# MICROBIOLOGICAL TREATMENT OF CHLOROPHENOLIC WASTES

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## Abstract.

Pentachlorophenol (PCP) containing wastes can represent an environmental hazard. Mineralisation of PCP by selected microorganisms offers a method for both disposal of pressure treated timber, and restoration of contaminated land. Three steps in the development of such bioremediation processes are considered in this paper: the selection of PCP-degrading organisms; the requirement for detailed metabolic studies in the laboratory; and the problems and limitations associated with the scale up of promising laboratory techniques to real-life waste treatment.

## Introduction.

Since the early 1950s, pentachlorophenol (PCP) has been used extensively in timber preservation for the control of both sap-staining and decay fungi. The use of PCP has however generated problems, such as the development of satisfactory waste disposal options for pressure-treated timber removed from service, and site contamination. Contamination of soil has occurred around both treating plants, where PCP in oil or creosote is used, and sawmills, which have used water-soluble chlorophenol salts in dip-tank treatments (12). Clean up of such sites may be required when site use changes, or ground water becomes threatened.

The waste disposal problems associated with PCP and other lower chlorinated phenols arise for two main reasons. Since aryl carbon-chlorine bonds rarely occur in natural compounds, very few organisms have evolved the necessary transport and degradative enzymes required for PCP metabolism, thus PCP is persistent in most environments. Pentachlorophenol also has a broad spectrum of toxicity (8). By un-coupling oxidative phosphorylation, the major energy producing mechanism for most oxygen-utilising organisms, PCP is toxic not only to fungi but also plants, wildlife and man. For these reasons, PCP is considered a serious environmental pollutant (24).

Currently, there are two major options for the disposal of PCP contaminated wastes, landfilling and incineration. Unfortunately, both these routes have associated problems, such as the limited capacity of landfill sites and the requirement for leachate monitoring and control, or the public

resistance to both landfilling and the construction of incineration facilities. With an estimated removal rate of 200,000 to 250,000 PCP-treated utility poles *per annum* in Canada alone, it is necessary to develop suitable disposal plans to prevent environmental damage from these materials.

In nature, the activities of microorganisms are ultimately responsible for the recycling of organic compounds (6). The harnessing of microorganisms to safely degrade PCP in soil, or wood, offers a means of removing the chemical before unwanted affects occur. Much research into the bioremediation of PCP-contaminated soil has been carried out over the past decade (14, 23, 5). This paper describes the steps involved in the development of such processes such as the selection of suitable organisms, the requirement for detailed metabolic studies, and the scale-up of successful laboratory systems, with reference to their applications in disposal of PCP-pressure treated utility poles.

## Selection of chlorophenol degrading organisms.

Karns *et al*. (10) identified three problems which must be overcome before microbial inoculants can be used successfully as decontamination agents. They described the foremost difficulty as finding a strain capable of the degradation of the pollutant chemical; other difficulties identified included the availability, in any waste treatment system, of carbon and energy sources other than the target chemical and the competition for space and nutrients from the indigenous microflora. There are now however several reports of microbial species capable, under laboratory conditions, of PCP degradation (2, 22); and more recent work has shown that achieving the survival and activity of such strains in actual waste-treatment situations is becoming the more challenging problem (4, 23).

The most common, and powerful, technique to obtain micro-organisms with the potential to degrade chlorophenols and other xenobiotics is that of enrichment. Enrichment techniques select the population(s) of interest by encouraging its growth, at the expense of other organisms, by manipulation of the enrichment culture environment (20, 29). In most cases, the initial inoculum is obtained from soil with a history of exposure to the target chemical, and the chemical is supplied as the sole source of carbon and/or energy, under otherwise ideal growth conditions, thus enriching for populations able to degrade the chemical.

Enrichment procedures can use either closed or open culture methods (19, 25). In a closed or batch system, after the initial conditions are set, there is no further input of nutrients or removal of cells and waste metabolites, although

provision for gas exchange is usually made. Batch systems start under non-limiting conditions therefore high initial substrate concentrations are required, and are characterised by continuously changing conditions as nutrients are used and metabolic products accumulate. Due to the high concentrations of substrates provided, batch enrichments select for organisms with the highest growth rates (r-selected). Such organisms can be likened to opportunists, rapidly able to exploit non-limiting conditions, but unsuited for long term survival in changing surroundings.

Open or continuous culture, on the other hand, has both inputs of fresh nutrients and outlets of cells and their products. The most common form is the chemostat, where growth is limited by one known compound, usually the xenobiotic carbon source. At a constant volume, the growth rate of the selected organisms is fixed by the rate of dilution, consequently the chemostat can be used to select for organisms with high affinities rather than high growth rates (K-selected; Figure 1).

Most enrichments for PCP-degrading organisms have been batch or closed cultures, which, under aerobic conditions, commonly yield r-selected, Gram negative organisms (19); the activity and survival of these types in waste treatment soil may be poor as concentrations of pollutant available for growth may be low (1, 9). Thus a more suitable choice for a microbial inoculant could be either a K-selected organism, requiring continuous culture enrichment, or mixtures of K- and r- selected organisms.

**The requirement for metabolic studies.**

Table 1 lists some of the organisms which have been obtained for PCP degradation by both enrichment and screening methods. Detailed studies on the metabolism of PCP by two of these organisms, *Flavobacterium* sp. and *Rhodococcus chlorophenolicus*, have been carried out (26, 3). In order to understand why such intensive studies are required before use of microbial treatment of chlorophenols on a large scale, it is important to distinguish between disappearance and mineralisation of the pollutant. Mineralisation can be defined as the complete conversion to mineral elements. In the case of aerobic mineralisation of PCP, these are water, carbon dioxide and inorganic chloride. Mineralisation to harmless components is therefore the ideal for any proposed microbial treatment. High rates of PCP disappearance, not matched by high mineralisation, would suggest biotransformation, rather than biodegradation, of PCP, which may result in the accumulation of chlorinated metabolites, of equal or greater environmental concern than PCP itself.

An example of this is the metabolism of PCP by the white-rot fungus *Phanerochaete chrysosporium*. Lamar, Glaser and Kirk (15) noted that inoculation of PCP-contaminated soil with this fungus resulted in a 98% loss of PCP, but only 2% mineralisation (Table 1), with the unaccounted PCP being converted to non-volatile bound intermediates. Recent attempts (17) to use this fungus for the remediation of pressure-treated timber, also confirmed extremely high disappearance of PCP (95% in two weeks from a starting concentration of 500 ppm). Gas chromatography linked mass spectrometry studies showed however that 70-80% of PCP had been O-methylated to pentachloroanisole, with a tetrachloroanisole as a minor (< 1ppm) metabolite (Figure 2). Pentachloroanisole is considerably more lipophilic and volatile than PCP, and thus is far more liable to bioaccumulation in the fatty membranes of living organisms, with possible deleterious environmental consequences.

The metabolic studies of both *Flavobacterium* sp. and *R. chlorophenolicus* have shown 30 to 60% mineralisation of PCP, with the rest being converted to biomass. It seems therefore that these would be ideal candidates for a biological treatment of soil or timber.

**Scale up of suitable laboratory species to real situations.**

Under ideal conditions in the laboratory *Flavobacterium* sp. is capable of rapid PCP degradation (30-50 ppm in less than six hours; 27). In attempts to remediate PCP-treated wood, this same bacterium was only capable of 20-25% degradation in 8 weeks. There are a number of factors which influence the transfer from ideal conditions to soil, water or wood (Table 2). Some of these factors are intuitive, for example biological temperatures and pH ranges are necessary; others are less obvious, such as competition for space, or predation, from other microflora. Yet others vary from situation to situation, for example the addition of a labile carbon source, such as simple sugars or amino acids may stimulate the degrading population or be used in preference to the target pollutant. In the case of *Flavobacterium* sp. the minimal rate of PCP in wood appears to be due to the inaccessiblity of the PCP to bacterial enzymes. In a recent development, a suspended solids model allowing continuous extraction and degradation of PCP by this species allowed 99% degradation of PCP in 4-6 days (17).

**Limitations of microbial treatments.**

Most methods of bioremediation to date depend on the degrading organisms gaining a growth advantage by breaking down the pollutant. In practice, therefore, microbial treatments will be limited to point sources containing

relatively high concentrations of pollutants, such as industrial effluents, accident and spill sites, and ground water plumes (16). Biological treatment may still be possible where pollutants are present at extremely low concentrations and/or adsorbed to a matrix by leaching of the contaminated site or material, with subsequent treatment of the leachate (5). Concentration of pollutants from dilute aqueous solutions is possible, for example Valo et al. (28) concentrated chlorophenols in groundwater by filtration through polyurethane at the natural groundwater temperature of 4°C. The polyurethane also contained immobilised cells which degraded the adsorbed, concentrated chlorophenols when heated to 25°C. High concentrations, if too toxic for bioremediation, can also be lowered into a suitable range by dilution with clean material, a physico-chemical extraction step, or the creation of low concentration microenvironments with semi-permeable membranes (13).

Actual contaminated sites and materials will always contain a mixture of pollutants, since, at best, technical grade chemicals with associated impurities will have been applied or spilt. Chlorophenol-contaminated sites, for example, contain most isomers of mono-, di-, tri-, tetra-, and pentachlorophenol, together with impurities such as poly-chlorinated phenoxyphenols, dibenzo-furans and dioxins, and other wood preservatives or carriers such as creosote and oil (11). In such cases, application of a single strain may not be sufficient and a mixture of strains capable of mineralising structurally different compounds may be necessary.

Most reports on the remediation of soil state that approximately $10^6$ cells $gram^{-1}$ soil are required. The costs of large-scale culture of the inoculum can be significant, and more recent reports have focused on the use of inocula immobilised on inert materials, such as polyurethane granules (18, 23). Immobilisation permits both high densities of cells $gram^{-1}$ support, and may enhance both storage and activity capacities.

## Conclusions.

Unquestionably, as pollution regulations tighten, biological treatment methods will become increasingly important. They should not however be considered in isolation, but rather in conjunction with novel and traditional physico-chemical waste treatment practices. All proposed methods must undergo thorough laboratory and pilot plant examination to prevent possible worsening by biotransformation rather than degradation.

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Table 1. Some PCP-degrading microorganisms used for remediation of soil.

| Species | PCP conc (mg/kg) | Time scale | Degree of degradation | Reference |
|---|---|---|---|---|
| *Arthrobacter* sp. | 150-200 | 12 days | 85% loss | Edgehill and Finn, 1983 |
| *Rhodococcus chlorophenolicus* | 600 | 47 days | 20-35% mineralisation | Salkinoja-Salonen et al. 1989 |
| *Flavobacterium* sp. | 100 | 12 days | 60% mineralisation | Crawford and Mohn, 1985 |
| *Phanerochaete chrysosporium* | 50 | 60 days | 98% disappearance 2% mineralisation | Lamar et al. 1990 |

Table 2. Factors affecting the survival and activity of xenobiotic-degrading microorganisms introduced into a waste treatment system.

(From Acea, Moore and Alexander, 1988; Roszack and Colwell, 1987; Goldstein, Mallory and Alexander, 1985).

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1. Presence of other carbon/energy sources which may:

   be used as cosubstrates, hence aid degradation;

   be used in preference to xenobiotic;

   lead to competition from native populations.

2. Nutrient limitation, *e.g.* availability of N, P, and S.

3. Temperature, pH, $E_H$, water activity.

4. Presence of interfaces enabling microbial attachment.

5. Presence or production of inhibitors and toxins.

6. Amount and method of inoculation, *e.g.* immobilised cells

7. Degree of mixing applied to heterogeneous systems.

8. Type of inoculum, *e.g.* Gram positive or negative, hyphal growth, mixed culture or sporing species, and ability to store carbon reserves.

9. Growth kinetics to minimise competition from native microflora.

10. Predation by protozoa.

11. Motility, with possible chemotaxis to xenobiotic.

12. Genetic stability of degradation capacity.

13. Degree of toxicity of xenobiotic.

14. Concentration in the environment may be:

    too low to support growth of inoculum;

    too high and therefore inhibitory to inoculum.

15. Degree of adsorption or incorporation of xenobiotic.

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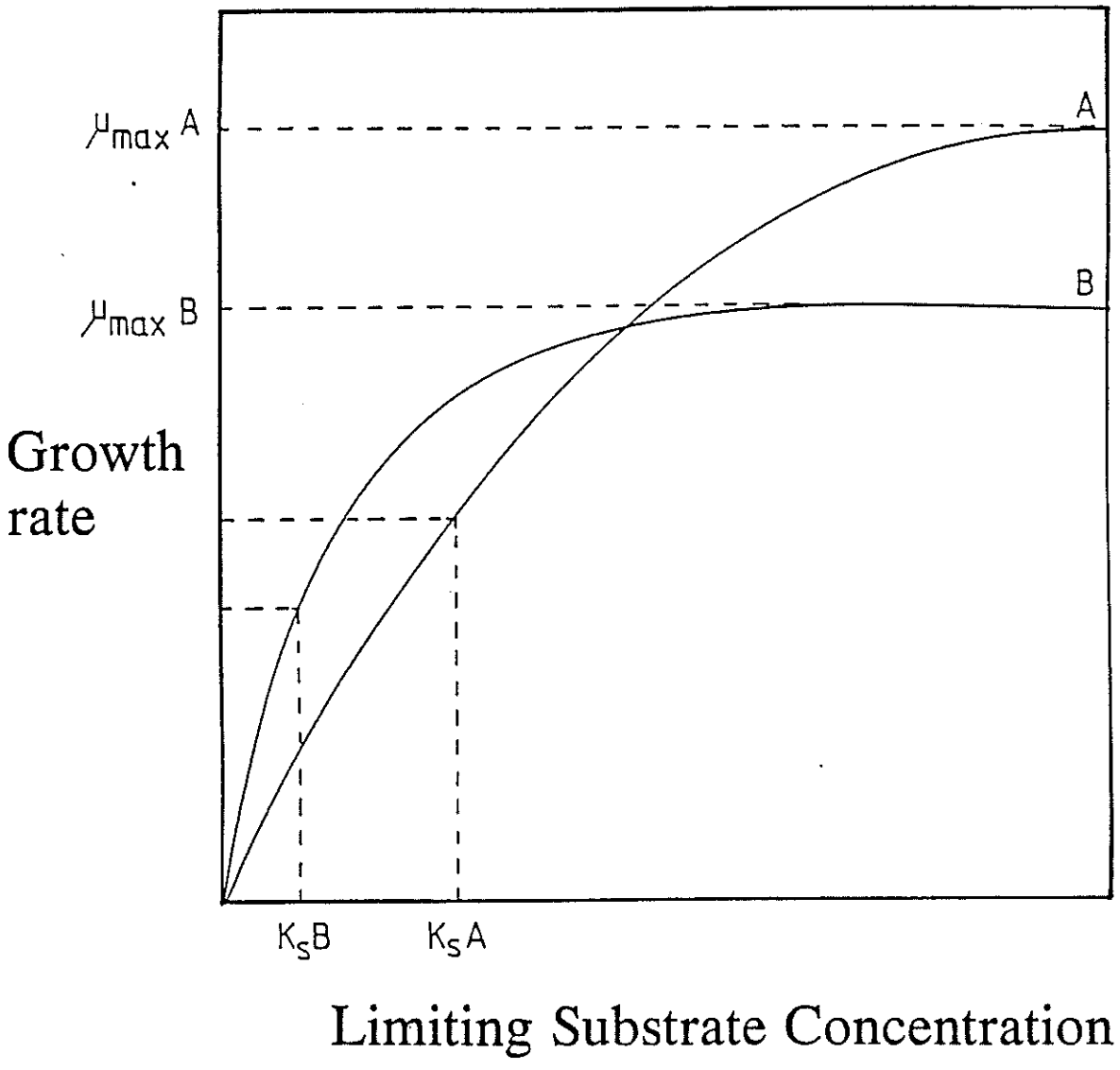
Figure 1. Theoretical growth kinetics of organisms from open and closed enrichments (Veldkamp and Jannasch, 1972).

Closed enrichments will be dominated by type A organisms, with the fastest growth rate ($\mu$ max).

In open enrichment, the growth rate can be controlled by the rate of dilution such that type B organisms with lower maximum growth rates, but high substrate affinities (low $K_s$).



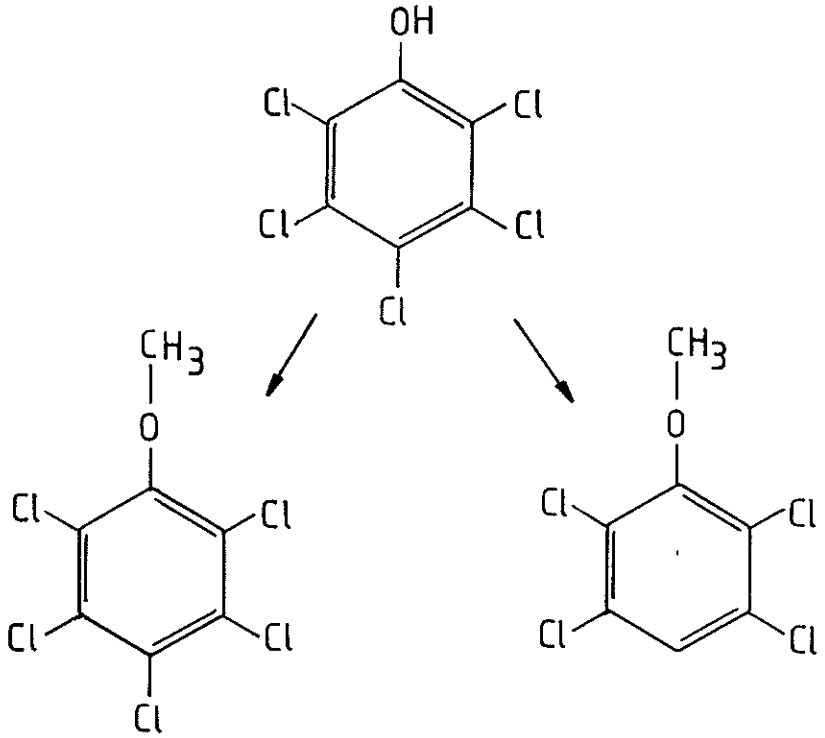



Figure 2. Formation of chloroanisoles from pentachlorophenol by Phanerochaete chrysosporium in treated pine.