

## Microbial reductive dechlorination of PCBs\*

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### Abstract

Reductive dechlorination is an advantageous process to microorganisms under anaerobic conditions because it is an electron sink, thereby allowing reoxidation of metabolic intermediates. In some organisms this has been demonstrated to support growth. Many chlorinated compounds have now been shown to be reductively dechlorinated under anaerobic conditions, including many of the congeners in commercial PCB mixtures. Anaerobic microbial communities in sediments dechlorinate Aroclor at rates of  $3 \mu\text{g Cl/g sediment} \times \text{week}$ . PCB dechlorination occurs at  $12^\circ \text{C}$ , a temperature relevant for remediation at temperate sites, and at concentrations of 100 to 1000 ppm. The positions dechlorinated are usually meta > para > ortho. The biphenyl rings, and the mono-ortho- and di-orthochlorobiphenyls were not degraded after a one year incubation. Hence subsequent aerobic treatment may be necessary to meet regulatory standards. Reductive dechlorination of Arochlors does reduce their dioxin-like toxicity as measured by bioassay and by analysis of the co-planar congeners. The most important limitation to using PCB dechlorination as a remediation technology is the slower than desired dechlorination rates and no means yet discovered to substantially enhance these rates. Long term enrichments using PCBs as the only electron acceptor resulted in an initial enhancement in dechlorination rate. This rate was sustained but did not increase in serial transfers. Bioremediation of soil contaminated with Aroclor 1254 from a transformer spill was dechlorinated by greater than 50% following mixing of the soil with dechlorinating organisms and river sediment. It is now reasonable to field test reductive dechlorination of PCBs in cases where the PCB concentration is in the range where regulatory standards may be directly achieved by dechlorination, where a subsequent aerobic treatment is feasible, where any co-contaminants do not pose an inhibitory problem, and where anaerobic conditions can be established.

### Introduction

Chlorinated organic chemicals probably constitute half of the environmental organic pollutant problems in the world today. The ability of aerobic microorganisms to degrade these chemicals is often limited by chlorine blocking the site of enzymatic attack. Consequently, some of these compounds are persistent in the environment. In the last decade, the ability of anaerobic microorganisms to reductively dechlorinate some of

these chemicals has become more widely recognized. This process has the following advantages: (i) reduction in the degree of chlorination making the product more susceptible to mineralization by aerobic microorganisms if it is not completely degraded by the anaerobic community, (ii) reduction in toxicity of the parent compound, and (iii) the relative ease of establishment of the appropriate *in situ* conditions conducive of dechlorination in many environments that contain these pollutants, i.e., principally, establishment of anaerobic conditions, which usually occurs following water saturation of soils and sediments. Hence, reductive dechlorination is important and often an economically

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feasible technology to be considered for remediation of chlorinated pollutants.

Bioremediation is a much simpler technology to manage if the pollutant provides a strong natural selection for growth of the biodegrading organisms. The more challenging situation is for co-metabolic processes in which substrates other than the pollutant are needed to stimulate a secondary and often indirect biodegradation process. Which case describes reductive dechlorination? Evidence suggests that reductive dechlorination does provide a selective advantage to at least some dechlorinating organisms, although the resultant growth advantage is not as great as for many aerobic organisms that grow on the pollutant chemical. The advantage of reductive dechlorination to anaerobes is not as a carbon and energy source, but as an electron acceptor (oxidant). The most limiting resource to microorganisms in anaerobic environments is not carbon substrates for growth, as it is in the aerobic environment, but for electron acceptors to allow these organisms to oxidize the abundant reduced substrates that usually accumulate in anaerobic environments. Dechlorination consumes electrons and, thus, chlorinated substrates represent a resource for any microorganism that has enzymatic capacity to transfer electrons to these compounds. It has recently been shown at the physiological level that an anaerobic organism, *Desulfomonile tiedjei* strain DcB-1, gains ATP for growth by using aromatic dechlorination as its sole electron acceptor (Dolfing & Tiedje 1987; Dolfing 1990; Mohn & Tiedje 1991). This organism can be grown on  $H_2$  or  $HCOOH$  as its sole electron donor using the conversion of 3-chlorobenzoate to benzoate and  $HCl$  as its electron acceptor (Dolfing 1990; Mohn & Tiedje 1990). This is accomplished by a chemiosmotic mechanism in which a proton gradient generated from the dechlorination drives ATP synthesis (Mohn & Tiedje 1991). This finding is particularly important because it established the basic precedent and underlying biochemical mechanism which shows that microorganisms can grow using chlorinated substrates as their sole electron acceptor. This demonstrates that there is a selective growth advantage provided by chlorinated chemicals in anaerobic environments. What is unknown is for what chemicals and how frequent are organisms that can take advantage of the organochlorine resource?

It is now becoming the rule rather than the exception to find that an organochlorine compound is reductively dechlorinated. More than 50 organochlorine compounds have now been shown to be reductively

dechlorinated, including many organochlorine pesticides (e.g., DDT, heptachlor, alaclor, 2,4-D), chlorinated solvents (e.g. PCE, TCE,  $CHCl_3$ , TCA), and arylhalides (e.g., PCB, chlorobenzenes, chloroguaiacols, chloroanilines) (Kuhn & Suffita 1989; Mohn & Tiedje 1992; Vogel et al. 1987). From these studies and others, the following important generalizations can be made:

- Microorganisms capable of reductive dechlorination seem to be relatively ubiquitous in nature, at least in anaerobic environments. The presence of these organisms in aerobic environments is not yet well investigated.
- Reductive dechlorination requires anaerobic conditions, with a few exceptions.
- Reductive dechlorination is a relatively slow process, with rate measurements often in terms of days to weeks, but it is not so slow as to be considered unfeasible, especially for *in situ* treatment.
- In general, there appears to be a specificity between particular dechlorinating populations and particular chemicals. There is yet no evidence for broad spectrum dechlorinators.
- Most dechlorinating cultures are consortia (mixed natural communities) and it has been very difficult to isolate in pure culture active dechlorinators from such communities. Thus, fermentor scale growth for inoculum to stimulate bioremediation is not yet feasible.

## Reductive dechlorination of PCBs

### Background

Polychlorinated biphenyls (PCBs) are one of the two chlorinated chemical groups that exhibit the most extensive environmental contamination. These chemicals were used primarily in the 1950s, 1960s, and 1970s in electronic equipment such as capacitors and transformers; hydraulic fluids and pump oils; and in non-contained uses such as adhesives, dyes, inks, and in carbonless copy papers. Some PCBs are still in service, particularly in transformers; others are in landfills, and in soils and sediments near where they were used or disposed. PCBs have very low solubility in water and, thus, should remain near the site of contamination. Unfortunately, because of the wide spread PCB use and significant disposal through municipal waste treatment systems, environmental distribution of PCBs in river systems, and thus in aquatic food chains,

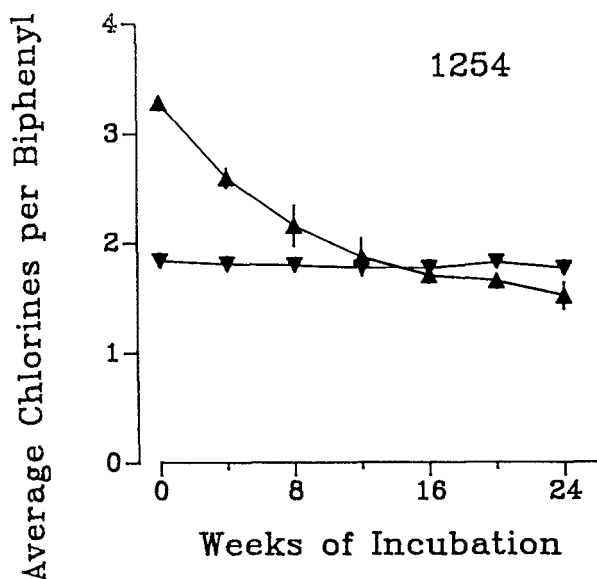


Fig. 1. Dechlorination of chlorines from the *meta* and *para* positions (▲) of 500 ppm Aroclor 1254 added to Hudson River sediment after inoculation with organisms from a site contaminated with Aroclor 1260. The *ortho* chlorines (▼) were not removed.

is more widespread than might have been expected for a water insoluble chemical.

Commercially used PCBs were complex mixtures of chlorinated biphenyls made up of different congeners resulting from the different number and position of chlorines on the two biphenyl rings. Commercial mixtures were sold under tradenames such as Aroclor, Kanechlor, Phenoclor, and Clophen followed by a number, larger numbers indicate a higher degree of chlorination. 209 different PCBs are possible and a commercial mixture has more than 50 different components. Historically, commercial PCB products were identified by attempting to match the major congeners in a environmental sample to those in manufactured mixtures; this assumes that the PCBs were not extensively metabolized. In a number of cases of PCB contaminated sediments, these assignments are likely in error because reductive dechlorination has substantially altered the congener profile from the original commercial mixture. Thus, it is important to consider the impact of reductive dechlorination on the congener profile before making an assignment about the particular type of commercial PCB contamination.

#### Rates and products of PCB dechlorination

Studies on PCB dechlorination have been conducted under three related conditions: (i) extent of *in situ* dechlorination (Brown et al. 1987), (ii) mixing sediments with indigenous PBC dechlorinating populations with other sediments or PCBs, and (iii) eluting PCB-degrading populations from sediments and using this population to inoculate other PCB contaminated soils or sediments. The latter approach was also used to prove that the observed transformation of PCBs to lesser chlorinated products was dependent on the microbial inoculant (Quensen et al. 1988; Quensen et al. 1990). This approach was used to obtain the results shown below.

The basic features of Aroclor dechlorination are shown for one of the more heavily chlorinated Aroclors, 1254, in Fig. 1. The PCB analytical data shown here and in subsequent tables and figures are from congener-specific analyses of the PCB components which are then corrected to molecular weight and summarized for all products based on those with chlorines in the *meta* + *para* positions versus those with chlorine in the *ortho* position (Quensen et al. 1990). As is true for all Aroclors, chlorine is preferentially removed from *meta* and *para* positions while chlorines in the *ortho* position are preserved (Quensen et al. 1990). Thus, complete dechlorination of PCBs is unlikely, although Van Dort and Bedard 1991) have recently shown that dechlorination can be observed from the *ortho* position when a single congener, 2,3,5,6-chlorobiphenyl was added to a dechlorinating sediment community. Figure 1 illustrates the typical time course of PCB dechlorination under laboratory conditions. In general, the rates of PCB dechlorination decrease as the degree of chlorination increases (Table 1). There is also a specificity that apparently is developed by the enriched population for the particular type of PCB at that site. For example, the population from the 1242-contaminated site was inefficient in dechlorination of Aroclor 1260 while the population from the 1260 site was active on 1260. Similarly, the 1260-exposed population showed less extensive dechlorination of 1242 than was observed with the 1242-exposed population (Table 1).

The rate of PCB dechlorination is affected by a number of factors (Tiedje et al. 1991). Those that have been studied can be summarized as follows.

– *PCB concentration*: Optimum rates of PCB dechlorination usually occur for concentrations in the range of several hundred to 1,000 ppm

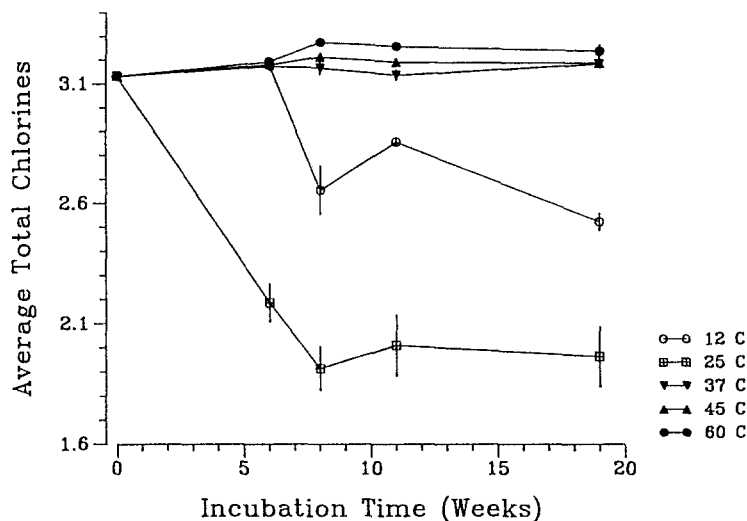


Fig. 2. Effect of incubation temperature on the dechlorination of Aroclor 1242 in Hudson River sediments.

Table 1. Maximum observed dechlorination rates of Aroclors for microorganisms collected from a site contaminated with Aroclor 1242 and from a site contaminated with Aroclor 1260<sup>a</sup> (From Quensen et al. 1990).

Aroclor at site	Aroclor added	Mean rate $\pm$ SD ( $\mu$ g-atoms of Cl <sup>-</sup> removed/g of sediment per week)	Period of rate (weeks)	% meta and para chlorine removed
1242	1242	0.31 $\pm$ 0.03	0–8	85 (12 wk) <sup>b</sup>
	1248	0.34 $\pm$ 0.01	0–8	75 (12 wk)
	1254	0.22 $\pm$ 0.02	0–8	63 (25 wk)
	1260	0.00 $\pm$ 0.03	0–25	0 (25 wk)
	1260	0.04 $\pm$ 0.005	16–24	15 (50 wk)
1260	1242	0.30 $\pm$ 0.02	0–4	46 (16 wk)
	1260	0.21 $\pm$ 0.01	12–16	19 (16 wk)

<sup>a</sup> Microbial population was eluted from the contaminated sediment and inoculated into clean sediment amended with the indicated Aroclor.

<sup>b</sup> Extent of dechlorination at indicated length of incubation.

(w/w) of sediment (Quensen et al. 1988). Below 50 ppm, dechlorination is often very slow or non-measurable.

– **Bioavailability:** PCBs may be dissolved in the organic phase of soils or sediments and are perhaps also protected under waxy layers of aged organic matter and do not reach biodegrading organisms. The requirement for moderately high concentrations of PCBs for a more rapid dechlorination may be partially a result of the lesser availability of the lower PCB concentrations.

– **Inhibitors:** PCBs are often not the sole contaminant present. Other contaminants such as oil and grease, heavy metals, and solvents can be toxic to organisms and restrict or eliminate dechlorination. We have found large concentrations of oil and grease to be most commonly associated with lower rates of dechlorination in sediments.

– **Temperature:** As shown in Fig. 2, 12 and 25°C temperatures support Aroclor 1242 dechlorination. Temperatures of 37°C or above showed no dechlorination, providing further support for the micro-

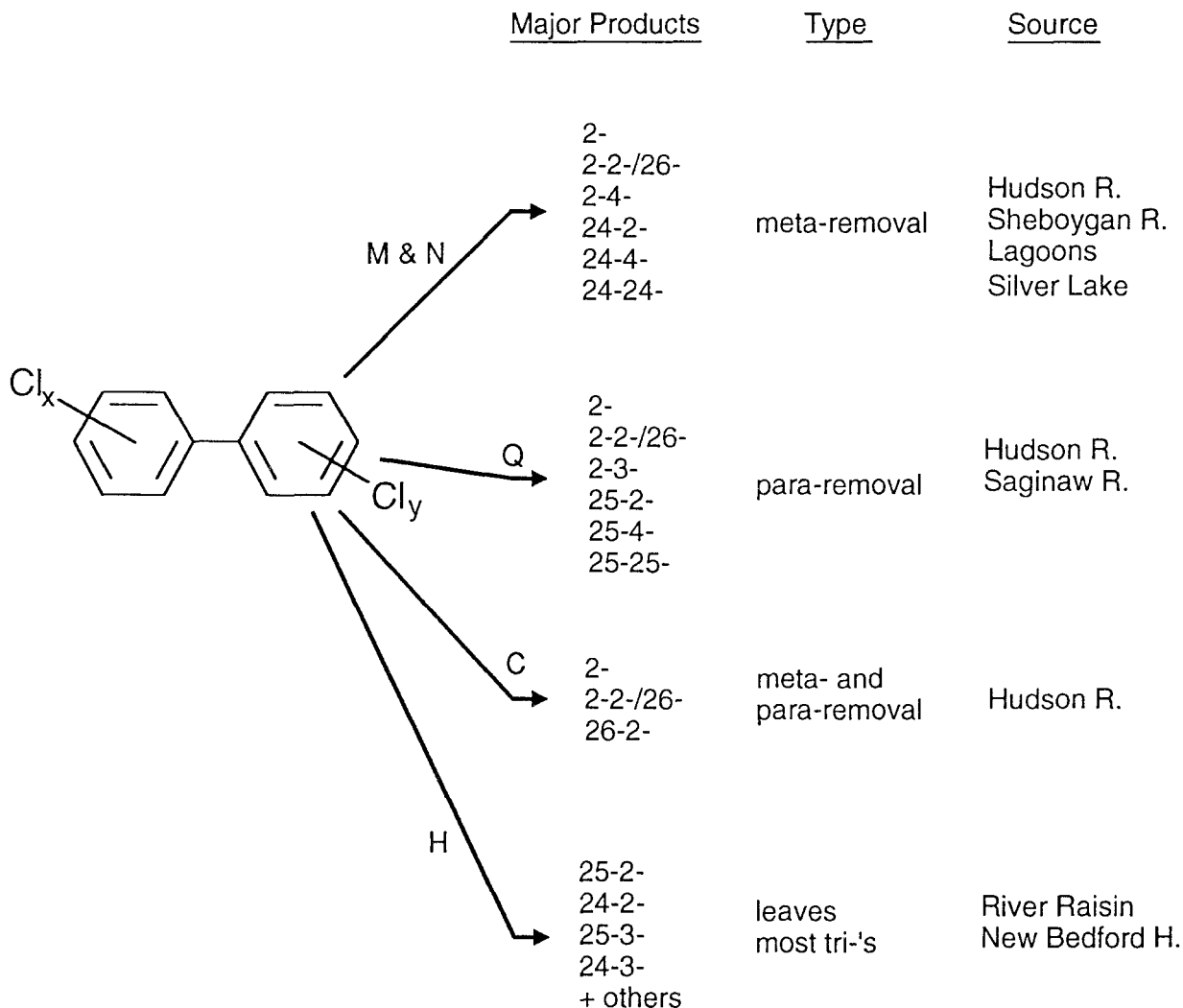


Fig. 3. Different sediments show different dechlorination products. Shown are the most common dechlorination patterns (shown by letter next to arrow), the major products, the principle type of dechlorination, and the habitat from which the inoculum was obtained that exhibited this type of dechlorination.

bial and enzymatic nature of this process. Dechlorination at 12°C is an important result because this temperature is a reasonable environmental temperature in temperate regions.

– *Nutrients*: Electron donors and mineral nutrients are needed for microbial populations. In an anaerobic environment mineral nutrients are rarely limiting, but quality electron donors may become limiting in deep sediments because these zones are usually isolated from a new supply of carbon from the water column.

Research has shown that new carbon additions do stimulate PCB dechlorination rates at least in some cases. This has been achieved in two ways: First, adding

a readily available carbon source to sediments such as methanol, glucose, acetone or acetate stimulated dechlorination rates by a factor of two to three (Nies & Vogel 1990). The second approach is by mixing or other physical disruption of sediments so that native carbon is made more available. In this case, added carbon usually provides no further enhancement of the dechlorination rate. A number of investigators have made many attempts to add various carbon, mineral and co-factor amendments in an attempt to stimulate dechlorination. So far, only marginal increases in dechlorination have been noted at best. For implementation of an invasive bioremediation process, stimu-

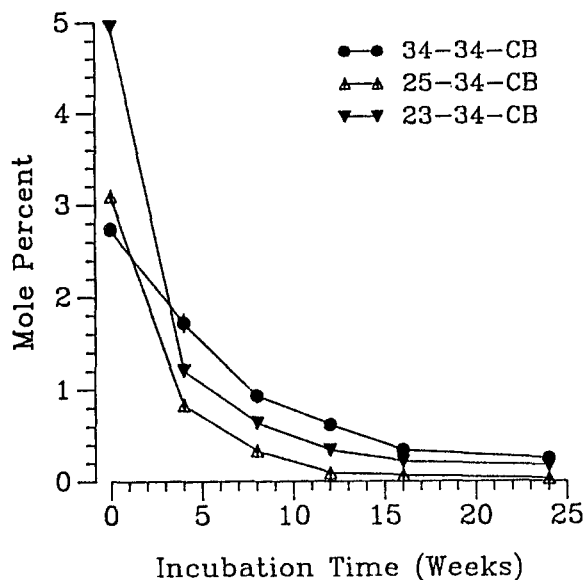


Fig. 4. Comparison of the dechlorination rates of a toxic coplaner tetrachlorinated biphenyl (34-34-CB) with non-toxic tetra-chlorinated biphenyls (25-34-CB, 23-34-CB) present in Aroclor 1242. Additional 34-34-CB was added to Aroclor 1242 to provide a more sensitive measurement of its dechlorination.

Table 2. Lack of further metabolism of lower chlorinated biphenyls after one year of incubation with dechlorinating sediments<sup>a</sup>.

Substrate	Treatment	nmoles recovered			
		Biphenyl	2-CB	2,6-CB	2,2'-CB
Biphenyl	autoclaved	505		—	—
	live	598	—	—	—
2-C1	autoclaved	0	440	—	—
	live	0	502	—	—
2,6-C1	autoclaved	0.1	7.7	433	—
	live	0.3	8.8	403	—
2,2'-C1	autoclaved	0	8.6	—	440
	live	0	12.6	—	524

<sup>a</sup> Single congeners at 100 µg/g sediment were incubated in clean sediment inoculated with an active Aroclor 1242 dechlorinating inoculum from the Hudson River.

lation of the PCB dechlorination rate by an order of magnitude likely would be necessary.

The type and extent of dechlorination varies with site. Figure 3 summarizes the major patterns of dechlorination observed with inocula taken from the indicated sediment. The pattern designations (letters) are described by Brown (Brown et al. 1988) based on characteristic products observed in different types of sedi-

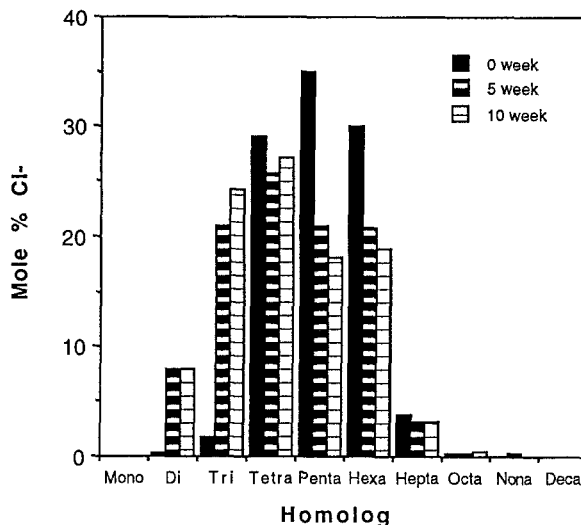


Fig. 6. Ability to dechlorinate Aroclor 1254 after 5 and 10 weeks by an enriched culture serially transferred seven times in the presence of Aroclor 1254.

ments. Three of the basic patterns are removal of *meta* chlorines (which is the most commonly observed pattern), removal of *para* chlorines, and removal of both *meta* and *para* chlorines. The most common but not unequivocally proven explanation for the different patterns is that they are the result of different populations with different congener specificity. If this explanation is true, it should be possible to inoculate sediment with different consortia of different specificities and achieve more extensive PCB dechlorination. We were unsuccessful in achieving this result when inocular with different apparent specificities were mixed together, but we were successful when the two different inocula were introduced sequentially after one-phase of dechlorination was complete.

The accumulation of lesser PCB products suggested that these may not be further degraded by anaerobes. We investigated this directly by incubating biphenyl and the predominant mono- and di-chlorinated PCB products with an active dechlorinating population in sediment for up to one year. We found no evidence that any of these products were further metabolized (Table 2). We measured change in substrate concentration as well as possible dechlorination products; the latter is a more sensitive indicator of lower amounts of dechlorination. Because of the low degree of chlorination, these chemicals were quantified by GC-mass spectrometry.

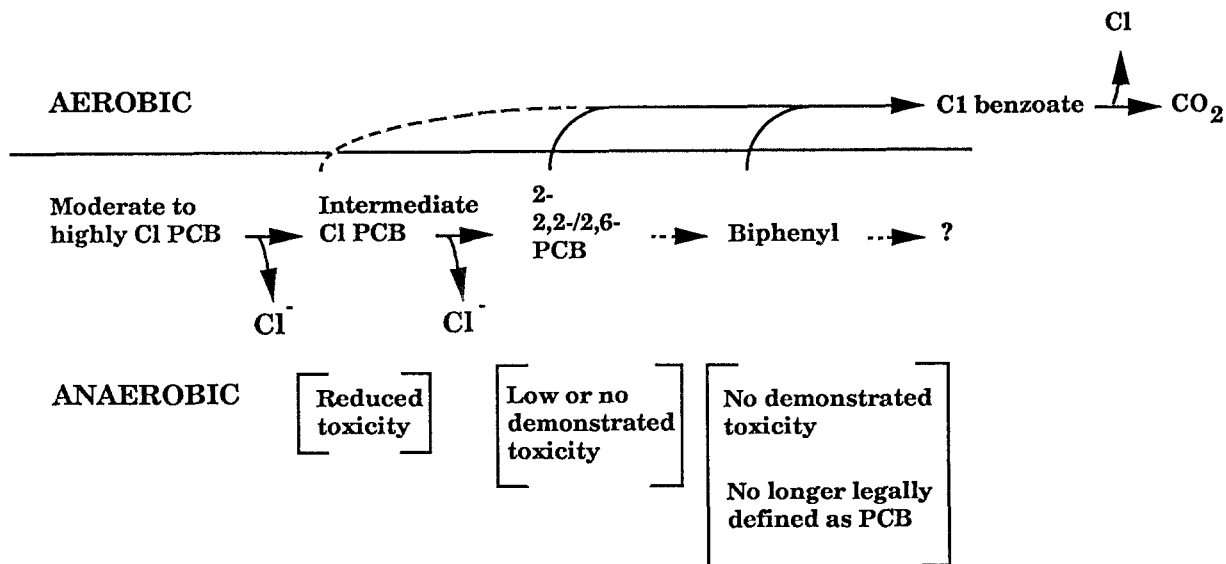


Fig. 5. Scheme summarizing evidence anaerobic and aerobic metabolism of PCB and where the processes can be coupled. Also shown are the changes in risk. Dashed lines indicate that these transformations are more rare.

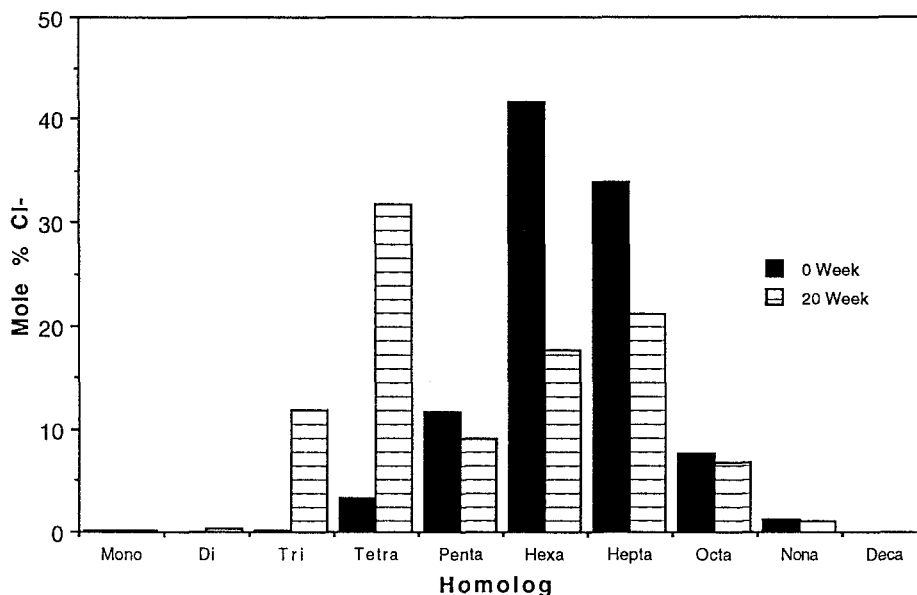


Fig. 7. Dechlorination of 5,000 ppm of Aroclor 1260 in a clean Hudson River sediment inoculated with a population enriched from an industrial sludge lagoon.

#### Value of partial dechlorination

The PCB dechlorination, although incomplete, does result in risk reduction. The coplaner PCBs are the components of commercial mixtures with the greatest demonstrated toxicity. These are structurally similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and exhibit

dioxin-like toxicity. However, this toxicity is dependent on chlorines in the *meta* and *para* positions of the PCBs, and it is these chlorines that we would expect to be removed. To investigate this directly, we measured the dechlorination of a toxic tetrachlorobiphenyl (34-34-CB) together with other tetrachlorinated biphenyls present in Aroclor 1242. All three tetrachlorobiphenyls

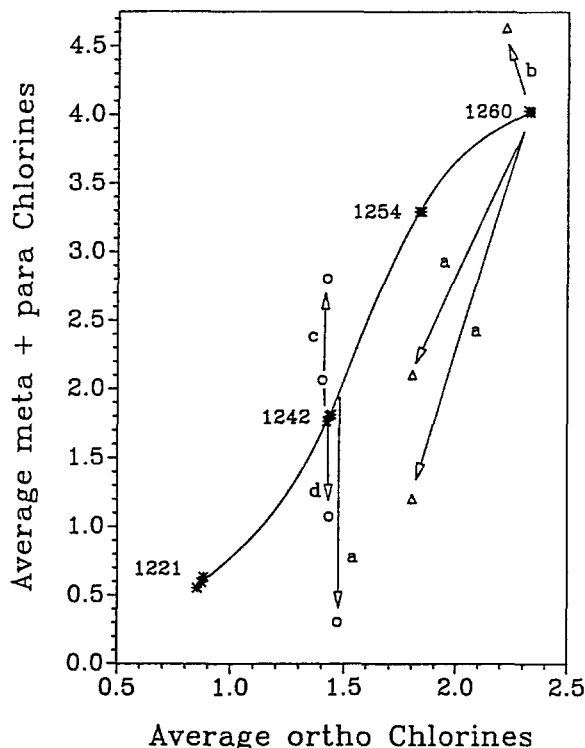


Fig. 8. Transformations of Aroclors 1260 ( $\Delta$ ) and 1242 ( $\circ$ ) as revealed by plotting the average number of ortho versus meta plus para chlorines. Unaltered Aroclors (\*) fall on an S-shaped line. Most transformations result in points that fall off the line. Transformations are: a – anaerobic dechlorination; b – P-450 mediated metabolism; c – evaporation; d – extraction into water. Figure and data adapted from Brown et al. (1988).

measured were removed at equivalent rates (Fig. 4). We have since confirmed that toxicity is reduced by toxicity bioassays (Giesy et al. 1992) and by congener specific tandem quadrupole mass spectrometry (Quensen et al. 1992). Thus, reductive dechlorination does reduce PCB risk, even though complete degradation does not occur. It should also be pointed out, however, that the *ortho* enriched PCBs are not major components of Aroclor mixtures and, therefore, have not been subject to extensive toxicology testing.

Despite reduction in toxicity, legal statutes may dictate removal of PCBs beyond that achieved by anaerobic organisms. Because the dechlorinated products can be metabolized by aerobic microorganisms (Abramowicz 1990; Bedard et al. 1987) it is feasible to sequentially couple anaerobic and aerobic treatment (Fig. 5). Substantial reduction of PCBs by the sequential treatment has been demonstrated in the laboratory (Nies et al. 1990; Abramowicz et al. 1990; Morris et al. unpub-

lished data). Successful field application of this concept depends upon the following: (i) extensive dechlorination of the PCBs so that the aerobic organisms can more efficiently mineralize the residual PCBs, (ii) most known aerobic PCB degraders grow on biphenyl and not the chlorinated PCBs, so the substrate to enrich the appropriate aerobic PCB-degrading population may not be present. Since biphenyl degrading enzymes are close relatives of those involved in toluene and naphthalene degradation, co-contamination with petroleum derivatives may actually aid the aerobic phase of the PCB treatment, and (iii) successful distribution of air or  $H_2O_2$  in the anaerobic matrix to support sufficient aerobic metabolism. Since these limitations can be minimized, it is now possible to attempt field tests of the sequential anaerobic-aerobic concept.

#### Enrichment of PCB dechlorination

For practical application of PCB dechlorination, it is important to evaluate the degree of enrichment that can be obtained using Aroclors as the sole electron acceptor and natural sediment carbon as the electron donor. We evaluated this principle using high concentrations of PCBs to maximize selection (5,000 ppm). We were able to enhance activity on Aroclor 1254 and 1260 and successfully transferred each population through seven serial transfers (Fig. 6). Activity increased with the first serial transfer, but after that transfer the activity was sustained at that level and not further increased. This suggests that enrichment towards a highly selected, very active population is not occurring in a manner expected for conventional enrichments. Thus, enrichment in nature may not be a feasible approach.

An enrichment from an industrial lagoon that was transferred as described above was tested for its ability to dechlorinate the most highly chlorinated Aroclor, 1260. As shown in Fig. 7, tri- and tetrachlorinated PCBs were produced at the expense of hepta- and hexachlorinated PCBs. Aroclor 1260 congeners are completely resistant to metabolism by any known aerobic biphenyl degraders and, thus, the anaerobic community is the only known biotransformation mechanism for this product.

#### PCB remediation

Remediation by reductive dechlorination involves insuring that the contaminated soils or sediments are anaerobic. In the case of sediments, this may naturally be the case. In the case of soils, it may be necessary

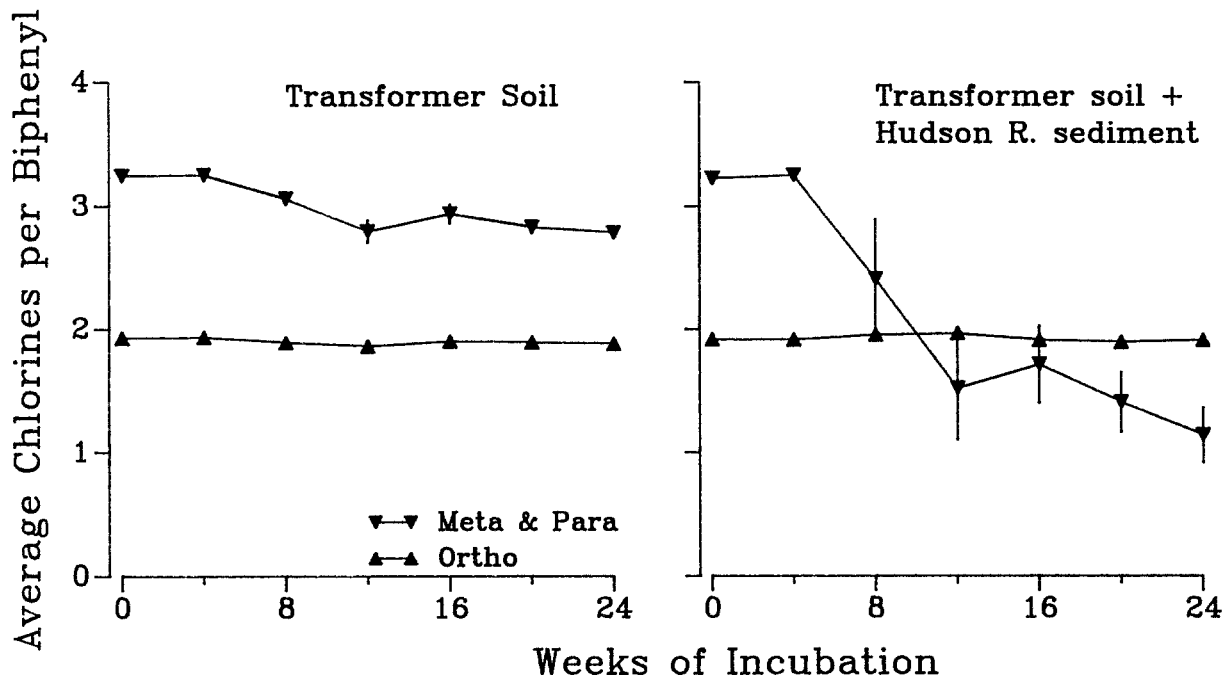


Fig. 9. Anaerobic reductive dechlorination of a soil contaminated by a transformer spill of Aroclor 1254. The left figure shows dechlorination when the soil was made anaerobic and the right figure shows dechlorination when the soil was inoculated with PCB-dechlorinating organisms eluted from the Hudson River and mixed 1 : 1 with clean Hudson River sediment.

to flood the soils if leaching can be prevented or may be partially achieved by adding a cheap available carbon source. For a habitat that is naturally anaerobic, such as sediments, it has been important to estimate the existing rate and extent of dechlorination. Remediation schemes may be: (i) to continue the *in situ* incubation, (ii) to attempt to stimulate the dechlorination rate by mixing the sediment and/or adding soluble carbon, or (iii) to cap the site to ensure anaerobiosis and containment.

To evaluate the processes that have affected the PCBs in nature and to determine if reductive dechlorination has occurred, one can map the measured PCB congener mixture on the S-shaped curve (Fig. 8) described by Brown et al. (1988). This curve is particularly helpful for mixtures of Aroclors, or if the type of PCB contamination is unknown, since all mixtures fall on the S curve. Since reductive dechlorination is the only mechanism that removes mainly *meta*- and *para*-chlorines, samples of residues from sediments that fall below the line are diagnostic for reductive dechlorination. The profiles resulting from other processes are also shown in Fig. 8 for comparison. The congener distributions in air and the remaining residue as a result of

evaporation should be complimentary. The samples for the residue should fall above the line as indicated in the figure while an air sample would fall below the line. The same should hold for the congener distributions in water and sediment as a result of dissolution. Water samples should result in the point falling below the line as shown in Fig. 8 while the PCBs remaining in the sediment should result in a point above the line. PCBs metabolized by aerobic oxidation by P-450 metabolism should fall above the line and those metabolized by aerobic microorganisms using 2,3-dioxygenases should fall to the right and upward from the line.

Remediation of soil by reductive dechlorination has been less well studied. However, in the one case we have studied, a soil contaminated with transformer oil (Aroclor 1254) was dechlorinated if PCB dechlorinating organisms from the Hudson River sediment were added to the soil and incubated under anaerobic conditions (Fig. 9). The transformer soil was also mixed 1 : 1 with clean upstream Hudson River sediment, which may have provided nutrients to the sediment community. If the soil was simply made anaerobic, very little dechlorination was observed within the 24-week incubation. The dechlorination of this naturally contami-

nated soil occurred at an equivalent rate constant to that found for Aroclor 1254 added to Hudson River sediment (Fig. 9 versus Fig. 1). Thus, the soil remediation was equal to the best rate of PCB removal we achieved in sediment.

## Conclusion

Reductive dechlorination of chlorinated organic compounds, including PCBs, is a remediation process that should be considered as it is often easy to achieve and may be the cheapest bioremediation technology. Before reductive dechlorination of PCBs can be a widely accepted treatment, it must be optimized and field tested so that the target concentrations can be achieved. The greatest needs are a means to enhance the rate of reductive dechlorination and schemes to insure success of the subsequent aerobic metabolism in a coupled anaerobic/aerobic process. In many cases, it may also be necessary to enhance the bioavailability of the PCBs to the PCB degraders. Nonetheless, research progress in recent years has now made it worthwhile to field test methods for PCB bioremediation.

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