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Photocatalytic inactivation of cyanobacteria with ZnO/γ-Al_2O_3 composite under solar light

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Abstract

Cyanobacteria were inactivated by using zinc oxide (ZnO) coated on the surface of γ-Al_2O_3 as a photocatalyst and combining with sunlight. In vitro experiments indicate that axenic cultures of planktonic cyanobacteria lost their photosynthetic activity after photocatalyzed with sunlight exposure exceeding 24 hr. As for Oscillatoria tenuisa, nearly 92% of the cells lost their photosynthetic activity and the cell morphology was severely damaged during 24 hr of the reaction. However, in the case of Microcystis aeruginosa, lower photocatalytic inactivity efficiency was observed, which was attributed to extracellular polymeric secretions (EPSs) surrounding the cells. With a high dosage ZnO catalyst, the dissolved organic carbon (DOC) concentration of the Oscillatoria tenuisa suspension was increased by up to about 190% during photocatalysis. The increased suspension of DOC was attributed to increase liberation of extracellular organic and cell-wall polysaccharides during photocatalysis.

Key words

Cyanobacteria, Inactivation, Photocatalysis, ZnO film in γ-Al_2O_3

Introduction

As the main source of drinking water in Taiwan, reservoirs may be contaminated by irregular agricultural activities and domestic wastewater discharges, leading to the blooding of algae. Large populations of algae and cyanobacteria in water supply reservoirs may block filters in drinking water supply facilities. The facilities may occasionally have to suspend operations for several weeks owing to the inability to treat the water. In particular, drinking water potentially threatens health when toxic cyanobacterial blooms appear in drinking water supplies (Lee *et al*., 1998). Many algae might release substances, subsequently inducing taste and odor problems in drinking water. Unpleasant earthy and musty odors, particularly those caused by geosmin (GSM) and 2-methylisoborneol (2-MIB) from cyanobacteria, account for the major source of public complaints to water authorities in Taiwan (Hu and Chiang, 1996). Furthermore, the cyanibacteria toxins in water have led to the upgrading of drinking water treatment facilities with chemical oxidation, carbon adsorption, and membrane filtration.

Conventional water treatment practices have been found to be ineffective in removing algae and cyanobacteria (Lam *et al*., 1995; Chow *et al*., 1998; Drikas *et al*., 2001; Teixeira and Rosa, 2006). However, reducing algae and cyanobacteria in water via algaecides such oxidants as chlorine, chlorine dioxide, ozone and potassium permanganate in a pre-disinfection stage allows algae and cyanobacteria to output physiologically toxins and release extracellular organic matters (Huang *et al*., 2006). Additionally, a high disinfectant dosage during water treatment to terminate bacteria and algae simultaneously produces highly concentrated of disinfection by-products (Chen *et al*., 2008; Hong *et al*., 2008). Therefore, the physiological effects of water treatment chemicals on cyanobacteria

must be evaluated, as well as cyanobacteria inhibition methods developed.

Photocatalysis was found to terminate microbial cells in water. (Robertson *et al*., 2005; Ryu *et al*., 2008). This finding gave us an insight that the massive growth of cyanobacteria in eutrophic water can be prevented *a priori* by applying the photocatalytic activity of a catalyst. Despite extensive study in this area, to our knowledge, the potential use of this technology for the inactivation of cyanobacteria has not been explored. In this syudy, the photocatalytic inactivation of cyanobacteria in water is performed with the zinc oxide (ZnO) thin film coated on the surface of γ-Al_2O_3.

Materials and Methods

Preparation of supported ZnO film on γ-Al_2O_3: Supported ZnO films on γ-Al_2O_3 were prepared by a modified sol-gel, process (Wang and Lin, 1998; Buelna and Lin, 2001) as follows. Zinc acetate [$Zn(CH_3COO)_2 \cdot 2H_2O$] was dissolved in 50 ml of absolute ethanol and then stirred with a magnetic stirrer (50°C) for thorough mixing. When the solution transformed into an emulsion, an amount of diethanolamine ($NH(CH_2CH_2OH)_2$; DEA) as the chelating agent and deionized water were added to the emulsion. The molar ratio of DEA/zinc acetate was 1:1 and deionized water/zinc acetate 2 : 1, respectively. When the emulsion became obvious after stirring, PEG of various contents were added and continually stirred for 2 hr until a transparent sol was obtained. Next, sol was coated on γ-Al_2O_3 using the dip-coating method, in which the drawing rate was around 6 cm min^{-1}. The coated films were dried at 100°C in air for 10 min. The above process was repeated six times to obtain the film thicknesses required. The obtained films were calcined in air up to 500°C at a heating rate of 2°C min^{-1} and left at 500°C for 1 hr. The ZnO film thickness was found to be 2.5 μm using stylus profilometry.

Cultivation of cyanobacteria: Axenic cultures of planktonic cyanobacteria *Oscillatoria tenuisa* and *Microcystis aeruginosa* were separated from Feng-San Reservoir (Kaohsiung, Taiwan). Cyanobacteria samples were separated using dissection phase contrast micro-scope (Model: SSM-422L, Microtech, Taiwan) and grown in standard culture media, which ASM-1 (Gorham *et al*., 1964) for *Oscillatoria tenuisa*, DY-3 (Lehman, 1976) for *Microcystis aeruginosa*.

The pH value was adjusted to 8.5 by buffering with bicine (*N,N*-2 bis (2-hydroxymethyl) glycine, $C_6H_{13}NO_4$). Cultures were grown in a mineral liquid medium at 25°C, bubbled with air containing 5% (v/v) CO_2 and continuously illuminated with cool white and daylight from fluorescent lamps (30-33 μE $m^{-2}s^{-1}$), at the surface of incubation chambers.

Photocatalytic inactivation of cyanobacteria: Photocatalytic reactions were performed in a semi-batch reactor (15 cm ID., 60 cm H.) The reactor was illuminated from the outside with a solar lamp (Hanau Suntest AM1). This lamp has a light spectral distribution of around 0.5% of the emitted photons at wavelengths shorter than 300 nm and around 7% between 300 and 400 nm. The emission spectrum between 400 and 800 nm follows the solar spectrum.

The applied concentrations of the photocatalyst were maintained with a range of 130-525 mg l^{-1} ZnO/γ-Al_2O_3, corresponding to dosages of 10-40 mg-ZnO. Samples were collected at predetermined times (t) in the dark. Then the solar lamp was turned on at an intensity of 1000 W m^{-2}, and samples were collected at predetermined times for 24 hr. Each experiment was repeated three times.

Photosynthetic efficiency of cyanobacteria was determined using the carbon-14 method (McConnaughey *et al*., 1997). Samples were withdrawn from the reactor during the reaction at various reaction times, and were charged into light and dark bottles. A solution of radioactive carbonate ($^{14}CO_3^{-2}$) was then added to the sample bottles. Following, incubation *in situ*, the cyanobacteria were collected on a membrane filter and then treated with hydrochloric acid (HCl) fumes to remove inorganic carbon-14, followed by assaying for radioactivity with a liquid scintillation counter. The quantity of carbon fixed by cyanobacteria is proportional to the fraction of radioactive carbon assimilated.

Analytical methods: Polysaccharides, amino acids, and carboxylic acids, were characterized in main identified extracellular polymeric secretions (EPSs). Extraction of the EPSs was prepared by the Folch, Lee & Sloane-Stanley method (Parrish, 1999). The total quantity of EPSs in cyanobacterial suspension was estimated using the parameter of dissolved organic carbon (DOC). The DOC in two culture chambers was determined daily from incubation day 0 to day 36. The DOC analysis utilized the combustion-infrared method with a total organic carbon analyzer (Model TOC-5000) (Shiamadzu, Tokyo, Japan).

Cyanobacterial cultures were counted in sedimentation chambers using a compound microscope Eclipse TS 100 MDS-3600 (Ching-Hsing Corporation, Taiwan) (Utermohl, 1958). Cyanobacteria biovolume was estimated with a geometrical program Opticount (PIA Image Analysis System). The chlorophyll-a was determined using a spectrophotometric method (APHA, 2005). The potessium ion (K^+) concentrations in the filtrates were measured with inductively coupled plasmaoptical emission spectrometer (ICP-OES) (Model; 2100 DV, Perkin-Elmer, Norwalk, CN).

Monosaccharides in aqueous phases were determined using high performance anion-exchange chromatography (HPAEC) (Model: DX-500, Dionex, Sunnyvale, CA) (Bruggink *et al.,* 2005) equipped with a gradient pump GP 40 and electrochemical detector ED 40 utilizing a Carbo-Pac PA1 (4 × 250 mm) pellicular anion-exchange column and with a Carbo-Pac guard column. Data were estimated using the Peak-Net 4.3 (Dionex, Sunnyvale, CA) software.

Pentafluorobenzylhydroxylamine (PFBHA) derivates were used to extract and assist in the identification of relatively polar carbonyl-containing compounds (carboxylic acids). Approximately 250 ml of culture filtrates were derived with PFBHA using the procedure developed by Richardson *et al*. (1999). The derivatized carboxylic acids were then extracted with hexane and concentrated

to 1 ml by rotary evaporation. The derivatives were analyzed by GC/EI-MS with a Varian 3700 GC connected to an ion trap MS detector (Walnut Creek. CA, USA) (APHA, 2005).

Results and Discussion

Inhibition of cyanobacteria growth: Axenic cultures of planktonic cyanobacterial suspension properties were determined before and after the photocatalytical reaction to determine how photocatalysis affects the inhibition of cyanobacteria growth. Experimentally, an *Oscillatoria tenuisa* suspension with $1.5x10^5$ cell ml^{-1} was photocatalyzed with a range of 130-525 mg l^{-1} ZnO/γ-Al_2O_3, corresponding to dosages of 10-40 mg-ZnO. Photocatalysis with 10-40 mg-ZnO decreased the chlorophyll content of the cyanobacterial suspension by 17-64% (Fig. 1a). The photocatalyzed

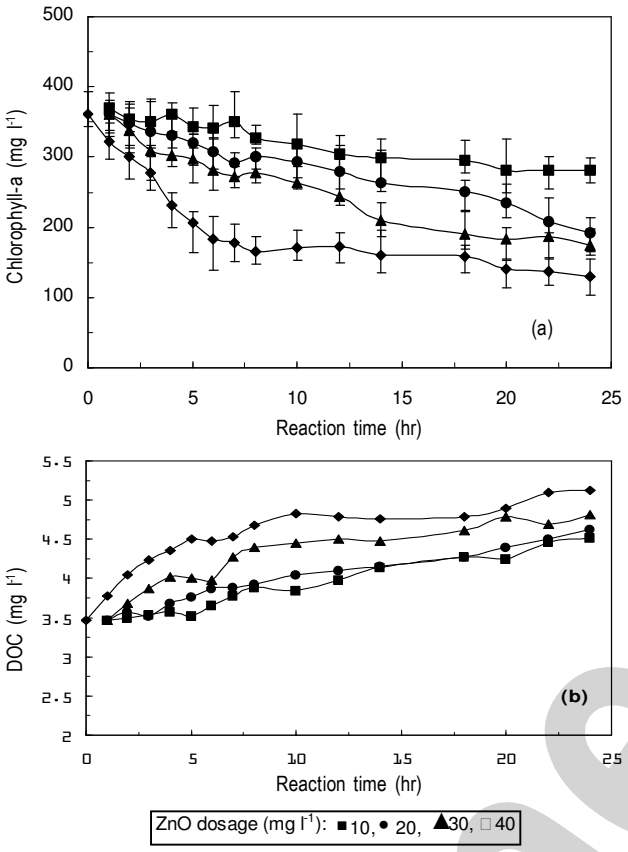

Fig. 1: The concentrations of chlorophyll-a (a) and DOC (b) as a function of photocatalytic reaction time in *Oscillatoria tenuisa* suspensions

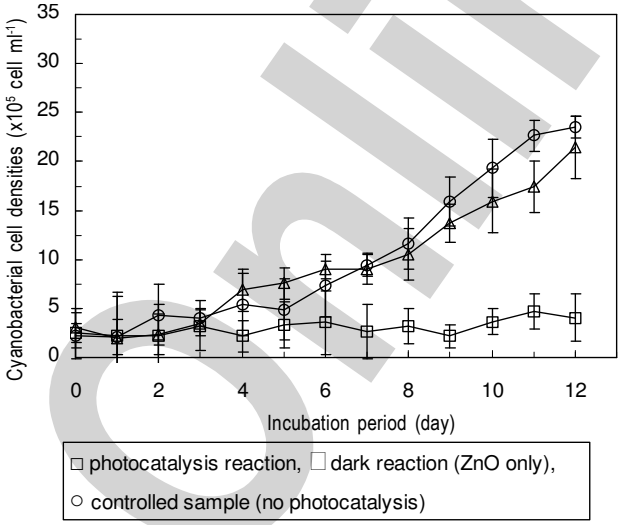

Fig. 2: Time courses of cell densities of *Oscillatoria tenuisa* cultures during incubaction period

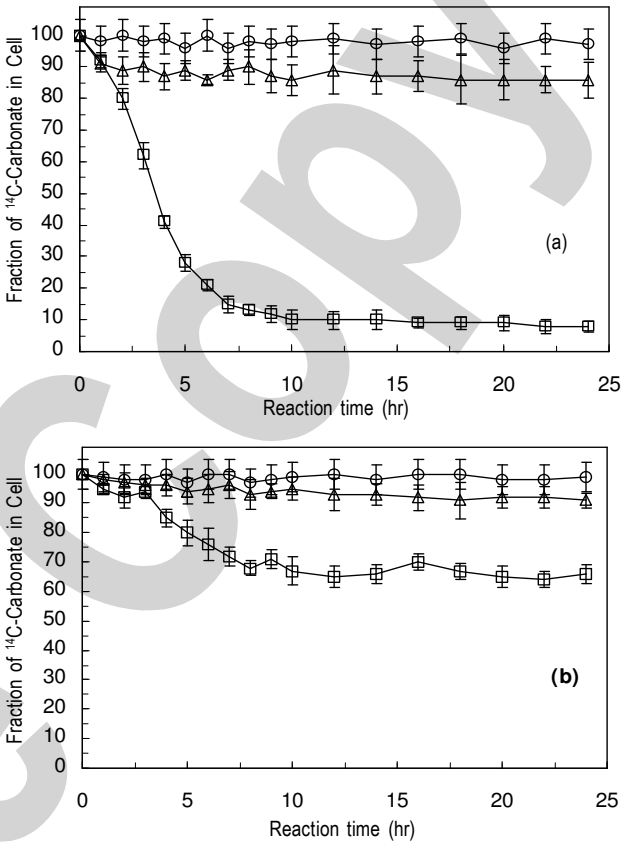

Fig. 3: Relative changes in photosyntheyic activity of *Oscillateria tenuisa* (a) and *Microcystis aeruginosa* (b) during photocatalytic reaction

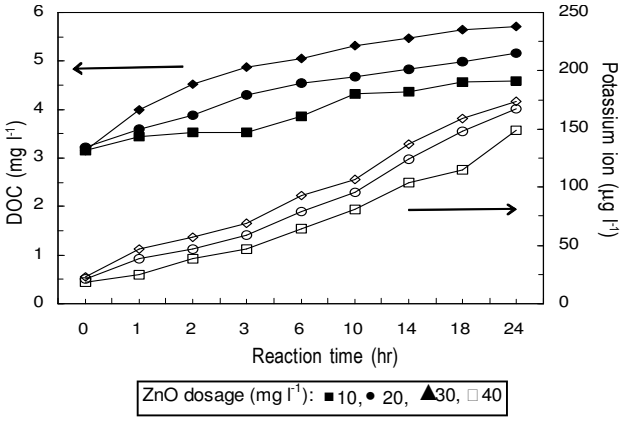

Fig. 5: The concentrations of DOC and potassium ion as a function of photocatalytic reaction time

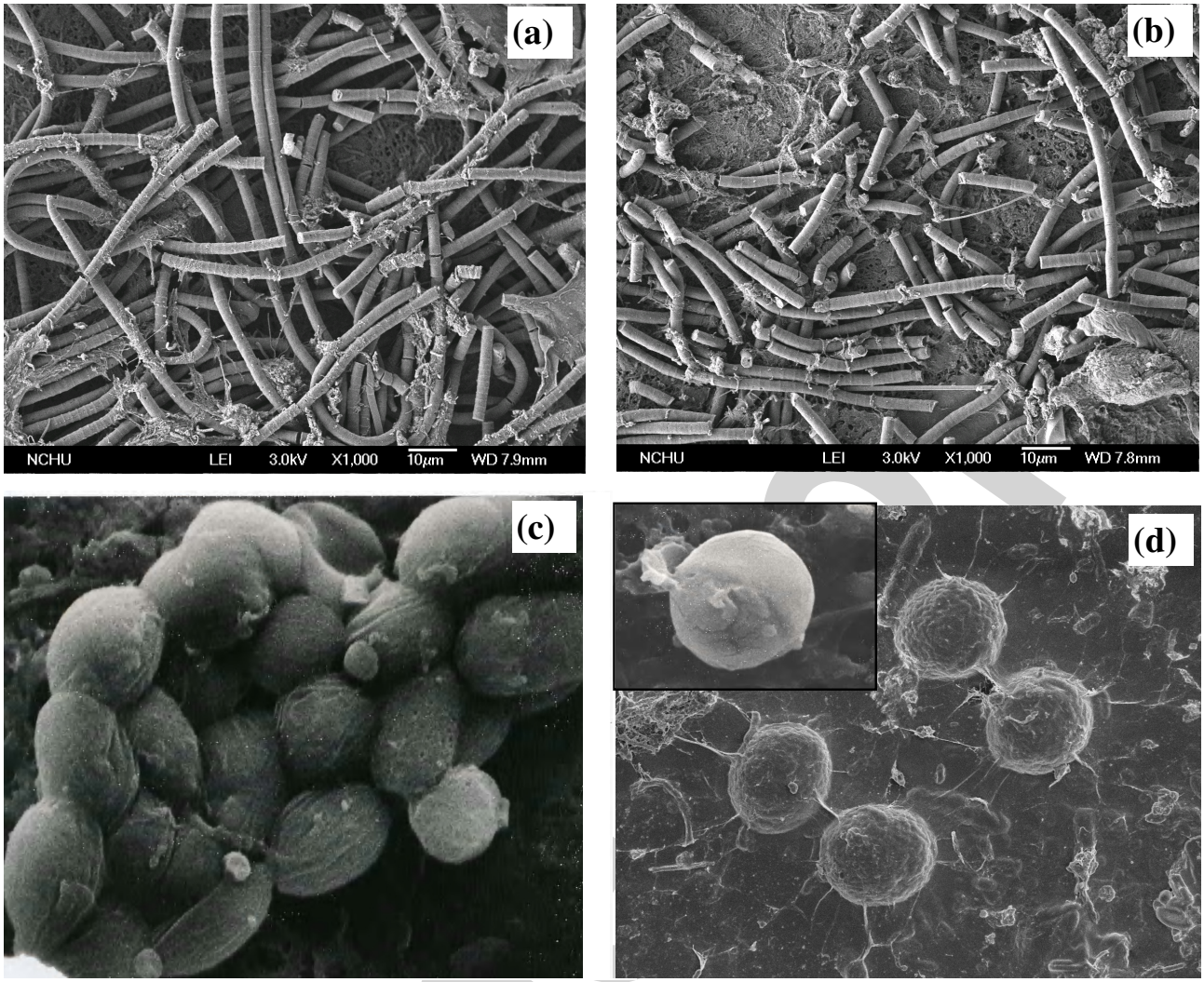

Fig. 4: Electron micrographs of cyanobacteria. (a) *Oscillateria tenuisa*: original (control sample), (b) *Oscillateria tenuisa*: after photocatalytic reaction, (c) *Microcystis aeruginosa*: original (control sample), and (d) *Microcystis aeruginosa*: after photocatalytic reaction

process was accompanied by a 15-47% increase in the dissolved organic carbon (DOC) content of the suspension (Fig. 1b). Normal sunlight exposure did not affect algal chlorophyll pigments, as expected (Liang *et al.,* 2006). Algal cells contain a large amount of green pigment in the chloroplasts of the cells. The loss of chlorophyll is attributed to the degration of algal pigment components such as chlorophyll-*a* and *b* and pheophytin-*a* by ZnO-promoted oxidation. The apparent change in colour from blue-green to brown in the photocatalyzed algal smaples points to a damaging oxidative process taking place at the ZnO surfaces that eventually leads to the degradation of the chlorophylls and other pigments, consistent with the data collected used TiO_2-mediated photocatalysis as by Hong and Otaki (2003).

Moreover, photocatalyzed samples were placed into cylinders and were maintained at 25°C under a fluorescent lamp in the incubation chamber with a 12/12hr light/dark cycle. Samples were periodically withdrawn for microscopic observation and accounting. According to Fig. 2, cyanobacterial cell density changes only slightly immediately after photocatalysis. However, after the fourth day of incubation under fluorescent light, the photocatalyzed and controlled samples (no photocatalysis) significantly differed in cell density. By the end of the twelfth day, the cell densities in all photocatalyzed samples were lower than 85% of those of the control group. After the fourth day, the green color of the cyanobacteria cells gradually started to fade and at the end of the twelfth day, all of these cells had either completely faded or their cell wells were broken. However, cells subjected to an addition of 40 mg l^{-1} ZnO without light illumination (dark reaction) continued to survive. The cell densities in these samples remained the same throughout the incubation period, whereas the cell density in the controlled samples increased from $(2.5 \pm 1.7) \times 10^5$ to $(2.3 \pm 1.6) \times 10^6$ cell ml^{-1}. Additionally, the ZnO only sample increased from $(2.1 \pm 1.3) \times 10^5$ to $(1.9 \pm 1.8) \times 10^6$ cell ml^{-1}. Notably, the no photolysis samples and the control group differed only slightly in terms of cell density after twelve days of incubation. From the variation of cell density and morphology, we can infer that

Table - 1: Concentrations of media nutrient during growth of cyanobacteria at 25°C

Cyanobacteria	Treatment	Nutrients			
		Light ($\mu E\ m^{-2}\ s^{-1}$)	NO_3^{-1}-N (mg l^{-1})	NH_3-N (mg l^{-1})	PO_4^{-3}-P (mg l^{-1})
Oscillatoria tenuisa	ASM-1[a]	30	32.8	—	3.2
Microcystis aeruginosa	DY-3[b]	32	6.8	—	0.7

[a] Source: cyanobacteria media adapted from Gorham *et al*. (1964), [b] Source: cyanobacteria media adapted from Lehman (1976)

Table - 2: Compounds identified by GC/EI-MS and HPAEC in cyanobacteria cultures, and percentage variation in photocatalyzed samples

Compounds	Cyanobacteria culture extracts (ppm)	Photocatalyzed samples (% variation) (ZnO dosage: 10 mg l^{-1})	Photocatalyzed samples (% variation) (ZnO dosage: 40 mg l^{-1})
Monosaccharides		0	
Rhamnose	0.08	+27.2	+28.7
Fucose	0.12	+16.4	+42.3
galactose	0.05	+9.6	+67.5
xylose	0.17	+26.7	+38.6
Amines and amino acids			
Ethylamine	0.27	+61.2	+88.4
Dimethylamine-	0.38	+23.6	+43.3
Trimethylamine	0.52	+79.4	+62.2
Cyclohexylamine	0.17	+9.6	+37.5
Aniline	0.08	+17.5	+15.4
Glycine	0.05	+26.7	+43.7
Alanine	ND[a]	ND	ND
Methionine	0.03	+0.8	+2.6
Phthalates			
Dimethyl phthalate	ND	+100[b]	+100
Diethyl phthalate	ND	+100	+100
Dibutyl phthalate	ND	ND	+100
1,2-Benzenedicarboxylic acid, bis (2-methoxyethyl) ester	ND	ND	+100
1,2-Benzenedicarboxylic acid, butyl-2-methylpropyl ester	ND	ND	+100
1,2-Benzenedicarboxylic acid, dipropyl ester	ND	ND	+100

[a] ND= Not detected, [b] +100 = Generated compound

the increase in DOC for photocatalyzed samples is attributed to increase liberation of extracellular organic matter or originate from lysed cyanobacterial cell.

Fig. 3 Illustrates the relative changes in photosyntheyic activity of *Oscillateria tenuisa* and *Microcystis aeruginosa* during photocatalytic reaction, respectively. The relative ^{14}C-assimilation recorded on the Y-axis refers to the ratio of the ^{14}C-assimilation before reaction and that during reaction (Kumar *et al.,* 2003; Andreasson and Wangberg, 2006). Most of the inactivation of the photosynthetic activity of *Oscillatoria tenuisa* occurred within 10 hr. However, in the case of *Microcystis aeruginosa*, significantly lower photocatalytic inactivation efficiency was observed. Approximately 35% of the cells lost their photosynthetic activity in 24 hr. We believe that the lower inactivation of *Micorcystis aeruginosa* is due to the presence of extracellular polymeric secretions surrounding the cells. EPS with a high molecular weight must have prevented the cyanobacterial cells from oxidation through the contact of ZnO photocatalyst. The SEM microscopic images of the two cyanobacteria types (Fig. 4), *Oscillatoria tenuisa* and *Micorcystis aeruginosa*, before reaction and after 24 hr of photocatalyzed reaction (ZnO dosage = 40 mg l^{-1}), significantly differed from each other. *Oscillatoria tenuisa* is a typical filamentous cyanobacterium, in which cells have scattered solitary granules and apical rounded cells; trichomes are straight, are not constricted at cross-walls, and are cylindrical. When the original *Oscillatoria tenuisa* cells undergo photocatalyzed reaction after around 10 hr, the cylindrical skeleton of *Oscillatoria tenuisa* broke. *Microcystis aeruginosa* is a typical nonfilamentous and a colonial cyanobacterium (Falconer and Yeung, 1992). Colonies of *Microcystis aeruginosa* consisted of hundreds of .spherical cells in a mucilaginous sheath. The spherical cell colonies were completely

isolated after the photocatalyzed reaction. Moreover, the literature reported that the extent of killing of microorganisms by a photocatalysis was inversely proportional to the thickness and complexity of the cell wall (Matsunaga *et al.,* 1988). The different sensitivities of *Oscillatoria tenuisa* and *Microcystis aeruginosa* to the photocatalytic reactions were considered to be one of the main reasons.

Fate of metabolic products during photocatalysis: The bulk properties of culture suspension were determined before and after the photocatalyzed reaction to understand how photocatalysis affects the dissolved organic characteristic of the suspension. According to Fig. 5, an *Oscillatoria tenuisa* suspension with 2.57 x 10^5 cell ml^{-1} was photocatalyzed under the following conditions: pH = 7.5; ZnO dosage is 10-40 mg l^{-1}; and the reaction time is 24 hr. The DOC concentration increased with the reaction time from 0 to 24 hr. High amounts of DOC was observed at a higher ZnO dosage. Above results attribute the increase in suspension of DOC to the increased liberation of extracellular organic matter and cell-wall polysaccharides during photocatalysis. Fig. 5 also illustrates the time-dose-response curves in relation to cell wall damage, as obtained by determining the cellular release of potassium. The release of potassium by cyanobacterial water increased steadily with ZnO dosage. The level of K^+ leakage likely is related to the degree of damage in the cell wall and thus could be used as a physiological indicator of toxic effects (Aliotta *et al.,* 1990; Schmitt *et al.,* 1999).

Metabolic products during photocatalysis were identified by subjecting photocatalyzed *Oscillatoria* cells to high-resolution GC/EI-MS and HPAEC (for polysaccharides analysis). Table 2 summarizes the classes of compounds that are identified in the photocatalyzed sample extractions. Column 2 displays the responses of the MS detector in terms of an estimated peak area for each compound that was extracted from cultures. Columns 3 and 4 present the relative concentrations in a given peak area following photocatalysis at ZnO dosages of 10 and 40 mg l^{-1}. The compound concentrations were determined from the area under the corresponding peaks in relation to the area under the peak for the internal standard anthracene D 10 (semi-quantitative estimation). This determination is based on a response of unity for all compounds related to the anthracene D 10 internal standard. The samples were spiked with an amount of internal standard that was equivalent to 1 mg l^{-1} in the original cyanobacteria suspensions. Table 2 reveals an increase in the amounts of carbohydrates (monosaccharides), amines/amino acids, and carboxylic acids (phethalate) during photocatalysis. Analyzing the data obtained by high-resolution GC/EI-MS and HPAEC reveals that the complete physiological inactivation, resulting from photocatalysis, was accompanied by extensive damage to the cell wall and membrane and the release of cell cytoplasm, including carbohydrates, proteins, and nucleobases. Carbohydrated, including polysaccharides and monosaccharides, are characterized by an extreme structural diversity; as a result they play very diverse roles in nature. Numerous algal polysaccharides and monosaccharides are potentially available, known to be involved in pathogenesis, protection from phagocytic predation and stress resistance in microorganisms (Sutherland, 2001; Vanhaverbeke *et al*., 2003). In previous studied, we demonstrated that the Microcystis carbohydrates in cell wall and membrane contain more that five species of monosaccharides, there are included hamnose, fucose, galactose, xylose and uronic acid, whereas the extract from *Oscillatoria* and *Anabaena* contained a small amount of rhamnose, fucose and galactose (Huang *et al*., 2007). This was similar with the composition of other cyanobacterial EPSs studied by Hy *et al.* (2003). They have reported the presence of 6-12 monosaccharides in EPS, produced by four filamentous cyanobacteria.

Above observations obviously indicate that photocatalysis processes could significantly inhibit the photosyntheyic activity of cyanobacteria under sunlight assisted by ZnO catalyst. Two species of cyanobacteria *Oscillatoria tenuisa* and *Microcystis aeruginosa* were taken for the inactivation experiments. The cylindrical skeleton of *Oscillatoria tenuisa* broke, and most of the isolated cells lost their photosynthetic activity. Although the colonies of *Microcystis aeruginosa* cells were completely separated into an individual spherical one, the inactivation efficiency for *Microcystis aeruginosa* was somewhat lower than the *Oscillatoria tenuisa*. We believe that the lower efficiency is due to EPSs surrounding the cells of *Microcystis aeruginosa*. Furthermore, organic substances are released from cyanobacteria cells during the photocatalyzed reaction. With a high dosage ZnO catalyst, the DOC concentration of the cyanobacteria suspension increased by up to about 190%. This additional DOC, apparently arising from the increased liberation of EPSs. Analysis results for identification of the liberation of EPSs indicated that the major compounds were carbohydrates, amino acids, and carboxylic acids.

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