Mineralization of Bacterial Cell Mass on a Photocatalytic Surface in Air

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Whole cells deposited on a titanium dioxide-coated surface have been oxidized in air to carbon dioxide via photocatalysis. This paper provides the first evidence that the organic matter in whole cells can be completely oxidized. Three experimental techniques were employed to monitor this reaction: scanning electron microscopy, ^{14}C radioisotope labeling, and batch reactor measurements. The scanning electron microscopy experiments illustrate the disappearance of *Escherichia coli* cell mass. The ^{14}C radioisotope labeling experiments establish that the carbon content of *E. coli* is oxidized to form carbon dioxide with substantial closure of the mass balance. The batch reactor experiments corroborate the mass balance and provide a preliminary indication of the rate of the oxidation reaction. These results provide evidence that a photocatalytic surface used for disinfection can also be self-cleaning in an air−solid system.

Introduction

Disinfection of air in a photocatalytic system has been reported (*1*), the photocatalytic deactivation of microorganisms has been demonstrated in the aqueous phase, and possible mechanisms have been postulated (*2*−*4*). Additional work is included in a recent review of photocatalytic chemistry (*5*). In an aqueous stream, the dead or damaged cells can be washed off the catalyst surface. However, in an air-phase system, the dead cells have the potential to accumulate and block the active surface. We report here the first evidence for the photocatalytic oxidation of whole cells to carbon dioxide. This is a key observation on the path to development of self-cleaning photocatalytic surfaces for air disinfection.

Bioaerosols are major contributors to indoor air pollution, and their impact on indoor air quality has been reviewed (*6*−*8*). More than 60 bacteria, viruses, and fungi are documented as infectious airborne pathogens. Diseases transmitted via bioaerosols include tuberculosis, Legionaries, influenza, colds, mumps, measles, rubella, small pox, aspergillosis, pneumonia, meningitis, diphtheria, and scarlet fever. A larger number of bioaerosols are allergens and may be responsible for a growing incidence of asthma and other respiratory illnesses.

Mineralization (e.g., complete oxidation) of low molecular weight organic molecules has been widely studied in gas and aqueous fluid-phase systems in which titanium dioxide is the photocatalyst. A survey of this chemistry and a discussion of the mechanisms that have been proposed are covered in a recent review (*5*). Oxidation is accomplished through the interaction between a photocatalyst and ultraviolet (UV) light. Titanium dioxide (TiO_2) is a semiconductor photocatalyst with a band gap energy of 3.2 eV. When this material is irradiated with photons with wavelengths of less than 385 nm, an electron is promoted from the valence band to the conduction band. The resulting electron−hole pair participates in chemical reactions that form hydroxyl radicals and superoxide ions. These highly reactive species oxidize organic compounds adsorbed on the catalyst surface. The technique has been explored as a means of removing volatile organic compounds (VOCs) from indoor air (*9*, *10*).

The photocatalytic deactivation of bacterial cells using TiO_2 has been reported in both the aqueous and gas phases, but the fate of the cell mass has not been addressed in previous studies. For disinfection of air, it is necessary to remove or deactivate bioaerosols (*7*). A practical device for this purpose should accomplish three sequential steps: (1) bioaerosols are separated from air and immobilized on the catalyst surface; (2) bioaerosols are killed on the catalyst surface; and (3) bioaerosols oxidatively decompose. The focus of this paper is on step 3, oxidative decomposition of cell mass to carbon dioxide and water vapor. Oxidation of cell mass is a critical step for the continuous operation of a photocatalytic reactor as a self-sterilizing and self-cleaning filter for bioaerosols. Ultimately, the rate of cell mass oxidation must be matched to the rate of cell mass deposition if a practical device is to be designed. The first steps on this path are presented here.

Cell mass oxidation was established using three experimental techniques: (a) scanning electron microscopy, (b) ^{14}C radioisotope labeling, and (c) batch reactor measurements of carbon dioxide evolution. The scanning electron microscopy experiments show the disappearance of *E. coli* cells deposited on the irradiated TiO_2 surface. The ^{14}C radioisotope labeling experiments show that the *E. coli* are the source of the observed carbon dioxide and provide substantial closure of the carbon mass balance. The batch reactor experiments corroborate the mass balance while providing an initial indication of the rate of the oxidation reaction.

Results and Discussion

Scanning Electron Microscopy Experiments. The disappearance of cell mass on an irradiated photocatalytic surface was shown by coating microscope slides with a thin film of TiO_2 (DeGussa P25). The slides were inoculated with a sterile water suspension of *E. coli* LE392 (grown in Luria broth) and dried in air. Inoculated slides were exposed to UV (∼254 nm) or near-ultraviolet (nUV, ∼356 nm) light for 75 h. A blank was kept in the dark. After sputter coating with gold, the slides were examined via SEM.

Figure 1 illustrates the results of this experiment. Figure 1a is a photomicrograph of a TiO_2 film cast on a glass slide. Figure 1b shows the appearance of *E. coli* on glass with no photocatalyst coating. The cylindrical shapes are the bacteria. Figure 1c is a catalyst-coated, bacteria-inoculated blank that received no irradiation. Figure 1d reveals that, without

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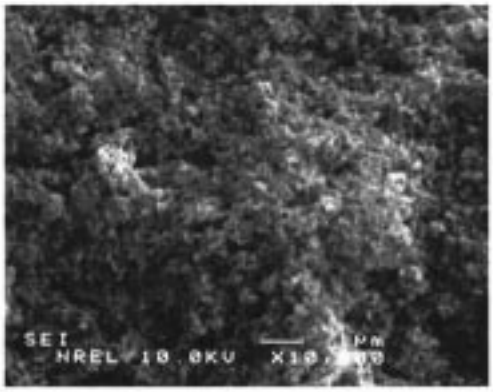
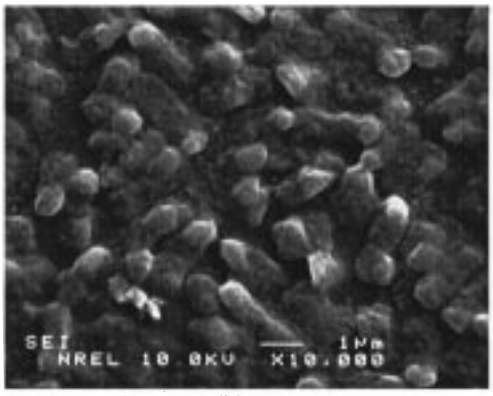
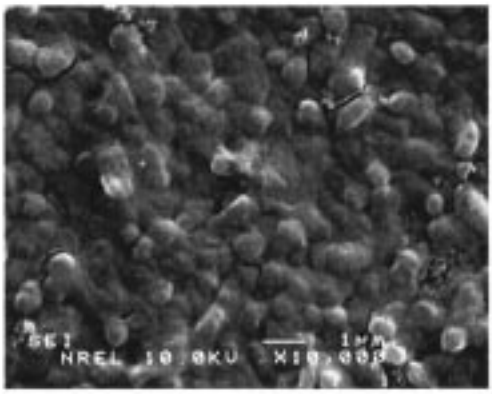


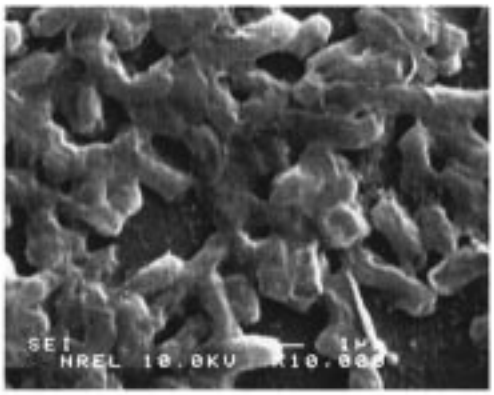
a) Titanium dioxide catalyst coated on a microscope slide.



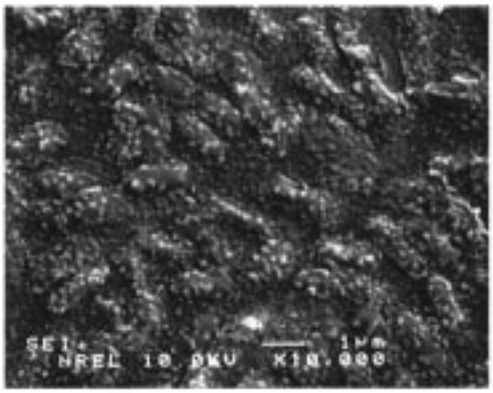
b) *E. coli* on a microscope slide, no catalyst, no exposure to light, cylindrical shapes are the bacteria.



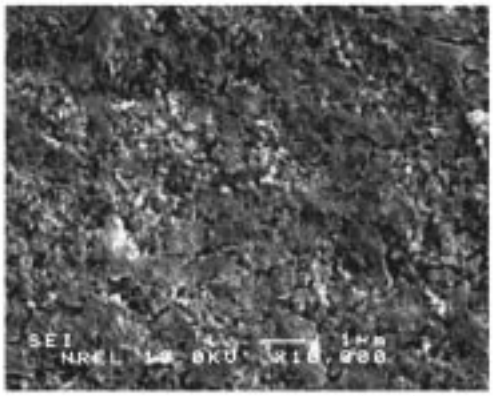
c) *E. coli* on a microscope slide coated with catalyst, no exposure to light.



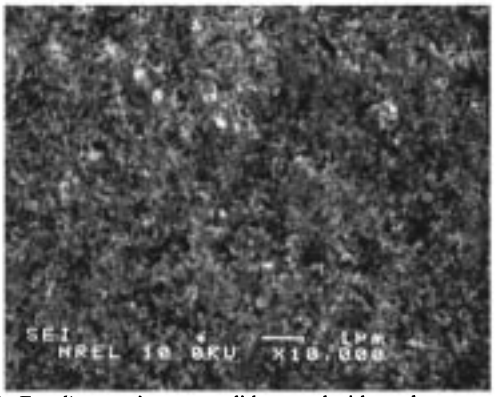
d) *E. coli* on a microscope slide, no catalyst, exposed to nUV light (356 nm) for 75 hours.



e) *E. coli* on a microscope slide, no catalyst, exposed to UV light (254 nm) for 75 hours.



f) *E. coli* on a microscope slide coated with catalyst, exposed to nUV light (356 nm) for 75 hours.



g) *E. coli* on a microscope slide coated with catalyst, exposed to UV light (254 nm) for 75 hours.

FIGURE 1. Scanning electron microscope images showing the disappearance of *E. coli* via heterogeneous photocatalysis.

catalyst, the nUV light has virtually no effect on the *E. coli* after 75 h of exposure. Exposure to UV radiation in the absence of catalyst for the same period effected some degree of decomposition, as shown in Figure 1e. In Figure 1, panels f and g, *E. coli* coated on irradiated films have decomposed almost completely after 75 h of exposure to nUV and UV radiation, respectively. These experiments were repeated four times using various TiO_2 coating techniques, and the results were consistent. In a fifth run, the films were cast onto quartz slides and the radiation was introduced from the reverse (uncoated and not inoculated) side of the slides. Similar results were again observed revealing that direct irradiation of the *E. coli* is not required to achieve decomposition of the microbes.

^{14}C Experiments. A flow-through photocatalytic reactor assembly was used to oxidize ^{14}C-labeled *E. coli* bacteria. The use of the radiolabeled bacteria provided the detection limits necessary to monitor the oxidation of cell mass and provide proof that the cell components are the source of the carbon dioxide. The ^{14}C-labeled cells were prepared by culturing *E. coli* K12 in fructose minimal medium (*11*) supplemented with 5 μCi of [U-^{14}C]fructose (New England Nuclear).

The photocatalytic reactor consisted of a fritted-glass disk (porosity E) with a total surface area of 12.6 cm^2 coated with 31.1 mg of TiO_2 catalyst (DeGussa P25). Approximately 0.33 mL of the ^{14}C-labeled *E. coli* K12 suspension in water was pipetted directly onto the disk and dried under a stream of nitrogen gas. The total dry cell mass loaded onto the frit was 1.5 mg containing 24 411 disintegration per minute (dpm). The reactor was irradiated by a nUV lamp that provided a light intensity at the photocatalyst surface of 4.1 mW/cm^2. The $^{14}CO_2$ produced was carried by a constant stream of zero-grade air flowing at 11.5 mL/min and subsequently trapped into a two-stage bubbler, each stage containing 65 mL of 0.2 N KOH solution. After 97 h of reaction, an aliquot of the KOH solution was pipetted into a scintillation vial with 8 mL of Optifluor scintillation cocktail (Packard) and counted in a Beckman LS 6000 scintillation counter. A dark control experiment was performed with a frit inoculated with labeled cells but not exposed to light.

Fifty-one percent of the radioactivity from the added bacterial cell mass was recovered in the KOH fraction as $^{14}CO_2$, a product of complete cell mass mineralization. Approximately 33% of the radioactivity still remained on the glass frit. This may be due to some cells being deposited in regions of the porous structure of the frit that were not accessible to photons. A negligible amount of $^{14}CO_2$ was detected in the dark control experiment. Preliminary experiments have also demonstrated mineralization of *Rhodobacter sphaeroides* SCJ, a Gram-negative, photosynthetic bacterium.

Batch Reactor Experiments. The goal of the batch mineralization experiments was to photocatalytically oxidize bacteria in a closed volume so that evolved CO_2 could build up to measurable concentrations and the rate of the oxidation reaction could be approximated. The experiments were performed using 250-mL gas sampling tubes that were equipped with high-vacuum valves and a septum port (Kontes Glass Company, Catalog No. 653150-250). In a typical experiment, a 1.0-mL aliquot of a 0.05 g/mL suspension of TiO_2 (DeGussa P25) was added to the gas sampling tube through the septum port, followed by a 1.0-mL aliquot of a 0.3 mg/mL *E. coli* K12 suspension. In blank control experiments, an equivalent amount of deionized water was added in place of the TiO_2 or *E. coli* suspensions. The *E. coli*/TiO_2 slurry was then dried with house air and moderate heating. After drying, the slurry formed an irregular film on the side of the sampling tube.

The tubes were purged with an 80:20 nitrogen:oxygen gas mixture and irradiated with an array of six evenly spaced nUV lamps, which provided a light intensity at the surface of the sampling tubes of approximately 3.5 mW/cm^2. The array heated the sampling tubes slightly above room temperature. The dark control experiments were performed by wrapping the sampling tube with aluminum foil before placing it under the light table.

Periodic samples were taken according to the following procedure. The sampling tube was removed from illumination and allowed to cool to room temperature. Triplicate 0.5-mL samples were removed from the tube with a gastight syringe and analyzed by gas chromatography (GC) (Hewlett-Packard model 5890 with a 6 ft $\times$ 1/8 in., Porapak Q column). The sampling tube was then repurged with a CO_2-free nitrogen:oxygen gas mixture, and illumination was continued. The GC was calibrated daily using a 998 ppm CO_2 calibration standard (Scott Specialty Gases).

Figure 2 shows representative kinetic data, including the control experiments. The data in curves 1 and 2 are experimental cases, where sampling tubes containing the *E. coli* and TiO_2 were illuminated. Three control experiments were also performed (curves 3−5), where *E. coli*, light, and TiO_2 were sequentially excluded from the gas sampling tube.

The greater cumulative yield of CO_2 among the experimental cases relative to the control cases shows evolution of CO_2 due to photocatalytic oxidation of cell mass. The statistical significance of this conclusion is established by the fact that each data point in Figure 2 represents between 6 and 15 replicate samples. Curve 1 includes representative error bars corresponding to 99% confidence intervals.

Curve 2 is similar in shape to curve 1 but exhibits a lower CO_2 flux. This may be due the degree of contact between the suspended *E. coli* and irradiated TiO_2. To test this, 23 h into experiment 2 the *E. coli*/TiO_2 film was resuspended in 2 mL of deionized water, redistributed over the sampling tube surface, and dried as before. An increase in the rate of CO_2 evolution is observed at 23 h in curve 2. The CO_2 evolution in the control experiments (curves 3−5) is likely due to leakage of CO_2 from the ambient air, although for the light/TiO_2 control experiment (curve 3), oxidation of trace organic impurities on the catalyst would also produce CO_2.

The carbon fraction of bacterial mass recovered as CO_2 was calculated by subtracting the 80 h data point from curve 3 (0.03 mg, no *E. coli*) from the mass average of the 80 h data points in curves 1 and 2 (0.11 mg) and then dividing by the initial bacterial mass loading (0.30 mg) to give a value of 27%. The literature values for carbon content of a typical bacterium range from 47% to 53% (*12*, *13*). If we assume 50% carbon content, the data in Figure 2 indicate 54% mineralization of the *E. coli* by photocatalytic oxidation. In this experiment, the amount of carbon remaining in the sampling tube could not be determined.

The literature contains references to cell killing via photocatalysis, but oxidation of cell mass has not been previously reported. Three different experimental approaches have demonstrated that a TiO_2 surface irradiated with nUV or UV illumination can oxidatively decompose a substantial portion of the bacterial cell mass in the presence of air. The carbon mass balances are not completely closed. The missing carbon could be due to cell material that is shaded from light and does not react or cell components that are refractory and do not react. The experiments reported here do not address the issue of the fate of mineral matter in the cells and the impact they might have on catalyst lifetime or activity. This inorganic matter could act to reduce the local photocatalytic activity at the site of the cell that is being decomposed.

Work is underway to further refine the carbon mass balances, to determine the fate of other elemental compo-

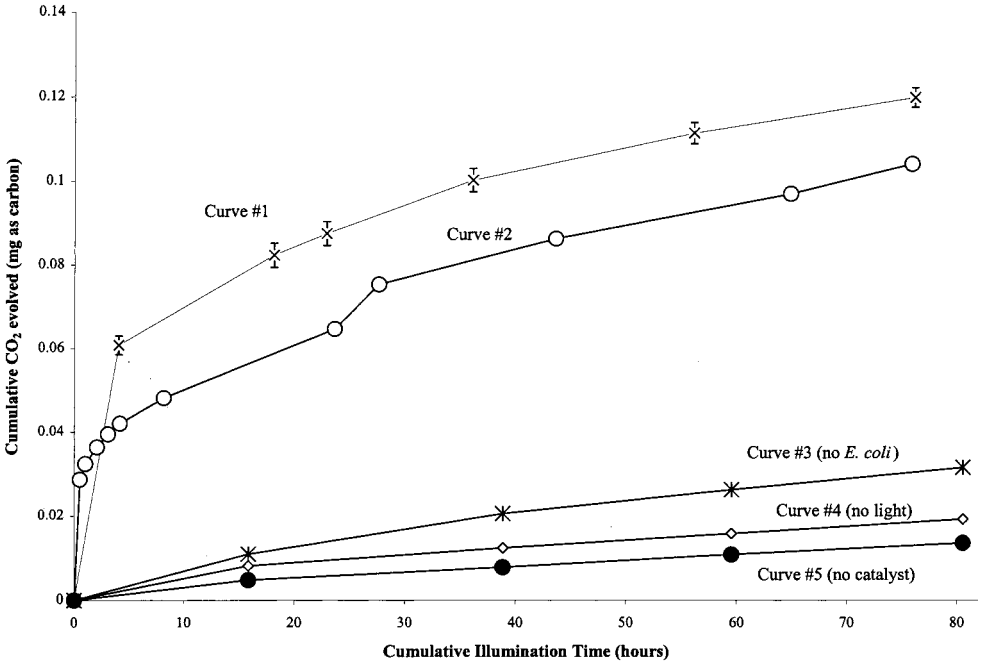


FIGURE 2. Cumulative CO_2 evolution as a function of irradiation time during the oxidation of cell mass via heterogeneous photocatalysis.

nents of mineralized cells, and to evaluate factors that control the rate and extent of cell mineralization. Variables to be investigated in this context include type of organism, light intensity, reactor design, and catalyst type and configuration.

Acknowledgments

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