USE OF ADVANCED OXIDATION PROCESSES FOR THE INACTIVATION OF *E. coli* BACTERIA WATER SUSPENSIONS

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EXTENDED ABSTRACT

The efficacy of various advanced oxidation processes based on ultraviolet and ultrasound irradiation to inactivate *E. coli* bacteria in sterile deionized water was evaluated. When an *E. coli* suspension in sterile deionized water was stirred for prolong time in the dark in the presence of 0.5 g/L Degussa TiO_2, *E. coli* population decreased at about 61% over the first 15 min and further decreased to 75% over the first 30 min beyond which the residual *E. coli* concentration remained stable, thus implying that adsorption of TiO_2 onto the surface of *E. coli* had occurred resulting in partial deactivation of bacteria. UV-A irradiation alone resulted in only about 40% *E. coli* bacteria reduction after 20 min. On the other hand, UV-A irradiation of an *E. coli* suspension in the presence of 0.25 g/L Degussa TiO_2 (i.e. photocatalysis) resulted in 99.5% reduction of *E. coli* population after 20 min, while the presence of peracetic acid (PAA) further increased *E. coli* population inactivation to 99.99%. In other words, disinfection efficiency follows the order: UV-A/TiO_2/PAA>UV-A/TiO_2>UV-A. PAA-assisted UV-A/TiO_2 photocatalytic inactivation of *E. coli* bacteria could generally lead to nearly complete *E. coli* destruction in 20 min contact time with the extent of inactivation depending on the photocatalyst type and loading (in the range 0-0.5 g/L), PAA concentration (in the range 0-2 mg/L) and temperature. Of the various commercially available TiO_2 tested Degussa P25 was found more efficient in terms of PAA-assisted *E. coli* photocatalytic inactivation than Hombicat UV 100 and Tronox A-K-1. Increasing catalyst loading from 0.1 g/L to 0.5 g/L increased *E.coli* inactivation leading to complete destruction of bacteria population at 0.5 g/L catalyst loading after 10 min contact time. When the temperature was decreased from 25±1 °C to 15±1 °C then *E. coli* deactivation slightly decreased. Moreover, *E. coli* inactivation increased by increasing PAA concentration leading to 99.999% removal after 15 min contact time at 2 mg/L PAA concentration and 0.25 g/L TiO_2. Low frequency (in the range 24-80 kHz), high power (in the range 150-450 W) ultrasound irradiation provided by a horn-type sonicator was less effective than photocatalysis requiring longer contact times for *E. coli* inactivation. When ultrasound and UV-A irradiation were applied simultaneously (i.e. sono-photocatalysis) PAA-assisted *E.coli* inactivation was greatly enhanced compared to the corresponding photocatalytic and sonolytic runs, thus demonstrating the beneficial and synergistic effect of the two modes of irradiation.

Keywords: *E.coli*, heterogeneous photocatalysis, TiO_2, peracetic acid, sonolysis, sono-photocatalysis

1. INTRODUCTION

Discharge of treated effluents, either municipal or industrial, to the water receiving bodies or reuse for irrigation or other applications, requires the proper disinfection and the degradation of various micro-pollutants present in the effluents to protect public health

and the environment. Chlorine, the most widely used disinfectant, has been associated with the formation of various undesirable chlorinated disinfection by-products (DBPs), some of which have been found carcinogenic and/or mutagenic [1].

Because of the concerns over the formation of DBPs, ongoing research focuses on the development of alternative disinfection methods. In recent years, advanced oxidation processes (AOPs) have been successfully employed for the degradation of a wide range of organic micro-pollutants as well as the deactivation of various pathogenic micro-organisms [2]. AOPs can be broadly defined as aqueous phase oxidation methods based primarily on the intermediacy of hydroxyl radicals $HO^{\bullet}$ in the mechanism leading to the destruction of the target compound. The hydroxyl radical $HO^{\bullet}$ is a powerful oxidant and a short lived, highly reactive and non selective chemical species. Hydroxyl radicals are able to oxidize a wide range of organic compounds regardless of the various functional groups present and to deactivate various pathogenic micro-organisms by oxidizing their cell components.

Among the various AOPs regarding water and wastewater disinfection, semiconductor photocatalysis using TiO_2 as a photocatalyst has received considerable attention with emphasis given on the inactivation of bacteria and, to a lesser extent, of viruses and protozoan parasites [3]. Moreover, ultrasound irradiation has also been used for water disinfection [4].

The aim of the present work was to investigate the efficiency of TiO_2 photocatalysis, sonolysis and their combination sono-photocatalysis, for the inactivation of *Escherichia coli* (*E. coli*) bacteria suspensions in sterile distilled water. *E. coli* bacteria were employed in this study because it is a model micro-organism widely used as an indicator of faecal contamination. In addition, the disinfection efficiency of peracetic acid (PAA, CH_3CO_3H), which is a new and alternative promising disinfectant, has been studied in combination with the abovementioned AOPs.

2. MATERIALS AND METHODS

2.1. Chemicals

The inorganic salts used in the present study, namely NaCl, KCl, Na_2HPO_4, K_2HPO_4, KH_2PO_4, $Na_2S_2O_3$, as well as agar and the catalase solution (273780 Units/mL) were purchased from Fluka, while peptone and meat extract were supplied by Merck. Peracetic acid (CH_3CO_3H, PAA) solution was kindly provided by OX-CTA (Zaragoza, Spain) and contains 5% wt peracetic acid, 25% wt hydrogen peroxide and 5-10% wt acetic acid. Three commercially available titanium dioxide TiO_2 samples were employed in this study, namely: (a) Aeroxide P 25 (Degussa P 25) supplied by Degussa AG (anatase:rutile 75:25, 21 nm particle size, 50 m^2/g BET area); (b) Hombicat UV 100 supplied by Sachtleben Chemie GmbH (anatase, 5 nm particle size, >250 m^2/g BET area); (c) Tronox A-K-1 supplied by Kerr-McGee Chemicals LLC (anatase, 20 nm particle size, 90 m^2/g BET area).

2.2. Bacterial strain

The bacterial strain used in the present study was *E. coli* K 12 (ATCC 23716, DSM 498) (DSMZ, German Collection of Microorganisms and Cell Cultures). *E. coli* was inoculated in 50 mL of the appropriate nutrient broth according to DSMZ catalogue (i.e. 5 g/L peptone, 3 g/L meat extract, 4 g/L K_2HPO_4, 1.5 g/L KH_2PO_4 and 5 g/L NaCl) and grown overnight at 37°C by constant agitation under aerobic conditions. The bacterial cells were

collected by centrifugation, washed two times with sterile phosphate buffered saline (8 g/L NaCl, 0.2 g/L KCl, 1.15 g/L Na_2HPO_4, and 0.2 g/L KH_2PO_4 at pH=7.3). Finally, the bacterial pellet was suspended in sterile deionized water and diluted to the required cell density corresponding to 10^4-10^5 CFUs/mL. Deionized water (18.2 MΩ cm at 25°C) used for solution preparation was prepared on a water purification system (EASYpureRF) supplied by Barnstead/Thermolyne (USA).

2.3. Photocatalytic and ultrasound disinfection of deionized sterile water spiked with E. coli

UV-A irradiation was provided by a 9 W lamp (Radium Ralutec, 9W/78, 350-400 nm). The photon flux of the lamp was determined actinometrically using the potassium ferrioxalate method and it was found 4.69 10^{-6} Einstein/s. Ultrasound (US) irradiation was provided by two horn-type sonicators operating at 24 kHz and a variable electric power output up to 450 W (Dr Hielscher UP400S, Germany) and at 80 kHz and a variable electric power output up to 150 W (LabPlant Ultrason 250, UK). Experiments were conducted in an immersion well, batch type, laboratory scale photoreactor, purchased from Ace Glass (Vineland, NJ, USA) which is described in detail elsewhere [5].

In a typical photocatalytic run, an *E. coli* suspension in sterile deionized water was introduced in the reaction vessel and the appropriate amount of TiO_2 was added to achieve the desirable catalyst loading. The resulting suspension was magnetically stirred for 30 min in the dark to ensure complete equilibration of adsorption/desorption of *E. coli* bacteria onto the catalyst surface. After that period of time, the UV-A lamp was turned on, while at the same time a measured volume of a diluted PAA solution was added dropwise with a peristaltic pump (slow addition of PAA was done over several minutes to avoid high local concentrations) to yield 0.5-2 mg/L PAA concentrations in the final solution. When the UV-A lamp was turned on, pure O_2 was continuously sparged in the liquid and the reaction mixture was continuously stirred. Unless otherwise stated, the temperature was maintained at 25±1 °C with a temperature control unit (Crioterm, Italy). The external reaction vessel was covered with aluminum foil to reflect irradiation exerting the outer wall of the reaction vessel. Similar procedures were followed for the sonochemical disinfection experiments.

At specific time intervals about 2 mL of the reaction solution were withdrawn and were immediately quenched adding 20 μL of 165 g/L sodium thiosulfate ($Na_2S_2O_3$) solution followed by 20 μL of a catalase solution. Sodium thiosulfate decomposes peracetic acid while catalase decomposes hydrogen peroxide. Prior to analysis, samples were not filtered to remove TiO_2 particles to avoid losses of bacteria during filtration. Following quenching, samples were analyzed with respect to viable *E. coli* cells employing the serial dilution-agar plate technique. 100 μL of diluted samples were spread over the surface of the appropriate solid agar medium (5 g/L peptone, 3 g/L meat extract, 15 g/L agar, 4 g/L K_2HPO_4, 1.5 g/L KH_2PO_4 and 5 g/L NaCl) in 90 mm Petri dishes and then incubated for 24 hr at 37°C. The method detection limit was 10 CFUs/mL. For the undiluted samples, 1 mL of sample was spread over the surface of four 90 mm Petri dishes (i.e. 250 μL of sample per Petri dish) and the *E. coli* colonies present were added after incubation for 24 hr at 37°C. Following this experimental procedure, the detection limit was reduced to 1 CFUs/mL for the undiluted samples. Finally, *E. coli* colonies were counted with an IUL Colony Counter (Spain).

3. RESULTS AND DISCUSSION

3.1. Photocatalytic disinfection experiments in the presence of peracetic acid

In a preliminary control experiment, a 10^4 CFUs/mL *E. coli* suspension in sterile deionized water was stirred for prolonged time in the dark at 25°C. It was found that *E. coli* population remained practically unchanged after 120 min, thus showing that the bacteria were stable at the conditions employed in the present study and cell damage due to osmotic effects was negligible. In addition, another control experiment was performed stirring an 8.75 10^4 CFUs/mL *E. coli* suspension in sterile deionized water in the presence of 0.5 g/L Degussa TiO_2 for prolonged time in the dark. It was found that cultivable *E. coli* population decreased to 3.45 10^4 CFUs/mL (i.e. 61% decrease) over the first 15 min and further decreased to 2.30 10^4 CFUs/mL (i.e. 75% decrease) over the first 30 min beyond which the residual *E. coli* concentration remained stable.

This result is in contrast with previous literature reports stating that *E. coli* population remained constant after stirring in the presence of Degussa TiO_2 for prolonged time in the dark [6]. The above result implies that *E. coli* bacteria interacted with the catalyst surface and this interaction resulted in loss of cultivability. It also implies that equilibrium between adsorption/desorption was achieved within 30 min. Recently, it has been reported that *E. coli* bacteria adsorb onto the surface of TiO_2 and this adsorption alters cell membrane integrity without affecting bacteria cultivability [7]. However, in our case it can be speculated that aggregation of titania clusters and their interactions with *E. coli* are responsible for reduced bacteria viability as expressed by the 75% reduction of cultivable *E. coli* population. If TiO_2 was actually toxic in the dark to all *E. coli* bacteria present in the solution, then a continuous decrease of *E. coli* population would be expected throughout the experiment.

In another experimental run, a 10^5 CFUs/mL *E. coli* suspension was UV-A irradiated. As can be seen in Figure 1 it was found that UV-A irradiation alone resulted in only about 40% bacteria reduction after 20 min. An additional dark run was performed with 0.5 mg/L PAA showing that, at the conditions in question, no inactivation occurred. The experiments were repeated in the presence of 0.25 g/L Degussa TiO_2 and as can be seen in Figure 1 the bacteria reduction after 20 min of treatment was 99.5 and 99.99% respectively for the UV-A/TiO_2 and UV-A/TiO_2/PAA systems. In summary, disinfection efficiency follows the order: UV-A/TiO_2/PAA>UV-A/TiO_2>UV-A.

In further experiments, the photocatalytic performance of various commercially available TiO_2 samples was evaluated, namely Degussa P25, Hombicat UV 100 and Tronox A-K-1 and the results are also shown in Figure 1. It was found that Degussa P25, one of the most commonly employed and effective TiO_2 photocatalysts was appreciably more active than the other two TiO_2 samples and all subsequent photocatalytic experiments were performed with Degussa P25 TiO_2.

TiO_2 loading in slurry photocatalytic processes is an important factor that can influence strongly the efficiency of the process. The effect of catalyst loading was also studied in the range 0.1 to 0.5 g/L Degussa P25 TiO_2 and the results are shown in Figure 2. As can be seen, increasing catalyst loading from 0.1 g/L to 0.25 g/L increased *E.coli* inactivation. For example, after 20 min contact time inactivation was 99.5% and 99.996% at 0.1 g/L TiO_2 and 0.25 g/L TiO_2 respectively.

Of particular interest is the experimental run performed at 0.5 g/L TiO_2 (data not shown in Figure 2 since the ordinate is in logarithmic scale). In this case, *E.coli* population was completely inactivated (i.e. 100%) after 10 min contact time. This very interesting result

indicates that increasing catalyst loading profoundly effects *E.coli* inactivation and at higher catalyst loadings complete inactivation can be achieved within minutes even at very low PAA concentrations. All previous experimental run were performed at 25±1 °C. An additional experiment was performed at 15±1 °C and the results are also shown in Figure 2. In this case, inactivation proceeded slightly slower. Therefore, all subsequent experimental runs were performed at 25±1 °C.

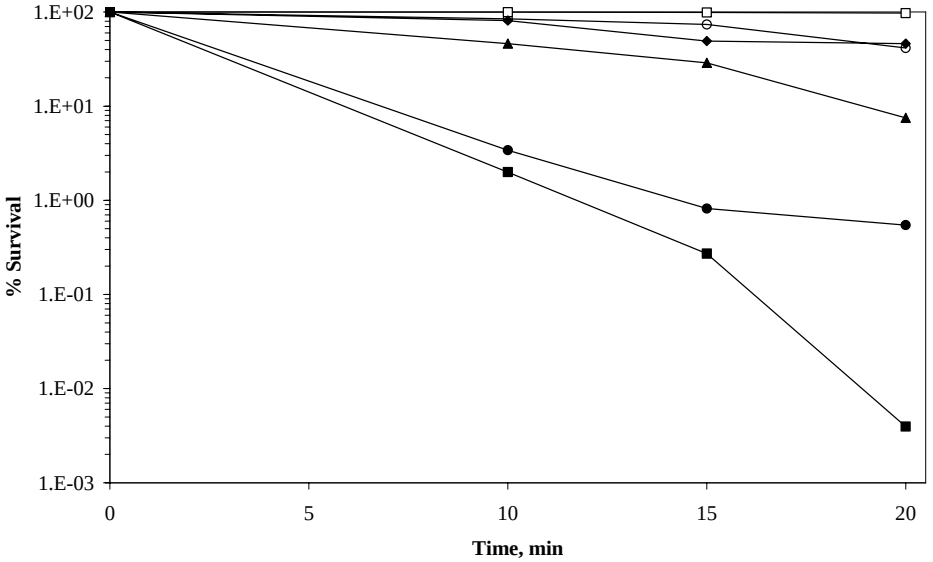

Figure 1: *E. coli* inactivation in water by ultraviolet irradiation. -○- UV-A alone; -□- PAA in the dark; -●- UV-A/TiO_2(Degussa); -■- UV-A/TiO_2(Degussa)/PAA; -◆- UV-A/TiO_2(Tronox)/PAA; -▲- UV-A/TiO_2(Hombicat)/PAA. [PAA]=0.5 mg/L; [TiO_2]= 0.25 g/L.

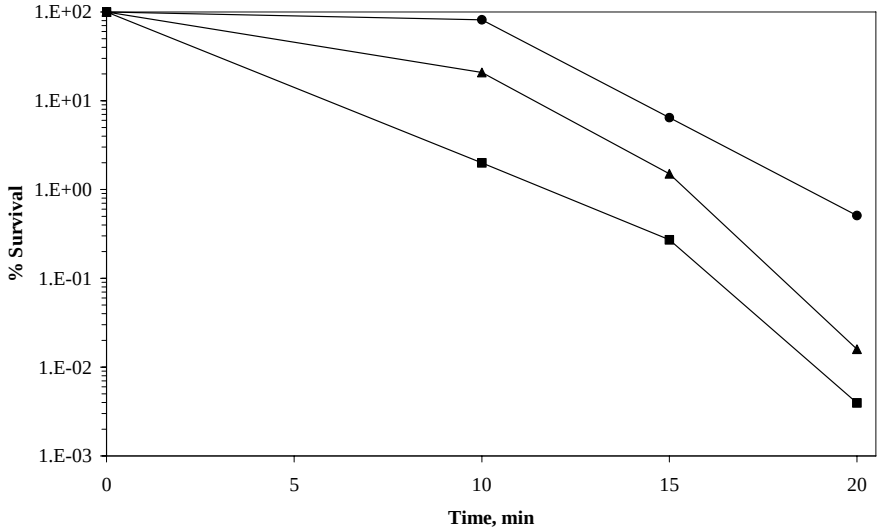

Figure 2: Effect of Degussa P 25 loading on *E. coli* inactivation in water during UV-A/TiO_2/PAA treatment. -●- 0.1 g/L; -■- 0.25 g/L; -▲- 0.5 g/L, temperature 15±1 °C; [PAA]=0.5 mg/L.

In further experiments the effect of peracetic acid concentration was studied in the range 0-2 mg/L, namely 0.5, 1, 1.5 and 2 mg/L, and the results are summarized in Figure 3. Blank runs were performed without catalyst and UV-A irradiation in order to evaluate the disinfection efficiency of PAA. As can be seen in Figure 3, not surprisingly, inactivation of *E.coli* population increased with increasing PAA concentration. When UV-A irradiation was applied in the presence of 0.25 g/L TiO_2 at an *E.coli* water suspension containing

PAA at various concentrations, higher inactivation of *E.coli* population was observed compared to the corresponding blank runs. As can be seen in Figure 3 inactivation proceeded faster in the case of 2 mg/L PAA and after 15 min contact time it was more than 99.999%. In the corresponding blank run inactivation was almost the same after 15 min contact time but inactivation rate was slower.

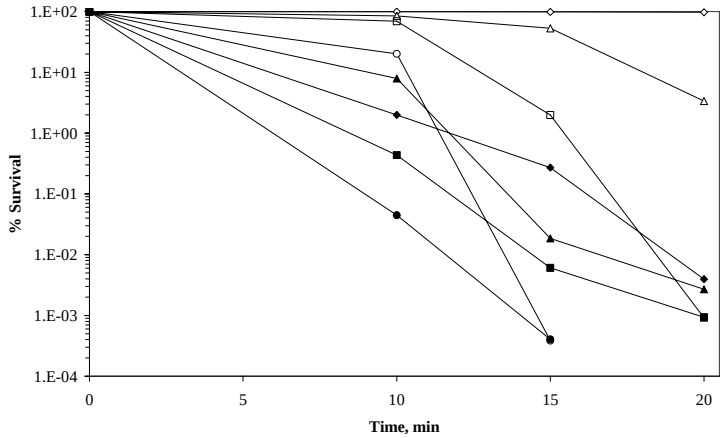

Figure 3: Effect of PAA concentration on *E. coli* inactivation in water during UV-A/TiO_2/PAA treatment. Open symbols: blank runs without UV-A and TiO_2; closed symbols: photocatalytic runs in the presence of TiO_2. -◆-, -◇- 0.5 mg/L; -▲-, -△- 1 mg/L; -■-, -□- 1.5 mg/L; -●-, -○- 2 mg/L. [Degussa TiO_2]= 0.25 g/L.

3.2. Ultrasound disinfection experiments in the presence of peracetic acid

In a preliminary run, a 10^5 CFUs/mL *E. coli* water suspension was ultrasound irradiated (160 W, 24 KHz) and it was found that *E. coli* population remained practically unchanged after 30 min, thus showing that the bacteria were stable under ultrasound irradiation. On the other hand, as can be seen in Figure 4, when the same experiment was performed in the presence of 0.5 mg/L PAA the *E. coli* population was inactivated at about 85% after 20 min contact time.

In additional experiments the effect of PAA was tested in the range 0-2 mg/L and the results are also shown in Figure 4. As can be seen, it was found that increasing PAA concentration increased *E. coli* inactivation relative to the corresponding blank runs (i.e. without ultrasound irradiation) and this was more pronounced at low PAA concentrations. Moreover, it was found that increasing US irradiation frequency from 24 kHz to 80 kHz marginally increased *E. coli* inactivation while increasing US irradiation power from 100 W to 120 W also increased *E. coli* inactivation.

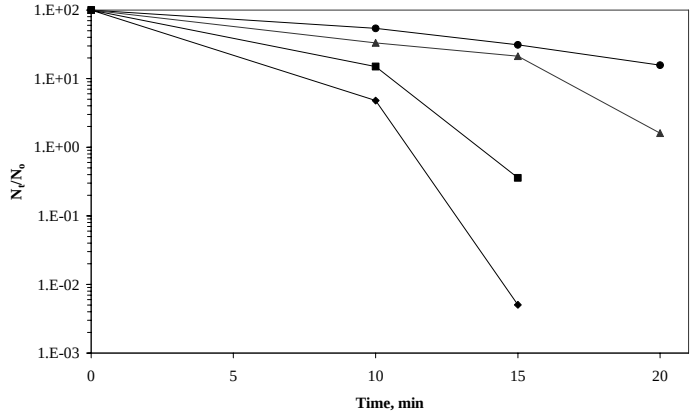

Figure 4: *E. coli* inactivation in water by ultrasound irradiation (24 kHz, 160 W) at various PAA concentration. -●- 0.5 mg/L; -▲- 1 mg/L; -■- 1.5 mg/L; -◆- 2 mg/L.

3.3. Sono-photocatalytic disinfection experiments in the presence of peracetic acid

In a final set of experiments the effect of simultaneous application of ultrasound and UV-A irradiation in the presence of TiO_2 (i.e. sono-photocatalysis) on the PAA-assisted inactivation of *E.coli* bacteria was studied and the results are shown in Figure 5.

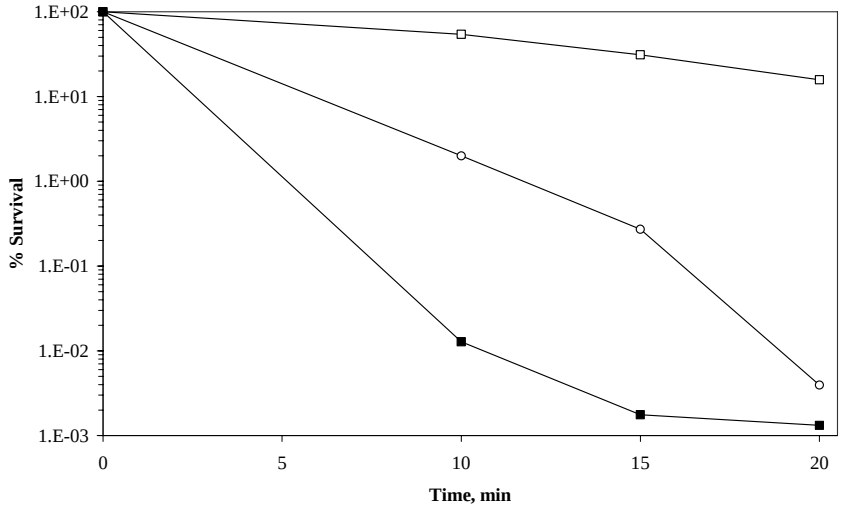

Figure 5: *E. coli* inactivation in water by ultrasound (24 kHz, 160 W) and UV-A irradiation. -○- photocatalysis; -□- Sonolysis; -■- sono-photocatalysis. [TiO_2]=0.25 g/L, [PAA]=0.5 mg/L

As can be seen, when ultrasound and UV-A irradiation were applied simultaneously, *E.coli* inactivation was greatly enhanced compared to the corresponding photocatalytic and sonolytic runs. These results clearly demonstrate the beneficial and synergistic effect of simultaneous application of the two modes of irradiation.

Ultrasound is able to inactivate bacteria and de-agglomerate bacterial clusters through a number of physical, mechanical and chemical effects arising from acoustic cavitation, namely: (i) chemical attack by the sonogenerated hydroxyl radicals, (ii) pressure and pressure gradients resulting from bubble collapse causing cell damage due to mechanical fatigue and (iii) shear forces induced by micro-streaming occur within and consequently damage bacterial cells.

4. CONCLUSIONS

The conclusions drawn from the present study can be summarized as follows:

- TiO_2 photocatalysis is capable of inactivating an *E. coli* water suspension. Addition of peracetic acid at low concentrations increased photocatalytic efficiency.
- Degussa P25 was found more efficient photocatalyst in terms of *E. coli* inactivation compared to other commercially available TiO_2 samples.
- Increasing both catalyst loading in the range 0.1-0.5 g/L TiO_2 and peracetic acid concentration in the range 0.5-2 mg/L increased photocatalytic efficiency.
- Ultrasound irradiation enhanced the disinfection efficiency of peracetic acid and this was more pronounced at low peracetic acid concentrations.
- Simultaneous application of ultrasound and UV-A irradiation in the presence of TiO_2 (i.e. sono-photocatalysis) increased the peracetic acid-assisted inactivation of *E. coli* bacteria population.

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