Photocatalytic inactivation of *Flavobacterium* and *E. coli* in water by a continuous stirred tank reactor (CSTR) fed with suspended/immobilised TiO$_2$ medium

Vered Cohen-Yaniv, Nava Narkis and Robert Armon

ABSTRACT

A photocatalytic continuous stirred tank reactor (CSTR) was built at laboratory scale to inactivate two environmental bacteria strains (*Flavobacterium* and *E. coli*) in tap water. Several parameters were found to impact reactor efficiency. Bacterial initial concentration is an important factor in inactivation rate. After 30 minutes of irradiation at $10^8$–$10^9$ CFU mL$^{-1}$ starting concentration, a >5 log reduction was achieved while at $10^4$–$10^6$ CFU mL$^{-1}$ only a 2 log reduction was observed. Water hardness and pH have an important influence on the photocatalytic inactivation process. Soft water, with low Ca$^{2+}$ and Mg$^{2+}$ at low pH ~ 5.3 resulted in increased inactivation of *Flavobacterium* reaching >6 orders of magnitude reduction. *E. coli* and *Flavobacterium* at pH 5 were inactivated by 3 logs more as compared to pH 7 under similar conditions. pH below TiO$_2$ isoelectric point (approximately 5.6) supports better contact between bacteria and anatase particles resulting in superior inactivation. TiO$_2$ powder suspension was compared with immobilised powder in sol-gel coated glass beads in order to exclude the need for particles separation from the treated water. TiO$_2$ suspension was more effective by 3 orders of magnitude when compared to coated glass beads. An interesting observation was found between the two bacterial strains based on their hydrophobicity/hydrophilicity balance. The more hydrophobic *Flavobacterium* compared to *E. coli* was inactivated photocatalytically by >3 logs more then *E. coli* in the first 30 minutes of irradiation interval. The results indicate the importance of the parameters involved in the contact between TiO$_2$ particles and microorganisms that govern the successful inactivation rate in CSTR.

**Key words** | CSTR, disinfection, *Flavobacterium*, photocatalysis, TiO$_2$

INTRODUCTION

Chlorination is actually the most common practice for water disinfection. In spite of its many advantages, chlorine has a major disadvantage of organo-chlorinated compounds formation during the process (Matamoros et al. 2007). These compounds are mutagenic and cancerogenic, especially the trihalomethanes (THMs). Therefore, the whole area of water disinfection looked upon new alternatives with similar or better efficiency in inactivating pathogenic microorganisms in potable water without DBPs (disinfection by products). Among these methods are: ozonation, use of ClO$_2$ (Narkis et al. 1994), UV irradiation at different wave lengths and intensity ranges and TiO$_2$ photocatalysis (Armon et al. 1998; Laot et al. 1999). Each of these methods has its advantages but there are also drawbacks that prevent their immediate implementation as the first line disinfection method for potable water. TiO$_2$ photocatalysis has been the focus of many water purification studies for the last three decades (Ollis 1985). It has a major advantage over the other methods: photocatalysis can mineralise both chemical and biological pollutants. Research is being conducted in order to make this process a commercially efficient technology for water treatment. The main goal of the present study was to...
investigate the bacteriocidal efficiency of TiO$_2$ suspended or immobilised under UV light (360 nm) irradiation at various intensities in a continuous stirred tank reactor (CSTR).

**MATERIALS AND METHODS**

**Photocatalytic continuous stirred tank reactor**

A continuous stirred tank reactor (CSTR) cylinder (28.3 cm × 6 cm × 50 cm) was made of quartz of 3.15 mm thickness. A similar cylinder reactor was made of borosilicate glass (27.3 cm × 5.9 cm × 70 cm). The reactors were placed in a plastic barrel, opened on two sides, covered with aluminium foil and holding 25 UV black light fluorescent lamps of 18 W power each (Phillips, Holland). The measured irradiance of each lamp was 0.9 mWcm$^{-2}$ and the distance from the reactor vertical axis was 11.5 cm. Each lamp was connected to an on/off button so that it could be activated manually and separately. The reactor was connected through a Tygon pipe (internal diameter 9.5 mm) to a magnetic pump (Model NH-100PX, PAN World, Japan) with a flow rate of 0.0016 m$^3$ min$^{-1}$ at 3,100 rpm. The flow velocity at the reactor inlet was 6.35 m min$^{-1}$. The inlet of the water flow was connected to the bottom part of the reactor and the outlet of the upper part of the reactor back to the pump (in a recirculatory mode). Two Nirosta filters of 40 mesh were located on the lower and upper part of the reactor at a distance of 38 cm to prevent particles escaping from the reactor. The whole system was equipped with a thermometer, pH meter and a ventilator at the bottom to prevent heating (Figure 1).

**Microorganisms tested in photocatalytic inactivation experiments**

*Flavobacterium* sp. was an isolate from our laboratory tap water (Environmental Microbiology Lab., Technion, Israel). *E. coli* CN$_{13}$ is an *E. coli* used for somatic bacteriophage detection that infects *E. coli* (from our laboratory collection) (*Armon et al. 1988; Starosvetsky et al. 2001*). Both microorganisms were grown on nutrient agar at 36°C for 24 hours before collection and batch preparation. Samples taken from the reactor during photocatalytic process were enumerated for both bacteria on R$_2$A agar by membrane filtration, as already described (*Armon et al. 1998*).

**Experimental water quality**

Different water compositions were used in the various experiments. Basically, distilled water was used as a primary medium at pH 6.3–7.5. In experiments with high levels of Fe$^{+2}$, 0.1 mg L$^{-1}$ FeSO$_4$ was added while in experiments with increased hardness CaCO$_3$ was Ca$^{+2}$ 62.5–185.2 mg L$^{-1}$, Mg$^{+2}$ 45 mg L$^{-1}$, SO$_4^{2-}$ 17.8 mg L$^{-1}$ Cl$^{-}$ 44.4 mg L$^{-1}$ at pH 5.2–8.6 accordingly.

**TiO$_2$ dopped sol-gel coated glass beads**

TiO$_2$-P25 was kindly supplied by Degussa Co. (Israel representative). The optimal concentration of 1 g L$^{-1}$ in powder form was used during the present study. Sol-gel was prepared according to already described methods (*Haruvy et al. 1992; Laot et al. 1999*). Briefly, methyltrimethoxysilane (MTMS) was used as a precursor for the sol-gel process. One hundred and twenty mL of previously prepared methanol solution was added to 75 mL MTMS while stirring for 2 minutes. An additional 4.5 mL of 0.1 M HCl was added and the mixture was stirred for an additional 5 minutes. 0.2 g of TiO$_2$ (P25) was added as powder (1 g L$^{-1}$ final concentration) to the sol-gel solution (final volume of 199.5 mL) and mixed to get a homogenous mixture, still liquid. Glass beads were introduced to the sol mixture and left to gelate as a coat on beads surface (approximately 8 minutes). The coated beads were left to dry for 24 hours at room temperature, followed by a drying period of 7 days in a
50°C incubator. A second coating process was performed similarly with these beads in order to increase coating efficiency.

**RESULTS AND DISCUSSION**

The optimal TiO$_2$ powder concentration for efficient photocatalysis is 1 g L$^{-1}$ (Armon et al. 1998). Experiments with various TiO$_2$ concentrations 0.25, 0.50, 1.00 and 1.50 g L$^{-1}$ revealed that 1 g L$^{-1}$ was the most efficient to inactivate *Flavobacterium* sp. Therefore, it was decided to use TiO$_2$ at this concentration for reactor process. As photocatalytic reaction is surface contact dependent, the effect of initial bacterial concentration was tested on TiO$_2$ inactivation (Figure 2). Initial bacterial concentrations from $10^4$ to $10^9$ CFU mL$^{-1}$ were subjected to photocatalytic process in a CSTR reactor with TiO$_2$ (P-25) suspension. As can be seen, low initial concentrations of bacteria have a slower inactivation rate and are less efficient. Pham et al. (1995) reported the same phenomenon of photocatalytic efficiency decrease with a lower initial concentration of bacteria. The explanation of this process is based on direct contact between photocatalyst and bacterial outer surface membrane and its potential to transfer the free radicals directly without intermediaries. As more bacteria will come in contact with TiO$_2$ nano-particles, with enough effective irradiation upon TiO$_2$ particles, more bacteria will be oxidised, damaged or killed during the process. Another explanation could be that under high bacterial load, more bacteria will be present within the reactor zone, close to water-air or water-solid interfaces, where irradiation is the most effective. Potable water designated for disinfection has a variety of minerals composition, basically defined as softness or hardness characteristics. In the present study, the reactor was tested under these conditions for its efficiency. Figure 3 represents the influence of Ca$^{+2}$ and Mg$^{+2}$ concentrations on photocatalytic inactivation efficiency of *Flavobacterium* bacteria at high and low pH. Reduced water hardness and low pH increased the inactivation efficiency. The inactivation process can be explained based on isoelectric point of TiO$_2$ (P25) that is below pH 5.8. At this pH, bacteria are still negatively charged while TiO$_2$ is positively charged. The opposite charges favour closer interactions of both particles and better free radicals flow between them. The positively charged cations Mg$^{+2}$ and Ca$^{+2}$ can react with bacterial negative surface, resulting in reduction of the net negative charge and cross-bridging between bacteria.

Both parameters decrease the efficiency of the process, as can be seen in Figure 3. The best inactivation rate was obtained with distilled water at pH 5.5. Vella & Veronda (1993) found the same phenomenon when studying TCE photocatalytic degradation in distilled and potable water. Carbonate ions (CO$_3^{2-}$ and HCO$_3^{-}$) are hydroxyl radicals scavengers, therefore reducing process efficiency.

Several other aspects were also tested in the present study. When comparing quartz with glass reactors, no significant different efficiency was found. Quartz has a minor advantage over glass through its better transparency.
towards UV\textsubscript{360} nm light, however, the high price of such a reactor does not justify its imperative use. The addition of 0.10 mg L\textsuperscript{-1} Fe\textsuperscript{+2} in the form of FeSO\textsubscript{4}·7H\textsubscript{2}O at pH 6.3–7.5 increased the photocatalytic efficiency against \textit{Flavobacterium}, starting at 30 minutes from experiment initiation. The overall inactivation was 1 order of magnitude higher in the presence of ferrous ions compared with distilled water. Fe\textsuperscript{+2} has some contribution, at the pH tested, to the Fenton reaction, however there is experimental proof that Fe\textsuperscript{+2} in Fenton reaction is much more efficient at lower pH (\textless 5.0) (Narkis & Shemer 2003).

Irradiation intensity was also studied with a different number of UV lamps (up to a total of 25 surrounding the reactor). Increasing the number of irradiating lamps raised the inactivation rate proportionally as already described by Laot \textit{et al.} (1999). Figure 4 represents photocatalytic inactivation experiments with TiO\textsubscript{2} (P-25) powder immobilised in sol-gel coated glass beads. As shown before, free TiO\textsubscript{2} powder suspension has a better contact with bacterial particles, due to its small size and dispersion. The suspension of TiO\textsubscript{2} powder inactivation of \textit{Flavobacterium} sp. bacteria was significantly higher (>3 orders of magnitude) compared to the immobilised one in the sol-gel layer. However, the immobilised TiO\textsubscript{2} revealed an adequate inactivation rate of 3 logs reduction in 30 minutes. It should be mentioned that further studies are needed to find an optimal method to enhance surface presence of anatase titanium dioxide particles on sol-gel coating. Recently, using direct titanium dioxide sol-gel methodology at low temperature, photocatalytic activity was significantly increased without the need of ormosils (MTMS) as glue mediator (Yun \textit{et al.} 2004).

During the whole study, lower pH levels favoured better photocatalytic inactivation of the selected bacteria. Figure 5 represents the inactivation kinetic of \textit{E. coli} and \textit{Flavobacterium} by TiO\textsubscript{2} suspension in the CSTR reactor. There are no significant differences between inactivation efficiency of the two bacteria at each pH range. Nevertheless, at pH \textless 5.1 a significant inactivation rate of >4 log reduction was observed with both bacterial strains. At pH below the isoelectric point of TiO\textsubscript{2} the photocatalytic activity is enhanced due to an increased charge gap between the bacteria and TiO\textsubscript{2} particles and a better free radical formation and retention.

Figure 3 | Photocatalytic inactivation efficiency on \textit{Flavobacterium} sp. in soft and hard water at various pH values.

Figure 4 | Comparison of photocatalytic inactivation efficiency of TiO\textsubscript{2} (P-25) powder suspension vs. immobilised in sol-gel coated glass beads on survival of \textit{Flavobacterium} sp. bacteria.

Figure 5 | Photocatalytic inactivation of \textit{E. coli} CN\textsubscript{13} and \textit{Flavobacterium} sp. bacteria at neutral and acidic pH.
An additional experiment was performed with immobilised TiO$_2$ in sol-gel coated glass beads (Figure 6). After 60 minutes of UV$_{360}$nm irradiation the reduction in viable count was almost similar at $\sim$3.5 logs from start to 30 minutes, *Flavobacterium* sp. was inactivated much faster while *E. coli* CN$_{13}$ count was almost unchanged. This behaviour can be explained by the hydrophobic difference between the two bacterial strains. From a previous study, it was found that *Flavobacterium* is more hydrophobic compared to *E. coli* (Starosvetsky et al. 2001). As sol-gel coating was prepared through highly hydrophobic MTMS precursor, that may explain the improved attachment of *Flavobacterium* to coated beads hydrophobic interaction compared to *E. coli*. The late start of *E. coli* inactivation can be explained by autophotolysis of methylic groups on the beads sol-gel coating and transfer to a more hydrophilic state enabling better contact with *E. coli* cells.

**CONCLUSIONS**

In summary, the photocatalytic reactor efficiency is governed by several parameters.

1. Initial low bacterial concentration will take a prolonged time interval to inactivate due to reduced contact probability.
2. Hardness reduces photocatalytic inactivation efficiency, therefore it is preferable to treat water chemically before disinfection.
3. Photocatalysis process should be performed at pH levels lower then 5.6, the isoelectric point of TiO$_2$.
4. Fe$^{3+}$ ions are required for the enhanced process linked to the Fenton reaction.
5. Imobilisation of TiO$_2$ should be performed through the sol-gel process of TiO$_2$ and not using ormosils precursors due to their organic content (methyl groups).
6. Hydrophobicity/hydrophylicity properties govern the attachment/contact between microorganisms and TiO$_2$ nanoparticles.

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**REFERENCES**


