinfection was investigated on *E. coli*. These experiments were performed with an initial cell concentration in the range of 10^2 to 10^3 cells in ml at pH 7, radiation time of 5 min, distance of 2 cm between the UV-A lamp and Zn/ZnO electrode, voltage 10 V, ZnO nanoparticles 5%wt, and LED UV-A lamp power 240 mw/cm^2. At a higher concentration, the efficiency started to lessen (Rezaee et al., 2011). The EPC reactor reached the highest efficiency (100%) at pH 8, radiation time of 7.5 min, and a cell concentration of 10^2 and 10^3 CFU/ml. The photocatalytic exposure time required for complete cell inactivation (10^2 and 10^3 in ml) was 7.5 min. This finding was in agreement with previously published data. These photocatalytic experiments were performed with an initial *C. perfringens* spore concentration in the range of 1×10^4 to 2×10^5 spores in ml at pH 7, and by a Degussa-TiO_2 alloy electrode. At a lower concentration, the photocatalytic exposure time required for complete spore inactivation started to lessen (Dunlop et al., 2008). Rapid death of *E. coli* cells using TiO_2 was reported. They indicated that ZnO photocatalyst nanoparticles killed 10^8/ml *E. coli* in 40 min (Liu and Yang, 2003).

Effect of pH

The pH was a significant operating variable affecting the performance of the EPC process. The bactericidal effect of this method was strongly dependent on pH, and was enhanced by an increase in pH. In the EPC process, different concentrations of $OH^{\cdot}$ radical from water were formed depending on the pH. These products played an important role in the removal of *P. aeruginosa* cells in the EPC process. This effect was attributed to an increase in the $OH^{\cdot}$ concentration at a higher pH. This observation was consistent with those of other previous studies (Khodja et al., 2002). The initial and final pH values were

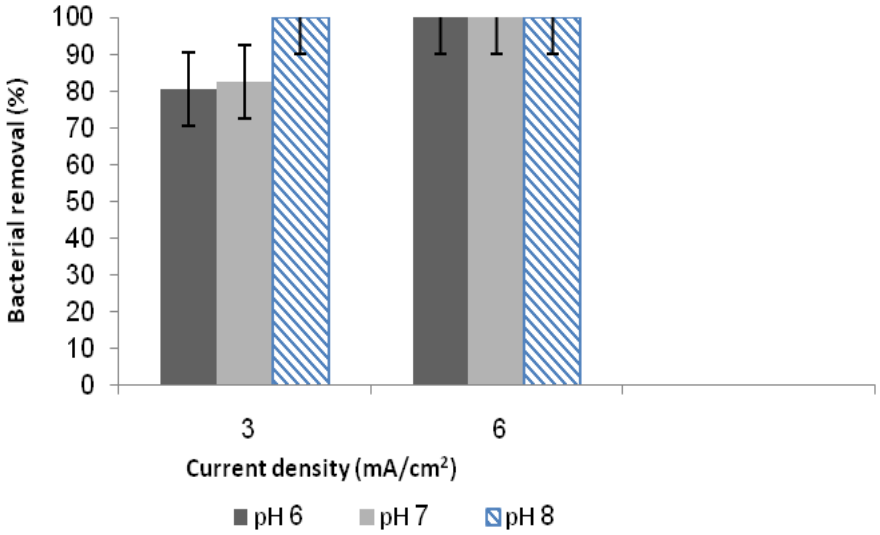

Figure 3. Effect of the pH on efficiency of bacterial removal (pH, 6-8; temperature, 25°C; radiation time, 7.5 min; UV-A lamp intensity, 480 mw/cm^2; initial bacterial number, 10^3; zinc oxide concentration, 10% wt).

measured in this study in order to investigate the effect of pH more effectively. The initial pH was enhanced during EPC studies. The effect of the pH on the removal efficiency of the EPC process is shown in Figures 2 and 3. The EPC reactor reached the highest efficiency (100%) at pH 8, radiation time of 15 min, ZnO nanoparticles 10%wt, distance of 1.5 cm between the LED UV-A lamp and Zn/ZnO electrodes, LED UV-A lamp intensity of 480 mw/cm^2, current density of 3 mA/cm^2, and a cell concentration of 10^2 and 10^3 CFU/ml. The pH 8 needed lower current density, compared with the two other current densities. It was concluded that the optimum pH for reaching microbial standard (MPN 0) was pH 8. It was expected that negative surface charge of *P. aeruginosa* logarithmic growth phase could affect the solution pH during photocatalytic oxidation. This finding was the same as the photocatalytic experiments were performed using ZnO activated with UV-A lamp (Rezaee et al., 2011). This observation was not consistent with the absence of the pH effect for *Bacillus subtilis* spore inactivation in a pH range of 6-8.2 as reported by Cho et al. (2006).

Effect of lamp intensity

The effect of the LED UV-A lamp intensity on the removal efficiency of the EPC process is shown in Figure 4. The removal percentage for *P. aeruginosa* cells (10^3 in ml) decreased from 95.5 to 91% as the LED UV-A lamp intensity increased from 360 to 480 mw/cm^2, with 7.5 min of radiation, and pH 8. The removal efficiency of *P. aeruginosa* was proportional to the LED UV-A lamp intensity and enhanced by an increase in the LED UV-A lamp intensity. This observation was consistent with previously published data. When primary wastewater samples were exposed to UV irradiation, the number of *P. aeruginosa* (ATCC 15442) cells progressively decreased from 10^7 cells in ml to 10^4 as the UV-C lamp dose increased from 0 to 500 mw·s/cm^2 (Melemeni et al., 2009; Mounaouer and Abdennaceur, 2012). At higher lamp intensity, the exposure time, and current density started to lessen. Optimum UV-A lamp intensity for reaching to microbial standard (MPN 0) was 480 mw/cm^2. The above increased optical activity was explained by higher formation of reactive oxygen species (ROS), such as electron donor $OH^{\cdot}$ radical from hydroxide anion of water, and superoxide radical anion ($O_2^{\cdot -}$). This finding was consistent with the photocatalytic experiments performed using a TiO_2 nanoparticle (Brunet et al., 2009).

Effect of ZnO and LED UV-A lamp

The effect of the ZnO, and LED UV-A lamp intensity on the removal efficiency of the EPC process is shown in Figure 5. The removal percentage for *P. aeruginosa* cells (10^2 and 10^3 in ml) dramatically increased in the presence of ZnO photocatalyst nanoparticles and the LED UV-A lamp. At higher lamp intensity along with higher amount of ZnO catalyst up to solution 10% wt, the exposure time and current density started to lessen. At fixed lamp intensity, it was observed that an optimum catalyst amount would be presented where the photocatalyst would form a maximum concentration of ROS which could be taken as part of the reaction at the outer film surface. The optimum amount of ZnO catalyst solution and optimum intensity of the LED UV-A lamp for reaching

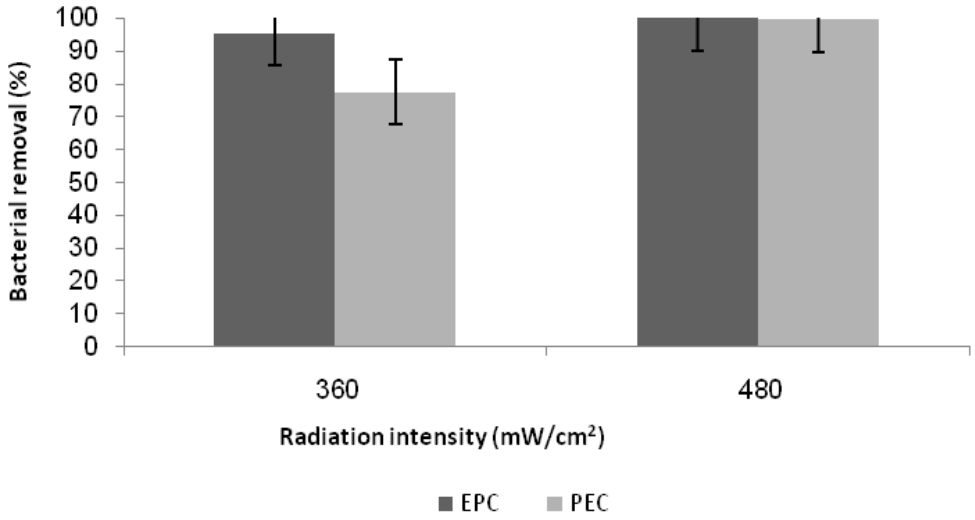

Figure 4. Effect of the UV radiation and catalyst on efficiency of bacterial removal (pH, 8; temperature, 25°C; radiation time, 7.5 min; UV-A lamp intensity, 360-480 mw/cm^2; initial bacterial number, 10^3; current density, 3 mA/cm^2).

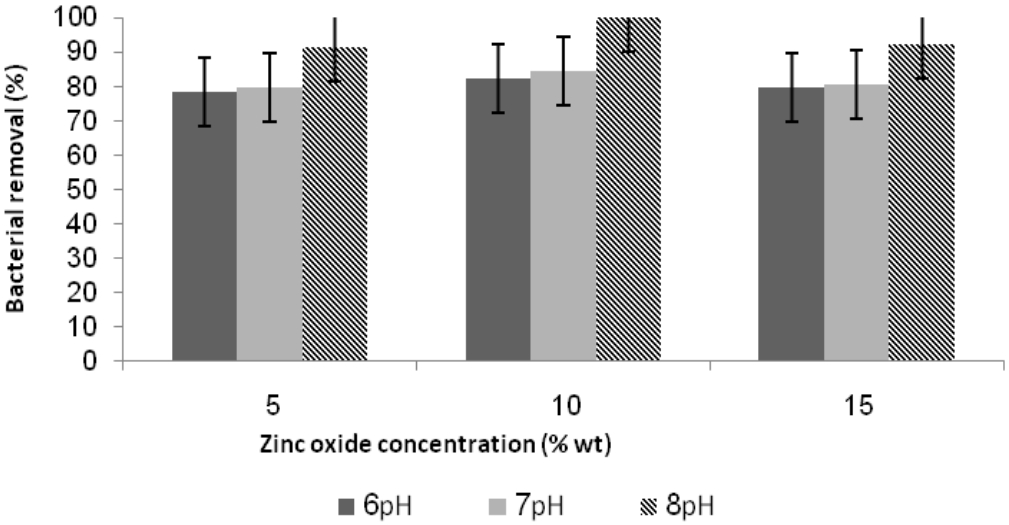

Figure 5. Effect of the catalyst layer on efficiency of bacterial removal (pH, 6-8; temperature, 25°C; radiation time, 7.5 min; UV-A lamp intensity, 480 mw/cm^2; initial bacterial number, 10^3; current density, 3 mA/cm^2).

to microbial standard (MPN 0) were 10% wt. and 480 mw/cm^2 respectively. While the removal efficiency decreased at the 1- and 3-layer Zno nanoparticle films, it reached the highest value (100%) at 2- layer Zno nanoparticle film. This finding was attributed to an increase in the surface area for inactivation of *P. aeruginosa* cells (10^2 and 10^3 in ml) and was consistent with the photocatalytic experiments performed using TiO_2 thin films. They concluded that the decay rate constants of red sulphonyl 3BL were proportional to the film thickness. The decay rate constants were enhanced with increasing film thickness. However, a limiting value was observed at thick films due to increase in opacity and light scattering leading to a decrease in the passage of irradiation through the film (Habibi et al., 2007). At higher catalyst loadings (that is, more than two layers), the removal efficiency of *P. aeruginosa* started to lessen. This phenomenon was attributed to a decrease in UV penetration to the outer layers of the film, and a decrease in protection effect of clusters blocking UV from reaching

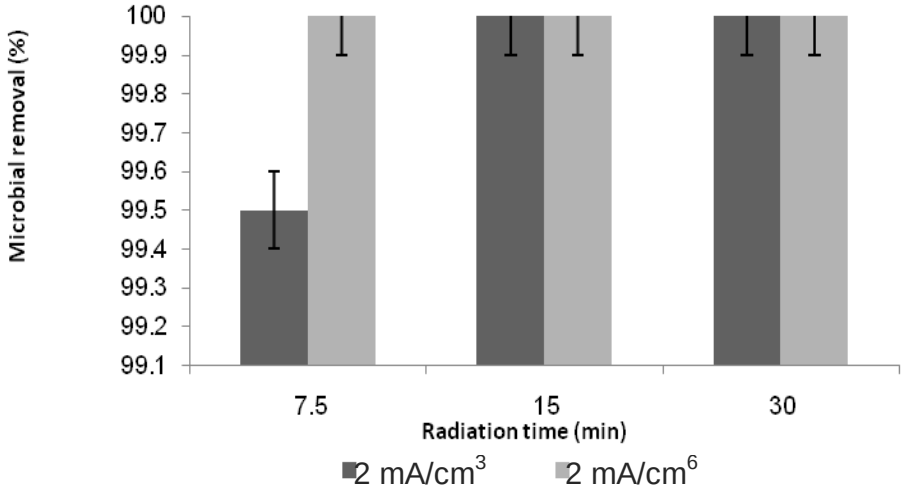

Figure 6. Effect of the current density on efficiency of bacterial removal (pH, 8; temperature, 25°C; radiation time, 7.5-30 min; UV-A lamp intensity, 480 mw/cm^2; initial bacterial number, 10^3; current density, 3-6 mA/cm^2; zinc oxide concentration, 10% wt).

the catalyst surface. The presence of ZnO photocatalyst nanoparticles and UV-A led to increase in the removal efficiency of *P. aeruginosa* due to the generation of $OH^{\bullet}$ radicals. This finding was consistent with the photocatalytic experiments carried out using TiO_2 (Daneshvar et al., 2007). $OH^{\bullet}$ radicals led to fat peroxidation of cellular membrane and degradation of the different compounds of the cell (Brunet et al., 2009). $O_2^{\bullet-}$ hydro peroxyl radical and hydrogen peroxide, generated by the reduction of dissolved oxygen in anode, was also fed into the photocatalytic disinfection mechanism. These species were responsible for the decaying of *P. aeruginosa*. It should be noted that the photocatalytic inactivation of gram-positive and negative bacteria in water had been reported by Blake et al. (1999). However, UV dosage required for 99.9% destruction of *P. aeruginosa* was 10500 μW·s/cm^2.

Effect of current density

A key variable parameter affecting the oxidation ability of EPC process was the applied current density since it regulated the amounts of generated $OH^{\bullet}$ radicals acting as oxidizing agents. The effect of the current density on the removal efficiency of the EPC process is shown in Figure 6. At lower current density, and lower radiation time, the removal efficiency of *P. aeruginosa* started to lessen. On the other hand, at higher current density, the radiation time started to lessen. The optimum current density for reaching microbial standard (MPN 0) was 3 mA/cm^2. At lower initial cell loadings, the photocatalytic treatment time required for complete cell inactivation started to lessen. The experimental results showed that the current density electrode enhanced the resulting gradient separated electron-hole, thereby diminishing its recombination rate, enhancing the photo current rate, and at length expediting the cell inactivation as shown in Figure 3.

Under higher applied current densities, the external electric field improved the direct and indirect electro-oxidation reactions at anode. The biocide efficiency was proportional to the specific surface area of photocatalysts and the quantum yield of photocatalytic system because the number of $OH^{\bullet}$ was proportional to the specific surface area and inversely proportional to the electron-hole recombination rate. The photo electro catalytic accelerated the mass transfer by electro-migration of negatively charged bacterium cells towards the electrode. This finding was the same as that of the photocatalytic experiments carried out using graphite-supported TiO_2 (Palmisano et al., 2009). The experimental results showed that the more the intensity of the radiation penetrating the photocatalytic electrode, the faster the cell inactivation progresses. As expected, the current density and exposure time was enhanced; accordingly the removal efficiency of *P. aeruginosa* was enhanced as shown in Figure 3. This finding was the same as that of the photocatalytic experiments carried out using TiO_2 reactor (Mounaouer and Abdennaceur, 2012). The increase in current density and exposure time led to faster generation of electrolysis products such as OH^- and Cl^- anions in cathode and anode electrodes, respectively. These products were responsible for *P. aeruginosa* inactivation. Increased current density led to an increased drift force on electrode surface, which was the main factor in the electrochemical processes. This finding was the same as that of the experiments performed using electrode (Rahmani et al., 2005). The

oxygen produced in anode electrode led to higher bactericidal effect against *P. aeruginosa*, because oxygen molecule played an important role in the photocatalysis stage, and the transformation of $O_2^{\bullet-}$ radical in capacity bond of ZnO photocatalyst nanoparticles. This finding was the same as that of the photocatalytic experiments performed using TiO_2 (Liu and CK-Yang, 2003). The efficiency of *P. aeruginosa* absorption by Zn electrode layered by ZnO nanoparticles as positive pole (anode) was directly related to an increase in current density, and exposure time. It was also explained that the Gram-negative bacterium of *P. aeruginosa* had a complex structure of cell wall, apeptidoglycan layer between the outer membrane and cytoplasmic membrane. The negative charge of lipopolysaccharide molecules for the outer membrane of the Gram-negative bacterium of *P. aeruginosa* led to its absorption by the Zn electrode. This finding was the same as that of the experiments performed using several nanoparticles (Yoon et al., 2007, 2008; Tam et al., 2008).

Conclusions

The experimental results suggest that ZnO thin layer nanoparticles immobilized on Zn in an EPC process is a promising method for the inactivation of *P. aeruginosa*. It is affected by pH, the number of bacteria, the lamp intensity, radiation time, the distance between lamp and electrode Zn/ZnO, the number of layers ZnO nanoparticles catalyst, and current density. The EPC treatments are capable of removing *P. aeruginosa* at the pH value of 8, with a radiation time less than 7.5 min. Enhanced *P. aeruginosa* removal is obtained with an increase in the pH, the lamp intensity, radiation time, and current density.

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