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NOTES

Inactivation of *Escherichia coli* by Titanium Dioxide Photocatalytic Oxidation

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Titanium dioxide in the anatase crystalline form was used as a photocatalyst to generate hydroxyl radicals in a flowthrough water reactor. Experiments were performed on pure cultures of *Escherichia coli* in dechlorinated tap water and a surface water sample to evaluate the disinfection capabilities of the reactor. In water devoid of significant amounts of inorganic-radical scavengers, rapid cell death was observed with both pure cultures and members of the indigenous flora in a natural water sample.

In recent years the hydroxyl radical (HO·) has become the focus of a significant body of research concerning the chemical oxidation of anthropogenic organic compounds in the environment (1, 3, 5–8, 12, 13, 15, 17, 18). In aqueous systems, complete mineralization of many organic substances is possible when a sufficient HO· flux can be generated in situ (13). Various water treatment technologies inherently produce HO· in relatively minuscule quantities (i.e., $<10^{-12}$ M) (6). Examples of such processes include ozonation, direct photolysis of hydrogen peroxide, and radiolysis. In contrast to the above systems, steady-state HO· concentrations of the order of 10^{-9} M can be generated in aqueous solutions over immobilized particles of UV-irradiated titanium dioxide (10).

The photocatalytic degradation of various organic compounds by illuminated TiO_2 is very well documented (1, 4, 9, 11, 14, 16, 19, 20; see also references cited in references 11, 14, and 19). However, we are not aware of any published studies concerning the use of TiO_2 for microbial inactivation (e.g., disinfection of potable water). We postulated that HO· might act as a potent biocide because of its high oxidation potential and nonselective reactivity. This study was designed to determine the inactivation of pure cultures of *Escherichia coli* in a TiO_2 photocatalytic "reactor."

Titanium dioxide in the anatase crystalline form behaves as a classical semiconductor. Illumination of TiO_2 in water with light of less than 400 nm generates excess electrons in the conduction band (e^-_{cb}) and positive "holes" in the valence band (h^+_{vb}): $TiO_2 + h\nu \rightarrow e^-_{cb} + h^+_{vb}$. At the TiO_2 particle surface the holes react with either adsorbed H_2O or surface OH^- groups to form HO· radicals: $h^+_{vb} + H_2O$ (ads.)$\rightarrow$HO· + H^+ or $h^+_{vb} + OH^-$(sur.)$\rightarrow$HO·. Excess electrons in the conduction band react with molecular oxygen to form superoxide ions, $e^-_{cb} + O_2 \rightarrow O_2\cdot^-$, which further disproportionate to form more HO· radicals: $2O_2\cdot^- + 2H_2O \rightarrow 2HO\cdot + 2OH^- + O_2$.

The photoreactor which generates HO· radicals consists of a steel jacket, a lamp, and a photocatalytic sleeve (Fig. 1). The lamp emits UV light in the 300- to 400-nm range and is mounted coaxially within the jacket. The lamp is covered with a sleeve formed of fiberglass mesh, which is coated with a firmly bonded layer of TiO_2. The TiO_2 layer is activated by the UV light. Water is pumped through the reactor parallel with the lamp.

For the disinfection experiments, a sterile plastic carboy was filled with 12 liters of water. Because the city tap water is chlorinated at levels great enough to cause significant bacterial inactivation, a 10-fold stoichiometric excess of sodium thiosulfate was added to dechlorinate the sample. In later experiments, the chlorine level was reduced by intentional exposure to UV light for 48 h when the addition of thiosulfate became problematic (as described below). In either case, no residual chlorine was detected by standard DPD titration methods. *Escherichia coli* cultures were grown in heart infusion broth for 18 to 24 h at 35°C. The cultures were concentrated and washed twice by centrifugation with standard methods buffer (2). Water samples of 12 liters each (seven experiments) were spiked with *E. coli* to yield starting concentrations of between 10^5 and 10^9 CFU/100 ml. Coliform-laden water in the reservoir was continuously mixed with a magnetic Teflon stirring bar and recirculated through the reactor at a fixed flow rate of 2 liters/min. After an initial mixing and recirculation period of about 6 to 10 min, a control sample was collected (time zero) before the UV lamps in the reactor were turned on. Samples (ca. 200 ml) were withdrawn from a spigot at the bottom of the reservoir at periodic intervals thereafter. Samples were analyzed for *E. coli* and total coliforms by the membrane filtration procedure with M-Endo LES agar (2). Heterotrophic plate count (HPC) was measured by the aerobic pour plate method, using plate count agar with incubation at 35°C for 48 h (2).

Table 1 presents typical *E. coli* inactivation data obtained with tap water dechlorinated with either excess sodium thiosulfate or UV light. It is evident from the essentially invariant number of viable bacteria counted over time in the thiosulfate system that little or no inactivation occurred. After the final sample collection at a cumulative exposure time of 44 min, an obvious "sulfurous" smell was evident in the reservoir. We postulate that virtually all generated HO· was consumed (quenched) by inorganic thiosulfate, probably

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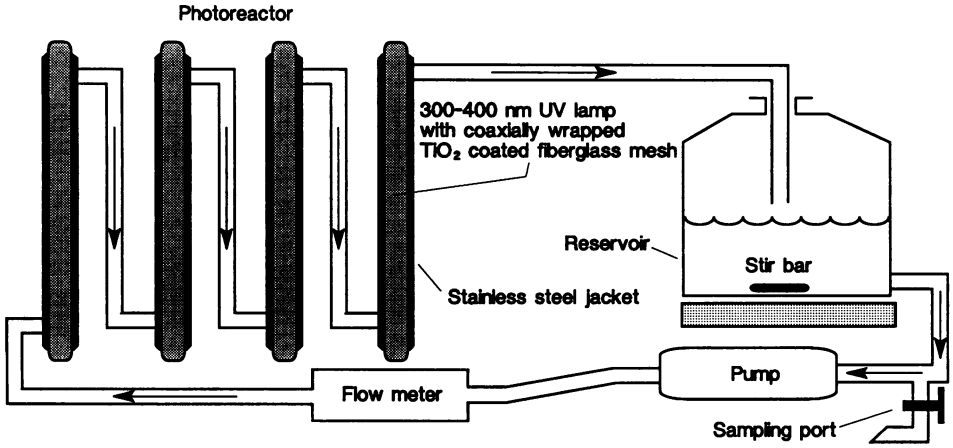

FIG. 1. Schematic diagram of the experimental TiO_2 photoreactor showing the flow configuration.

forming SO_2 gas and/or other sulfur species of intermediate oxidation state in the reactor. As such, the bacteria may have been effectively shielded from HO· attack.

In contrast, Table 1, column 2, presents typical results for the same experiment performed with tap water previously dechlorinated with UV light instead of thiosulfate. The initial concentration of organisms increased slightly following recirculation of the inoculated water through the reactor. Apparently *E. coli* had already populated the inside of the reactor from previous experiments, forming a biofilm on the porous fiberglass mesh. Combined with the spike coliform concentration, the total organism density prior to HO· exposure exceeded the upper measurement range of the analysis (reported in Table 1 as too numerous to count). After the first 6 min of exposure (lamps on, catalyst activated), we observed a reduction in the concentration of viable organisms of 7 orders of magnitude. *E. coli* counts in the 9-min-cumulative-exposure sample (and all subsequent samples) were below the detection limit, indicative of a total reduction of 9 to 10 $\log_{10}$ units.

TABLE 1. Inactivation of *E. coli* over photoactivated TiO_2

Sample description[a]	Concn of *E. coli* (CFU/100 ml)	
	With $S_2O_3^{2-}$	Without $S_2O_3^{2-}$
Control: uninoculated tap water dechlorinated with thiosulfate	<1	<1
Immediately after inoculation of *E. coli* into reservoir	3.4×10^7	2.0×10^9
Recirculation of inoculated reservoir water through reactor for 6 min with lamps off	3.1×10^7	TNTC[b]
3-min exposure with lamps on, TiO_2 photoactivated		TNTC[b]
6-min exposure	2.7×10^7	2.6×10^2
9-min exposure		<1
12-min exposure	2.7×10^7	<1
18-min exposure	2.7×10^7	
24-min exposure	2.4×10^7	
30-min exposure		<1
44-min exposure	1.7×10^7	
60-min exposure		<1

[a] Exposure times are cumulative.
[b] TNTC, too numerous to count.

Duplicate experiments with UV-dechlorinated tap water were performed with a lower initial concentration of *E. coli* more suited to valid method ranges. Prior to this experiment, the photoreactor was thoroughly disinfected with concentrated sodium hypochlorite for 24 h and flushed with 20 reactor volumes of distilled water. After inoculation and thorough recirculation with the lamps off, an initial concentration of 7.4×10^5 CFU of *E. coli* per 100 ml was present in the system. After 3 min of exposure to the activated catalyst, we observed a coliform inactivation of approximately 1 $\log_{10}$ unit, with 2.9×10^4 CFU/100 ml surviving. The next sample at 8-min cumulative exposure and all subsequent samples revealed complete inactivation within the limits of the analytical method (i.e., <1 CFU/100 ml).

We are presently expanding our investigation of the efficacy of HO·-mediated disinfection by studying samples of a highly colored surface water from a local pond. This pond water contains significant amounts of algae and a total organic carbon level of approximately 20 mg/liter. Table 2 presents typical inactivation data from recirculation mode experiments under conditions similar to those used for the pure-culture studies. It is evident from the results that little inactivation had been achieved as measured by HPC reduction (ca. 1 $\log_{10}$ unit), although the total-coliform count reduction was more significant.

Table 3 presents results from a second set of pond water experiments in which a chemical additive was used to increase the HO· radical flux (10, 13). Two hydrogen peroxide additions were made to the reservoir, the first after 9 min

TABLE 2. Inactivation of heterotrophic bacteria over photoactivated TiO_2 in a recirculating system

Sample description[a]	Concn of:	
	Total coliforms (CFU/100 ml)	HPC (CFU/ml)
Untreated pond water	1.7×10^2	3.4×10^3
6-min exposure with lamps on, TiO_2 photoactivated	1	2.2×10^2
12-min exposure	1	2.1×10^2
18-min exposure	<1	2.4×10^2
24-min exposure	<1	2.4×10^2

[a] Exposure times are cumulative.

TABLE 3. Inactivation of heterotrophic bacteria over photoactivated TiO_2 with addition of an irreversible electron acceptor

Sample description	Concn of:	
	Total coliforms (CFU/100 ml)	HPC (CFU/ml)
Untreated pond water control	2.4×10^2	4.8×10^3
Untreated pond water control plus 6.5 mM H_2O_2	1.7×10^2	2.1×10^3
3-min exposure with lamps on, TiO_2 photoactivated	4.0×10^1	3.6×10^2
6-min exposure	<1	23
9-min exposure and addition of 6.5 mM H_2O_2	<1	9
12-min exposure	<1	14
15-min exposure	<1	4
18-min exposure with second addition of 6.5 mM H_2O_2	<1	2
28-min exposure	<1	6
38-min exposure	<1	2

of exposure and the second after approximately 15 min. After the first 9 min of exposure, we observed a reduction in viable total coliforms and HPC of 3 orders of magnitude. After approximately 28 min of exposure, all visible (green) color had been removed from the pond water. Effective color removal did not begin until the second peroxide addition.

Hydrogen peroxide in this system acts as an irreversible electron acceptor which significantly reduces the semiconductor electron-hole recombination process and itself produces more HO· (1, 13) by the reactions $H_2O_2 + e_{cb}^- \rightarrow HO\cdot + OH^-$ and $H_2O_2 + O_2\cdot^- \rightarrow HO\cdot + OH^- + O_2$. It should be noted that this mechanism of HO· formation is fundamentally different from the direct photolysis of H_2O_2 by UV radiation, which is well known. The heterogeneous photocatalytic process on the semiconductor particle surface is significantly more efficient (13) and occurs at wavelengths at which the homogeneous photolysis of H_2O_2 is not observed. Interestingly, this concentration of H_2O_2 in the absence of photoactivated TiO_2 produced negligible inactivation (i.e., <0.5 log unit) in replicate control experiments.

In conclusion, our findings suggest that TiO_2 photocatalysis may be a viable process for disinfection of bacteria in water treatment systems. Inorganic-radical scavengers, though, can have a major negative impact on the efficacy of the process. The presence of organic matter also degrades the inactivation kinetics, ostensibly by competing with bacteria for the hydroxyl-radical oxidant. Addition of an irreversible electron acceptor compound at millimolar levels significantly improves the disinfection capability. Further research is required to produce a data base of sufficient size to enable meaningful statistical evaluation. We are also evaluating transient TiO_2 catalyst-fouling effects which are dependent on feed water quality.

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