REMOVAL OF PCDD/Fs IN FLY ASH BY *Sphingomonas wittichii* RW1

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Introduction

PCDD/Fs are known as dioxins, and are not produced with any specific purpose but are by-products of the chemical industry, incineration and natural phenomena such as forest fires[1,2]. Incineration of waste produces residual ash and flue gas which contain toxic materials. Although almost complete destruction of PCDD/Fs occurs during high temperature incineration of municipal waste, significant amounts of these toxic pollutants remain in fly ash. Currently the major fly ash treatments are reclamation or solidification. Although these methods are relatively cheap, they do not decrease the absolute amount of hazardous material such as PCDD/Fs[3,4].

Bioremediation may be an alternative waste management method as it is relatively inexpensive and has minimal impact on the environment. The nature of fly ash is such that it cannot support the usual indigenous microorganisms. In such instances where natural flora is unable to degrade target pollutants satisfactorily due to lack of necessary catalytic activity, bioaugmentation can be used, in which specialist organisms possessing the required activities are added to waste[5].

There are several reports regarding the isolation of microorganisms which biotransform dioxins, and on the bioremediation of soil using a well known dioxin degrader[6,7,8,9]. One such study examined degradation of DF and DD in soil microcosms in which *Sphingomonas* sp. strain RW1 was introduced. Although a number of studies have examined the use of microorganisms to degrade dioxins in soil, none have investigated the degradation of dioxins in fly ash. The objective of the present study was to investigate the effect of strain RW1 on the removal rate of PCDD/Fs in fly ash from incinerators.

Methods and Materials

Fly ash was obtained from a municipal waste incinerator in a city of Korea. The ash was sieved (1 mm mesh), homogenized by jar tester for 24 h, then stored at -20^{o}C. The strain *Sphingomonas* sp. RW1 employed in this experiments, has only recently proposed as a new species, *Sphingomonas wittichii* sp. RW1 and was purchased from DSMZ (DSM6014). Strain RW1 was pre-cultivated in 20 ml of minimum salts medium (MSM) as previously described[10]. The concentration of DF was 1 mM. The whole culture was transferred to 1 L of the same medium (250 ml × 4 in 2 L e. flasks) and incubated in a shaking incubator (160 rpm) at 28^{o}C for 2 days. In order to obtain resting cells of strain RW1, the cells were harvested by centrifugation (8,000 rpm at 4^{o}C), washed three times with 50 mM phosphate buffer, and resuspended in the same buffer. The final optical density of the cell culture solution at 578 nm was adjusted to 4.0.

Before the application of strain RW1 to remove dioxins of actual fly ash, the capability of the

strain RW1 degrading mixture of dioxins was determined with commercially available dioxin mixture and the extracts of the fly ash. For this experiment, the biomass and controls were obtained by the same methods described above. Ten microgram of dioxin mixture in 5 ml of toluene was applied to the 250 ml of e. flask and toluene was evaporated by the gentle blowing of N_2 gas. Then, the same amount of biomass was introduced into the e. flask and incubated for 10 days. The extracts were obtained by the extraction of the fly ash in soxhlet for 24 h. The extract was cleaned-up by the methods descried previously[11].

After 10 days incubation, the flasks were removed from the incubator and extracted. A mixture of $^{13}C_{12}$labeled PCDD/Fs (1 ng each) was added as an internal standard. Samples were centrifuged (3,000 rpm for 20 min) and the supernatant was extracted by liquid-liquid extraction five times using toluene. The remaining ash was dried in a 60^{o}C oven for 24 h, transferred to an accelerated solvent extractor (ASE200, DIONEX, Sunnyvale, CA, US), and extracted for 3 h with toluene. The extracted samples were treated as previously described[11]. PCDD/Fs were analyzed by HRGC/HRMS (JMS 700T, JEOL, Tokyo, Japan) with a DB-5MS column (60 m, 0.25 mm i.d., 0.25 μm film thickness) as previously described[11]. The leachate was filtered using 0.1 μm glass-wool fiber filter and analyzed by inductively coupled plasma-atomic emission spectroscopy (Thermo Elemental, Franklin, MA, US). Total organic carbon was measured using a total organic carbon analyzer (TOC-VCPH, Shimadzu Co., Kyoto, Japan) equipped with a solid sample module (SSM-5000A. Shimadzu Co., Kyoto, Japan). Glucose (40% for total carbon) and Na_2CO_3 (11.235% for inorganic standard) were employed as the standard materials.

Results and Discussion

The major components of the fly ash are CaO, Na_2O and K_2O. The total concentration of PCDDs was 28.85 ng/g ash, and the toxicity equivalency (TEQ) was 622.5 pg I-TEQ/g ash. Ash was analyzed for the presence of indigenous microorganisms which may have a similar catabolic activity as strain RW1. Ash (5 g) was added to 25 ml MSM with or without 1.0 mM DF. In order to observe the appearance of colonies, 100 μl of the solution was plated on nutrient agar without dilution for 5 days. No colonies were observed. These results suggested that no indigenous microorganisms could grow under these experimental conditions unless pre-treatment of ash, such as adjusting the pH, was performed. This result also indicated that there are no microorganisms that would compete with an introduced strain. It is usually assumed that TOC, the strong basic property of the ash solution (pH 13.0), heavy metals and the existence of other toxic organic compounds like PAHs could affect the degradation of organic substances. In the case of strain RW1, Halden et al. showed that the rate of removal of DD, DF and 2-MCDD decreased as TOC content increased[9]. The results of our analysis showed that the ash contained no significant amounts of organic carbon, therefore, we could assume that the level of ash TOC would not inhibit the activity of introduced RW1.

In the experiment the ash slurry was made using MSM and resting cells. Although strain RW1 removed 75.5% of total PCDDs, the control containing dead cells also removed significant amounts of PCDDs from ash (71.0%). Many studies have demonstrated the adsorption capacity of dead biomass, indicating that it may be a factor in toxin removal rates. The adsorption capacity of the same strain can be changed according to cell status. Table 1 shows that most PCDDs are removed by abiotic reactions like adsorption, and only a relatively small proportion are removed

by biological reactions. The data also indicate that more chlorinated PCDDs strongly adsorbed to the biomass. Although the proportion removed by live biomass or dead biomass was different according to the congener, a significant amount of TCDD was degraded (634 pg/g-ash, 20.1% of total PCDDs). Table 2 shows that proportion of congeners can be removed, although only relatively small amounts are degraded. In the case of 2,3,7,8-TCDD, 26 pg/g-ash and 10.7% of total 2,3,7,8-TCDD was degraded.

The initial attack by strain RW1 on the PCDDs or PCDFs was upon both halogenated and non-halogenated aromatic rings[7,9,12]. Theoretically, every PCDD or PCDF which has a TEF value can be transformed by strain RW1, except OCDD. The potential of strain RW1 for the productive conversion of PCDD/Fs were known to be restricted to less halogenated congeners because of the relatively narrow substrate range of the initial dioxygenase[9]. However, recent research showed that strain RW1 could transform 1,2,3,4-TCDD[10]. Our study showed that the concentration of total HpCDD and OCDD were virtually unchanged during incubation. This result seems reasonable considering HpCDD and OCDD are hardly attacked by initial dioxygenase from strain RW1 because no aromatic rings positions are free for chlorine substitution. Analysis of each isomer substituted with 2,3,7,8-positions also showed that more chlorinated PCDDs like hexa- to hepta-chlorinated dioxins were less preferred by RW1. Habe et al. also obtained similar results using *Pseudomonas* strain CA 10 applied to dioxin-contaminated soil[8]. Those results showed that strain CA10 had a potential to degrade tetra- to hepta-chlorinated congeners including the most toxic compound, 2,3,7,8-TCDD. This is noteworthy given there has not been any report of degradation of 2,3,7,8- or more chlorinated dioxins in liquid culture using bacteria, though some research groups succeeded using fungi. Both *Pseudomonas* sp. CA10 and *Sphingomonas wittichii* RW1 harbor regioselective dioxygenase that simultaneously hydroxylate an ether bond-carrying carbon and the adjacent unsubstituted carbon. Thus, theoretically, these strains can degrade all 15 highly chlorinated 2,3,7,8-substituted dioxins (except OCDD) because these dioxins have an unsubstituted carbon adjacent to an ether bridge, which presents a suitable target for the dioxygenation. However, in order to get complete destruction or to meet the regulatory requirements, other methods should be developed in order to destroy HpCDD and OCDD.

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References

1. LaFleur, L., Brunck, B., McDonough, T., Ramage, K., Gillespie, W., and Malcom, E. (1990) Chemosphere. 28, 1731.
2. Lamparski, L.L., Nestrick, T.J., and Stenger, V.A. (1984) Chemosphere. 13, 361.
3. Czuczwa, J.M., and Hites, R.A. (1984) Environ. Sci. Technol. 18, 444.
4. Shin, D.H., Choi, S.M., Oh, J.E., and Chang, Y.S. (1999) Environ. Sci. Technol. 33, 2657.
5. Comeau, Y, Greer, C.W., and Samson, R. (1993) Appl. Microbiol. Biotechnol. 38, 681.
6. Fortnagel, P., Harms, H., Wittich, R.M., Krohn, S., Meyer, H., Sinnwell, V., Wilkes, H., and Rancke, W. (1990) Appl. Environ. Microbiol. 56, 448.
7. Wittich, R.M., Wilkes, H., Sinnwell, V., Francke, W., and Fortnagel, P. (1992) Appl. Environ. Microbiol. 58, 1005.

8. Habe, H., Chung, J.S., Lee, J.H., Kanto, K., Takao, Y., Hiedaki, N., and Omori, T. (2001) Appl. Microbiol. Biotechnol. 656, 788.
9. Halden, R.U., Halden, B.G., and Dwyer, D.F. (1999) Appl. Environ. Microbiol. 65, 2246.
10. Hong, H.B., Chang, Y.S., Nam, I.H., Fortnagel, P., and Schmidt, S. (2002) Appl. Environ. Microbiol. 68:2584-2588.
11. Oh, J.E, Chang, Y.S., Kim, E.J. and Lee, D.W. (2002) J. Air Waste Manage. 52, 174.
12. Happe, B., Eltis, L.D., Poth, H., Hedderich, R., and Timmis, K.N. (1993) J. Bacteriol. 175, 7313.

Table 1. Removal of PCDDs by strain RW1 in fly ash from an incinerator. A= Control containing no biomass, B= Control containing dead cell, C=Sample containing live cell, STD= Standard Deviation, Unit of A, B and C = pg/g-ash, %[a] = [(A-C)/A]X100, %[b=] [(A-B)/A]X100 , %[c] = [((A-C)-(A-B))/(A-C)]X100

PCDDs	A±STD	B±STD	C±STD	Amount and Percentage decreased					
				By cells alive		By cells dead		Degradation	
				pg/g-ash	%[a]	pg/g-ash	%[b]	pg/g-ash	%[c]
TCDD	3,151±271	2,260±220	1,626±152	1,525	48.4	891	28.3	634.0	20.1
PeCDD	4,383±500	1,648±48	1,474±48	2,909	66.4	2,735	62.4	174	3.9
HxCDD	4,990±709	1,446±184	1,275±59	3,715	74.4	3,544	71.0	171	3.4
HpCDD	6,114±434	1,452±192	1,321±204	4,793	78.4	4,662	76.2	131	2.0
OCDD	10,214±1150	1,560±277	1,384±264	8,830	86.4	8,654	84.7	176	1.7
SUM	28,852	8,366	7,080	21,798	75.5	20,486	71.0	1,312	4.5

Table 2. Removal of 2,3,7,8-substituted congeners by strain RW1 in fly ash from an incinerator. A= Control containing no biomass, B= Control containing dead cell, C= Sample containing live cell, STD= Standard Deviation Unit of A, B, and C= pg/g-ash, %[a] = [(A-C)/A]X100, %[b=] [(A-B)/A]X100, %[c] = [((A-C)-(A-B))/(A-C)]X100

Congener	A±STD	B±STD	C±STD	Amount and Percentage decreased					
				By cells alive		By cells dead		Degradation	
				pg/g-ash	%[a]	pg/g-ash	%[b]	pg/g-ash	%[c]
2378	243±40	181.3±32	155±14	88	36.2	62	25.5	26	10.7
12378	509±132	59.3±24	39±18	470	92.3	450	88.4	20	3.9
123478	243±46	44.6±9	42±12	201	82.7	199	81.8	2	0.8
123678	371±27	95±11	93±14	278	74.9	276	74.4	2	0.4
123789	234±36	76±17	63±15	171	73.0	158	67.5	13	5.5
1234678	2991.5±248	697±34	628±43	2,363	79.0	2,294	76.6	69	2.3
OCDD	10214±1150	1560±277	1384±264	8,830	86.4	8,654	84.7	176.2	1.7
SUM	14,805	2,712	2404	12,401	83.7	12,093	81.7	308	2.1