Degradation Capacity of a 1,2,4-Trichlorobenzene Mineralizing Microbial Community for Traces of Organochlorine Pesticides

Article *in* Science of The Total Environment · July 2010

DOI: 10.1016/j.scitotenv.2010.04.009 · Source: PubMed

CITATIONS
13

READS
76

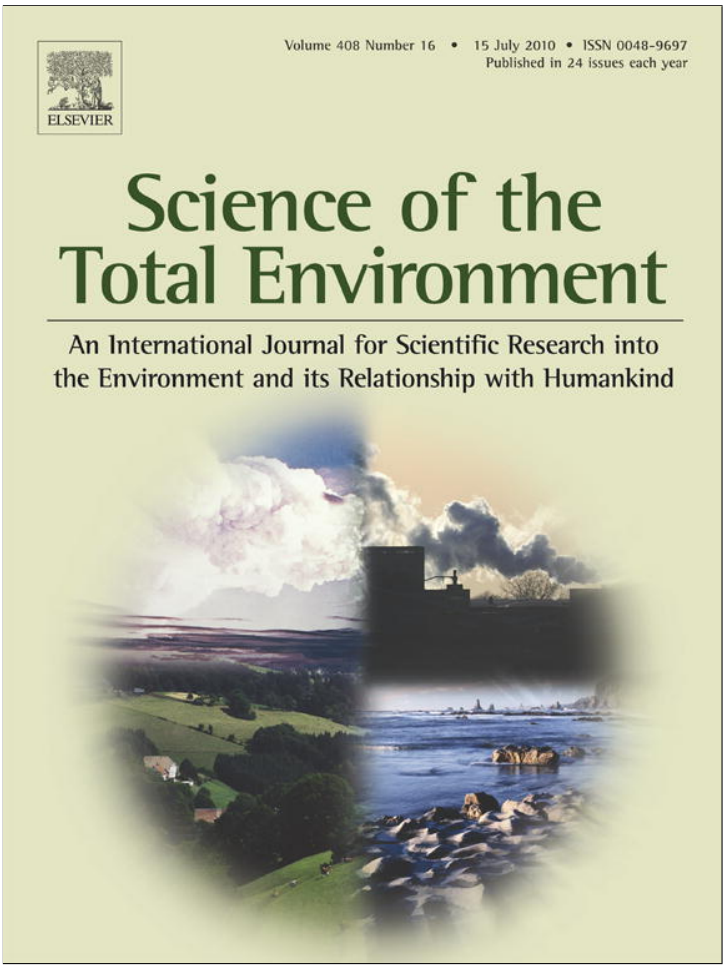
9 authors, including:

Fredrick Orori Kengara
Maseno University
52 PUBLICATIONS **653** CITATIONS
SEE PROFILE

Jean Charles Munch
Technische Universität München
362 PUBLICATIONS **10,621** CITATIONS
SEE PROFILE

Gerhard Welzl
126 PUBLICATIONS **2,375** CITATIONS
SEE PROFILE

Reiner Schroll
Helmholtz Zentrum München
96 PUBLICATIONS **1,599** CITATIONS
SEE PROFILE



Science of the Total Environment 408 (2010) 3359–3366

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Degradation capacity of a 1,2,4-trichlorobenzene mineralizing microbial community for traces of organochlorine pesticides

Fredrick Orori Kengara [a,e], Karl-Werner Schramm [b], Ulrike Doerfler [a], Jean Charles Munch [a], Bernhard Henkelmann [b], Gerhard Welzl [c], Silke Bernhoeft [b], Burkhard Hense [d], Reiner Schroll [a,*]

[a] Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Soil Ecology, 85764 Neuherberg, Germany
[b] Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Ecological Chemistry, 85764 Neuherberg, Germany
[c] Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Developmental Genetics, 85764 Neuherberg, Germany
[d] Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Biomathematics, 85764 Neuherberg, Germany
[e] Department of Chemistry, Maseno University, Private Bag Maseno, Kenya

ARTICLE INFO

Article history:
Received 21 September 2009
Received in revised form 25 March 2010
Accepted 7 April 2010
Available online 1 May 2010

Keywords:
OCPs
1,2,4-TCB
Persistence
Microbial degradation
HRGC-HRMS
PRC

ABSTRACT

A soil-borne microbial community isolated from a contaminated site was previously shown to mineralize 1,2,4-trichlorobenzene (1,2,4-TCB) under aerobic conditions. The key degrader in this community was identified as *Bordetella* sp. F2. The objective of the study was to test the capacity of the microbial community to degrade a complex mixture of 27 organochlorine compounds and pesticides (OCPs) commonly detected in the environment. The hypothesis was that the microbes would utilize the OCPs as carbon sources at the low concentrations of these compounds, found in natural waters and soil solution. The study was carried out in liquid culture and the changes in concentration of the OCPs were monitored using GC-MS. Data analysis was done using a multivariate analysis method similar to Principal Response Curve (PRC) analysis. Contrary to expectations, the data analysis showed a general trend where higher concentrations were observed in the microbially treated samples relative to the controls. The observed trend was attributed to decreased volatilization due to sorption of the chemicals by microbes since most of the compounds in the cocktail had high Kow values. Nevertheless, when using adequate statistical methods for analysing the very complex data set, correlation of Kow and K_H values with the loadings of the PRCs showed that three chlorinated mono-aromatics — pentachlorobenzene, pentachloroanisole and octachloroanisole — were amenable to degradation. This provided indications that the community could hold promise for the degradation of higher-chlorinated mono-aromatic OCPs.

1. Introduction

Organochlorine compounds are important ubiquitous environmental xenobiotics (Bignert et al., 1998). The fact that these compounds are highly persistent has led to their accumulation in various environmental compartments (Aislabie et al., 1997). They can be transported in the atmosphere to areas where they have never been used (Wania and Mackay, 1993). This transport is usually from the warm zones (tropics) to the cold ones (temperate) — therefore, organochlorine residues have been detected in the snowy Arctic and in regions of high mountains (Su et al., 2008; Shen et al., 2009).

The recalcitrant and ubiquitous nature of these compounds destines them to be a cause of environmental concern, and measures are being taken to restrict their use and release into the environment (UNEP, 2009). The ratification of the Stockholm Convention on persistent organic plollutants (POPs) underscores the global nature of this concern (Stockholm convention, 2009). The convention targeted 12 priority POPs, seven of which are included in this study, for restriction or complete phasing out. Even then, the compounds continue to be released into the environment as industrial wastes and from their use as pesticides in agriculture or vector control (UNEP, 2009).

It is therefore of great public concern to develop cheap yet effective and sustainable methods for enhancing the breakdown of organochlorine residues and their subsequent elimination from the environment. Various approaches have been tried to overcome the limitations afforded by the recalcitrant organochlorine compounds and pesticides (OCPs) (Miguel et al., 1993): catalysis, surfactants, hydrogenation etc. An increasingly utilized strategy has been the use of bioremediation (Iwamoto and Nasu, 2001; Singh, 2007). The lack of a proper indigenous population of microbial degraders can be overcome by inoculating foreign microorganisms into the system (Edgehill and Finn, 1983). This strategy, usually referred to as "bioaugmentation," is based on the inoculation of a pollutant-degrading microbial strain or a microbial

* Corresponding author. Tel.: +49 89 3187 3319; fax: +49 89 3187 3376.
E-mail address: schroll@helmholtz-muenchen.de (R. Schroll).

0048-9697/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2010.04.009

consortium into the contaminated system (Singh, 2007; Wang et al., 2010).

In spite of the ability of the diverse bacteria in the environment to degrade many pollutants, a variety of chemical structures of certain pollutants, especially some xenobiotics, are beyond bacterial biodegradation capabilities (Cao et al., 2009). This is especially the case where single bacterial strains are used for remediation (Diaz, 2004). This has led to the engineering of recombinant bacteria with desirable bioremediation properties (Stephenson and Warnes, 1996; Diaz, 2004). However, this approach is highly controversial because of biosafety issues associated with the release of such strains into the environment (Diaz, 2004). A less controversial strategy has been to develop syntrophic bacterial consortia whose members are specialized in certain catabolic steps or in the biodegradation of certain pollutants in complex pollutant mixtures, such as the commonly encountered benzene, toluene, ethylbenzene and xylene (BTEX) mixture (Diaz, 2004; Cao et al., 2009). Research work regarding such bacterial consortia is still in its infancy (Cao et al., 2009).

This study was designed to add to this nascent field. We sought to determine whether a specific microbial community could degrade a mixture of 27 environmentally relevant OCPs at ambient concentrations found in environmental waters and soil solutions. As a first step the study was carried out in a pure culture medium.

The microbial consortium used in this study was enriched from an organochlorines-contaminated site. In former experiments it was assessed that this community was able to mineralize 1,2,4-TCB very effectively (45% of the applied radioactivity after two weeks) at a high concentration of 15 ppm. *Bordetella* sp. F2 was identified as the key degrader (Wang et al., 2007). Few reports exist in literature on degradation of compounds by the genera Bordetella. *Bordetella petrii* has been shown to degrade naphthalene and toluene (Bianchi et al., 2005) while *Bordetella hinzii* has been shown to grow in PAH cultures (Eriksson et al., 2003).

The use of a microbial community in this study, rather than the pure *Bordetella* sp. F2, was designed to provide a microbial consortia to overcome the limitations of single strains in remediation, and the complexities involved in developing syntrophic bacterial consortia (Diaz, 2004; Cao et al., 2009). The use of a cocktail of pollutants was motivated by the fact that polluted sites are usually contaminated with a mixture of the selected OCPs (Miguel et al., 1993; Singh, 2007; Shen et al., 2009; Su et al., 2008; Wang et al., 2009) and also because it was not known before hand which of the compounds, if any, could be degraded by the microbes. The hypothesis was that given the diverse nature of compounds degraded by the species of the *Bordetella* family (Bianchi et al., 2005; Eriksson et al., 2003; Wang et al., 2007), the microbial community would have the capability to utilize compounds in the complex mixture as carbon sources hence degrading them. The success of this approach would offer a safer bioremediation option relative to the controversial recombinant strains (Diaz, 2004; Cao et al., 2009).

To the best of our knowledge, this is the first time that a study testing the capacity of a community — with a species of the *Bordetella* family as the key degrader — to degrade such a complex OCPs mixture, has been carried out.

2. Materials and methods

2.1. Chemicals

Uniformly ^{14}C-ring-labeled 1,2,4-TCB was obtained from International Isotopes (Munich, Germany). Non labeled 1,2,4-TCB and other non-labeled OCPs used in the cocktail (see Table 1) were purchased from Dr. Ehrenstorfer Laboratories (Augsburg, Germany). ^{13}C labeled internal standards were obtained from Cambridge Isotope Laboratories (Woburn, MA, USA). Scintillation cocktails were purchased from

Table 1
List of OCPs in the cocktail and some physical-chemical properties.

Compound	Molecular formula	MW	Solubility mg/L	Log Kow	*K_H Pa m^3 mol^{-1}
Alpha-HCH (αHCH)	$C_6H_6Cl_6$	290.85	10[c] (28 °C)	3.80[c]	0.695[c] (20 °C)
beta-HCH (β-HCH)	$C_6H_6Cl_6$	290.85	5[c] (20 °C)	3.78[c]	0.046[c] (20 °C)
Gamma-HCH (γ-HCH)	$C_6H_6Cl_6$	290.85	17[c] (20 °C)	3.71[a]	0.355[c] (20 °C)
delta-HCH (δ-HCH)	$C_6H_6Cl_6$	290.85	10[c] (20 °C)	4.14[c]	0.021[c] (20 °C)
epsilon-HCH (ε-HCH)	$C_6H_6Cl_6$	290.85			
Pentachlorobenzene (PCB)	C_6HCl_5	250.34	0.68[g] (25 °C)	5.19[g]	72.00[g] (25 °C)
Pentachloroanisole (PCA)	$C_7H_3Cl_5O$	280.36	0.2[d] (20 °C)	5.66[d]	196.57[d] (20 °C)
Octachlorostyrene (OCS)	C_8Cl_8	379.7	0.00174[b] (20 °C)	6.29[d]	23.30[d] (20 °C)
Hexachlorobenzene (HCB)	C_6Cl_6	284.8	0.006[c] (25 °C)	5.14[a]	58.77[c] (20 °C)
p,p'-DDT	$C_{14}H_9Cl_5$	354.48	0.003[b] (25 °C)	6.50[a]	0.841[c] (25 °C)
o,p'-DDT	$C_{14}H_9Cl_5$	354.48	0.085[c] (25 °C)	6.79[c]	0.060[c] (25 °C)
p,p'-DDD	$C_{14}H_{10}Cl_4$	320.1	0.05[b] (25 °C)	6.02[a]	2.128[c] (25 °C)
o,p'-DDD	$C_{14}H_{10}Cl_4$	320.1	0.10[c] (25 °C)	5.87[c]	1.824[c] (25 °C)
p,p'-DDE	$C_{14}H_8Cl_4$	318.1	0.065[b] (24 °C)	5.50[a]	0.405[c] (25 °C)
o,p'-DDE	$C_{14}H_8Cl_4$	318.1	0.0013[b] (20 °C)	6.00[c]	0.828[c] (25 °C)
trans-Chlordane (trans-CHL)	$C_{10}H_6Cl_8$	409.8	0.014[b] (25 °C)	6.07[a]	8.420[c] (25 °C)
cis-Chlordane (cis-CHL)	$C_{10}H_6Cl_8$	409.8	0.042[b] (20 °C)	6.11[a]	12.92[a] (25 °C)
Oxy-Chlordane (OC)	$C_{10}H_4Cl_8O$	423.8	0.023[f] (20 °C)	4.95[a]	7.48[a] (25 °C)
Heptachlor (HC)	$C_{10}H_5Cl_7$	373.3	0.05[c] (25 °C)	6.10[c]	29.79[c] (25 °C)
cis-Hexachloroepoxide (cis-HCE)	$C_{10}H_5Cl_7O$	389.4	0.275[c] (25 °C)	5.00[e]	1.665[c] (25 °C)
trans-Hexachloroepoxide (trans-HCE)	$C_{10}H_5Cl_7O$	389.4	0.275[c] (25 °C)	5.40[c]	3.242[d] (25 °C)
Aldrin	$C_{12}H_8Cl_6$	364.9	0.011[c] (20 °C)	6.50[c]	4.965[c] (20 °C)
Dieldrin	$C_{12}H_8Cl_6O$	380.9	0.14[b] (20 °C)	3.692-6,20[b]	0.527[c] (20 °C)
Endosulfan-I	$C_9H_6Cl_6O_3S_5$	406.9	0.32[b] (22 °C)	3.13[b]	1.013[c] (25 °C)
Endosulfan-II	$C_9H_6Cl_6O_3S_5$	406.9	0.33[c] (20 °C)	3.52[c]	1.935[c] (25 °C)
Methoxychlor	$C_{16}H_{15}Cl_3O_2$	345.65	0.10[b] (25 °C)	4.68-5.08[c]	1.621[c] (25 °C)
Mirex	$C_{10}Cl_{12}$	545.59	0.0000005[b] (22 °C)	5.28[c]	52.284[c] (25 °C)

* Some of the K_H values are given in M/atm in the references and have been converted to the SI unit of Pa m^3 mol^{-1} using the relation 1 Pa m^3 mol^{-1} = 9.8692 × 10^{-6} M/atm.

[a] Paasivirta et al. (1999).
[b] ARS - Agricultural Research Service (2009).
[c] ATSDR - Agency for Toxic Substances and Disease Registry (2009).
[d] HSDB - Hazardous Substances Data Bank (2009).
[e] Mackay et al. (1997).
[f] PPDB - Pesticide properties database (2009).
[g] Shen et al. (2005).

Packard (Dreieich, Germany). Alumina B super 1 and Silica gel 60 were obtained from LGC Standards (Wesel, Germany), and Isolute HM-N (diatomaceous earth) from Separtis (Grenzach-Wyhlen, Germany). All other chemicals and solvents were of analytical grade and were purchased from Sigma-Aldrich (Germany).

2.2. Degrading bacterial community

The soil-borne degrading bacterial community, used in this study, was enriched from a site in Hungary which had been polluted with chlorobenzenes for 25 years (Schroll et al., 2004). From this community, *Bordetella* sp. F2 was isolated and identified as the key degrading organism able to use 1,2,4-TCB as the sole energy and C source (Wang et al., 2007). The phylogenetic description of the enriched microbial community is given in Wang et al. (2010). The active degrading bacterial community which was stored at −80 °C, was thawed and after thawing, its 1,2,4-TCB mineralization ability was confirmed by biomineralization experiments.

2.3. Biomineralization experiments

Besides the confirmation of the mineralizing capacity, the biodegradation experiments served as a measure to find out whether the microbial community could remain active over the entire 30 weeks study period, without changing the liquid culture media. The microbial community (1.71×10^6 CFU mL^{-1}) was spiked into 50 mL of liquid media (see section 2.4) in special incubation flasks (Brahushi et al., 2004). The starting concentration of microbial cells was similar to that in the cocktail culture (1.23×10^6 CFU mL^{-1}). ^{14}C-1,2,4-TCB was mixed with non-labeled 1,2,4-TCB to give a specific radioactivity of 6.66 Bq μg^{-1}. 25 µL of the standard solution, corresponding to 5 kBq was applied to the liquid media which resulted in a 1,2,4-TCB-concentration of 15 µg mL^{-1} in the liquid culture. After inoculation and application the samples were incubated on a shaker (120 rpm) in the dark at 20 ± 1 °C (Wang et al., 2007). Filters (0.20 µm, Sartorius, Göttingen, Germany) were installed at the air inlet and outlet of the flasks. The flasks were connected to a closed laboratory trapping system and aerated twice per week for 1 h at an air exchange rate of 1 L h^{-1} to trap $^{14}CO_2$ and volatile ^{14}C-substances separately. The trapping system and sampling of the trapping solutions are described in Schroll et al. (2004). In order to keep the 1,2,4-TCB concentration in the liquid culture nearly constant, 25 µL ^{14}C-1,2,4-TCB was reapplied after each aeration (Wang et al., 2007). All radioactivity measurements were done in a liquid scintillation counter (Tricarb 1900 TR, Packard, Dreieich, Germany).

2.4. OCPs degradation experiment: design, sampling and cell counting

Microbial degradation experiments were performed in a mineral medium (van der Meer et al., 1987) which contained 2.9 g $Na_2HPO_4\cdot 2H_2O$, 1.5 g KH_2PO_4, 1 g NH_4NO_3, 100 mg $MgSO_4\cdot 7H_2O$, 50 mg $Ca(NO_3)_2\cdot 4H_2O$ and 1 mL trace metal solution (Zehnder et al., 1980) per liter of double distilled water. 100 mL of the autoclaved mineral medium was put into each of 10 smooth-necked 250 mL autoclaved conical flasks, under sterile conditions under a flow bank. 100 µL of the OCPs cocktail which contained 10,000 ng mL^{-1} of each compound, was added into the 10 flasks, resulting in a concentration of 10 ng mL^{-1} medium for each compound. 0.5 mL of the microbial consortium was spiked into 5 of these incubation flasks to give a starting microbial concentration of 1.23×10^6 CFU mL^{-1}. The other 5 flasks served as controls. The flasks were stoppered with autoclaved corks that allow air flow into the flasks while maintaining sterile conditions. The flasks were shaken for 1 h on an orbital shaker (120 rpm) to mix the OCPs and the mineral media. The first sampling was then done and thereafter the flasks were returned on the shaker (120 rpm) and shaken continuously in the dark at 20 ± 1 °C. Subsequent sampling was conducted on days 2, 5, 8, 16, 32, 64, 97, 164 and 186 during the first experimental phase.

On day 186 the cocktail was reapplied to the liquid media. The microbial culture (1.71×10^6 CFU mL^{-1}) was also respiked into the five microbial flasks. This marked the beginning of the second phase of the experiment which lasted for four weeks. Apart from confirming the results of the first phase, the second phase experiment was also supposed to establish whether the trend witnessed at the end of the first phase would continue. Like in the first phase, the first sampling was done after 1 h of shaking. Subsequent samplings were done after one week intervals.

Sampling was always done under sterile conditions under a flow bank. 1 mL was sampled for ultimate GC–MS analysis of OCPs while 100 µL was sampled for determination of colony forming units (CFU). Cell counting was performed by spreading serial dilutions of the liquid culture on Nutrient Broth (Sigma-Aldrich, Taufkirchen, Germany) agar plates and incubating them at 30 °C. CFU were determined after 48 h. Cell counting was done for the microbially treated samples at all the sampling points and on days 32, 64, 97, 164, 186 and 216 in the controls to check for sterility.

2.5. Extraction of OCPs, clean-up, analysis and quantification

10 µL of ^{13}C-OCP internal standard was added to 10 g of Isolute HM-N (diatomaceous earth) in 250 mm long columns with an inner diameter of 24 mm, followed by 1 mL of the liquid culture sample. The columns were allowed to stand undisturbed for 1 h and then extracted with 50 mL of acetone:hexane (1:3) at a flow rate of about 0.1 mL s^{-1}. The extract was rotary evaporated at 50 °C and a rotation speed of 100 rpm, to a final volume of about 1 mL.

To remove interferences, the concentrated crude extracts were cleaned-up by column chromatography. The solvents used were of residue quality. The glass columns were 250 mm long with an inner diameter of 24 mm. Elution flow rates were about 0.1 mL s^{-1}. The clean-up column stationary phase consisted of 5 g silica gel, 2.5 g alumina (basic, super active) + 3% H_2O, followed by topping with Na_2SO_4. The silica gel, alumina and Na_2SO_4 had been pre-heated at 600 °C for 24 h. The columns were conditioned with 30 mL hexane: dichloromethane (1:1). The crude extract was introduced into the column and eluted with 60 mL hexane:dichloromethane (1:1). The cleaned extract was rotary evaporated at 50 °C and a rotation speed of 100 rpm, to a final volume of about 1 mL. Then it was put into brown vials (placed in a Barkey evaporator at 30 °C) to which ^{13}C internal standards of the 27 compounds had been added to establish the method efficiency, and evaporated to 50 µL under a gentle flow of nitrogen. The vials were sealed with caps having Teflon septa. The sealed vials were stored at −20 °C for subsequent high resolution gas chromatography-high resolution mass spectrometry (HRGC-HRMS) analysis. An Agilent 6890 GC coupled to a MAT 95 S (Thermo) MS was used (for details on analysis see Wang et al., 2009). The MS was operated in SIM mode and the two most intense ions of the molecular ion cluster or of an abundant fragment ion cluster were monitored for the unlabeled and labeled isomers. Identification and quantification were carried out by applying the isotope dilution method.

2.6. Data analysis

A multivariate analysis of the log transformed data centered over time was performed. Values below the detection limit were substituted for by uniformly distributed random values between zero and the minimum analyzed value. To analyze the differences in the chemical concentrations between the controls and treated samples, a derivation of the principal response curve method was used — an ordination method based on redundancy analysis (RDA), (Van den Brink and Ter Braak, 1998). Principal response curve analysis (PRC) is a multivariate technique which is suitable to investigate the effects of species (e.g. chemicals) and their changes over time (Moser et al., 2007), and is increasingly being used in data analysis (Cuppen et al., 2000; Hense

et al., 2005; Hense et al., 2008; Schramm et al., 2008). The method makes it possible to summarize the effects of all the species and to display them in a single diagram. The environmental variable is the concentration of species (chemicals) in the samples, with sampling time as the co-variable (Van den Brink and Ter Braak, 1999). The focus is on the deviation of the concentrations of the species in the treated samples from those in the controls (Moser et al., 2007). For the experimental design, this method is equivalent to a two-step procedure which involves the transformation of the data (centering with respect to sampling days) and a principal component analysis (Van den Brink and Ter Braak, 1999).

In this study, the species were the 27 chemicals in the OCPs cocktail. In contrast to the principal response curve method, the groups (controls and treatments) were not averaged. This enabled a better visibility of differences within each group. A linear combination of variables (changes in the abundance of chemical concentrations) was calculated to determine the strength of the differences in the chemical composition between the samples at each sampling date, expressed as canonical coefficients (c_{dt}) (Hense et al., 2005). The curves were derived by plotting the c_{dt}-values against time. The line at $y = 0$ represents the mean of both the controls and treatments, while the c_{dt}'s represent the deviation of the controls and treatments from this mean, for each sampling date.

The weights (loadings) of the linear combination of variables are also important for the interpretation of the results. These loadings indicate the direction and strength of the change in concentration for each chemical (Hense et al., 2005). For samples with positive c_{dt}-values, chemicals with positive loadings tend to have higher concentrations, while chemicals with negative loadings tend to have lower concentrations (Moser et al., 2007). The concentrations of chemicals (for samples with positive c_{dt}-values) increase with increasing loading and c_{dt}-values. For samples with negative c_{dt}-values, chemicals with positive loadings tend to have lower concentrations, while chemicals with negative loadings tend to have higher concentrations. The concentrations of chemicals (for samples with negative c_{dt}-values) increase with decreasing loading and c_{dt}-values (or increasing negative absolute values).

Apart from the PRC, a PRC statistic (declared variance) is given (Moser et al., 2007). Declared variance is the power of a canonical axis (component) to explain the data. It shows the contribution of the axis as a percentage of the sum of all axes. Several canonical axes (components) can be used to discriminate between the groups, but usually the first canonical axis (first component) is the most important. Lastly, the significance of the differences between the groups in the PRC curve is also reported. Significance was tested for by a Monte-Carlo permutation test (Hense et al., 2008; Moser et al., 2007).

In terms of interpretation, in a PRC of treatments and controls — with the treatments having positive c_{dt}-values and the controls having negative c_{dt}-values — chemicals with positive loadings will have higher concentrations in the treatments while chemicals with negative loadings will have higher concentrations in the controls (Hense et al., 2005; Moser et al., 2007). Chemicals with loadings of zero will either be present in equal concentrations in both controls and treatments, or will be absent from both controls and treatments. Therefore a zero loading means that these chemicals do not contribute to the differences between the controls and treatments.

3. Results

3.1. Ability of the microbial community to mineralize ^{14}C-1,2,4-TCB

The mineralization and volatilization of ^{14}C-1,2,4-TCB in liquid culture were monitored over the 30 week period the OCPs degradation experiment lasted (Fig. 1). The results show that the community could remain active over the entire study period without changing the liquid culture media. Considering the total quantity of substrate added due to continuous application twice every week, up to 20 mg (corresponding to 88% of the applied 22.5 mg ^{14}C) was mineralized. Volatilization of up to 1.82 mg (corresponding to about 8% of the applied ^{14}C) was observed.

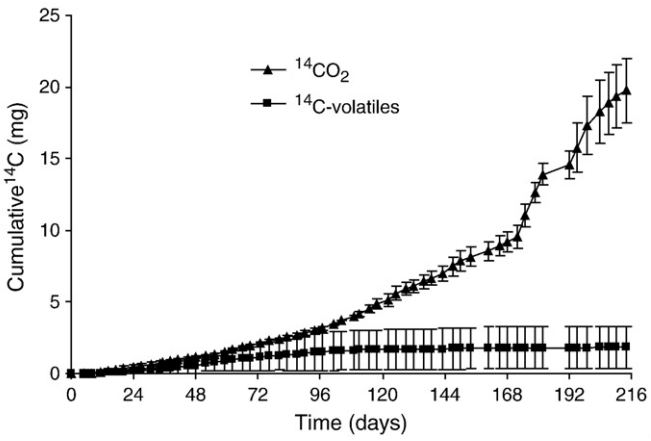


Fig. 1. Mineralization and volatilization of ^{14}C-1,2,4-TCB during the period of the OCPs degradation experiment.

3.2. Ability of the microbial community to degrade OCPs

All the data of the OCPs degradation experiment were analyzed statistically to enable an evaluation of the results.

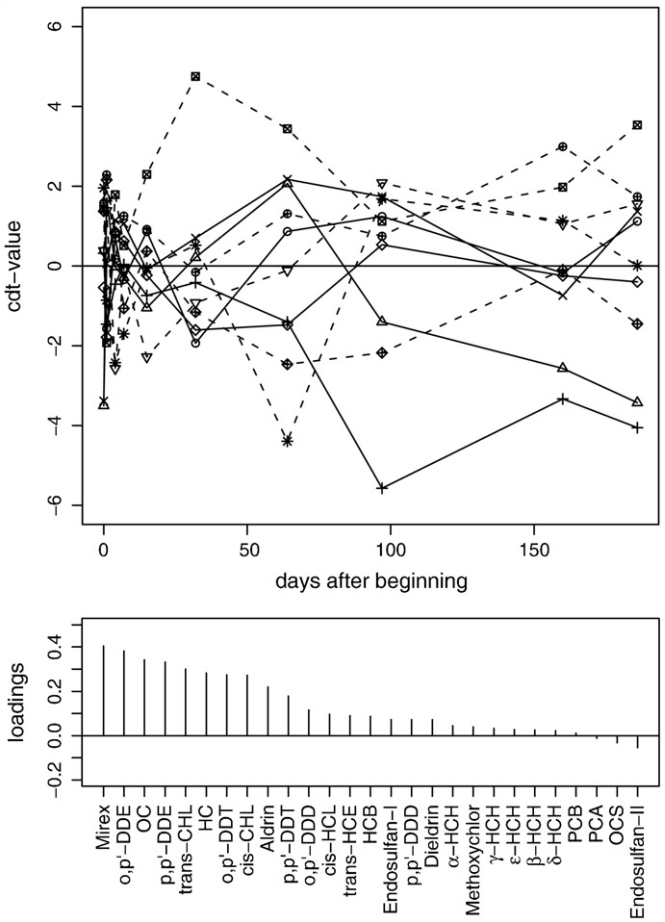


Fig. 2. Principal response curve (first component) and loadings for the OCPs during the first phase of the experiment. Continuous line = control, Dotted line = Samples treated with microbes.

Fig. 2 shows the principal response curve for the dissipation of the OCPs with time during the first phase of the experiment and the loadings for the PRC. As can be seen, there was no significant difference in response between the samples treated with microbes and the controls during the first 60 days of the experiment ($p = 0.37$). The declared variance of the first component was 36.5%, and a second component was therefore necessary. However, since the second component explained much less variance (declared variance = 13.4%), and there were still no significant differences between the groups ($p = 0.37$), the results for the second component are not shown.

However, beginning around day 60, there seemed to be a tendency towards separation between the two sets. This tendency increased with time, and was strongest on day 164 with the controls tending towards lower chemicals concentrations — but it remained a tendency at the end of this phase on day 186.

The second phase of the experiment was necessitated by two reasons: (i) to confirm the results observed during the first phase — especially the first days where no difference in response was observed, and (ii) to find out whether the tendency in response witnessed at the end of the first phase would continue. To achieve conditions similar to the initial conditions in the first phase the OCPs were respiked and the microbial community was reapplied to the liquid cultures. As can be seen from Fig. 3, there was a clear difference between the two experimental phases ($p<0.001$). The tendency that was witnessed at the end of the first phase of the experiment became a clear trend in the second part of the experiment (Figs. 2 and 3). The first component was adequate to explain the data (declared variance = 78.4%).

The loadings curves (Figs. 2 and 3) show all the 27 compounds, arranged along the x-axis in order of decreasing loading values from left to right. The *y*-axis shows the loading of each component. In the first phase, there was no clear separation between the two groups (Fig. 2), so the loadings cannot be used to interpret the c_{dt}-values. In the second phase, however, there was clear separation between the two groups (Fig. 3) and the loadings can therefore be used to interpret the c_{dt}-values. Compounds with positive loadings e.g. aldrin, heptachlor, o,p-DDE, HCB, p,p-DDE, mirex etc. were higher in the microbially treated samples (which had positive c_{dt}-values). Aldrin and heptachlor had the highest loadings, and therefore had the highest concentrations in the microbially treated samples. This means that aldrin and heptachlor contributed the most to the observed trend of higher concentrations in the treatments relative to the controls. Only PCA had a negative loading and was therefore higher in the controls (which had negative c_{dt}-values). Compounds with loadings of zero or around zero e.g. the HCH isomers, PCB and OCS, had comparable concentrations in the controls and treatments. Therefore they did not contribute to the differences between the two groups.

The second experimental phase showed a trend where the controls had lower concentrations relative to the microbially treated

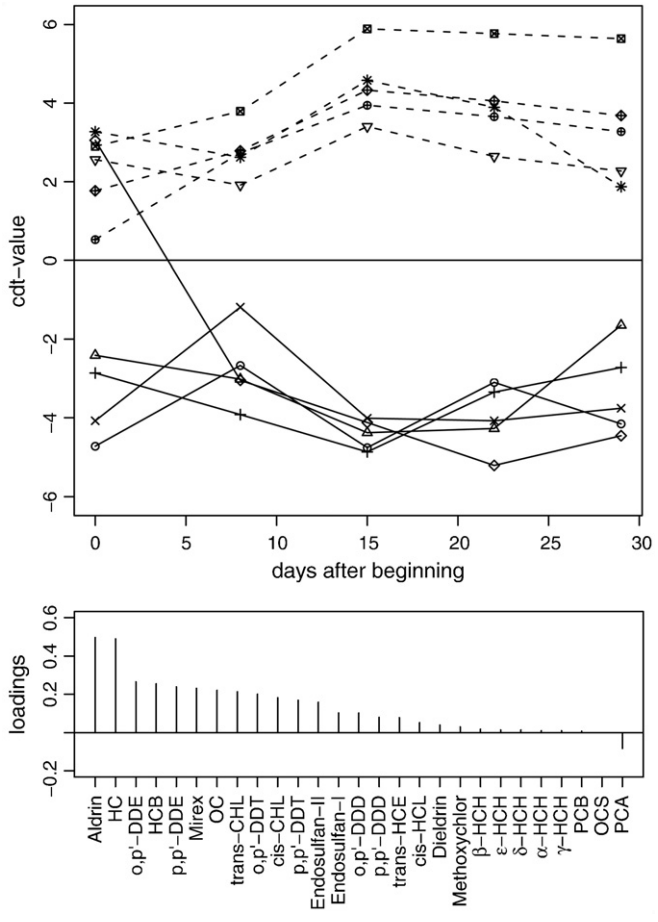


Fig. 3. Principal response curve (first component) and loadings for the OCPs during the second phase of the experiment. Continuous line = control, Dotted line = Samples treated with microbes.

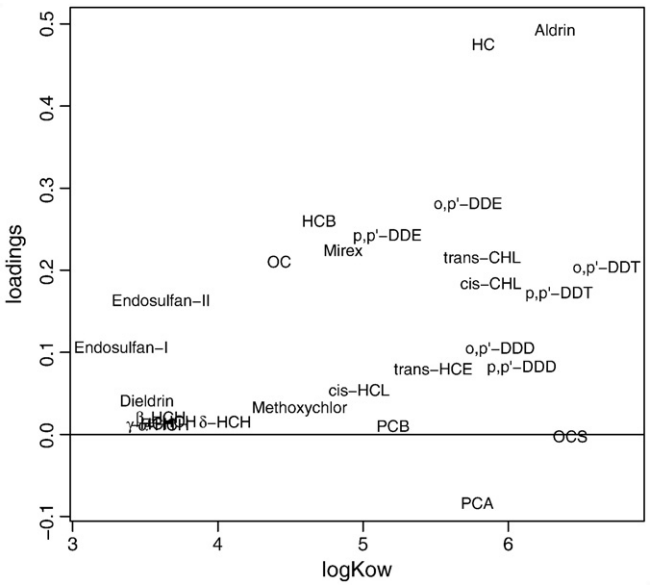


Fig. 4. Correlation of Log Kow and the loadings of the second phase of the experiment.

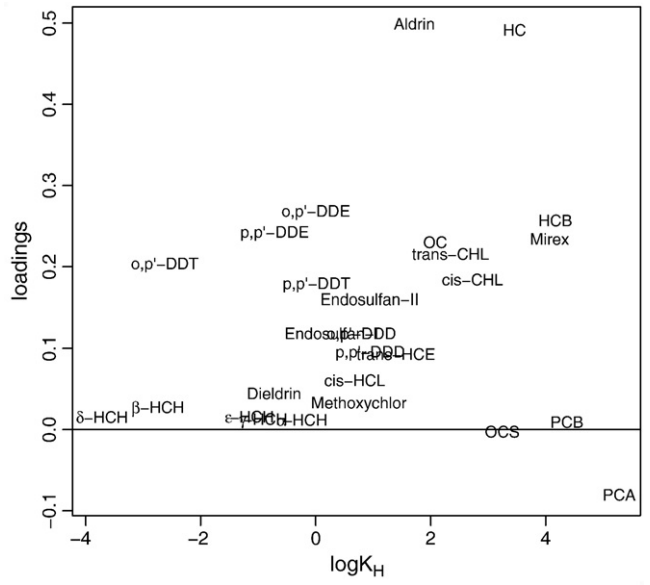


Fig. 5. Correlation of Log K_H and the loadings of the second phase of the experiment.

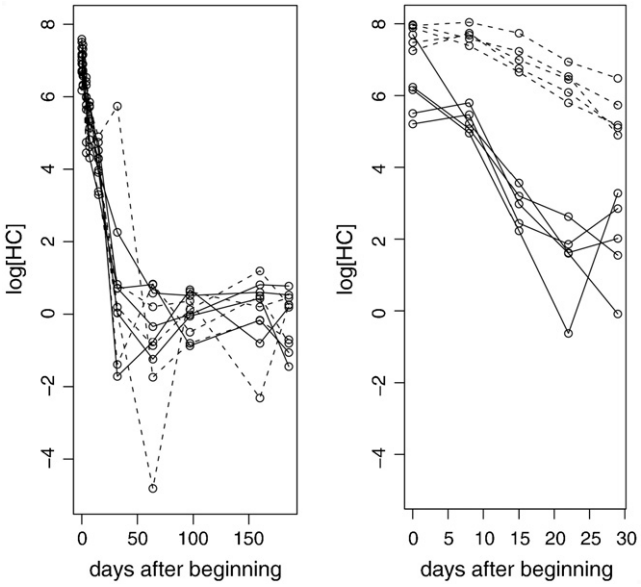


Fig. 6. Changes in concentrations of Log transformed heptachlor concentrations during the first (left curve) and second (right curve) experimental phases. Continuous line = control, dotted line = samples treated with microbes.

samples (Fig. 3). To further evaluate this result, the loadings were compared with the log Kow and log K_H of the OCPs (Figs. 4 and 5). Fig. 4 shows that there was a general increase in loadings with increasing log Kow, with aldrin and heptachlor showing the highest effect. The correlation was significant ($r = 0.44$ and $p = 0.02$). The endosulfans, with low log Kow showed higher loadings relative to other compounds with similar log Kow values. The HCHs, dieldrin and methoxychlor had low loadings consistent with their Kow values. PCB, PCA and OCS, however, had low loadings in spite of their high log Kow values. These facts can account for the lack of a higher correlation coefficient. Fig. 5 shows a similar trend as Fig. 4: increasing loadings with increasing log K_H values. However, there was no significant correlation ($r = 0.18$ and $p = 0.38$). This could indicate that there was no monotonic linear relationship for most of the chemicals. Heptachlor and aldrin again had the highest loadings, while PCB, PCA and OCS had low loadings despite their high log K_H values.

Fig. 6 shows the univariate curve of the log transformed data for heptachlor concentrations during the two experimental phases. It illustrates the general trend shown by most of the chemicals i.e. no clear differences between the controls and treated samples during the first experimental phase but clear differences during the second experimental phase, with the treatments having higher concentrations relative to the controls. This is the same trend that was observed in the PRCs (Figs. 2 and 3). At the end of the first phase, the concentrations of heptachlor and most of the other OCPs in the liquid medium were very low or below detection limit. Since heptachlor showed the same trend as that of the PRCs, it means that it contributed strongly to the overall trend of the PRC curves. Accordingly it had a high loading during both experimental phases (Figs. 2 and 3).

3.3. Growth of the microbial community

After the first application of OCPs, microbes grew rapidly to a maximum after 8 days (Fig. 7). Thereafter there was a continuous decrease to a minimum on day 186. The slight increase observed on day 64 could be an artifact because no further increase was noted on the next sampling point. After day 186 there was a respiking of the liquid culture with the OCPs. Since the microbial population had decreased considerably to levels far below the initial microbial concentration, it was necessary to add the microbial culture, to achieve microbial concentrations similar to those at the start of the first phase. As can be seen from

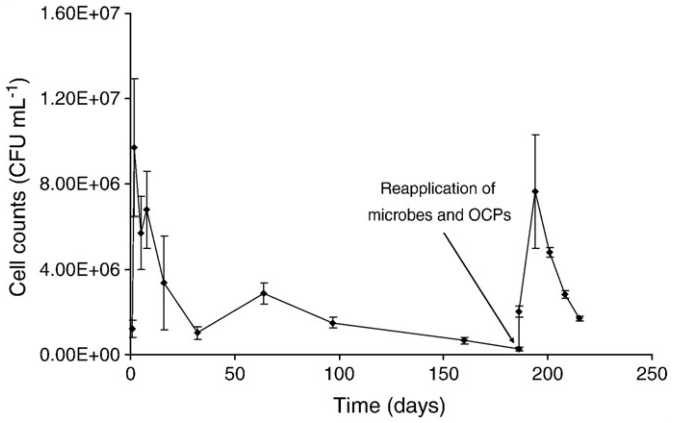


Fig. 7. Cell counts during the first and second phases of the OCPs degradation experiment.

Fig. 7, there was once again a sharp increase in population in the first days after respiking followed by a decrease in population. This is the same trend that had been witnessed at the beginning of the first spiking (Fig. 7).

There was no growth of microbes in the controls. This confirmed that sterile conditions were maintained throughout the experimental period.

4. Discussion

The ability of the used microbial consortium to aerobically degrade 1,2,4-TCB makes it a potentially important resource for the bioremediation of sites contaminated with aromatic chlorinated compounds. Wang et al. (2007) had shown that the same community could mineralize 1,2,4-TCB in liquid culture, but the experiments typically lasted two to four weeks in the same media. Our experiments were conducted with the same 1,2,4-TCB concentration used by Wang et al. (2007) of 15 μg mL^{-1} and we could show that the community remained active in the same liquid culture over the whole experimental period of 216 days.

Unlike the 1,2,4-TCB experiment, the OCPs experiment was carried out at low and realistic ambient concentrations. Though this was in part due to the solubility limitation of the compounds, the primary motivation was the desire to find out if the microbes could degrade environmentally relevant OCPs by utilizing them as the sole carbon sources at the low concentrations found in environmental waters and soil solution.

There was a decrease in concentrations of OCPs with time in both the controls and treated samples (illustrated by Fig. 6). PRC analysis showed a clear difference in response between the treatments and the controls in the second phase (Fig. 3), with the controls having lower chemicals concentrations relative to the treatments. This was contrary to the expected results based on the experimental hypothesis: that the microbes would utilize the OCPs as carbon sources and therefore lower concentrations should be measured in the treated samples relative to the controls.

Dissipation of pollutants can occur through various processes such as sorption, volatilization, biodegradation and abiotic degradation — photolysis and catalysis (Guan et al., 2009). The difference in dissipation of OCPs between the controls and treatments was mainly due to three processes: sorption by microbes, volatilization and biodegradation. Sorption is dependent on Kow, volatilization is dependent on K_H, while biodegradation depends on the availability of substrate, the capability of the microbes to degrade certain compounds and the population density of the degraders.

Compounds with high log Kow, being hydrophobic, would partition more into the microbial phase while those with high log K_H would dissipate more because of volatilization. These compounds would thus be expected to be higher in the microbially treated samples (positive

loadings), and hence contribute to the observed trend. Compounds with low log Kow, being more hydrophilic and soluble, would not be sorbed considerably by the microbial phase; and if such compounds also have low log K_H, they would not volatilize to a high extent. These compounds would thus have comparable losses via volatilization and sorption in the controls and treatments. This would result in similar concentrations in the two groups, hence loadings of zero, because they would not contribute to the differences between the two groups. Where such compounds undergo microbial degradation, it would result in lower concentrations in the treatments relative to the controls. This is the opposite of the observed trend i.e. higher concentrations in the microbially treated samples relative to the controls. Accordingly, their loadings would be negative and they would not contribute to the observed trend.

The findings in our study that the chemicals concentration in the controls was lower than in the treated samples could therefore be due to sorption and volatilization behavior of the compounds because the presence of microbes could have stemmed the tide of volatilization in the treated samples by providing sorption sites for the OCPs. This line of reasoning is commended by the high Kow values of most of the chemicals in the cocktail, which favours their partitioning into the organic (microbial) phase. This is consistent with the findings of Wang et al. (2007) who showed that in a liquid culture inoculated with the same community, the volatilization of 1,2,4-TCB decreased from 45.38% in the control to only 0.73% in the inoculated samples. Rapp and Timmis (1999) had earlier shown that 1,2,4,5-TeCB adsorbed to *Burkholderia* sp. strain PS14 in liquid culture, with a sorption coefficient (*Kp*) that was approximately 4 orders of magnitude stronger than adsorption to a soil used in the same study. Secondly, the fact that the microbes were not filtered off prior to extraction could also explain the higher concentrations noted in the treated samples. It means that the amounts assimilated and adsorbed by the microbes, though removed from the aqueous media, were nevertheless analyzed as present in the treated samples. Wang et al. (2007) showed that the concentration of ^{14}C in ^{14}C-1,2,4-TCB-spiked liquid media decreased from 14.38% to 2.36% after the microbes were filtered off.

The loadings reveal the contribution of the individual compounds to the observed trend. Based on these loadings and the properties of the compounds, it is possible to explain the influence of individual compounds to the trend and categorize the OCPs into three broad groups. Group 1 contains those compounds which have low log Kow and low K_H values relative to the other compounds in the cocktail. These compounds are therefore relatively more soluble and less volatile, and should thus be more bioavailable for microorganisms. This means that they should be degraded more relative to the other compounds in the cocktail, and therefore lower concentrations should be expected in the microbially treated samples. However, the loading values for these compounds were zero or close to zero in both experimental phases, indicating that the concentrations of these compounds were similar in the controls and in the treated sets. This means that minimal, if any, of the expected biodegradation took place. Besides a lacking degradation ability of the microbial community, the missing biodegradation could be due to (i) the low concentrations used because it has been shown that low concentrations may hamper biodegradation (Gianfreda and Rao, 2008; Johnsen et al., 2005); (ii) the use of a complex mixture because complex phenomena inhibiting the degradation of xenobiotics may arise when more polluting compounds are simultaneously present (Gianfreda and Rao, 2008).

The HCH isomers are the main compounds in group 1. Dieldrin and methoxychlor do not have the relatively high solubilities of the HCHs (Table 1). However, based on the loadings and their properties, they had little influence on the observed trend and can therefore be placed in the first group.

The second group of compounds is the major contributor to the observed trend. These have low solubility and high Kow values. Therefore these compounds volatilized considerably, with volatility increasing with increasing K_H values. The high Kow values caused partitioning of the compounds to the microbial phase hence reducing their dissipation in the treated sets relative to the controls. Most of the compounds fall into this category (Fig. 2, 3, 4 and 5), with the main contributors being heptachlor and aldrin. Others in the group are the DDTs, the chlordanes, mirex, HCB, the endosulfans and the heptachlor epoxides. The univariate curves of these compounds would show the same trend as that of heptachlor (Fig. 6).

The third group is interesting because it consists of compounds with high Kow and high K_H values. However their loadings are around or below zero (Figs. 2, 3, 4 and 5). These compounds are PCA, PCB and OCS. This means that these compounds acted against the observed trend. The lack of correlation between loadings and Log K_H (Fig 5) could in great part be due to these compounds, because of their high K_H values (Table 1). Given their high K_H values, one would expect greater losses in the controls as was the case with the compounds discussed in group 2 above. This indicates that the microbes did act on these compounds to the extent that the concentrations in the treated samples were as low as those in the controls, in spite of the higher volatilization in the latter. Therefore, these are the compounds that were most probably responsible for the microbial growth noted at the beginning of the experiments in both phases (Fig. 7), besides the nutrients introduced by the repetition of inoculation. *Bordetella petrii* has been shown to possess a large number of genes coding for enzymes of chloroaromatic metabolism and also complete pathways for the degradation of these compounds (Gross et al., 2008).

The compounds in group 3 have one property in common: they all contain a single aromatic ring. This could explain the observed results, because the community used mineralizes 1,2,4-TCB, a lower-chlorinated mono-aromatic compound. Furthermore, this community was isolated from a site polluted with chlorobenzenes (Schroll et al., 2004). From all these facts it was expected that the community is adapted to degrading chlorobenzenes and that it could also degrade higher chlorinated mono-aromatic compounds as used in the cocktail. This is also supported by the fact that related microbes have the ability to degrade aromatic compounds (Bianchi et al., 2005; Eriksson et al., 2003; Gross et al., 2008) and that other microbes have been shown to degrade some chlorobenzenes at low concentrations (Van der Meer et al., 1987; Rapp and Timmis, 1999).

5. Conclusion

These results illustrate the challenge that studying microbial degradation of compounds at low ambient concentrations presents. The microbial consortium could not degrade most of the compounds in the OCPs cocktail such as the DDTs, Chlordanes and Heptachlors. The reasons for this can be many as discussed above and much remains unknown about the mechanisms underlying the limiting factors for biodegradation (Singh, 2007). However, the application of adequate statistical methods gave additionally important information and we received indications that the community could be able to degrade mono-aromatic OCPs like PCA, PCB and OCS. Further studies with monoaromatic OCPs are needed to verify these indications.

Acknowledgement

I would like to acknowledge the German Academic Exchange Service (DAAD) for funding my stay in Germany hence enabling the undertaking of this study.

References

Aislabie JM, Richards NK, Boul HL. Microbial degradation of DDT and its residues — a review. NZ J Agr Res 1997;40:269–82.

ARS - Agricultural Research Service, U.S. Department of Agriculture. Pesticide Properties Database. Accessed in August 2009 at http://www.ars.usda.gov/Services/docs.htm?docid.

ATSDR - Agency for Toxic Substances and Disease Registry. Accessed in August 2009 at http://www.atsdr.cdc.gov/.
Bianchi F, Careri M, Mustat L, Malcevschi A, Musci M. Bioremediation of toluene and naphthalene: development and validation of a GC-FID method for their monitoring. Ann Chim-Rome 2005;95:515–24.
Bignert A, Olson M, Persson W, Jensen S, Zakrison S, Litzen K, et al. Temporal trends of organochlorines in northern Europe, 1967-1995. Relation to global fractionation, leakage from sediments and international measures. Environ Pollut 1998;99:177–98.
Brahushi F, Doerfler U, Schroll R, Munch JC. Stimulation of reductive dechlorination in soil by inducing the native microbial activity. Chemosphere 2004;55:1477–84.
Cao B, Nagarajan K, Loh K-C. Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. Appl Microbiol Biot 2009;85:207–28.
Cuppen JGM, Van den Brink PJ, Camps E, Uil KF, Brock TCM. Impact of the fungicide carbendazim in freshwater microcosms. I. Water quality, breakdown of particulate organic matter and responses of macroinvertebrates. Aquat Toxicol 2000;48:233–50.
Diaz E. Bacterial degradation of aromatic pollutants: a paradigm of metabolic versatility. Int Microbiol 2004;7:173–80.
Edgehill RU, Finn RK. Microbial treatment of soil to remove pentachlorophenol. Appl Environ Microb 1983;45:1122–5.
Eriksson M, Sodersten E, Yu ZT, Dalhammar G, Mohn WW. Degradation of polycyclic aromatic hydrocarbons at low temperature under aerobic and nitrate-reducing conditions in enrichment cultures from northern soils. Appl Environ Microb 2003;69: 275–84.
Gianfreda L, Rao MA. Interactions between xenobiotics and microbial and enzymatic soil activity. Crit Rev Env Sci Technol 2008;38:269–310.
Gross R, Carlos AG, Mohammed S, Vítor AP, Martins DS, Dietmar HP, et al. The missing link: *Bordetella petrii* is endowed with both the metabolic versatility of environmental bacteria and virulence traits of pathogenic Bordetellae. BMC Genomics 2008;9:449.
Guan Y-F, Wang Ji-Zhong, Ni H-G, Zeng Eddy Y. Organochlorine pesticides and polychlorinated biphenyls in riverine runoff of the Pearl River Delta, China: assessment of mass loading, input source and environmental fate. Environ Pollut 2009;157:618–24.
Hense BA, Welzl G, Severin GF, Schramm K-W. Nonylphenol induced changes in trophic web structure of plankton analysed by multivariate statistical approaches. Aquat Toxicol 2005;73:190–209.
Hense BA, Jaser W, Welzl G, Pfister G, Woehler-Moorhoff GF, Schramm K-W. Impact of 17α-ethinylestradiol on the plankton in freshwater microcosms—II: Response of zooplankton and abiotic variables. Ecotox Environ Safe 2008;69:453–65.
HSDB - Hazardous Substances Data Bank, National Library of Medicine. Accessed in August 2009 at http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.
Iwamoto T, Nasu M. Current bioremediation practice and perspective. J Biosci Bioeng 2001;92:1–8.
Johnsen AR, Wick LY, Hams H. Principles of microbial PAH degradation in soil. Environ Pollut 2005;133:71–84.
Mackay D, Shiu W-Y, Ma K-C. Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Pesticide Chemicals, 5. New York: Lewis Publishers; 1997. 812 pp.
Miguel A, Providenti HL, Jack TT. Selected factors limiting the microbial degradation of recalcitrant compounds. J Ind Microbiol 1993;12:379–95.
Moser T, Roembke J, Schallnass H-J, Van Gestel CAM. The use of the multivariate principal response curve (PRC) for community level analysis: a case study on the effects of carbendazim on enchytraeids in terrestrial model ecosystems (TME). Ecotoxicology 2007;16:573–83.
Paasivirta J, Sinkkonen S, Mikkelson P, Rantio T, Wania F. Estimation of vapour pressures, solubilities and Henry's law constants of selected persistent organic pollutants as functions of temperature. Chemosphere 1999;39:5.
PPDB - Pesticide properties database. Accessed in July 2009 at http://sitem.herts.ac.uk/aeru/iupac/.
Rapp P, Timmis KN. Degradation of chlorobenzenes at nanomolar concentrations by *Burkholderia* sp. strain PS14 in liquid cultures and in soil. Appl Environ Microb 1999;65:2547–52.
Schramm K-W, Jaser W, Welzl G, Pfister G, Woehler-Moorhoff GF, Hense BA. Impact of 17α-ethinylestradiol on the plankton in freshwater microcosms—I: Response of zooplankton and abiotic variables. Ecotoxicol Environ Safe 2008;69:437–52.
Schroll R, Brahushi F, Doerfler U, Kuehn S, Fekete J, Munch JC. Biomineralization of 1, 2, 4-trichlorobenzene in soils by an adapted microbial population. Environ Pollut 2004;127:395–401.
Shen L, Wania F, Lei YD, Teixeira C, Muir DCG, Bidleman TF. Atmospheric distribution and long-range transport behaviour of organochlorine pesticides in North America. Environ Sci Technol 2005;39:409–20.
Shen H, Henkelmann B, Levy W, Zsolnay A, Weiss P, Jakobi G, et al. Altitudinal and chiral signature of persistent organochlorine pesticides in air, soil, and spruce needles (*Picea abies*) of the Alps. Environ Sci Technol 2009;43:2450–5.
Singh DK. Biodegradation and bioremediation of pesticides in soil: concept, method and recent developments. Indian J Microbiol 2007;48:35–40.
Stephenson J, Warnes A. Release of genetically modified micro-organisms into the environment. J Chem Technol Biot 1996;65:5-14.
Stockholm convention on persistent organic pollutants. Accessed in August 2009 at http://chm.pops.int/.
Su Y, Hayley H, Pierrette B, Gregory P, Roland K, Alexei K, et al. A circumpolar perspective of atmospheric organochlorine pesticides (OCPs): results from six Arctic monitoring stations in 2000-2003. Atmos Environ 2008;42:4682–98.
UNEP – United Nations Environmental Program. Action plans and studies to replace/reduce release of POPs. Accessed in August 2009 at http://www.chem.unep.ch/pops/actplan.html.
Van den Brink PJ, Ter Braak CJF. Multivariate analysis of stress in experimental ecosystems by Principal Response Curves and similarity analysis. Aquat Ecol 1998;32:163–78.
Van den Brink PJ, Ter Braak CJF. Principal response curves: analysis of time-dependent multivariate responses of a biological community to stress. Environ Toxicol Chem 1999;18:138–48.
Van der Meer JR, Roelofsen W, Schraa G, Zehnder AJB. Degradation of low concentrations of dichlorobenzenes and 1, 2, 4-trichlorobenzenes by *Pseudomonas* sp. P51 in non sterile soil columns. FEMS Microbiol Ecol 1987;45:333–41.
Wang F, Grundmann S, Schmid M, Doerfler U, Roehrer S, Munch JC, et al. Isolation and characterization of 1, 2, 4-trichlorobenzene mineralizing *Bordetella* sp. and its bioremediation potential in soil. Chemosphere 2007;67:896–902.
Wang J, Bi Y, Pfister G, Henkelmann B, Zhu K, Schramm K-W. Determination of PAH, PCB, and OCP in water from the three gorges reservoir accumulated by semipermeable devices (SPMD). Chemosphere 2009;75:1119–27.
Wang F, Dörfler U, Schmid M, Fischer D, Kinzel L, Scherb H, et al. Homogeneous inoculation vs. microbial hot spots of isolated strain and microbial community: what is the most promising approach in remediating 1, 2, 4-TCB contaminated soils? Soil Biol Biochem 2010;42:331–6.
Wania F, Mackay D. Global fractionation and cold condensation of low volatility organochlorine compounds in polar regions. Ambio 1993:22–7.
Zehnder AJB, Huser BA, Brock TD, Wuhrmann K. Characterization of an acetate-decarboxylating nonhydrogen-oxidizing methane bacterium. Arch Microbiol 1980;124:1-11.