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A comparison of the effectiveness of TiO_2 photocatalysis and UVA photolysis for the destruction of three pathogenic micro-organisms.

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Abstract

When exposed to UVA light TiO_2 demonstrates an effective bactericidal activity. The mechanism of this process has been reported to involve attack by valence band generated hydroxyl radicals. In this study when three common bacterial pathogens, *Escherichia coli*, *Salmonella enterica* serovar Enteritidis and *Pseudomonas aeruginosa*, were exposed to TiO_2 and UVA light a substantial decrease in bacterial numbers was observed. Control experiments in which all three pathogens were exposed to UVA light only resulted in a similar reduction in bacterial numbers. Moreover exposure to UVA light alone resulted in the production of a smaller than average colony phenotype among the surviving bacteria, for all three pathogens examined, a finding which was not observed following treatment with UVA and TiO_2. Small slow growing colonies have been described for several pathogenic bacteria and are referred to as Small Colony Variants. Several studies have demonstrated an association between Small Colony Variants and persistent, recurrent and antibiotic resistant infections. We propose that the production of Small Colony Variants of pathogenic bacteria following UVA treatment of drinking water may represent a health hazard. As these Small Colony Variants were not observed with the UVA/TiO_2 system this potential hazard is not a risk when using this technology. It would also appear the bactericidal mechanism is different with the UVA/TiO_2 process compared to when UVA light is used alone.

1. Introduction

The spread of water borne infection is a problem in both developed and underdeveloped countries. However it is a greater problem in the latter for several reasons including the lack of adequate sanitary conditions and insufficient health care. Many infectious diseases are transmitted via the faecal-oral route and in countries where sanitation practices are less than adequate, faecal contamination of water supplies is a common occurrence. Thus the presence of individuals in a community with active disease (or in a carrier state) often results in contamination of the water supply. Most cases of water borne infection are acquired via the subsequent consumption of this water. However in addition, the use of such water for bathing or even inhalation of water aerosols may also cause infection.

In recent years the use of titanium dioxide (TiO_2) as a photocatalyst for water treatment has been extensively reported. When TiO_2 is illuminated with light of wavelength less than 380 nm it generates highly active reagents such as valence band holes, h^+, or hydroxyl radicals generated via oxidation of water by the valence band holes. These species have been demonstrated to mineralise a wide range of organic compounds including aromatics and aliphatics, dyes, pesticides and herbicides [1-4]. TiO_2 is especially suitable as a photocatalyst for water treatment, compared to other semiconductors, because it is highly photo-reactive, cheap, non-toxic, chemically and biologically inert, and photostable [5]. Due to the effectiveness of this process as a water treatment technology, the suitability of TiO_2 photocatalysis for water disinfection has been investigated by a number of authors, who have reported vary degrees of efficacy of this technique [6-14].

The disinfecting properties of UV light alone (photolysis) have also been documented [15, 16, 17]. While this technique has been shown to be very effective for disinfection purposes, there are certain factors which need to be considered prior to use. Firstly, the sensitivity of the target micro-organism to UV light, since it is now known that different micro organisms respond to the lethal effects of UV light in different ways [16, 18, 19]. Furthermore, the choice of UV wavelength is important since the mechanism of UV light induced inactivation differs with different wavelengths used. In addition, both UV photolysis and TiO_2 photocatalysis have been reported to suffer from the possibility of photo reactivation i.e. the process by which UV inactivated micro organisms use sunlight energy and the enzyme photolyse to repair UV induced DNA lesions and hence regain their viability. Dark repair mechanisms i.e. repair in the absence of light can also take place, however photo reactivation is considered to be the more problematic of the two methods since it can in some cases reverse the effects of UV disinfection within several hours of treatment [15].

In this work we have compared the effectiveness of TiO_2 photocatalysis and UVA photolysis for the destruction of three important human waterborne bacterial pathogens, *Escherichia coli*, *Salmonella enterica* serovar Enteritidis and *Pseudomonas aeruginosa.*

2. Materials and Methods.

2.1 Bacterial strains and culture conditions

Stock cultures of *Escherichia coli* K12, *Salmonella enteritidis* strain S1400/95 and *Pseudomonas aeruginosa* (NCTC 10662) were sub-cultured on to nutrient agar plates (Oxoid, UK) and grown at 37 ^{o}C overnight (16 hours). Several colonies were removed from each plate and used to inoculate 20 ml of Nutrient broth (Oxoid, UK) and these were grown overnight at 37 ^{o}C. Overnight cultures were washed twice in sterile distilled water by centrifuging at 4,500 rpm for ten minutes and were finally re-suspended in 10 ml of sterile distilled water to a concentration of approximately $1x10^{8}$ colony forming units (CFU) ml^{-1}.

2.2 Photocatalytic reaction

A stock solution (1 g L^{-1}) of Titanium dioxide (P-25 Degussa, UK) was freshly prepared in sterile distilled water. Aliquots (100 ml) of the solution were inoculated with 1 ml of the appropriate bacterial culture. Samples were irradiated for 2 hours using a xenon UVA lamp (480 W UVA spot 400 lamp, UVA light Technology Ltd; spectral output 330-450 nm) placed at a distance of 10 cm from the reaction vessel. The light intensity at this distance was calculated to be 3.42×10^{-5} Einsteins s^{-1} using ferrioxalate actinometry. Reactions were carried out in sterile 125 ml glass beakers with continuous stirring to ensure adequate mixing of TiO_2 and bacteria. Samples of these solutions were collected in triplicate at half hourly intervals and bacterial numbers were assessed by means of a viable count [20]. Control samples which consisted of bacterial suspensions exposed to UVA light in the absence of TiO_2 and bacterial suspensions containing TiO_2 in the dark were run in parallel. Temperature and pH were monitored at half hourly intervals but did not appear to have any

influence on experimental outcome. None of the samples were aerated either prior to or during the experimental procedure.

3. Results and Discussion.

For each of the species under investigation the reduction in colony forming units following irradiation, in the presence and absence of TiO_2, are displayed in figures 1-3. A relatively high rate of bacterial inactivation was observed for all three bacterial samples (Table 1) when irradiated in the presence of TiO_2. The viable cell count decreased with time and after 120 minutes a four log order reduction in bacterial numbers was observed for all three strains. When the bacteria were exposed to TiO_2 in the dark no reduction in viable counts was observed.

Exposure of all three pathogens to UVA light alone also resulted in a significant reduction in bacterial numbers. In the case of *Ps. aeruginosa*, however, the rate of UVA inactivation was greater than that observed for TiO_2 photocatalysis. Moreover, the exposure of all three pathogens to UVA light only resulted in the production of a smaller than average colony phenotype as well as the expected phenotype among the surviving bacteria (figure 4). These small colony phenotypes were not observed in the samples that were irradiated in the presence of the TiO_2 (figure 5).

The effects of temperature and pH on experimental outcome have been examined by several authors [6, 21, 22]. On the whole, pH changes in the range 5-8 do not appear to have any influence on experimental outcome [21]. Results from the current study show that the average starting and finishing pH values fell within this range i.e. 5.7-4.9 respectively. With respect to temperature effects, the rate of bacterial disinfection

has been shown to increase with increasing temperature [6, 13]. Wei *et al* [6] reported that the bacterial inactivation they observed in solar disinfection studies was due to a rise in the temperature of their water samples, in excess of 40^{o}C. In the current study the average initial starting temperatures were around 21^{o}C. The highest temperature recorded at the end of any irradiation period was found to be 29^{o}C thus disinfection due to temperature is highly unlikely.

The results obtained in this study for the photocatalytic destruction of bacterial pathogens are similar to those reported by several other authors [7,8,10,13,23,24]. The true significance of these results, however, is unclear since exposure of contaminated water to UVA light alone also resulted in a strong bacterial disinfection. These results are similar to those of Herrera-Melián *et al* [22] who found little difference between TiO_2 photocatalysis and direct UVA light irradiation of urban waste waters. Although care should be taken when making such comparisons since, as several workers have highlighted, significant differences exist between the photocatalytic response of microbes in natural water conditions and those under simulated laboratory conditions [23-25].

The mechanism for bacterial destruction by TiO_2 has been proposed to occur via attack by hydroxyl radicals generated on the photocatalyst surface [8]. Recent works concentrating on the mode of microbial destruction suggest that initial target for photocatalytic attack is the bacterial cell wall [8, 9, 26, 27, 28]. On the other hand the mechanism of destruction of bacterial pathogens by UV light only varies with UV wavelength. Thus while UVB and UVC inactivate the organism by producing DNA lesions in the organisms genome which inhibit normal DNA replication, UVA

damage occurs following excitation of photosensitive molecules within the cell resulting in the production of active species such as $O_2^{\cdot-}$, H_2O_2, and $^{\cdot}OH$. These species can have both lethal and sub lethal effects on the bacterial genome and other intracellular molecules resulting in physiological alterations, growth delay and oxidative disturbances of bacterial membranes resulting in growth inhibition [29, 30]. The lethal and mutagenic effects of UVA (320-400 nm), in particular, on bacterial cells has been investigated using *E. coli* as a model organism [31, 32].

In this work the production of a smaller than average colony phenotype among the surviving bacterial population is likely to be the result of a UVA induced mutation resulting in reduced cell viability particularly since this colony phenotype is not observed in the case of the TiO_2/UVA system. Small colony phenotypes have previously been observed in *S. typhimurium* [33], *Ps. aeruginosa* [34], *Burkholderia psuedomallei* [35] and *Staphlyococcus aureus* [36, 37, 38]. They were first described over eighty years ago and are referred to as Small Colony Variants (SCV's). Many studies have demonstrated an association between SCV's and persistent, recurrent and antibiotic resistant infections [33, 36, 37, 38].

SCV's of several pathogenic bacteria have been phenotypically characterised as hyperpilated, slow growing, non-pigmented strains which produce less toxin than and exhibit a different pattern of carbohydrate utilisation to the parent strain. [34]. A typical feature of SCV's is that they revert to the parent phenotype when sub-cultured onto nutrient agar. This complex phenotype is due to deficiencies in electron transport, specifically mutations in the genes responsible for menaquinone or heme biosynthesis [34, 36, 37]. Several studies have shown that SCV's are highly invasive

for host cells but due to the reduction in toxin production these variants do not damage such cells and thus may persist for long periods within them [33]. Problems arise however because as already stated, SCV's can revert to the parent colony phenotype and this can result in the production of disease. Furthermore SCV's of *S. aureus* and *Ps. aeruginosa* have been isolated from patients with persistent and relapsing infections and represent a significant problem when it comes to treatment [36-39].

The likelihood of an infection occurring and it's severity in a particular host is a multifactorial event depending on a number of host and pathogen factors. Thus there is no tolerable lower limit for pathogens, even very small numbers present a serious risk, to certain members of the population, i.e. immunocompromised individuals. Hence water intended for human use should be pathogen free. While treatment of contaminated water with UVA irradiation alone appears to be a very effective disinfection method, the possible production of SCV's of target pathogenic organisms represents an increased risk of producing infection with a pathogen that is more difficult to treat.

Moreover, it is now apparent that different bacteria respond differently to the effects of UVA [18]. Recent studies have shown that *Ps. aeruginosa* is more susceptible to the effects of UVA irradiation than other Gram negative bacteria [16, 19]. Thus while *E. coli* has been shown to suffer merely from growth delay following UVA treatment, the effects of such treatment on *Ps. aeruginosa* appear to be lethal [16]. This growth delay has been proposed to represent a phase of DNA repair taking place before any further cell division can occur [17]. Further more the repair mechanism in *E. coli* is

thought to operate at much lower fluences than the similar system in *Ps. aeruginosa* [16]. Fernandez *et al* showed that while UVA induced membrane damage undoubtedly contributed to cell death in *Ps. aeruginosa* they proposed that UVA also produced DNA lesions which resulted in cell death due to inherent deficiencies in the DNA repair mechanisms [16]. The results obtained in the current study show that the rate of destruction of *Ps. aeruginosa* in the presence of UVA light is greater than that of either *E. coli* or *S. typhimurium*. Alternatively Oppezzo and Pizarro [18] showed that *Enterobacter cloacae* was more resistant to the effects UVA than several other Gram negative bacteria. These authors suggested that this increased resistance was due to an increased ability of *En. cloacae* to overcome oxidative stress during exposure to UVA. Hence the effects of UVA irradiation on different bacterial species warrants closer investigation.

The use of solar water disinfection processes in underdeveloped countries e.g. SODIS and of UV home water treatment systems, is rapidly gaining in popularity not least because of the fact that such treatment systems do not involve the use of chemicals and the production of their associated hazardous by-products but also due to factors such as ease of installation and use and lack of interference with the taste, colour and odour of the treated water. It should however, be noted that most home water disinfection units utilise UVC sources and that to date no reports have been made on the production of SCV's with this method.

The success of any UV water treatment system method depends upon UV intensity and the amount of time the micro-organisms are exposed to the radiation. Other limitations associated with this form of water treatment include a dependence on the

characteristics of the wastewater being treated i.e. concentration of colloidal and particulate constituents in the wastewater which can shield target organisms against the light and the possibility that UV treated organisms can sometimes repair and reverse the damaging effects of UV. Hence it is recommended that community potable water treated in this way is already partially treated with e.g. reduced chlorination, prior to passing through the UV disinfecting system. Findings from the current work not only suggest further limitations for the use of UVA light as a method of water disinfection but may actually represent a detrimental effect of this process. Further work on is required to establish whether or not this is the case.

4. Conclusion.

TiO_2 photocatalysis is a more effective technology than UVA irradiation, for disinfection of water contaminated by *E. coli* and *S. enteritidis.* Direct UVA irradiation, however, appears to be more effective for removal of *Ps. aeruginosa*. UVA irradiation, however, results in the generation of small colony variants which are not observed when the TiO_2 photocatalyst is present. These results suggest that when UVA light is used alone the micro organisms may reactivate, which does not appear to occur in the TiO_2 photocatalytic system. This potentially may be a limitation for the use of UVA light as a disinfection method.

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REFERENCES

[1] M. R. Hoffmann, S. T. Martin , W. Choi, D. F. Bahnemann , Chem. Rev. 95 (1995) 69.

[2] L. Linsebigler, G. Lu, J. T. Yates Jr., Chem. Rev. 95 (1995) 735.

[3] P. K. J. Robertson, J. Cleaner Prod. 4 (1996) 203.

[4] A. Mills, S. Morris, J. Photochem. Photobiol. A: Chem. 71 (1993) 75.

[5] Mills, R. H. Davies, D. Worsley, Chem. Soc. Rev. (1993) 417.

[6] C. Wei, W. Lin, Zainal, N.E. Williams, K. Zhu, A.P. Kruzic, R.L. Smith, K. Rajeshwar, Env. Sci. Technol., 28 (1994) 934-938.

[7] J.C. Ireland, P. Klostermann, E. Rice, R. Clark, Appl. Environ. Microbiol. (1993) 1668-1670.

[8] T. Matsunaga, R. Tomoda, T. Nakajima, H. Wake, FEMS Microbiology Letters, 29 (1985) 211-214.

[9] K. Sunada, T.Watanabe, K. Hashimoto, J. Photochem. Photobiol. A: Chem., 156 (2003) 227-233.

[10] M. Bekbölet, Wat. Sci. and Technol., 35 (1997) 95-100.

[11] X.Z Li, M. Zhang, H.Chua, Wat. Sci. and Technol., 33 (1996) 111-118.

[12] N. Huang, Z. Xiao, D. Huang, C. Yuan, Supramol. Science, 5 (1998) 559-564.

[13] G. Rincón, C. Pulgarin, Appl. Catal. B: Environ., 44 (2003) 263-284 A.

[14] D. M. Blake, P-C. Maness, Z. Huang, E. J. Wolfrum, J. Huang, 28 (1999) 1-50

[15] A. M. Shaban, G. E. El-Taweel, G. H. Ali, Water Science and Technology, 35, (1997),107-112.

[16] R.O.Fernandez, R.A. Pizarro, Photochem. Photobiol., 64 (1996) 334-339.

[17] A Caldeira de Araujo, A Fauvre. EMBO J, 5 (1986) 175-179

[18] O. J. Oppezzo, R.A. Pizzarro. 2001. J. Photochem. Photobiol. B: Biol., 62 (2001) 158-65.

[19] R.O. Fernandez, R.A. Pizarro, J. Photochem. Photobiol. B: Biol., 50 (1999) 59-65.

[20] A. A. Miles, S.S Misra, J.O. Irwin, J. Hyg., 38 (1938) 732-749.

[21] R.J. Watts, S. Kong, M.P. Orr, G.C. Miller, B.E. Henry, Water Research, 29 (1995) 95-100.

[22] J. A. Herrera Melián, J. M.Doña Rodríguez, A.Viera Suárez, E.Tello Rendón, C.Valdés do Campo, J. Arana, J. Pérez Peña, Chemosphere, 41 (2000) 323-327.

[23] R. Dillert, U. Siemon, D. Bahnemann, J. Adv. Oxid. Technol., 4 (1999) 55.

[24] A. G. Rincón, C. Pulgarin, Appl. Catal. B: Environ., 49 (2004) 99-112.

[25] J. Wist, J. Sanabria, C. Dierolf, W. Torres, C. Pulgarin, J. Photochem. Photobiol. A: Chem., 147 (2002) 241-246.

[26] T. Saito, T. Iwase, J.Horie, T. Morioka, J. Photochem. Photobiol. B: Biol., 14 (1992) 369-379.

[27] P. C. Maness, S. Smolinski, D.M. Blake, Z. Huang, E.J. Wolfrum, W.A. Jacoby, Appl. Environ. Microbiol., 65 (1999) 4094-8.

[28] Z. Huang, P-C. Maness, D.M. Blake, E.J. Wolfrum, S.L. Smolinski, W.A. Jacoby, 130 (2000) 163-170.

[29] R. A. Pizarro, L.V. Orce. Photochem. Photobiol., 47 (1988) 391-397.

[30] R. A. Pizarro, Int. J. Radiat. Biol. 68 (1995) 293-299.

[31] A. Eisenstark, Adv. Genet., 26 (1989) 99-147.

[32] A. Favre, E. Hajnsdorf, K. Thiam, A. Calderia de Araujo, Biochimie, 67 (1985) 335-342.

[33] D. A. Schiemann, FEMS Microbiol. Letters, 130 (1995) 45-49.

[34] C. von Eiff, C. Heilmann, R.A. Proctor, C. Woltz, G. Peters, F. Gotz, J. Bacteriol., 179 (1997) 4706-4712.

[35] S. Häußler, B. Tümmler, H. Weißbrodt, M. Rohde, I. Steinmetz, Clin. Infect. Dis., 29 (1999) 621-625.

[36] R. A. Proctor, J. M. Balwit, O. Vesga, Infect. Agents Dis., 3(6) (1994) 302-12.

[37] R. A. Proctor, P. van Langevelde, M. Kristjansson, J.N. Maslow, R.D. Arbeit, Clin. Infect. Dis., 20 (1995) 95-102.

[38] P. J. McNamara, R.A. Proctor, Int. J. Antimicrob. Agents, 14 (2000) 117-122.

[39] S. Häußler, M. Rohde, I. Steinmetz, Med. Microbiol. Immunol. 188 (1999) 91-97.

List of captions for Figures

Figure 1 – Effect of UVA light and TiO_2 on viability of *Escherichia coli*

Figure 2 - Effect of UVA light and TiO_2 on viability of *Salmonella enteritidis*

Figure 3 - Effect of UVA light and TiO_2 on viability of *Pseudomonas aeruginosa*

Figure 4. *Escherichia coli* colonies from UVA treated sample showing both regular and small colony phenotypes.

Figure 5. *Escherichia coli* colonies from UVA/TiO_2 treated sample showing uniform colony phenotype

Table 1. Bacterial counts (CFU $ml^{-1} \pm$ SD) before and after 2 hours exposure to TiO_2 and UVA light or UVA light alone.

Bacteria	**TiO_2 photocatalysis**		**UVA light only**	
	Initial count	**Final count**	**Initial count**	**Final count**
E. coli	$5.2x10^6$	150	$8.0x10^6$	50
S. enteritidis	$1.7x10^7$	825	$1.3x10^7$	$4.0x10^3$
Ps. aeruginosa	$1.0x10^7$	1016	$1.1x10^7$	50

Table 1.

Figure 1.

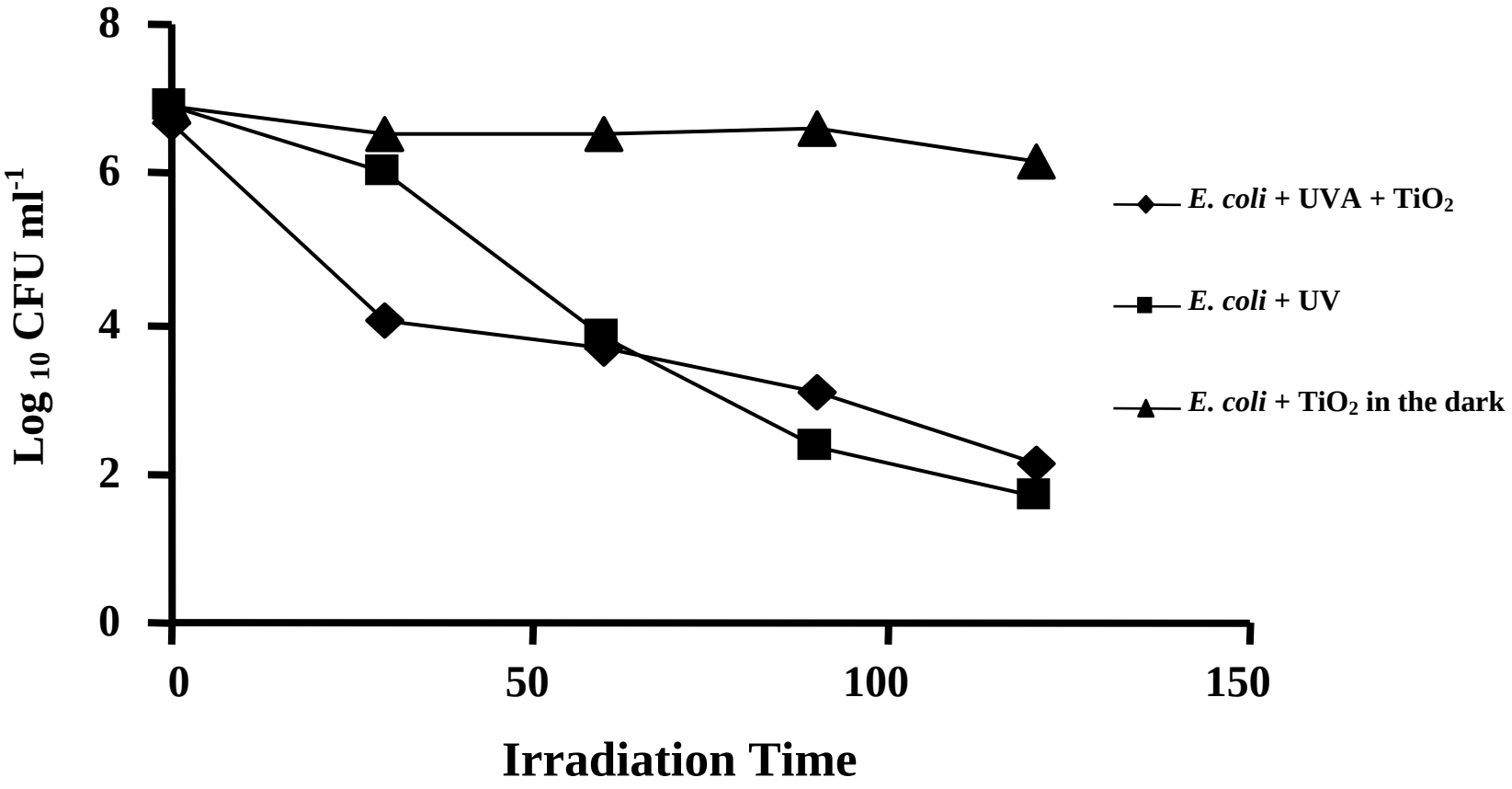
8
6
4
2
0
Log 10 CFU ml-1
0
50
100
150
Irradiation Time
E. coli + UVA + TiO2
E. coli + UV
E. coli + TiO2 in the dark

Figure 2.

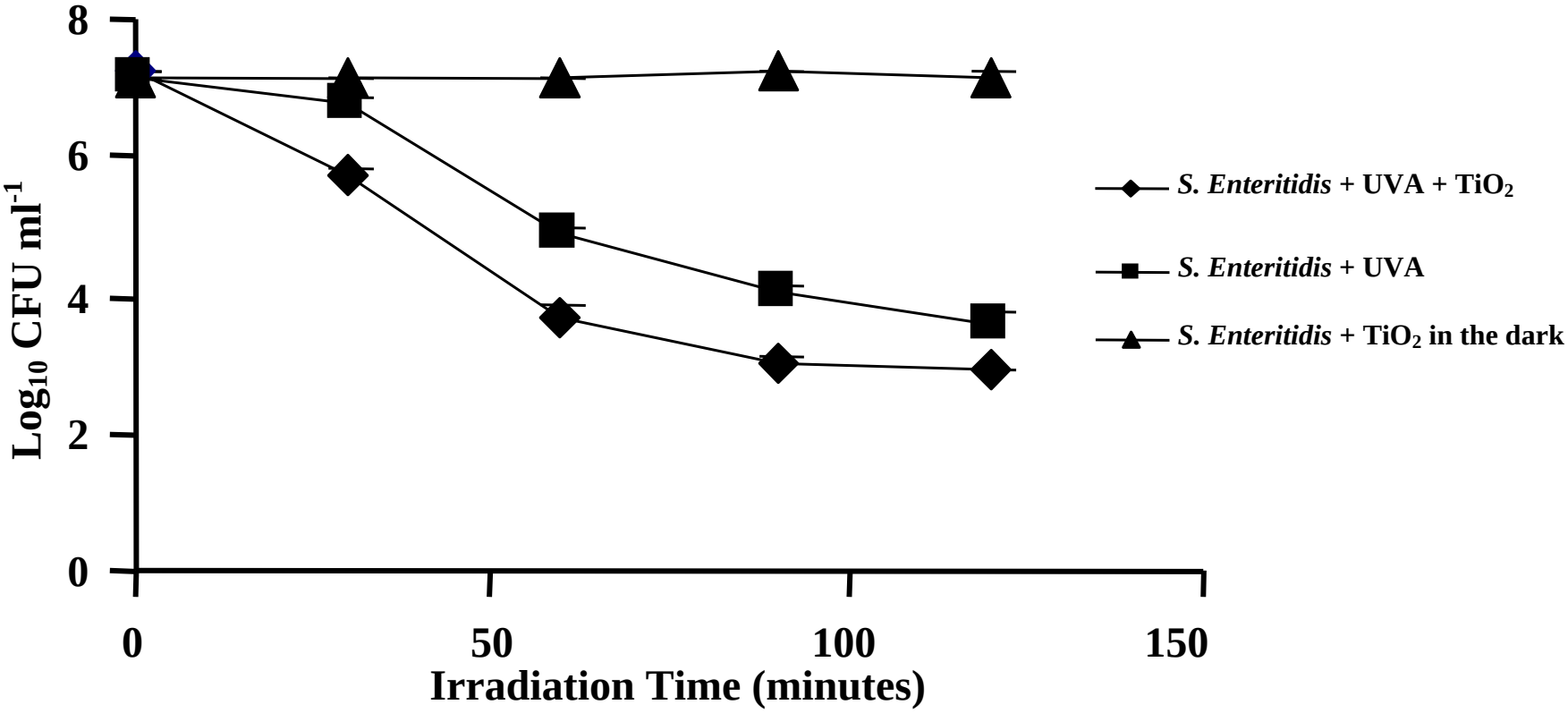
8
6
4
2
0
Log10 CFU ml-1
0
50
100
150
Irradiation Time (minutes)
S. Enteritidis + UVA + TiO2
S. Enteritidis + UVA
S. Enteritidis + TiO2 in the dark

Figure 3.

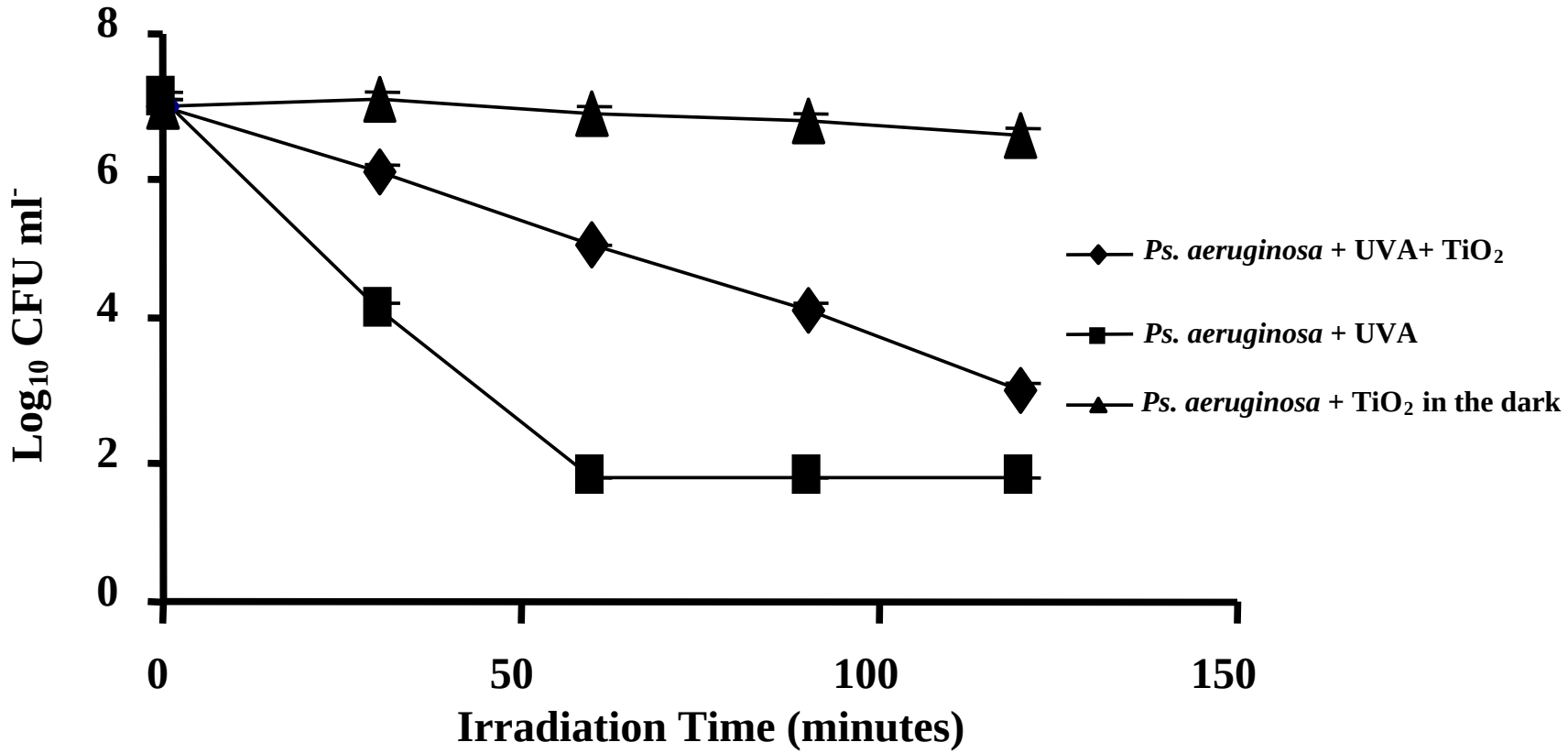
8
6
4
2
0
Log10 CFU ml-
0
50
100
150
Irradiation Time (minutes)
Ps. aeruginosa + UVA+ TiO2
Ps. aeruginosa + UVA
Ps. aeruginosa + TiO2 in the dark

Figure 4

Figure 5