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Removal of Hazardous Phenols by Microalgae under Photoautotrophic Conditions

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Various algae were screened for their ability to decrease the concentration of 2,4-dinitrophenol (DNP), as a model compound of hazardous phenols, under photoautotrophic conditions. *Chlorella fusca* var. *vacuolata* and *Anabaena variabilis* grew well and showed high DNP removal ability over the concentration range of 5 to 40 μM. Their abilities to remove various phenols were investigated. More than 90% of 40 μM *o*- and *m*-nitrophenol and DNP was removed during the cultivation period of 5 d. *o*-, *p*-Chlorophenol and 2,4-dichlorophenol could be removed, but not to a significant extent. *C. fusca* also removed 85% of bisphenol A, suspected to be an endocrine disrupter. It was found that microalgae would be applicable to the removal of hazardous phenols without the addition of any organic carbon sources.

[**Key words:** green algae, cyanobacteria, nitrophenol, chlorophenol, bisphenol A, photoautotrophic conditions]

Microalgae have actually been used in tertiary treatment processes for removing nitrogen and phosphorus from wastewater (1) and have the potential to be used to remove various pollutants, such as heavy metals (2) and NOx (3, 4). Phenol and its derivatives are commonly found in industrial wastewater from the production of pharmaceuticals, agricultural chemicals, dyes, and plastics (5–8). Phenol is known to be toxic to fish at concentrations of 5 $mg \cdot l^{-1}$ to 25 $mg \cdot l^{-1}$ (9). It was also reported that nitrophenols and chlorophenols are toxic to aquatic organisms over the same concentration range (10–12). The emission standard for phenols in industrial effluent after wastewater treatment is set at 5 $mg \cdot l^{-1}$ by the Ministry of the Environment in Japan. In this study, therefore, phenols at a concentration of 40 μM, approximately corresponding to the emission standard for phenols, were used. Some of the phenolic compounds are suspected to be endocrine disrupters and have adverse effects on humans and other organisms in the natural ecosystem at concentrations lower than the emission standard for phenols (13–15). Therefore, the low concentrations of these phenols need to be removed from industrial effluents for aquatic environmental protection. If bacteria are used for the removal of phenols from effluent, it is necessary to add an external organic carbon source to maintain biomass and the ability to remove these compounds. On the contrary, as microalgae use CO_2 as a carbon source, they can grow photoautotrophically without the addition of an organic carbon source. We therefore focused on the utilization of microalgae for removing low concentrations of hazardous phenols from industrial effluents. The objective of this study was to obtain microalgae with the ability to remove various phenols at the concentration of the emission standard under photoautotrophic conditions.

Chlamydomonas reinhardtii IAM C-238, *Chlorella ellipsoidea* IAM C-87, *Chlorella fusca* var. *vacuolata* IAM C-28 (*C. fusca*), and *Chlorella sorokiniana* IAM C-212 were cultivated in modified Bristol medium (16), the pH of which was adjusted to 6.0. *Carteria inversa* NIES 422, *Chlamydomonas fasciata* NIES 437, and *Chlamydomonas moewusii* IAM C-259 were cultivated in C-medium (17), the pH of which was adjusted to 7.5. Cyanobacteria, *Anabaena cylindrica* NIES19 and *Anabaena variabilis* NIES23 were cultivated in MDM (16), in which 1.60 $mg \cdot l^{-1}$ $Fe_2(SO_4)_3 \cdot nH_2O$ and 4.32 $mg \cdot l^{-1}$ EDTA were used instead of $FeSO_4 \cdot 7H_2O$. The pH was adjusted to 7.5. *Microcystis aeruginosa* f. *aeruginosa* NIES 44 was cultivated in M-18 medium (18), the pH of which was adjusted to 8.0. The strains were pre-cultivated in the respective media at 27.5°C under illumination with white fluorescent light (14 $W \cdot m^{-2}$), and aerated with air containing 1% CO_2. After 7 d, cells were harvested by centrifugation (845 × *g*, 5 min, 15°C). The cell pellet was re-suspended in fresh medium and the optical density of cells at 680 nm (OD_{680}) was adjusted to 1.0. For the removal of nitrophenols including *o*-, *m*- and *p*-nitrophenol, 2,4-dinitrophenol (DNP), and 2,4,6-trinitrophenol, and bisphenol A [2,2-bis(4-hydroxyphenyl)propane], the cell suspension (50 ml) was transferred into a 100-ml glass test tube. Algal cells were cultivated under the same conditions as those for pre-cultivation. For chlorophenols, including *o*- and *p*-chlorophenol, and 2,4-dichlorophenol, a small closed-type photobioreactor was used, because these compounds are easily volatilized from the medium by aeration. The photobioreactor consisted of a 100-ml Erlenmeyer flask sealed with a silicon rubber cap that was covered with aluminum foil. Algal cells were cultivated at 25°C on an orbital

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TABLE 1. Removal of 2,4-dinitrophenol by microalgae

Strain	Removal (%)
Green algae	
Carteria inversa NIES 422	38
Chlamydomonas fasciata NIES 437	20
Chlamydomonas moewusii IAM C-259	3
Chlamydomonas reinhardtii IAM C -238	1
Chlorella ellipsoidea IAM C-87	5
Chlorella fusca var. *vacuolata* IAM C-28	57
Chlorella sorokiniana IAM C-212	26
Cyanobacteria	
Anabaena cylindrica NIES 19	20
Anabaena variabilis NIES 23	86
Microcystis aeruginosa f. *aeruginosa* NIES 44	63

[a] Initial concentration of 2,4-dinitrophenol was 40 μM. Microalgae were cultivated with 2,4-dinitrophenol for 72 h.

shaker for 120 h under illumination (14 $W \cdot m^{-2}$). The initial concentration of phenols was adjusted to 40 μM. The cell growth was evaluated by the OD_{680}, which was measured using a spectrophotometer (U-2000; Hitachi, Tokyo). The concentrations of phenols were measured using a high performance liquid chromatography (HPLC) system (D-7000 series; Hitachi) with a diode array detector (L-4500; Hitachi) at 280 nm using a reversed-phase column (Mightysil RP-18 GP250-4.6; Kanto Chemical, Tokyo). Acetonitrile/50 mM potassium dihydroxy phosphate buffer (pH 2.5) (50/50, v/v) was used as the mobile phase at a flow rate of 0.7 $ml \cdot min^{-1}$. The detection limit of phenols was about 0.5 μM.

We attempted to select freshwater microalgae exhibiting the ability to remove 40 μM DNP, which was employed as a model compound, because DNP exhibits toxicity to microalgae by inhibiting oxidative phosphorylation and photosynthesis and is often contained in the wastewater from dye production. At first, the concentration change in DNP in uninoculated media was tested, and the concentration was found not to decrease. Table 1 shows the removal of DNP during the cultivation period of 72 h. *C. fusca* and *A. variabilis* removed 57% and 86% of the DNP, respectively, which are the highest removal levels among the seven green

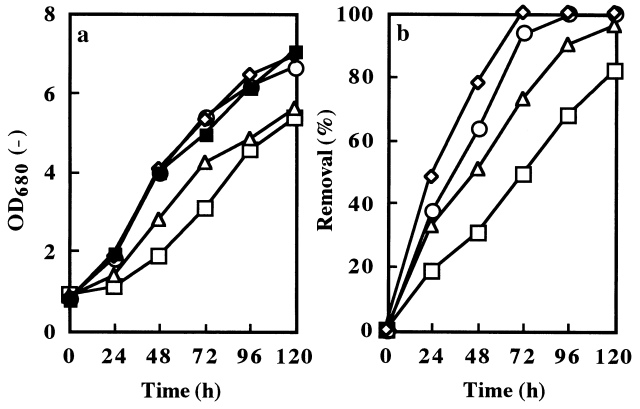

FIG. 1. Effects of initial 2,4-dinitrophenol concentration on cell growth (a) and its removal (b) by *C. fusca*. Symbols: closed squares, 0 μM; diamonds, 5 μM; circles, 10 μM; triangles, 20 μM; open squares, 40 μM.

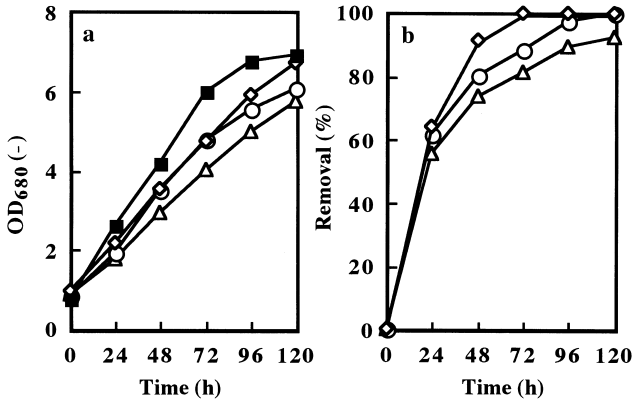

FIG. 2. Effects of initial 2,4-dinitrophenol concentration on cell growth (a) and its removal (b) by *A. variabilis*. Symbols: squares, 0 μM; diamonds, 5 μM; circles, 20 μM; triangles, 40 μM.

algal strains and three cyanobacterial strains investigated here. Therefore, these strains were used for further studies.

These selected strains grew well in the DNP concentration range of 5 to 40 μM (Figs. 1a and 2a). *A. variabilis* had the ability to remove slightly higher levels of DNP than did *C. fusca*. The lower the initial DNP concentration, the higher the level of removal obtained (Figs. 1b and 2b). These results suggest that both strains grow well photoautotrophically and can remove DNP efficiently even in the low concentration range without the addition of any organic carbon sources.

The removal of nitrophenols, bisphenol A, and chlorophenols, which are contained in some industrial wastewaters and landfill leachates, was investigated. Light illumination during cultivation is one important factor for treatment of organic pollutants by microalgae, because they transform and degrade the pollutants using energy generated by photosynthesis (19, 20). Therefore, the effect of light on the removal of phenols was also determined. Table 2 shows the removal of these phenols by *C. fusca* and *A. variabilis* at 120 h under light or dark conditions. The concentration of nitrophenols and bisphenol A did not decrease in the absence of algal cells. *o*-Nitrophenol and DNP were removed efficiently by the two strains both in the light and dark. *m*-Nitrophenol was also efficiently removed by *A. variabilis* (light, 100%; dark, 84%), whereas *C. fusca* removed 60% only occurred under light conditions. *p*-Nitrophenol was ef-

TABLE 2. Removal of nitrophenols and bisphenol A by *C. fusca* and *A. variabilis* under light and dark conditions

Phenols	Removal[a] (%)			
	C. fusca		*A. variabilis*	
	Light	Dark	Light	Dark
o-Nitrophenol	100±0	95±10	100±0	97±5
m-Nitrophenol	60±1	0±1	100±0	84±5
p-Nitrophenol	77±1	10±6	4±1	4±0
2,4-Dinitrophenol	90±9	68±2	95±4	81±14
2,4,6-Trinitrophenol	0±0	0±0	51±3	0±0
Bisphenol A	85±7	22±3	23±6	0±0

[a] Initial concentration of nitrophenols and bisphenol A was 40 μM. Microalgae were cultivated with a supply of CO_2 and air. Values indicate the mean±SD of three independent experiments.

ficiently removed only by *C. fusca* and 2,4,6-trinitrophenol by *A. variabilis*. Some physical and chemical characteristics of these nitrophenols are similar. For example, the octanol/water partition coefficients (log P) of these nitrophenols, which is commonly used as an index of the permeability of chemicals through cell membranes, are very close in value as follows; trinitrophenol (2.03), *m*-nitrophenol (2.00), *p*-nitrophenol (1.91), *o*-nitrophenol (1.79), and DNP (1.67) (21). However, the removal patterns of nitrophenols by these strains were not correlated with the log P of the nitrophenols. Furthermore, the removal pattern was variable for each strain and differed in the light and dark. Therefore, removal of nitrophenols was thought not to be the result of simple uptake into cells by permeation, but to be related to some specific intracellular reaction, such as an enzymatic degradation process.

Bisphenol A is a phenolic endocrine disrupter that has been frequently detected in industrial wastewaters (8) and the aquatic environment (15). *C. fusca* showed the high ability to remove bisphenol A (85%) at 120 h, whereas *A. variabilis* removed it to a small extent (Table 2).

The removal of chlorophenols was investigated using the small closed-type photobioreactor to suppress volatilization of chlorophenols from the medium. Figure 3 shows the removal of chlorophenols in the presence and absence of cells. Although volatilization of chlorophenols was not suppressed completely, a significant decrease in *o*- and *p*-chlorophenol and 2,4-dichlorophenol were observed for both *C. fusca* and *A. variabilis*. The removal of chlorophenols was not observed in the dark (data not shown).

C. fusca and *A. variabilis* were found to have high DNP removal ability under photoautotrophic conditions. These selected strains could remove nitrophenols, bisphenol A and chlorophenols. There have been a few studies on the removal of phenols by microalgae. Klekner and Kosaric reported that DNP was removed by *Senedesmus obliquus*, but the level of removal of *o*-chlorophenol and 2,4-dichlorophenol was unclear because of their volatilization from the medium (22). We showed the ability of microalgae to remove chlorophenols by improving the photobioreactor used to suppress volatilization. Furthermore, to the best of our knowledge, this is the first report to show the removal of bisphenol A by microalgae. From these results, a microalgal culture system using these strains would be useful to treat various hazardous phenols without the addition of any organic carbon sources.

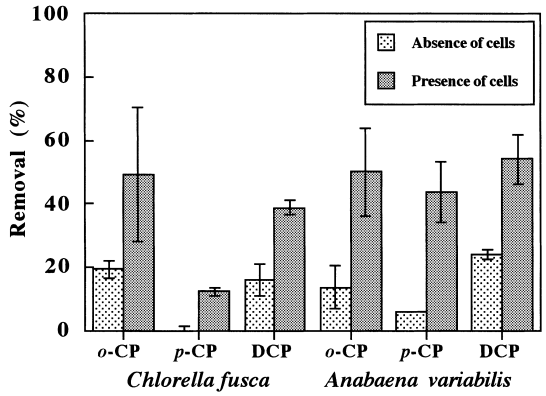

FIG. 3. Removal of chlorophenols by *C. fusca* and *A. variabilis*. The initial concentration was 40 μM. Removal of *o*-chlorophenol (*o*-CP), *p*-chlorophenol (*p*-CP), and 2,4-dichlorophenol (DCP) was measured after incubation for 120 h and was compared in the presence and absence of cells. Values indicate the mean±SD of three independent experiments.

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