Aerobic Biodegradation of Polychlorinated Biphenyls

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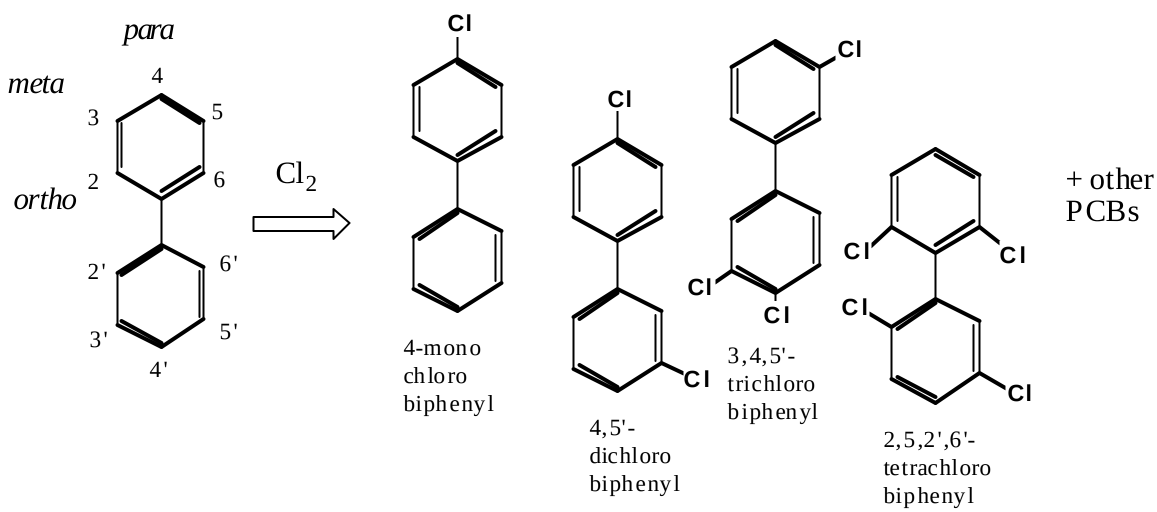
Composition and properties of PCBs

PCBs are produced by direct chlorination of biphenyl:



209 combinations, or congeners are possible, up to decachlorobiphenyl, but only about half were produced in commercial PCB synthesis. The commercial PCB mixtures were sold under the names Aroclor (Monsanto, US), Phenochlor, Clophen, and Kanechlor with the degree of chlorination indicated by a number. Thus Aroclor 1254 had 12 carbons and 54% chlorine by weight.

PCBs were widely used as transformer dielectric fluids, heat transfer fluids, hydraulic fluids, plasticizers due to their properties of low vapor pressure, low aqueous solubility, excellent dielectric properties, stability and inertness.

PCBs accumulate in biological tissues and have been associated with decreased wildlife populations, particularly birds (bioaccumulation). Carcinogenicity and teratogenicity of some highly chlorinated PCB congeners and mixtures has been observed but is still controversial.

Most environmental contamination by PCBs is as complex highly chlorinated mixtures: e.g. Aroclors 1242, 1254, and 1260. Due to their low aqueous solubility, low volatility, and high octanol/water partitioning coefficients, PCBs are generally concentrated on sediment and soil surfaces, both organic and inorganic.

Biodegradation of PCBs

Biodegradation of PCB mixtures requires broad specificity and multiple types of attack. PCBs are known to biodegraded in 3 general ways:

Aerobically as a growth substrate. Bacterial. Less chlorinated PCB degraded faster

Aerobically by cometabolism. Either by bacteria that grow on other PCBs or by white-rot fungi (e.g., *Phanerochaete chrysosporium*)

Anaerobically by reductive dehalogenation. Replacement of chlorines by hydrogen in anaerobic sediments. Unknown organisms and mechanism.

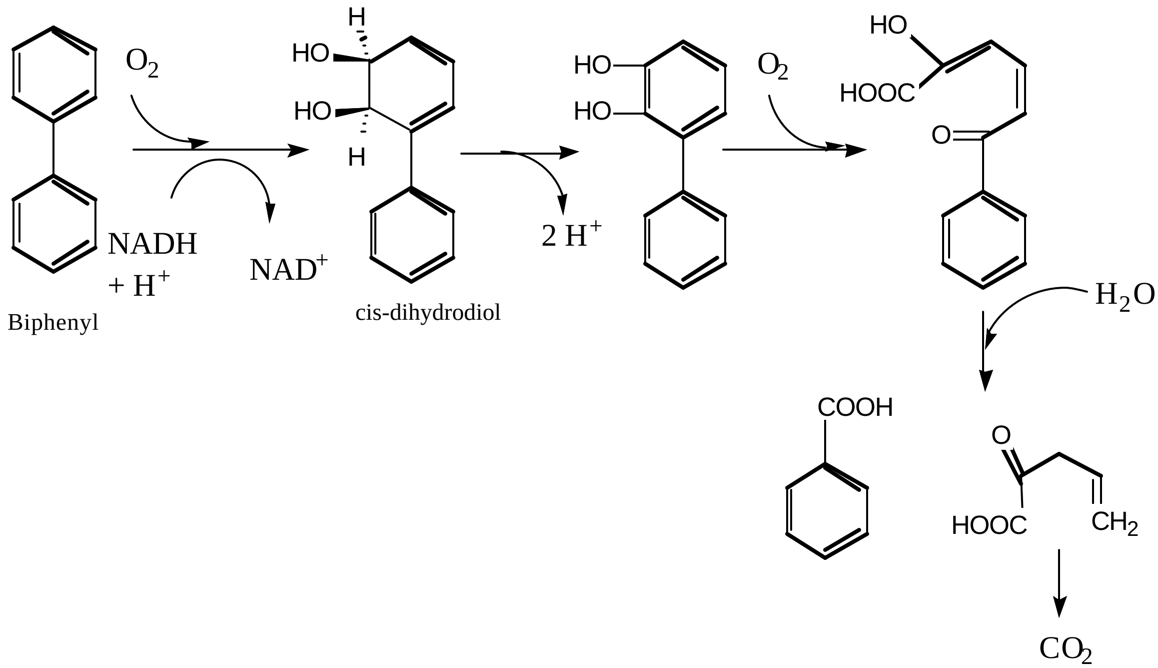
Bacterial Degradation of Biphenyl as a growth substrate

Most aerobic PCB degraders are obligate aerobes, motile, gram negative rods, enriched by growth on **biphenyl** and selected for by their ability to grow on biphenyl or PCB agar plates and by clearing of biphenyl or PCB films around colonies on agar.

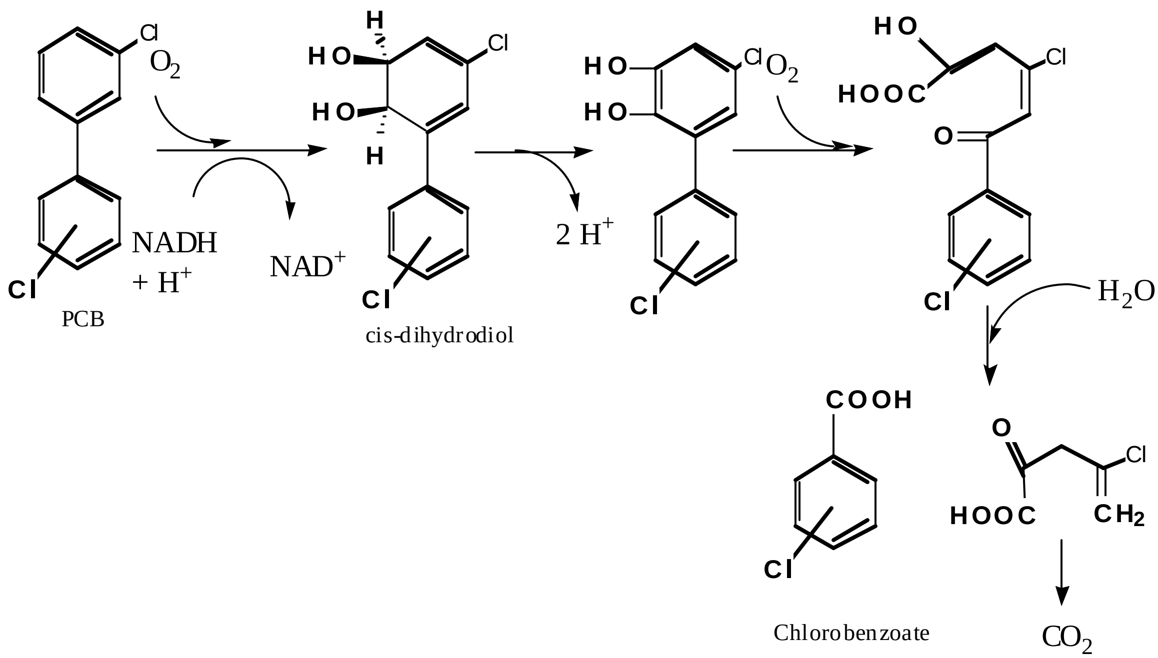
Degradation of mono-, di-, and tri-chlorinated biphenyls is relatively common as shown in the accompanying table from Bedard et al. [1]. Only a few strains are capable of degrading PCBs with more than 4 chlorines.

Pathways of aerobic PCB degradation

Biphenyl is degraded by a large variety of bacteria capable of degrading aromatics. Attack is by a **2,3-dioxygenase** attack on the 2,3 carbons and a **metacleavage**, producing benzoate:



Similarly there are many bacterial strains from the genera, *Pseudomonas, Vibrio, Aeromonas, Micrococcus, Acinetobacter, Bacillus,* and *Streptomyces* that degrade mono-, di-, tri-, and some tetrachlorinated PCBs by meta-cleavage of unchlorinated 2,3-carbons:

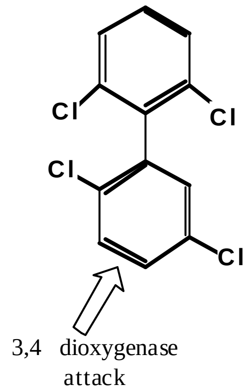


Oxidation may also take place on the chlorinated ring, if the 2,3 carbons are not obstructed. This pathway for PCB degradation was worked out in the labs of Focht, Furukawa, Gibson, in the early 70s, and is the same as the pathway for toluene and other aromatics.

Some strains are exceptional PCB degraders: *Pseudomonas* LB400, *Alcaligenes eutrophus* H850, *Corynebacterium* MB1 and *Acinetobacter* P6.

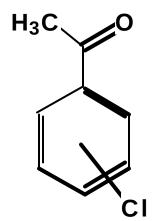
The specificity of the dioxygenases in these organisms differs greatly. Strains P6 and MB1 are particularly active against double *para* chlorinated PCBs.

H850 and LB400 preferentially express a 3,4-dioxygenase, forming a *cis*-dihydrodiol from 2,5,2',6'-tetrachlorobiphenyl, which is degraded faster by H850 than PCBs with an unchlorinated ring [2].



2,5,2',6'-tetrachlorobiphenyl

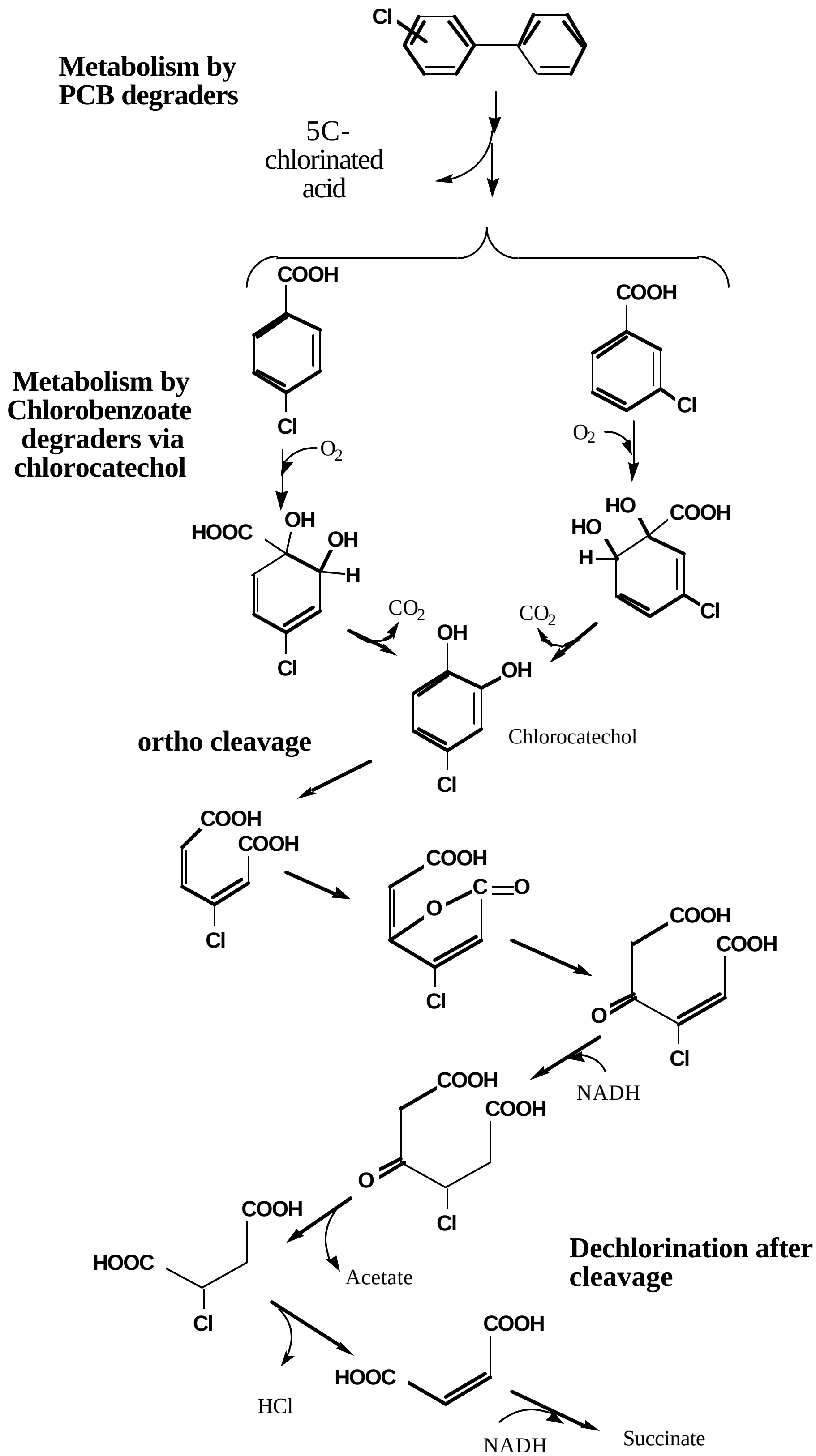
Both types of attack also yield chloroacetophenones by a pathway that is not understood:



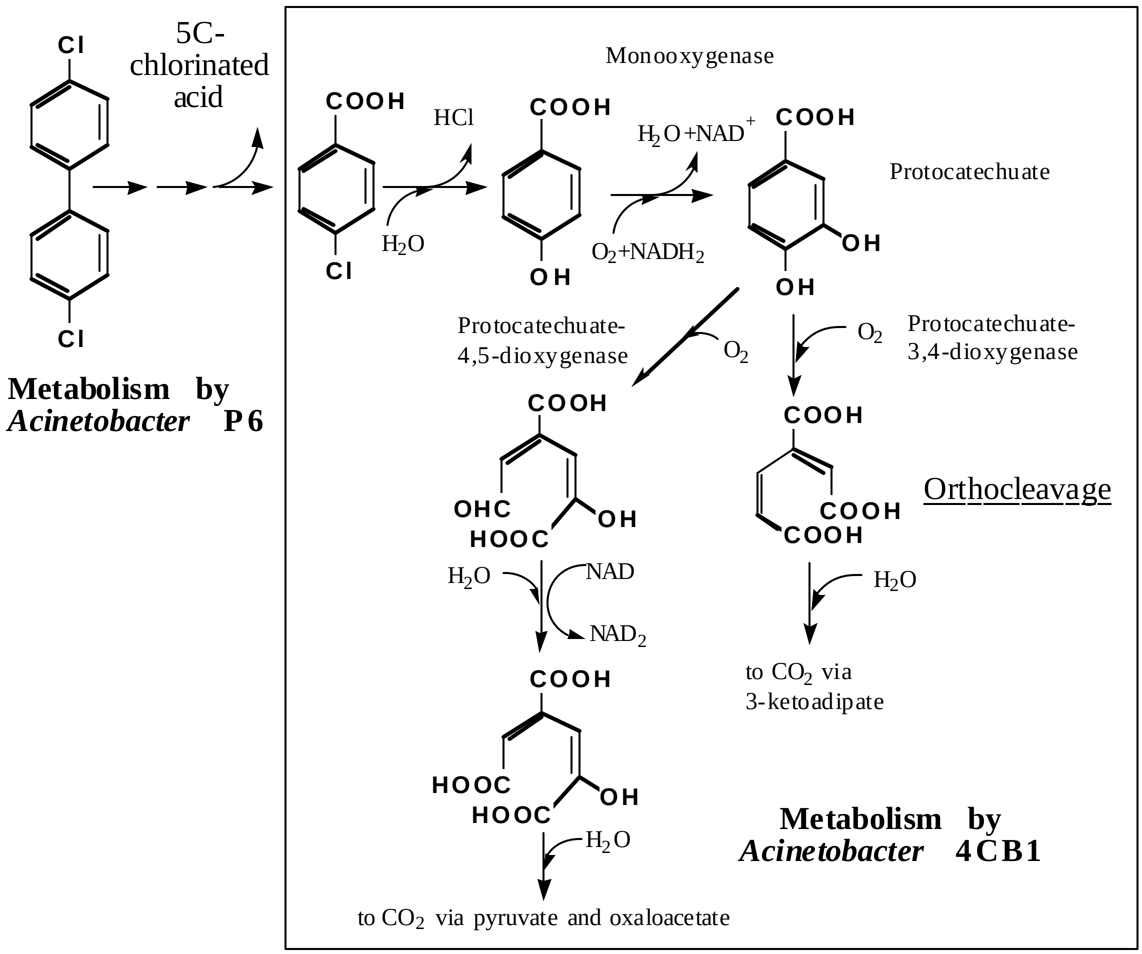
<u>Degradation of PCB cleavage products</u>

The chlorobenzoic acids are not further degraded by most PCB-degrading bacteria. An exception is the degradation of 2,3'-dichlorobiphenyl by LB400. For other cultures, a consortium of bacteria is necessary to completely mineralize PCBs.

Most chlorobenzoates are degraded via chlorocatechol [3, 4].

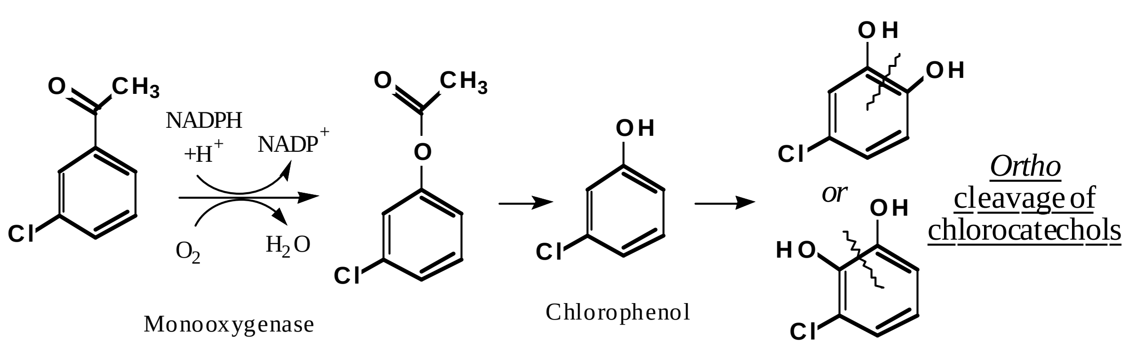

Metabolism by PCB degraders
5C- chlorinated acid
Metabolism by Chlorobenzoate degraders via chlorocatechol
ortho cleavage
Chlorocatechol
NADH
Acetate
Dechlorination after cleavage
HCl
NADH
Succinate

A coculture of strain P6 and *Acinetobacter* strain 4CB1, isolated on 4-chlorobenzoate, was able to completely degrade 4,4'-dichlorobiphenyl [5].



Degradation of chloroacetophenones

A *Alcaligenes* ACA, which was enriched on acetophenone, could cometabolically degrade several chloroacetophenones via biological Baeryer-Villiger reaction [6]:



Fungal Degradation of PCBs by cometabolism

Aspergillus niger , a filamentous fungus with cytochrome P-450 activity, is able to attack lowr chlorinated PCBs.

Phanerochaete chrysosporium, the white-rot fungus, are able to attack even highly chlorinated PCBs, but only at very low concentrations (<500 ppb), while the aerobic bacteria are able to degrade PCBs at levels of 10 ppm.

Bacterial PCB degradation genetics

Genes involved in PCB degradation are similar across strains and environments, implying genetic transfer.

Recombinant strains have been constructed using the 2,3-dioxygenase pathway and a broad-host-range plasmid. 3 of the 4 enzymes involved are on a 7.9kb DNA fragment. The 2,3-dioxygenase gene, *bphC*, has been sequenced.

The broad PCB degradative capability of LB400 has been duplicated in *E. coli* recombinants which contained all 4 of the PCB degradative genes on a 12.4kb fragment. The recombinant did not require biphenyl for optimal PCB degradation.

Application to PCB degradation in soils and sediments

Degradation of PCB is inducible in known PCB degraders, so presence of other carbon sources like glucose and low levels of PCBs and biphenyl may result in repression of PCB degradative pathways. PCB degradation in soils is increased by adding biphenyl to soils.

PCB degradation rates in soils may be >50 times slower than in laboratory cell suspensions due to binding of PCBs to soil surfaces. The use of surfactants or chitin can increase the availability of the PCBs and their degradation rate in soils and sediments

Summary

Aerobic bacteria attack mono-, di-, tri-, and some tetrachlorobiphenyls using 2,3-dioxygenase and meta cleavage; the more chlorines the lower the rate.

Some bacteria (H850, LB400) are able to use 3,4-dioxygenase to attack a few highly chlorinated PCBs

PCB degrading aerobic bacteria grow on biphenyl and require it or similar compounds as an inducer.

Other bacteria attack products of PCB cleavage: chlorobenzoates and chloroacetophenones.

These bacteria are widely distributed in contaminated soils.

Genes involved in PCB degradation are similar across strains and environments, implying genetic transfer.

References

1. Bedard, D.L., *et al.*, *Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls.* Appl. Environ. Microbiol., 1986. **51**: p. 761-768.

2. Bedard, D.L., *et al.*, *Evidence for novel mechanisms of polychlorinated biphenyl metabolism in Alcaligenes eutrophus H850.* Appl. Environ. Microbiol., 1987. **53**: p. 1103-1112.

3. Hickey, W.J. and D.D. Focht, *Degradation of monohalogenated, dihalogenated, and trihalogenated benzoic acids by Pseudomonas aeruginosa jb2.* Applied and Environmental Microbiology, 1990. **56**: p. 3842-3850.

4. Neilson, A.H., *The biodegradation of halogenated organic compounds - a review.* Journal of Applied Bacteriology, 1990. **69**: p. 445-470.

5. Adriaens, P., *et al.*, *Bacterial dehalogenation of chlorobenzoates and coculture biodegradation of 4,4'-dichlorobiphenyl.* Appl. Environ. Microbiol., 1989. **55**: p. 887-892.

6. Higson, F.K. and D.D. Focht, *Bacterial degradation of ring-chlorinated acetophenones.* Applied and Environmental Microbiology, 1990. **56**: p. 3678-3685.

Reviews

Abramowicz DA (1990) Aerobic and anaerobic biodegradation of PCBs: a review. Critical Rev. Biotechnol. 10, 241-251

Furukawa K (1986) Modification of PCBs by bacteria and other microorganisms. PCBs and the Environment, Volume II, Chapter 6, Waid JS, Ed., CRC Press, Inc., Boca Raton, Florida, pp 89-100.