MODELING FOR BIODEGRADATION OF 2,3,7,8-TRICHLORODIBENZO-P-DIOXIN BY USING *Pseudallescheria boydii*

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Introduction

Soil contamination in and around sites of incinerators for municipal solid waste (MSW) incinerator sites caused by dioxins is a concern in Japan. For example, the scattering of wastewater from a wet gas scrubber at a MSW incinerator in Nose, Osaka, caused contamination of both soil and surface water, and the concentration of dioxins in the soil at the site was approximately 8,000 pg-TEQ/g.

We have developed bioreactor systems for dioxins-contaminated water and soil[1, 2], because biological methods are inexpensive, and have a low potential to produce toxic by-products. We have shown that a fungus, *Pseudallescheria boydii (P. boydii)*, isolated from activated sludge used to treat wastewater containing dioxins can degrade highly chlorinated dioxins[1]. One reaction product of octachlorinated dibenzo-p-dioxin (OCDD) was identified as heptachlorinated dibenzo-p-dioxin[1]. However, the quantitative characteristics about dioxin degradation using *P. boydii* have not yet been clarified. To design bioreactors, the fundamental characteristics of the relationship between cell growth and dioxin degradation, and the influence of dioxins on cell growth need to be investigated. Modeling based on these characteristics will provide an estimation of the amount of treated dioxins per cell weight, nutrient demand for degrading dioxins, and time required for treatment, which are useful parameters for designing a bioreactor.

In this study, we have investigated the fundamental characteristics of 2,3,7,8-trichlorodibenzo-p-dioxin (TCDD) degradation by *P. boydii*. Based on the degradation characteristics, an existing mathematical model was applied to TCDD degradation by *P. boydii*.

Materials and Methods

Fungus

The fungus was isolated from activated sludge from a leachate treatment facility associated with MSW landfill sites in Japan[2]. The leachate contained dioxins, and the fungus was identified as *P. boydii* from 18S rDNA and morphological analysis[3].

Cultivation conditions

The medium contained 1.0 g of glucose, 0.2 g of $(NH_4)_2SO_4$, 0.2 g of NaCl, 0.1g of K_2HPO_4, 0.1g of $MgSO_4 \cdot 7H_2O$, 0.2 g of $CaCO_3$, and 0.1 mL of a trace element solution (0.01 g of $FeSO_4 \cdot 7H_2O$, 0.01 g of $MnCl_2 \cdot 4H_2O$, and 0.01 g of $ZnSO_4 \cdot 7H_2O$ per 10 mL of distilled water) per 100 mL of distilled water. Before the experiments commenced, precultivation was conducted in 100 mL of the medium for a period of 48 h.

Resting cells

The cells after 20 h cultivation were separated from the medium by centrifugation, and rinsed in 25 mM phosphate buffer (pH=7.3).

Dioxins degradation tests

The resting cells or precultivated cells and 50 ng of TCDD were injected into a 50 mL Erlenmeyer flask, and the solution was shaken while being maintained at 30 °C. Triplicate samples were prepared for analysis at the same time as sampling. In the analysis, after the addition of 13C-labeled TCDD into the Erlenmeyer flask, the concentration of cells was determined using an absorptiometer (620 nm). The total organic carbon (TOC) concentration was measured using the phenol-sulfuric acid method, and the dioxins including TCDD

were extracted three times using 5mL of toluene, followed by the addition of sulfuric acid (3 mL) and nitric acid (3 mL) to break down the cells.

Analysis of dioxins
The toluene phase was analyzed using a GC-MS/MS apparatus (ThermoQuest GCQ plus ion trap mass spectrometer and TRACE GC 2000 gas chromatograph) in accordance with the method of Kemmochi and Tsutsumi[4].

Chemicals
The TCDD and 13C-labeled TCDD used, ED-901 and ED-900, respectively were purchased from Cambridge Isotope Laboratories. All the other chemicals used were laboratory grade.

Results

Growth rate of P. boydii
The *P. boydii* grew with decreasing glucose concentration, as shown in Figure 1. A logarithmic growth phase was observed over a period of 20 h, and thereafter, the growth rate became more stable. It should be noted that the cells were measured using their dry weight.

TCDD degradation tests using resting cells
The resting cells did not grow using only TCDD, as shown in Figure 2, because either TCDD is not a carbon source for *P. boydii*, or because the concentration of TCDD was too low. No decrease in the concentration of TCDD was observed.

TCDD degradation tests using glucose as a carbon source
The cultivation of *P. boydii* using glucose and TCDD showed that the concentration of TCDD decreased with decreasing glucose concentration. This observation indicates that the concentration of TCDD decreased with the growth of *P. boydii*, i.e., the TCDD was metabolized during the logarithmic growth phase of *P. boydii*.

Effect of TCDD on the growth rate of *P. boydii*
Figure 4 shows the effect of TCDD on the growth rate of *P. boydii*. There was no effect on the growth rate of *P. boydii* during the logarithmic growth phase, and therefore, a concentration of 10 ng/mL of TCDD has no effect on the growth rate of *P. boydii*.

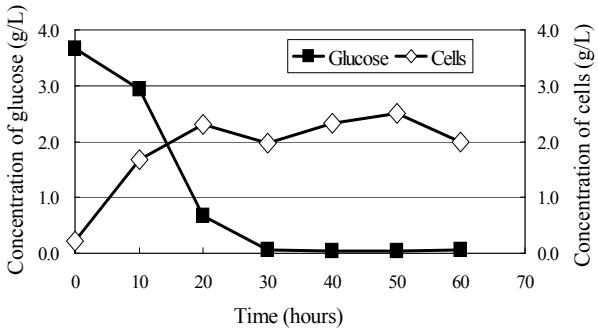

Figure 1 Growth curve of *P. boydii* with glucose

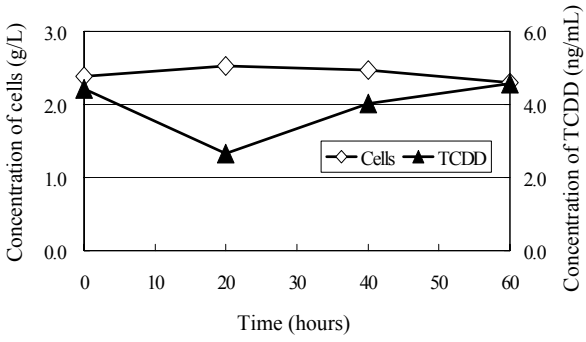

Figure 2 Time course of TCDD with resting cells and no glucose

Modeling of the TCDD degradation rate

TCDD degradation rate
The above experimental results indicated that the concentration of TCDD decreased with the growth of *P. boydii*. Therefore, we assumed that the TCDD degradation rate was proportional to the growth rate of *P.*

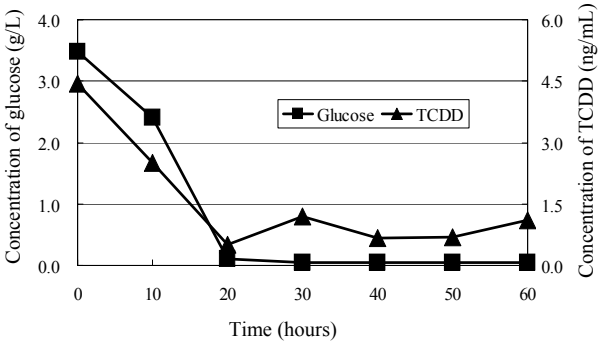

Figure 3 Time course of TCDD during cultivation of *P. boydii* with glucose

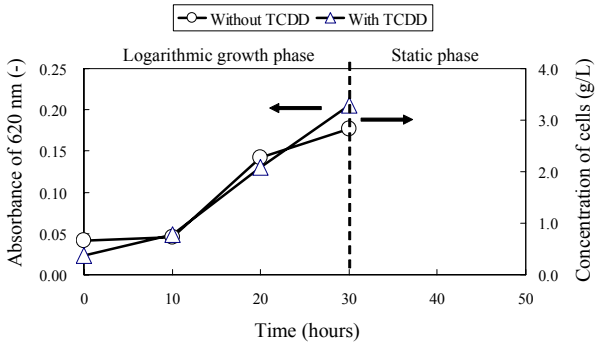

Figure 4 Effect of TCDD on the growth of *P. boydii*

boydii, as shown in Eq. (1):

$$\frac{dC}{dt} = -T_C \frac{dX}{dt} \quad \text{— (1)}$$

where T_C is the transformation capacity [ng-TCDD/mg-cell],
C is the concentration of TCDD [ng/mL],
X is the cell concentration [g/L], and
t is the time [hours].

Growth of *P. boydii* and the consumption of glucose

With regard to the growth rate of *P. boydii* and the rate of consumption of glucose, this was analyzed using a Monod type equation, shown in Eqs. (2) and (3):

$$\frac{dX}{dt} = \frac{\mu_{max} S}{S + K_S} X \quad \text{— (2)}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \frac{dX}{dt} \quad \text{— (3)}$$

where μ_{max} is the maximum microbial growth [hour^{-1}], K_S is the glucose saturation constant [g/L], S is the concentration of glucose [g/L], and $Y_{X/S}$ is the yield coefficient [g-cell/g-glucose].

Determination of parameters

The growth rate of *P. boydii* and the rate of consumption of glucose, as shown in Figure 1, was initially determined using Eq. (2) and (3). The fitting curves are shown in Figure 5, and the calculated parameters are shown in Table 1.

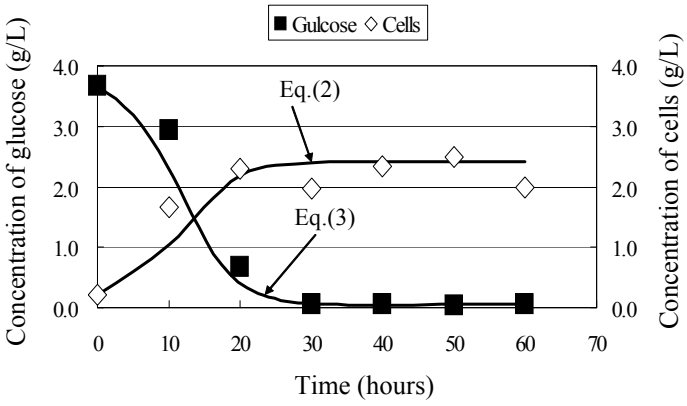

Figure 5 Curve fitting of Eqs. (2) and (3) with observed data on the growth of *P. boydii* and glucose consumption

Table 1 Parameters on the growth of *P. boydii* and glucose consumption

Parameter	Value
μ_{max}, the maximum microbial growth [hour^{-1}]	0.56
K_S the glucose saturation constant [g/L]	7.53
$Y_{X/S}$, the yield coefficient [g-cell/g-glucose]	0.61

Next, we estimated the growth rate of *P. boydii* with TCDD and glucose using the glucose consumption curve shown in Figure 3. The broken line shown in Figure 6 shows the estimated growth curve of *P. boydii*. In addition, using this growth curve, we determined the TCDD degradation curve employing Eq. (1), as shown in Figure 6. The value of T_C was determined to be T_C=0.57 ng-TCDD/mg-cell. Other experiments showed the same behavior, and T_C was determined to be T_C= 0.62 ng-TCDD/mg-cell. It should be noted that the initial concentration of TCDD was 6.2 ng/mL. Consequently, in general, the degradation rate of TCDD by *P. boydii* was determined using Eqs. (1-3).

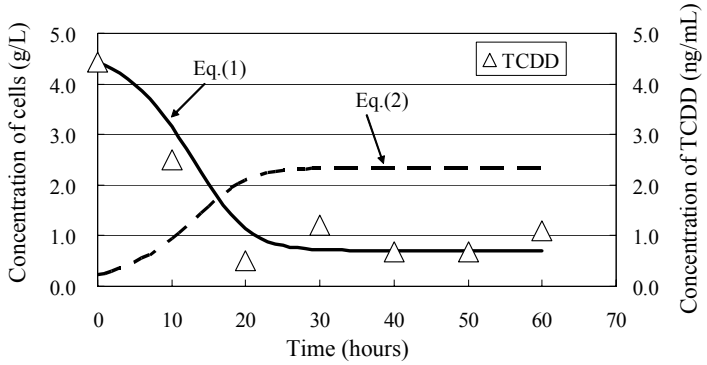

Figure 6 The TCDD degradation rate presented by Eq. (1)

Acknowledgments

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