Growth and Some Enzymatic Responses of *E. coli* to Photocatalytic TiO_2

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ABSTRACT

The effects of photocatalytic nano-TiO_2 on the survival or die-off of *E coli* (e.g. TB1) were investigated under ambient conditions. Experimentally, 18-h *E. coli* culture was exposed to photocatalytic nano-TiO_2 at various concentrations, e.g., 0 to 1,000 mg/L and particle sizes, e.g., 3 to 55 nm both in darkness and the presence of several light sources including a simulated solar light. Preliminary results indicated that there was bacteria die-off in the presence of nanoscale TiO_2 in dark. Generally it appears that the growth rate decreases as the particle size decreases. The presence of light irradiation significantly enhanced the killing of *E. coli* due to additional photocatalytic activity. Upon exposure of *E. coli* to nano-TiO_2 the photocatalytic activity that was generated has markedly increased the production of MDA, TTC and GST. SEM observations vividly indicate cell wall damages.

Keywords: *E.coli*, TiO_2, toxicity, die-off, photocatalysis

1 INTRODUCTION

The photocatalytic destruction of organic compounds in polluted air and water has been extensively studied. Following the work of Matsunaga, et al [1], an interest has grown in using this process for water disinfection [2 - 8]. Different mechanisms involved in the bactericidal action of TiO_2 photocatalysis have been proposed [1, 9 - 12]. The above studies suggest that the cell membrane is the primary target of reactive photogenerated oxygen species attack. Oxidative attack of the cell membrane leads to lipid peroxidation. The combination of cell membrane damage and oxidative attack of internal cellular components, results in cell death.

TiO_2 in the anatase crystal form is the most used semiconductor with a band gap of 3.2 eV or more. Upon excitation by light whose wavelength is less than 385 nm, the photon energy generates an electron hole pair on the TiO_2 surface. The hole in the valence band can react with H_2O or hydroxide ions on the TiO_2 surface to produce hydroxyl radicals (OH·), and the electron in the conduction band can reduce O_2 to produce superoxide ions ($O_2^{\bullet -}$). Both holes and OH· are extremely reactive with organic compounds including microbial tissues. Matsunaga, et al. [1,2] reported that microbial cells in water could be killed by contact with a TiO_2-Pt catalyst upon illumination with near-UV light for 60 to 120 min. The findings of Matsunaga, et al [1,2] have prompted various researchers to use TiO_2 as a sterilization agent to disinfect drinking water. Most notably, Cai,et al. have attempted to kill cancer cells with the TiO_2 photocatalyst [13]. Blake et al. (1999) have reviewed the medical application of photocatalysts [14].

Several researchers have proposed the mechanisms of the killing of microorganisms using photocatalytic TiO_2. Matsunaga, et al. were the first to believe that direct photochemical oxidation of intracellular coenzyme A to its dimeric form was the cause of decreases in respiratory activities that led to cell death [1,2]. Saito, et al. proposed that the photocatalytic TiO_2 was disrupted the cell membrane and the cell wall of *Streptococcus sobrinus* AHT, as evident of leakage of intracellular K^+ ions that caused cell death [9]. Sakai, et al. reported the leakage of intracellular Ca^{2+} ions from cancer cells [15,16]. Sunada, et al. reported direct damage to outer membrane of *E. coli* as evident of the destruction of the endotoxin, an outer membrane component [6].

Although studies have demonstrated the importance of the bactericidal affects of the TiO_2 photocatalyst, the main mechanism causing the photocatalytic killing process has not been well-established yet. In this paper we compared the toxicity of TiO_2 toward bacteria exemplified by *E. coli* under dark and light conditions. Additionally we also studied the enzymatic responses of E. coli in terms of MDA formation and TTC reduction analysis.

2 MATERIALS AND METHODS

2.1. Culture of microorganisms:

E. coli TB1 strain was grown aerobically in 100 mL of Luria-Bertani (LB) broth at 37°C on a rotary shaker (200 rpm) for 18 h. The cells were harvested by centrifugation at 7,800g for 10 min, and resuspended in 10% glycerol and LB Broth mixture. The final optical density at 660 nm of the suspension was determined by measuring the turbidity with a Hach DR/2000 spectrophotometer to calculate the growth rate of the cells.

2.2. Experimental setups: Five experimental runs were conducted in the laboratory (Table 1).

2.2. Photocatalytic reaction

Four different sizes of TiO_2 were used in experiments; P25 Degussa (75% anatase, 25% rutile, 30 nm), R5 Reade (99% anatase, 5 nm), R10 Reade (99% anatase, 3 nm), U100 (75% anatase, 25% rutile, 100 nm) particles. A stock suspension (10 g L^{-1}) in four different particle sizes was prepared with LB Broth media and kept refrigerated in the dark until use. *E.coli* was added to an aliquot of the stock suspension immediately prior to toxicity run. The final concentrations ranged from 0.01 to 1 g L^{-1}. All experiments were conducted in continuously stirred aqueous slurry solutions to ensure maximal mixing and to prevent possible settling of the TiO_2 particles.

Table 1: Experiments conducted

Run #	Culture / Media	Initial Conc.	Light Source	Instru-ment	Incuba-tion
I	2 mL / 1 L	10^9 CFU mL^{-1}	No Light	Jacketed beaker, 200 rpm	37 °C for 24 h
II			Halogen (100 W)		
III	0.1 mL / 1 L		UV (100 W)		
IV			Solux (70 W)	Shaker, 200 rpm	
V			Agrosun (40 W)		

2.3. Cell viability:

The numbers of viable cells in cell suspensions that were subjected to the light and dark treatments were determined by plating serially diluted suspensions onto LB agar plates. The plates were incubated at 37°C for 24 h, and then the numbers of colonies on the plates were counted by using Fisher Acculite 133-8002 model colony counter.

2.4. Determination of lipid peroxidation:

Lipidperoxidation was determined by monitoring the formation of Malondialdehyde (MDA). Quantification of MDA was done following the methods described by Esterbauer, et al. [17] and Maness, et al. [10]. The method is based on the formation of pink MDA- Thiobarbituric Acid (TBA) adducts which has an absorption maximum in acidic solution at 532 nm. The concentrations of the MDA formed were calculated based on a standard curve for the MDA (Sigma Chemical Co.) complex with TBA. The extent of lipid peroxidation was expressed in nanomoles of MDA per milligram (dry weight) of cells.

2.5. Determination of cellular respiration:

The reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to its reduced product, 2,3,5-triphenyltetrazolium formazan (TTF), was measured as described by Maness et al (1999), with minor modifications. The concentrations of the TTF formed were determined based on a standard curve for freshly prepared TTF (Sigma Chemical Co.) in methanol. The rate of O_2 or TTC reduction was expressed in nanomoles of O_2 or TTF per minute per milligram (dry weight) of cells.

3 RESULTS

3.1. Effects of TiO_2 concentrations on cell viability

In order to study the killing mechanism, a high concentration (10^9 CFU ml^{-1}) of *E. coli* cells and TiO_2 concentrations ranging from 0.1 to 1 g L^{-1} were used to examine any change in cellular processes resulting from TiO_2 biocidal action. (Fig. 1, 2 and 3). The results indicated that *E. coli* underwent a two-stage response to TiO_2 particles; a rapid decrease in population within the first 15 min followed by a slow decrease upon extended treatment time, e.g., 30-45 min. No significant difference in terms of bacterial die-off was observed in the size range of 3 to 55 nm. An optimal particle concentration of 0.01g L^{-1} for the survival of *E. coli* was observed.

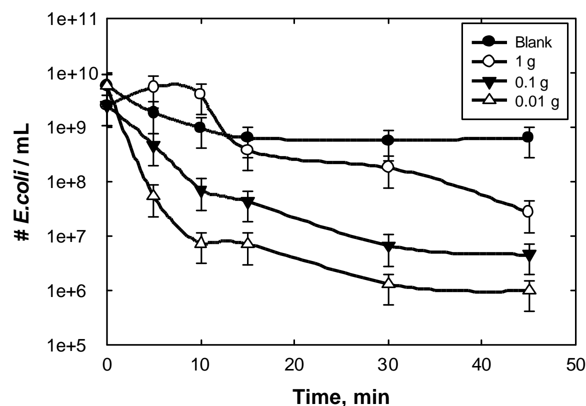


Fig 1 : Concentration effect of R10 TiO_2 nanoparticles on the die-off of *E.coli* under dark condition (Run I).

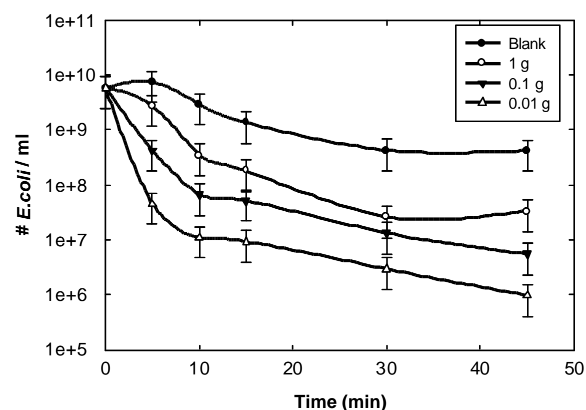


Fig 2: Concentration effect of P25 TiO_2 nanoparticles on the die-off of *E. coli* under dark condition (Run I).

3.2. Effect of irradiated TiO_2 on lipid peroxidation

To estimate membrane damage, we examined the production of MDA, a product of lipid peroxidation, by *E. coli* cells. The effects of irradiated TiO_2 on MDA formation in *E. coli* cells under dark and light conditions were also determined (Figure 6). *E. coli* cells were incubated with 0.001 to 0.3 g L^{-1} of TiO2 and were irradiated with Solux70 (8 W m^{-2}). Figure 6 shows that cells in the dark produced comparably low levels of MDA than in the light.

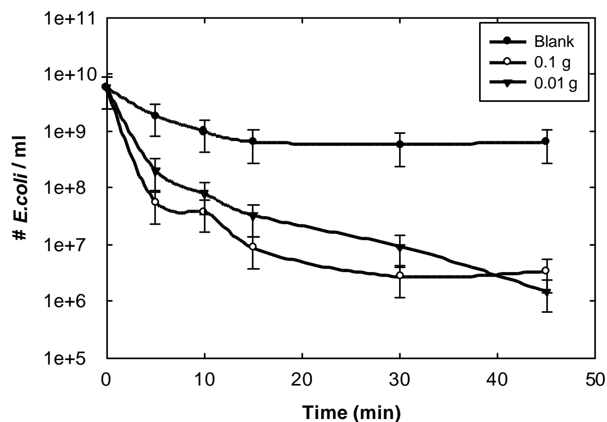


Fig 3: Concentration effect of U100 TiO_2 nanoparticles on the die-off of *E.coli* under dark condition (Run I).

Table 2: The percent kill of *E. coil*

Time, Min	Particle size				
	0	R10 3 nm	R5 5 nm	P25 25nm	U100 55 nm
5	68.15	91.87	90.13	99.84	92.86
30	90.12	99.89	99.64	99.91	99.77

Experimental conditions: 0.1g L^{-1}; particles sizes 5 nm; reaction time: 30 min; under dark (Run I)

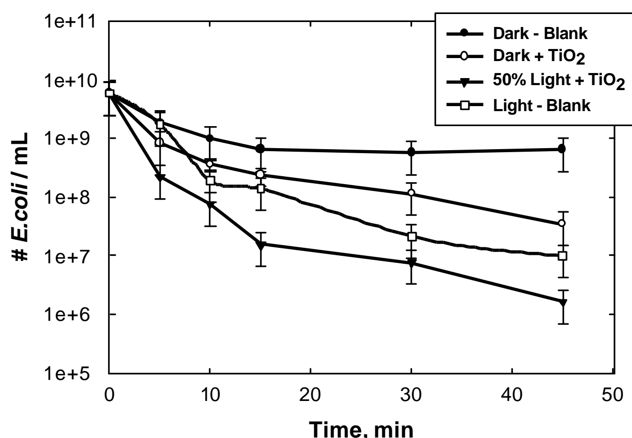


Fig 4: Die-off of E. coli as affected by Concentration. Experimental conditions: particle concentration: 0.5 g L^{-1} Particle: R5 TiO_2 ; light: Halogen (Run II)

3.3. Effect of TiO_2 on cellular respiratory activity

Since the bacterial cell membrane contains essential components of the respiratory chain, it was reasonable to investigate the effect of TiO_2 photocatalysis on cellular respiratory activities. Respiration was monitored by studying the reduction of TTC to TTF. Succinate was used as the electron donor in both assays. Figure 7 shows the reduction of TTC to TTF in 2 h under dark and light conditions.

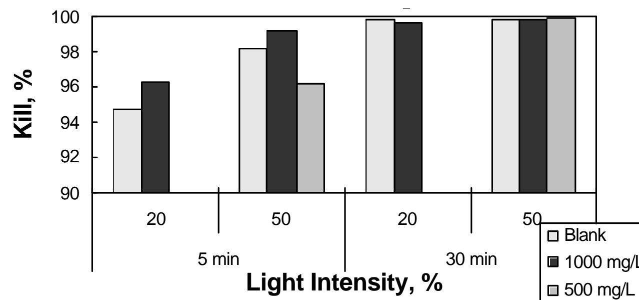


Fig 5: Stage-II Die-off of E. coli. Experimental conditions: Particle: R5 TiO_2; light: Halogen (Run II).

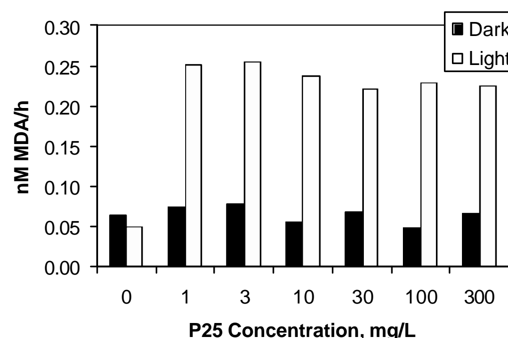


Fig 6: The effects of irradiated TiO_2 on MDA formation in *E. coli* cells under dark and light conditions (Run IV).

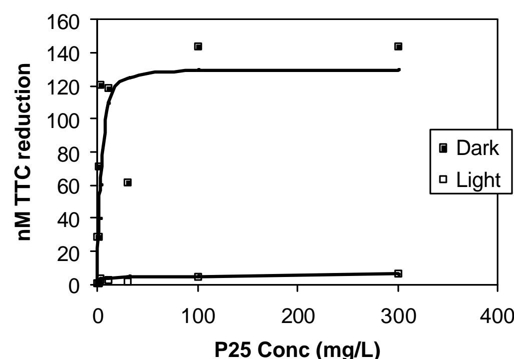
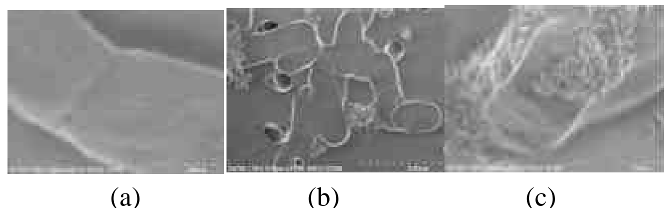


Fig 7: The reduction of TTC to TTF under dark and light conditions (Run IV)

Cryo-SEM images were taken in order to see the physical damage of the cells under dark and light conditions. Figure 8 shows that the effect of irradiated TiO_2 on the cell membrane is higher than the effect of TiO_2 under dark condition.

4 CONCLUSIONS

Results show that in darkness, nanoparticles still exhibited adverse effect on the growth of bacteria. The die-off of bacteria takes in two stages; fast die-off followed by a second slow killing. The smaller particle concentrations and larger primary particle size appear to be more damaging to the bacteria under dark conditions.



(a) (b) (c)

Fig 8: The SEM images of *E.coli* with no treatment (a), in the presence of P25 TiO_2 at 0.1 g L^{-1} under dark (b) and in the presence of P25 TiO_2 at 0.1 g L^{-1} P25 TiO_2 under light (c).

In the presence of light, results show that the light source plays an important role on the toxicity of TiO_2 to bacteria. Among four different light sources used, the presence of halogen light was adequate to kill bacteria without involving photo-catalyst, TiO_2. Larger concentrations of TiO_2 appear to be beneficial to the bacteria, since the concentrated solution would shield the bacteria from long wavelength UV and other activated TiO_2 complex.

Results of MDA analysis show that the lipid peroxidation process was dependent on the presence of both light and TiO_2. Under dark, the effects of irradiated TiO_2 on MDA formation in *E. coli* cells were lower than the MDA production in the light. The results also showed that small concentrations of TiO_2 caused a high lipidperoxidation.

Cellular respiratory activity was examined by TTC reduction to TTF. The results indicated that in 2 h under light condition, the bacteria became unhealthy and TTC reduction in light was lower than in dark.

Overall, results demonstrated that TiO_2 was able to deactivate common infectious water-borne microorganisms such as *E. coli*.

5 ACKNOWLEDGEMENTS

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