Journal of Microbiology (2014) Vol. 52, No. 9, pp. 801–804
DOI 10.1007/s12275-014-3424-6

NOTE

Is The Biotransformation of Chlorinated Dibenzo-*p*-dioxins by *Sphingomonas wittichii* RW1 Governed by Thermodynamic Factors?

In-Hyun Nam[1]*, Hyo-Bong Hong[2], and Stefan Schmidt[3]

[1]*Geologic Environment Division, Korea Institute of Geoscience and Mineral Resources (KIGAM), Daejeon, 305-350, Republic of Korea*
[2]*Future Technology Research Group, Electronics and Telecommunications Research Institute (ETRI), Daejeon 305-700, Republic of Korea*
[3]*Discipline of Microbiology, School of Life Sciences, University of KwaZulu-Natal, Pietermaritzburg 3201, South Africa*

(Received Aug 6, 2013 / Revised Nov 21, 2013 / Accepted Nov 27, 2013)

Density functional theory (DFT) calculations were used to explore the relationship between the biotransformation of dibenzo-*p*-dioxin and selected chlorinated derivatives by resting cells of *Sphingomonas wittichii* RW1 and measuring the thermodynamic properties of the biotransformation substrates. *Sphingomonas wittichii* RW1 can aerobically catabolize dibenzo-*p*-dioxin as well as 2,7-dichloro-, 1,2,3-trichloro-, 1,2,3,4-tetrachloro-, and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin; however, neither the 2,3,7-trichloro- nor the 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin was transformed to its corresponding metabolic intermediate. The experimental biotransformation rates established were apparently governed by the selected thermodynamic properties of the substrates tested.

***Keywords*:** dibenzo-*p*-dioxin, biotransformation rates, *Sphingomonas wittichii* RW1, DFT calculation, correlation analysis

Polychlorinated aromatic compounds are among the most problematic environmental pollutants because of their chemical inertness, lipid solubility, and toxicity (Meharg and Osborn, 1995; Brzuzy and Hites, 1996; Reineke, 1998; Jones *et al*., 2001; Jeon *et al*., 2013). Naturally occurring microorganisms have evolved to degrade and mineralize many, but by no means all, of these compounds. For example, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans (PCDD/Fs), and highly chlorinated biphenyls are degraded only very slowly or not at all (McLachlan, 1996; Jones *et al*., 2001). In recent years, numerous studies have attempted to elucidate the mechanism by which aerobic bacteria degrade PCDD/Fs produced by incineration processes or as unwanted by-products of the synthesis of pesticides and herbicides (Wittich, 1998; Chang, 2008; Jeon *et al*., 2013). PCDD/Fs have attracted particular interest on account of the environmental concerns associated with their persistence and toxicity (Bünz and Schmidt, 1997).

Our previous studies have demonstrated that *Sphingomonas wittichii* RW1 (Hong *et al*., 2002; Nam *et al*., 2006) can transform dibenzo-*p*-dioxin, 2,7-dichlorodibenzo-*p*-dioxin, 1,2,3-trichlorodibenzo-*p*-dioxin, 1,2,3,4-tetrachlorodibenzo-*p*-dioxin, and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin; however, some dioxin congeners, such as 2,3,7-trichlorodibenzo-*p*-dioxin and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin were surprisingly transformed to their corresponding metabolites by the same strain at rates not significantly different from the rate of transformation of the control compound. Here, we describe a comparison between the aerobic biotransformation rates established for dibenzo-*p*-dioxin and six selected chlorinated derivatives in the laboratory and the rates predicted by modeling studies. In addition, we present data illustrating the potential impact of certain substitution patterns on the degradability of the compounds. A thermodynamic argument is proposed to account for the inability of strain RW1 to transform the two congeners 2,3,7-trichloro- and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin. Density functional theory (DFT) calculations (Mhin *et al*., 2002; Dorofeeva and Yungman, 2003; Lee *et al*., 2003; Thomson and Ewan, 2007; Wang *et al*., 2008; Jansson *et al*., 2009) were employed to investigate the mechanisms underlying the biotransformation rates established for mixtures of dibenzo-*p*-dioxin and several selected chlorinated congeners, such as 2,7-dichloro- and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin. The identified structure-reactivity indicator was then used to predict the biotransformation ability.

The biotransformation rates of the individual dibenzo-*p*-dioxin congeners have been reported previously (Hong *et al*., 2002; Nam *et al*., 2006); however, the biotransformation experiments involving dioxin mixtures were performed using resting *Sphingomonas wittichii* RW1 cells produced using dibenzofuran as the sole source of carbon and energy, as reported previously (Keim *et al*., 1999; Hong *et al*., 2002; Nam *et al*., 2006). An appropriate aliquot of the required congener mixture stock solution prepared using a toluene-nonane mixture (1:1 by volume) was added to sterilized replicate Erlenmeyer flasks, and the solvent was evaporated by flushing the flask with N_2. The biodegradabilities of dibenzo-*p*-dioxin and the selected chlorinated congeners were compared by examining substrate mixtures containing equal amounts (0.5 mg of each compound) of dibenzo-*p*-dioxin, 2,7-di-, 1,2,3-tri-, 2,3,7-tri-, 1,2,3,4-tetra-, 1,2,3,7,8-penta-,

*For correspondence. E-mail: nih@kigam.re.kr; Tel.: +82-42-868-3164; Fax: +82-42-868-3414

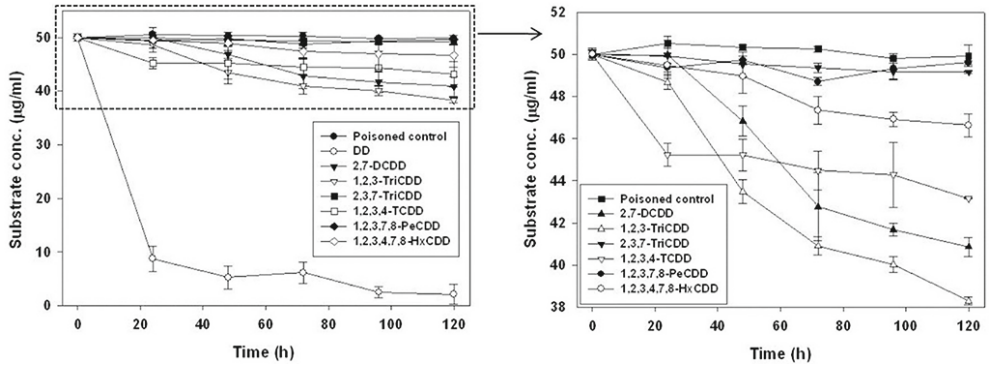

Fig. 1. Biotransformation kinetics associated with the transformation of a mixture of 7 selected dibenzo- *p*-dioxin congeners by resting *S. wittichii* RW1 cells.

and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin. The biotransformation was monitored and quantified by employing replicate 100 ml Erlenmeyer flasks. Each flask contained 10 ml of a cell suspension (OD_{578} = 8.0), with each substrate present at 50 μg/ml. Every 24 h, a set of triplicate flasks was removed, 2 ml of *ortho*-phosphoric acid was added to each flask to stop the reaction, and the flasks were immediately frozen and stored at -70°C. After incubation for 120 h, all flasks were thawed, and the contents were extracted, as described previously (Hong *et al.*, 2002). The recovery rates were routinely determined by adding 500 μg 2-chlorodibenzo-*p*-dioxin and 5 ng 2,3,4,5-tetrachlorophenol to each flask prior to extraction. Extracts obtained from the flasks and the corresponding controls were dried over anhydrous sodium sulfate, and the solvent was evaporated under reduced pressure. Aliquots were analyzed directly using a gas chromatography-mass spectrometry system, as described below.

The concentrations of dibenzo-*p*-dioxin and the chlorinated dioxin congeners were determined using high-resolution gas chromatography-ion trap mass spectrometry. Gas chromatography-mass spectrometry measurements were carried out using a Trace GC 2000 system (Thermoquest, USA) linked to a Finnigan Polaris Q mass spectrometer (Thermoquest) with a 60 m DB-5 column. The initial temperature, 60°C, was maintained for 2 min, increased to 310°C over 10 min, and then held at that temperature for an additional 10 min. The formation of metabolic intermediates in these biotransformation experiments was confirmed using the same approach or using the method reported previously (Hong *et al.*, 2002; Nam *et al.*, 2006). In all experiments, which were generally performed in triplicate, heat-inactivated (75°C for 20 min) and poisoned cell suspensions (10 mM NaN_3) were employed as controls. Generally, metabolites were identified and quantified by comparing the mass spectra, retention times, and peak areas with the values obtained from authentic standards. Dibenzofuran and 2,3,4,5-tetrachlorophenol were obtained from Sigma-Aldrich (USA). Dibenzo-*p*-dioxin, 2-mono-, 2,7-di-, 1,2,3-tri-, 2,3,7-tri-, 1,2,3,4-tetra-, 1,2,3,7,8-penta-, and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin were purchased from Accustandard (USA). The solvents and *ortho*-phosphoric acid were obtained from Merck (Germany). All chemicals employed were of the highest quality commercially available.

To estimate ΔH_f and ΔG_f as well as the other reported thermodynamic properties of dibenzo-*p*-dioxin and its selected chlorinated congeners, DFT calculations were carried out at the level of hybrid B3LYP density functional theory with 6-31G** basis sets using a Gaussian 98 suite of programs, as described previously (Mhin *et al.*, 2002; Lee *et al.*, 2003).

In the dibenzo-*p*-dioxin congener mixture biotransformation experiments, 7 selected dioxin congeners were tested using resting cells of the strain *Sphingomonas wittichii* RW1 (DSM 6014). Within 5 days, the concentrations of DD, 2,7-DCDD, 1,2,3-TriCDD, 2,3,7-TriCDD, 1,2,3,4-TeCDD, 1,2,3,7,8-PeCDD, and 1,2,3,4,7,8-HxCDD were reduced by, respectively, 93.82%, 16.32%, 22.46%, 1.10%, 16.31%, 0.86%, and 11.72% relative to the initial concentration present. During the biotransformation experiments, the catabolism of the chlorinated congeners present gave rise to the expected key metabolites reported previously for these congeners (data not shown) (Hong *et al.*, 2002; Nam *et al.*, 2006). The formation of the expected metabolic intermediates from the chlorinated congeners was confirmed (i.e. 4-chlorocatechol for 2,7-DCDD; 3,4,5-trichlorocatechol for 1,2,3-TriCDD;

Table 1. Thermodynamic properties of dibenzo-*p*-dioxin and the selected chlorinated congeners tested in this study. All values (E_e (Hartree), ΔE_e^{rel} (kJ/mol), ZPE (kJ/mol), ΔE_T (kJ/mol), S_{tot} (J/mol·K), ΔH_f (kJ/mol), and ΔG_f (kJ/mol)) were calculated at 301.15 K and 101.3 kPa according to the hybrid B3LYP density functional theory with 6-31G** basis sets using the Gaussian 98 suite of programs.

Congener	E_e	ΔE_e^{rel}	ZPE	$\Delta E_{T=301}$	$S_{tot,301}$	$\Delta H°_{f,301}$	$\Delta G°_{f,301}$
DD	-612.54108	-	441.16	466.43	394.84	-59.20	59.12
2,7-DCDD	-1531.72290	0.00	390.03	422.08	461.24	-110.54	16.01
1,2,3-TriCDD	-1991.30502	23.77	364.89	400.24	494.17	-100.83	30.04
2,3,7-TriCDD	-1991.31327	2.09	364.59	399.99	496.01	-122.76	7.61
1,2,3,4-TeCDD	-2450.88835	36.48	339.53	378.32	516.26	-101.55	36.69
1,2,3,7,8-PeCDD	-2910.48422	4.44	313.63	355.85	553.17	-135.81	5.44
1,2,3,4,7,8-HxCDD	-3370.06714	6.11	288.32	333.93	575.51	-135.44	13.18

Symbols; E_e (Hartree), Electronic energy; ΔE_e^{rel} (kJ/mol), Relative electronic energy; ZPE (kJ/mol), Zero-point energies; ΔE_T (kJ/mol), Thermal energy; S_{tot} (J/mol·K), Total entropy; ΔH_f (kJ/mol), Enthalpy of formation; ΔG_f (kJ/mol), Gibbs free energy of formation.

Table 2. Removal rates measured for dibenzo-*p*-dioxin and the selected chlorinated congeners, and their calculated Gibbs free energies of formation

Congener	Removal rate ($ng \times ml^{-1} \times min^{-1}$)[a]	ΔG_f (kJ/mol)[b]
DD	6.5153	59.12
2,7-DCDD	1.1331	16.01
1,2,3-TriCDD	1.5599	30.04
2,3,7-TriCDD	0.0761	7.61
1,2,3,4-TeCDD	1.1324	36.69
1,2,3,7,8-PeCDD	0.0595	5.44
1,2,3,4,7,8-HxCDD	0.8139	13.18
Poisoned control	0.0430	-

[a] Each removal rate represents the mean of three independently performed measurements using resting cells.
[b] ΔG_f values at 301.15 K and 101.3 kPa are taken from Table 1.

3,4,5,6-tetrachlorocatechol for 1,2,3,4-TeCDD and 1,2,3,4,7,8-HxCDD) by mass spectrometry, as reported previously (Hong *et al*., 2002; Nam *et al*., 2006). All percentage removal values and the specific biotransformation rates represent the mean obtained from running three independently performed measurements (Fig. 1). As shown in Table 2, the removal rates that had been established analytically for dibenzo-*p*-dioxin and the chlorinated congeners employed appeared to be correlated with the calculated values for the Gibbs free energy of formation. Thus, a linear regression analysis (data not shown) using the $\log_{10}$ normalized Gibbs free energy of formation data and the biotransformation rates indicated that about 85% of the variation observed could be predicted (r^2=0.85). As demonstrated earlier (Nam *et al*., 2006), the aerobic catabolism of 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin by *Sphingomonas wittichii* RW1 gave rise to two polar metabolites (i.e. 3,4,5,6-tetrachlorocatechol and 2-methoxy-3,4,5,6-tetrachlorophenol) whereas the less chlorinated 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin was not transformed to any detectable extent by the same strain. Hong *et al*. (2002) showed previously that the non-substituted angular sites of the benzene rings in 1,2,3,4-tetrachlorodibenzo-*p*-dioxin were potential targets for an initial oxygenolytic attack. It was therefore surprising that the 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, which is chlorinated to a lesser degree than the catabolized 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin, was not transformed to detectable metabolites. In the case of 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin, the chlorine substituents did not occupy all available sites on the higher chlorinated ring and, thus, were not expected to block an angular oxygenolytic attack. Thus, in principle, both of the rings were potential targets for an initial angular attack, as reported for the non-chlorinated dibenzo-*p*-dioxin (Wilkes *et al*., 1992); however, only one benzene ring in 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin included unoccupied angular sites that would enable oxygenation. The reported detection and identification of 3,4,5,6-tetrachlorocatechol as an oxidation product derived from 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin confirmed that an initial attack could take place at the less substituted ring and, thus, the biotransformation could take place (Fig. 1). The ability of the substitution patterns to impact the biodegradability of these compounds was tested using two different trichlorinated congeners, namely 1,2,3- and 2,3,7-trichlorodibenzo-*p*-dioxin, in our dibenzo-*p*-dioxin congener mixture (Fig. 1). The general molecular properties that governed the transfer of the substrates into the bacterial cells, such as the molecular weight, which increased with increasing chlorine substitution, and the sizes and substitution patterns of the molecules, were expected to limit the turnover rate (Providenti *et al*., 1993; Schreiner *et al*., 1997). Nevertheless, because 1,2,3- and 2,3,7-trichlorodibenzo-*p*-dioxin exhibited identical molecular weights, these factors could not sufficiently explain the transformation of 1,2,3-trichlorodibenzo-*p*-dioxin and the lack of transformation (relative to the controls) of 2,3,7-trichorodibenzo-*p*-dioxin. Similarly, whereas the smaller and lighter 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin was transformed at rates comparable to those of the abiotic controls, the larger and heavier 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin was transformed. Hence, the biotransformation rates may be governed by the inherent thermodynamic properties of the recalcitrant pollutants in addition to the structural molecular properties. The thermodynamic properties of the chlorinated dioxin congeners were recently calculated using density functional theory (Mhin *et al*., 2002; Dorofeeva and Yungman, 2003; Lee *et al*., 2003; Thomson and Ewan, 2007; Wang *et al*., 2008; Jansson *et al*., 2009); the corresponding data for the congeners tested in the mixture biotransformation experiments in the present study are summarized in Table 1.

All chlorinated dioxin congeners reported in Table 1 have substantially more negative ΔH_f-values than those exhibited by the nonchlorinated dibenzo-*p*-dioxin. Based on the calculated ΔG_f-values, the nonchlorinated dibenzo-*p*-dioxin was expected to be the least stable among the dioxin congeners tested, whereas 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin (ΔG_f = 5.44 kJ/mol) was expected to be the most diffi-

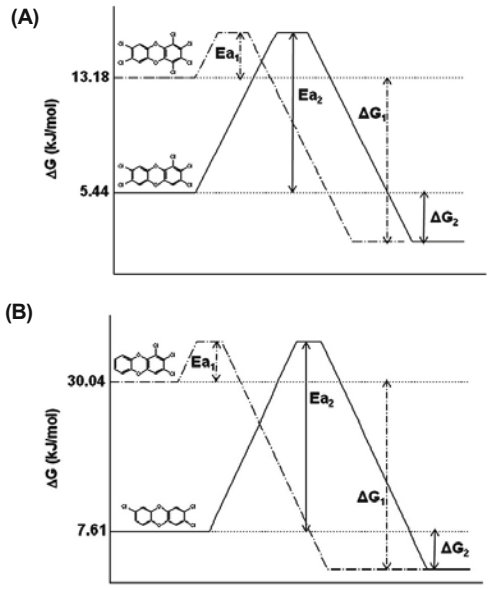

Fig. 2. Calculated energy requirements for the transformation of the selected dioxin congeners by *S. wittichii* RW1. (A) The activation energy (Ea_1) and Gibbs free energy of formation (ΔG_1) for 1,2,3,4,7,8-HxCDD (catabolized), and the activation energy (Ea_2) and Gibbs free energy of formation (ΔG_2) for 1,2,3,7,8-PeCDD (not catabolized). (B) The activation energy (Ea_1) and Gibbs free energy of formation (ΔG_1) for 1,2,3-TriCDD (catabolized), and the activation energy (Ea_2) and Gibbs free energy of formation (ΔG_2) for 2,3,7-TriCDD (not catabolized).

cult substrate to transform. This hypothesis was supported by the results obtained in the present study (Fig. 1 and Table 2) and agreed with previous findings (Hong *et al*., 2002; Nam *et al*., 2006), confirming that 2,7-dichlorodibenzo-*p*-dioxin (ΔG_f = 16.01 kJ/mol), 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (ΔG_f = 36.69 kJ/mol), and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin (ΔG_f = 13.18 kJ/mol) were transformed by *S. wittichii* RW1. Finally, the transformation of 1,2,3-trichlorodibenzo-*p*-dioxin (ΔG_f = 30.04 kJ/mol) and the lack of transformation of 2,3,7-trichlorodibenzo-*p*-dioxin, which displayed a much lower ΔG_f–value (ΔG_f = 7.61 kJ/mol) and was not catabolized to yield detectable metabolites, further supported the notion that the thermodynamic properties contributed to the biodegradability of the compounds. A preliminary hypothetical explanation for these effects, based on the calculated Gibbs free energy of formation, is presented in Fig. 2. Figure 2 indicates that the lower ΔG_f–values for the recalcitrant 2,3,7-trichloro- and 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin may have led to much higher biotransformation activation energy requirements, thereby potentially limiting the aerobic biodegradability of these congeners on thermodynamic grounds. Both the experimental results and the thermodynamic data suggested that the observed transformations of 1,2,3,7,8-pentachlorodibenzo-*p*-dioxin and 2,3,7-trichlorodibenzo-*p*-dioxin by *Sphingomonas wittichii* RW1 at rates not significantly different from the controls may have resulted from the structural (i.e., substitution pattern and size) and inherent thermodynamic properties of these compounds. The biotransformation experiments conducted using a mixture of 7 individual dibenzo-*p*-dioxin congeners with different ΔG_f–values revealed that the molecules were transformed with different efficiencies when present at the same concentration (Fig. 1); however, the predicted outcome did not entirely reflect the expected biotransformation rates if only the ΔG_f –values were considered. The predicted results suggested that the 2,7-dichlorodibenzo-*p*-dioxin (ΔG_f = 16.01 kJ/mol) would be transformed much slower than the 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (ΔG_f = 36.69 kJ/mol), which was not the case.

A complex system of effects appeared to govern the relationship between the biotransformation rates of several dioxin congeners via aerobic bacterial catabolism and the congeners' inherent thermodynamic and structural properties. In addition to the molecular size, which impacted the transfer of the substrates into the bacterial cell, the thermodynamic characteristics of the compounds (i.e., ΔG_f) appeared to influence the degradability. Additional experimental data from similar recalcitrant pollutants, such as the polychlorinated dibenzofurans or biphenyls, would enable a more in-depth analysis of the relationship between the inherent molecular properties and the biotransformation rates.

This subject is supported by Korea Ministry of Environment (MOE) as "GAIA program" (Project No. 2013-000550003).

References

Brzuzy, L.P. and Hites, R.A. 1996. Global mass balance for polychlorinated dibenzo-*p*-dioxins and dibenzofurans. *Environ. Sci. Technol.* **30**, 1797–1804.

Bünz, P.V. and Schmidt, S. 1997. The microbial degradation of halogenated diaryl ethers. *Biotechnol. Adv.* **15**, 621–632.

Chang, Y.S. 2008. Recent developments in microbial biotransformation and biodegradation of dioxins. *J. Mol. Microbiol. Biotechnol.* **15**, 152–171.

Dorofeeva, O.V. and Yungman, V.S. 2003. Enthalpies of formation of dibenzo-*p*-dioxin and polychlorinated dibenzo-*p*-dioxins calculated by density functional theory. *J. Phys. Chem. A* **107**, 2848–2854.

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

Jansson, S., Antti, H., Marklund, S., and Tysklind, M. 2009. Multivariate relationships between molecular descriptors and isomer distribution patterns of PCDD/Fs formed during MSW combustion. *Environ. Sci. Technol.* **43**, 7032–7038.

Jeon, J.R., Murugesan, K., Nam, I.H., and Chang, Y.S. 2013. Coupling microbial catabolic actions with abiotic redox processes: A new recipe for persistent organic pollutant (POP) removal. *Biotechnol. Adv.* **31**, 246–256.

Jones, J.P., O'Hare, E.J., and Wong, L.L. 2001. Oxidation of polychlorinated benzenes by genetically engineered CYP101 (cytochrome P450(cam)). *Eur. J. Biochem.* **268**, 1460–1467.

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

Lee, J.E., Choi, W., and Mhin, B.J. 2003. DFT calculation on the thermodynamic properties of polychlorinated dibenzo-*p*-dioxins: intramolecular Cl-Cl repulsion effects and their thermochemical implications. *J. Phys. Chem. A* **107**, 2693–2699.

McLachlan, M.S., Sewart, A.P., Bacon, J.R., and Jones, K.C. 1996. Persistence of PCDD/Fs in a sludge-amended soil. *Environ. Sci. Technol.* **30**, 2567–2571.

Meharg, A.A. and Osborn, D. 1995. Dioxins released from chemical accidents. *Nature* **375**, 353–354.

Mhin, B.J., Lee, J.E., and Choi, W. 2002. Understanding the congener-specific toxicity in polychlorinated dibenzo-*p*-dioxins: chlorination pattern and molecular quadrupole moment. *J. Am. Chem. Soc.* **124**, 144–148.

Nam, I.H., Kim, Y.M., Schmidt, S., and Chang, Y.S. 2006. Biotransformation of 1,2,3-tri- and 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin by *Sphingomonas wittichii* RW1. *Appl. Environ. Microbiol.* **72**, 112–116.

Providenti, M.A., Lee, H., and Trevors, J.T. 1993. Selected factors limiting the microbial degradation of recalcitrant compounds. *J. Ind. Microbiol.* **12**, 379–395.

Reineke, W. 1998. Development of hybrid strains for the mineralization of chloroaromatics by patchwork assembly. *Annu. Rev. Microbiol.* **52**, 287–331.

Schreiner, G., Wiedmann, H., Schimmel, H., and Ballschmiter, K. 1997. Influence of the substitution pattern on the microbial degradation of mono- to tetrachlorinated dibenzo-*p*-dioxins and dibenzofurans. *Chemosphere* **34**, 1315–1331.

Thomson, D. and Ewan, B.C.R. 2007. A group additivity algorithm for polychlorinated dibenzofurans derived from selected DFT analyses. *J. Phys. Chem. A* **111**, 5043–5047.

Wang, L., Heard, D.E., Pilling, M.J., and Seakins, P. 2008. A Gaussian-3X prediction on the enthalpies of formation of chlorinated phenols and dibenzo-*p*-dioxins. *J. Phys. Chem. A* **112**, 1832–1840.

Wilkes, H., Francke, W., Wittich, R.M., Harms, H., Schmidt, S., and Fortnagel, P. 1992. Mechanistic investigation on microbial degradation of diaryl ethers-analysis of isotope-labeled reaction products. *Naturwissenschaften* **79**, 269–271.

Wittich, R.M. 1998. Degradation of dioxin-like compounds by microorganisms. *Appl. Microbiol. Biotechnol.* **49**, 489–499.