



Photocatalytic Removal of *Pseudomonas aeruginosa* from Water Using Titanium Dioxide Nanoparticles and UV Irradiation

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ABSTRACT

Background: Titanium dioxide (TiO₂)-mediated photocatalysis has been found to be an efficient method of water treatment and is capable of degrading a wide range of organic pollutants and microbial agents with high efficiency. The microorganism *Pseudomonas aeruginosa* is resistant to chemicals and UV irradiation. Bacteria which are resistant to UV-induced oxidative damage of the cell membrane are susceptible to photocatalytic technology. The main objective of this research was to examine the photocatalytic removal of *P. aeruginosa* upon UV irradiation in the presence of TiO₂ nanoparticles.

Methods: Polluted water samples were prepared by the addition of *P. aeruginosa* colonies into water, followed by contact with UV, TiO₂, or a combination of the two in separate stages. The effect of various parameters, including contact time, pH, and TiO₂ concentration, on reaction efficiency was examined.

Results: The highest efficiency of bacterial disinfection was achieved with UV irradiation in the presence of TiO₂ nanoparticles, with complete (100%) removal of *P. aeruginosa* observed upon irradiation for 60 min in the presence of 0.4 g/L TiO₂ under conditions of neutral pH.

Conclusions: The results of this study suggest that from a technical and economic perspective, the UV/TiO₂ process may be effectively applied for the disinfection of polluted water. This process could be considered a promising method of cleaning and purification, and applicable for water disinfection.

1. Introduction

Water quality and human health are fundamental for disease prevention and promoting the quality of life. There is an

increasing demand for clean water resources, and the shortage of such resources due to rapid industrialization, population growth,

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and long-term droughts has become a global issue. Water industries and governments in certain arid areas with abundant sunlight, less rainfall, and long-term droughts face the challenging task of seeking viable water resources. Approximately 4 billion people worldwide have been estimated to have little or no access to clean water resources, and millions die every year due to severe waterborne diseases [1].

Chlorine, chlorine dioxide, ozone, and UV irradiation are commonly employed for the disinfection of drinking water. Chlorine and chlorine dioxide are employed as final disinfectants because of the assured maintenance of a detectable disinfectant residual within the distribution network, which renders drinking water safe from a microbiological perspective. In contrast, UV irradiation and ozone treatment can only be employed for primary disinfection, as they fail to ensure the maintenance of a detectable residual [2]. Photocatalysis has recently emerged as an alternative technology for bacterial inactivation [1,3] and organic compound oxidation [4, 5].

In recent years, the use of TiO_2 as a photocatalyst for water treatment has been extensively reported. The illumination of TiO_2 with light of wavelength less than 380 nm results in the generation of highly reactive holes (h^+) in the valence band as well as hydroxyl ($\cdot\text{OH}$) radicals generated *via* the oxidation of water molecules by the valence band holes. These species have demonstrated ability to decompose a wide range of organic compounds (both aromatic and aliphatic), dyes, pesticides, and herbicides. Compared to other semiconductors, TiO_2 is particularly suitable as a photocatalyst for water treatment because it is highly photo-reactive, cheap, non-toxic, chemically and biologically inert, and photostable [4]. The effectiveness of this process in water treatment has prompted numerous studies on the suitability of TiO_2 -

mediated photocatalysis for water disinfection, and varying degree of efficacy of this technique has been reported [6].

The first report on photocatalytic disinfection of water was published by Matsunaga *et al.* in 1985; they showed that the disinfection process followed second order kinetics in the presence of high concentrations of microorganisms, with complete microbial inactivation achieved upon 60 min of irradiation [7].

Several subsequent studies investigated the use of TiO_2 along with UV irradiation for the inactivation of different microorganisms [1, 6, 8-12]. *Pseudomonas aeruginosa*, which belongs to the genus *Pseudomonas*, is a highly adaptable human pathogen capable of utilizing 80 organic compounds. This bacterium is the third most common pathogenic agent encountered in hospitals, after *Staphylococcus aureus* and *Escherichia coli* [13].

The aim of this study was the evaluation of TiO_2 -mediated heterogeneous photocatalysis in the presence of UV irradiation for the removal of *P. aeruginosa* from water samples under environmental conditions.

2. Materials and Methods

2.1. Bacterial culture

A culture of *P. aeruginosa* was provided by the faculty of Microbiology Department of Medicine in Hamadan University of Medical Sciences. Polluted water samples were prepared by the addition of a single colony of *P. aeruginosa* from blood agar plates into 2500 ml of water.

TiO_2 (Degussa, P-25), as a catalyst was employed as the photocatalyst in a batch-type reactor from Evonik-Industries, Germany. The sample used in the present study had a BET surface area of $50 \text{ m}^2/\text{g}$, average particle diameter of 30 nm, and a density of 130 g/L.

Other chemicals were of analytical grade, and were used without further purification. Deionized and double-distilled water was used throughout the present study. The pH of solutions was adjusted using NaOH and H₂SO₄, as needed.

2.2. UV source and light intensity

A low-pressure UV lamp of 40 W and 80 cm length was installed 5 cm above the sample surface. The UV light intensity, as measured by a radiometer in the 300–400 nm range was 0.9 J/s.m².

2.3. Experimental setup

The photochemical cell consisted of sterile 250-ml beakers and magnetic stirrers used for stirring the samples. The reactor temperature was controlled at 20 °C. During the first phase of the run, the beakers were filled with 200 ml of polluted water, and in separate stages, were subjected to UV irradiation, TiO₂ treatment, or a combination of these two. The effect of various parameters, including pH (5.1, 7.0, and 8.5) and the concentration of TiO₂ (0.2–6 g/L), on the reaction process was examined after 60 min of treatment. Polished aluminum was employed as the reflective material for the protection of samples that were not subject to irradiation. During the second phase of the run, the optimal values of pH and TiO₂ concentration obtained from the first stage were employed for the determination of optimal contact time (0, 15, 30, 45, and 60 min).

In the third phase of the run, the optimal values obtained from the previous stages were employed for determining the effect of TiO₂ concentration on the efficiency of the disinfection process. The disinfection efficiency, E, was calculated as follows:

$$E = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

C_i and C_f correspond to the initial and final most probable number (MPN)/100 ml, respectively. A schematic of the experimental details of the reactor is presented in Fig. 1.

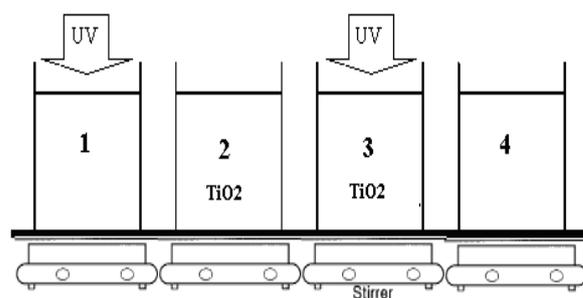


Fig. 1: Schematic of the Experimental Set-up Analysis.

Samples were withdrawn from the sample port of the reactor at predetermined time intervals, and filtered using membrane filter (0.45 µm pore-size) for the removal of TiO₂ particles. *P. aeruginosa* contamination in the samples was measured as MPN/100 ml before, during, and after the treatments using presumptive and confirmation tests that employed fermentation technique. Tubes were incubated at 35 °C to 37 °C for 48 h. Asparagine and acetamide broths were employed in the presumptive and confirmation tests, respectively. All analyses were carried out according to standard methods [14].

3. Results

3.1. Effect of operational conditions

Prior to conducting photocatalytic oxidation runs, several blank experiments were performed in order to assess bacterial removal through mechanisms other than photocatalytic reactions. The adsorption of *P. aeruginosa* from the water sample was found to be less than 5% under dark conditions and 60-min treatment duration. All the reactor parts were made of glassware to minimize the effect of adsorption. The role of UV irradiation *alone* as a function of time brought about moderate changes in the number of bacteria. Moreover, near-complete removal of *P. aeruginosa* was achieved upon simultaneous treatment of the water sample with 0.4 g/L TiO₂ and UV irradiation. The combination of these two processes (the UV/TiO₂ system) resulted in further

improvement in bacterial removal to 100%. Fig. 2 shows the profile of *P. aeruginosa* removal under different reaction conditions.

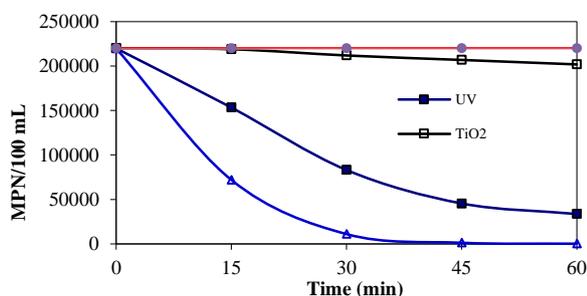


Fig. 2: Effect of UV Irradiation and TiO₂ on the Removal of *P. aeruginosa*. (TiO₂ = 0.4 g/L, pH=7).

3.2. Effect of TiO₂ dosage on the removal of *P. aeruginosa*

The optimal concentration of TiO₂ was determined by mixing various amounts of the catalyst (0.2–6 g/L) with a constant MPN/100 mL of *P. aeruginosa*. Figure 3 shows that the addition of TiO₂ increased the efficiency of bacterial removal by the UV/TiO₂ system, with a TiO₂ dosage of 0.4 g/L resulting in complete (100%) removal of *P. aeruginosa* from the water sample. However, further addition of TiO₂, even doubling of the catalyst concentration or beyond, resulted in slightly decreased extent of *P. aeruginosa* removal (less than 93.4%). The optimal amount of TiO₂ for the experiment was confirmed to be 0.4 g/L.

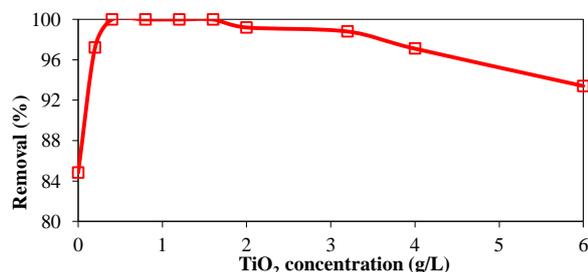


Fig. 3: *P. aeruginosa* Removal as a Function of the Initial Concentration of TiO₂. (Initial MPN/100 ml=220000, pH=7, t=60 min).

3.3. Influence of pH

Examination of the effects of pH is important, given that it is a main operating parameter that governs surface properties of the catalyst in aqueous phase. Figure 4 shows that the performance of the UV/TiO₂ system was independent of pH; significant variations were not observed in the pH range of 5.1–8.5, but a slight decrease was observed in acidic pH. The best results were obtained at neutral pH, where bacteria were completely eliminated upon irradiation lasting less than 60 min.

3.4. Order of the reaction

The removal of bacterial population was observed as a function of irradiation time, and data were fitted to the first-order rate equation (1):

$$\ln \frac{C}{C_0} = -kt \quad (1)$$

Where C_0 and C correspond to the number of bacteria at irradiation times 0 (initial) and t (final), k is the first-order rate constant (min^{-1}), and t , the irradiation time (min). The process under optimum conditions of operation was used for the illustration of first order kinetics, as shown (Fig. 5).

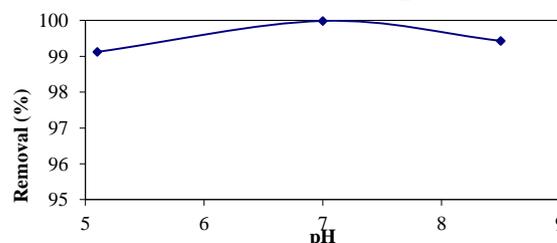


Fig. 4: *P. aeruginosa* Removal as a Function the pH Value. (Initial MPN/100 ml=220000, TiO₂ = 0.4 g/L)

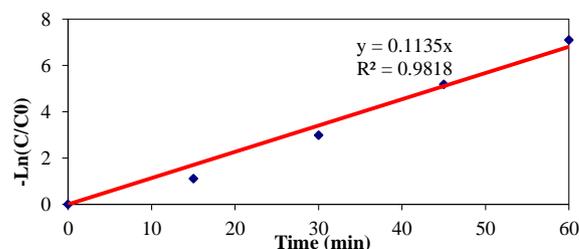


Fig.5: Constant Reaction Rate (k) Using UV/TiO₂ Process. (Initial MPN/100 ml=220000, TiO₂ = 0.4 g/L, pH=7)

4. Discussion and Conclusion

Disinfection has become a challenging aspect of water treatment due to the rapid elevation of health standards and a growing requirement for pollution-free water resources. TiO₂-mediated photocatalysis appears to be an effective method for water disinfection, with the type and concentration of catalyst playing an important role in treatment efficiency [15]. The present study revealed that the UV/TiO₂ system was appropriate for the removal of *P. aeruginosa* from contaminated water resources. While UV irradiation alone resulted in a moderate reduction in bacterial numbers, the resistance of *P. aeruginosa* to UV irradiation resulted in low rates of decrease in bacterial MPN over time in the absence of TiO₂ [16]. A high rate of bacterial destruction was observed in samples irradiated in the presence of TiO₂ (Fig. 2), due to the generation of hydroxyl radicals. Similar results have been reported in other studies [8, 17, 18].

The amount of catalyst is a significant parameter of the photocatalytic process. As shown in Figure 3, a TiO₂ dosage of 0.4 g/L showed comparable efficiency of bacterial removal with a treatment duration of 30 min. Several studies have shown that bacterial removal rates are strongly influenced by the number of active sites and photo-absorption property of the catalyst employed. An adequate dosage of the catalyst increases the rate of generation of electron-hole pairs, and thereby, the formation of hydroxyl radicals that promote photodegradation. However, an overdose of the catalyst decreases UV penetration due to opacity caused by excess catalyst clusters and increased scattering effect [4, 8, 17].

The UV/TiO₂ system failed to reveal significant effects of pH on the removal of *P. aeruginosa*. As shown in Figure 4, pH values in the range of 5.1–8.5 support near-complete removal of bacteria within 60 min, although a slight decrease in the extent of removal was

observed in acidic pH. This decrease is mostly attributable to the slight loss of TiO₂ at low pH, and the higher removal rates at higher pH, to sustained TiO₂ stability. This observation supports an earlier study, where the use of another system revealed complete removal of bacteria within 30 min over the pH range 4–10 [17, 19]. Cho et al., (2004) reported that *E. coli* has a net negative surface charge, while the TiO₂ particle surface has a point of zero charge at a pH of 6.3. Thus, greater electrostatic repulsion is expected between the TiO₂ particles and coliform bacteria at high pH due to negative surface charges on both; the TiO₂/water interface is likely to be important in several instances of photocatalytic degradation of charged substrates, albeit it is a relatively weak force which is easily overshadowed by other factors [19, 20].

An exponential decrease in residual *P. aeruginosa* was observed with time, as shown in Figure 2; this suggests that the reaction is likely to have followed pseudo-first order kinetics. As shown in Figure 5, the log transformation of the UV/TiO₂-mediated removal of *P. aeruginosa* confirmed that the reaction closely followed pseudo-first order kinetics. The reaction rate constant (*k*) was found to be 0.113 min⁻¹ under optimal conditions. These observations support previous studies which reported complete removal of bacteria by photocatalytic systems following pseudo-first order kinetics [18]. In contrast, other studies showed that the disinfection process followed second order kinetics in the presence of high concentrations of microorganisms [21]. Saito et al., (1992) showed that dissolved salts affect disinfection rates and inhibit the photocatalytic reaction, with phosphate and chloride having the highest and lowest inhibitory effects, respectively. This can be explained by the adsorption of anions onto the surface of TiO₂, which inhibits its photocatalytic activity [22].

The results obtained in the present study indicate that the UV/TiO₂ process is effective and reliable for the removal of *P. aeruginosa* from water samples, with complete removal achieved with UV irradiation for 60 min in the presence of 0.4 g/L TiO₂ at pH 7.

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