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Association of Photosynthesis and Photocatalytic Inhibition of Algal Growth by TiO_2

Jinglan Hong[1]* and Masahiro Otaki[1]

Department of Human Environmental Sciences, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan[1]

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The relationship of photosynthesis and photocatalytic inhibition of algal growth (*Chroococcus* sp.) by TiO_2 was investigated by an ATP assay under ultraviolet (UV) and fluorescent light (FL) irradiations. The adverse effects of what on *Chroococcus* sp. growth in the TiO_2+FL+UV run are larger than those in the TiO_2+UV run. The difference is considered to be caused by oxygen generated by *Chroococcus* sp. under FL irradiation. A rate equation for the relationship of light irradiation intensity to rate of decrease in the *Chroococcus* sp. population on the basis of the Langmuir–Hinshelwood model has been proposed. The experimental data are in good agreement with the proposed model. In this study, the optimum FL intensity is 0.51 mW/cm^2.

[**Key words:** photosynthesis, ATP assay, photocatalytic, algal growth]

Large volumes of effluent wastewater from textile industries containing a high amounts of nitrogen and dye chemicals are produced, and are considered as one of the major industrial pollutants (1). Traditionally, physical and chemical treatment methods (2–4) have been applied to the removal of dyes from textile wastewater. However, because of the high costs of these techniques, the development of more economical favorable methods has attracted increasing attention from the dye industry.

Photodependent denitrifying sludge (PDDS), a type of stable denitrifying sludge containing photosynthetic bacteria, was previously used to investigate the simultaneous removal of dyes and nitrate in batch and continuous systems (5–8). It was found that PDDS is capable of removing the color from dyes utilizing methanol as an organic carbon source for conventional denitrification. However, in the continuous treatment of dyes and nitrogen with PDDS, problems of algal adhesion and growth on the walls of the reactor were observed. Excessive algal growth in the reactor caused filter clogging, inhibited light penetration and deteriorated PDDS activity. The reduction in specific surface area with increasing stocks of photosynthetic bacteria and alga and the drop in bacterial activity caused by oxygen generated by algae were found to be the main reasons for the deterioration in decolorant efficiency (9).

During TiO_2 excitation by light of less than 385 nm wavelength, the photon energy generates electron hole pairs on the TiO_2 surface. Holes in a valence band can react with H_2O or hydroxide ions adsorbed on the surface to produce hydroxyl radicals ($OH^{\bullet}$), and electrons in a conduction band can reduce O_2 to produce superoxide ions ($^{\bullet}O_2^-$) (10). These are highly reactive with organic compounds and microorganisms (11).

We previously reported the use of a laboratory-scale photocatalytic reactor for controlling excessive algal growth and adhesion, thus improving the penetration of optical light (12). Moreover, the effects of photocatalysis on a biological decolorant reactor and the biological activity of isolated photosynthetic bacteria were investigated (13). The decoloration efficiency in the reactor with photocatalysts was significantly higher than that in the reactor without photocatalysts. The volumetric loading rate of the reactor and the specific loading rate of sludge were improved about 4- and 5-fold, respectively. These studies suggested the possibility of using thin-film photocatalysis for controlling algal growth and enhancing the decolorant efficiency of photosynthetic bacteria. In addition, the different sensitivities of alga (*Chroococcus* sp.) and photosynthetic bacteria to photocatalysis were measured by an ATP assay (9). Matsunaga *et al*. reported that the mortality rate of microorganisms in a photocatalytic reaction is inversely proportional to the thickness and complexity of the cell wall (14, 15). The difference in structure between photosynthetic bacteria and *Chroococcus* sp. is considered to be the reason for this. In addition, as opposed to photosynthetic bacteria, *Chroococcus* sp. can generate oxygen under FL irradiation. Oxygen can be transformed to superoxide ions on the surface of TiO_2 film by UV light irradiation, therefore, oxygen generated by *Chroococcus* sp. is considered to be one of the causes of the increased mortality rate of *Chroococcus* sp. In this study, we investigated the effects of oxygen generated by *Chroococcus* sp. on its control by TiO_2 by observing viability.

MATERIALS AND METHODS

Reactor, substrate and operating conditions The influence of photocatalysis on algal growth was investigated using a glass

* Corresponding author. e-mail: jlhong@cc.ocha.ac.jp
phone/fax: +81-(0)3-5978-5747

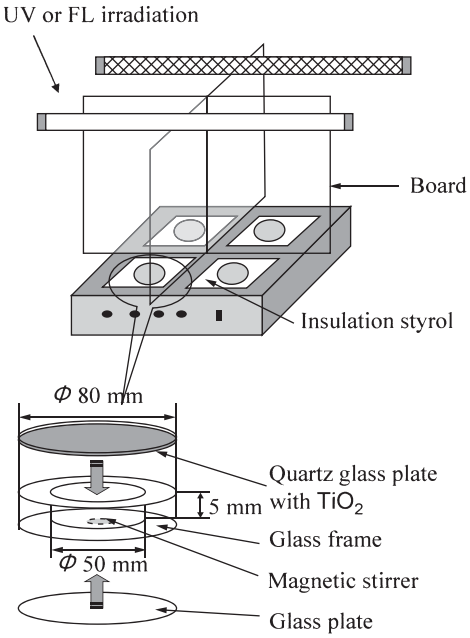

FIG. 1. Reactor.

frame with a height and a diameter of 5 mm and 5 cm, respectively, covering a quartz glass plate with and without TiO_2 (Fig. 1). *Chroococcus* sp. was used in our experiments as the model alga. The cultivation of *Chroococcus* sp., and the reactor, TiO_2 coating method, operating conditions and analytical methods used have been described in detail in our previous papers (9, 13).

Analytical methods Light intensity was measured using a UVX radiometer (UVX-25; Ultraviolet, Upland, CA, USA) or a lux meter (LM-332; As One, Osaka) with a measurable wavelength range of 400–700 nm. The cell concentration of *Chroococcus* sp. was quantified by a direct total cell count assay using a hemocytometer (no. 8100210; LO-Laboroptik, Friedrichsdorf, Germany) and an ATP count assay using a luminescencer-JNR II (AB-2300; Atto, Tokyo) with an ATP assay system (LL-100-1; Toyou Ink, Tokyo). Moreover, untreated and treated culture samples were filtered through the membrane filter and then measured spectrophotometrically at 260 nm to investigate the changes in dissolved organic materials before and after the treatment. Experimental grade oxygen and nitrogen (Kenis, Tokyo) were sparged into the solution to provide different levels of dissolved oxygen in solution. Dissolved oxygen was measured using a dissolved oxygen meter (810-Aplus; Beverly, MA, USA).

RESULTS AND DISCUSSION

Effects of photocatalytic reaction under different optical irradiations on *Chroococcus* sp. growth The effects of the photocatalytic reaction on *Chroococcus* sp. growth are shown in Fig. 2a. As shown, there was a significantly decrease in ATP-dependent luminescence in the ultraviolet (UV) and UV+fluorescent light (FL) runs. This indicates that the growth of *Chroococcus* sp. could be controlled in UV+FL and UV runs. Interestingly, a difference in ATP-dependent luminescence between the UV and UV+FL runs was observed. The difference was thought to be caused by oxygen generated by *Chroococcus* sp. by photosynthesis under FL irradiation. Oxygen was transformed to superoxide ions on the surface of TiO_2 film by UV light irradiation,

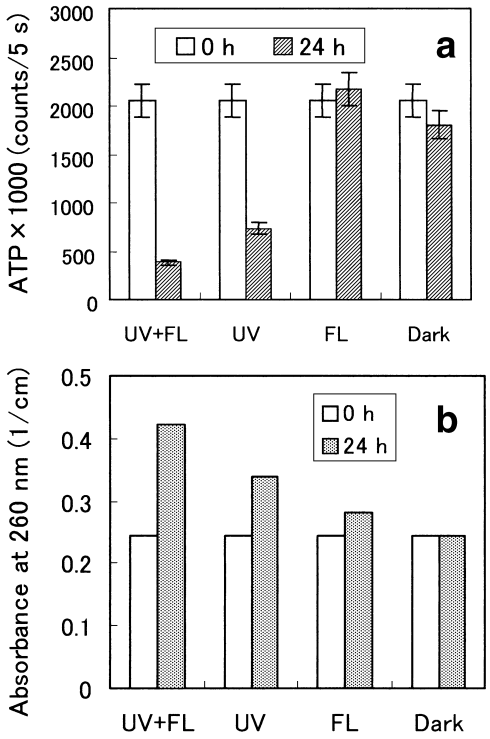

FIG. 2. Effects of photocatalysis on growth of *Chroococcus* sp. under different optical conditions: (a) ATP dependent luminescence and (b) UV absorbance at 260 nm in culture solution after filtration (0.45-μm pore size membrane filter; FL=0.51 mW/cm^2; UV=0.70 mW/cm^2).

and the mortality rate of *Chroococcus* sp. was increased. Figure 2b shows a comparison of UV absorbances at 260 nm between cases with and without photocatalytic reactions under various light irradiations. The increases in UV absorbance in the UV and UV+FL runs are larger than those in the FL and dark runs. This result indicates that the photocatalytic reaction activated the algal cells and destroyed the cell surface architecture, as was observed by microscopy (9). In addition, the UV absorbance in the UV+FL run is significantly higher than that in the UV run. This indicates that the level of destruction of the cell surface architecture of *Chroococcus* sp. in FL+UV run is higher than that in UV run.

Effects of dissolved oxygen on *Chroococcus* sp. growth Dissolved oxygen is well known for scavenging photogenerated electrons for use in further reactions. To confirm this, the effect of dissolved oxygen concentration on *Chroococcus* sp. growth in TiO_2+UV only run was investigated. The specific variation rate of ATP–dependent luminescence can be expressed as

$$Y = \frac{\ln C_t - \ln C_0}{t} \qquad (1)$$

where Y is the specific variation rate of ATP-dependent luminescence (h^{-1}), C_t is the ATP-dependent luminescence after light irradiation (counts/5 s), C_0 is of ATP-dependent luminescence before light irradiation (counts/5 s), and t is the light irradiation time (h).

With bubbling with nitrogen, oxygen or open air, the ini-

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