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Effect of heavy metals on the biodegradation of dibenzofuran in liquid medium

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Abstract

The effect of heavy metals on the degradation of dibenzofuran by *Sphingomonas wittichii* RW1 was determined in liquid cultures. The results showed that 10 mg/L cadmium, mercury and copper not only affected the growth of RW1 with dibenzofuran but also the ability of resting cells to degrade this compound. Growth and degradation were strongly inhibited by mercury, even at 1 mg/L, while the inhibitory effect of cadmium and copper at the same concentration or at 5 mg/L were negligible. In contrast, arsenic and lead did not affect degradation or growth, even at very high concentrations of 100 mg/L. Subsequent analyses additionally revealed that concentrations of arsenic and lead remained unchanged following incubation, while those of cadmium, mercury and copper decreased significantly.

Keywords: Biodegradation; Heavy metals; *Sphingomonas wittichii* RW1; Dibenzofuran

1. Introduction

Polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), generally dioxins, are ubiquitous due to considerable increases in the incineration of wastes and the production of chemical compounds [1–3]. Combustion processes result in the major emission of dioxins into the environment. Moreover, incineration releases a significant amount of these pollutants into the environment *via* the generation of ash. The concentration and toxic equivalency (TEQ) values of dioxins from fly ash are the highest among other solid matrices, such as soil or sediment [2,4]. Currently, the most cost-effective ways of removing dioxins from contaminated ash or soil involve reincineration or reclamation. Although these procedures are costly favorable than other methods, the reincineration poses a risk of dioxin regeneration and the reclamation does not decrease the absolute amount of dioxins. Consequently, a lot of efforts have been undertaken in trying to remove these compounds from the environment even by evaluating the potential of biological adsorption [5]. However, biological degradation appears to be a promising method although several obstacles are yet to be overcome as previous studies and research reports clearly demonstrated that several environmental factors, including pH, heavy metals, content of organic compounds, temperature, and humidity affect the degradation of such organic pollutants by bacteria [6–8].

Fly ash comprises considerable amounts of heavy metals such as cadmium, lead, mercury, arsenic, and copper [4]. Although some of these metals are necessary for biological life, excessive quantities often result in the inhibition of essential biological reactions *via* numerous pathways [9]. A number of reports collectively show that various metals, such as Al, Co, Ni, Cu, Zn, Pb, and Hg at a range of concentrations have adverse effects on the biochemical oxygen demand (BOD) or the degradation of organic compounds [6,7,10,11]. However, at present there is only limited information on the effect of individual heavy metals on the biological degradation of polychlorinated dibenzofurans and dibenzo-*p*-dioxins. Most heavy metals are adsorbed on soil particles, and some heavy metals such as arsenic and chromium can be oxidized to anionic forms that freely move in the soil

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environment. Currently, we are developing a fly ash detoxification system, which includes a slurry-based fermentation reactor for dioxin degradation employing single or mixed microbial inocula. Accordingly, examination of the effect of heavy metals frequently found in fly ash on the degradation of dioxins is highly needed.

In this study, the model compound dibenzofuran in the liquid phase by *Sphingomonas wittichii* RW1, which has been known as the most well known dioxin degrader, was tested with five important metal species. This bacterium could completely mineralize dibenzofuran (DF) and dibenzo-*p*-dioxin (DD) and catabolizes several chlorinated congeners [12–16]. The strain, therefore, exhibits an important catabolic potential and is currently the subject of intensive efforts to evaluate its ability to remediate contaminated environmental compartments. However, although a previous report does suggest that, in addition to other physicochemical factors, heavy metals present in contaminated soil may affect the biodegradation of dibenzofuran by this strain [8] and inhibition effects caused by cadmium, copper, and mercury are well-known [9], there are no reports available to specifically elucidate and confirm their effect on the strain. Therefore our results imply that specific heavy metals at certain concentrations in the environment should affect the degradation of dioxins by microorganisms.

2. Materials and methods

2.1. Chemicals

ICP standard solutions (SPEX plasma standard, 1000 mg/L of H_3AsO_4, Cd, Cu, $Pb(NO_3)_2$, and Hg) in 2% nitric acid or 10% for mercury were purchased from SPEX (Metuchen, NJ, USA) and employed as the source of heavy metals and standard solution for inductively coupled plasma (ICP) spectrometry (Thermo Elemental, Franklin, MA, USA). Dibenzofuran (DF) and other chemicals were purchased from Sigma (St. Louis, MO, USA). Ethyl acetate and phosphoric acid (85%) were purchased from Merck (Darmstadt, Germany). Chemical compounds used were of the highest grade commercially available.

2.2. Effect of heavy metals on the growth of S. wittichii RW1

Cells were pre-grown in mineral salts medium with DF (1 mM) in Erlenmeyer flasks at 28 °C (160 rpm) until an optical density (OD_{578}) of about 0.5 was reached. For the generation of growth curves, cells pre-grown with DF were inoculated into Erlenmeyer flasks containing 1 mM DF and heavy metals. The concentrations of heavy metals were adjusted to 10 or 100 mg/L by addition of an appropriate volume of 100 or 1000 mg/L stock solution. One hundred milligrams per litre of stock solutions were prepared by 10 times dilution of 1000 mg/L solution with 2% or 10% nitric acid solution (for mercury). The initial OD at 578 nm was adjusted to about 0.05. The controls used included cultures containing 2% nitric acid solution without heavy metals, biomass inactivated by heating (70 °C for 30 min) and poisoned controls (10 mM sodium azide). The OD at 578 nm was measured every 4 h for 24 h. The optical density values of live and inactivated cells were adjusted to the control containing only heavy metal and inactivated biomass.

2.3. Effect of heavy metals on the degradation of dibenzofuran by resting cells of strain RW1

Resting cells of *S. wittichii* RW1 were prepared as described previously [12,13]. The harvested biomass was dissolved in phosphate buffer (20 mM, pH 7.2) and the optical density at 578 nm was adjusted to 2.0. A stock solution of dibenzofuran (DF) was prepared by dissolving 500 mg DF in 1 mL *N*,*N*-dimethylformamide (DMF), subsequently sterilized by filtration using 0.45 μm PTFE membrane filter. Autoclaved heavy metal solution (2 mL) was added to 18 mL culture solution in a 250 mL Erlenmeyer flask. The final concentration of DF in the cultures was 1.0 mM. Flasks were incubated on an orbital shaker for 5 days (28 °C, 160 rpm). The controls included inactivated (boiled and poisoned) biomass with the same concentration of heavy metal and DF or only heavy metal. The concentrations of heavy metals present in flasks after incubation and prepared under the same conditions were measured using inductively coupled plasma (ICP) spectrometry (Thermo Elemental, Franklin, MA, USA).

2.4. Extraction and analysis

After incubation, 200 μg of 2-chlordibenzo-*p*-dioxin (2-MCDD) in acetone was added and the reaction stopped by freezing the culture solution at −20 °C for 24 h. Thawed samples were extracted four times with 10 mL ethyl acetate. Extracted samples were dried using anhydrous sodium sulfate and the remaining organic phase was evaporated. Residues were dissolved in 10 mL acetonitrile. Aliquots of them were analyzed by reverse-phase HPLC using an Agilent liquid chromatograph (Agilent 1100, Agilent, CA, USA) equipped with a Lichrocart RP-18 column (125 mm × 30 mm, 5 μm, Merck, Darmstadt, Germany) by using UV–vis detection at 232 nm. The aqueous solvent system (flow rate of 1.0 mL/min) contained 0.1% (wt./vol.) phosphoric acid and 80% acetonitrile.

3. Results and discussion

The addition of 10 mg/L cadmium, copper, and mercury inhibited the growth of this strain, as shown in Fig. 1. In contrast, arsenic did not have any inhibitory effect, while lead only had a slight effect on growth. Experiments with 100 mg/L heavy metal present revealed similar results (Fig. 2), thus confirming that arsenic and lead do not exhibit adverse effects upon the growth of this strain. Metal ions may inhibit the metabolic activity of microorganisms involved in the degradation of organic compounds and thus affect the biodegradation rate [6,17]. The level of inhibition depends on the concentration and availability of heavy metals and the action of complex processes controlled by multiple factors, including the nature of metals, media and microbial species [18,19]. Further, several microorganisms exhibit a high level of resistance against heavy metals [20]. Although no specific experiments concerning the resistance of

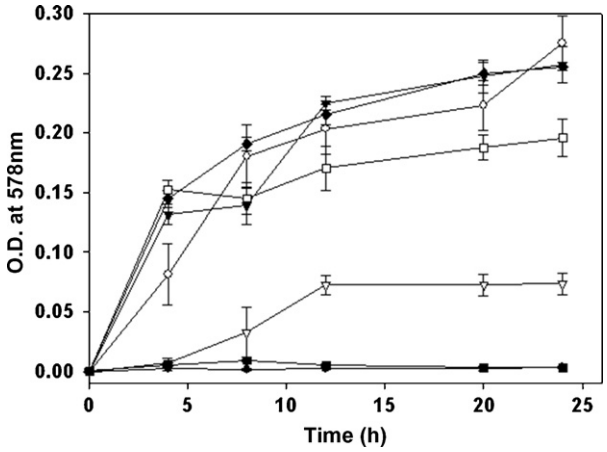

Fig. 1. The effect of 10 mg/L heavy metals on the growth of *Sphingmonas wittichii* RW1. Growth of RW1 without heavy metals (▼), with 2% nitric acid solution without heavy metals (♦), arsenic (◯), lead (□), cadmium (▽), copper (●) and mercury (■). The corrected values represent the mean of three independent measurements.

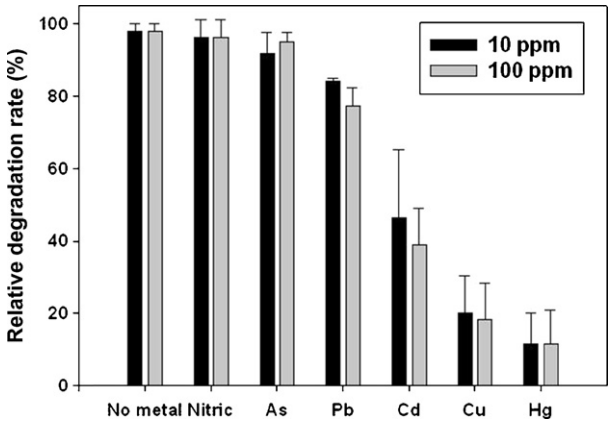

Fig. 3. The effect of 10 and 100 mg/L heavy metals on the degradation of dibenzofuran by resting cells of *S. wittichii* RW1. Shown are the relative degradation rates. A DF-control without heavy metals present was taken as 100%.

RW1 against arsenic were performed, our results clearly showed that the strain RW1 was not significantly affected by this heavy metal. Additionally, arsenic did not have any inhibitory effect on the degradation of DF by resting cells of RW1 (Fig. 3). These incubations were performed as described in Section 2. Data provided in square brackets state the relative amount of heavy metal detected after incubation compared to the initial amount of heavy metal taken as 100%.

Lead is also a heavy metal toxic to a range of micro- and higher organisms. Lead is one of the most ubiquitous pollutants, released from chemical industry, automobiles, and various other inventories. Thus, if a pollutant degrader like *S. wittichii* RW1 is to be employed under field conditions, the effect of lead on this strain should be estimated prior to application. Fortunately this strain remains unaffected when employed in liquid medium, even at relatively high concentrations of lead in the phosphate buffered condition. However, this could be due to the reduced bioavailability of lead which is certainly governed by the low solubility of side product with phosphate in the liquid media (the solubility of lead phosphate is about 10^{-54}). Subsequent analysis of heavy metal concentrations by ICP revealed that after 5 days of incubation with resting cells the levels of these two heavy metals (arsenic and lead) were not significantly changed (Table 1). These results suggest that adsorption, uptake, or metabolic activation of heavy metals should be important factors in the process of biodegradation [11,17,18].

The other possible explanation of the detrimental effect of metals on the BOD is oxygenation of the metal itself. Dissolved oxygen (DO) in the aqueous medium may be consumed in two ways, either during the oxidation of organic matter or by reacting with metal ions resulting in oxide formation [10]. Therefore, the oxidation potential of metal ions may affect the degradation rate or the growth of strain. However, we found no evidence of oxidation potential effect of the five metal ions tested. For example, while mercury has the smallest oxidation potential value (only minute consumption of oxygen under aqueous conditions and around neutral pH at 20 °C), it displays the highest inhibitory effect on the biodegradation of dibenzofuran by our strain. And, even though there was a possibility that copper and mercury are oxidized to precipitate under liquid conditions, its possibility could be eliminated by reanalysis of reaction solutions after dissolution with strong acids [data not shown].

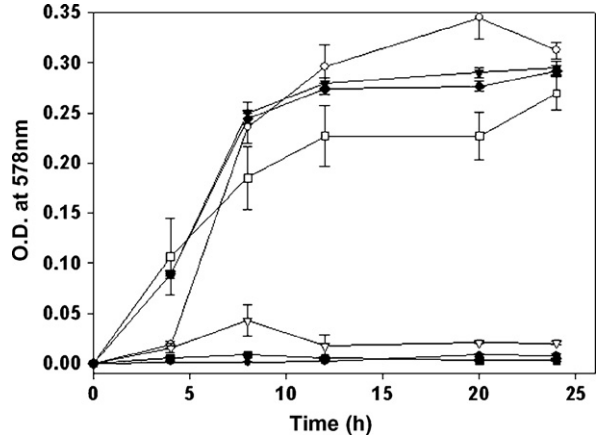

Fig. 2. The effect of 100 mg/L heavy metals on the growth of *S. wittichii* RW1. Growth of RW1 without heavy metals (▼), with 2% nitric acid solution without heavy metals (♦), arsenic (◯), lead (□), cadmium (▽), copper (●), and mercury (■). The corrected values represent the mean of three independent measurements.

Table 1
The change of heavy metal concentrations after incubation of resting cells of strain RW1 in the presence of 10 or 100 mg/L of heavy metals for 5 days

Heavy metal	10 mg/L	100 mg/L
Arsenic	7.0 [70%]	97.7 [97.7%]
Lead	9.2 [92%]	93.6 [93.6%]
Cadmium	2.5 [25%]	13.0 [13%]
Copper	N/D[a]	58.5 [58.5%]
Mercury	N/D[a]	N/D[a]

Values shown are the average of three independent measurements and are corrected for the poisoned and/or inactivated controls.
[a] Not detectable.

4. Conclusion

Our results showed that specific heavy metals affected the degradation of DF and growth of *S. wittichii* RW1 with this carbon source. The heavy metals of Hg, Cu, and Cd affected the growth of strain RW1, even at relatively low concentrations. Thus, our data indicate that heavy metals present in a target matrix such as fly ash at similar concentrations would interfere most certainly with the degradation of dibenzofuran by the strain RW1, and may thus be one of the factors controlling the degradation rate. In summary, while the addition of 10 mg/L cadmium, copper, and mercury inhibited the growth of this strain, addition of 10 mg/L arsenic did not cause such an inhibition and lead exhibited only a slight inhibition of growth. Experiments with a higher concentration (100 mg/L) of heavy metals present revealed similar results. Arsenic did not have any inhibitory effect on the degradation of DF by resting cells of strain RW1, while lead had a slight effect on the degree of degradation. Cadmium, mercury, and copper clearly inhibited the degradation of dibenzofuran. These data suggest the potential inhibition of dioxin degradation in fly ash, the major source of dioxin degradation, but which also would limit the applicability of a slurry-based fermentation reactor for dioxin degradation. Therefore, further study should be performed to elucidate whether these conditions are effectively imitating those of contaminated fly ash in a slurry system, which are very complicated chemical and physical phenomena.

Acknowledgement

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References

[1] D. Shin, S. Choi, J.E. Oh, Y.S. Chang, Evaluation of polychlorinated dibenzo-*p*-dioxin/dibenzofuran (PCDD/F) emission in municipal solid waste incinerators, Environ. Sci. Technol. 33 (1999) 2657–2666.

[2] R. Addink, E.R. Altwicker, Formation of polychlorinated dibenzo-*p*-dioxins and dibenzofurans on secondary combustor/boiler ash from a rotary kiln burning hazardous waste, J. Hazard. Mater. B 114 (2004) 53–57.

[3] C.H. Wu, G.P. Chang-Chien, W.S. Lee, Photodegradation of tetra- and hexachlorodibenzo-*p*-dioxins, J. Hazard. Mater. B 120 (2005) 257–263.

[4] K.J. Shin, Y.S. Chang, Characterization of polychlorinated dibenzo-*p*-dioxin, dibenzofurans, biphenyls, and heavy metals in fly ash produced from Korean municipal solid waste incinerators, Chemosphere (1999) 2655–2666.

[5] H.B. Hong, S.H. Hwang, Y.S. Chang, Biosorption of 1,2,3,4-tetrachlorodibenzo-*p*-dioxin and polychlorinated dibenzofurans by *Bacillus pumilus*, Water Res. 34 (1999) 349–353.

[6] L. Amor, C. Kennes, M.C. Veiga, Kinetics of inhibition in the biodegradation of monoaromatic hydrocarbons in presence of heavy metals, Bioresource Technol. 78 (2001) 181–185.

[7] E. Baath, Effect of heavy metals in soil on microbial processes and population, Water Air Soil Poll. 47 (1989) 335–379.

[8] K. Figge, U. Metzdorf, J. Nevermann, J. Schmiese, M. Keskin, P. Fortnagel, R.-M. Wittich, Bakterielle Mineralisierung von Dibenzofuran, Dibenzo-*p*-dioxin und 1,2,4,5-Tetrachlorbenzol in Böden, UWSF-Z. Umweltchem. Ökotox. 5 (1993) 122–130.

[9] D.H. Nies, Microbial heavy-metal resistance, Appl. Microbiol. Biotechnol. 51 (1999) 730–750.

[10] S.K. Mittal, R.K. Ratra, Toxic effect of metal ions on biochemical oxygen demand, Water Res. 34 (2000) 147–152.

[11] J. Sokhn, F.A. De Leij, T.D. Hart, J.M. Lynch, Effect of copper on the degradation of phenanthrene by soil microorganisms, Lett. Appl. Microbiol. 33 (2001) 164–168.

[12] H.B. Hong, Y.S. Chang, I.H. Nam, P. Fortnagel, S. Schmidt, Biotransformation of 2,7-dichloro- and 1,2,3,4-tetrachlorodibenzo-*p*-dioxin by *Sphingomonas wittichii* RW1, Appl. Environ. Microbiol. 68 (2002) 2584–2588.

[13] T. Keim, W. Francke, S. Schmidt, P. Fortnagel, Catabolism of 2,7-dichloro- and 2,4,8-trichlorodibenzofuran by *Sphingomonas* sp. strain RW1, J. Ind. Microbiol. Biotechnol. 23 (1999) 359–363.

[14] P.V. Bünz, S. Schmidt, The microbial degradation of halogenated diaryl ethers, Biotechnol. Adv. 15 (1997) 621–632.

[15] R.-M. Wittich, H. Wilkes, V. Sinnwell, W. Francke, P. Fortnagel, Metabolism of dibenzo-*p*-dioxin by *Sphingomonas* sp. strain RW1, Appl. Environ. Microbiol. 58 (1992) 1005–1010.

[16] H. Wilkes, R.-M. Wittich, K.N. Timmis, P. Fortnagel, W. Francke, Degradation of chlorinated dibenzofurans and dibenzo-*p*-dioxins by *Sphingomonas* sp. strain RW1, Appl. Environ. Microbiol. 62 (1996) 367–371.

[17] H. Hattori, Influence of heavy metals on soil microbial activities, Soil Sci. Plant Nutr. 38 (1992) 93–100.

[18] G. Tyler, Heavy metal pollution and soil enzymatic activity, Plant Soil 41 (1974) 303–311.

[19] S.Y. Chen, J.G. Lin, Effect of substrate concentration on bioleaching of metal-contaminated sediment, J. Hazard. Mater. B 82 (2001) 77–89.

[20] S. Sultan, S. Hasnain, Pseudomonad strains exhibiting high level Cr(VI) resistance and Cr(VI) detoxification potential, Bull. Environ. Contam. Toxicol. 71 (2003) 473–480.