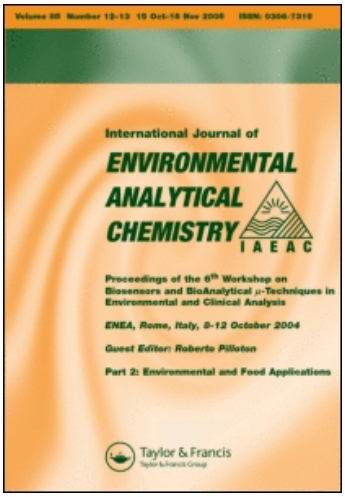


International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Polychlorinated biphenyls fractioning in aqueous bioremediation assay with *Phanerochaete chrysosporium*

Matthieu Sangely[abcd]; Caroline Sablayrolles[ab]; Claire Vialle[ab]; Pierre Strehaiano[c]; Laurent Thannberger[d]; Mireille Montrejaud-Vignoles[ab]
[a] Université de Toulouse, INP, LCA (Laboratoire de Chimie AgroIndustrielle), ENSIACET, F-31029 Toulouse, France [b] INRA, LCA (Laboratoire de Chimie AgroIndustrielle), F-31029 Toulouse, France [c] Laboratoire de Génie Chimique, UMR CNRS 5503, 31106 Toulouse, France [d] VALGO, 31100 Toulouse, France

Online publication date: 22 September 2010

To cite this Article Sangely, Matthieu , Sablayrolles, Caroline , Vialle, Claire , Strehaiano, Pierre , Thannberger, Laurent and Montrejaud-Vignoles, Mireille(2009) 'Polychlorinated biphenyls fractioning in aqueous bioremediation assay with *Phanerochaete chrysosporium*', International Journal of Environmental Analytical Chemistry, 89: 8, 849 — 856
To link to this Article: DOI: 10.1080/03067310903015763
URL: http://dx.doi.org/10.1080/03067310903015763

Intern. J. Environ. Anal. Chem.
Vol. 89, Nos. 8–12, 15 July–15 October 2009, 849–856

Taylor & Francis
Taylor & Francis Group

Polychlorinated biphenyls fractioning in aqueous bioremediation assay with *Phanerochaete chrysosporium*

Matthieu Sangely[abcd]*, Caroline Sablayrolles[ab], Claire Vialle[ab], Pierre Strehaiano[c], Laurent Thannberger[d] and Mireille Montrejaud-Vignoles[ab]

[a]*Université de Toulouse, INP, LCA (Laboratoire de Chimie AgroIndustrielle), ENSIACET, 4 Allées Emile Monso, F-31029 Toulouse, France;* [b]*INRA, LCA (Laboratoire de Chimie AgroIndustrielle), F-31029 Toulouse, France;* [c]*Laboratoire de Génie Chimique, UMR CNRS 5503, 5 rue Paulin Talabot, 31106 Toulouse, France;* [d]*VALGO, 81 rue Jacques Babinet, 31100 Toulouse, France*

(*Received 19 November 2008; final version received 13 April 2009*)

The white rot fungus *Phanerochaete chrysosporium* is able to degrade PCBs in water and in soil. This study aims at estimating the fractioning of truly degraded PCBs, adsorbed PCBs and residual PCBs in an aqueous bioremediation assay with *Phanerochaete chrysosporium*. Di-, tri- tetra-, penta-, hexa-, hepta-chlorinated biphenyls (IUPAC numbers: PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180) were extracted from 500 mL aqueous bioremediation assays using a liquid–liquid extraction with *n*-hexane. Analyses were performed by gas chromatography coupled to mass spectrometry. The study reveals that the adsorbed PCBs fraction ranges from 42% to 54%, whereas the degraded one ranges from 39% to 49%. No PCBs were detected in the residual water (limit of detection: 13 ng L^{-1}).

Keywords: bioremediation; polychlorinated biphenyl; *Phanerochaete chrysosporium*; adsorption

1. Introduction

Polychlorinated biphenyls (PCBs) are known to be worldwide-spread persistent organic pollutants (POPs) [1,2]. PCBs are chemically, physically and biologically stable and have low solubility in water, especially the more chlorinated members [3]. These properties made their success in the past in heat transfer and dielectric fluids uses. Moreover, they are soluble in oils and most organic solvents and have a very high environmental mobility due to their high vapour pressure [4]. Due to inadequate storages, industrial incidents or handling oversights, PCBs have contaminated the environment and are considered as one of the priority hazardous substances for monitoring [5].

The French Environment Ministry, through the BASOL database (http://basol.environnement.gouv.fr/home.htm), lists more than 240 PCBs contaminated sites. Public Authorities from the Rhône rivers area have forbidden the consumption of fish caught in the Rhône river due to high levels of PCBs in the flesh of fishes [6].

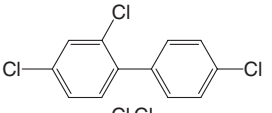
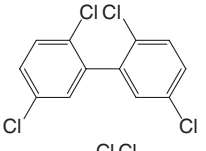
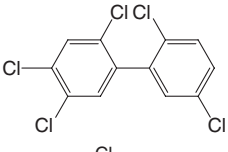
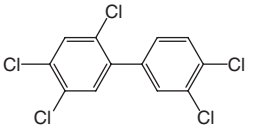
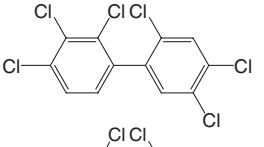
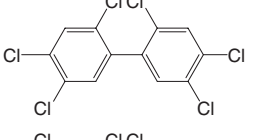
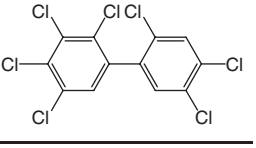
*Corresponding author. Email: matthieu.sangely@ensiacet.fr

ISSN 0306–7319 print/ISSN 1029–0397 online

DOI: 10.1080/03067310903015763
http://www.informaworld.com

The main destruction process for PCBs is high temperature incineration which can cause releases of highly toxic compounds such as Polychlorodibenzodioxins (PCDDs) and Polychlorodibenzofurans (PCDFs). Developing alternative treatment for PCBs-contaminated soils has become an environmental challenge. Biologic processes are environmentally friendly and low cost. They can be a good alternative to incineration for PCBs-contaminated soil. Some micro-organisms, such as *Burkholderia xenovorans* [7,8] or *Phanerochaete chrysosporium* [9,10], are known to degrade PCBs in the presence of an additional source of carbon. Some studies report bioremediation assays but little is known about PCBs sorption onto fungus with degradation activity like *Phanerochaete chrysosporium*. Consequently, this work aims to assess PCBs partition within an aqueous bioremediation assay leaded with *Phanerochaete chrysosporium*. The formulae of the 7 PCBs studied are given in Table 1.

Table 1. PCB congeners studied: molecular and structural formula and IUPAC numbers.

Compound	IUPAC number	Molecular formula	Structural formula
2,4,4′trichlorobiphenyl	PCB 28	$C_{12}H_7Cl_3$	
2,2′,5,5′tetrachlorobiphenyl	PCB 52	$C_{12}H_6Cl_4$	
2,2′,4,5,5′pentachlorobiphenyl	PCB 101	$C_{12}H_5Cl_5$	
2,3′,4,4′,5pentachlorobiphenyl	PCB 118	$C_{12}H_5Cl_5$	
2,2′,3,4,4′,5′hexachlorobiphenyl	PCB 138	$C_{12}H_4Cl_6$	
2,2′,4,4′,5,5′hexachlorobiphenyl	PCB 153	$C_{12}H_4Cl_6$	
2,2′,3,4,4′,5,5′heptachlorobiphenyl	PCB 180	$C_{12}H_3Cl_7$	

2. Experimental

2.1 *Materials*

2.1.1 *Reagents*

Mineral salts K_2HPO_4, KH_2PO_4, NH_4Cl, $MgSO_4$, and $CaCl_2$ came from Sigma-Aldrich (Steinheim, Germany) and were at least 98% pure. $FeSO_4$ purity 95% came from Prolabo (Fontenay-sous-bois, France). A stock solution of liquid mineral medium containing K_2HPO_4: 4.4 g L^{-1}, KH_2PO_4: 1.7 g L^{-1}, NH_4Cl: 2.14 g L^{-1}, $MgSO_4$: 0.2 g L^{-1}, $FeSO_4$: 0.01 g L^{-1} and $CaCl_2$: 0.003 g L^{-1} was prepared [11]. Yeast extract and D(+)-Glucose-monohydrate came from Merck (Darmstadt, Germany).

PCBs standards of di-, tri- tetra-, penta-, hexa-, hepta-chlorinated biphenyls (PCB28, PCB52, PCB101, PCB118, PCB138, PCB153, PCB180) (10 mg L^{-1} in acetone) and 2, 4, 5, 6 tetrachlorometaxylene (TCMX) were provided by Cluzeau Info Labo (200 mg L^{-1} in acetone) (Sainte-Foy La Grande, France).

Extraction solvent was *n*-hexane suprasolv Merck (Darmstadt, Germany). Clean-up was performed using 1g SPE Florisil cartridges from Supelco (Bellefonte, USA). Samples filtrations were made thanks to 11 μm-diameter filter Whatman purchased from Bioblock France (Illkrich, France).

2.1.2 *Fungus strain*

Phanerochaete chrysosporium was provided by the Laboratory of Cryptogamy from the National Museum of Natural History (Paris, France), under LCP 51 995 nomenclature. Malt extract was provided by Merck (Darmstadt, Germany). *Phanerochaete chrysosporium* was grown in a liquid malt medium (malt extract 3%) at 35°C on rotary shaker. Pellets were divided in 8 parts to provide inoculii.

2.2 *Methods*

2.2.1 *Bioremediation assay*

500 mL of liquid mineral medium were placed in 1 L glass Erlenmeyer and sterilised by autoclaving at 120°C, under 1 bar during 25 minutes. D(+)-Glucose-monohydrate (2 g L^{-1}), yeast extract (0.5 g L^{-1}) and PCBs (10 μg L^{-1} for each of the seven congeners) were sterilely added. Amended liquid mineral medium was inoculated with *Phanerochaete chrysosporium* as described previously. 100 mL of liquid mineral medium were taken at $t = 0$ day. Cultures were placed at room temperature under magnetic stirring for 7 days. Experiments were performed in triplicate.

2.2.2 *Samples preparation and PCBs extraction*

After reactions, the remaining media were filtrated through filter Whatman (pore size 11 μm) to separate the fungus from the aqueous phase.

Aqueous samples were extracted by liquid–liquid extraction with *n*-hexane (ratio sample : solvent = 2.5 : 1). A 10-minute period of manual stirring was repeated three times. Both of organic and mineral phases were collected in the same 1 L-glass vessel and were sonicated during 5 minutes. Organic phase was dried by freezing and concentrated with rotary evaporation then under nitrogen flow to a final volume of 1 mL. The fungal organic matter was dried overnight at room temperature. Dry mycelia were separated from filters

to be weighted. Both of mycelia and filters undergo a solvent extraction with *n*-hexane. A 10-minute period of manual stirring was repeated three times. The organic phase was sonicated during 5 minutes. Both of residual mycelia and organic phase was frozen to separate them.

All samples were purified through a Superclean LC-Florisil SPE tube conditioned by 10 mL of *n*-hexane [12]. The extract was deposed in column head. The elution was made with 8 mL of *n*-hexane at 5 mL min^{-1} rate. The elution product was concentrated under nitrogen flow to a final concentration of 1 mL. Fifty μL of TCMX solution (10 μg L^{-1} in *n*-hexane) were added as internal standard before analysis.

2.2.3 *PCB analysis*

PCBs extracts were analysed by high resolution gas chromatography coupled with low resolution mass spectrometry (HRGC-LRMS) on electron impact mode. It is the most widely used technique for these compounds [13–17]. Ionisation by this technique is the most sensitive and reproducible. The apparatus was a Thermo-Finnigan Trace 2000 series coupled with a Thermo-Finnigan Trace MS with a quadrupole type analyser, entirely computer-controlled with data acquisition and processing using XCalibur software. The chromatograph was fitted with a Restek RTX-5MS (5% diphenyl; 95% dimethylpolysiloxane) column 30 metres long, 0.25 mm in diameter and with a 0.25 μm film thickness. Helium (Alpha 2, Air Liquide, France) carrier gas was used, flow rate 1.2 mL min^{-1}. A 1 μL sample was injected into the split/splitless inlet in splitless mode at 250°C. The temperature of the HRGC-LRMS interface was 250°C, and the oven temperature programme starts at 50°C for 1 minute followed by a rise of 20°C min^{-1} to 310°C, this temperature being maintained for 6 minutes. The full scan electron impact data was obtained under the following conditions: solvent delay 5 minutes, electron impact energy 70 eV, source temperature 200°C, emission current 150 μA, detector voltage 350 V.

The HRGC-LRMS temperatures ramp, injection and detection parameter are listed in Table 2. Single ion monitoring method for detection is detailed in Table 3.

3. Results and discussion

3.1 *PCB analysis and degradation rate*

The limit of detection (LOD) (3 standard deviations) for each PCBs was 13 ng L^{-1} for final aqueous samples and 150 ng L^{-1} for initial aqueous samples. LOD for organic matter was 13 ng g^{-1} dry matter. The limits of quantification (LOQ) (10 standard deviations) were 42 ng L^{-1} for final aqueous samples and 500 ng L^{-1} for initial aqueous samples. LOQ for organic matter was 42 ng g^{-1} dry matter.

The analysis of the initial aqueous sample (Figure 1a) revealed a total amount of 14.34 ± 3.45 mg PCBs. After 7 days, no PCBs were longer detectable in the aqueous sample (Figure 1c) whereas 6.95 ± 2.81 mg were quantified onto organic matter (Figure 1b). Figure 2 presents the percentage of every fraction for each PCB. It shows that our experiment does not induce selectivity toward any PCB. We can assume that the adsorption process is not influenced by the numbers of chlorine atoms.

After 7 days, the fungus has been weighted at 400 ± 115 mg of dry matter. The specific sorption rate of total PCB average 17,375 mg g^{-1} DM. The average of specific sorption rate per day was 248 mg g^{-1} d^{-1}.

Table 2. HRGC-LRMS analysis parameters.

Injection parameters	
Temperature	250°C
Volume	1 µL
Mode	Splitless 1min then split 50 mL min^{-1}
GC parameters	
Gas flow	1.2 mL min^{-1}
Interface temperature	250°C
Temperature ramp	Initial step: 50°C – 5 min Ramp 1: 16°C min^{-1} – 230°C Ramp 2: 5°C min^{-1} – 280°C Final step: 280°C – 1 min
Detection parameters	
Delay	10.5 min
Source temperature	200 C
Emission current	150 µA
Detection voltage	350 V
Sweep speed	4 m s^{-1}
Electrons impact	70 eV

Table 3. MS detection method.

	Time (min)	Compound	Characteristic mass (m/z)
1	10.50–12.5	TCMX	136; 171; 244
2	12.50–14.5	PCB 28	150; 186; 258; 292
		PCB 52	
4	14.5–15.65	PCB 101	184; 254; 326
5	15.65–18.0	PCB 118	184; 218; 254; 290; 326; 360
		PCB 138	
		PCB 153	
6	18.0–20.0	PCB 180	162; 252; 324; 394

3.2 *Fractioning assessment*

A LOQ of 42 ng L^{-1} was taken for the determination of PCBs amount in final aqueous sample. Degraded PCBs were deduced according to this calculation:

$$\text{Degraded PCBs} = \text{Initial PCBs} - (\text{Adsorbed PCBs} + \text{Residual PCBs})$$

The results presented in Figure 3 reveal that the adsorbed PCBs fraction is the main fraction with percentage ranging between 42% and 54%. The second fraction is the degraded one with percentage ranging from 39% to 49%. The residual fraction represents only 5% to 9% of initial PCBs content. These results indicate that despite the well-known ability of *Phanerochaete chrysosporium* to degrade organic compounds by extra cellular oxidation, a large part of them can just be adsorbed onto fungus mycelium. This fact is often overlooked in the literature by extracting both water and fungus.

These results are consistent with former studies. Ruiz-Aguilar *et al.* [18] show degradation rate ranging from 34% to 73% for 10 days' incubation, on high concentration

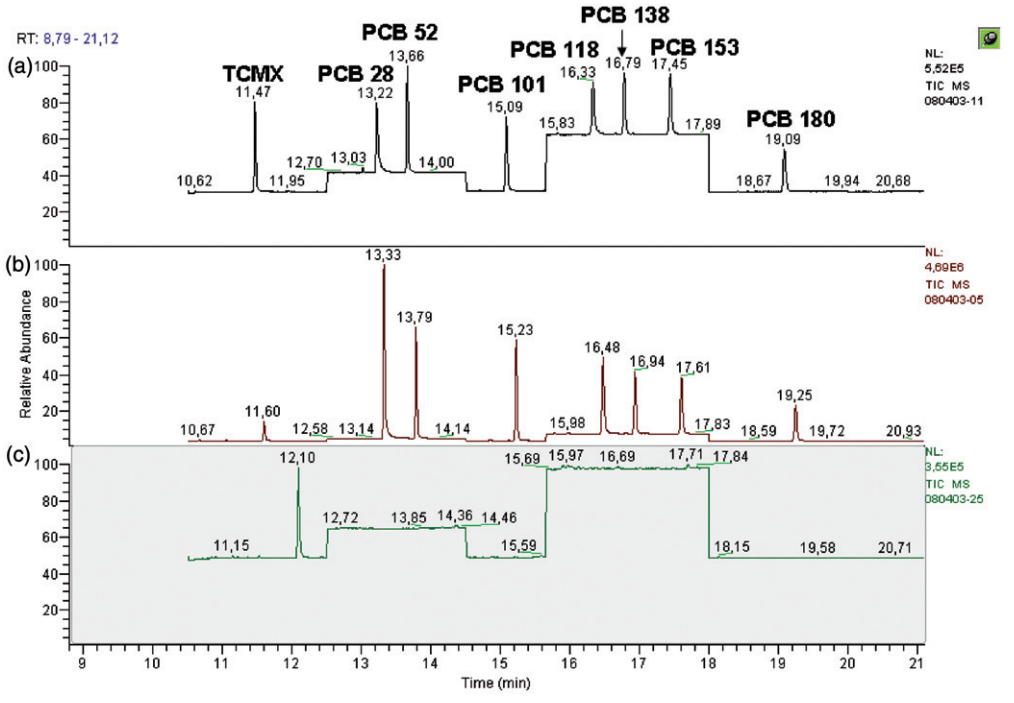


Figure 1. Chromatograms from (a) initial aqueous sample, (b) final organic matter sample, and (c) final aqueous sample.

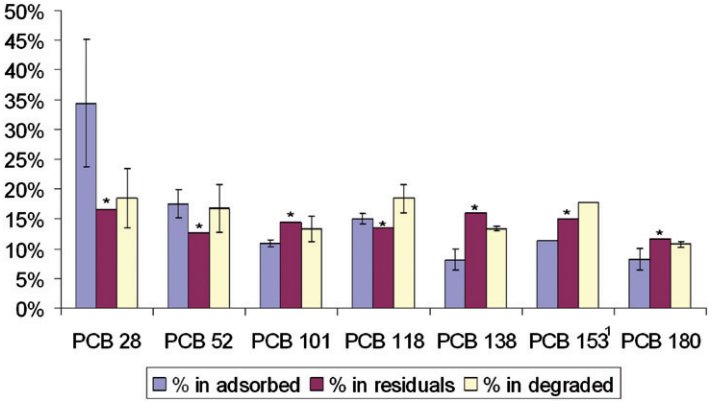


Figure 2. Individual PCB fractioning in analysed and calculated compartments.
Note: *Estimated with LOD.

of soil extracted PCBs (from 600 mg L^{-1} to 3000 mg L^{-1}). Nevertheless, no estimation of adsorbed fraction is made. Solvent from fungal mycelium washing phase is mixed with solvent from water extraction to form the residual fraction. Kamei *et al.* [19] found a degradation rate about 50% for 14 days on low concentration of 4,4′-dichlorobiphenyl (about 5 mg L^{-1}). Fungal mycelium was separated from liquid by centrifugation but

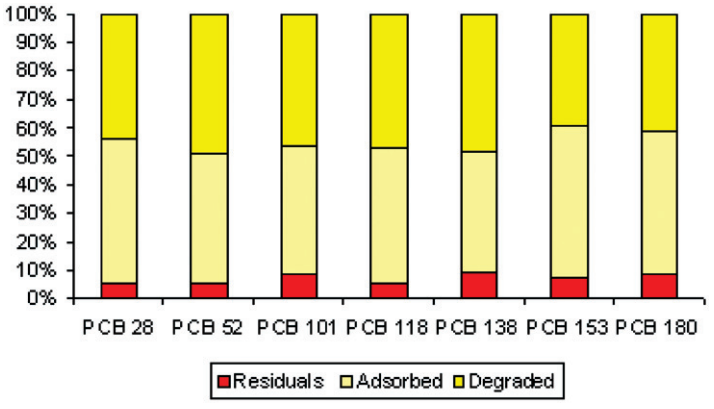


Figure 3. PCBs fractioning results.

organic matter and aqueous phase are extracted with the same solvent. No information on adsorption ability from *Phanerochaete chrysosporium* is shown. In an industrial process, residual and adsorbed fractions have to be handled. The sorption capacity can be used to quickly remove PCBs from water as the degradation ability could be used in long-term bio treatments. *Phanerochaete chrysosporium* shows efficiency and wide range of degradation abilities.

These results confirm that *Phanerochaete chrysosporium* can degrade highly chlorinated PCBs. De *et al.* [9] reported that the nitrate reductase gene from *Phanerochaete chrysosporium* could be involve in dechlorination of PCB 153 under non-ligninolytic condition (nitrogen-rich condition) which is the case in our study.

Besides, such calculations are useful to understand extra cellular degradation of PCBs by *Phanerochaete chrysosporium*. This phenomenon has to be explored to understand the degradation rate limits. Are adsorbed PCBs available to *Phanerochaete chrysosporium* extra cellular enzymes?

4. Conclusion

This study has assessed the bioremediation of PCBs in an aqueous assay with *Phanerochaete chrysosporium*. Truly degraded PCBs, adsorbed PCBs and residual PCBs have been monitored. This work underlines the fact that fungus based bioremediation in aqueous media involve two phenomena (i) biofiltration; (ii) biodegradation. After 7 days of process, an important fraction of PCBs was adsorbed onto organic matter. Further studies are conducted to check if a longer exposition would induce better degraded/adsorbed PCBs ratio. In the case that PCBs are not degraded, an additional process using this fungus must be added to remove the adsorbed PCBs.

Acknowledgements

This work is supported by Agence National de la Recherche Technologique with Grant No. 46/2007 and by OSEO with Grant No. A0703011. We would like to thank Ecole National de Formation Agronomique for their technical support.

References

[1] J.S. Waid, *PCBs and the Environment* (CRC Press, Boca Raton, FA, USA, 1986).
[2] J. Albaigés, Ed. *Environmental Analytical Chemistry of PCBs* (Gordon & Breach, Reading, UK, 1993).
[3] French Environment and Energy Management Agency, ADEME editions no. 1799, 223 (1996).
[4] K. Jones and J. Stevens, *Organic Contaminants in Sewage Sludge Applied to Agricultural Land* (Water Industry Research Ltd. 81, London, UK, 2002).
[5] P. Whylie, J. Albaigés, R. Barra, H. Bouwman, P. Dyke, F. Wania, and M. Wong, *Global Assessment of Persistent Toxic Substances* (United Nations Environment Programme, Geneva, Switzerland, 2003).
[6] M. Babut and C. Miege, *Contamination des poissons et des sediments du Rhône par les Polychlorobiphényles – Synthèses des données disponibles* (CEMAGREF, Lyon, France, 2007).
[7] L. Rehmann and A.J. Daugulis, Biochem. Eng. J. **38**, 219 (2008).
[8] W.-R. Abraham, B. Nogales, P.N. Golyshin, D.H Pieper, and K.N Timmis, Curr. Opin. Microbiol **5**, 246 (2002).
[9] S. De, M. Perkins, and S.K. Dutta, J. Hazard. Mater. B **135**, 350 (2006).
[10] G.V. Bhasker Reddy and M.H. Gold, Microbiol **146**, 405 (2000).
[11] D.L. Bedard, R. Unterman, L.H. Bopp, M.J. Brennan, M.L. Haberl, and C. Johnson, Appl. Env. Microbiol **51**, 761 (1986).
[12] C. Sablayrolles, Ph.D. thesis, Institut National Plytechnique, 2004.
[13] G.A. O'Connor, D. Kiehl, G.A. Eiceman, and J.A. Ryan, J. Environ. Qual. **19**, 113 (1990).
[14] J.F. Müller, D.W. Hawker, M.S. McLachlan, and D.W. Connell, Chemosphere **43**, 507 (2001).
[15] E. Eljarrat, J. Caixach, J. Rivera, M. de Torres, and A. Ginebreda, Env. Sci. Technol. **35**, 3589 (2000).
[16] C. Bandh, E. Björklund, L. Mathiasson, C. Näf, and Y. Zebühr, Env. Sci. Technol. **34**, 4995 (2000).
[17] P. Kömp and M.S. Lachlan, Sci. Total Env. **63**, 250 (2000).
[18] G.M.L. Ruiz-Aguilar, J.M. Fernandez-Sanchez, R. Rodriguez-Vazquez, and H. Poggi-Varaldo, Adv. Env. Res. **6**, 559 (2002).
[19] I. Kamei, R. Kogura, and R. Kondo, Appl. Microbiol. Biotechnol. **72**, 566 (2006).