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Disinfection of bacterial suspensions by photocatalytic oxidation using TiO_2 nanoparticles under ultraviolet illumination

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Titanium dioxide nanoparticles are prepared using sol-gel hydrolysis, autoclaving and condensation of titanium isopropoxide in acetic acid solution. Structure and properties of the films are characterized by X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM). The films exhibit anatase phase and the mean particle size of the as-prepared powder is approximately 9 nm. The photocatalytic reaction is carried out with various TiO_2 concentrations and Ultraviolet (UV) illumination time. A feasible synergistic effect exhibited that the bactericidal effect of TiO_2 on *Acinetobacter* suspension after UV light irradiation is much higher than that without TiO_2. As the concentration of TiO_2 increased to 10.0 mg/ml, the bactericidal effect is increased. However, the bactericidal effect is rapidly abbreviated at TiO_2 concentration higher than 4.0 mg/ml for Acinetobacter suspensions. UV illumination time affected significantly the viability of bacterial cells with different death rate. The optimum exposure time of the disinfection process is in the range of 60-80 minutes at TiO_2 concentration of 8-10 mg/ml.

يتلخص هذا البحث فى تحضير مركب ثانى أكسيد التيتانيوم ذى الأبعاد النانومترية بإستخدام طريقة المحلول-الجيل والتى تتميز بإمكانية الحصول على حبيبات دقيقة جدا بإستخدامها . وقد تم فحص المركب الناتج عن طريق تقنية حيود الأشعة السينية لدراسة التركيب البللورى والميكروسكوب الإلكترونى الماسح لقياس التركيب المجهرى حيث تم تحديد حجم الحبيبات لتتراوح مابين ٩ نانومتر للمسحوق الناتج و ١٨ نانومتر بعد معالجته حراريا عند ٥٠٠ درجة مئوية لمدة ساعة .أيضا فقد تم تحضير محلول معلق من هذا المركب لدراسة تأثير الأكسدة الضوئية على قتل سلالة معينة من البكتيريا وقد تم الحصول على كفاءة تقارب من ١٠٠ % عند كثافة محلول تصل إلى ١٠ مللى جرام لكل مللى لتر من مادة ثانى أكسيد التيتانيوم .

Keywords: TiO_2, Antibacterial, Disinfection, Photocatalytic oxidation

1. Introduction

Titanium dioxide (TiO_2) photocatalysts have attracted great attention as an alternative material to aid in the purification of water and air [1-5]. TiO_2 photocatalysts generate strong oxidizing power when illuminated with UV light at wavelength of less than 385 nm [6]. Illuminated TiO_2 photocatalysts decompose organic compounds by oxidation, with holes (h^+) generated in the valence band and with conduction hydroxyl radical ($^{\bullet}OH$) produced by the oxidation water.

Wastewater from hospital, food factories, and contaminated sites sometimes contains microorganisms, virus, and organic compounds. One of the typical sterilization methods is the photocatalytic sterilization by UV illumination at wavelength of 254 nm, which provides a high rate of sterilization at room temperature. Alternately, it is well known that the TiO_2 in anatase form is capable of oxidizing and then decomposing various kinds of compounds [7-9].

Acinetobacter species are widespread in nature, and can be obtained from water, soil, living organisms and even from human skins. They are oxidase-negative, non-motile, strictly aerobic and appear as gram-negative shortbacilli in pairs under the microscope. They can use various carbon sources for growth, and can be cultured on relatively simple media, including nutrient agar or trypticase soya agar. Also, most members of Acinetobacter show good growth on MacConkey agar with the exception of some A. lwoffii strains [10].

TiO_2 particles catalyze the killing of bacteria [11] and cancer cells [12] by near-UV light, probably due to the generation of free radicals by photoexcited TiO_2 particles. Reports have appeared concerning the bactericidal effects of TiO_2 powder, often referring to OH^- as the toxic agent [13]. In 1985, Matsunaga and coworkers reported that microbial cells in water could be killed by contact with a

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TiO_2–Pt catalyst upon illumination with near-UV light for 60–120 min [14]. Since then, research work on TiO_2 photocatalytic killing has been intensively conducted on a wide spectrum of organisms including viruses, bacteria, fungi, algae, and cancer cells.

In this study, we selected activated sludge dominant bacteria, Acinetobacter strain DF4/PUTK2 in order to investigate the bactericidal effect with various near-UV illumination time and TiO_2 concentrations.

2. Materials and methods

2.1. Titanium dioxide

Anatase TiO_2 colloids were prepared from sol-gel hydrolysis, autoclaving, and condensation of titanium isopropoxide in acetic acid solution described as follows; 160 ml of H_2O and 50 ml acetic acid were poured into flask in an ice bath and stirred. Six ml of isopropanol was added to a dropping funnel followed by 24 ml titanium isopropoxide. The titanium isopropoxide / isopropanol solution was dripped into the acetic acid solution with rate of 2 drops per second. After refluxing at 80 °C for overnight, the colloidal solution was loaded into autoclave equipped with a Teflon beaker at 100 °C for 12 h, and then condensed using a Buchi rotary evaporator.

X-ray diffraction patterns were obtained from Shimadzu 7000 X-ray diffractometer using Cu- $K\alpha$ radiation at 40 kV, 30 mA and scanning speed 0.02 degree per second. The SEM was carried out using JEOL (JSM 6700) instrument embedded with Energy Dispersive X-ray analysis (EDAX).

2.2. Microorganism and culture conditions

The test microorganism used is Acinetobacter strain DF4/PUTK2 that had been genetically modified by conjugation to contain the plasmid PUTK2 [15], with the Tn4431 lux transposon downstream of a putative plasmid maintenance promoter to produce continuous visible light so-called bioluminescence. The culture medium for stock cultures of Acinetobacter strain DF4/PUTK2 was LB medium (Merck, Germany). The working culture was prepared by transferring 2 ml bacterium suspension from stock culture to 50 ml conical flasks containing 20 ml of medium. Culture flasks were aerobically incubated on a rotary shaker at 30 °C for overnight.

2.3. Cell preparation, bioluminescent and growth assays

Acinetobacter strain DF4/PUTK2 cells grown from working culture were harvested by centrifugation at 4000 rpm for 15 min then washed twice using a sterile PHOSPHATE-BUFFER Saline (PBS, pH 7.2) and resuspended with PBS. Subsequently, two milliliters of the culture were then added to 20 ml scintillation vials containing 2.0 ml Minimal Salts Medium (MSM) supplemented with titanium dioxide at final concentrations ranging from 0 to 10 mg/ml.

Aliquots of 200 μl were removed from each scintillation vial and transferred to low-fluorescence black 96-well Microfluor microtiter plates (Dynex Technologies, Chantilly, VA) to produce 8 replications of each dilution. An additional 8 replications consisting of the bacterial strain without titanium dioxide were runnied.

At various incubation periods ranging from 10 - 80 min, the UV exposure experiment was carried out by exposuring the microtiter plates containing Acinetobacter cells prepared as described above to UV lamp with peak wavelength of 360 nm. Plates were then taken in intervals up to 80 min. Subsequently, the exposed plates were placed in a Luminometer (Lumistar Galaxy, BMG, Germany) for luminescence detection at room temperature. At the same time, the growth inhibition experiment was performed. Ready prepared microtiter plates containing 100 micro liter of LB medium were inculcated with 10 micro liter of the bacterial suspension from previously UV exposure plates for each exposure interval time. Subsequently, plates were covered with transparent plate sealer and incubated for overnight at 30°C. In the next day, optical density were measured in an ELISA microtiter plates reader at 600 nm.

3. Results and discussions

Fig. 1 shows the XRD patterns of the as prepared and annealed TiO_2 powder at 500 °C for 1 hr. The as prepared powder (ig. 1-a shows the Anatase phase (JCPDS card no. 21-1272) with a broad and small intensity of (101) peak. The annealed powder fig. 1-b shows a strong orientation of (101) peak and the other peaks are also increased in intensity. The crystallite size are calculated from the X-ray diffraction data, FWHM (Full Width Half Maximum) using the Scherer equation [16]:

$$L = 0.9\ \lambda_{K\alpha 1} / (B_{2\theta} \cos \theta_B)$$

Where L is the average particle size, $\lambda_{K\alpha 1}$ is the X-ray wavelength (1.54056 Å for Cu $K_{\alpha 1}$ radiation), $B_{2\theta}$ is the peak broadening and θ_B is the angle corresponding to the maximum peak. The calculated particle size of (101) peak for the as prepared powder is 9 nm increased to 18 nm after the heat treatment at 500 °C for 1 hr.

The morphology of the annealed powder is investigated by scanning electron microscopy as shown in fig. 2. The film exhibits a regular grain distribution of about 20 nm size. The large grains are composed of agglomerated small grains. This could be investigated at higher magnification.

The effect of titanium dioxide, concentration on the bioluminescence of the Acinetobacter strain DF4/PUTK2 is shown in fig 3. No bioluminescence inhibition was observed with TiO_2 concentration of 4 mg/ml after UV exposure period of 20 min. However, the most significant decrease in the bioluminescence, was observed at concentrations above 4 mg/ml for UV exposure time greater than 30 min. It was also noted that the bioluminescence inhibition value was increased by increasing the concentration of TiO_2 .

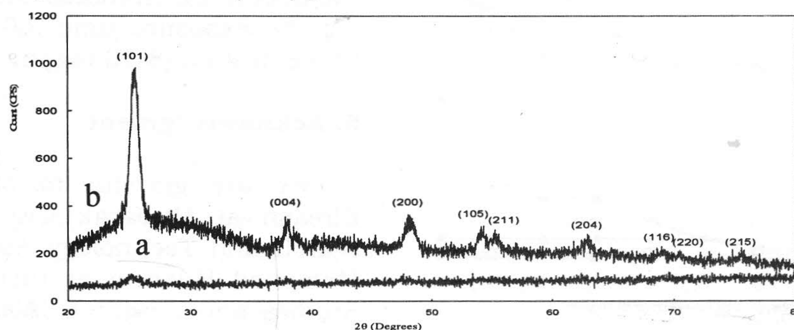

Fig. 1. XRD Patterns of as-prepared TiO_2 (a) and annealed at 500 °C for 1 hour (b).

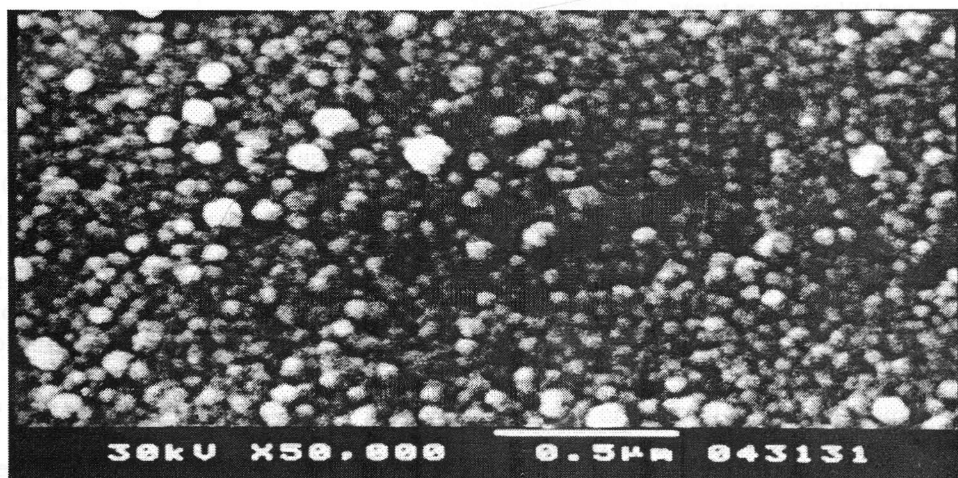

Fig. 2. SEM annealed TiO_2 powder at 500 °C for 1 hour.

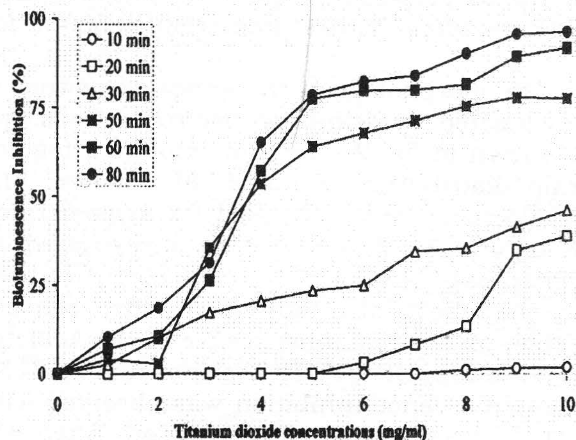

Fig. 3. The bioluminescence inhibition percentage of strain DF4/PUTK2 in response to different TiO_2 concentrations and UV exposure times.

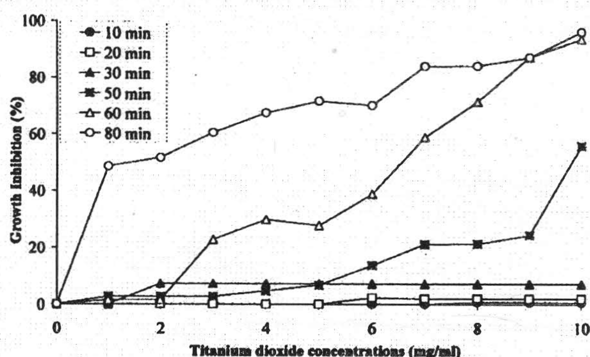

Fig. 4. The growth inhibition percentage of strain DF4/PUTK2 in response to different TiO_2 concentrations and UV exposure times.

The growth inhibition of Acinetobacter strain DF4/PUTK2 shown in fig. 4 indicated that the bactericidal effect of TiO_2 at all tested concentrations (1 to 10 mg/ml) and UV exposure times (10 to 80 min) was less significant than that recorded with the bioluminescence assay. This may be due to the timing of measurement, since the bioluminescence assay was measured directly after exposing the cells to the UV light. While, growth inhibition experiments were conducted after at least 18 h of incubation which may allow the growth inhibited cells to re-growth again. This agreed with the fact that, by prolonging the UV exposure time to 80 min, a significant increase in the growth inhibition was observed at the lowest TiO_2 concentration (1 mg/ml) exhibiting inhibition value of 48.615%. However, at low exposure time, the significant inhibition of growth 58.89% was noted only at concentration 10 mg/ml after exposure time for 50 min.

4. Conclusions

TiO_2 nanoparticles were prepared using sol-gel method. The as prepared powder exhibited very small particles with 9 nm size. In conclusion, it was obviously seen the higher photocatalytic bactericidal effect of UV exposure time with different TiO_2 concentrations on the *Acinetobacter* strain in compare that without using TiO_2. The bactericidal effect was rapidly increased at TiO_2 concentration higher than 4.0 mg/ml. UV illumination time affected significantly the viability of all bacterial cells with different bacterial cells death rate according to the exposure time. The efficiency of the disinfection process increased by increasing the exposure time (60-80 min) and TiO_2 concentration (8-10 mg/ml).

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References

[1] D.F. Ollis and H. El-Akabi, Editors, "Photocatalytic Purification and Treatment of Water and Air", Elsevier Science, Amsterdam, p. 405 (1993).

[2] D.M. Blake, "Bibliography of Work on the Photocatalytic Removal of Hazardous Compound from Water and Air", NREL/TP-430-6084, National Renewable Energy Laboratory, Golden, Co., p. 2 (1994).

[3] D.M. Blake, "Bibliography of Work on the Photocatalytic Removal of Hazardous Compounds from Water and Air", NREL/TP-473-20300, National

Renewable Energy Laboratory, Golden, Co., p. 4 (1995).
[4] D.M. Blake, "Bibliography of Work on the Photocatalytic Removal of Hazardous Compounds from Water and Air", NREL/TP-473-22197, National Renewable Energy Laboratory, Golden, Co., p. 35 (1997).
[5] M.M. Halmann, "Photodegradation of Water Pollutants", CRC Press, Boca Raton, p. 18 (1996).
[6] Fujishima and K. Honda, "Electrochemical Photocatalysis of Water at Semiconductor Electrode", Nature Vol. 238, p. 26 (1972).
[7] I. Sopyan, S. Murasawa, K. Hashimoto and A. Fujishima, "Highly Efficient TiO_2 Film Photocatalyst: Degradation of Gaseous Acetaldehyde", Chem. Lett. p. 723 (1994).
[8] M.L. Sauer and D.F. Ollis, "Photocatalyzed Oxidation of Ethanol and Acetaldehyde in Humidified Air" J. Catal. Vol. 158, p. 570 (1996).
[9] A.V. Vorontsov, E.N. Savinov, G.B. Barannik, V.N. Troitsky and V.N. Parmon, "Quantitative Studies on the Heterogeneous Gas-Phase Photooxidation of CO and Simple VOCs by Air Over TiO_2", Catal. Today, Vol. 39, p. 207 (1997).
[10] E. Bergogne-Bérézin and K. Towner, "Acinetobacter Spp. as Nosocomial Pathogens: Microbiological, Clinical, and Epidemiological Features", Clin. Microbiol. Rev. Vol. 9 p. 148 (1996).
[11] H.N. Pham, T. McDowell and E. Wikins, "Photocatalytically-mediated Disinfection of Water Using TiO_2 as a Catalyst and Spore-Forming Bacillus Pumilus as a Model", J. Environ. Sci. Health A, Vol. 30 (3), p. 627 (1995).
[12] R. Cai, Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto and A. Fujishima, "Induction of Cytotoxicity by Photo Excited TiO_2 Particles", Cancer Res. Vol. 52, p. 2346 (1992).
[13] J.C. Ireland, P. Klostermann, E.W. Rice, and R.M. Clark, "Inactivation of Escherichia Coli by Titanium Dioxide Photocatalytic Oxidation". Appl. Environ. Microbiol. Vol. 59, p. 1668 (1993).
[14] T. Matsunaga, R. Tomada, T. Nakajima and H. Wake, "Photochemical Sterilization of Microbial Cells by Semiconductor Powders" FEMS Microbiol. Lett. Vol. 29, p. 211 (1985).
[15] G. Abo El-Reish, "Genetically Modified Biosensor to Detect Environmental Pollutants", M. Sc. Thesis, Faculty of Science, Al-Azhar University, p. 35, (2005).
[16] C. Hammond, "The Basic of Crystallography and Diffraction", International Union of Crystallography, Oxford Science Publications, p. 147 (1997).

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