Photocatalytic Inhibition of Algae Growth Using TiO$_2$, WO$_3$, and Cocatalyst Modifications

CLOVIS A. LINKOUS,*,[#]
GLENDA J. CARTER,[#]
DAVID B. LOCUSON,[†]
ANTHONY J. OUELLETTE,[‡]
DARLENE K. SLATTERY,[#] AND
LISA A. SMITHA[§]

Department of Chemical Engineering, Florida Institute of Technology, Melbourne, Florida 32901, Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455, Florida Department of Law Enforcement, Tallahassee, Florida 32308, and Florida Solar Energy Center, University of Central Florida, 1679 Clearlake Road, Cocoa, Florida 32922-5703

TiO$_2$ and WO$_3$, with and without noble metal cocatalysts, were employed as photocatalytic surfacing agents to inhibit the attachment and growth of *Oedogonium*, a sessile, filamentous algae. It was demonstrated that coating a cement substrate with a dispersion of TiO$_2$ powder held in a 10 wt % binder and irradiating with a combination of black light and fluorescent lamps could effect a 66% reduction in the growth of algae in comparison to the unprotected cement surface. Adding a 1.0 wt % loading of a noble metal such as Pt or Ir to the photocatalyst enabled an 87% reduction. The extent of inhibition was shown to be related to the amount of near-UV light contained in the irradiation source. The ability of the photocatalysts to inhibit algae correlated well with their ability to photooxidize d-(+)-glucose, building block of numerous biochemical polysaccharides, suggesting a nonspecific mechanism in the breakdown of cellular structures.

Introduction

Wide band gap metal oxides such as TiO$_2$ and WO$_3$ (E_g = 3.1 and 2.7 eV, respectively) have been well studied as photoanode materials in photoelectrochemical cells (*1–5*). During this development, it was observed that certain photooxidative chemistries could be performed simply by irradiating the loose, suspended powder in the presence of the reagent of interest. Initially, inorganic species were investigated (*6–9*), but soon after organic compounds were studied as well (*10–14*). Wastewater detoxification via photocatalytic chemistry became an established field by itself (*15, 16*).

The ability to photooxidize organics led naturally to disinfection, the idea that light-activated photocatalysts, principally TiO$_2$, could kill bacteria or other dangerous pathogens in aqueous systems. Most work in this area has concentrated on *Escherichia coli* (*17*), although other mi-

* Corresponding author phone: (321)638-1447; fax: (321)638-1010; e-mail: calink@fsec.ucf.edu.
† Florida Institute of Technology.
‡ University of Minnesota.
§ Florida Department of Law Enforcement.
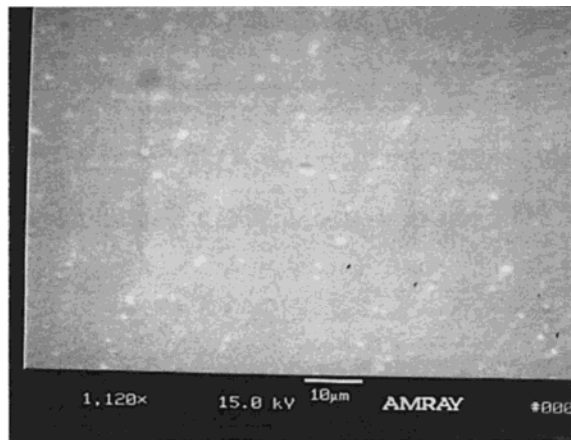# Florida Solar Energy Center, University of Central Florida.

FIGURE 1. Electron micrograph of acrylic substrate after 3 day's exposure to an algae-rich environment.

croorganisms such as Baker's yeast (*Saccharomyces cerevisiae*) and *Lactobacillus acidophilus* have been studied as well (*18*).

There are many other types of microorganisms besides bacteria to which this technology may be applied. While not of particular concern from a public health standpoint, algae growth on exposed sunlit surfaces is a form of biofouling that can have great aesthetic and economic consequences. Attachment to the hulls of watercraft is a serious problem in terms of increased drag and additional fuel consumption. Excessive algae build-up can occlude drainage pipes and foul interlocking mechanisms. In general, algae are more resistant to the more common chemical oxidants than are bacteria (*19*), and so from a photocatalytic control perspective, they offer a greater challenge. On the other hand, the photosynthetic algae can only grow where light is available, so that photocatalytic technology represents an ideal approach to their control. Hashimoto et al. demonstrated that an aquarium could be kept algae-free by circulating the water through an external photocell consisting of a UV-photolyzed TiO$_2$ support (*20*). This approach can only remove free floating or motile species, however. There are many species of algae that are affixed to a surface for most of their life cycle, propagating themselves by a number of methods, such as scission of plant segments, rhizoidal outgrowths, or production and release of zoospores.

Our objective was to investigate whether a metal oxide photocatalyst could be applied to a surface vulnerable to algal biofouling and offer a means of protection. *Oedogonium* is a genus of common freshwater, filamentous algae found in lakes, ponds, and aquariums (*21*). These are algae that prefer to live in an immobilized state but are able to propagate and spread through the release of zoospores into the open water. These zoospores are able to attach and adhere to a suitable surface and grow into hair-like filaments that can be many centimeters long. In Figures 1–3, electron micrographs are shown that depict different stages of algae growth. In Figure 1, an acrylic substrate is shown to be initially smooth, even at 1500× magnification. Within days, the tiny zoospores are able to attach themselves. A micrograph of a single basal cell is shown, along with its adhesive "foot", or holdfast. To prevent incipient algae growth, presumably oxidative photochemistry must be performed on either the zoospore or the holdfast of the germling. After 2 weeks, the algae on an unprotected substrate has grown quite thick, as shown in Figure 3.

As the field of photocatalytic chemistry was developing, it became apparent that photocatalyst activity could be
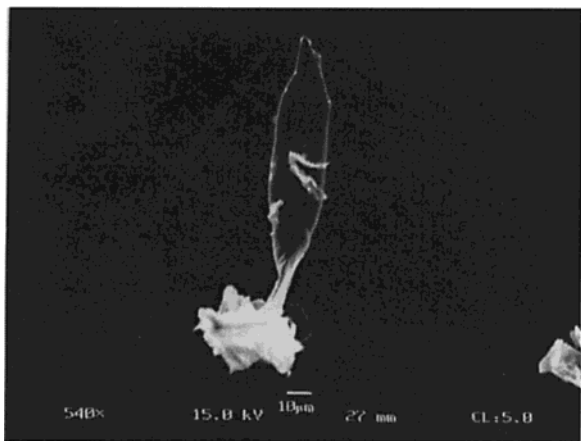
FIGURE 2. Electron micrograph of young *Oedogonium* germling with developed holdfast.
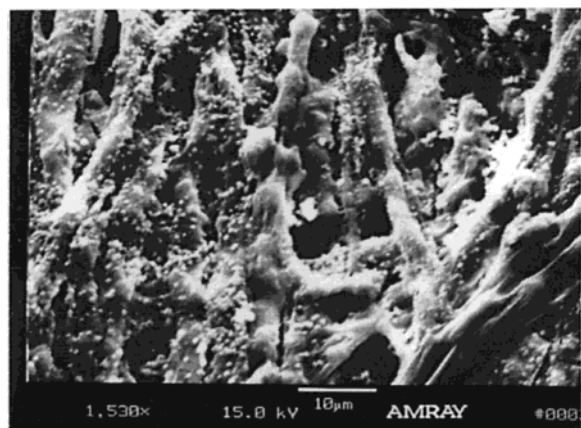

FIGURE 3. Electron micrograph of *Oedogonium* algae growth on an acrylic substrate after 2 week's exposure to an algae-rich ennvironment.

improved by adding small amounts of other catalytic substances, or cocatalysts, to the light-absorbing particle surface. The role of the cocatalyst is to facilitate the rate-limiting half reaction. Depending on specific conditions, the rate limitation can be either oxidation or reduction. In particular, a loading of Pt on the order of 1 wt % can lead to a rate increase due to faster reduction of dissolved molecular oxygen (*10*, *11*, *22*). The ability of noble metal cocatalyst additions to facilitate photocatalytic algae inhibition is also an objective of this work.

Experimental Section

Photocatalyst Preparation. TiO$_2$ was obtained from Degussa (P25). WO$_3$ was obtained from Fisher as yellow green powder. Pt and Ir cocatalyst modification was performed according to the method of Cook et al. (*23*), where metal deposition is accomplished via borohydride reduction of the respective chloride complex. For Pt, 50 g of the metal oxide was suspended in 150 mL of water and 1.327 g of H$_2$PtCl$_6$·6H$_2$O were added. A 0.4848 g quantity of NaBH$_4$ was dissolved in 100 mL of water and slowly added via an addition funnel to the metal oxide-Pt suspension, while stirring under a blanket of inert gas. The suspension was allowed to stir for 12 h before isolating the solids via vacuum filtration. For Ir, a similar procedure was employed, except that the metal complex solution was made by gently heating 0.78 g of IrCl$_3$. After dissolution, the solution was allowed to cool before the addition of the borohydride.

The metallic state of the Pt cocatalyst was confirmed by X-ray photoelectron spectroscopy. The state of the Ir could not be accurately determined, due to the overlap of the Ti 3s and Ir 4f transitions.

Glucose Photocatalysis. One hundred milligrams of photocatalyst was added to 50 mL of a 10.0 mM glucose (dextrose; Sigma) solution, placed in front of a 1000 W Xe lamp filtered by a 10 cm water filter, and photolyzed for an hour while stirring. Then 15 mL of the photolyzed solution would be removed, placed in a centrifuge tube, and spun for 15 min. The centrifugate would then be sampled with a microliter syringe for glucose assay.

Glucose concentration after photolysis was determined via the hexokinase, or HK method (*24*). Glucose is converted by hexokinase in the presence of ATP into glucose-6-phosphate, which then reacts with NAD$^+$ via glucose 6-phosphate dehydrogenase (G-6-PDH) to form 6-phospho-gluconate and NADH. Glucose concentration is then determined by NADH optical absorption at 340 nm. A kit containing all the necessary reagents was obtain from Sigma. Standard solutions gave results within 2% of theory.

To correct for adsorption effects by the photocatalyst, a parallel test in the dark would be run and analyzed for glucose. The absorbance of the irradiated solution would then be subtracted from the dark experiment to derive the free glucose concentration. The adsorbed glucose typically accounted for 12% of the total amount initially present. This approach also corrected for background light scattering by the residual photocatalyst in the cuvette, about 2% of the total absorbance.

The percent of glucose consumed during the photolysis was then determined as

$$\% \text{ glucose consumption} = \frac{A_{\text{dark}} - A_{\text{light}}}{A_{\text{g}} - A_{\text{HK}}} \times 100$$

where A_{dark} = absorbance of centrifuged solution containing glucose and photocatalyst but not photolyzed; A_{light} = photolyzed solution absorbance; A_{g} = absorbance of HK-converted glucose solution, without photocatalyst or light; and A_{HK} = background absorbance of hexokinase solution.

Algae Inhibition. The algae inhibition experiments were performed by coating 5 × 5 cm white Portland cement substrates with a photocatalyst formulation, consisting of the inorganic photocatalyst powder suspended in a dichloroethane solution of poly(methyl methacrylate), or PMMA. The dried films were nominally 90% photocatalyst by weight. The substrates were mounted vertically on a plastic rack leaning against the back face of a 40 L, rectangular glass tank. Samples of *Oedogonium* were obtained from local aquarists and propagated in the tank. While the front and back faces of the tank were kept algae-free, the sides were allowed to cover over with algae growth, thus providing a steady supply of zoospores to attack the photocatalyst samples.

Samples were irradiated through the front face with a 4-element light bank consisting of two black light lamps and two conventional fluorescent lamps, alternately mounted to give as uniform a light flux as possible. The black light lamp had most of its irradiance concentrated in a single peak centered at 365 nm. This wavelength was short enough that most of its spectral irradiance could be absorbed by the wide band gap photocatalysts but long enough that it could not perform direct photolytic damage on the algae. The radiant power of the lamps was measured with a photomultiplier. The fluorescent lamps had a broad spectral response across the visible range, along with a few sharp Hg emission lines, providing 54% of the total integrated light flux, which was estimated to be 12 W/m^2 using a LI-COR pyranometer. A 250 cm^2 rectangular test region of uniform light intensity was defined along the back face of the tank, so that up to 10 substrates could be tested at a time.
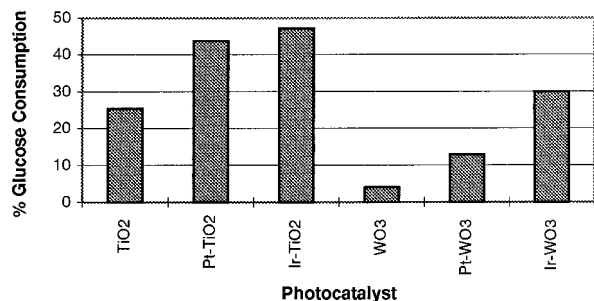
FIGURE 4. Percent glucose consumption by various photocatalysts.

FIGURE 5. Algae growth for various photocatalysts normalized to an unprotected cement substrate; mixed lamp irradiation.

The extent of algae growth was determined via quantification of chlorophyll content of the cellular growth on the surface. A spectroscopic method based on that of Lorenzen was employed (25). Each cement substrate was extracted with 20 mL of a 90% alkaline acetone solution and scrubbed with a metal brush until all cellular growth had been removed. The loosened material plus the aqueous acetone extract were sonicated together for 5 min and then centrifuged at 5000 rpm for 10 min. A 2.0 mL solution volume was then decanted into a standard 1.0 cm quartz cuvette. Chlorophyll content was determined by measuring solution absorbance at 665 nm and correcting for any residual turbidity by subtracting out absorbance at 750 nm.

Results and Discussion

To determine whether our photocatalyst samples were active, they were initially screened against a 10.0 mM glucose solution. The ability of $Pt-TiO_2$ powders to facilitate the photooxidation of glucose had already been demonstrated (26). The following photocatalytic coatings were examined: WO_3, TiO_2, $Pt-WO_3$, $Pt-TiO_2$, $Ir-WO_3$, and $Ir-TiO_2$.

Percent glucose consumption by each photocatalyst after a 1 h photolysis is shown in Figure 4. Glucose concentration was determined from NADH absorbance, after correcting for turbidity and adsorption effects, as described in the Experimental Section. In all cases, photolysis in the presence of a photocatalyst resulted in net glucose consumption. Photolysis in the absence of photocatalyst produced negligible glucose decomposition.

It was observed that cocatalyst modification did indeed improve photocatalytic activity. The amount of glucose consumed increased from 26% for unmodified TiO_2 to 44% for $Pt-TiO_2$ and 47% for $Ir-TiO_2$. The consumption of glucose by WO_3 was significantly less, the unmodified WO_3 consuming only 4% of the initial glucose present, while the Pt and Ir modifications consumed 13% and 30%, respectively.

These same photocatalyst preparations were then tested in an immobilized state against *Oedogonium* algae. Also included were a bare, uncoated cement substrate and one coated only with PMMA binder as control samples. The extent of algae growth after a week's exposure under irradiation with the mixed light source is shown in Figure 5. Growth is plotted relative to that obtained on the unprotected cement substrate. All results are reported as an average of three or more experiments. Relative standard deviations of 20–30% were obtained. Most of the photocatalyst formulations exhibited an inhibitory effect. Even the PMMA binder alone enabled a 19% reduction in algae growth. On the other hand, unmodified WO_3 exhibited a negative effect, i.e., 67% more algae grew on the WO_3-coated cement sample than on the cement substrate itself.

The negative effect observed for WO_3 suggests that algae growth factors in addition to photocatalysis are associated with photocatalyst application on a surface. One must consider toxicity, surface energy, ablative, and morphology effects as well. Toxicity effects are unlikely, since TiO_2 and
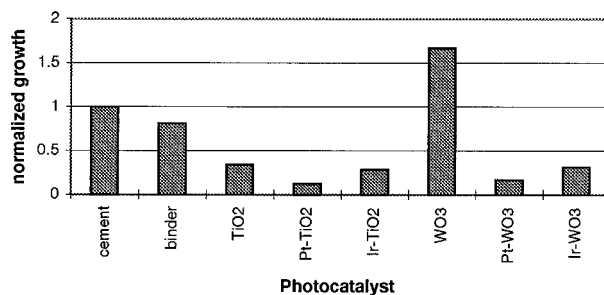
WO_3 are largely inert substances under the stated experimental conditions. Moreover, TiO_2 is commonly used in residential paints, lotions, and other personal hygiene formulations. The addition of cocatalysts once again presents the possibility of toxicity effects, but as will be seen below, any possible toxicity introduced by Pt or Ir is far outweighed by their promotion of the photocatalytic effect.

Surface energy can also be an issue. It has been shown that zoospore attachment in other algae species can be mitigated by coating with a low surface energy material such as fluoropolymers, silicones, or poly(ethylene oxide)s (27, 28). However, as can be seen in Figure 5, the PMMA binder by itself, a coating which would present the lowest surface energy of those examined, exhibits only a modest inhibitory effect.

Ablative effects describe a situation where the force of water or other perturbations against a weaker surface cause it to wear away in a predictable manner. Some marine paint manufacturers employ this approach to foil attachment of microorganisms. In our case, however, even though the binder/photocatalyst ratio was kept deliberately low (1:9 on weight basis) to maximize any photocatalytic effect, in no instance was erosion of a surface through the course of an experiment observed. Moreover, except for the bubbling action of a single aerator, the algae test system was kept quiescent, minimizing any ablative effect.

Promotion of algae growth after photocatalyst application is best explained as a morphology effect. The white Portland cement used as a support is exceedingly fine grained, on order of 0.1 μm and less. The nominal particle size for P-25 TiO_2 was 30 nm, so that, even after allowing for some particle agglomeration, the TiO_2 could be deposited as a smooth surface over the cement substrate. On the other hand, the WO_3 particles were 2–5 μm in diameter, as measured by scanning electron microscopy. As a result, the WO_3 morphology was of the same dimensions as the attacking zoospore. The *Oedogonium* zoospores are on the order of 30 μm in width (21), so that they could easily wedge into crevices between particle aggregates. In the fluorescent control experiments, where there was minimal UV radiation to stimulate the photocatalysts, positive growth effects were exhibited by TiO_2, $Pt-WO_3$, and $Ir-WO_3$ in addition to WO_3. Thus whenever a photocatalyst is applied to a surface, there is an interplay of independent factors, of which photocatalytic activity is only one, that will determine whether the coating promotes or inhibits the attachment and growth of microorganisms.

Normally, a photocatalytic effect would be confirmed by comparing reactivity in the light versus dark. However, since the algae themselves are photosynthetic and require light that their chloroplasts can absorb and convert to chemical energy, one cannot perform the simple "dark" control experiment. Instead we took advantage of the differing spectral distributions of two light sources and the differing photoaction spectra of the algae versus that of the metal oxides. While the algae has an array of chlorophylls, xan-
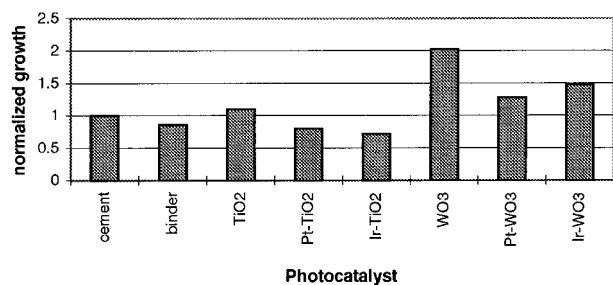
FIGURE 6. Algae growth for various photocatalysts normalized to an unprotected cement substrate; fluorescent lamp only.

thophylls, carotenoids, and phycobilins that enable it to absorb light across the visible spectrum and into the near-ultraviolet, the metal oxides under study here can only absorb photons whose energies exceed the band gap energy, so that they can only utilize blue to ultraviolet light. For anatase TiO_2, the band gap energy is 3.1 eV, while for WO_3 it is 2.7 eV. This corresponds to cutoff wavelengths of 400 and 460 nm, respectively. By turning off the black lights and irradiating with the twin fluorescents only, it was possible to remove most of the light utilized by the metal oxides, while continuing to supply sustaining light to the algae.

Repeated tests on cement blanks showed that turning off the black lights actually improved algae growth by 15%, indicating that UV illumination did stress the algae somewhat, but since growth is measured relative to the cement control, this factor has been taken into account. Based on the absorption band threshold, it was estimated that the fluorescent lamps alone provided only 31% as much usable light to the WO_3-based photocatalysts as the black light/fluorescent combination. For the TiO_2-based photocatalysts, the effective light intensity fell to 7%.

The effect of illumination by fluorescent lamps only on algae growth is shown in Figure 6. The immediate observation is that the variation in relative algae growth rates is much reduced. Four of the photocatalysts (TiO_2, WO_3, Pt—WO_3, and Ir—WO_3) now exhibited negative inhibition effects. Even the Pt—TiO_2 and Ir—TiO_2 samples were only capable of 19% and 28% inhibition, respectively. The value for the binder-only sample is essentially unchanged, consistent with the idea that the PMMA/cement system is indifferent to the illumination source, at least in the near UV.

Some researchers have sought to attribute the action of irradiated TiO_2 to prevention or interruption of a specific chemical step or compound that is vital for cellular well being. Matsunaga et al. attributed the demise of *S. cerevisiae* cells to photocatalytic decomposition of coenzyme A (29).

Most researchers, however, point to the generation of hydroxyl radicals as a nonspecific oxidant. Spin trapping studies have shown that HO• is present as an intermediate during the photolysis of water on TiO_2 (30). The method of action is quite similar to that provided by hydrogen peroxide, H_2O_2. Rate constants for the attack of HO• on hundreds of organic molecules have been tabulated (31). Izumi et al. irradiated aqueous benzene solutions in the presence of platinized anatase TiO_2 and obtained phenol as a primary degradation product (11). Hong et al. irradiated 2-chloro-biphenyl in the presence of TiO_2 and saw that the initial degradation products were a mix of the seven isomers of 2-chlorobiphenyl-ol and biphenyl-2-ol (32). Given the non-specific nature of hydroxyl radical in organic systems and the ubiquity of d-(+)-glucose in cellular structures, it would seem unlikely that the photogenerated hydroxyl radical is seeking out a specific chemical moiety for attack.

A problematic aspect of the general reactivity of HO• on organic substrates is the difficulty in finding a polymeric binder for the photocatalyst. While we saw no evidence of photocatalytic binder degradation in this work, most organic polymer binders, including PMMA, are ultimately susceptible to photocatalytic attack in TiO_2/water systems. This issue has been addressed by Murasawa (33) et al. who found that various perhalogenated polymers could withstand TiO_2-generated HO• attack and by Heller (34) et al. who found that UV-transmitting silicones showed good stability.

It has been established that other oxygen species are generated that contribute to cell mortality on a secondary level (35, 36). Hydroperoxyl radical, $HO_2•$, is thought to be generated via reduction of dissolved O_2, while hydrogen peroxide, H_2O_2, can be generated by either H_2O oxidation or O_2 reduction processes.

Similar trends were seen for the glucose decomposition and the algae inhibition experiments; those photocatalysts most effective at glucose photooxidation were also most effective toward algae inhibition. Cell walls of plant organisms, including algae, are made largely of cellulose, of which d-(+)-glucose is a primary building block, so that one might expect comparable photocatalytic behavior. Indeed, one objective apart from the algae inhibition studies was to determine whether the glucose photolysis test itself could be used as an indicator of photocatalyst effectiveness, since the entire analysis could be performed in ~1.5 h, as opposed to a week or more of exposure to *Oedogonium*.

It may also be true that the photocatalytic oxidation is not performed on the outer cell wall but rather on the sticky mucilage secreted by the germling to affix itself onto a surface. Rather little is known about the biochemical adhesives secreted by sessile algae forms. Studies on *Enteromorpha* suggested that zoospore adhesion occurs via a muco-polysaccharide substance consisting of both proteinaceous and carbohydrate segments (37), which may account for the slightly differing activities of Ir and Pt photocatalysts toward glucose and *Oedogonium*.

Huang et al. treated *E. coli* with TiO_2 and near-UV light in the presence of *o*-nitrophenol-β-d-galactopyranoside (38). By observing the rate of conversion of the pyranoside to *o*-nitrophenol, they were able to monitor changes in cell wall permeability and correlate it with cell death. The results were consistent with a sequential decomposition of the outer cell wall and cytoplasmic membrane, followed by leakage and decomposition of intracellular components. There is also the work of Maness et al. who irradiated *E. coli* in the presence of TiO_2 and measured the rate of production of malondialdehyde due to lipid membrane peroxidation (39). Once again, the rate of HO• radical reaction correlated well with the loss of respiratory activity and cell viability.

Recent work by Jacoby et al. has shown that, at least in airborne species, complete mineralization of the microorganism can occur (40). This was demonstrated for an *E. coli* cell mass, but preliminary work on the photosynthetic bacterium *Rhodobacter sphaeroides* was mentioned as well. Complete mineralization is an unlikely scenario in the specific case of *Oedogonium*, since it has been observed that germlings unable to make firm attachment can release, float to the water surface, differentiate back into zoospores, and go off again in search of a more benign supporting surface (41).

Despite the poor photocatalytic inhibition demonstrated by WO_3, the Pt- and Ir-modified WO_3 samples performed nearly as well as those of TiO_2. Relatively little work on Pt—WO_3 with regard to organic photooxidation has appeared in the literature, most likely because researchers did not anticipate the extent of improvement after cocatalyst addition. Pichat et al. utilized an earlier study (42) to compare WO_3 to TiO_2 in the oxidation of propene and found a 10% quantum yield for TiO_2 in comparison to 0.03% for WO_3 (43). A subsequent study on oxalic acid reported a mean photocatalytic activity of 21 μmol/h for TiO_2 versus 9.6 μmol/h for

WO$_3$ (*44*). Maldotti et al. may have obtained an effect similar to the present work when they found that Pt–WO$_3$ was more effective than Pt–TiO$_2$ in the cyanation of methoxybenzene to make cyanoanisole (*45*), even though it has been established that WO$_3$ shows a much lower activity than TiO$_2$ for CN$^-$ oxidation to cyanate (*8*). Perhaps the most effective use of WO$_3$ in photocatalytic detoxification work has been as a 3.0 wt % cocatalyst for TiO$_2$, which enabled a doubling of activity (*22*).

That the algae-inhibiting ability demonstrated by Pt–WO$_3$ was essentially the same as Pt–TiO$_2$ points to the role of Pt in accelerating a rate-limiting process to the same level, presumably reduction of dissolved O$_2$. The vigorous attack of *Oedogonium* zoospores on a fresh surface can be approximated as a highly concentrated organic background. The quantum efficiency of hole transfer in this situation can approach unity, provided an electron acceptor is present in sufficient quantity. Molecular oxygen solubility typically lies in the single millimolar range, so that in the absence of other electron acceptors, O$_2$ reduction becomes the rate-limiting step. It has been suggested that reduction of O$_2$ is often the limiting factor in determining the efficiency of photoelectrochemical processes on semiconductor particles (*46, 47*). For example, Leng (*48*) replaced air sparging with pure O$_2$ in a TiO$_2$-based photocatalytic system and saw a 2-fold increase in the rate of oxidation of aniline.

On the other hand, it was also observed that upon switching to fluorescent light only, Pt–WO$_3$ was much less effective than Pt–TiO$_2$, even though it was ostensibly absorbing several times as much radiation. This points to the superior ability of TiO$_2$ to absorb near band gap light energy, generate charge carriers, and trap conduction band electrons near the particle surface until they can react (*49*). In the case of glucose photooxidation, a different situation is present where the oxidizable substrate is only present at 10.0 mM concentration, and so hole-capturing processes exert some control over the net reaction.

While total inhibition of algae growth was not achieved, it may be possible that more active photocatalysts and more uniform application of the photocatalyst would be successful. It may also be necessary to restrict application of photocatalytic coatings to thoroughly filtered aqueous environments, since build-up of sediments on the coatings can block the light while providing attachment sites for algae growth. At the least, photocatalytic coatings could substantially reduce the regimen of chemical oxidants needed to control microbial growth.

Acknowledgments

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