Bactericidal and detoxification effects of irradiated semiconductor catalyst, TiO₂

C. Srinivasan* and N. Somasundaram
Department of Materials Science, Madurai Kamaraj University, Madurai 625 021, India

The successful photochemical splitting of water with a TiO₂ electrode has established that TiO₂ has to be considered as one of the most important semiconductor photocatalysts. This photocatalyst has been shown to be efficient to mineralize several organic compounds and thereby remove a variety of pollutants and wastes. For safe drinking water, the present environmental conditions demand new technologies for the disinfection and treatment of water. One of the promising applications of TiO₂ photocatalysis is the photocatalytic removal of bacterial pollutants from drinking water. This semiconductor photocatalyst has been employed as a suspended powder or thin film on a suitable substrate or as a coated electrode, and the irradiated TiO₂ has been found to be efficient to sterilize water containing E. coli. Cyanobacterial toxins such as microcystins present in drinking water are also destructed using TiO₂ photocatalysis.

Since the spectacular discovery of photocatalytic cleavage of water on TiO₂ electrodes by Fujishima and Honda¹, interest in the use of photocatalysts, particularly TiO₂ has received greater attention in recent years². Three types of TiO₂ are abundant in nature: brookite (orthorhombic), anatase (tetragonal) and rutile (tetragonal). Two well-known commercially available TiO₂ powders used extensively in photocatalytic studies are Degussa P-25 (~75% anatase, ~25% rutile) and Aldrich (~99.9% anatase). TiO₂ in the anatase crystal form is a semiconductor with a band gap of 3.2 eV or more. When TiO₂ particles are irradiated with near UV irradiation (λ>350 nm), electron–hole pairs are generated on the photocatalyst surface. It has been reported that the hole in the valence band has a positive redox potential and is capable of oxidizing an organic substrate (RX) adsorbed on the catalyst surface and also water or hydroxide ions to form hydroxyl radicals in water³ (eqs (2–4)).

\[
\text{TiO}_2 + h\nu \rightarrow \text{TiO}_2 (e^- + h^+) , \quad (1)
\]

\[
\text{TiO}_2 (h^+) + RX_{ad} \rightarrow \text{TiO}_2 + RX_{ad}^{++} , \quad (2)
\]

\[
\text{TiO}_2 (h^+) + H_2O_{ad} \rightarrow \text{TiO}_2 + ^{\prime}\text{OH}_{ad} + H^+ . \quad (3)
\]

\[
\text{TiO}_2 (h^+) + ^{\prime}\text{OH}_{ad} \rightarrow \text{TiO}_2 + ^{\prime}\text{OH}_{ad} . \quad (4)
\]

The electron promoted from the valence band to the conduction band is readily available for transfer, and it reduces O₂ to superoxide, \(O_2^-\) (Figure 1).

\[
\text{TiO}_2 (e^-) + O_2 \rightarrow \text{TiO}_2 + O_2^- , \quad (5)
\]

\[
O_2^- + H^+ \rightarrow \text{HO}_2^- , \quad (6)
\]

\[
O_2^- + \text{HO}_2^- \rightarrow O_2 + H_2O_2 , \quad (7)
\]

\[
\text{TiO}_2 (e^-) + H_2O_2 \rightarrow \text{TiO}_2 + \text{OH}^- + ^{\prime}\text{OH} . \quad (8)
\]

Other reactive species, perhydroxy radical, \(^{\prime}\text{OOH}\) and H₂O₂ are also formed during the illumination of TiO₂ (eqs (6–8)). These reactive oxygen species can interact with the surrounding organic molecules or species and yield a variety of products. The \(^{\prime}\text{OH}\) radicals are particularly highly active for both the oxidation of organic substances and the inactivation of bacteria and viruses. The hydroxyl radicals are far more oxidizing (2.8 V) than many materials that are commonly employed for disinfection of water, including ozone (2.07 V), hydrogen peroxide (1.78 V), hypochlorous acid (1.49 V) and chlorine (1.36 V)⁴. The detailed investigations of photocatalytic reactions involving TiO₂ have established their

*For correspondence. (e-mail: ceesri@yahoo.com)
practical importance in areas such as solar energy conversion, organic functional group transformations, environmental clean-up and self-cleaning. TiO₂ is widely employed as the semiconductor photocatalyst due to its nontoxic nature, chemical stability, availability at a reasonable cost and capability of repeated use without substantial loss of catalytic activity. Further, TiO₂ photocatalysis does not require the addition of consumable chemicals and does not produce hazardous waste products. In recent years it has been shown that by utilizing semiconductor particles as photocatalysts, harmful organic contaminants such as aromatic and haloaromatic compounds and other pollutants can be degraded and mineralized. The purification of water for use as safe drinking water necessitates the evolvement of new technologies for the disinfection and treatment of water. The use of free chlorine as a disinfectant will have to be limited to high-quality groundwater (or water treated to reduce the total organic carbon) in order to meet the disinfection by-product (DBP) regulations. Chlorination of groundwater high in total organic carbon would produce unacceptable levels of trichloromethanes and other carcinogenic DBP as secondary pollutants. Ozonization is also not effective for the inactivation of some pathogens like Cytosporidium. Therefore, alternative technologies are required for water purification, and for this purpose semiconductor photocatalysts will be attractive agents. Since most of the organic compounds are decomposed on photoexcited TiO₂, it is expected that microorganisms which also contain organic molecules should also be destroyed on it, regardless of the fact that species and disinfection approach through this route will be more advantageous. Therefore, photocatalysts are expected to play a major role in the destruction of bacteria and also in causing detoxification. This review briefly highlights some of the important studies in recent years on the bactericidal and detoxification effects of illuminated TiO₂, which has been beneficially employed as powder or thin film on a suitable substrate or as a coated electrode.

**Sterilization on irradiated TiO₂ powders**

As early as 1985, Matsunaga et al. discovered that Lactobacillus acidophilus, Saccharomyces cerevisiae and Escherichia coli were completely sterilized when incubated with platinum-loaded TiO₂ particles under metal halide lamp irradiation for 60–120 min. This has opened a new avenue for sterilization and motivated attempts to use this novel photocatalytic technology for disinfecting water and in the removal of bioaerosols from indoor environments. In this and other reports of photocatalytic treatment, TiO₂ particles are dispersed in the water to be treated and illuminated with light. Ireland et al. have also confirmed the application of TiO₂ for the inactivation of E. coli, employing UV-light in the wavelength range of 300–400 nm.

A detailed study by Wei et al. on solar-assisted water disinfection system using TiO₂ photocatalyst corroborates the findings of Matsunaga et al. and Ireland et al. on the bactericidal activity, and established that irradiation of suspensions of E. coli and TiO₂ with UV-visible light of wavelengths longer than 380 nm resulted in the complete killing of the bacteria within minutes. Studies with different compositions of the gas (O₂-N₂) flowing in the solutions exerted a dramatic effect on the bactericidal activity of irradiated TiO₂. There was no bactericidal activity in the presence of 100% N₂, and the presence of O₂ was found to be a prerequisite for the bactericidal activity as it will favour formation of reactive oxygen species. Kinetic studies revealed that the bacterial killing follows first order in E. coli concentration. The disinfection rate constant was proportional to the square root of the concentration of TiO₂ and proportional to the incident light intensity. If electron–hole, i.e. charge recombination is the dominating process, it would lead to 0.5 dependence on the intrinsic catalytic efficiency. Though Rajeshwar and co-workers considered the formation of •OH radicals, they are of the opinion that the mechanistic details associated with the further fate of •OH radicals and their role in the destruction of E. coli require further investigation. The generation of H₂O₂ at the TiO₂/water interface may also complicate the process. The adverse influence of free radicals on cell DNA replication and the modification of cellular membranes (e.g. lipid peroxidation) have already been documented. On the other hand Matsunaga et al. suggest an alternative mechanism involving the direct oxidation of intercellular coenzyme A to its dimeric form, which was the root cause of decrease in respiratory activity that led to cell death.

Recently, employing Degusa P-25 TiO₂ as a suspension and a Hanau Suntest lamp for the photocatalytic inactivation of E. coli K12, Rincoin and Pulgarin observed that some parameters such as light intensity, extent of continuous irradiation, catalyst concentration and temperature have a positive effect on disinfection. In order to mimic solar-UV power which varies with time, especially when cloud passes, intermittent illumination was employed and the results indicated that an increase in time was required for E. coli inactivation. The interruption of illumination provides time for bacteria to recover by a self-defense mechanism against oxidation stress, resulting in the production of superoxide dismutase (SOD) enzymes and in some cases, catalyse. SOD enzyme accelerates the disproportionation of O₂⁻ (precursors of •OH) into H₂O₂ and molecular oxygen (eq. (9)) and catalyse eliminates the photogenerated H₂O₂ (eq. (10)).

\[ 2O_2^- + 2H^+ \xrightarrow{\text{SOD}} O_2 + H_2O_2, \quad (9) \]
\[ H_2O_2 + H_2O \xrightarrow{\text{catalyse}} O_2 + 2H_2O. \quad (10) \]
Another important finding is that no bacterial growth was observed after illumination in suspended TiO$_2$ and then keeping in the dark for 3 h. This is in contrast to the complete recovery of bacteria on illumination in the absence of the catalyst and after 3 h in the dark.

While confirming the previous findings that illuminated TiO$_2$ exhibits bactericidal activity$^{17,19,22,24}$, Mannes et al.$^{25}$ report that disinfection is positively correlated with the TiO$_2$ dose used up to a concentration of 1 mg ml$^{-1}$. For the first time, these authors presented evidence that the lipid peroxidation reaction is the underlying mechanism of death of *E. coli* K-12 cells that are irradiated in the presence of TiO$_2$ catalyst. In order to estimate cell-membrane damage, the production of malondialdehyde (MDA), a product of lipid peroxidation by *E. coli* cells, was employed as an index. They observed that there was an exponential increase in the production of MDA, whose concentration reached 1.1 to 2.4 mol mg$^{-1}$ (dry weight) of cells$^{-1}$ after 30 min of illumination, and the kinetics of this process paralleled cell death. Under these conditions, concomitant losses of 77 to 93% of the total lipid, 86 to 97% of the cell respiratory activity were detected, as measured by both oxygen uptake and reduction of 2,3,5-triphenyltetrazolium chloride from succinate as electron donor. It was also observed that there was a decrease in MDA on prolonged irradiation, after about 30 min. This has been attributed to two factors: (i) as MDA is quite reactive, it is able to modify proteins via carboxylation or form protein–MDA adducts, and (ii) MDA is oxidatively destroyed by TiO$_2$ photocatalysis. The authors concluded that the various reactive oxygen species such as *OH, O$_2^*$ and H$_2$O$_2$ generated on the irradiated TiO$_2$ surface in concerted operation, attack polyunsaturated phospholipids in *E. coli*. The lipid peroxidation reaction that subsequently causes a breakdown of the cell-membrane structure and therefore its associated functions, is the mechanism underlying cell death.

In all the above studies, TiO$_2$ powder is commonly employed in the form of slurry or suspension. In spite of the fact that this method provides a high catalyst surface area to volume ratio for pollutant hydroxyl radical interaction, the catalyst has to be removed by a post-treatment separation stage, which may not be cost-effective on a large scale. This led to development of methods wherein the removal of the catalyst is avoided.

**Photocatalytic bactericidal effect of TiO$_2$ thin films**

Supported photocatalyst is advantageous under continuous flow, as the recovery steps such as filtration and decantation can be avoided. However, the following factors that influence the photocatalytic activity of immobilized TiO$_2$ should also be taken into consideration:

(i) Diminution of specific surface catalyst accessible to light and bacteria; (ii) TiO$_2$ support could enhance the recombination of photo-generated electron–hole pairs; (iii) limitation of oxygen diffusion in the deeper layers of TiO$_2$; (iv) mean distance between bacteria and immobilized TiO$_2$ increases and causes a diminution of the probability of attack by *OH compared to suspended TiO$_2$, and (v) due to catalytic fixation, there is no penetration of the little TiO$_2$ beads (30–50 nm) into bacteria (~1 μm) to cause intercellular damage.

Matsunaga et al.$^{22}$ successfully constructed a practical photochemical device in which TiO$_2$ powder was immobilized on an acetylcellulose membrane.

Fujishima and co-workers$^{26}$ have extensively studied photocatalytic effect of TiO$_2$ thin films. TiO$_2$ thin films were prepared by a conventional dip-coating technique on silica-coated soda-lime glass plates. These films are transparent in the visible region and their high photocatalytic efficiency has been demonstrated. In their study on the bactericidal effect of TiO$_2$ thin films (Figure 2), Fujishima and co-workers$^{26}$ observed that the survival ratio for *E. coli* in the liquid film on the illuminated (1 mW/cm$^2$) TiO$_2$ film decreases to a negligible level (i.e. essentially complete sterilization) within 1 h. In the absence of TiO$_2$, UV illumination caused only 50% sterilization in 4 h. To know which active oxygen species are responsible for the bactericidal effect and the possible mecha-
nism, the reactions were carried out in the presence of mannitol, catalyse, etc. The addition of mannitol, a scavenger for •\( \text{OH} \) radical, suppresses the bactericidal effect. Though this may lead one to conclude that the main bactericidal reagent is \( \cdot \text{OH} \), one has to be cautious in arriving at the conclusion, as mannitol also inhibits the formation of \( \text{H}_2\text{O}_2 \) via \( \cdot \text{OH} \). Also, some kinetic considerations support the view that two molecules of mannitol are required to inhibit \( \text{H}_2\text{O}_2 \) formation from two molecules of \( \cdot \text{OH} \).

When the bactericidal effect was studied in a system in which the \( E. \text{coli} \) suspension was separated from the \( \text{TiO}_2 \) surface by a porous 50 \( \mu \text{m} \) thick PTFE membrane (pore size 0.4 \( \mu \text{m} \); Figure 2), the activity was observed with an efficiency similar to that found without the membrane. The half-length of \( \cdot \text{OH} \) [the length \( \cdot \text{OH} \) can travel with its half-life \((2D_{\text{TiO}_2})^{1/2}\), where \( D \) is the diffusion coefficient for \( \cdot \text{OH} \) assumed to be \( 2.3 \times 10^{-9} \text{ m}^2 \text{s}^{-1} \)] is so small (~2.0 \( \mu \text{m} \)), that \( \cdot \text{OH} \) radical species will be deactivated before traversing the 50 \( \mu \text{m} \) thickness. Therefore, other active oxygen species will be responsible for the sterilization effect. Addition of catalyse, a well-known enzyme that decomposes \( \text{H}_2\text{O}_2 \) to water and \( \text{O}_2 \), inhibits the photocatalytic bactericidal effect in both the liquid film and membrane-separated systems. While catalyse has a significant effect in the membrane-separated system, that due to mannitol is small. These results suggest that the main bactericidal agent is not \( \cdot \text{OH} \) but \( \text{H}_2\text{O}_2 \) in this system. Both oxidation and reduction sites in photocatalysis produce \( \text{H}_2\text{O}_2 \).

**Reductive reactions:**

\[
\begin{align*}
\text{O}_2 + e^- & \rightarrow \text{O}_2^- \quad (11) \\
\text{O}_2^- + \text{H}^+ & \rightarrow \cdot \text{OH}_2 \quad (12) \\
\cdot \text{HO}_2 + e^- + \text{H}^+ & \rightarrow \text{H}_2\text{O}_2 \quad (13)
\end{align*}
\]

**Oxidative reactions:**

\[
\begin{align*}
\text{OH}^- + \text{h}^+ & \rightarrow \cdot \text{OH} \quad (14) \\
\cdot \text{OH} + \cdot \text{OH} & \rightarrow \text{H}_2\text{O}_2 \quad (15)
\end{align*}
\]

While the estimated concentration of \( \text{H}_2\text{O}_2 \) produced during photocatalysis in the membrane-separated system was \( 2 \times 10^{-7} \text{ mol L}^{-1} \), \( 10^{-7} - 10^{-4} \text{ mol L}^{-1} \) of \( \text{H}_2\text{O}_2 \) solution exhibits no killing effect. Though this may contradict the conclusion that \( \text{H}_2\text{O}_2 \) is the active species, the observed results have been explained by the cooperative effect of other oxygen species. Generally, antibacterial reagents inactivate cell viability, but pyrogenic and toxic ingredients such as endotoxins remain even after the bacteria are destroyed. Nearly 1800 people, including many children were hospitalized and twelve died in the summer of 1996 in Western Japan due to food poisoning by the toxin of \( E. \text{coli} \). The poisoning was caused by O-157 endotoxin and this inspired Fujishima’s group to examine \( \text{TiO}_2 \) photocatalysis as a means of decomposing this deadly toxin.

Investigations with the reagent endotoxin alone indicate that its concentration on the \( \text{TiO}_2 \) film decreased under black-light illumination, and its concentration was unaffected either on the \( \text{TiO}_2 \) film in the dark or on a soda-lime glass plate under black-light illumination. Main detoxification proceeds on the surface of \( \text{TiO}_2 \) thin film, as the lifetime of \( \cdot \text{OH} \) is very short. Some inactivation reactions are also likely to proceed in the solution phase by \( \text{H}_2\text{O}_2 \) (ref. 28).

It has also been observed that the survival rate of \( E. \text{coli} \) generally decreased under black-light illumination without \( \text{TiO}_2 \) (photodynamic action), but the concentration of endotoxin increased due to the fact that endotoxin is exhausted from cells when they are killed. On the other hand, on illuminated \( \text{TiO}_2 \) film the concentration of endotoxin in \( E. \text{coli} \) suspension decreased with concomitant decrease in the survival ratio of \( E. \text{coli} \). The decomposition of endotoxin from \( E. \text{coli} \) cells certainly points out that the \( \text{TiO}_2 \) photocatalyst destroys the outer membrane of the \( E. \text{coli} \) cell. Therefore, the antibacterial effects of \( \text{TiO}_2 \)-coated materials involve not only nullification of the viability of the bacteria, but also destruction of the bacterial cell.

Another study by Sunada et al., demonstrated that the antibacterial effect of \( \text{TiO}_2 \)-coated materials is not a simple bacteriostatic action, but a bactericidal action that involves the decomposition of the cell wall. Irrespective of the initial cell concentration, the destruction of intact cells involved two steps, an initial lower rate photokilling step followed by a higher rate one. With an initial cell concentration of \( 2 \times 10^3 \text{ cfu/ml} \), the rate constants of the first and second steps were 0.015 and 0.085 min\(^{-1} \), respectively, which are closer to those obtained in the powder system.

The cell envelope of \( E. \text{coli} \) consists of three layers, viz. a cytoplasmic membrane, a monolayer of peptidoglycon and an outer membrane (starting from the inside and moving outward, Figure 3). Spheroplasts, which lack the peptidoglycon and part of the outer membrane of the...
cell envelope, were prepared by Sunada et al. who found that their decay follows a single exponential decay process with a higher rate constant than that for the intact cells. These observations suggest that the cell wall (the outermost membrane and the peptidoglycan layer) may block the reactive species produced during TiO<sub>2</sub> photocatalysis (e.g. hole, •OH, O<sub>2</sub>−, H<sub>2</sub>O<sub>2</sub>), and that the cell wall of the intact cell is damaged during the initial step with lower photokilling effect.

The outermost layer of the wall consists of phospholipid, protein and lipopolysaccharide (LPS) as major constituents. If the assumption that the outermost layer is affected is correct, then the concentration of LPS should decrease during the photocatalytic process. Indeed, the authors observed a decrease in concentration of LPS with time. Atomic force microscopy (AFM) studies also support this view. AFM shows that the outermost layer which was clearly observed before illumination, disappeared after 24 h of illumination on a TiO<sub>2</sub> film. These authors are of the opinion that a combination of TiO<sub>2</sub> with some antibacterial reagents that can permeate the outer membrane could show a far superior photokilling effect.

The mechanism of photokilling initially involves decomposition of the outer membrane by the reactive species, and partial decomposition of the membrane allows the permeability of reactive species to easily reach the cytoplasmic membrane, which is attacked leading to the peroxidation of membrane lipid. Lipid peroxidation causes structural and functional disorders of the cytoplasmic membrane that leads to the loss of cell viability and cell death.

It has also been reported that TiO<sub>2</sub> immobilized on Nafion membranes deactivates E. coli with efficiencies close to those observed for bacterial suspension containing the same concentration of suspended TiO<sub>2</sub>. However, bactericidal effect for fixed TiO<sub>2</sub> on glass is diminished compared to that with the suspended one.

It has also been demonstrated that TiO<sub>2</sub> nanoparticles embedded in a hybrid organic/inorganic membrane can have bactericidal activity on E. coli under UV-light illumination.

Recently, Muraleedharan et al. have reported that a thin film of anatase-type TiO<sub>2</sub> developed on an anodized Ti6Al4V alloy can be employed as a photocatalyst for the sterilization of Pseudomonas sp., a film-forming bacteria. They have shown that there is a three order of magnitude decrease in the total viable count of bacteria (TVC) on the anodized surface under UV-light illumination with black-light blue-fluorescent lamps. They believe that the reactive free radicals like •OH, and O<sub>2</sub>− will sterilize microbial cells attached to the surface, accounting for the reduced TVC on the anodized surface. As bacterial attachment on a substratum is a dynamic process involving adhesion of bacterial cells, growth and detachment, it is quite likely that cells damaged due to photocatalytic activity of the surface get detached and actively dividing cells adhere to the surface. By this continuing process of surface attachment, photocatalytic oxidation and detachment, most of the cells in the liquid medium are also sterilized, as revealed from the very low bacteria counts in the liquid medium.

**Photoelectrocatalytic disinfection**

There are reports that degradation of formic acid and 4-chlorophenol on illumination on immobilized TiO<sub>2</sub> increases the rate with applied potential, i.e. photoelectrocatalytic process enhances the rate of degradation of organic compounds. The use of an applied field reduces recombination of UV-generated charge carriers.

To assess the potential of photocatalytic and electrochemically-assisted photocatalytic disinfection, Dunlop et al. studied the disinfection of water containing E. coli K12 using TiO<sub>2</sub> electrodes prepared by the electrophoretic immobilization of TiO<sub>2</sub> powder (Aldrich or Degussa P-25) onto titanium foil. In the sterilization studies, an initial lag phase was observed in the first 20 min. The authors concluded that the combination of increased initial bacterial stability towards disinfection, anion concentration decreasing the efficiency of photocatalytic mechanism (Ringer’s solution was employed in the study) and the provision for bacterial repair and recovery could be responsible for the lag period observed during the first 20 min of the experiments. Under open-circuit conditions, bacterial disinfection occurred at a faster rate with Degussa P-25-coated electrodes compared to the Aldrich electrodes. The higher rate with the former electrode is due to efficient charge separation as a result of defects in the crystal structure and surface morphology caused by preparation method. The rate of disinfection on both films was increased with applied positive potential. The effect of applied potential was significant under conditions of high initial cell loading and high incident light intensity. It was demonstrated by Christensen et al. that photoelectrochemical disinfection at TiO<sub>2</sub> electrodes was superior to photodisinfection by particulate slurries of Degussa P-25 TiO<sub>2</sub>. They also used in their photoelectrocatalytic studies two types of electrodes, viz. ‘thermal’ electrodes made by oxidation of titanium metal mesh and ‘sol-gel’ electrodes prepared by depositing and heating a layer of titanium gel on titanium mesh. The performance of thermal electrodes was significantly more effective than the sol-gel electrode at killing E. coli suspension in a gas-sparged reactor. For instance, even with an applied potential of 1.3 V, more than 95% of the bacteria were killed, and much more than the 15% destroyed with the sol-gel electrode at the same potential. Thus the thermal film is a more active photoelectrocatalyst but a less active photocatalyst, i.e. in the absence of an applied potential, the sol-gel electrode is more active.
ence in the behaviour of the two electrodes has been attributed to the depletion layer thickness. The application of a small potential in thermal films allows the development of a depletion layer in which the effective charge separation can occur with more effective reduction of charge-carrier combination. In the sol-gel electrode, the particles are significantly smaller than the depletion layer thickness\textsuperscript{40}, and hence are too small to show an appreciable potential drop across their width.

**Destruction of toxins by semiconductor photocatalysis**

In the earlier part of this article, the work of Fujishima’s group on the degradation of endotoxin\textsuperscript{27} of *E. coli* was discussed. Microcystins are toxins produced in freshwater systems as secondary metabolites by various cyanobacteria (blue-green algae) belonging to the genera *Microcystis*, *Anabaena*, *Nostoc* and *Oscillatoria*. The increasing eutrophication of natural water has led to an increase in the incidence of algae blooms and the consequent increased risk of microcystin contamination of water. It has been reported that the presence of microcystins in water bodies has caused illness and death of wild and domestic animals\textsuperscript{41}; and recently their presence in dialysis water resulted in human fatalities\textsuperscript{42}. Microcystins are cyclic heptapeptides and generally contain five invariant amino acids (or derivatives of them) and two variable L-amino acids, whose one-letter nomenclature abbreviations are used to name the various analogues (Figure 4). Thus, microcystin-LR contains leucine and arginine, microcystin-YR, tyrosine and arginine and microcystin-YA, tyrosine and alanine. They are hepatotoxic due to their inhibition of protein phosphates\textsuperscript{43} 1 and 2A and are potent promoters of liver cancer\textsuperscript{44}. Microcystins are chemically very stable\textsuperscript{45} over a wide range of pH and temperature due to their cyclic structure, and conventional water treatment with chlorine and ozone has limited degree of success for the destruction of microcystins in drinking water. The photolysis of microcystin using sunlight resulted in relatively slow decomposition in pure solution\textsuperscript{46}. The use of powdered and granulated, activated carbon has only limited efficacy\textsuperscript{47}. In 1999, WHO suggested a guideline\textsuperscript{47} value of 1 µg l\textsuperscript{-1} for microcystin-LR. It is essential to establish a reliable treatment strategy that will cause their removal from potable water.

The most commonly occurring cyanobacterial toxin, microcystin-LR on irradiation in the presence of TiO\textsubscript{2} resulted in the effective destruction of the toxin\textsuperscript{48}. Though the concentrations employed (µM) in the photocatalytic destruction of this toxin exceeded those occurring naturally (nM–pM), it has been shown that the much lower concentrations which can be present in drinking water will be rapidly removed by this technique. The samples that were illuminated without TiO\textsubscript{2} displayed no degradation.

The initial rate of disappearance of the substrate follows the Langmuir–Hinshelwood kinetics and the rate constant (\(k\)) and the Langmuir adsorption constant (\(K\)) have been determined as 19.23 µM min\textsuperscript{-1} and 2.9 × 10\textsuperscript{-2} dm\textsuperscript{3} µmol\textsuperscript{-1}, respectively. It is interesting to note these rates and adsorption constants are comparable with those reported for the photocatalytic destruction of other organic compounds. However, the \(K\) value for adsorption in the dark is 2.9 × 10\textsuperscript{-4} dm\textsuperscript{3} µmol\textsuperscript{-1}, which is 100 times lower than the value obtained from the initial rate of destruction. This difference has been attributed to the photoadsorption reactions at the TiO\textsubscript{2} surface or that the decomposition of the substrate may not occur exclusively at the catalyst surface, but may be promoted by hydroxyl radicals released from the catalyst surface.

In order to determine the influence of a single amino acid substitution on both the initial rates of destruction and dark adsorption, the TiO\textsubscript{2} photocatalysis of microcystin-LR, -RR, -LW and -LF was investigated\textsuperscript{49}. In each case, it was found that they could all be successfully des-

![Figure 4. Generic structure of microcystins; X and Z represent variable amino acids.](image-url)
troyed in the presence of TiO$_2$ and UV-light, and no destruction was observed in any control experiment. It was observed that dark adsorption and rate of destruction were influenced both by pH and the amino acid composition of the microcystins. The pH dependence has been associated with both changes to surface charge of the photocatalyst and altered hydrophobicity and net charge on the toxin. The rate of photocatalytic destruction has also been affected by the different amino acid compositions of the microcystin, through charge and hydrophobicity influences on the toxin molecule. Though the most rapid destruction occurred when there was high dark adsorption, dark adsorption is not essential for the photocatalytic decomposition as demonstrated by the behaviour of microcystin-LR.

Shephard et al.$^{50}$ reported the rapid decomposition of microcystins-LR, -YR and -YA from contaminated water using an experimental laboratory-scale photocatalytic ‘falling film’ reactor in which an oxygen purge, UV-radiation and TiO$_2$ were used to oxidatively decompose the microcystin pollutants. The decomposition followed first order kinetics with half-life of less than 5 min, with the reactor operating in a closed-loop mode. It was also observed that the reaction rates were strongly dependent on the amount of TiO$_2$ catalyst (1–5 g/l), but only marginally influenced by a change in gas purge from oxygen to compressed air. It is generally believed that the hydroxyl radicals generated at the semiconductor surface may be responsible for the photo-destruction process.

Generally, photocatalytic reactions are enhanced by the addition of chemical reagents such as H$_2$O$_2$, an alternative electron acceptor.$^{51-54}$ It has been reported by Rositano et al.$^{55}$ that though little toxin degradation occurred in the presence of H$_2$O$_2$, it was effective in combination with ozone. Cornish et al.$^{56}$ found that H$_2$O$_2$ with UV-illumination alone was capable of decomposing microcystin-LR, although at a much slower rate than found for TiO$_2$. When the TiO$_2$/UV/H$_2$O$_2$ system was used, the method was more effective than TiO$_2$/UV alone. Though H$_2$O$_2$ enhanced photocatalytic destruction, there was a marked decline in the dark adsorption of microcystin to the surface of the catalyst and it reduced to as little as 15%. There is a strong competition between the added H$_2$O$_2$ and the microcystin-LR for active sites on the TiO$_2$ catalyst surface. However, it has been realized that the inhibition of adsorption of microcystin-LR by the addition of H$_2$O$_2$ does not appear to affect the rate of photocatalytic toxin destruction. It has been shown that H$_2$O$_2$ can act as an alternative electron acceptor to oxygen (eq. (16)), which is thermodynamically more favourable than oxygen reduction.$^{57,58}$ \[ E^\circ = -0.13 \text{ V for O}_2 \text{ reduction, } \ E^\circ = 0.72 \text{ V for H}_2\text{O}_2 \text{ reduction.} \] Therefore, the reduction of H$_2$O$_2$ at the conduction band will produce hydroxyl radicals for attack on the toxin.$^{58}$ Hydroxyl radicals are also produced by the transfer of electron from superoxide to H$_2$O$_2$ (eq. (17)).

\[ \text{e}^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \cdot\text{OH}, \] (16)

\[ \text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \cdot\text{OH} + \text{O}_2. \] (17)

This is another instance to show that H$_2$O$_2$ greatly enhances the photocatalytic oxidation of microcystin-LR.

**Conclusion**

There are several efforts in discovering alternative technology for the sterilization of water intended for human consumption, as the conventional methods used for potable water disinfection are not efficient. With the emergence of environmental problems, simple and reliable methods to clean-up gain greater priority. The work briefly described in this article points out the potential of photocatalytic decomposition using TiO$_2$ as a means of killing bacteria and destroying microcystins in contaminated water. In recent years, various photocatalyst-based products are available in the market for applications such as indoor air-cleaning, etc. TiO$_2$ tiles with self-cleaning and bactericidal function have already been commercialized.$^{59}$ Typical room-lighting illuminations will be sufficient to kill several bacteria. Thus ~80% of *E. coli* on TiO$_2$-coated tile was destroyed on exposure to an ordinary fluorescent lamp for 3 h under such low-intensity UV-light as 0.8 μW/cm$^2$ or to illumination with an incandescent lamp.$^{60}$
