Biosorption of Polychlorinated Dibenzo-*p*-dioxins and Dibenzofurans by *Bacillus pumilus*

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Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) have recently been of great concern because of the extreme toxicity and persistency in the environment (6). However, the fate of the molecules already being discharged into the environment has not been well understood and the interactions with the microorganisms, which are responsible for degradation and transfer of the molecules in the environment, have been unknown. Many scientists emphasized the significance of adsorption by the interactions with microorganisms not only because it occurs before the metabolic reactions but also is involved in the movement of the molecules in the environment (1). Adsorption of the other hazardous molecules by selected live and dead microorganisms has been investigated by several researchers (5,7). These studies show that the understanding of the adsorption mechanism is significant for the understanding of the ultimate fate of such organics, and for the establishment of proper biological sludge disposal guide lines (2).

Nevertheless, adsorption of PCDDs and PCDFs by microorganisms has rarely been studied. It first had been thought that these kinds of large hydrophobic compounds penetrate into cell membranes with easy. However, experiments with octachlorodibenzo-p-dioxin (OCDD) showed that it had very low transport kinetics (9). As many reports suggested, that might be due to the alteration of cell membrane structures by the compounds, and the interference with biological functions of the membrane (8). In addition, other studies with polychlorinated biphenyls (PCBs) and small unilamellar vesicles of four saturated phosphatidyl cholines also suggested that the fluidity of the membrane, which can be affected by temperature, could be one of the factors determining the adsorption ratio of organic pollutants (9). These may explain why some dead biomass accumulate or adsorb more organic pollutants than live biomass. In addition to the attachment to microorganisms itself, extracellular polymeric substances (EPS) might also be involved in the adsorption process (4). Although the general aspects of EPS of bacterial origin are well studied, the involvement of EPS in the accumulation of xenobiotic compounds is not clearly understood and the information on the mechanisms is rarely obtainable. However, the study on the biosorption of phenanthrene by microorganisms from coal tar waste site showed that some EPS of bacterial origin enhance polynuclear aromatic hydrocarbons (PAHs) transport in natural system (4). The function of these kinds of polymers may also explain parts of some differences of adsorption ratio according to the cell conditions (dead or live).

Thus, the intent of this work is to show whether (1) biosorption of TCDDs and PCDFs can be accounted for the significant portion of removal of these molecules and (2) some extracellular proteins, of which production is increased during the boiling, are mainly responsible for the removal of the molecules.

Material and Methods

Materials and organisms

Chemicals were of the highest purity commercially available. Dibenzofuran (DBF) was obtained from Sigma (St.Louis, MO, U.S.A) and 1,2,3,4-TCDD was obtained from Wellington Lab (Ontario, Canada). The mixture of PCDFs was synthesized by molecular chlorination of dibenzofuran (3). UV-VIS spectrophotometer (Carry 3 bio,Varian, Victoria, Australia), HRGC/MS (Platform, Micromass, Chelshire, WA, UK) and GC-ECD with autosampler (HP-6890, Hewlett-Packard, Willington, DE, U.S.A)were used in the analysis. *Bacillus Pumilus* was isolated from the laboratory hood which is being used for the analysis of PCDDs and PCDFs at Pohang University of Science and Technology, Korea and characterized at the Korea Research Institute of Bioscience and Biotechnology (Taejeon, Korea).

Adsorption by live and dead biomass

One hundred microliter of the pre-cultured broth was inoculated on 100 milliliter of nutrient broth and cultivated for overnight. The culture was washed with minimal salt medium (MSM) and the pellets were re-dissolved to 10 milliliter of MSM. The pellets were prepared by centrifugation (3,000 rpm for 10 min). Dead biomass was prepared by heating half of the culture at 90℃ for 20 min. About 100㎕ of dead biomass was smeared to nutrient solid medium to confirm sterilization and incubated at 30℃ for 3 days. To determine the effect of biomass amount, seven tubes of samples containing different amount of biomass were prepared. Among them, three tubes contained dead biomass according to the following ratio (concentrated dead biomass / minimal salt media: 1.0/4.0, 0.3/4.7, 0.5/4.5 milliliter) and so did the other three tubes containing live biomass. One of them was a control excluding the biomass. The volume of sample was 5 milliliter. Five hundred nanogram of 1,2,3,4-TCDD or PCDFs dissolved in acetone was added to all samples. All samples including the control were agitated vigorously for 2 or 3 min and incubated at 30℃ with shaking (160 rpm for 20 min). Then, these samples were extracted with toluene and analyzed by HRGC/MS.

Effect of extracellular protein

To determine the effect of extracellular protein, the samples were prepared as following. The concentrated live and dead biomass were obtained by the same method described for determination of adsorption. However, in this case, the highest concentration of biomass (concentrated culture: MSM 0.5:4.5) was used. After 30 minutes of incubation without 1,2,3,4-TCDD or PCDFs, the six milliliter of medium was filtered by microsyringe filter with pore size was 0.25㎛. The amount of protein in the sample was measured by Bradford method with bovine serum albumin as standard. The adsorption ability of protein was confirmed by adding the proteinase K. The molecular weight of protein was measured by conventional electrophoresis and MALDI-mass spectrometry (DESTR, Perseptive Biosystems, Framingham, MA, U.S.A). In order to determine the protein adsorbing TCDFs, the centrifugal filtration system that separates the compounds based on size was used.

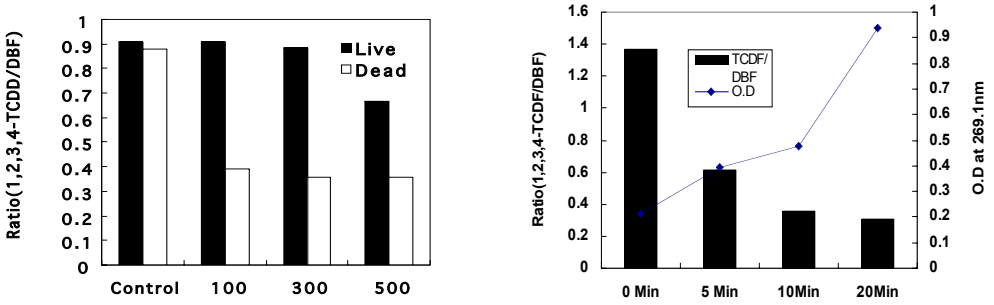
Results and Discussion

Adsorption by live and dead biomass

A change of each amount of 1,2,3,4-TCDD and PCDFs in the sample solution was measured by comparing with the peak area of dibenzofuran that had been added as internal standard or with the peak area of 1,2,3,4-TCDF in the case of GC-ECD. The number for the Y-axis was obtained by the following equation.

Y = peak area of 1,2,3,4-TCDD or PCDFs/ Peak area of DBF

Sorption of 1,2,3,4-TCDD and PCDFs in this study showed that dead biomass was more effective than live biomass [Fig. 1]. Other studies also showed that higher degree of sorption by dead biomass could occur for some organic compounds (7).



(a) (b)

Fig. 1. The degree of biosorption for 1,2,3,4-TCDD by (a)live and dead biomass and (b) biosorption of 1,2,3,4-TCDF by filtrate of dead biomass and the production of biocompound(s) according to heating time .

Although they could not derive any strong explanation for this phenomenon, they suggested that the change of cell membrane structures or missing the ability of permeability control could be the factors.

As in the case of dead biomass, the adsorption of 1,2,3,4-TCDD was not directly proportional to the amount of dead biomass [Fig.1a]. This indicates that there might be other mechanisms in addition to attaching to microbial cell itself. With above observations, we suspected that there might be other mechanisms or compounds involved that can be responsible for this phenomenon.

Effect of extracellular protein

During the UV-VIS analysis of our samples, production of unknown biocompound(s) was observed at its λ max of 260 nm . Although the production of this compound was also observed in the sample of live biomass, that of dead biomass was much greater. The GC/MS and GC-ECD analysis showed that the decrease of 1,2,3,4-TCDF was related to the amount of unknown biocompound[Fig 1b].

The subsequent studies using proteinase k showed that the unknown biocompounds accounting the adsorption is a protein of which molecular size is first determined by electrophoresis for about 5,000 daltons and further confirmed by MALDI mass spectrometry at m/z 5318. [Fig 2]. We also

are currently investigating the detailed structure by conventional peptide sequencing and advanced MALDI-MS techniques.

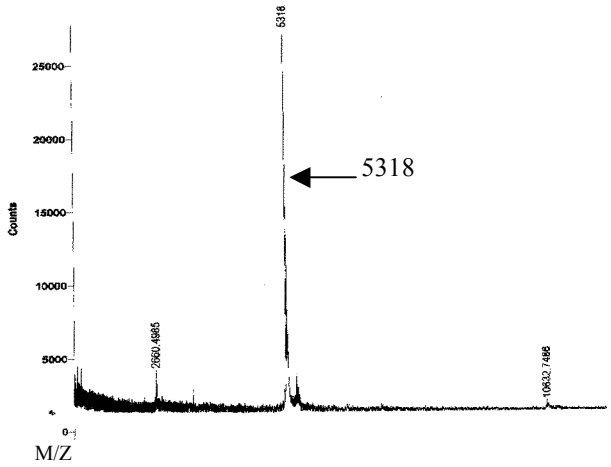


Fig 2. Molecular weight of the extracellular protein determined by MALDI mass spectrometry

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