

Synthesis of TiO₂ Nanosized Powder Photocatalyst Via Sol-gel Method for Bacteria Inactivation Application

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Titanium dioxide (TiO₂) is the most widely used photocatalyst and has been applied for water and air purification and disinfection of microorganism. Anatase structured TiO₂ is a wide bandgap (3.2 eV) semiconductor which, under UV light, is able to degrade chemicals and cell components of microorganisms. In this study, TiO₂ nanopowder photocatalyst was synthesized by sol-gel process using a titanium chelate compound, titanium (di-isopropoxide) bis(acetylacetonate) (PTP) as the precursor. The precursor was hydrolyzed under an acidic catalyst condition, followed by reflux for 15 minutes and 1 hour. The precipitate powder obtained after filtration was calcined at 400°C. XRD analysis confirmed that the TiO₂ powders A (15 minutes reflux) and B (1 hour reflux) have strongest peak at 25° which indicates anatase crystal structure. The particle size of powder A was smaller than the powder B as proved by calculation using Scherrer's equation. The crystallization temperature of TiO₂ powder A was higher due to its smaller particles that needed more heat to crystallize than powder B as confirmed by TG/DTA. FESEM analysis showed powder A have homogenous spherical shape, contrast to powder B showing inhomogenous spherical shape and connected particle. The TiO₂ photocatalyst activity in killing bacteria was investigated for 5 hours by varying the concentration of TiO₂ and UV light intensity.

Keywords: TiO₂ photocatalyst, sol-gel, nanopowder, bacteria killing

INTRODUCTION

Semiconductor-mediated photocatalytic oxidation has been acknowledged as a promising technique for the removal of pollutants [1]. Titanium dioxide (TiO₂), due to chemical stability, high photosensitivity, non-toxicity, strong oxidizing power, and low cost has been extensively utilized as a photocatalytic material. TiO₂ have three crystal structures which are anatase, rutile and brookite. Rutile is used in light scattering application but anatase used as photocatalysis and photon-electron transfer [2]. It has long been examined in environmental purification as one of the

most popular photocatalysts. Current studies demonstrate that photocatalytic activity of TiO₂ depends on its grain size. The decrease of grain size involved the enhance of surface area and high redox potential, which leads to high photocatalytic activity [3].

Existing bulk semiconducting materials have low surface area, less adsorption property and fast electron-hole recombination. In order to avoid such problems researchers are interested in the synthesis of nanomaterials for environmental applications. Nanocrystalline materials reveal unique properties, such as high surface area, short interface migration distance,

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quantum size effect, and visible light active, all of which attain enhanced photocatalytic performance. Particle size is another significant parameter for photocatalysis as it directly impacts the specific surface area of a catalyst. With a small particle size, the number of active surface sites increases and so does the surface charge carrier transfer rate in photocatalysis [1]. Consequently, much more awareness have been focused on the research of nanocrystalline TiO_2 , the exclusive properties of nanoparticles and their relations to photocatalytic properties [3].

There are several techniques to synthesize TiO_2 including precipitation methods, solvothermal methods, combustion synthesis, sol-gel methods, microwave methods, and sol-hydrothermal methods [2]. Recently, sol-gel process became a versatile technique for the preparation of nanocrystalline TiO_2 . Sol-gel process is a wet-chemical technique for the fabrication of materials (typically a metal oxide) which involves the transition of system from liquid "sol" (mostly colloidal precursor) into a solid "gel" phase. For synthesis of TiO_2 powder, this process involves the formation of a TiO_2 sol or gel or precipitation by hydrolysis and condensation of titanium alkoxides [4]. Acid-base catalysis can also be utilized to allow separation of hydrolysis and condensation steps. It has been revealed that acid catalysis enhances hydrolysis rates and eventually crystalline powders are formed from fully hydrolyzed precursors [4].

It has been demonstrated that through sol-gel process, the physico-chemical and electrochemical properties of TiO_2 can be modified to improve its efficiency. Sol-gel method also provides a simple and easy ways of synthesizing nanoparticles at ambient temperature under atmospheric pressure and this technique does not involve a complicated set-up. As this technique is a solution process, it has all the advantages over other preparation techniques in terms of purity, felicity and flexibility in introducing dopants in large concentrations, stoichiometry control, ease of processing and composition control.

In this present research, TiO_2 photocatalyst powders are synthesized via sol-gel method by using titanium (di-isopropoxide)bis(acetyl-acetonate) (PTP) as the precursor and hydrochloric acid as catalyst. The synthesized TiO_2 powders was characterized physically and chemically to determine crystal size, surface area, crystal structure and others properties which affect the photocatalyst activity. The photocatalytic performance of prepared TiO_2 powders was investigated in inactivation of bacteria.

METHOD AND MATERIALS

Synthesis of TiO_2 by Sol-gel Process

Titanium (di-isopropoxide) bis(acetyl-acetonate) (PTP, Strem Chemicals, 75% in isopropanol) was used as precursor to synthesize nanosized TiO_2 powders via sol-gel method. Methanol solution (SYSTEM Malaysia) was used as organic solvent for the synthesis of TiO_2 nanopowder. Distilled water and 1N of hydrochloric acid (HCl) was used as hydrolyzing agent to form the TiO_2 nanosized powder. Acetone and hexane were used to produce the powder via dissolution and filtration process. TiO_2 powders were synthesized by mixing PTP precursor and 30 ml of methanol into the round-bottomed flask at 0°C with stirring condition. Organic solvent of methanol was used to carry out the hydrolysis process to form a formable solution (sol) and a loosely cross-link matrix (gel). After the temperature reached 0°C , the solution are kept for 30 minutes. Then, a mixture of 1 N of HCl, distilled water and 30 ml of methanol was added dropwise by using pipette with stirring condition to hydrolyze the PTP precursor. The heating process was started and continued until the reflux temperature reached about 65°C (methanol boiling point) and held for 15 minutes and 1 hour. A white sol was obtained after reflux process and then evaporated. Subsequently, 25ml of acetone was added into the solution formed. About 5 to 10 ml of the yellow translucent solution was added dropwise into 200 ml hexane with vigorous stirring condition.

The light yellow precipitate was obtained and followed by filtration process to get the gel powder. The gel powder was dried overnight in drying cabinet at 80°C and calcined at 400°C for 1 hour of holding time.

Characterization of TiO₂ Powder

The nanosized TiO₂ powders were characterized by x-ray diffraction (XRD) machine from Shimadzu (XRD 6000) to determine crystal structure of the powders. The scanning range started from 20° to 50° in 2θ and this machine used copper Kα radiation to examine the powder samples with the scanning step of 2° in 2θ/min. The powders also characterized by Thermogravimetric and Differential Thermal Analyzer (TG/DTA) (PYRIS DIAMOND, Perkin Elmer) at ambient temperature with heating rate of 2°C/min from 30°C to 1100°C. For surface area of the powders, the Brunauer-Emmet-Teller (BET) surface analyzer was used. Particle size of the powders was investigated by using the Zetasizer (Nano S, Malvern Instrument). The Field Emission Scanning Electron Microscope (FESEM-JSM 6700F, JOEL) was used to determine the surface morphology of the powders.

Bacteria Killing Experiment

Stock culture of *E. coli* carrying Nucleocapsid (NP) of Newcastle Disease Virus (NVD) gene was used for inactivation studies. Degussa P25 (Aeroxide TiO₂ P25 made in Japan) was employed as the reference TiO₂. Whereas, the TiO₂ prepared by sol-gel method was used for comparison with Degussa P25 in photocatalytic activity. The TiO₂ photocatalyst concentration used are ranged between 0.25 to 3.0 mg/ml. The initial concentration of *E. coli* was 10⁹ cell/ml which have been prepared through bacteria culture steps, i) Preparation of starter culture which is called inoculum, ii) Cell cultivation in thermostated rotary shaker at 250 rpm and 37°C for 5 hours. The photocatalytic activity was carried out under two different ultraviolet (UV) intensity, low UV (LUV: 0.3 mW/cm²)

and high UV (HUV: 2.8 mW/cm²). The UV light (UVA) model F20T9/BL of 18 Watt was used as the source of UV light. The light intensity was monitored by a digital UV meter (Solartech Inc, USA) with the peak response of 370 nm. The TiO₂-cell slurry was placed on a magnetic stir plate with continuous stirring and illuminated with 1 bulb (LUV intensity) and 3 bulbs (HUV intensity) at above. All experiments were conducted in continuously stirring aqueous slurry of TiO₂/*E. coli* with a magnetic stirrer (Heidolph, MR Hei-Mix S, Germany) to ensure a complete mixing and to prevent settling of the nanoparticles. *E. coli* has been counted under microscope using the THOMA counting chamber.

RESULT AND DISCUSSION

Fig. 1 shows the XRD pattern for TiO₂ powder A (15 min reflux) and powder B (60 min reflux), both powders have a strong peak at 25° which shows that powders are purely anatase phase. The diffraction peaks of TiO₂ powder A are more broadened compared to powders B, which indicates a decrease in crystal size. The average crystal sizes of the samples was calculated using Scherrer equation. The particle size of TiO₂ Powders A and B calculated by Scherrer's equation is presented in Table 1. From the table, it seems that the crystal size increased with increasing the reflux times. Therefore, the suitable reflux time for small particle size was at 15 minutes. Furthermore, smaller powder particles will present shorter distance to be traveled by the electrons from the valence band to the catalytic site. Therefore, more holes will be left at the valence band by the electrons that travel to the conduction band. The conduction band electrons are the reduction centers that will react with oxygen while the valence band holes become the oxidation centers that will react with water to produce more hydroxyl radicals.

Figs. 2 and *3* show the characterization of thermal properties of the TiO₂ powders by TG-DTA analysis. The amorphous powder was heated at room temperature to 1100°C with the rate of 2°C/min. From the TG curve, it showed

that the first weight loss occurs at temperature ~100 °C which is attributed to the evaporation of water molecules. While, the second weight loss at temperature of ~400 °C is attributed to the removal of inorganic compounds. From DTA curve, exothermic reaction peak appeared at temperature of 371°C for powder A and 366°C for powder B indicating a phase change of powder from amorphous to anatase phase. This is the crystallization temperature. Hence, this result shows that powder A have smaller particle since it need more heat to crystallize compared to powder B.

TABLE 1
Particle size of TiO₂ powders using Scherrer's equation

Samples of TiO ₂	Angle (2θ)	Particle size (nm)
Powder A	25.3235	14.8
Powder B	25.2566	15.0

Fig. 4 illustrates the N₂ adsorption-desorption isothermal analysis curve of TiO₂ powder (a) using BET surface area analyzer. The N₂ adsorption-desorption isotherms of TiO₂ Powder can be attributed to their pore connectivity effects [5]. This BET surface area

analysis shows that the sol-gel TiO₂ powder has Isotherm type II which was evaluated from the adsorption curve shown in the figure. Type II isotherms are normal form of isotherms obtained with a nonporous and macroporous adsorbent. From BET surface area analysis, TiO₂ powder A has a surface area of 41.24 m²/g.

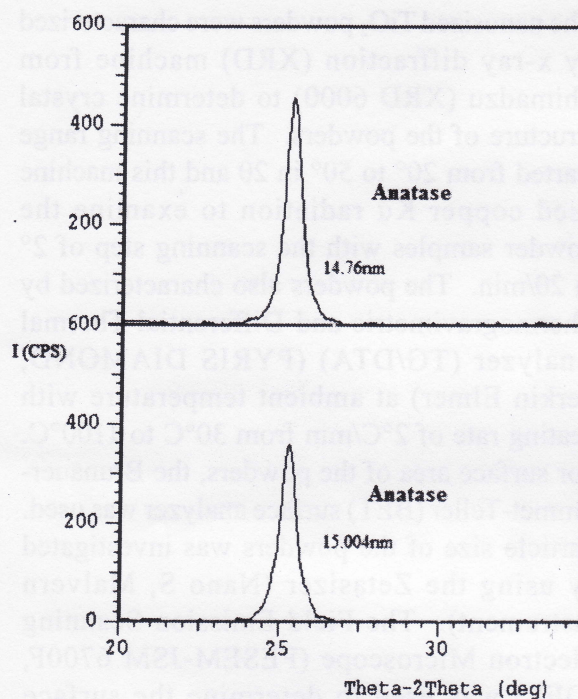


Fig. 1: XRD patterns of TiO₂ Powder A and Powder B

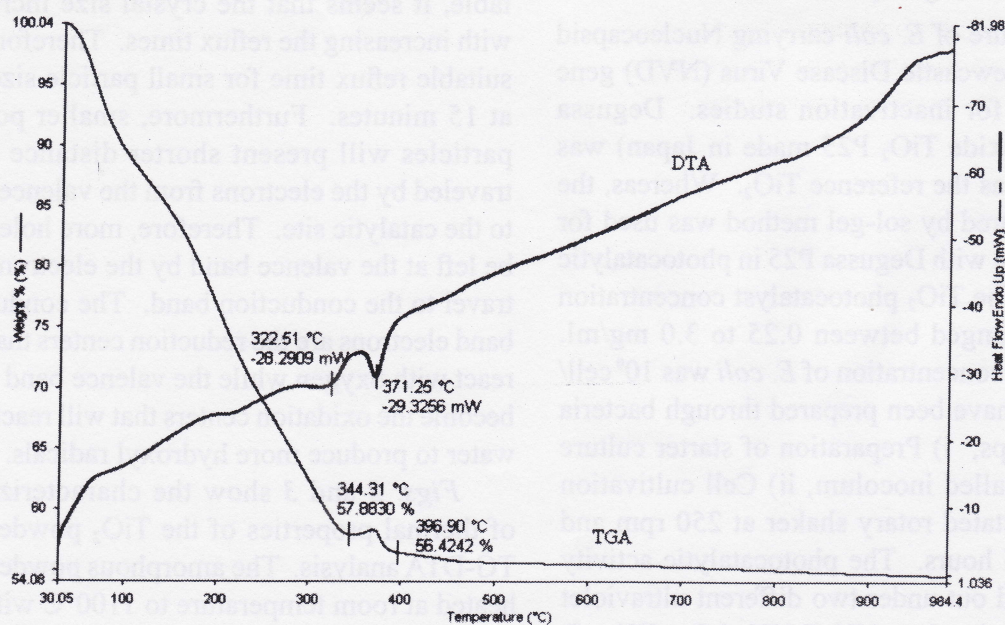


Fig. 2: TG/DTA of TiO₂ Powder A

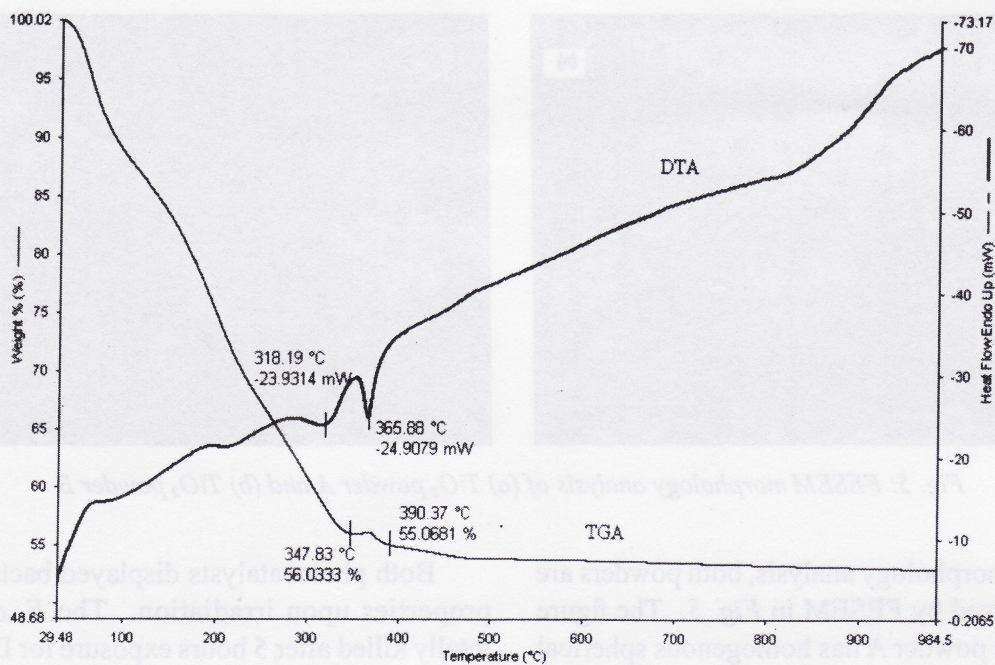


Fig. 3: TG/DTA of TiO₂ Powder B

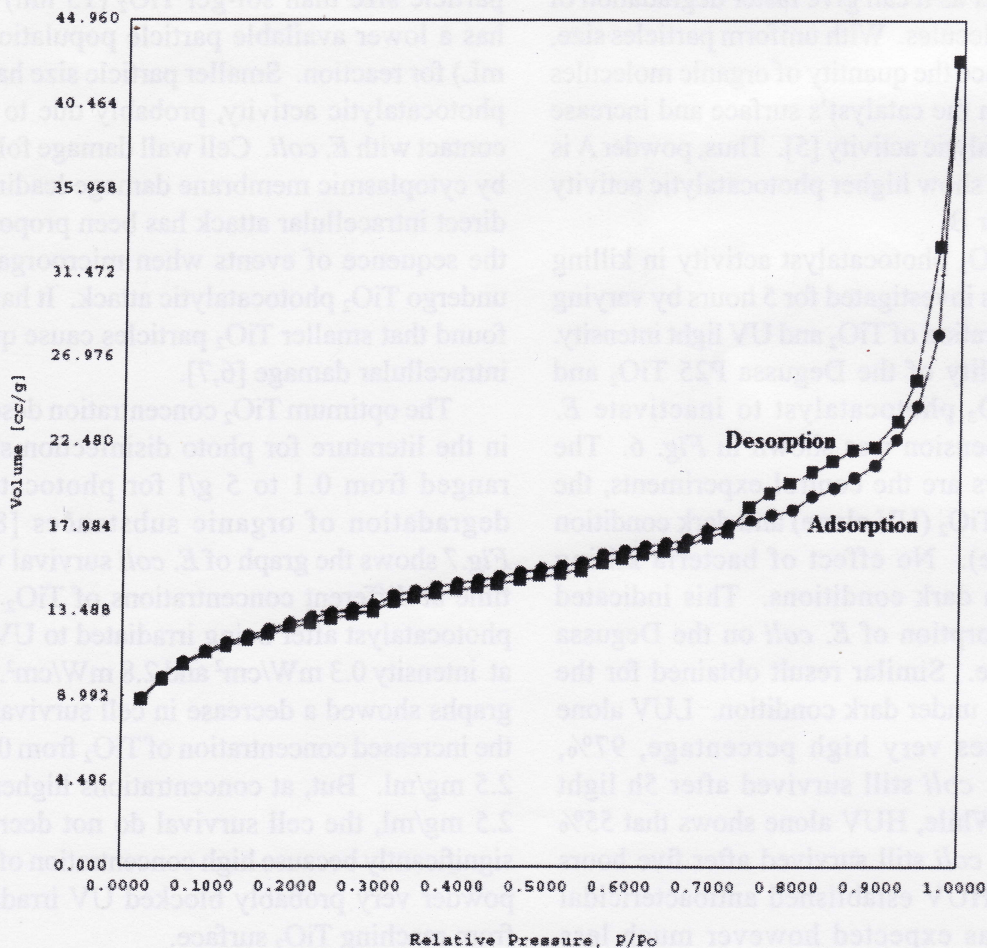


Fig. 4: N₂ adsorption-desorption isothermal analysis of nanosized TiO₂ Powder A

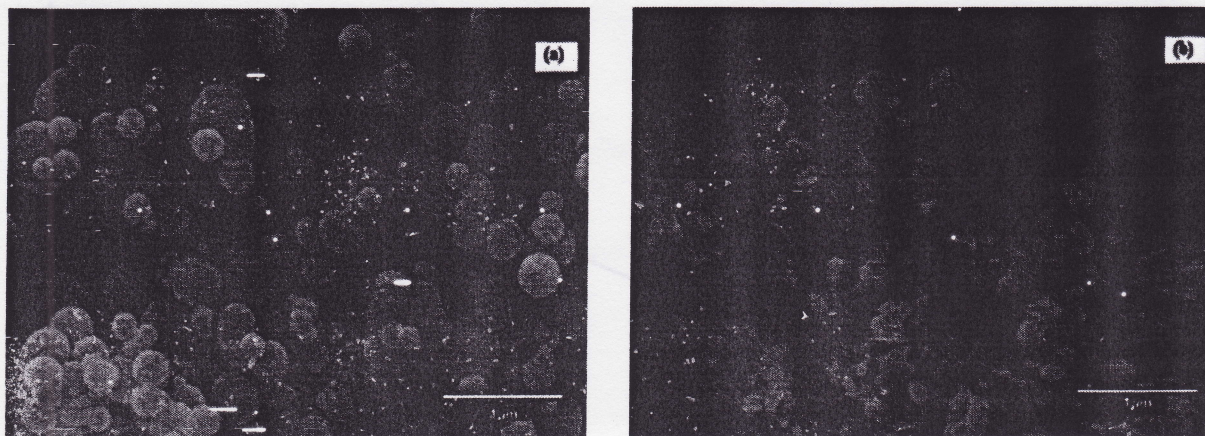


Fig. 5: FESEM morphology analysis of (a) TiO_2 powder A and (b) TiO_2 powder B

For morphology analysis, both powders are characterized by FESEM in Fig. 5. The figure shows the powder A has homogenous spherical shape particles. Powder B shows inhomogenous non-spherical shape and connected particle. It is desirable to have a homogeneous size of TiO_2 powder as it can give faster degradation of organic molecules. With uniform particles size, it can enhance the quantity of organic molecules adsorbed on the catalyst's surface and increase its photocatalytic activity [5]. Thus, powder A is expected to show higher photocatalytic activity than powder B.

The TiO_2 photocatalyst activity in killing bacteria was investigated for 5 hours by varying the concentration of TiO_2 and UV light intensity. The capability of the Degussa P25 TiO_2 and sol-gel TiO_2 photocatalyst to inactivate *E. coli* in suspension was shown in Fig. 6. The figure shows are the control experiments, the absence of TiO_2 (UV alone) and dark condition (TiO_2 alone). No effect of bacteria killing observed in dark conditions. This indicated that no absorption of *E. coli* on the Degussa TiO_2 surface. Similar result obtained for the sol-gel TiO_2 under dark condition. LUV alone demonstrates very high percentage, 97%, of initial *E. coli* still survived after 5h light exposure. While, HUV alone shows that 55% of initial *E. coli* still survived after five hours exposure. HUV established antibactericidal properties as expected however much less efficient than photocatalysis [6].

Both photocatalysts displayed bactericidal properties upon irradiation. The *E. coli* are totally killed after 5 hours exposure for Degussa P25 TiO_2 and the sol-gel TiO_2 photocatalyst. The observed results can be related to the particle size of the catalysts. P25 (20 nm) has a larger particle size than sol-gel TiO_2 (15 nm) hence has a lower available particle population (per mL) for reaction. Smaller particle size has high photocatalytic activity, probably due to better contact with *E. coli*. Cell wall damage followed by cytoplasmic membrane damage leading to a direct intracellular attack has been proposed as the sequence of events when microorganisms undergo TiO_2 photocatalytic attack. It has been found that smaller TiO_2 particles cause quicker intracellular damage [6,7].

The optimum TiO_2 concentration described in the literature for photo disinfection studies ranged from 0.1 to 5 g/l for photocatalytic degradation of organic substances [8-10]. Fig. 7 shows the graph of *E. coli* survival versus time at different concentrations of TiO_2 (P25) photocatalyst after being irradiated to UV light at intensity 0.3 mW/cm² and 2.8 mW/cm². Both graphs showed a decrease in cell survival with the increased concentration of TiO_2 from 0.25 to 2.5 mg/ml. But, at concentrations higher than 2.5 mg/ml, the cell survival do not decreased significantly because high concentration of TiO_2 powder very probably blocked UV irradiation from reaching TiO_2 surface.

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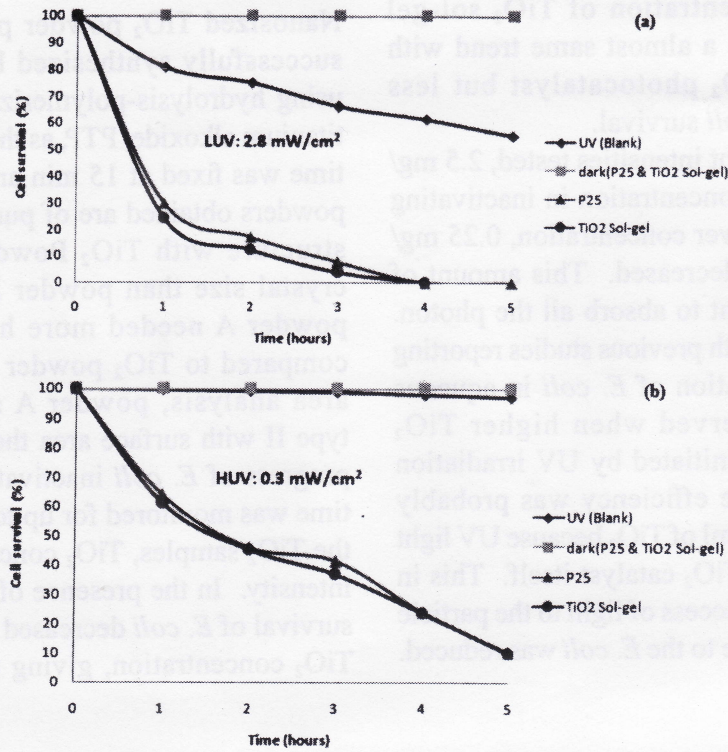


Fig. 6: Cell survival under UV irradiation only without both TiO₂ powders, dark condition with the TiO₂ powders, and under UV irradiation with the TiO₂ powders. UV intensities used are (a) 2.8 and (b) 0.3 mW/cm²; catalyst concentration: 2.5 mg/ml

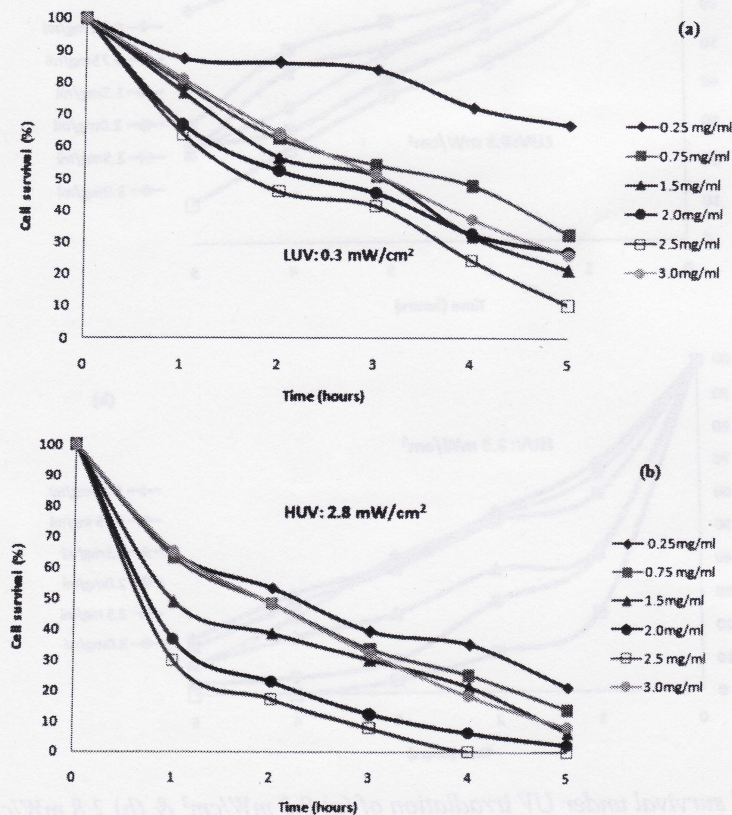


Fig. 7. Cell survival under UV irradiation of (a) 0.3 mW/cm² and (b) 2.8 mW/cm² intensities with various Degussa TiO₂ concentrations

Fig. 8 revealed that the *E. coli* survival at different concentration of TiO₂ sol-gel photocatalyst have a almost same trend with Degussa P25 TiO₂ photocatalyst but less percentage of *E. coli* survival.

For the two light intensities tested, 2.5 mg/ml is the optimal concentration in inactivating bacteria. At the lower concentration, 0.25 mg/ml, the efficiency decreased. This amount of TiO₂ is not sufficient to absorb all the photon. This is consistent with previous studies reporting that higher inactivation of *E. coli* in aqueous medium was observed when higher TiO₂ concentration was initiated by UV irradiation [11]. However, the efficiency was probably limited over 2.5 mg/ml of TiO₂ because UV light can be blocked by TiO₂ catalyst itself. This in turn will reduce the access of light to the particle and access of particle to the *E. coli* was reduced.

CONCLUSION

Nanosized TiO₂ powder photocatalyst were successfully synthesized by sol-gel process using hydrolysis-polymerization of a chelated titanium alkoxide, PTP, as the precursor. Reflux time was fixed at 15 min and 1 hr. Both TiO₂ powders obtained are of purely anatase crystal structure with TiO₂ Powder A has smaller crystal size than powder B. The reason is powder A needed more heat to crystallize compared to TiO₂ powder B. From surface area analysis, powder A showed Isotherm type II with surface area the 44.24 m²/g. The progress of *E. coli* inactivation as function of time was monitored for up to 5 h depending on the TiO₂ samples, TiO₂ concentrations and UV intensity. In the presence of UV light, the cell survival of *E. coli* decreased with the increased TiO₂ concentration, giving the best results at

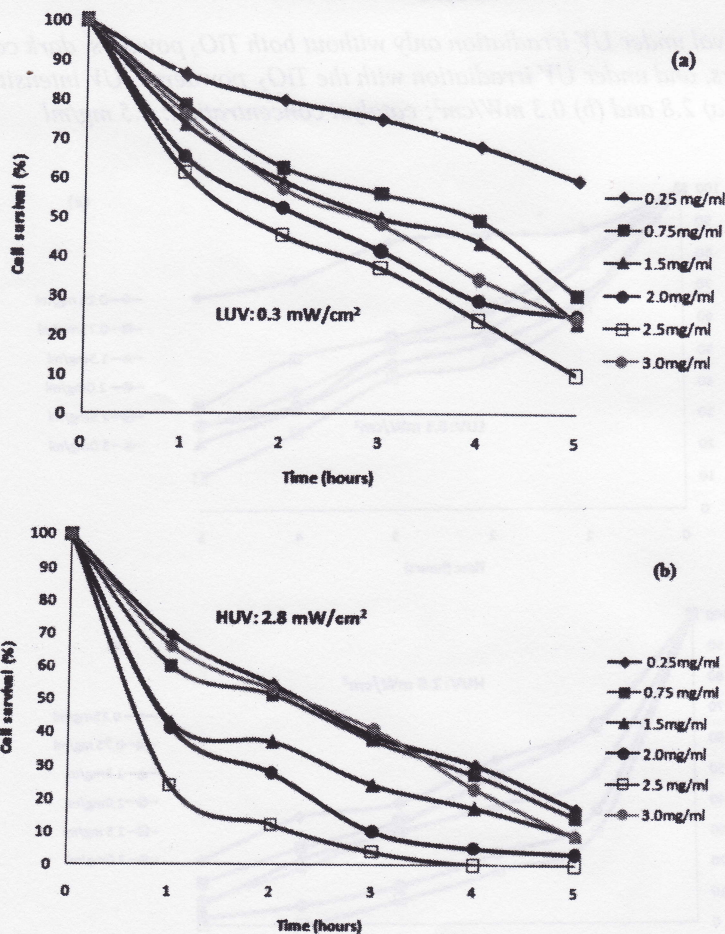


Fig. 8. Cell survival under UV irradiation of (a) 0.3 mW/cm² & (b) 2.8 mW/cm² intensities with various sol gel TiO₂ concentration

2.5 mg/ml, with complete inactivation achieved after 5 hours exposure. TiO₂ concentration higher than 2.5 mg/ml resulted in significantly lower cell survival due to the excessive concentration of TiO₂ that reduced UV light penetration to the bacteria suspension.

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