New Microbial Agent for Removal of PCDD/Fs from Municipal Solid Waste Incinerator Fly Ash

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Introduction

Incineration is increasingly being used to dispose of municipal waste.[1,2] However, the fly ash created during incineration contains pollutants such as dioxins, known environmental hormones that disturb ecosystems and induce cancers and behavior disorders in living organisms, including humans.[2,3] In fact, various polychlorinated compounds may be generated during incineration, leading to contamination of the environment around incineration facilities. Since dioxins are known to be recalcitrant contaminants, their toxic effects last for long periods of time in soils after contamination.[1-3] In Korea, incinerator fly ash has generally been buried in landfill sites, leading to serious dioxin contamination of soil leachates from those sites and strident objections against the use of this approach to treat fly ash.[3,4] These objections were given further weight by the finding of significant amounts of dioxins in the bottom layer of buried fly ash, indicating that these compounds should no longer be buried in landfill sites. Other approaches that have been used to treat fly ash include solidification by cementing, stabilization by chemical treatment, stabilization using solvent for acid extraction and neutralization of exhaust gas, and stabilization by thermal treatment.[2,3] The chemical treatment of fly ash generates significant amounts of byproducts that act as secondary contaminants, which must in turn be treated. None of the above processes actually remove the dioxin from the ash, leaving open the possibility that it will subsequently escape from the site. Compared to the other approaches, the thermal treatment approach uses more energy, costs more, and requires an additional facility for exhaust gas treatment. In contrast to the physical and chemical processes for treating fly ash outlined above, biological treatment is an eco-friendly and cheaper treatment method that does not cause secondary contamination. Currently, several countries are attempting to develop a viable biological dioxin treatment method; however the technology is still in its infancy. The objective of the present study was to develop an efficient and cost-effective biological process to treat dioxin-contaminated fly ash from incinerators using a manufactured microbial agent containing various dioxin-degrading microorganisms.

Materials and Methods

The fly ash used in this study was obtained from a municipal solid waste incinerator in Daejon, Korea. The fly ash samples were sieved through a 1 mm mesh, mixed using a jar tester and tubular mixer for 24 h, and then stored at -20°C until further analysis. The microbial agents used in this study comprised 4 bacterial strains and 5 fungal strains (Table 1). For seed culture of bacterial strains, each strain was grown in minimal media containing 1 mMdibenzofuran as a sole carbon source, and was then transferred to 1 L medium to prepare seed culture. Cells were harvested using a continuous high speed centrifuge (8,000 g at 4°C for 20 min), suspended in 700 ml of the media without dibenzofuran, mixed with vermiculite, and then incubated for 24 hours at 30°C.To prepare the fungal inocula, the 5 strains of fungi were grown separately in 100 ml of potato dextrose broth for seed culture and transferred to 500 ml potato dextrose agar. After combination of cultures, 250 ml broth was filtered for harvesting of cell mass. Harvested cells were mixed with 10 g of sawdust and minimal media containing 10% molasses to make 280 g of fungal inoculum. Each fungal and bacterial inoculum was mixed with 280 g of vermiculite to make a 560 g sample containing the microbial agent and then incubated for 21 days at 30°Cwith fly ash prepared as described above. To identify the evolution of the microbial community, denaturing gradient gel electrophoresis (DGGE) analysis was performed every 7 days during the incubation process.[5] After 21 days of incubation, the samples were transferred to an accelerated solvent extractor (ASE200, DIONEX, Sunnyvale, CA) and extracted for 3 h with toluene. Before extraction, a mixture of ^{13}C-labeled PCDD/Fs (1 ng each) was added as an internal standard. The extracted samples were treated and analyzed as previously described.[4] 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 density and particle size of the municipal waste incinerator ash used in this study were 2.971 g/cm^3 and 0.3-1.0 μm (85%), respectively. Concentrations of total dioxins were determined using the ash from a municipal solid waste incinerator. The total concentration of PCDDs was 27,253 pg/g-ash and the toxicity equivalency (TEQ) was 1.018 I-TEQ pg/g-ash. The fly ash contained 0.0325 ± 0.008% total carbon (TC) and 0.00143 ± 0.004% total organic carbon (TOC). A past study found that the high TOC content in soil correlated with a lower removal rate of dioxins from the soil by the bacterium *Sphingomonas* sp, strain RW1.[6] That study also showed that high pollutant concentrations decreased the efficacy of biodegradation. In the present experiments, however, the TOC content of the fly ash tested was low and the PCDDs concentration in the fly ash was considered an acceptable level for bacterial degradation. The TOC levels were also sufficiently low that we could assume that the activity of the introduced microbial agents would not be inhibited by organic carbon in the fly ash.[6] To create a microbial agent for treating dioxins in incinerator ash, we mixed the 4 bacterial and 5 fungal dioxin-degrading strains listed in Table 1. A mixture of highly active bacteria and fungi was used to enhance the dioxin degradation rate. To increase the efficiency of mixing of the microorganisms and to maintain aeration, vermiculite was supplemented as an additive. To increase the degradation rate achieved by a given amount of the microbial agent, the mixture of agents was subjected to a solid-state post-fermentation process. During the operation of the process, the microbial consortium in fly ash was analyzed using denaturing gradient gel electrophoresis (DGGE). The DGGE analysis showed that all of the fungal and bacterial strains in the dioxin-degrading microbial mixture were maintained during reactor operation, and thus that the microbial strains were stable under the dioxin treatment conditions. In the present experiments, the microbial agent reduced the total concentration of PCDD/Fs from 27.25 to 9.97 ng/g of ash, which corresponds to the removal of 63.4% of total PCDD/Fs by biological adsorption and degradation. Importantly, this study employed a short incubation period, and the microbial strains could survive in the harsh conditions arising with fly ash. The data obtained in the present work thus indicate that the microbial agent described here may serve as a potential candidate for the industrial elimination of PCDD/Fs in fly ash, and that the combination of several dioxin degraders into a single microbial agent may be beneficial in the treatment of fly ash contaminated with dioxins.

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Table 1. List of bacteria and fungi with high dioxin-degrading activity combined into a single microbial agent

Microorganism	Strain	Source
Bacterium	*Sphingomonas* sp. DSM 7135	DSMZ (Germany)
	Sphingomonas sp. RW1	POSTECH (Korea)[9]
	Pseudomonas veronii PH-03	POSTECH (Korea)[10]
	Paenibacillus sp. VSE5L	Isolated in this study
	Phanerochaetechrysosporium DSM 6909	DSMZ (Germany)
	Phanerochaetechrysosporium DSM 1556	DSMZ (Germany)
Fungus	*Irpex* sp. KW3	KRIBB (Korea)
	Strain CH2	Isolated in this study
	Strain VSO7	Isolated in this study

Table 2. Removal of PCDD/Fs by the newly manufactured microbial agent (containing five fungi and four bacteria) from incinerator fly ash after 21 days of incubation

Homolog	Congener	Total conc. (pg/g-ash)		I-TEQ (pg/g-ash)	
		Sample	Control	Sample	Control
TCDD	2378	31.4	70.4	31.4	70.4
PeCDD	12378	81.8	192.6	40.9	96.3
HxCDD	123478	57.6	122.0	5.7	12.2
HxCDD	123678	459.3	1044.6	45.9	104.4
HxCDD	123789	311.1	696.6	31.1	69.6
HpCDD	1234678	3384.2	8363.4	33.8	83.6
OCDD	12346789	3675.6	10813.8	3.6	10.8
TCDF	2378	87.0	185.9	8.7	18.5
PeCDF	12378	81.0	189.4	4.0	9.4
PeCDF	23478	133.5	707.2	66.7	353.6
HxCDF	123478	129.0	380.7	12.9	38.0
HxCDF	123678	136.3	392.6	13.6	39.2
HxCDF	123789	262.9	738.9	26.2	73.8
HxCDF	234678	68.9	190.0	6.8	19.0
HpCDF	1234678	521.1	1511.1	5.2	15.1
HpCDF	1234789	90.9	237.4	0.9	2.3
OCDF	12346789	461.6	1416.2	0.4	1.4
TOTAL		9974.3	27253.5	338.5	1018.3

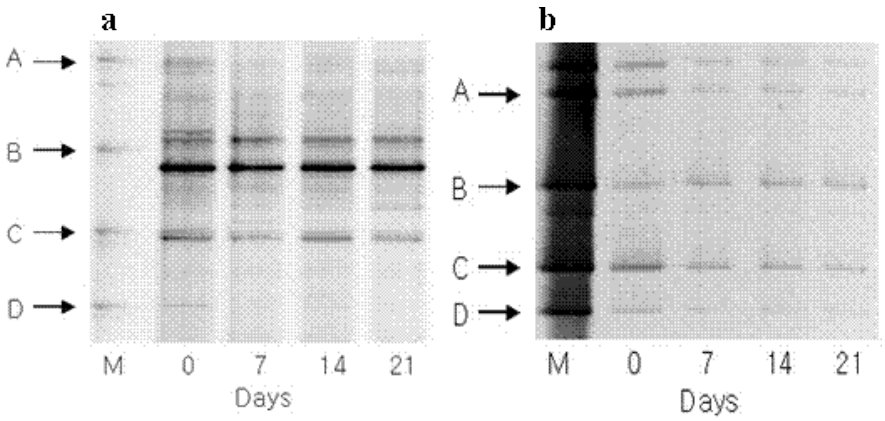

Figure 1.Denaturing gradient gel electrophoresis (DGGE) analysis of bacteria (a) or fungi (b) specific genes in fly ash treating reactor. In the left figure (a), A; PH-01, B; DSM 7135, C; RW-1, D; VSE5, and M; Marker. In the right figure (b), A;*Irpex* sp. KW3, B;*Trametes* sp. CH2, C;*Phanerochaete* sp. DSM 1556, D;*Phanerochaeta* sp. DSM 6909, and M; Marker.