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Inactivation of Phage MS2 by Iron-Aided Titanium Dioxide Photocatalysis

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Near-UV photocatalytic disinfection was accomplished in aqueous titanium dioxide suspensions. A level of inactivation of phage MS2 of 90% increased to 99.9% after 2 μM ferrous sulfate was added. Hydroxyl radical oxidation, with Fenton reaction enhancement, is suspected to be primarily responsible for the viral degradation observed.

Titanium dioxide photocatalysis has been the focus of much water purification research during the past decade (15). Despite a broad spectrum of investigation, the potential use of this technology for water disinfection has been essentially unexplored. Matsunaga et al. (13, 14) and Ireland et al. (9) have described the photocatalytic destruction of microbial cells, but our literature search uncovered no studies directed at the inactivation of viruses.

Mindful of previously described reactions (2, 6, 8, 10, 19, 21, 24), we hypothesized that TiO_2 photocatalytic viral inactivation occurs and is enhanced by iron. We tested this hypothesis by documenting the inactivation of phage MS2, a single-stranded RNA bacteriophage with icosahedral morphology and a diameter 26.0 to 26.6 nm (22).

All reaction solutions were prepared with Milli-Q-treated water (Millipore Corp.) and were buffered (3.780 g of $Na_2HPO_4 \cdot 7H_2O$ per liter of H_2O and 1.063 g of $NaH_2PO_4 \cdot H_2O$ per liter of H_2O) at pH 7.2. There was no change in the pre- and postreaction pH values in any solution. Titanium dioxide (type P25 powder; Degussa Corp.) and iron ($FeSO_4 \cdot 7H_2O$) were added at concentrations of 1 g liter^{-1} and 2 μmol liter^{-1}, respectively. An iron concentration of 2 μM (0.1 mg liter^{-1}) was used to comply with recommended United States and international drinking water levels (0.3 mg liter^{-1} [18]). The reaction solutions also contained ~5 mg of Cl^- per liter from the delivery of a saline Tris buffer with the MS2 inoculum. We used the materials and technique described by Bales et al. (3) to prepare the inoculum and for the viral assay. Phage MS2 (= ATCC 15597B1) was grown on host lawns of *Escherichia coli* ATCC 15597, and the number of phage was determined by the PFU method (1). For each experiment, the average MS2 concentrations at various contact times were determined by using sample dilutions that were plated in duplicate.

Experiments were conducted in a continuously stirred batch reactor under irradiated and dark conditions. A 100-ml volume of reaction solution and a 7.6-cm Teflon-coated stir bar were placed in a 1,000-ml glass beaker. The beaker was placed on top of a magnetic stirrer below a two-tube UV lamp (model J-205 tubes; wavelength, 365 nm [unfiltered]; 15 W; UVP, Inc.). When the lamp was switched on, the lamp irradiance was 2 mW cm^{-2}, as determined with a model UVX radiometer equipped with a type UVX-36 365-nm sensor (UVP, Inc.), at the reaction solution surface. This UV intensity was selected so test results would be relevant to solar-excited treatment designs (the intensity of Tucson sunlight was 3 mW cm^{-2} on a cloudless, 98°F [ca. 37°C] day). The reaction solution temperature rose <1°C (from 25°C initially) during each experiment. The stirring rate (65 rpm) was rapid enough to suspend the TiO_2 powder and slow enough to prevent notable MS2 inactivation by surface tension (20). We centrifuged the water samples at 16,000 × *g* for 7 min in an Eppendorf model 5415 microcentrifuge to remove the TiO_2. No centrifugal inactivation or settling of the bacteriophage was observed when centrifuged and noncentrifuged samples were compared.

The results of control experiments (averages of two experiments per phage-spiked reaction solution) are shown in Table 1. UV radiation was applied for a 10-min time interval that began 55 min after additions were made. With no irradiation, the MS2 concentrations stabilized within 15 to 45 min after the addition of TiO_2 and iron. The hypothesis that photocatalytic inactivation occurred was supported by the observation that only the two solutions containing TiO_2 exhibited plaque reductions after UV exposure. The results obtained with the reaction solution containing no additives confirmed that no MS2 inactivation occurred because of UV irradiation and stirring. On the basis of the information in Table 1, samples (Fig. 1) were removed during 10-min irradiations, with UV irradiation started 45 min after the addition of TiO_2 and iron. Figure 1 shows the inactivation profiles as best-fit lines (intercepts, 0; slopes, 0.116 and 0.317) drawn through datum points that were the averages from triplicate experiments.

Photocatalytic production of oxidants. UV irradiation of TiO_2 excites valence band electrons into higher conduction band energy levels. In an aqueous environment, these conduction band electrons (e^-_{CB}) are available for electron donation to reducible species that have adsorbed to the semiconductor surface. The valence band holes (h^+_{VB}), created by the promotion of the valence band electrons, are able to accept electrons from adsorbed oxidizable species. The thermodynamic feasibility of such a redox reaction is determined by the reduction potentials of the TiO_2 and adsorbed constituents. The reaction rate of redox candidates

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TABLE 1. MS2 inactivation during control experiments

MS2 reaction solution addition(s)	Avg MS2 concn (PFU ml^{-1})				
	5 min before addition	15 min after addition	45 min after addition	55 min after addition	After 10 min of UV irradiation and 65 min after addition[a]
None	6×10^4	6×10^4	6×10^4	6×10^4	6×10^4
$FeSO_4$	6×10^4	4×10^4	4×10^4	4×10^4	4×10^4
TiO_2	6×10^4	9×10^3	1×10^3	1×10^3	1×10^2
TiO_2 + $FeSO_4$	6×10^4	2×10^4	6×10^3	6×10^3	4×10^0

[a] UV exposure was started 55 min after compounds were added to the reaction solution.

is controlled by factors that include TiO_2 surface conditions and adsorbate transfer to the surface.

In aerated aqueous solutions at neutral pH, hydroxide ions and dissolved oxygen are prominent electron donors (donating to h^+_{VB}) and e^-_{CB} acceptors, respectively; they are favored for these roles because of oxygen adsorption onto Ti^{3+} centers (21) of the readily hydroxylated, metal oxide surface.

Reduction of oxygen by e^-_{CB} produces superoxide radicals (O^-_2) at a neutral pH (2, 21): $O_2 + e^-_{CB} \rightarrow O^-_2$. The two-electron reduction of oxygen produces hydrogen peroxide (H_2O_2) (10): $O_2 + 2H^+ + 2e^-_{CB} \rightarrow H_2O_2$. Superoxide radicals and H_2O_2 can react to form the hydroxyl radical ($OH^\cdot$), which has been proposed as the primary oxidant (21) in the TiO_2 photocatalytic system (2): $H_2O_2 + O^-_2 \rightarrow OH^- + O_2 + OH^\cdot$. Reduction of adsorbed H_2O_2 produces additional $OH^\cdot$ (2): $H_2O_2 + e^-_{CB} \rightarrow OH^- + OH^\cdot$.

h^+_{VB} can actuate $OH^\cdot$ and H_2O_2 formation by a more direct route. Electron donation by surface hydroxide ions creates $OH^\cdot$ (21): $OH^- + h^+_{VB} \rightarrow OH^\cdot$. Hole injection by adsorbed water molecules can produce both $OH^\cdot$ (21) and H_2O_2 (8): $H_2O + h^+_{VB} \rightarrow H^+ + OH^\cdot$ and $2H_2O + 2h^+_{VB} \rightarrow 2H^+ + H_2O_2$.

Fenton type supplementation of $OH^\cdot$. Fujihira et al. (4) observed an increase in TiO_2 photocatalytic oxidation of toluene after ferrous sulfate was added. Sclafani et al. (19) described accelerated destruction of phenol in UV-irradiated aqueous TiO_2 dispersions to which ferrous sulfate was added. These phenomena were attributed to supplemental hydroxyl radical oxidations that were enabled by Fenton reactions, such as (6): Fe(II) + $H_2O_2 \rightarrow$ IC $\rightarrow$ Fe(III) + OH^- + $OH^\cdot$, where IC is an intermediate complex and Fe(III) and Fe(II) are Fe^{3+} and Fe^{2+} and complexes thereof. The H_2O_2 was thought to have been produced by the e^-_{CB} reduction of oxygen.

The iron-catalyzed Fenton reaction requires ferrous iron. Although the ferric state usually predominates at a neutral pH, there are ways by which Fe(II) levels can be boosted photocatalytically. One alternative involves direct reduction of adsorbed ferric ions by e^-_{CB} (19): $Fe^{3+} + e^-_{CB} \rightarrow Fe^{2+}$, where the standard reduction potential of this half-reaction is 0.77 V (versus the normal hydrogen electrode) (12). Sclafani et al. confirmed (19) the e^-_{CB} scavenging ability of Fe^{3+}. Support for this reaction also comes from the TiO_2 photocatalytic work of Prairie et al. (16), who concluded that direct e^-_{CB} reduction of metals was possible for metals having half-reaction standard potentials of >0.3 V (normal hydrogen electrode).

Zepp et al. (24) described a photo reduction of iron: Fe(III)L_n + light $\rightarrow$ Fe(II), where Fe(III)L_n is a photoreactive complex of Fe^{3+} (L_n is an abbreviation for ligands such as phosphates, citrates, oxalates, etc.). Zepp et al. thought that this is an important precursory pathway in permitting Fenton type $OH^\cdot$ formation in sunlit natural waters.

Iron may also be reduced by superoxide radicals (6): Fe(III) + $O^-_2 \rightarrow$ Fe(II) + O_2. Combining this equation with the Fenton reaction yields the $OH^\cdot$-generating pathway called the iron-catalyzed Haber-Weiss reaction (6): $H_2O_2 + O^-_2 \rightarrow O_2 + OH^- + OH^\cdot$.

Photocatalytic oxidation of MS2. Our MS2 inactivation hypothesis was founded on the reaction possibilities summarized in the discussion above, while we recognized the proven (23) vulnerability of viral components (protein capsids and nucleic acids) to $OH^\cdot$ degradation. We postulated that iron-enhanced phage inactivation would occur by rationalizing a Fenton type augmentation of $OH^\cdot$ levels.

There are additional inactivation pathways that deserve consideration. Direct oxidation of MS2 by capsid group hole injection is possible. Ozone causes an oxidative capsid alteration that prevents virus uptake into susceptible cells (17). Titanium dioxide has a thermodynamic capacity for this action, with a reduction potential at its valence band of 2.6 V (21) (normal hydrogen electrode; at neutral pH), compared with ozone's standard reduction potential of 2.07 V (normal hydrogen electrode; $O_3 + 2H^+ + 2e^- \rightarrow O_2 + H_2O$) (12).

For a significant amount of MS2 direct oxidation to have occurred, the phage would have had to outcompete OH^- for electron donation to h^+_{VB}. This dominance seems doubtful when surface charge is considered. At pH 7.2, the P25 TiO_2 surface is predominantly neutral (11), while the MS2 surface has both hydrophobic (3) and negatively charged (pH_{iep} 3.9 [25]) hydrophilic regions. Although MS2-TiO_2 attraction was not anticipated at a neutral pH, the pre-UV treatment plaque reductions observed with the TiO_2 and TiO_2-$FeSO_4$ controls (Table 1) indicate that adsorption occurred. This adsorption probably resulted from a nonattractive partitioning of the phage onto the semiconductor that was ascribable to exclusion of the hydrophobic MS2 portions by water. Under these conditions, the phage would have overlaid the hydroxylated

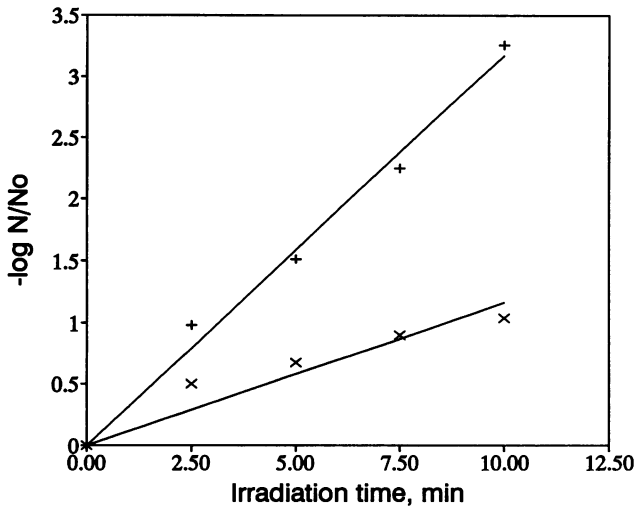

FIG. 1. Comparison of bacteriophage MS2 inactivation in near-UV-excited TiO_2 suspensions with (+) and without (×) added $FeSO_4$. N, MS2 concentration; No, initial MS2 concentration.

TiO_2 surface and resided at locations unfavorable for rivaling OH^- hole injection.

Another inactivation possibility includes a non-Fenton pathway, by which ferrous ions might have served as additional oxidants (e^-_{CB} scavengers). This could have facilitated greater hole availability and escalated phage oxidation by delaying e^-_{CB}-to-h^+_{VB} recombination. Support for this hypothesis can be obtained from the study of Prairie et al. (16). Prairie et al. surmised that photocatalytic oxidation of salicylic acid was oxidant controlled (in other words, controlled by the rate at which oxidants accepted electrons from the TiO_2 conduction band).

Substantial inactivation by the non-Fenton route described above is not supported by the results shown in Fig. 1. For this mechanism to have exerted a major influence, we think that the >170% increase in the rate (compared with the best-fit line slopes) would have demanded a commensurate oxidant addition. Even if all of the iron was reduced by e^-_{CB}, the 2 μM concentration would have contributed an increase in the e^-_{CB} acceptor of <1% (2 μM iron e^-_{CB} acceptor added to the >250 μM oxygen e^-_{CB} acceptor assumed to be present in the air-equilibrated system).

The greatest inactivation was most likely caused by $OH^\cdot$ oxidation. This mechanism accommodates iron-aided enhancement by Fenton reactions. Iron catalysis is implied by the marked rate change that accompanied the trace salt addition. If the iron was recycled, as simultaneous execution of the Fenton reaction with any of the iron reduction reactions suggests, then a small amount could have catalyzed this large effect.

Enhanced MS2 destruction might also be explained by efficient targeting of $OH^\cdot$ oxidation directed by ferrous iron adsorbed to MS2 sites. Fenton reactions can be generated by the reaction of H_2O_2 with iron that has complexed to various chemical structures (5, 6). The resulting $OH^\cdot$ oxidizes molecules in the immediate vicinity and thus causes site-specific destruction. In a photocatalytic environment, this mechanism could supply a means by which species less reactive than $OH^\cdot$ (such as O^-_2 and H_2O_2) could diffuse away from the TiO_2 surface and initiate $OH^\cdot$ oxidation at locations furnishing iron.

Participation of this reaction scheme in the MS2 inactivation experiments would have necessitated iron adsorption onto MS2. The fact that this occurred is substantiated by the $FeSO_4$ solution data (Table 1). Table 1 shows that all of the inactivation occurred within 15 min after iron was added. If the direct oxidation by iron species was insignificant, the decrease in PFU was probably caused by iron-assisted MS2 sedimentation (hastened by centrifuging). This implies that iron complexation occurred at the phage surface.

If iron-enhanced MS2 inactivation revealed the expression of site-specific reactions, such a mechanism might be useful in photocatalytic disinfection. $OH^\cdot$ oxidation of microorganisms is a low-yield process when dissolved species intercept $OH^\cdot$ before it encounters microbes (7, 9). This unwanted scavenging could be decreased by fostering the photocatalytic production of selectively reactive species. Site-specific destruction could then be triggered by the ultimate reaction of the diffusing intermediates with recipient constituents that are tailor made for viral attachment.

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