การยับยั้งเชื้อแบคทีเรียในน้ำของไทเทเนียมไดออกไซด์โฟโตแคทตาไลซิสโดยแสงยูวีและแสงอาทิตย์

Titanium Dioxide-Photocatalytic Inactivation of Bacteria in Water by UV and Solar Irradiation

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บทคัดย่อ

การพัฒนาด้วยเทคนิคที่เป็นมิตรต่อสิ่งแวดล้อมมีความสำคัญต่อการรักษาคุณภาพน้ำ ไทเทเนียมไดออกไซด์ (TiO_2) เป็นสารเคมีอนุภาคนาโนที่มีประโยชน์และใช้ในเชิงพาณิชย์อย่างกว้างขวาง วัตถุประสงค์การศึกษานี้เพื่อประเมินผลของแสงยูวีที่ช่วยไทเทียมไดออกไซด์โฟโตแคททาไลซิสต่อการยับยั้งการเจริญของเชื้อแบคทีเรียในน้ำโดยใช้แสงยูวี (เอ และบี) และแสงอาทิตย์ เครื่องปฏิกรณ์ประกอบด้วยแผ่นเคลือบและไม่เคลือบด้วยไทเทเนียมไดออกไซด์ กล่องแก้วปฏิกรณ์เคลือบไทเทเนียมไดออกไซด์จะเคลือบที่แผ่นกระจกด้านล่างผลการทดลองแสดงว่าทั้งยูวีเอร่วมกับไทเทเนียมไดออกไซด์โฟโตแคทตาไลซิสและยูวีเออย่างเดียวมีผลให้ลดจำนวนเชื้อแบคทีเรียได้ประมาณ 3 log CFU ต่อมิลลิลิตร และ 1 log CFU ต่อมิลลิลิตรในระยะเวลา 120 นาทีตามลำดับ ทั้งยูวีบีร่วมกับไทเทเนียมไดออกไซด์โฟโตแคททาไลซิสและยูวีบีอย่างเดียวมีผลให้ลดจำนวนเชื้อแบคทีเรียได้ประมาณ 8 log CFU ต่อมิลลิลิตร และ 1 log CFU ต่อมิลลิลิตรในระยะเวลา 30 นาทีและ 60 นาทีตามลำดับ สำหรับแสงอาทิตย์ การใช้แสงอาทิตย์ร่วมกับไทเทเนียมไดออกไซด์โฟโตแคทตาไลซิสและยูวีบีอย่างเดียวมีผลให้ลดจำนวนเชื้อแบคทีเรียได้ประมาณ 1 log CFU ต่อมิลลิลิตรในระยะเวลา 120 นาทีในขณะที่แสงอาทิตย์อย่างเดียวไม่มีผลต่อการทำลายเชื้อแบคทีเรีย ผลการทดลองสรุปว่าการใช้โฟโตแคททาไลซิสในการทำลายเชื้อโดยใช้พลังงานแสงอาทิตย์หรือแสงยูวีให้ประสิทธิภาพสูง การศึกษานี้แสดงว่าไทเทเนียมไดออกไซด์เป็นตัวเร่งโฟโตแคทตาไลซิสที่สามารถนำมาใช้ในการบำบัดน้ำได้

คำสำคัญ: โฟโตแคททาไลซิส ไทเทเนียมไดออกไซด์ ของเสีย

Abstract

There is great importance in developing more environmental friendly treatment techniques to provide good quality and safe water. Titanium dioxide (TiO_2), a chemically inert nanoparticle, is commercially manufactured worldwide for a wide range of applications. The objective of this study aimed to evaluate the effect of UV-assisted TiO_2-photocatalysis inactivation of bacteria in water using different UV (A and B) irradiation and solar irradiation. The experimental reactor set-up consisted of the coated and uncoated titanium dioxide reactor. The TiO_2 glass box reactor consisting of TiO_2 was coated on the lower plate. The results showed that both UVA-TiO_2-photocatalysis and UVA alone resulted in bactericidal phase of approximately 3 log CFU/mL and 1 log CFU/mL in 120 min, respectively. Both UVB-TiO_2-photocatalysis and UVB alone resulted in bactericidal phase of approximately 8 log CFU/mL and 1 log CFU/mL in 30 min and 60 min, respectively. For solar irradiation, solar irradiation-TiO_2-photocatalysis resulted in bactericidal phase of approximately 1 log CFU/mL in 120 min while the solar irradiation alone resulted no effect to bactericidal activity of the bacteria. In conclusion, photocatalytic water disinfection using solar energy or UV light are effective. This study suggests that TiO_2 is one of a promising photocatalyst for water treatment catalyst

Keywords: photocatalysis, titanium dioxide, wate

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Introduction

Solar disinfection involves storing microbially contaminated water in transparent containers such as plastic bags or plastic or glass bottles. These are placed in direct sunlight for periods of up to 8 h before consumption.[1] The biocidal effect of sunlight is due to optical and thermal processes, and a strong synergistic effect occurs for high water temperatures[2] and UV discharge tubes;[3] validation of the inactivation kinetics of some pathogens was tested using natural sunlight. Photocatalysis is a cost-effective technique and offers a simple solution for water purification since it involves the acceleration of photoreaction with a semiconductor and can be employed under solar irradiation. The generation of electron-hole pairs is the principal step in photocatalysis. TiO_2 semiconductor exhibits strong photoactivity since the radicals (•OH, O_2•-, HO_2•-, etc), formed by the electron-hole pairs under UV light, are capable of oxidizing organic substrates and inactivate bacteria in water. It has high photocatalytic and chemical activity, non-toxicity, wide availability and low cost.[4] TiO_2 is considered to be the most potential material for the photocatalytic purposes because of its exceptional optical and electronic properties.[5] TiO_2 films can be prepared by different deposition techniques such as the reactive sputtering, chemical vapor deposition and sol–gel process. The sol–gel process is the most popular method because of its simplicity and low costs. The properties of the film, and consequently its applications, will strongly depend on the nature of the preparation process as well as on its operational parameters.[6] Many studies have shown that activation of TiO_2 and TiO_2 with Ag photocatalysts with ultraviolet A (UVA) light is a highly effective process for complete inactivation of bacterial cells.[7] The photocatalytic effect depends on the wavelength of light.[8] UV is separated into UVA, UVB, and UVC based on wavelength. In this study, bacterial cells of *E. coli* and *S. typhimurium* which are gastrointestinal pathogens that cause diarrhea and enteritis in humans were used as models to investigate the disinfective capacities of TiO_2 in suspension and films with natural sunlight and different UV wavelengths.

Materials and Methods

Preparation of catalysts

TiO_2 suspension and TiO_2 films were prepared using the following chemicals: tetra-isopropoxytitanium $Ti(i\text{-}OC_3H_7)_4$, ethanol (C_2H_5OH), hydrochloric acid (HCl), and water. The TiO_2 sol was composed of 29.0 g $Ti(i\text{-}OC_3H_7)_4$, 100 mL ethanol, 2.5 mL water and 0.5 mL HCl. The solution was mixed for 5 min at room temperature (for TiO_2 film). The diameter of nanoparticles in the paste was 20 nm. The glass substrates (4.5 x 2.4 cm, 1.4 mm thick) were immersed in sol solution at the rate of 2 mm/sec and dried at room temperature for 10 min. The sol films were heated at 250 ºC for 60 min in air to achieve cohesion and adhesion of the film to the substrate.

UV-photocatalysis

Photocatalysis disinfection Ultraviolet A-light emitting diodes (UVA) with peak irradiance at 365 nm were used to create the sterilization device and UVA intensity was 9 mW/cm^2. In all cases, the light was switched on 30 min before the start of the reaction to stabilize the emission power and spectrum. The distance between the UVA and the surface of the bacterial solution was 1.5 cm. UVA irradiation was performed in a dark room at 25 ºC for various time periods. UVB irradiation, a low-pressure UV lamp (8 W) was used to irradiate at 302 nm (UVB). Intensity was adjusted by changing the distance between the lamp and the bacterial solution. Intensity at 302 nm was 0.09 mW/cm^2. In all cases, the light was switched on for 30 min before the start of the reaction to stabilize the emission power and spectrum. UVB irradiation was performed in a dark room at 25 ºC for various time periods.

Preparation of bacterial cells

E. coli strain KM74 and *S. typhimurium* strain KM7555 were used as model microorganisms for the disinfection experiments. The bacteria were cultured in Luria-Bertani (LB) broth (1% tryptone, 0.5% yeast extract, 1% NaCl) at 37ºC with rotary shaking for 18 h. The overnight culture (2 mL) was centrifuged at 12,000 rpm for 5 min. The supernatant was discarded and the bacterial pellet was washed three times with steriled phosphate buffer saline (PBS) and suspended in PBS at an initial concentration of $4.2x10^7$ CFU/mL. Apparatus for Petri dish test consisted of UV lamp, Petri dish (DI 87 mm) and supporter. For TiO_2 suspension, the experiment was carried out by transferring the washed bacterial cells (about $4.2x10^7$ CFU/mL) into the Petri dish containing 18 mL of the sterilized deionized water containing TiO_2 0.18 g. A magnetic stirrer agitated the TiO_2–cell mixture with UVA and UVB lamps or solar light radiated vertically. For TiO_2 immobilized surface, the experiment was carried out transferring washed bacterial cells (about $4.2x10^7$ cfu/ml) while two of UV lamps or solar light were radiated vertically. The control was treated either without TiO_2 at the same condition as mention above or with TiO_2 in the dark. Samples were taken in triplicates at 15 min intervals for 120 min. After irradiation, the sample was placed in a Petri dish with 5 mL of PBS and shaken for 10 min. Dilutions were placed on LB agar plates and incubated at 37ºC for 24 h before bacterial colonies were counted. Survival of the bacterial population was calculated using the equation:

$$\text{survival ratio} = \text{Log}\ (N_t/N_0)$$

Where N_0 represents the initial population and N_t represented the population after irradiation time (t). All results were calculated with data from three independent experiments.

Results and Discussion

Inactivation kinetics of *E. coli* and *S. typhimurium* exposed to UVA, UVB and solar TiO_2 photocatalytic processes

Accordingly, in Figure 1a, the inactivation kinetics of *E. coli* strain by applying natural solar, UVA and UVB with and without driven TiO_2 photocatalytic process was evaluated. The process was operated with 0.1 gL^{-1} TiO_2, approximately $4.2x10^7$ CFU/mL initial bacterial density and 120 min total irradiation time. The inhibition growth of *E. coli* strain was observed in the early 15 min of irradiation in both processes. However, total inactivation was observed after 30 min and 45 min irradiation time for UVB with and without TiO_2 photocatalysis, respectively. The solar and UVA with TiO_2 photocatalysis processes did not result in total bacteria inactivation, but 99.75% (2.59 log) and 99.99% (4.41 log) removal of initial bacterial density ($1.07x10^5$ and $1.62x10^3$ CFU/mL residual density, respectively) was observed after 120 min irradiation time. The UVA and solar without TiO_2 photocatalysis processes did not result in total bacteria inactivation, but 95.45% (1.34 log) and 31.43% (0.16 log) removal of initial bacterial density ($1.91x10^6$ and $2.88x10^7$ CFU/mL residual density, respectively) was observed after 120 min irradiation time. The similar pattern was found in the inactivation kinetics of *S. typhimurium* strain (Figure 2) but the inactivation was less than that of *E. coli*. This might be suggested that *S. typhimurium* has higher complexity and density of the cell wall than that of *E.coli*. In Figure 1b, the inactivation kinetics of *S. typhimurium* strain by applying natural solar, UVA and UVB with and without driven TiO_2 photocatalytic process was evaluated. The processes was operated with 0.1 gL^{-1} TiO_2, approximately 10^7 CFU/mL initial bacterial density and 120 min total irradiation time. The inactivation of *S. typhimurium* strain colonies was inactivated in the early 15 min of irradiation in both processes. However, total inactivation was observed after 45 min and 60 min irradiation time for UVB with and without TiO_2 photocatalysis, respectively. The solar and UVA with TiO_2 photocatalysis processes did not result in total bacteria inactivation, but 97.51% (1.60log) and 99.97% (3.46 log) removal of initial bacterial density ($1.05x10^6$ and $1.45x10^4$ CFU/mL residual density, respectively) was observed after 120 min irradiation time. The UVA and solar without TiO_2 photocatalysis processes did not result in total bacteria inactivation, but 88.34% (0.93 log) and 45.46% (0.26 log) removal of initial bacterial density ($4.90x10^6$ and $2.29x10^7$ CFU/mL residual density, respectively) was observed after 120 min irradiation time. In Figure 2a, the inactivation kinetics of *E. coli* strain by applying natural solar, UVA and UVB with driven TiO_2 film photocatalytic process was evaluated. The process was operated with TiO_2 film, approximately $4.2x10^7$ CFU/mL initial bacterial density and 120 min total irradiation time. The inactivation of *E. coli* strain colonies was inactivated in the early 15 min of irradiation in both processes. However, total inactivation was observed after 45 min irradiation time only for UVB with TiO_2 film photocatalysis. The solar and UVA with TiO_2 film photocatalysis processes did not result in total bacteria inactivation, but 95.97% (1.39 log) and 99.94% (3.21 log) removal of initial bacterial density ($1.70x10^6$ and $2.57x10^4$ CFU/mL residual density, respectively) was observed after 120 min irradiation time. The similar pattern was found in the inactivation kinetics of *S. typhimurium* strain (Figure 2b) but the inactivation was less than that of *E. coli*. This might be noted that *S. typhimurium* has higher complexity and density of the cell wall than that of *E. coli*. In Figure 2b, the inactivation kinetics of *S. typhimurium* strain by applying natural solar, UVA and UVB with driven TiO_2 film photocatalytic process was evaluated. The process was operated with TiO_2 film, approximately $4.2x10^7$ CFU/mL initial bacterial density and 120 min total irradiation time. The inactivation of *S. typhimurium* strain colonies was inactivated the early 15 min of irradiation in both processes. However, total inactivation was observed after 45 min irradiation time only for UVB with TiO_2 film photocatalysis. The solar and UVA with TiO_2 film photocatalysis processes did not result in total bacteria inactivation, but 92.12% (1.10 log) and 99.66% (2.46 log) removal of initial bacterial density ($3.31x10^6$ and $1.45x10^5$ CFU/mL residual density, respectively) was observed after 120 min irradiation time.

The bacteria *E. coli* and *S. typhimurium*, which are relevant for hygiene, were illuminated on the sample solution for 120 min. The survival ratio is achieved, and the experimental conditions are shown in Table 1. The survival ratio of *E. coli* decreased more than that of *S. typhimurium*. It might be noted that *E. coli* has complexity and density of the cell wall less than that of *S. typhimurium*. This precedence appears reasonably if it is assumed that the primary step in photocatalytic decomposition consists of an attack by OH radicals on the cell wall, leading to punctures.[9] In Figure 1a and 1b, *E. coli* exposed to UVA without photocatalyst showed little reduction after 30 min, or after as little as 60 min in the case of *S. typhimurium*. This might be UVA which is relatively low energy, damages cells by oxidative stress caused by oxygen radicals within the cells.[10] If the stress on the cell exceeds a certain threshold, the cell dies or becomes incapable of further division. The extent of this effect depends on the kind of bacterium and growth condition (growth phase, status of nutrition). A UV light inactivates microorganisms by damaging deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). When DNA and RNA absorb UV light that damage results from the formation of dimers (covalent bonds between the same nucleic acids). Dimers cause faults in the transcription of information from DNA to RNA, which in turn results in disruption of microorganism replication. The microorganism continues to live, but it can not reproduce and therefore is not infective. Different kinds of DNA,[10] such as single-strand were broken or caused photomodified bases. However, this damage may be repaired by the cell to a certain extent. From Figure 1a and 1b, for UVB photocatalyzed oxidation with TiO_2 suspensions, *E. coli* at 7 log were inactivated within 30 min. The required value 30 min is not met for *S. typhimurium*, but exposure times up to 45 min for inactivating 7 log stages are satisfactory. For UVA photocatalyzed

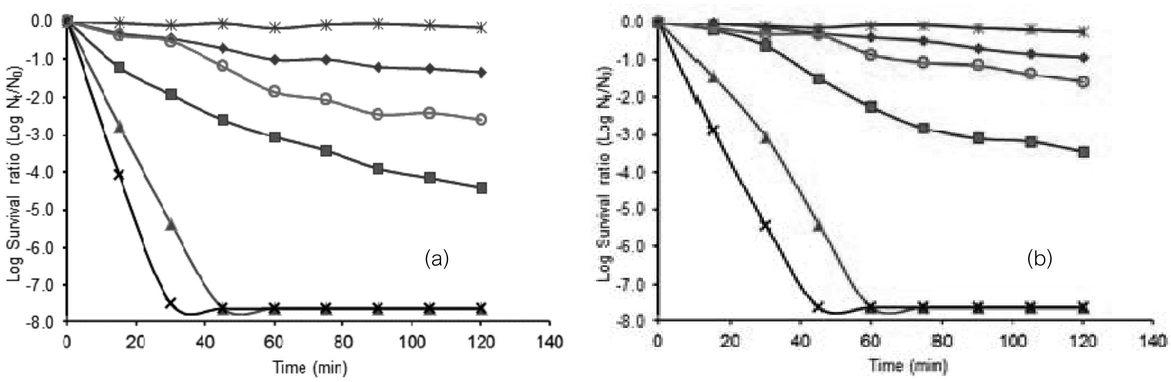

Figure 1 Inactivation of *E. coli* (a) and *S. typhimurium* (b) by solar radiation (✻), solar irradiation with TiO_2 suspension (◆), UVA (○), UVA with TiO_2 suspension (■), UVB (▲) and UVB with TiO_2 suspension (×).

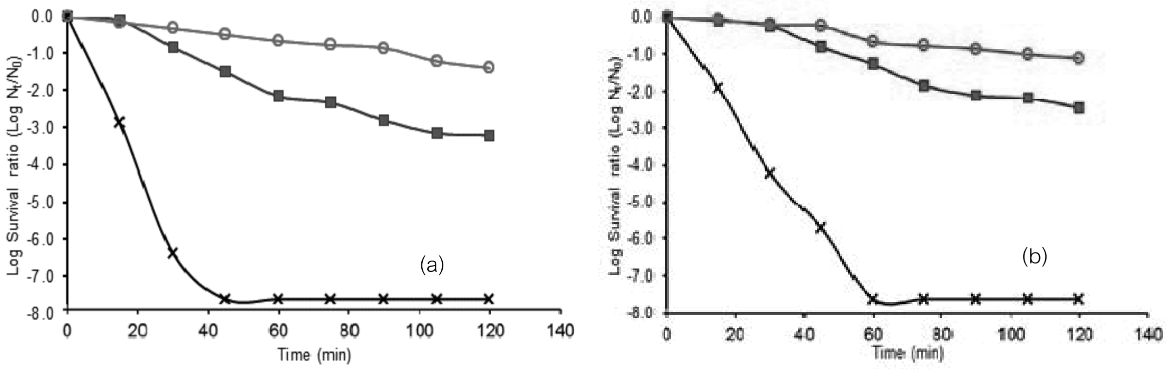

Figure 2 Inactivation of *E. coli* (a) and *S. typhimurium* (b) by solar irradiation with TiO_2 film (○), UVA with TiO_2 film (■), and UVB with TiO_2 film (×).

Table 1 Photoinactivation of *E. coli* and *S. typhimurium* by UVA, UVB and solar irradiation with and without TiO_2 in suspension for 30 min.

Treatment	*E. coli*	*S. typhimurium*
	Final cell count ± error (CE in CFU/mL)	**Final cell count ± error (CE in CFU/mL)**
UVA	$8.1x10^6±1.4x10^6$	$2.1x10^7±0.3$ $x10^7$
UVA+TiO_2	$1.0x10^6±0.2$ $x10^6$	$1.2x10^7±0.6x10^7$
UVB	$3.2x10^1±0.4x10^1$	$1.7x10^2±0.2x10^2$
UVB+TiO_2	0	0
Solar radiation	$3.7x10^7±0.6x10^7$	$3.0x10^7±0.5x10^7$
Solar radiation +TiO_2	$2.7x10^6±0.4x10^6$	$1.9x10^7±0.6$ $x10^7$

oxidation with TiO_2 suspensions, *E. coli* at 4 log stages need to be inactivated within 96 min. The required 96 min value is not met for *S. typhimurium*, but exposure times over 120 min for inactivating 4 log stages are satisfactory outside risk zones.[11] The behaviour of *S. typhimurium* during extended exposure time by UVA photocatalyzed oxidation will be examined for the further study. From Figure 1-2, the survival ratios were found to decrease in the following order: UVB with TiO_2 suspension > UVB > UVA with TiO_2 suspension > solar irradiation with TiO_2 suspension > UVA > solar irradiation. UV irradiation can inactivate bacterial growth depending on its wavelength and intensity. Due to shorter wavelength, UVB inactivated bacterial growth more than UVA. Due to solar irradiation containing lower intensity of UVA and UVB, sunlight alone inactivated bacterial growth less than UVA and UVB lamps. From Figure 2a-2b, TiO_2 photocatalytic surfaces inactivated bacterial cells lower than that of the suspension power. One possible application of the photoactive TiO_2 surface might be using for permanent maintenance of bacteria-free conditions on surfaces that have been disinfected already, e.g. using conventional wiping with disinfectant. The aim of disinfection is to reduce the number of infectious bacteria in an area or an object, preventing it from providing a starting-point for infection.[11] The disinfectants traditionally used are of chemical origin, with risk of allergy and toxicity.[12] Regular application of disinfection procedures is necessary in areas of antibacterial protection. Compared to the general disinfectants, TiO_2-coated surfaces do not involve aerosol formation. Another disadvantage of conventional disinfection methods is poor validation. By contrast, the procedure presented here is easier to apply. Although disinfection requires inactivation of pathogenic bacteria, in a reduction of around 3–5 log stages is sufficient about 99.90–99.99% in comparison with complete sterilization.[11] The requirement for a reduction of 3–5 log10 stages is achieved by the present method. To allow widespread use of the method, it will certainly be necessary to validate its optimal conditions, particularly with dried microorganisms and with high levels of protein.[13] This bacteria-reducing process would ideally be usable in fields in which flat working areas are used: in the medical field, food processing, in the pharmaceutics and industrial field and aquiferous systems in which reliable prevention of contamination and biofilm formation is needed. Although the higher inactivation efficiencies of *E. coli* were observed with the UV lamp, the high energy consumption was used compared to the conventional UV lamps. Solar driven photocatalytic treatment has been increasingly investigated because it is an attractive technology on economical energy consumption.[14]

Conclusion

The TiO_2 suspension and TiO_2 film were efficiently synthesized using the sol–gel method. It was found that TiO_2 powders are more efficient than TiO_2 film for the photocatalytic degradation. The loading of TiO_2 powders showed that UVA and solar radiation of the microbial activity was reduced over 90% after 15 min. Beside UVB that is harmful to human, UVA and solar irradiation with TiO_2 photocatalysis are effective solutions to solve the problems of water pollution.

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