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PHOTOCATALYTIC DISINFECTION OF WATER POLLUTED BY PSEUDOMONAS AERUGINOSA

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ABSTRACT
Photocatalysis by titanium dioxide (TiO_2) is a water treatment method. Pseudomonas aeruginosa is a microorganism resistant to chlorine and UV-C irradiation. TiO_2 photocatalytic technology can destroy bacteria, which are resistant to oxidative destruction of cell membrane caused by sole UV irradiation. This study aims to investigate the total mineralization of the bacterium (P. aeruginosa) to the extent of death and cell-mass destruction using TiO_2 photocatalytic oxidation process. In this work the effects of parameters such as amount of TiO_2, irradiation time, initial concentration of bacterium, presence of ascorbic acid and effect of cell wall on removal of P. aeruginosa were studied. The data, which were obtained in this study, showed that the optimum concentration of TiO_2 was 325 ppm. Also at the initial concentration of TiO_2 equal to 325 ppm and initial microorganism MPN / 100 ml equal to 300 and after 75 min UV irradiation time, P. aeruginosa removal efficiency was 94.3 %. Removal efficiency of P. aeruginosa in the absence of TiO_2 or UV irradiation was very low. Decreasing the concentration of microorganisms increased its removal efficiency. Removal efficiency of spheroplast cells was more than intact cells of P. aeruginosa, which shows the important role of cell wall on cell resistance against chemical agents. Ascorbic acid had inhibitory effect on this process.

KEYWORDS: Advanced Oxidation Processes, Disinfection, Photooxidation, Pseudomonas Aeruginosa

1. INTRODUCTION

Chemical oxidizing agents such as chlorine and its compounds, ozone, hydrogen peroxide and potassium permanganate are used to treat microbial pollution of waters, but they are not completely efficient on some of resistant microorganisms. Therefore, it is necessary to use more advanced and efficient methods to disinfect waters and wastewaters. Some of the methods that are recently studied for this purpose are advanced oxidation processes (AOPs), which use UV-C irradiation along with a photocatalyst such as TiO_2 to destroy the microorganims. When irradiated TiO_2 particles are in direct contact with or close to microbes, the microbial surface becomes the primary target of the initial oxidative attack [1]. The wavelength range of UV-C irradiation is from 100 nm to 290 nm (UV-C) [2].

Some metallic oxides and sulfides are used as environmental photocatalysts. They have a completed valence band and an empty conduction band. When the energy of irradiated photons are being equal or more than the split between these two bands, an electron is

excited from valence to conduction band and in this way the photocatalytic property is appeared [3].

The most studies in this field have been done on bacteria, especially e. coli, while viruses and yeasts have been least studied. Matsunaga and Tomodo published first report about photocatalytic disinfection on 1985 [4]. They found that in the presence of high concentrations of microorganisms, disinfection process follows the second order rate equation. Saito *et al.* (1992) [5] showed that dissolved salts affect on disinfection rate and inhibit the photocatalytic action. Phosphate has the highest and chloride has the lowest inhibitory effect. This can be explained by adsorption of anions on the surface of TiO_2, which inhibits its photocatalytic action.

Pseudomonas aeruginosa is a species of pseudomonas genus and has the highest pathogenic effect in human. After staphylococcus aureus and escherichia coli, it is the third pathogen agent in hospitals [6]. Pseudomonas aeruginosa is very adaptable and can consume 80 organic compounds for its metabolism. It can be alive in distilled water. It can be found in all parts of hospitals and is resistant to chemical disinfectants. It can be also found in ammonium quarternary compounds, hexachlorophene soaps and iodine solutions. It is resistant to many of conventional antibiotics especially penicillins and first generation of cephalosporins, too [7].

Since it is resistant to each of chemical oxidizing agents and sole UV irradiation, the aim of this work is to study the efficiency of photocatalytic oxidation in the presence of TiO_2 to remove P. aeruginosa.

2. EXPERIMENTAL

2.1. Apparatuses and materials

Pseudomonas aeruginosa was prepared from microbiology division of Imam Khomeini hospital in Tabriz. All the compounds were used to prepare the culture medias were from Merck company. TiO_2 was from Degussa (Degussa P-25). Prepared culture medias were sterilized using autoclave (H+P, 15 psig, 121 ^oC, Germany) for 15 min. The prepared medias were cultured in an incubator (Memmert, Germany) for 24 h to 48 h at 35±0.5 ^oC. All TiO_2 suspensions were sterilized at 121 ^oC and 15 psig for 15 min. TiO_2 suspensions were prepared using double distilled water.

2.2. Reactor

All experiments were done in a batch wooden chamber with a cover of aluminum foil and equipped with a UV-C lamp (30 W, Philips). Air stream was used to prevent from excess heating of the chamber. Distance between UV lamp and reaction glass chamber was 0.2 m and the intensity of UV irradiation in reaction chamber was 0.365 klx.

2.3. Detection of pseudomonases in water

One of the methods was used to detect pseudomonases was membrane filter method [8], which is fast and applicable for large volumes of samples. In this method, 100 ml of the sample is used. The equipments, which are used in this method, should be inert and sterilized. Proper culture medias are used to detect P. aeruginosa.

Also, multiple tube fermentation method [9] was used to detect P. aeruginosa. In this method, the bacteria can be detected by production of fluorescent pigments, which are detectable by UV irradiation. Some pigments are not fluorescent and dispersed in the culture media and make it bluish purple. This method reports the number of microorganisms as MPN (Most Probable Number). In this method, gas production confirms the presence of microorganisms.

3. RESULTS and DISCUSSION

3.1. Effect of UV irradiation and TiO_2 on P. aeruginosa removal

Figure 1 shows the effect of UV irradiation and TiO_2 on removal of P. aeruginosa. The experiments were done at initial MPN / 100 ml of 300 and TiO_2 concentration of 0 and 325 ppm. The experiments were continued up to 75 min. It is evident that in the absence of each of TiO_2 or UV irradiation, decrease in microorganism MPN with time was low, while in the

presence of both of them, microorganism MPN efficiently decreased. In the presence of both of TiO_2 and UV irradiation, hydroxyl radicals ($^{\cdot}OH$) are produced, which increase the efficiency of the process. In the absence of UV irradiation, some of microorganisms can be adsorbed on TiO_2 particles, which causes low decrease in the amount of microorganisms with time. Pseudomonas aeruginosa is resistant to UV irradiation [10] and therefore, decrease in microorganism MPN with time in the absence of TiO_2 was low.

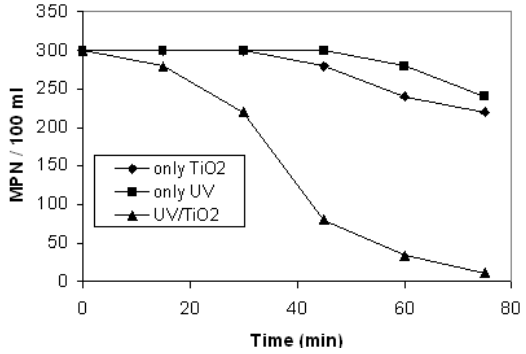

Figure 1. Effect of UV irradiation and TiO_2 on the removal of P. aeruginosa, initial MPN / 100 ml=300, $[TiO_2]_0$=325 ppm

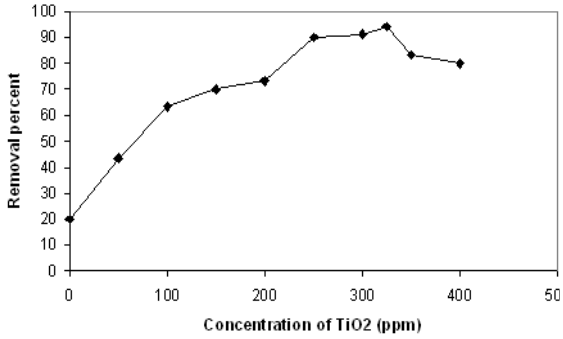

Figure 2. Effect of TiO_2 on P. aeruginosa removal, initial MPN /100 ml=300, irradiation time=75 min

3.2. Effect of amount of TiO_2 on removal of P. aeruginosa

Different amounts of TiO_2, from 0 to 400 ppm, were added to the samples with initial MPN / 100 ml of 300 and the results are shown in figure 2 at 75 min UV irradiation time. It can be seen that to some extent, increasing the TiO_2 concentration increased the removal of P. aeruginosa. At high TiO_2 concentrations, turbidity of the solution prevents from the effect of UV irradiation and therefore, the removal efficiency decreases [11].

3.3. Effect of initial concentration of P. aeruginosa on its removal

These experiments were done in TiO_2 concentration of 325 ppm and microorganism MPN / 100 ml of 50 to 1600 at various UV irradiation times. The results are shown in figure 3 and it can be seen that increasing the initial concentration of microorganism, increased its residual concentration. It is because of increasing cell numbers against constant photocatalytic sites and UV irradiation.

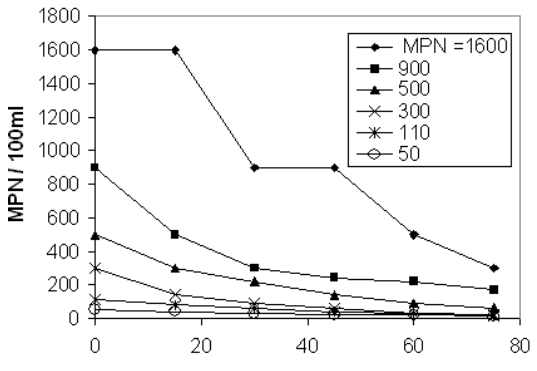

Figure 3. Effect of initial concentration of P. aeruginosa, $[TiO_2]_0$=325 ppm

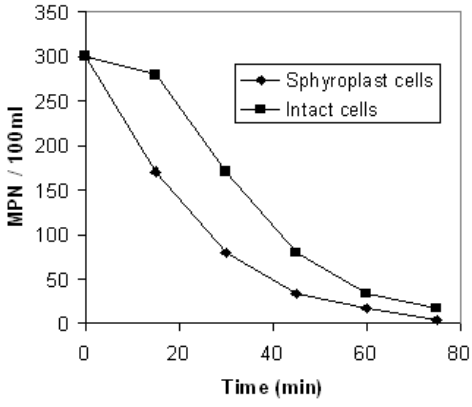

Figure 4. Effect of cell wall on removal of P. aeruginosa at various times (min), initial MPN /100 ml=300, $[TiO_2]_0$=325 ppm

3.4. Effect of cell wall on P. aeruginosa removal

Figure 4 compares the effect of UV/TiO_2 process on removal of intact cells and spheroplast cells of P. aeruginosa at initial microorganism MPN / 100 ml of 300 and TiO_2 concentration of 325 ppm at various times. The removal rate of spheroplast cells was higher than intact cells since, spheroplast cells do not have complete layers of cell wall. Spheroplast cells were prepared by soaking intact cells with solutions consisted of some compounds such as saccharose, EDTA and so on [12]. This shows the effect of cell wall on preventing from entrance of active chemical species into the cell.

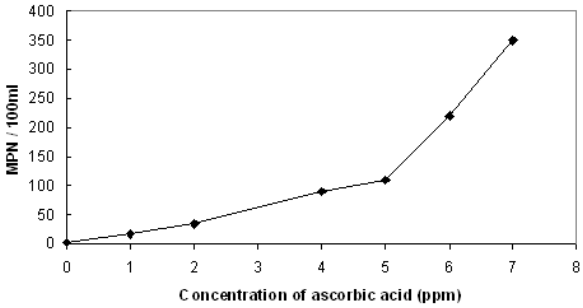

Figure 5. Effect of ascorbic acid on P. aeruginosa removal, $[TiO_2]_0$=325 ppm, initial microorganism MPN / 100 ml=350

3.5. Effect of ascorbic acid on P. aeruginosa removal

In cells, vitamins such as ascorbic acid are employed as added defense mechanisms against free radical formation [13]. Figure 5 shows that ascorbic acid had inhibitory effect on the photocatalytic removal of P. aeruginosa by UV/TiO_2 process. The result is related to scavenging of free radicals, which attack the cell wall of the microorganisms.

4. CONCLUSION

The results of this study showed that UV/TiO_2 process efficiently removed Pseudomonas aeruginosa. This process needed joint action of UV irradiation and TiO_2. Increasing the concentration of TiO_2, first increased the microorganism removal efficiency, but further increase in photocatalyst concentration resulted in a decrease in process efficiency. Also it is resulted that microorganism cell wall has significant effect on its resistant against UV/TiO_2 disinfection process. The results showed that ascorbic acid has inhibitory effect on the process.

5. ACKNOWLEDJEMENT

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6. NOMENCLATURE

EDTA: Ethylene Diamine Tetra Acetatic Acid
klx: kilolux (unit of illuminance)
MPN: Most Probable Number
ppm: parts per million

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