



**HAL**  
open science

# Impact of anaerobic and aerobic processes on PolyChloroBiphenyl removal in contaminated sewage sludge

Dominique Patureau, Eric Trably

► **To cite this version:**

Dominique Patureau, Eric Trably. Impact of anaerobic and aerobic processes on PolyChloroBiphenyl removal in contaminated sewage sludge. *Biodegradation*, 2006, 17 (1), pp.9-17. 10.1007/s10532-005-1920-4 . hal-02660240

**HAL Id: hal-02660240**

**<https://hal.inrae.fr/hal-02660240>**

Submitted on 9 Aug 2023

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

**Impact of anaerobic and aerobic processes  
on PolyChloroBiphenyl removal in contaminated sewage sludge**

Patureau D. & Trably E.

INRA Laboratoire de Biotechnologie de l'Environnement, avenue des étangs, 11100 Narbonne, France

Tel: 33 (0) 468 425 169, Fax: 33 (0) 468 425 160, Email: patureau@ensam.inra.fr

**Keywords:** Bioavailability; Biodegradation; Bioprocesses; PCB; Sewage sludge; Xenobiotics.

**Abstract**

Aerobic and anaerobic biodegradation of six priority PCBs was investigated in continuous stirred tank reactors fed with naturally contaminated sewage sludge. Anaerobic and aerobic abiotic losses were higher for the lightly chlorinated PCBs but remained for all PCBs below 20%. Under strict methanogenic conditions, PCB removals were about 40% whatever PCB molecular weight or their degree of chlorination. However, considering abiotic losses, the heaviest PCBs were more efficiently anaerobically biodegraded probably because of higher dechlorination rates. The aerating sludge process enhanced removal of the lightest chlorinated PCBs from 40% up to 100%, while removal rates of the heaviest PCBs remained around 40%. Although the mesophilic aerobic process exhibits better removal efficiencies because of operating conditions, the results suggest that PCB biodegradation was strongly limited by their bioavailability in naturally contaminated sludge, under both redox conditions. Indeed, since PCB removal was closely linked to the solid reduction rates, PCB bioavailability was likely the limiting factor for biodegradation. As a consequence, the raw PCB concentrations (in  $\text{mg.kg}_{\text{dry weight}}^{-1}$ ) which are concerned by legislative procedures did not decrease sufficiently in both processes to reach a limit value fulfilling the current French / European regulation about PCB contents in sewage sludge before spreading on agricultural land.

## **Introduction**

Since World War II, human activity has introduced on a very large scale a variety of xenobiotic chemicals into the environment. Every year, some 1000 new chemicals are introduced on the market. Many of them have a rather poor biodegradability and accumulate in many compartments of our environment such as water, soils, plants, animals (Alexander, 1981). The Polychlorobiphenyls (PCBs) are one of these most persistent classes of pollutants. Because of their chemical and thermal stability, low flammability and high permittivity, they have been widely used over the last 50 years in industrial applications as hydraulic fluids, heat transfer fluids, plasticizers, or as flame retardants (Abramowicz, 1990). Unfortunately, such properties have led as well to the persistence and the accumulation of the PCBs in the environment because of their low biodegradability, with a range of concentrations from several  $\mu\text{g.kg}_{\text{dw}}^{-1}$  in sediments and soils, up to more than  $\text{mg.kg}_{\text{dw}}^{-1}$  in sewage sludge. Significant amount of PCBs have also been found in animal and human lipidic tissues by accumulation throughout the food chain (Przyrembel *et al.*, 2000). Moreover, they are well known to have adverse effects on biological life and to be carcinogenic and mutagenic which makes them a serious environmental problem (Przyrembel *et al.*, 2000). For these reasons, the study of the different ways of elimination is of great importance, and one crucial process is their microbial degradation. Anaerobic biodegradation of PCBs in highly contaminated sediments and soils or in pure culture was recently reported by several authors (Mohn and Tiedje, 1992; Bedart and Quensen, 1995; Natarajan *et al.*, 1999). It consists of a reductive dechlorination of the PCBs leading to less chlorinated congeners, repetitively up to the non-chlorinated biphenyl molecule. Furthermore, Natarajan *et al.* (1999) reported the possibility of full mineralization of the biphenyl congener by a PCB-dechlorinating anaerobic consortium.

An interesting point is that dechlorination occurs only on meta- and para-chlorine positions and can consequently affect the toxic properties (Lang, 1992).

Aerobic degradation of PCBs has also been extensively studied over the past ten years. PCB degradation was observed in highly contaminated ecosystems and in pure cultures (Abramowicz, 1990; Camara *et al.*, 2004). The aerobic metabolic pathways as well as the genes have been described (Abramowicz, 1990; Chaudhry and Chapalamadugu, 1991; Mukerjee-Dhar *et al.*, 1998). Mainly, PCB aerobic pathway involves a biphenyl-dioxygenase, which converts PCBs to chlorinated benzoic acids and chlorocatechols (Abramowicz, 1990). Bedard *et al.* (1987) also reported the production of a 3,4-dioxygenase by *Pseudomonas* sp. LB400 and *Alcalignes eutrophus* H850. Both enzymes need the presence of high amount of oxygen to be efficient. Such aerobic microbial processes were successfully used for bioremediation of highly contaminated sites (Adriaens and Focht, 1990; Shi, 1998), and were combined with anaerobic bioprocesses (Anid *et al.*, 1991; Ng *et al.*, 1999). However, PCB bioavailability seems to be the main limiting factor affecting PCB removal in contaminated sites. Indeed, the addition of sediments as well as the increase of solid concentration in sludge reduces the PCB removal rates by enhancing the adsorption surface (Chang *et al.*, 1999; Hartkamp-Commandeur, 1996). Furthermore, Bedard and Quensen (1995) demonstrated that the hard-adsorbed fraction increases over the time when soils or sediments are exposed to contamination.

Wastewater treatment plants (WWTPs) may play an important role in environmental PCB decontamination as these xenobiotics could transfer throughout the urban system. Due to their hydrophobic properties, they accumulate by sorption onto sludge particles in the settling processes. Since 60% of the sludge produced in France is currently spread on agricultural land and half-lives of PCBs in soils are between 2 to 4 years, the detection of such compounds in sludge is of great importance. This concern has led the European Union to regulate the PCB

contents in sewage sludge before spreading on land by fixing limits for the future common EU Sewage Sludge Directive. The proposed limit values are of  $0.2 \text{ mg.kg}_{\text{dw}}^{-1}$  for each PCB n°28 (2,4,4'-trichlorobiphenyl), n°52 (2,2',5,5'-tetrachlorobiphenyl), n°101 (2,2',4,5,5'-pentachlorobiphenyl), n°118 (2,3',4,4',5-pentachlorobiphenyl), n°138 (2,2',3,4,4',5'-hexachlorobiphenyl), n°153 (2,2',4,4',5,5'-hexachlorobiphenyl) and n°180 (2,2',3,4,4',5,5'-heptachlorobiphenyl). The current limit value in France is of  $0.8 \text{ mg.kg}_{\text{dw}}^{-1}$  for the sum of the seven PCBs. By treating biologically the contaminated sludge, the WWTPs could constitute a convergence point where the wide diversity of microbial processes could help in minimizing the release of PCBs into the receiving environment. However, PCB concentrations in such naturally contaminated sludge are quite lower than in usual ecosystems previously studied. The purpose of the present study was to investigate the natural potential of microbial ecosystems to degrade PCBs at trace levels in naturally contaminated sewage sludge. Continuous stirred tank reactors (CSTR) were inoculated with long-term acclimated anaerobic and aerobic ecosystems, and were fed with a mixture of primary and secondary sewage sludge naturally contaminated by PCBs.

## **Material and methods**

*1. Chemicals.* All chemicals were of analytical grade or better. All solvents were provided by J.T.BAKER-MALLINKRODT (Noisy le Sec, France). Prior to analysis, the borosilicate glassware and experimental apparatus were rinsed with a solvent mixture of acetonitrile:acetone (50:50, v:v).

*2. Source of biological material.* The anaerobic ecosystem corresponded to the outlet of an industrial anaerobic digester located in an urban wastewater treatment plant (WWTP) contaminated by PCBs for more than 10 years. The aerobic ecosystem corresponded to the outlet of the activated sludge pond of the same WWTP. The reactors were fed with a mixture

of primary and secondary sludge sampled from the same PCB-contaminated WWTP. The level of PCB contamination (around  $1.74 \text{ mg.kg}_{\text{dw}}^{-1}$ ) corresponded to twice the French allowed concentrations for spreading sludge on land.

*3.Experimental design.* Four laboratory-scale continuous stirred tank reactors were implemented to simulate traditional full-scale mesophilic sludge digesters. Two experimental conditions corresponded to methanogenic conditions of anaerobic sewage sludge digesters, and two others corresponded to aerobic conditions. All bioreactors worked under perfectly mixed continuous conditions with a hydraulic retention time of 40 days (anaerobic) and 20 days (aerobic), a mesophilic temperature regulated at  $35^{\circ}\text{C}$  (temperature probe coupled to thermic resistance), a reactional volume of 5 litres, and a daily organic load about one  $\text{kg}_{\text{COD.m}^{-3}.\text{d}^{-1}}$ . The TS and VS concentrations in the feeding mixture are respectively of 32 and  $26 \text{ g.l}^{-1}$ . pH was not regulated, but did not evolved significantly between the assays, with  $7.6 \pm 0.1$  and  $7.2 \pm 0.1$  pH units for, respectively, the anaerobic and aerobic reactors. The biogas outlet was cooled to avoid water losses. The 2 L feeding tanks were cooled at  $4^{\circ}\text{C}$  to keep the sludge properties, and were filled once a week. Magnetic stirring was used to agitate anaerobic reactors (250 rpm). Mechanistic stirring at 250 rpm was used to mix the aerobic ones. All of these performing conditions simulated closely the behaviour of theoretical continuous stirred perfectly mixed tank reactors.

One anaerobic and one aerobic reactor were chemically sterilized by addition of 0.2 g sodium azide/g TS ( $\text{NaN}_3$  - minimal purity over 99% - Riedel de Haën<sup>TM</sup>) which stops bacterial activity. These control reactors were implemented to assess PCB abiotic losses.

*4.Analytical methodology.* After reaching steady state (which corresponds to 4 HRT and no variation of the outlet concentration), samples were directly taken from reactor outlets and inlets. All analyses were carried out in triplicates. After sample homogenization, total solid (TS) was determined by 24 hours drying of 20 mL sample in ventilated oven at  $105^{\circ}\text{C}$ .

Volatile solid (VS) was determined by overnight drying in furnace at 550°C (Clesceri *et al.*, 1985). Total Chemical Oxygen Demand (COD) was measured by sludge sample mineralization according to the Standard Methods for Examination of Water and Wastewater (Clesceri *et al.*, 1985). Rates of CH<sub>4</sub> and CO<sub>2</sub> in biogas were determined by gas chromatography (SHIMADZU) equipped with two columns (molecular sieved and Haye sep Q) and catharometric detection. Columns and injector temperatures were maintained at 30, 100°C respectively. Detector current was maintained at 70 mA. Argon was used as a carrier gas at 2.6 bars.

*5.PCB analysis.* PCB analysis of sludge sample was carried out with 350 ml sample from the reactor outlet or from the feeding tank. The samples were first centrifuged (20 000 g, 25 min.). Aqueous supernatant was stored in cool chamber (-20°C) for further Solid-Phase Extraction. Solid pellets were ground with glass beads (diameter 4 mm) to dissociate sludge fibers and were dried during 60 hours in ventilated oven (40°C). Dry samples were sieved on 2mm diameter grid and were stored in cool chamber (-20°C) for further Accelerated Solvent Extraction. Solid-Phase Extraction and Accelerated Solvent Extraction methods for PCB analysis in sludge sample were performed according to Trably *et al.* (2004a). PCB extraction from aqueous phase was performed with a 6mL Supelco ENVI-18<sup>TM</sup> column (0.5 g). The extraction column was conditioned with 2\*6 ml toluene:methanol (50:50 v:v), then 3\*6 ml methanol and 3\*6 ml deionized water. 200 mL of aqueous sample was then passed three times through the column under vacuum. After drying, PCBs were eluted with 2mL of toluene:methanol. The extract was then evaporated under nitrogen flow to dryness. Dry residues were dissolved in 2mL of hexane for further analysis. No PCBs were measured in the aqueous phase. PCBs were extracted from dry samples by the ASE-200 system (DIONEX<sup>TM</sup>). The extracting cells were filled with 1g of activated copper bars, 0.5g of the dried and sieved sludge sample, 0.5g of alumina (SIGMA<sup>®</sup> A-1522) and 1.5g of hydromatrix-celite dispersant

(VARIAN<sup>®</sup>). The extracting solvent was a mixture of hexane:acetone (50:50, v:v). Extracting parameters were as follows: temperature, 120°C; pressure, 100 bars; 2 cycles of extraction; static time, 5 min.; cell flush, 60% and purge time, 120 sec. The extract was evaporated under nitrogen flow to dryness. Residues were dissolved in 5mL of hexane. The extract was then purified on florisil (60/100 mesh) column. The florisil is previously dried (16 h at 130°C) and activated with water (2ml for 100g). A column is filled with the activated florisil (5g) and then with Na<sub>2</sub>SO<sub>4</sub> (5mm), and conditioned with hexane. The extract is purified and the PCBs were finally eluted with hexane and concentrated under a stream of nitrogen to an appropriate volume. An internal standard (PCB n°202 at 50µg.L<sup>-1</sup>) was added for internal calibration, and the sample was analysed by GC-ECD (VARIAN3400) on a DB column (530m×0.25mm×0.25µm). The carrier gas was helium and the temperature program was as follow: 1 min at 70°C, from 70 to 250°C at 18°C.min<sup>-1</sup>, 5 min at 250°C, from 250 to 280°C at 5°C.min<sup>-1</sup>, 20 min at 280°C. PCB recoveries of the extraction-purification and analytical procedures from sludge samples were previously determined, and were higher than 95% in all cases. Only the PCB n°28 was altered by the method, and was completely lost during the drying steps.

## **Results and discussion**

### *1. Solid reduction rate.*

Overall objective of anaerobic and aerobic sludge treatments is the efficient reduction of organic matter. Total Solids (TS) and Volatile Solids (VS) are commonly used as indicators of organic matter reduction. In this study, TS, VS, total COD (carbon oxygen demand) contents as well as biogas composition were determined. In the anaerobic reactor, biogas production rate was of 0.4L per gram of COD degraded, with a methane content of 68%. The TS and VS reduction rates were respectively of 35% and 49%. All parameters were highly characteristic for an efficient methanogenic activity (Ross *et al.*, 1992; Arundel, 2000). In the



aerobic reactor, TS and VS reduction rates were respectively of 28% and 23%. These rates are lower than under anaerobic conditions because of higher biomass yield of aerobic bacterial consortium ( $0.4$  to  $0.6 \text{ g}_{\text{dw}} \cdot \text{g}_{\text{degraded COD}}^{-1}$  versus  $0.02$  to  $0.15 \text{ g}_{\text{dw}} \cdot \text{g}_{\text{degraded COD}}^{-1}$  for anaerobic ecosystems). These aerobic reduction rates are close to values published in the literature for same bioreactors, and are highly typical of aerobic sludge processes (Water Agency, 1994). In contrast, no biological activity was observed in the control reactors. Since no biogas was produced and TS reduction rates were non-significant, it was concluded that the controls were well sterilized and were representative of the abiotic losses.

## *2. PCB abiotic losses.*

*[Figure 1: PCB abiotic losses in anaerobic and aerobic control reactors at steady state.]*

Abiotic losses were assessed with the control reactors (Figure 1). Because of the low fusion/ebullition temperatures and the semi-volatile properties of the PCBs, abiotic losses mainly result of volatilization, photodegradation or chemical combination with organic matter. Under anaerobic conditions, the highest abiotic losses were observed for the lightest chlorinated PCBs with about 20% of losses for the tetra- and penta-PCB (n°52, n°101, n°118). The heaviest PCBs presented lower losses with 5% for the hexa-PCB (n°138 and n°153), except for the hepta-PCB (n°180) with around 20% of abiotic losses. Moreover, these losses were more dependent of molecular weight and, consequently, of number of chlorine, than the highly variable concentration of each PCB (Table 1). This result is consistent with the statement that light PCBs are more volatile and less adsorbed than the highly chlorinated ones. Moreover, PCB abiotic losses under anaerobic conditions are limited to an average value of 20%, which can be considered as a significant but not a main effect for PCB

removal. Usually, experiments on anaerobic PCB microbial reductive dechlorination in contaminated sediments are performed with control cultures where no release of chlorine is shown in the medium (Wu *et al.*, 1998). However, no measurements of the original PCB molecule are carried out. Only one study reported the absence of abiotic losses through PCB quantification during incubation of spiked sewage sludge, but this work was conducted under batch conditions without shaking (Chang *et al.*, 1999). Therefore, the values reported in the present study might represent more precisely the actual abiotic losses expected in case of anaerobic digestion of naturally contaminated sludge, with around 20% maximum of non biological losses for all PCBs.

*[Table 1: Average PCB concentrations in inlet and in outlet of the control and biological reactors, at steady state.]*

In contrast, abiotic losses under aerobic conditions were globally lower than under anaerobic conditions. As well as for the anaerobic control reactor, these losses were more dependent of the molecular weight and the number of chlorine, than the highly variable concentration of each PCB in inlet (Table 1). This result is surprising because mechanistic stirring combined to high aeration rate should have enhanced the availability of the semi-volatile compounds implying higher abiotic losses than under anaerobic conditions. In previous work, similar results were described in case of heavy PAHs (Trably *et al.*, 2004b). As well, PCB amounts in the aqueous phase were surprisingly negligible implying that abiotic phenomena occurred *in situ* or in the interface between the solid and the aqueous phases. Since the physicochemical properties of the PCBs are close to the highest PAHs in terms of hydrophobicity and volatility, our result suggest that PCB mass transfer occurred during the process from non-extractable to extractable fraction within the solid phase. Higher concentrations and lower

removal rates are therefore expected in the outlet of the reactors. Actually, the global observation of this phenomenon is counterbalanced by the high volatilization in the aerobic process. Abiotic losses measured in aerobic reactor consequently result from these two phenomena. Such diffusion-volatilisation effect was already observed with other hydrophobic compounds such as PAHs, under the same conditions (Trably *et al.*, 2003).

### *3. PCB removal under anaerobic and aerobic conditions*

Performances of the anaerobic and aerobic processes on PCB removal are presented in Figure 2. Anaerobic PCB removal rates were around 35% to 45% from PCB n°52 to n°180, whatever the molecular weight. Significant biological removal was here observed for all PCBs, in comparison with the control reactors.

*[Figure 2 : PCB removal efficiency in anaerobic and aerobic biological reactors, at steady state.]*

This anaerobic biological degrading ability is consistent with general findings from the literature (Abramowicz, 1990; Anid *et al.*, 1991). Nevertheless, this result represents the first observation of an anaerobic PCB-degrading activity at such low PCB level in non-spiked sludge sample, i.e. at trace level concentrations from 1 to 20 $\mu\text{g.L}^{-1}$ . Such biological degradation activity is very surprising because PCB amounts only represented less than 1% of the total COD, and such activity certainly requires a long-term acclimation of the biological ecosystem. Inversely, other authors demonstrated that dechlorination of a PCB mixture can only occur by decreasing the total concentration from 140 to 4 $\text{mg.L}^{-1}$  because of PCB inhibitory effect (Quensen *et al.*, 1988). In addition, an ANOVA statistical test on the average removal of the seven PCBs was performed. No significant difference was observed between

the different PCB removal rates with an average percentage of  $38.7 \pm 10\%$  (ANOVA factor F of 2.81 lower than critical factor of 3.1 at 5%). Moreover, the TS reduction rate was of  $35.7 \pm 2.1\%$ . A second statistical comparison of these two averages showed no difference, and it can therefore be concluded that PCB removal was strongly linked to the solid reduction rate (value of the T-test factor is 1.32 and is lower than the critical value of 2.92 at 5%). Therefore, anaerobic PCB removal from sludge seems to be limited by their bioavailability, as previously shown for other hydrophobic xenobiotic compounds (Trably *et al.*, 2003). Moreover, Chang *et al.* (1999) demonstrated an inverse correlation between solid concentrations and PCB removal performances, and concluded that higher solid concentrations increase sorption and consequently reduce biodegradation. This correlation between TS and PCB removal rates explains why raw PCB concentrations in solids ( $\text{mg} \cdot \text{kg}_{\text{dw}}^{-1}$ ) did not decrease significantly even if biological process was efficient enough to reduce more than 40% of the total amount (Table 1).

By considering the abiotic losses, the actual biological degradation of PCB can be calculated. Corrected results are presented in Figure 3. We observed that biological removal rate increases with PCB molecular weight, except for the hepta-PCB n°180. Therefore, the highest chlorinated PCBs seemed to be preferentially degraded under anaerobic conditions. However dechlorination of the heaviest PCB results in the production of lightest one : the biological removal rate for the lightest PCB is thus a result of production by dechlorination and degradation. This result is in accordance with previous findings published in the literature. Indeed, since the discovery of PCB dechlorination in anaerobic ecosystem (Brown *et al.*, 1984), several authors concluded to a preferential dechlorination of hepta-, hexa- and penta-CB with an increase proportion of mono- and diCB (Quensen *et al.*, 1990; Wu *et al.*, 1998). Surprisingly, Chang *et al.* (1999) reported no dechlorination of hexa-, hepta and octo-PCB in anaerobic batch experiments, probably due to the relative short incubation period (4 months)

of the sludge inoculum compared to the time need for the adaptation of the microflora. Indeed, Wu *et al.* (1998) observed dechlorination of Aroclor 1260 only after a lag phase of more than 3 months. In our study, anaerobic sludge ecosystem has been acclimated to PCB trace levels for more than 10 years in the contaminated WWTP, and the long acclimation time can explain the high PCB removal efficiency measured, even for trace level PCB concentrations.

Under aerobic conditions, significant higher removal rates were measured for the two lightest chlorinated PCBs (t-test values of 12.4 (n°52) and 2.4 (n°101) for, respectively, a critical t value of 2.35 and 2.15 at 5%) (see Figure 2). The final outlet concentration of the tetra-PCB n°52 was indeed under the analytical limit of detection ( $0.01 \text{ mg.kg}_{\text{dw}}^{-1}$ ). The removal rates for the PCBs from n°118 to n°180 were statistically non different than those obtained under anaerobic conditions, with an average of  $41.1 \pm 3.3\%$  (ANOVA tests of both averages led to F factor value of 1.93 lower than critical F value of 2.65 at 5%). However, after correction by abiotic losses, the PCB biological removal part decreases with the molecular weight implying that the less chlorinated compounds are better degraded under aerobic conditions (Figure 3). A statistical test comparing biological removal averages between both processes showed a statistically enhancement of biodegradation of all PCBs under aerobic conditions (t-test values of 14.4 (n°52), 5.62 (n°101), 2.6 (n°118), 3.64 (n°138), 3.92(n°153), 2.81(n°180) higher than critical t value of 2.13 at 5 %).

*[Figure 3 : PCB biodegradation efficiency after correction by the abiotic losses in anaerobic and aerobic biological reactors, at steady state.]*

This result is in agreement with previous results reported in the literature (Abramowicz, 1990; Mohn & Tiedje, 1992). Furthermore, total TS removal rate is quite lower under aerobic than

under anaerobic conditions. That implies that aerobic conditions enhanced PCB bioavailability and, consequently, PCB biodegradation by increasing mass transfer from the sludge matrix to more bioavailable fraction. Calculation of the ratio between PCB removal rates and associated TS removal rate –called efficiency factor - confirms that bioavailability of the PCBs was enhanced in the aerobic process (Figure 4). Similar results were found under same culture conditions with PAHs as organic pollutants naturally present in low contaminated sludge (Trably *et al.*, 2004b). Since bioavailability of xenobiotic hydrophobic compounds such as PAHs or PCBs limits their biodegradation, aerobic process is more appropriated by enhancing transfer phenomena likely because of the operating conditions (mixing, aeration) or the production of biosurfactants by aerobic specialized microorganisms, as previously shown by Deziel *et al.* (1996) and Zhang *et al.* (1997).

*[Figure 4 : Efficiency factors in anaerobic and aerobic biological reactors, at steady state.]*

In addition, aerobic efficiency factors were significantly higher than 1 for all PCBs, i.e. PCB removal rates were higher than TS reduction. The raw PCB concentrations were therefore lower in the outlet of the aerobic reactor than in the sewage sludge (Table 1). The raw PCB concentrations can be reduced from around 20% with a final value of  $1.36 \text{ mgPCBs.kg}_{\text{dw}}^{-1}$ , which is unfortunately still higher than allowed concentrations for spreading sludge on land in France and Europe (see Table 1).

## **Conclusions**

The main objective of this study was to assess the fate of seven priority PCBs during anaerobic and aerobic sludge treatment. A naturally contaminated sludge was chosen as a model of the strong sorption of an old PCB contamination. In this paper, an efficient

biodegradation under anaerobic and aerobic conditions has been demonstrated for all PCBs. However, PCB biodegradation of naturally contaminated sludge seems to be limited by the strong sorption of PCBs on sludge particles, and consequently by their bioavailability. Due to the operating conditions and a higher biological potential, the aerobic process presented the highest removal rates with a decrease of 20% of the raw PCB concentrations. However, aerobic or anaerobic sludge treatments were not yet sufficiently efficient to reach allowed PCB concentrations for sludge spreading on agricultural land. Nevertheless, our results are very promising as a first basis for further process optimization focusing on the enhancement of PCB bioavailability to reach the requirements. Further experiments could be carried out base on an increase of temperature, addition of surfactant or solvent that could be beneficial for mass transfer enhancement and, therefore, *in situ* PCB biodegradation by adapted anaerobic or aerobic microorganisms.

### **Acknowledgments**

The French Agency for Environment and Energy Management (ADEME, Angers, France) and the National Institute for Agronomic Research (INRA, Narbonne) are especially thanked for the support of Eric Trably's PhD work. Marie Curie actions from the European Commission are also thanked for the grant to E. Trably on his current project about the "characterization of the microbial communities degrading xenobiotics", in collaboration with Assoc.Prof. J.E.Schmidt and Assoc.prof. DJ Batstone from the Technical University of Denmark – Kgs Lyngby (DK) (Framework Program 6 – Intra European Fellowship – MEIF-CT-2003-500956-Xenomic project).

### **References**

- Abramowicz DA (1990) Aerobic and anaerobic biodegradation of PCBs: a review. Crit. Rev. Biotechnol. 10:241-251
- Adriaens P & Focht DD (1990) Continuous coculture degradation of selected polychlorinated biphenyl congeners by *Acinetobacter spp.* in an anaerobic reactor system. Environ. Sci. Technol. 24:1042-1049
- Alexander M (1981) Biodegradation of chemicals of environmental concern. Science 211:132-138

- Anid PJ, Nies L & Vogel TM (1991) Sequential anaerobic-aerobic biodegradation of PCBs in the river model. In: Butterworth-Heinemann (Ed) On-site bioreclamation, (pp 428-436), Boston
- Arundel J. (2000) Sewage and industrial effluent treatment. Blackwell Science, Oxford
- Bedard DL, Haberl ML, May RJ & Brennan J (1987) Evidence for novel mechanisms of polychlorinated biphenyl metabolism in *Alcaligenes eutrophus* H850. Appl. Environ. Microbiol. 53:1103-1112
- Bedard DL & Quensen III JF (1995) Microbial reductive dechlorination of polychlorinated biphenyls. In: Young LY & Cerniglia CE (Ed) Microbial transformation and degradation of toxic organic chemicals, Wiley-Liss, Inc., New-York
- Brown JF, Wagner RE, Bedard DL, Brennan MJ, Carnahan JC, May RJ & Tofflemire TJ (1984) PCB transformations in upper Hudson sediments. Northeast. Environ. Sci. 3:167-179
- Camara B, Herrera C, Gonzalez M, Couve E, Hofer B & Seeger M (2004) From PCBs to highly toxic metabolites by the biphenyl pathway. Environ. Microbiol. 6:842-50
- Chang BV, Chou SW & Yuan SY (1999) Microbial dechlorination of polychlorinated biphenyls in anaerobic sewage sludge. Chemosphere 39:45-54
- Chaudhry GR & Chapalamadugu S (1991) Biodegradation of halogenated organic compounds. Microbiol. Rev. 55:59-79
- Clesceri LS, Franson MAH, Greenberg AE & Trussell RR (1985) Standard methods for the examination of water and wastewater. 16<sup>th</sup> edition. American Public Health Association. ISSN 8755-3546
- Deziel E, Paquette G, Villemur R, Lepine F & Bisailon JG (1996) Biosurfactant production by a soil *Pseudomonas strain* growing on polycyclic aromatic hydrocarbons. Appl. Environ. Microbiol. 62:1908-1912
- Hartkamp-Commandeur LCM, Gerritse J, Govers HAJ & Parsons JR (1996) Reductive dehalogenation of polychlorinated biphenyls by anaerobic microorganisms enriched from dutch sediments. Chemosphere 32:1275-1286
- Lang V (1992) Polychlorinated biphenyls in the environment. J. Chromatography 595:1-43
- Mohn WW & Tiedje JM (1992) Microbial reductive dehalogenation. Microbiol. Rev. 56 :482-507



- Mukerjee-Dhar G, Hatta T, Shimura M & Kimbara K (1998) Analysis of changes in congener selectivity during PCB degradation by *Burkholderia* sp. strain TSN101 with increasing concentrations of PCB and characterization of the *bphBCD* genes and gene products. Arch. Microbiol. 169:61-70
- Natarajan MR, Wu WM, Sanford R & Jain MK (1999) Degradation of biphenyl by methanogenic microbial consortium. Biotechnol. Lett. 21:741-745
- Ng WJ, Hu JY, Ong SL & Aziz MA (1999) Effect of acidogenic stage on aerobic toxic organic removal. J. Environ. Engineering. 125:495-500
- Przyrembel H, Heinrich-Hirsch B & Vieth B (2000) Exposition to PCBs and health effects of residues in human milk. Adv. Exp. Med. Biol. 478:307-25
- Quensen III JF, Tiedje JM & Boyd SA (1988) Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. Science 242:752-754
- Quensen III JF, Boyd SA & Tiedje JM (1990) Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. Appl. Environ. Microbiol. 56:2360-2369
- Ross WR, Novella PH, Pitt AJ, Lund P, Thomson BA, King PB & Fawcett KS (1992) Anaerobic digestion of wastewater sludge : operating guide. Project n°390. Report to the Water Research Commission of Pretoria, South-Africa
- Shi Z (1998) Biodegradation of UV-irradiated polychlorinated biphenyls in surfactant micelles. Wat. Sci. Technol. 38:25-32
- Trably E, Patureau D & Delgenès JP (2003) Enhancement of Polycyclic Aromatic Hydrocarbons (PAHs) removal during anaerobic treatment of urban sludge. Wat. Sci. Technol. 48:53-60
- Trably E, Delgenès N, Patureau D & Delgenès JP (2004a) Optimization and validation of a highly reproducible method for Polycyclic Aromatic Hydrocarbons (PAHs) analysis in sludge samples. Int. J. Environ. Anal. Chem. (In press)
- Trably E, Delgenès N, Patureau D & Delgenès JP (2004b) Comparison of Polycyclic Aromatic Hydrocarbons (PAHs) removals during anaerobic and aerobic sewage sludge treatment. In: Lichtfouse E, Dudd S & Robert D (Ed) Environmental Chemistry. Springer-Verlag, Heidelberg, Germany (in press)

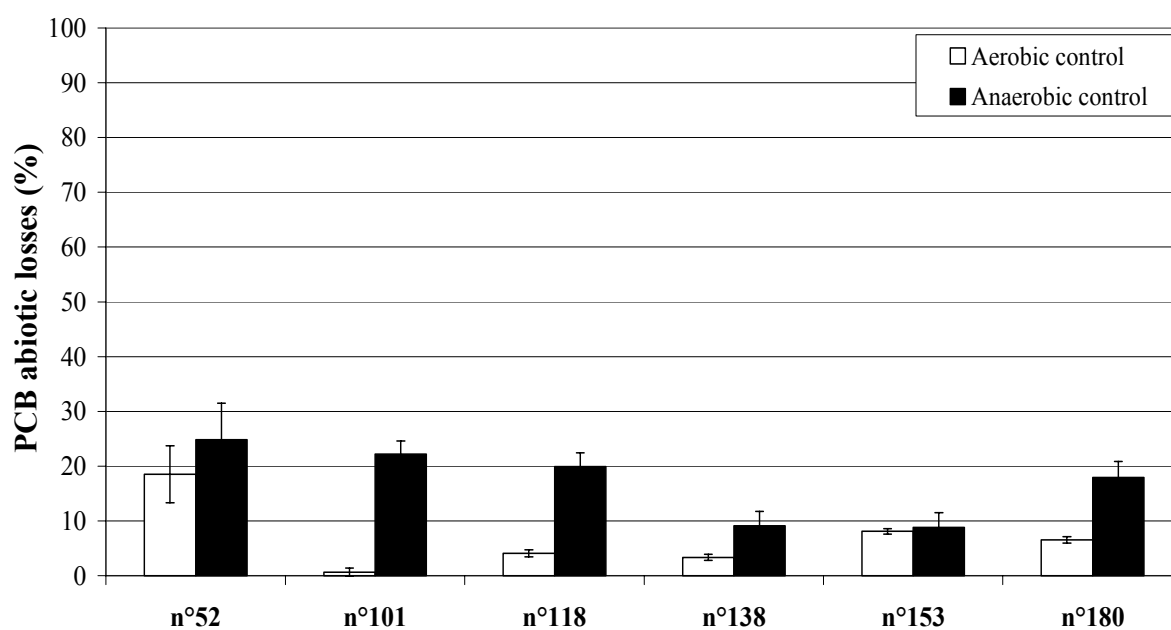
Water Agency (1994) Urban networks : current techniques of treatment and evolution. Inter-agencies study n°27.  
Ed.French water agency

Wu Q, Sowers KR & May HD (1998) Microbial reductive dechlorination of aroclor 1260 in anaerobic slurries of estuarine sediments. *Appl. Environ. Microbiol.* 64: 1052-1058

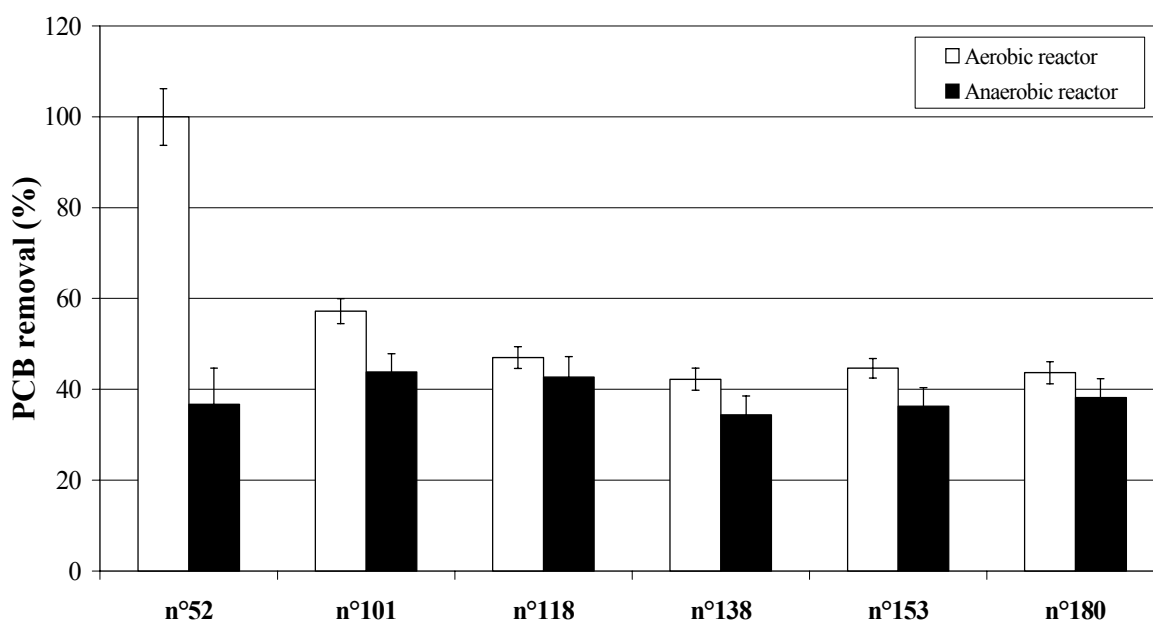
Zhang Y, Maier WJ & Miller RM (1997) Effect of rhamnolipids on the dissolution, bioavailability, and biodegradation of Phenanthrene. *Environ. Sci. Technol.* 31:2211-2217

**Table 1:** Average PCB concentrations in inlet and outlet of control and biological reactors at steady state under anaerobic and aerobic conditions. (maximum standard deviation 10 %). nd : not detected (limit of detection 0.010 mg.kg<sub>dw</sub><sup>-1</sup>). The French limit value for sludge spreading on land is 0.8 mg.kg<sub>dw</sub><sup>-1</sup>.

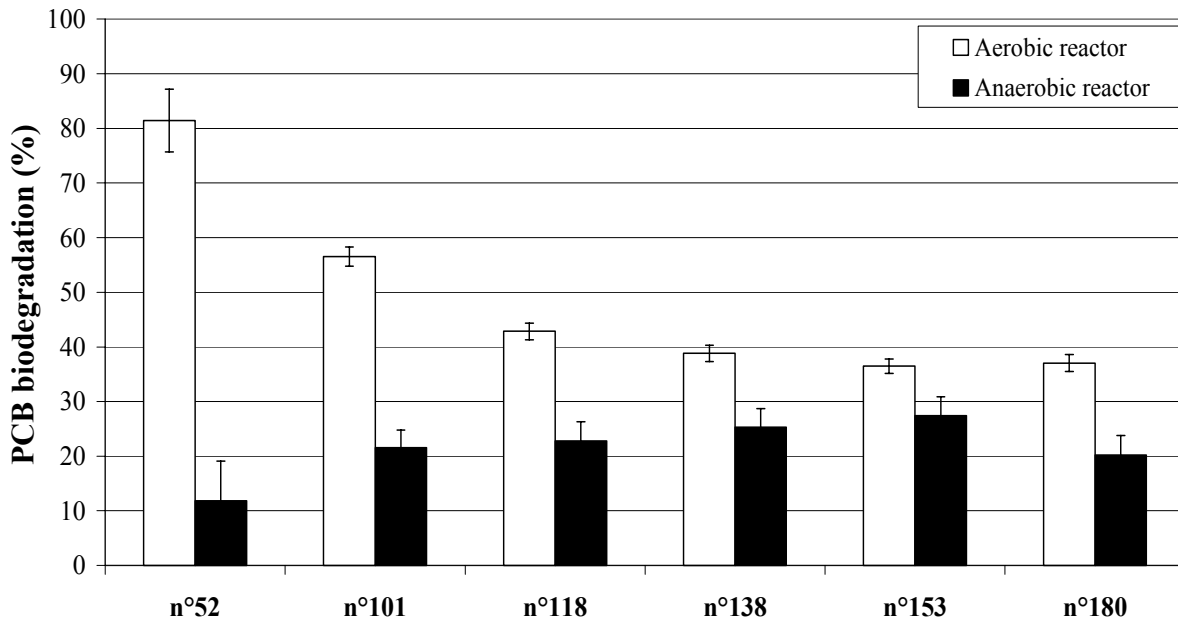
PCBs :		N°28	N°52	N°101	N°118	N°138	N°153	N°180	SUM
<b>INLET Sewage Sludge</b>	<b>(mg.kg<sub>DW</sub><sup>-1</sup>)</b>	nd	0,024	0,217	0,184	0,429	0,477	0,308	<b>1,639</b>
	<b>(μg.Γ<sup>l</sup>)</b>	nd	1	8,98	7,58	17,71	19,70	12,71	67,7
<b>Anaerobic Control reactor</b>	<b>(mg.kg<sub>DW</sub><sup>-1</sup>)</b>	nd	0,019	0,180	0,157	0,415	0,463	0,269	<b>1,503</b>
	<b>(μg.Γ<sup>l</sup>)</b>	nd	0,75	6,98	6,07	16,10	17,95	10,43	58,3
<b>Anaerobic Biological reactor</b>	<b>(mg.kg<sub>DW</sub><sup>-1</sup>)</b>	nd	0,024	0,190	0,164	0,438	0,473	0,296	<b>1,585</b>
	<b>(μg.Γ<sup>l</sup>)</b>	nd	0,63	5,04	4,34	11,61	12,55	7,86	42,0
<b>Aerobic Control reactor</b>	<b>(mg.kg<sub>DW</sub><sup>-1</sup>)</b>	nd	0,021	0,232	0,187	0,441	0,467	0,306	<b>1,655</b>
	<b>(μg.Γ<sup>l</sup>)</b>	nd	0,82	9	7,3	17,12	18,1	11,88	64,18
<b>Aerobic Biological reactor</b>	<b>(mg.kg<sub>DW</sub><sup>-1</sup>)</b>	nd	nd	0,145	0,151	0,386	0,411	0,270	<b>1,363</b>
	<b>(μg.Γ<sup>l</sup>)</b>	nd	nd	3,84	4,02	10,23	10,91	7,17	36,18



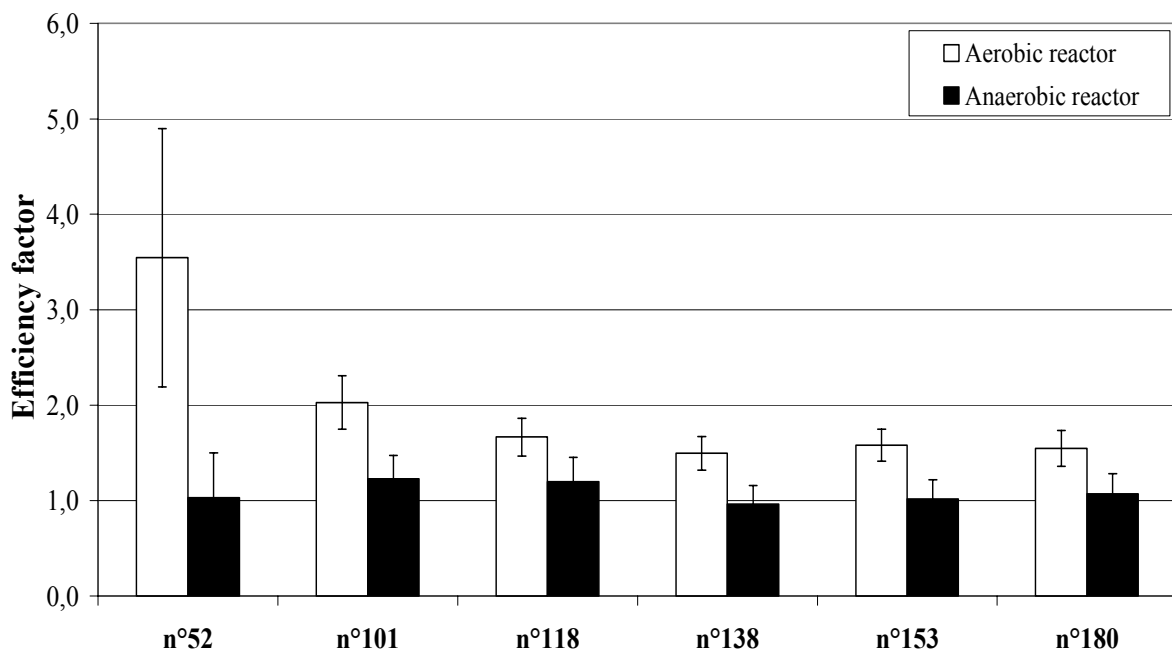
**Figure 1:** Abiotic losses of six priority PCBs in anaerobic and aerobic control sludge reactors at steady state.



**Figure 2:** PCB removal efficiency in anaerobic and aerobic biological reactors at steady state.



**Figure 3:** Actual PCB biodegradation efficiency after correction by the abiotic losses in anaerobic and aerobic biological reactors at steady state.



**Figure 4:** Efficiency factors in anaerobic and aerobic biological reactors at steady state. Efficiency factors correspond to the ratio of PCB removal efficiency (%) on TS reduction rate (%). High standard deviation for PCB n°52 in aerobic reactor results from the very low concentration after treatment (below detection limit).