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Inactivation of *Escherichia coli* Under Fluorescent Lamp using TiO_2 Nanoparticles Synthesized *Via* Sol Gel Method

(Penyahaktifan *Escherichia coli* di bawah Lampu Pendarfluor Menggunakan Nanozarah TiO_2 yang Disintesis Melalui Kaedah Sol Gel)

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ABSTRACT

Titanium dioxide nanoparticles were synthesized by using sol gel method and their physico-chemical properties were characterized by transmission electron microscopy (TEM), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and UV-Vis spectrophotometer. The photocatalytic property of TiO_2 nanoparticles was investigated by inactivation of Escherichia coli *under irradiation of fluorescent lamp. The results showed that the size of TiO_2 was in the range of 3 to 7 nm with high crystallinity of anatase phase. The sharp peaks in FTIR spectrum determined the purity of TiO_2 nanoparticles and absorbance peak of UV-Vis spectrum showed the energy band gap of 3.2 eV. Optimum inactivation of* E. coli *was obtained at 1.0 g/L TiO_2 nanoparticles, with 80% of* E. coli *population was inactivated. The light scattering effect and insufficient concentration are the factors that cause the less effective inactivation reaction for 2.5 g/L and 0.1 g/L TiO_2 concentration.*

Keywords: E. coli*; photocatalyst; sol gel; TiO_2 nanoparticles*

ABSTRAK

Nanozarah titanium dioksida telah disintesis dengan menggunakan kaedah sol gel dan sifat fizik-kimia telah dicirikan dengan menggunakan mikroskop transmisi elektron (TEM), pembelauan sinar-X (XRD), spektroskopi inframerah transformasi Fourier (FTIR) dan UV-Vis spektrofotometer. Sifat fotomangkin nanozarah TiO_2 telah dikaji terhadap penyahaktifan Escherichia coli *di bawah sinaran lampu pendarflour. Hasil kajian menunjukan saiz nanozarah TiO_2 adalah dalam julat 3 ke 7 nm dengan habluran fasa anatase yang tinggi. Puncak tajam pada spectrum FTIR menunjukan ketulenan nanozarah TiO_2 dan serapan UV-Vis menunjukan jurang petala tenaga adalah 3.2 eV. Penyahaktifan* E. coli *yang optimum diperolehi pada 1.0 g/L kepekatan TiO_2 dengan 80% populasi* E. coli *dinyahaktifkan. Kesan serakan cahaya dan kepekatan yang tidak mencukupi adalah faktor kepada kurang efektif tindak balas penyahaktifan pada 0.1 g/L dan 2.5 g/L kepekatan TiO_2.*

Kata kunci: E. coli*; fotomangkin; nanozarah TiO_2; sol gel*

INTRODUCTION

Titanium dioxide (TiO_2) nanoparticles have been of interest in a wide range of applications such as photocatalyst (Carp et al. 2004), dye-sensitized solar cells (Lee et al. 2008), gas sensor (Mohammadia et al. 2007) and nanomedicine (Wang et al. 2008). TiO_2 nanoparticles have been synthesized using various methods such as hydrothermal (Jitputti et al. 2009), sonochemical (Mizukoshi et al. 2007), solvothermal (Zhanga et al. 2009), reverse micelles (Anwar et al. 2010), and sol gel reaction (Nga et al. 2008) for those applications. Recently, sol gel process has been used for the preparation of TiO_2 nanopowder. Experimental results have shown that this method had successfully produced uniform size, unagglomerated state, high purity and homogeneous nanoparticles (Liu et al. 2000; Kao et al. 2007). Sol gel method had also been used to synthesize nanoparticles at low preparation temperature (Li et al. 2005; Han et al. 2005). Mahshid et al. (2007) have shown that by using sol gel method, controlled size and narrow size distribution of prepared powder can be obtained under optimized preparation condition.

It is also well known that TiO_2 is one of the most superior semiconductor materials for decomposing organic materials due to its strong photocatalytic property. TiO_2 semiconductor becomes a photocatalyst when exposed to ultraviolet or near-visible light ($E \geq E$ band gap) with wavelengths shorter than 390 nm. If this light is adsorbed by the semiconductor surface, it will have enough energy to overcome the energy barrier and excite an electron to transfer the electron from the filled valence band (VB) to the empty conduction band (CB), leaving an electron deficiency (hole) in the valence band. Once the charge carriers are generated across the band gap, they may transfer to the semiconductor surface and be adsorbed by the reactants. The generation of charge carrier (electron-hole pairs) leads to the formation of highly oxidizing hydroxyl and superoxide radicals. These two species are capable of oxidizing practically all organic matter including cell

component of microorganisms to innocuous products, mainly water and carbon dioxide (Wolfrum et al. 2002). This process is known as photocatalysis reaction.

The photocatalytic biocidal effect of TiO_2 was first reported by Matsunaga et al. (1985). They observed that when TiO_2–Pt catalyst is in contact with the microbial cells and exposed to near-ultraviolet light, the microbial cells in water could be killed. Since then, numerous studies related to the bactericidal effect of TiO_2 photocatalyst have been reported including the successful killing of cancer cells, bacteria, viruses, fungi and algae under UV illumination (Ji et al. 2008; Kuhn et al. 2003; Thevenot et al. 2008; Zan et al. 2007; Seven et al. 2004) observed that the killing rate of bacteria were dependent on the thickness and structure of the cell walls. According to Sunada et al. (1998), not only bacteria are killed on the TiO_2 surface by photocatalytic action, but the toxic ingredient of bacteria can also be decomposed. If the UV illumination continued for a sufficient time, the bacteria were found to mineralize completely into CO_2, H_2O and other mineral substances (Jacoby et al. 1998). This complete oxidation process is referred as mineralization.

Study by Kikuchi et al. (1997) showed that *E. coli* cells were killed completely after 1 h of UV illumination on TiO_2 thin films. By using commercial TiO_2 nanopowder, Degussa P25, and filtered UV light, only 0.25 g/L is needed to inactivate *E. coli* cells (Benabbou et al. 2007). In the present work, we have synthesized TiO_2 nanopowder by using the sol gel method (Mahshid et al. 2007). The aim of this study was to determine the survival of *E. coli* in TiO_2 nanoparticles suspension in the presence of fluorescent light. *E. coli* was chosen as the microorganism because it is an accepted indicator microorganism for the existence of faecal contamination in water (Grieken et al. 2009).

Materials and Methods

Preparation of TiO_2 nanoparticles

In this work, TiO_2 nanoparticles were prepared by the sol gel method according to the method of Mahshid et al. (2007). About 5 mL titanium isopropoxide (TTIP, 97%, Aldrich Chemical) and 15 mL isopropyl alcohol (99%, System) were added into 250 mL distilled water. The pH was adjusted by adding nitric acid in the range for 2.0 to 2.5. The resulting suspension was heated and stirred at 60-80°C for 24 h. The product was washed with distilled water and dried in vacuum system at 100°C for 3 h. The white TiO_2 powder was later obtained. The TiO_2 powder was analyzed by using XRD, TEM, FTIR and UV-Vis spectrophotometer.

Preparation of standard inoculums

E. coli were used for the photocatalytic degradation study. Preparation of standard inoculums was done using method as described by Ainon et al. (2010).

Photocatalytic study

In photocatalytic experiments, 0.1, 1.0 and 2.5 g/L of aqueous TiO_2 suspension in normal saline was prepared prior to photocatalytic reaction and kept in the dark. About 10% of fresh standard inoculums of *E. coli* ($\approx 10^8$ cfu/mL) were inoculated in 80 mL sterilized normal saline and 10 mL TiO_2 suspension in a 200 mL glass beaker. The slurry of TiO_2-cells was mixed with magnetic stirrer and illuminated with fluorescent light (240 V, 50 Hz) at 20 cm from the surface of the medium (Figure 1). An *E. coli* suspension without TiO_2 was also illuminated and used as a control. *E. coli* suspension was removed at 1 h interval for 6 hours. Viable concentration of *E. coli* was enumerated with spreading plate method on nutrient agar (NA) after a serial of dilutions of the sample in normal saline. All the plates were incubated at 37°C for 18-24 h and the colonies were counted using colony counter. The survival percentage of bacteria was determined as follows:

$$Survival\ percentage = \frac{x - y}{x} \times 100, \quad (1)$$

where x and y are the number of colony (cfu/mL) before and after exposure, respectively.

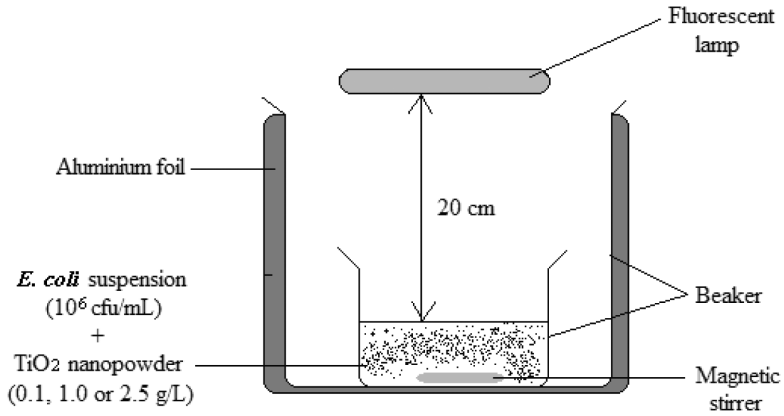

FIGURE 1. Experimental setup for photocatalysis reaction under fluorescent lamp

RESULTS AND DISCUSSION

The morphology and size distribution of TiO_2 nanoparticles indicated the non-uniform structure of tetragonal anatase phase in the range from 3 to 7 nm (Figure 2 (a) and (b)). The XRD patterns showed this sample have four sharp peaks 2θ angle at 25.24°, 37.76°, 48.04° and 54.36° with (101), (004), (200) and (105) diffraction planes, respectively (Figure 3) (Trung & Ha 2004). This pattern also demonstrated anatase phase of TiO_2 nanoparticles with superior catalytic effects for photocatalytic activity (Seo et al. 2007). The mean size of the ordered anatase TiO_2 nanoparticles have been estimated from full width at half maximum (FWHM) and Debye-Sherrer formula as follows:

$$D = \frac{0.89\lambda}{B\cos\theta}, \quad (2)$$

where, 0.89 is the shape factor, λ is the x-ray wavelength, B is the line broadening at half the maximum intensity (FWHM) in radians, and θ is the Bragg angle. The mean size of TiO_2 nanoparticles was 2.03 ± 0.05 nm from this Debye-Sherrer equation.

The infrared spectrum of the synthesized TiO_2 nanoparticles was in the range of 400-4000 cm^{-1} wavenumber which identify the chemical bonds as well as functional groups in the compound (Figure 4). The large broad band at 3400 cm^{-1} is due to the OH stretching. These bands correspond to O-H vibration of the Ti-OH group and H_2O molecules (Velasco et al. 1999). The sharp bands at 1644 cm^{-1} and 1386 cm^{-1} can be assigned to the bending mode of nitrate group as a result of nitric acid addition. The low energy region (below 1000 cm^{-1}) at 715 cm^{-1} and 432 cm^{-1} indicated the stretching mode of Ti-O and Ti-O-Ti bond of a TiO_2 network (Kanna & Wongnawa 2008). These two bonds are important functional group that is related to the photocatalytic activity.

The quantum confinement of TiO_2 nanoparticles was determined by using UV-Vis spectrum (Calandra et al. 1999) and analysis in the optical absorption spectrum can defined the energy band gap of the TiO_2 nanoparticles by using the equation below:

$$\alpha h\nu = K(h\nu - E_g)^{1/2}, \quad (2)$$

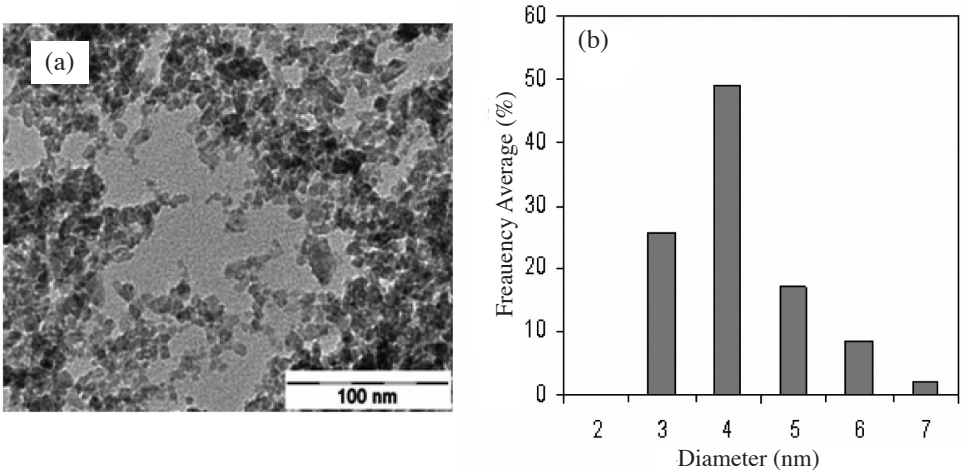

FIGURE 2. (a) Transmission electron micrograph (TEM) image of TiO_2 nanoparticles powder heated at 100°C and (b) distribution size of TiO_2 nanoparticles

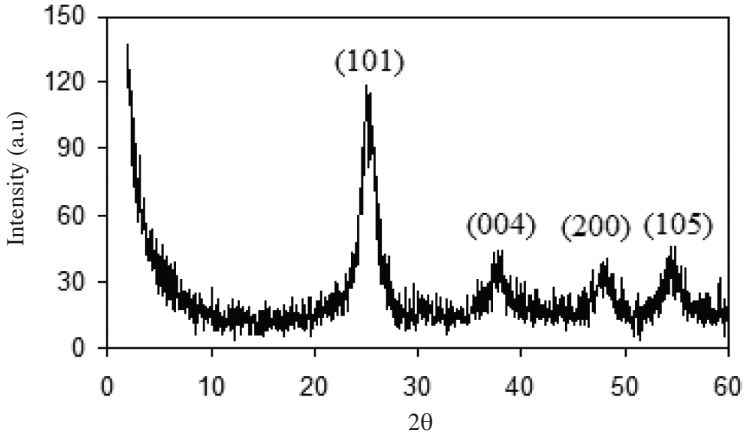

FIGURE 3. XRD pattern of TiO_2 anatase phase which have intensity at peak (101), (004), (200) and (105)

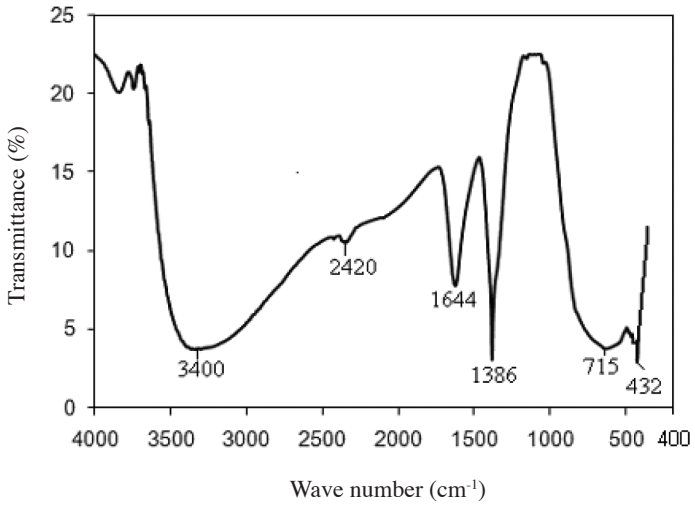

FIGURE 4. FTIR spectrum of TiO_2 sample

where, α is absorption coefficient, $h\nu$ is photon energy, K is constant and E_g refer to band gap energy of the nanoparticles (Sartale 2001). The energy band gap of TiO_2 nanoparticles is defined by extrapolating $(\alpha h\nu)^2$ vs $h\nu$ at zero absorption coefficient which is 3.2 eV as well as its band gap energy as shown in Figure 5.

The survival percentage of *E. coli* was decreasing with illumination time for three different TiO_2 concentrations (Figure 6). It was shown that the optimum inactivation of *E. coli* ($\approx 10^8$ cfu/mL) was achieved in the presence of 1.0 g/L TiO_2 where 80% of *E. coli* was inactivated. The 0.1 g/L of TiO_2 concentration is insufficient for inactivation reaction of *E. coli*. Meanwhile, at 2.5 g/L of TiO_2 concentration, it becomes saturated in the suspension, thus give scattering effect in the medium. Coleman et al. (2005) suggested that at high concentration of TiO_2, shadowing effect is dominant, thus reducing the extent of fluorescence light can reach all the particles in suspension.

It is possible to increase the percentage of bacterial inactivation by increasing the crystallinity of TiO_2 nanoparticles via calcinations (Benabbou et al. 2007) which we have not done here.

Sunada et al. (2003) found that the mechanism of photocatalysis on *E. coli* illumination with TiO_2 could be divided into three stages: (1) the outer membrane of *E. coli* was attacked and partially decomposed by reactive species such as $\cdot OH$, $O_2^{\cdot -}$ and H_2O_2; (2) disordering of the inner membrane leading to the peroxidation of the lipid membrane thus killing the cell and; (3) decomposition of the dead cell. According to Fujishima et al. (2000), if the fluorescent illumination continues for a sufficient time, *E. coli* will be completely mineralized into CO_2, H_2O and other mineral compounds.

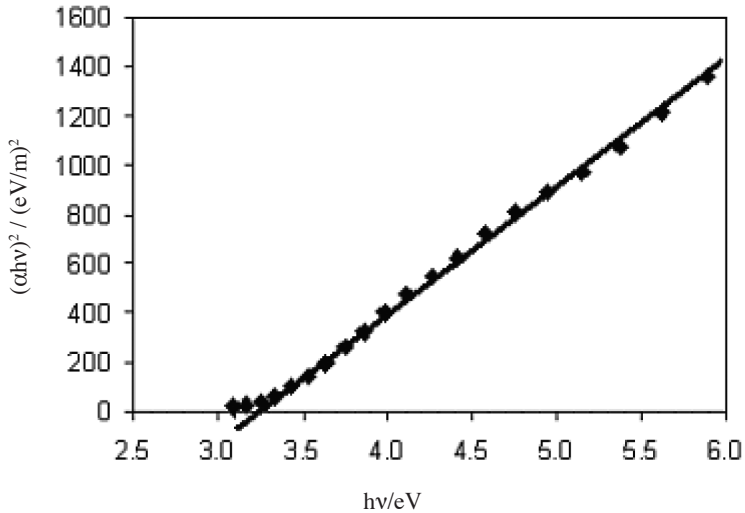

FIGURE 5. Extrapolation of graph $(\alpha h\nu)^2$ vs $h\nu$ for TiO_2 nanoparticles with 3.2 eV band gap energy

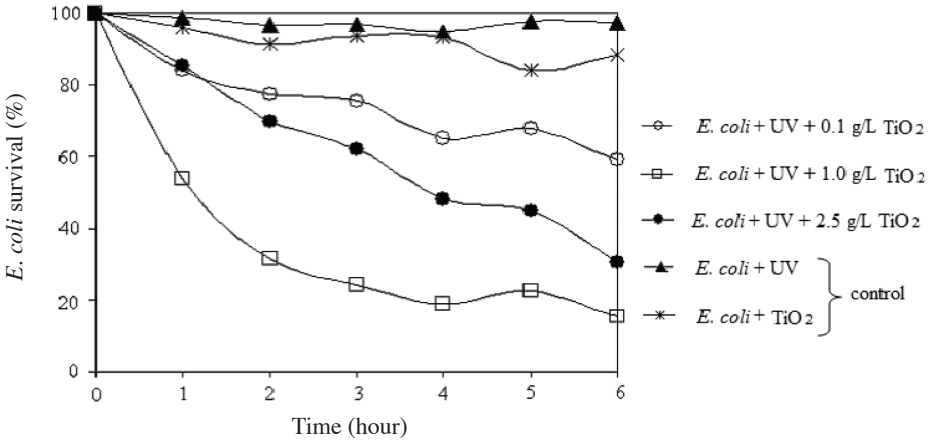

FIGURE 6. The effect of TiO_2 nanoparticles towards survival of *E. coli* during photocatalytic process

CONCLUSION

The TiO_2 which was synthesized by sol gel process is a viable material for inactivation of *E. coli* by photocatalysis. The TiO_2 was tetragonal anatase phase with 4-7 nm in size. The anatase phase was confirmed by X-ray diffraction pattern with sharp peak at $2\theta=25.5^o$ and 3.2 eV energy band gap, E_g. The IR spectrum at 432 cm^{-1} indicated the Ti-O and Ti-O-Ti bonds due to stretching mode of a TiO_2 network which was important for photocatalytic process. TiO_2 at 0.1 g/L is not enough and at 2.5 g/L is too high to give scattering effect and both concentrations are not effective to inactivate *E. coli*. However, TiO_2 at 1.0 g/L to sufficient to inactivated 10^8 cfu/mL of *E. coli*.

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