Degradation of selected PCB congeners by chemical and enzymatic methods

Gábor Köller, M. Möder, J. Mattusch
Centre for Environmental Research Leipzig-Halle Ltd., Department of Analytical Chemistry, Permoserstrasse 15, D-04318 Leipzig , Germany
K.Czihal
Scientific-technical Company , Permoserstrasse 15, D-04318 Leipzig , Germany
W.Engewald
University of Leipzig, Institute of Analytical Chemistry, Linnèstrasse 3, D-04103 Leipzig, Germany

1. Introduction

The special properties of Polychlorinated Biphenyls (PCBs) like their stability against chemicals and heat as well as their electrical resistance account for a former use in a wide range of products. But these properties are also responsible for the persistence of this substance class in the environment and aggravate their degradation. The actual favoured processes for destruction of the partially highly toxic compounds are high temperature incineration and hydrogenation techniques. But the public acceptance of these methods is limited. Searching for alternative degradation methods the interest in microbiological technologies is permanently increasing.
Unfortunately, such hazards like PCBs, Chlorophenols or PCDD/Fs are hardly attacked by bacteria [1,2]. However, white-rot basidiomycete fungi as natural wood-decay organisms are able to degrade biphenyl systems which belong to the basic constituents of the wood polymer lignin. So these micro-organisms become interesting for degradation studies of PCBs. Up to now, most of the work has been done with the white-rot fungus *Phanerochaete chrysosporium* [3]. But the required culture conditions of this fungus restrict a wider application for remediation. Therefore, we tested the capability of *Trametes multicolor* for PCB transformation. This kind of fungus is growing under middle European climate and has a comparable enzymatic system capable of degrading biphenyl molecules [4].
The aim of this work was to examine the kinetic of the PCB degradation and to study the nature of the metabolites. In order to obtain more details about the rather unknown mechanism we started with experiments using chemical reactions (Fenton's reagent [5]) for the destruction of selected PCB isomers. Furthermore, enzymatic supported transformations of PCBs (No. 9, 138) were examined as another approach to fungal processes , described previously [6] . For this purpose horseradish peroxidase (HRP) was applied as catalytic-active system.

2. Experimental

The PCB congeners (Balschmitter-No.: 9, 138) as well as the horseradish peroxidase were purchased from Merck and were applied without further purification. The components of Fenton's reagent (Fe(II)-Ions, Hydrogen Peroxide) were delivered by Merck and mixed before use .
The horseradish enzyme was stabilised by the following buffer system: 0,1M phosphate buffer (pH=7)
The water as well as the buffer solution were saturated with the corresponding PCB congeners by stirring in a brown bottle for two days. After determining the initial PCB concentrations by later described methods the solutions were treated with Fenton's reagent and the enzyme, respectively.
The decrease of the initial PCB content was detected by SPME (Polydimethylsiloxane Fibre , SUPELCO) in combination with GC-MS (HP 5890 II/MSD, Hewlett Packard).

In this instrument a capillary column with a liquid film of HP-5 , film thickness of 0.25 µm, and dimensions of 30m x 0.25 mm was used in splitless mode. The GC- oven was programmed from 50 to 200 ^{0}C with the rate of 10 K/min held 5min, then with 5 K/min to 260 ^{0}C, held 20 min. Electron impact ionization was at 70 eV. The detection of characteristic trace compounds were realised by target ion analysis (SIM) and the metabolites were identified using full scan mode in the mass range from 50 to 500 daltons.
According to the above described parameters the metabolites of the degradation experiments were determined after liquid-liquid extraction with toluene (two times at neutral and acid conditions, pH 2) , evaporation of the solvent to a volume of 10 µl and injection of 1 µl to the GC-MS .
The detection of dissolved ions like chloride and carboxylated compounds were carried out with an ion chromatograph DX-100 equipped with an Ion Pac ICE-AS1 column. The analytes were eluted with 1.6 mM heptafluorobutyric acid and detected by conductivity. An AMMS-ICE unit was used as supressor working with 5 mM tetrabutylammonium hydroxid.
The white-rot fungus *Trametes multicolor* was cultured relating to the conditions described by Farhaeus and Reinhammar [7,8].

3. Results and Discussion

We treated a water sample containing individual PCB congeners with peroxide and Fe(II)-sulphate in order to study the radical oxidation steps which belong to the basic mechanisms of active peroxidases. First the degradation of 2,5-dichlorobiphenyl (PCB9) at a concentration of 5 µM was examined. The content of the present PCB was detected by head-space SPME and following GC-MS analysis. The Figure 1 shows the decrease of PCB9 in dependence on the reaction time.

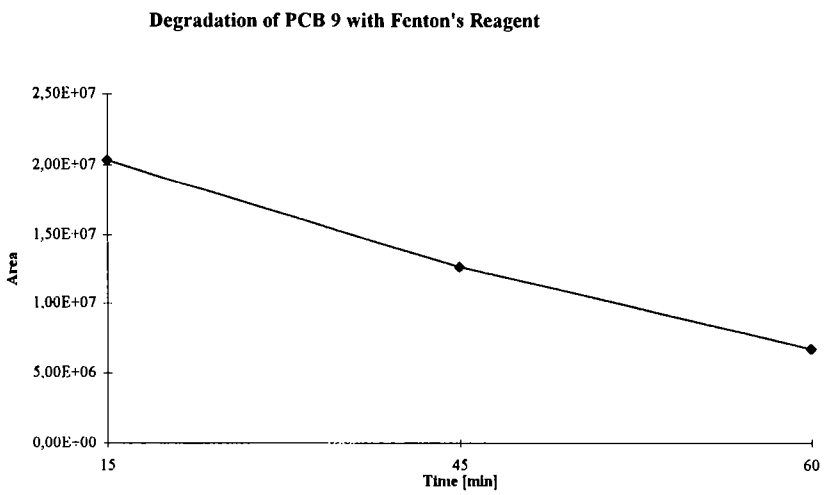

Figure 1

The main reaction was completed after 24 hour and nothing of the PCB9 amount was found. Toluene extracts of intermediate states at 15, 45 and 60 minutes were used for the identification of metabolites. Some of the most abundant degradation products were also time-depended detected. The

corresponding diagram in Figure 2 indicates the formation and the final destruction of the major by-products biphenyl and 2- monochlorosubstituted one (MCB).

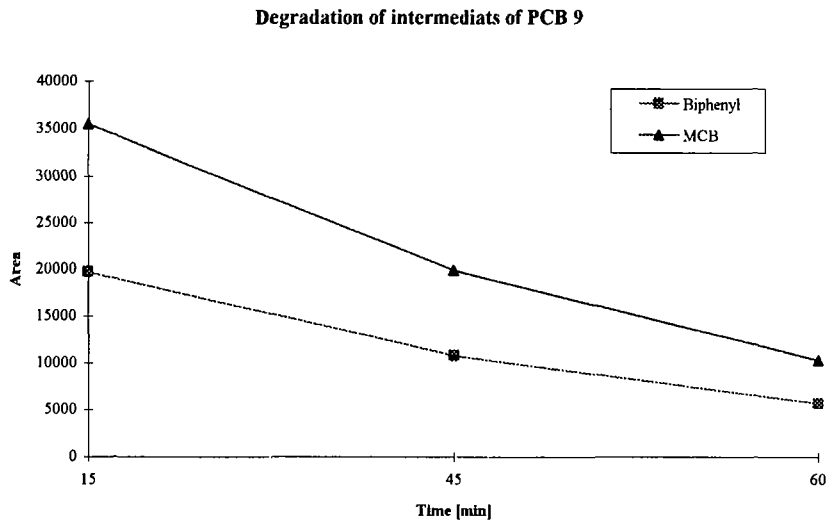

Figure 2 Formation and degradation of Biphenyl and Monochlorobiphenyl during the reaction of PCB9 with Fenton's reagent

Simultaneously , the release of chloride was just as proved by ion chromatography as the formation of carboxylated compounds.
The experiments were continued with the application of horseradish peroxidase for PCB9 transformation.
According to the degradation with Fenton's reagent an accelerated reaction could be observed. The main decrease of the PCB9 happens within the first 30 minutes (Fig. 3).

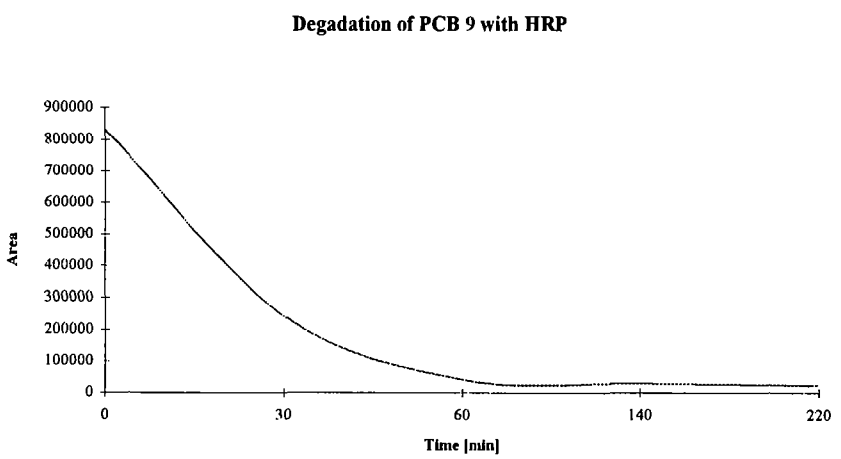

Figure 3 PCB 9 degradation supported by horseradish peroxidase

The initial PCB content was reduced to 7 % . Possibly the drop of enzyme activity prevents a complete mineralization of the PCB. A renewed addition of fresh enzyme can continue the reaction.
Going over to a higher chlorinated biphenyl we studied the enzymatically mediated degradation of the 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138). The PCB-spiked water solution (60 µg/l) was treated with HRP for 16 hours. The flat gradient of the curve in Figure 4 indicate a slower and more incomplete degradation process. Moreover, the final concentration was still 11,5 % of the initial PCB amount.

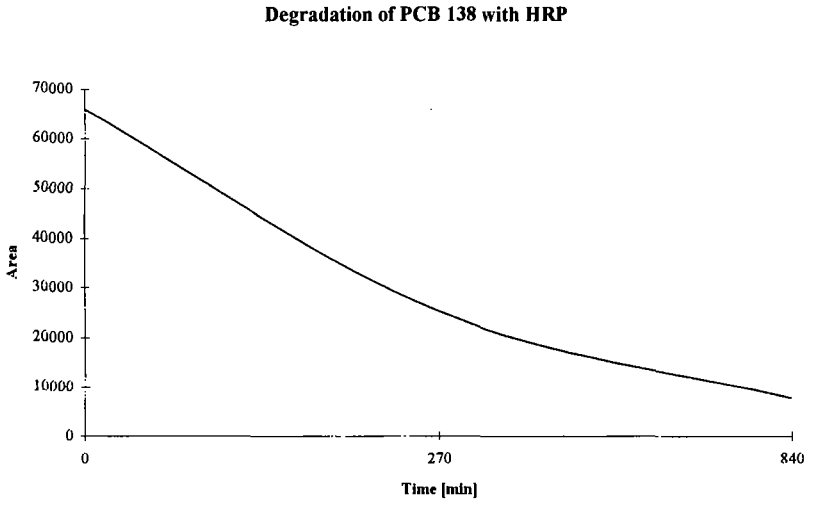

Figure 4 Degradation of PCB138 catalyzed by horseradish peroxidase

The range of degadation product reflects the stepwise release of chlorine in order to form tri-, di- and monochloro biphenyls. Some other hydroxylated as well as carboxylated substances as cyclohexanon derivatives or lactones could be identified.
The application of the fungus *Trametes multicolor* to the degradation of the PCB9 was also successful regarding the decrease of the congener. Within a week the spiked PCB9 disappeared out of the solution. But accompanying formation reactions led to higher chlorinated products besides smaller molecules related to the degradation. Mainly penta-, hexa- and heptachlorobiphenyls were determined with the centre at hexachlorobiphenyls. But nothing pointed to the presence of the most toxic isomeres.

4. Conclusion

The chemical and enzymatic catalyzed degradation of some selected PCB congeners of different homologues series (PCB9,138) were studied. Furthermore, a fungus, *Trametes multicolor*, was applied to destruct 2,5-dichlorobiphenyl (PCB9) at liquid culture conditions.
The results have shown that the chemical degradation using Fenton's reagent is a convenient method , whereby it took the higher chlorinated biphenyl (PCB138) much longer to degrade.
The same ratio was found for the enzymatic supported reaction of the individual PCBs. Compared to the chemical reactions the molecular changes occurred faster but more incomplete caused by the loss of enzymatic activity. The nature of products varied widely. In the case of PCB9 degradation higher chlorinated biphenyls with five till seven chlorine substituents were identified besides smaller molecules partially hydroxylated and/or chlorinated.

These kind of higher chlorinated by-products also appeared while the transformation of PCB9 by *Trametes multicolor.* Using the micro-organisms for degradation the speed and the resulting conversion of the PCB depend on the exact observance of the culture conditions .

5. References

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