Sensitivity of bacteria to photoactivated titanium dioxide in comparison with UV irradiation

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Titanium dioxide was used as a photocatalyst to generate hydroxyl radicals in a flowthrough water reactor. Experiments were performed with cultures of Aeromonas hydrophila AWWX1 and Pseudomonas fluorescens R6 to evaluate the disinfection capabilities of the reactor. Although a decrease in viable counts was observed with long-wavelength (λ=370 nm) irradiated TiO₂ pellets, direct UV₂₅₄ irradiation seems a superior technology for the disinfection of transparent potable water since the viable counts of the test strains declined stronger (2-5 logs) and faster (20×) in UV₂₅₄-treated water than in photoactivated TiO₂-treated water. Outdoor tests conducted in the summer noonday sun showed that the viable counts of Aeromonas hydrophila AWWX1 decreased strongly (ca 5 log units) in transparent and turbid water samples (750 NTU) exposed to natural sunlight (47,000 lux). The addition of TiO₂ to the solar irradiated waters did not influence the die-off of the strain. These observations indicate that the photocatalytic approach does not offer real prospects as an alternative technology for the disinfection of drinking water.

Generally, chlorine is used for the disinfection of drinking water. Although this method is effective and cheap, its use is best restricted to high quality groundwater (i.e. water low in total organic carbon) since chlorine has been found to react with a variety of organic impurities in water, generating trihalomethanes and other (carcinogenic) disinfection by-products (DBPs). Regulations proposed by the EEG² to limit the concentrations of DBPs necessitate the evaluation of new technologies for the disinfection and treatment of water.

One alternative technology for disinfection of water is UV irradiation. Low pressure mercury vapour burners, which emit UV light with a predominant wavelength of 254 nm, are generally the most favoured UV sources for the inactivation of water-borne microorganisms. UV₂₅₄ light produces photochemical changes in the pyrimidine bases of DNA that hinder the bacteria from normal reproduction³. Likewise, free radicals might act as a biocide. In contrast to the damage on nucleic acids wrought by UV light at 254 nm, the impact of free radicals is relatively broad, affecting also lipids and proteins in addition to nucleic acids⁴. Various water treatment technologies produce hydroxyl radicals (OH⁻) in minuscule quantities (i.e. < 10⁻¹² M)⁵. These treatments include ozonation, direct photolysis of hydrogen peroxide and radiolysis. Free radicals are also generated when semiconductor powders, such as titanium dioxide (TiO₂) are irradiated with near-UV light⁶. The photocatalytic degradation of various harmful-organic and inorganic compounds by illuminated TiO₂ is well-documented⁷. Recently, the bactericidal properties of TiO₂ photocatalysts were studied by Ireland et al.⁶, Biguzzi and Shama⁸ and Wei et al.⁹. They observed rapid cell death of pure bacterial cultures when TiO₂ was illuminated with near-UV light (wavelength of less than 400 nm). Hence, this mechanism might provide a suitable alternative for the disinfection of drinking water. Moreover, such an approach could be applicable in developing countries for the disinfection of drinking water since the UV light required to activate the catalyst is the natural UV component of sunlight.

In this study, the effect of photoactivated TiO₂ on the survival of two prokaryotic test organisms
was examined and further compared with their sensitivity to UV<sub>254</sub> irradiation. *Aeromonas hydrophila* was chosen as a test organism because of its frequent occurrence in various aquatic environments<sup>2,13</sup> and its association with a broad spectrum of human diseases<sup>14</sup>. The survival of the *A. hydrophila* strain was further compared with that of *Pseudomonas fluorescens*, a strain isolated from grassland rhizosphere<sup>15</sup>. Outdoor tests with TiO<sub>2</sub> were also performed to examine the practical feasibility of a solar-assisted TiO<sub>2</sub> catalytic disinfection approach.

**Materials and Methods**

**Titanium dioxide**

TiO<sub>2</sub> pellets comprising principally the anatase form, were donated by Shell, Gent.

**Bacterial strains**

Disinfection studies were performed with genetically manipulated strains of *A. hydrophila* and *P. fluorescens*. *A. hydrophila* AWWX1 is a kanamycin and tetracycline resistant derivative of *Aeromonas* strain AWW88 which was isolated from a sand filter in the Oelegem (Belgium) drinking water production plant<sup>16</sup>. *P. fluorescens* strain R<sub>f</sub>f is a kanamycin resistant derivative of a *Pseudomonas* strain isolated from grassland rhizosphere soil<sup>15,17</sup>. Stock cultures of *A. hydrophila* strain AWWX1 were maintained at -70°C in tryptic soy broth (Difco) containing 15% (v/v) glycerol, 300 mg L<sup>-1</sup> tetracycline (Tc) and 300 mg L<sup>-1</sup> kanamycin (km). Stock cultures of *P. fluorescens* strain R<sub>f</sub>f were maintained at -70°C in tryptic soy broth (Difco) containing 15% (v/v) glycerol and 50 mg L<sup>-1</sup> km.

**Plate counts**

Viable counts were determined by either the spread plate procedure or the membrane filtration technique, using 0.45-μm filters (Millipore). When necessary, samples were serially diluted in 0.85% (w/v) NaCl and 100 μL-samples were plated on the appropriate media. The medium described by Havelaar et al.<sup>18</sup> supplemented with 300 mg km L<sup>-1</sup> and 300 mg Tc L<sup>-1</sup> instead of 10 mg ampicillin L<sup>-1</sup>, was used for the isolation of *A. hydrophila* AWWX1. The plates were incubated at 28°C and colonies were counted after 24 h. *Pseudomonas fluorescens* R<sub>f</sub>f was enumerated after 3 h of incubation at 28°C on casamino acid agar supplemented with 50 mg km L<sup>-1</sup>. Casamino acid agar consisting of casamino acids (Difco), 5 g; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.25 g; K<sub>2</sub>HPO<sub>4</sub>·3H<sub>2</sub>O, 1.18 g; agar, 18 g and 1000 mL of distilled water.

**Reactor configuration**

The photoreaction systems used for this study consisted of one continuous UV<sub>254</sub> flow reactor and two continuous UV<sub>370</sub> flow reactors. Equipment employed for UV<sub>254</sub> irradiation consisted of a glass column and a UV lamp. The source of UV light was a blacklight blue TUV 30W G30T8 UV-C tube (Philips Lighting, Brussels) with a maximum peak emissivity at 254 nm. The lamp was mounted coaxially within the glass column. The same reactor configuration was used for studying the bactericidal properties of TiO<sub>2</sub> photocatalysts illuminated with near-UV light. One UV<sub>370</sub> reactor was filled up with TiO<sub>2</sub> pellets to generate OH<sup>-</sup> radicals, whereas the control reactor was used for studying the bactericidal effect of near-UV light. A cross-section of a UV<sub>370</sub> reactor with TiO<sub>2</sub> pellets is shown in Fig. 1. Two tubular low-pressure mercury-vapour fluorescent lamps emitting UV radiation between 300 and 460 nm, with a maximum at 365 nm were used as sources of near-UV light (TL 40W/05, Philips Lighting, Brussels). All reactors were fed with varying water flow rates to obtain different residence times in the systems.

![Fig. 1—Cross-section of a UV<sub>370</sub> reactor with TiO<sub>2</sub> pellets; (1) influent; (2) effluent; (3) UV<sub>370</sub> lamp; (4) TiO<sub>2</sub> pellets and (5), silicon layer](image-url)
Inoculum preparation
The test strains were inoculated into tryptic soy broth, incubated at 28°C on a shaker (100 rpm) and harvested after an 18 h incubation by centrifugation (14,000×g, 5 min). The supernatants were discarded and the pellets resuspended in 0.85% (w/v) NaCl. This procedure was repeated three times. Following the final resuspension, the cultures were inoculated in tap water.

Disinfection experiments
For the disinfection experiments, a plastic carboy was filled with 10 L of tap water and inoculated with A. hydrophila AWWX1 and P. fluorescens R2f to yield starting concentrations of 10^5 to 10^6 cfu mL^-1. Control samples (UV dose zero) were collected from the reservoir before the water entered the continuous flow reactors. The possibility that bacterial cells were being adsorbed onto the surface of the TiO_2 pellets, rather than being inactivated was examined by repeating the experiment in the absence of UV light. Samples (ca. 300 mL) were taken at the outlet of the glass columns and analyzed for Aeromonas hydrophila AWWX1 and Pseudomonas fluorescens R2f.

Outdoor experiments
A 2-liter Aeromonas suspension was irradiated in uncovered Pyrex pots (140×200 mm), containing either 2 g of flattened TiO_2, 1 g CaCO_3, 1 g CaCO_3 and 2 g TiO_2, or no compounds at all. The suspension became highly turbid after the addition of CaCO_3 (750 NTU) or TiO_2 (430 NTU). A magnetic stir bar was used to mix the slurry in the pots in order to keep the TiO_2 and CaCO_3 from settling down. The pots were irradiated with sunlight for 90 min, with samples being taken just before and after 30, 60 and 90 min of irradiation. Sunlight intensity was measured using a radiometer (Metrux K, Metrawatt). The experiment was repeated in the absence of sunlight.

Data analysis
Graphs of fractional survival were plotted against contact time (min). Fractional survival is defined as the number of viable cells remaining at any time divided by the initial viable cell count. The notation 1e-1 means 10 exponent -1 = 0.1, etc.

Results
Effect of near-UV light on the bactericidal activity of TiO_2
The fractional survival curves of A. hydrophila AWWX1 and P. fluorescens R2f, both exposed to near-UV light and irradiated TiO_2 are shown in Fig. 2. The number of viable Aeromonas cells decreased when the cell suspension was pumped through the photoactivated TiO_2 reactor. The decline in cell numbers increased proportionately with contact time. Aeromonas counts declined approximately 4 log units after 1 h irradiation with 1.2×10^5 cfu mL^-1 surviving. Photoactivated TiO_2 showed also bactericidal activity towards P. fluorescens R2f. The number of pseudomonads declined approximately 1 log unit with 1.2×10^5 cfu mL^-1 surviving (Fig. 2). No bactericidal activity towards A. hydrophila AWWX1 and P. fluorescens R2f was observed when the cells were exposed to near-UV light in the absence of TiO_2 (Fig. 2). The possibility that cells were being adsorbed onto the surface of the TiO_2 pellets, rather than being suppressed could be ruled out as the decrease in cell counts achieved in the absence of UV light (data not shown) was the same as shown for bacterial cells exposed to the UV source in the absence of TiO_2 (Fig. 2).

Bactericidal activity of UV_254 irradiation
The effect of UV_254 light on the survival of A. hydrophila AWWX1 and Ps. fluorescens R2f is shown in Fig. 3. The number of viable Aeromonas and Pseudomonas cells declined rapidly when the
Fig. 3—Bactericidal activity of UV_{254} irradiation. Cell suspensions of *Aeromonas hydrophila* AWWX1 (10^6 cfu mL^{-1}) and *P. fluorescens* RSf (10^6 cfu mL^{-1}) were irradiated with UV_{254} light. (○), *P. fluorescens* RSf; and (■), *A. hydrophila* AWWX1.

Fig. 4—Effect of supplementation of tap water (TW) with TiO_2 and CaCO_3 on the survival of *A. hydrophila* AWWX1 during irradiation with natural sunlight. The incident photon flux was 47,000 lux (470 W m^{-2}). (○), TW; (△), TW + TiO_2; (■), TW + CaCO_3; (●), TW + CaCO_3 + TiO_2; (---), sunlight; and (---), darkness.

Cell suspensions were irradiated with UV_{254} light. A reduction of 4 and 6 logs was achieved after 1 min irradiation for *P. fluorescens* RSf and *A. hydrophila* AWWX1, respectively.

Effect of TiO_2 on the survival of *A. hydrophila* AWWX1 irradiated with natural sunlight

Outdoor experiments were performed on a terrace of the Faculty of Agricultural Sciences under summer (August) noonday sun in Gent. The incident photon flux was 47,000 lux. *Aeromonas* counts decreased ca. 5 log units when TiO_2 was illuminated with natural sunlight for 90 min. A similar decrease in bacterial counts was observed in the other examined waters (tap water; tap water supplemented with (i) CaCO_3 and (ii) CaCO_3 + TiO_2). No bactericidal activity was shown by exposing the *Aeromonas* cells to TiO_2 and CaCO_3 in the dark (Fig. 4).

Discussion

Light-sensitized photochemical reactions involving TiO_2 have been successfully used for the degradation of contaminants in water^{19}. This study investigated the use of this process for the disinfection of water.

The viability of the examined test strains *A. hydrophila* AWWX1 and *P. fluorescens* RSf in water flowing through the UV_{370} reactor with photoactivated TiO_2 pellets decreased significantly (Fig. 2). Wavelengths in the near-UV region could not be responsible for this phenomenon as cell numbers remained constant upon exposing the microorganisms to near-UV light in the absence of TiO_2 (Fig. 2). Attachment of bacteria to TiO_2 pellets could equally be discounted as the reduction in cell counts in the absence of UV_{370} was similar to that shown for bacterial cells exposed to the UV_{370} source in the absence of TiO_2. It could therefore be concluded that long-wavelength (370 nm) irradiated TiO_2 pellets cause deactivation of bacteria. This corroborates the findings of Biguzzi and Shama^{16}, who reported a reduction in the concentration of viable *Pseudomonas stutzeri* of 6 orders of magnitude after 6 h irradiation when cultures were treated with 4 g TiO_2 L^{-1} at an incident UV_{370} intensity of 8.1 W m^{-2}. Matsunaga et al.^{20,21} found that cultures of *Saccharomyces cerevisiae* and *E. coli* were completely eliminated after 120 min when treated with 1 g TiO_2 L^{-1} (platinized) at a light intensity of 1200 W m^{-2}. The initial cell count in their experiments was only 10^2-10^3 cfu mL^{-1}. At concentrations above 10^4 cfu mL^{-1}, the survival of cells increased with initial cell concentration. They concluded that hygienisation with photosemiconductor particles was only suitable for samples containing a low concentration of bacteria (below 10^3 cfu mL^{-1}). The incomplete elimination
of the test strains in this study may therefore be attributed to the high initial concentration of bacteria in the examined waters (2.2×10^6 pseudomonads mL^-1 and 3.5×10^6 aeromonads mL^-1, respectively). Survival characteristics were clearly organism-dependent as the present results show that P. fluorescens was more resistant to the bactericidal properties of illuminated TiO₂ than Aeromonas hydrophila. Matsunaga et al. also found that the sensitivity to photoactivated TiO₂ differed for various organisms.

A mechanism by which illuminated TiO₂ generates chemical species (free radicals and hydrogen peroxide), that are highly toxic towards living cells, has been outlined by Teichner and Formenti. Irradiation of TiO₂ with light of less than 400 nm results in electrons in the conduction band (e^-cb) and positive holes in the valence band (h^+vb):

\[
\text{TiO}_2 + h\nu \rightarrow e^-_{\text{cb}} + h^+_{\text{vb}}
\]

At the TiO₂ particle surface the holes react with either adsorbed H₂O or surface OH groups to form HO' radicals:

\[
h^+_{\text{vb}} + \text{H}_2\text{O (ads.)} \rightarrow \text{HO}^+ + \text{H}^+ \text{ or } h^+_{\text{vb}} + \text{OH (sur.)} \rightarrow \text{HO}^-
\]

The electrons will react with molecular oxygen to form superoxide ions,

\[
e^-_{\text{cb}} + \text{O}_2 \rightarrow \text{O}_2^-
\]

which further leads to the production of either HO' radicals:

\[
2 \text{O}_2^- + 2 \text{H}_2\text{O} \rightarrow 2 \text{HO}^+ + 2 \text{OH}^- + \text{O}_2
\]

or hydrogen peroxide according to the following reactions:

(i) \[
\text{O}_2^- + \text{H}^+ \rightarrow \text{HO}_2^-
\]

(ii) \[
\text{HO}_2^- + e^- \rightarrow \text{HO}_2^-
\]

(iii) \[
\text{HO}_2^- + \text{H}^+ \rightarrow \text{H}_2\text{O}_2
\]

Matsunaga et al. reported that intracellular coenzyme A was photoelectrochemically oxidized with semiconductor powders, resulting in both inhibition of respiration and death of microbial cells.

To investigate the potential of the photocatalytic approach as an alternative technology for the disinfection of drinking water, the bactericidal properties of illuminated TiO₂ were compared with those of UV₂₅₄ irradiation. Pseudomonas and Aeromonas appeared to be much more sensitive to UV₂₅₄ than to photoactivated TiO₂ (Figs 2 and 3). These findings show that straight UV₂₅₄ light is unequivocally preferable to illuminated TiO₂ pellets for the disinfection of transparent potable water. Nevertheless, the semiconductor might be useful for the disinfection of (turbid) drinking water in developing countries since (i) according to Lund and Hongve only waters with very low and consistent turbidity can be effectively treated with UV₂₅₄ and (ii) no energy source would be required in these hot climates because the catalyst may be activated by natural sunlight. However, outdoor experiments with A. hydrophila AWWX1 showed that cell viability in the waters with TiO₂ decreased as much as in the solar irradiated controls (transparent tap water, and turbid tap water due to the addition of CaCO₃) (Fig. 4). Results clearly show that photocatalysis did not have a significant germicidal effect in solar irradiated waters. In contrast with these observations, Wei et al. found that the combination of heat, natural sunlight and photocatalysis resulted in a faster killing of E. coli in comparison to the control without photocatalyst. A survey of the literature indicated that solar radiation exerts a great influence on culturable densities of bacteria in open waters, bringing about a higher die-off than other environmental factors (i.e. suboptimal water temperature, nutrient deficiencies, predation by protozoa, lysis by bacteriophages). Gameon and Gould reported that the inactivation rate in sunlight is typically 2 or more orders of magnitude greater than in the dark. Findings show that solar irradiation is the dominant factor that causes the die-off of the test strain in the transparent and turbid water samples (with CaCO₃ and/or TiO₂) since no bactericidal activity was observed in the dark (Fig. 4). The finding that natural sunlight has a germicidal effect in highly turbid water samples contradicts with the results of Fujioka et al., who found that the mechanism of solar disinfection requires low-turbidity water samples (< 200 NTU).

In conclusion, long-wavelength irradiated TiO₂ pellets caused deactivation of bacteria. However, UV₂₅₄ is preferable to photoactivated TiO₂ for the disinfection of transparent potable water since the test organisms were much more sensitive to UV₂₅₄ irradiation. Disinfection of (turbid) drinking water at sunny sites in developing countries by solar irradiated TiO₂ suspensions seems useless since the
supplementation of the photocatalyst to solar radiated waters did not cause an additional die-off of the test strain compared to natural sunlight. These observations allow to conclude that the photocatalytic approach does not offer real prospects as an alternative technology for the disinfection of drinking water.

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