PROTEIN ADSORPTION OF PCDD/Fs ISOLATED FROM FLY ASH

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Introduction

The degradation of PCDD/Fs by microorganism has been interesting topic of environmental study. However, the degradation efficiency of PCDD/Fs is low compared with the degradation of other chemicals, and metabolites may have more toxicity than mother molecules. Therefore, our research group proposed the adsorption of PCDD/Fs by microorganism. The adsorption can be more efficient removal mechanism and important to the biodegradation because biodegradation may occur in concert with adsorption.

Generally, the adsorption by microorganism means attachment of chemicals to outer cell wall or penetration through cell wall or membrane. In previous studies, the amount of adsorbed chemicals by cell itself was measured but studies of adsorption by biocompounds released from microorganisms are rare. Because biodegradation may occur in concert with adsorption and desorption, thereby making the observed data difficult to interpret[1]. So, dead or chemically inactivated cells were frequently used. The possibility of changes in the biomass during experimentation caused by cell growth or decomposition is also minimized by the use of dead biomass[2]. In general, the adsorption amount of dead cell is greater than or equal to that of live cell. By autoclaving, dead biomass can be made. During autoclaving, cell lysis can be occurred. The colloidal materials may be released during autoclaving remained associated with the aqueous phase following centrifugation and filtration, thereby interfering with measurement of the true aqueous phase concentration[3,4].

In previous studies, researchers have tried to understand the adsorption mechanism through experiments with various cell conditions. Methods using autoclave or specific chemicals to kill or deactivate cells have been mainly used [1,5]. Mutated microorganism lost degradation ability to specific chemical was also used.[6] The adsorption mechanism of organic chemical was suggested to be not active adsorption by microorganism but passive physico-chemical phenomenon by adsorption sites[5]. But, the relationship between the cell conformation change and adsorption behavior of dead biomass has not been clearly examined.

In our previous studies, cell lysis was not observed when *Bacillus pumilus* was boiled at 100 _ for 20 min. However, 1,2,3,4-TCDF was adsorbed on the biocompound released from cell[7]. This biocompound was identified as a protein[8]. In this research, adsorption characteristics of PCDD/Fs mixture from standard and fly ash extract are investigated.

Materials and methods

To harvest the biomass, *Bacillus pumilus* was inoculated on 300 mL of nutrient broth and incubated for overnight. Culture medium was centrifuged at 3000 rpm for 15 min. After discard of the supernatant, 0.05 M phosphate buffer (pH 7) was added and vortex was followed. This washing procedure was repeated for two times and final volume was adjusted to 20 mL.

To get protein solution, concentrated cell solution was boiled at 100°C for 20 min and then centrifuged at 3000 rpm for 20 min. The supernatant was filtered by membrane filter (0.2 μm pore

size). The protein concentration was measured by the Bradford method.

Two mL of protein solution and 100 ng of 1,2,3,4-TCDF or PCDD/Fs mixture standard (all isomers have same concentration) dissolved in acetone were mixed into glass centrifuge tube. 1,2,3,4-TCDF and PCDD/Fs mixture standards were purchased from Accustandard (New Haven, USA). PCDD/Fs extracted from fly ash by EPA method 1613 was also used. The sample solution was shaken at 160 rpm for 2 hours and extracted with 2 mL of toluene. The adsorption amount of PCDD/Fs was analyzed by GC-ECD (HP6890, Hewlett-Packard, USA) and HRGC/HRMS (JMS-700T, JEOL, Japan).

To investigate adsorption characteristics according to the cell condition, the supernatant of normal cells, chemically inactivated cells by inactivation of electron transport system by addition of sodium azide (NaN_3) 0.1% (w/v), osmotic shocked cells by addition of NaCl 5% (w/v), autoclaved cells at 120_ for 20 min, boiled cells at 100°C for 20 min and sonicated cells for 40 min were used. After centrifugation and filtration, 1,2,3,4-TCDF was added. The following procedure was carried out as described earlier.

Results and Discussion

We compared the adsorption amount of 1,2,3,4-TCDF according to the cell conditions of *Bacillus pumilus*. There were no significant differences among the adsorption amounts by cell itself according to the cell conditions (Data not shown). However, the adsorption amount by supernatant was much different from each other (Figure 1). It is considered that the increased adsorption amount of 1,2,3,4-TCDF by dead cell was not by the increase of cell adsorption capacity, but by the increase of adsorption sites like released proteins. Therefore, the adsorption mechanism may be just passive physico-chemical phenomenon in this experiment.

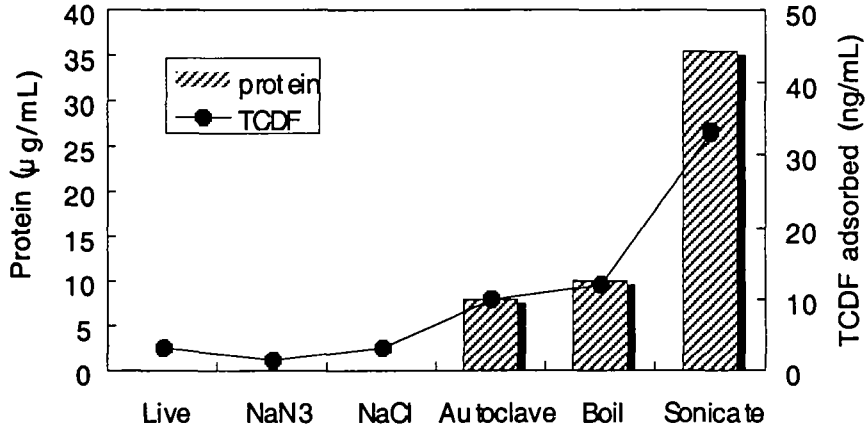

Figure 1. Released protein concentration and amount of adsorbed 1,2,3,4- TCDF according to cell conditions.

It was found that h igh chlorinated PCDD/Fs isomers were more adsorbed than low chlorinated ones (Figure 2). This can be explained by the fact that as the chlorine substitution number is increased the water solubility is decreased and octanol/water partition coefficient is increased[9].

But, more precise explanation may be possible only after understanding of the structure and properties of protein.

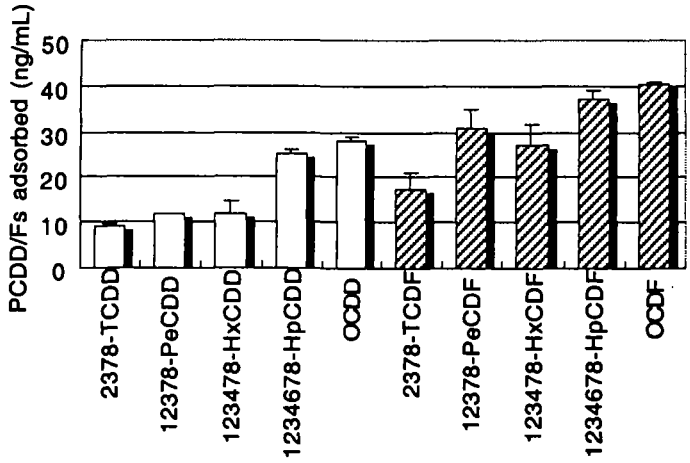

Figure 2. Adsorption amounts of PCDD/Fs congeners by protein

In case of PCDD/Fs extracted from fly ash, the adsorption amounts of 17 toxic isomers was analyzed. Figure 3 shows the adsorption amounts of 17 isomers. There is a trend that high-chlorinated isomers were more adsorbed. Under the maximum equilibrium concentration, however, the adsorption amounts will be proportional to the initial concentration. For more exact interpretation, adsorption rate (adsorbed amount / initial amount) should be calculated. The adsorption rates of high-chlorinated isomers were higher than those of low chlorinated ones and adsorption rates of PCDDs and PCDFs were similar (Figure 4).

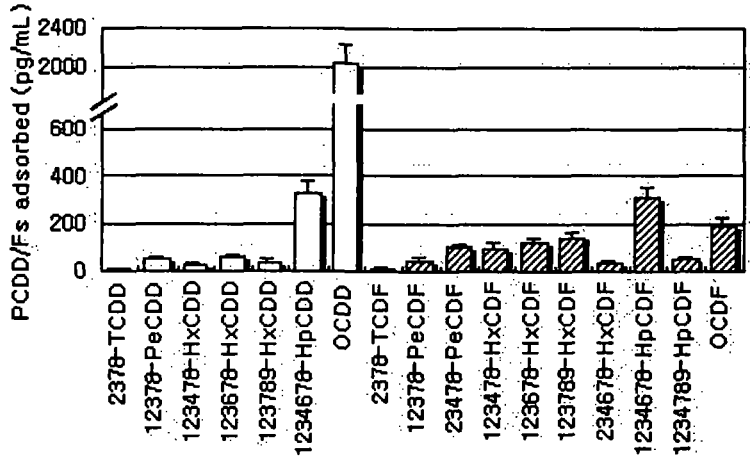

Figure 3. Adsorption amounts of PCDD/Fs extracted from fly ash

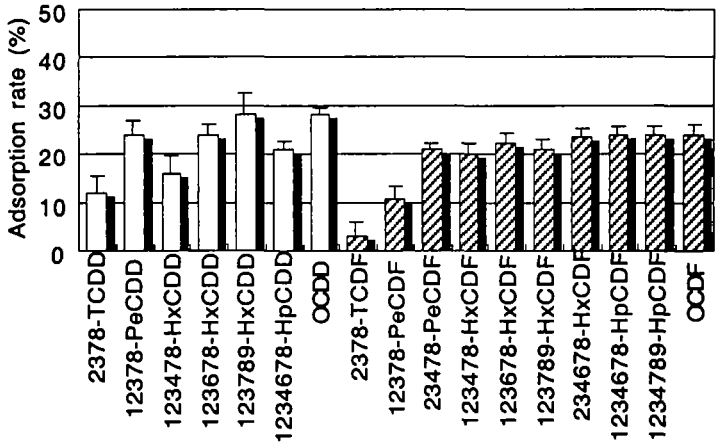

Figure 4. Adsorption rate of PCDD/Fs extracted from fly ash

The amount of PCDD/Fs mixed with protein solution was 6.94 pg-TEQ and adsorption amount was 1.17 pg-TEQ. Therefore, about 17% of TEQ value was decreased.

Acknowledgements

This work was supported in part by the Korea Science and Engineering Foundation (KOSEF) through the Advanced Environmental Monitoring Research Center at Kwangju Institute of Science and Technology.

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