

Microbial Degradation of Chlorophenols

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1 Introduction

Chlorophenols (CPs) are common environmental pollutants that are usually introduced into the environment as a result of chemical and pharmaceutical industrial activities (Jensen 1996; Czaplicka 2004; Michałowicz and Duda 2007). The prevalence of these pollutants in the environment is associated with the production, use and degradation of numerous pesticides which can easily find their ways into the environment (Hale et al. 1994). The main potential environmental sources of chlorophenols include: (a) direct soil application as biocides; (b) synthesis during engineering processes where chlorine is used; (c) leaching and vaporizing from treated wood items; (d) release or discharge from factories into air and water; and (e) combustion of organic material in the presence of chloride.

Chlorophenols have become today important pollutants in the environment and their treatment, disposal, and general management have become serious challenges to environmental agencies in most parts of the world (Weber et al. 2008). The diversity, versatility, adaptability, and metabolic potentials of a number of microbes have been harnessed and applied in the bioremediation of numerous environmental contaminants. In an effort to remediate the effects of chlorophenols, bioremediation using microorganisms has been suggested by many workers (Pandey et al. 2003; Czaplicka 2004). However, a number of contaminants have been shown to be refractory to microbial degradation; hence they are either not metabolizable or are

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transformed into other metabolites that accumulate in the environment (Esteve-Núñez et al. 2001).

Phenol and its derivatives, such as chlorophenols are among the most common environmental pollutants that are often used as agricultural pesticides. They are also produced during the preparation of paper and the burning of organic materials in the presence of chloride (Antizar-Ladislao and Galil 2004). Microorganisms, such as bacteria, fungi, actinomycete, protozoa, etc. have been found to be effective in the degradation and recycling of organic pollutants. Microorganisms, that are capable of degrading organic contaminants, can be isolated, cultured, adapted, and enriched under laboratory conditions. Indigenous microbial populations, especially heterotrophic bacteria and fungi, can play a key role in the biodegradation process of toxic and recalcitrant pollutants.

It has been reported that a fraction of the soil biota containing microorganisms, used to bio convert chlorophenols rapidly and also develop the ability to degrade certain pesticides, when they are continuously applied to the soil. These chemicals provide adequate carbon source and electron donors to certain soil microorganisms which are required for the bioconversion of pesticide-contaminated sites (Qiu et al. 2007; Araya and Lakhi 2004). It was also observed that microorganisms, that have the ability to degrade pesticides, can also bio-remediate other chemical compounds as well (Singh and Walker 2006). The transformation of such contaminants depends not only on the presence of microorganisms with appropriate degrading enzymes, but also on a wide range of environmental parameters (Aislabie and Lloyd-Jones 1995). Moreover, the ecological, biochemical, molecular and physiological factors play a significant role in the biodegradation process of such pollutants (Iranzo et al. 2001; Vischetti et al. 2002).

This chapter addresses the state of chlorophenolic compounds in the environment with special emphasis on their microbe-induced degradation. The biodegradation of CPs has been reviewed with respect to CPs degrading microbes, factors for degradability and degradation mechanisms. The enzymes involved in the microbial degradation of CPs have also been discussed. However, this chapter mostly focuses on the degradation mechanism of 2,4-dichlorophenol and pentachlorophenol.

2 Structures of Chlorophenols

Chlorophenols (CPs) are a group of chemicals in which chlorines are attached between C1 and C5 of phenol, which is an aromatic compound derived from benzene by adding a hydroxyl group to a carbon to replace hydrogen. There are five basic types of CPs: monochlorophenols, dichlorophenols, trichlorophenols, tetrachlorophenols, and pentachlorophenols. In all, there are 19 different CPs which have been shown in Fig. 1 (Van Agteren et al. 1998).

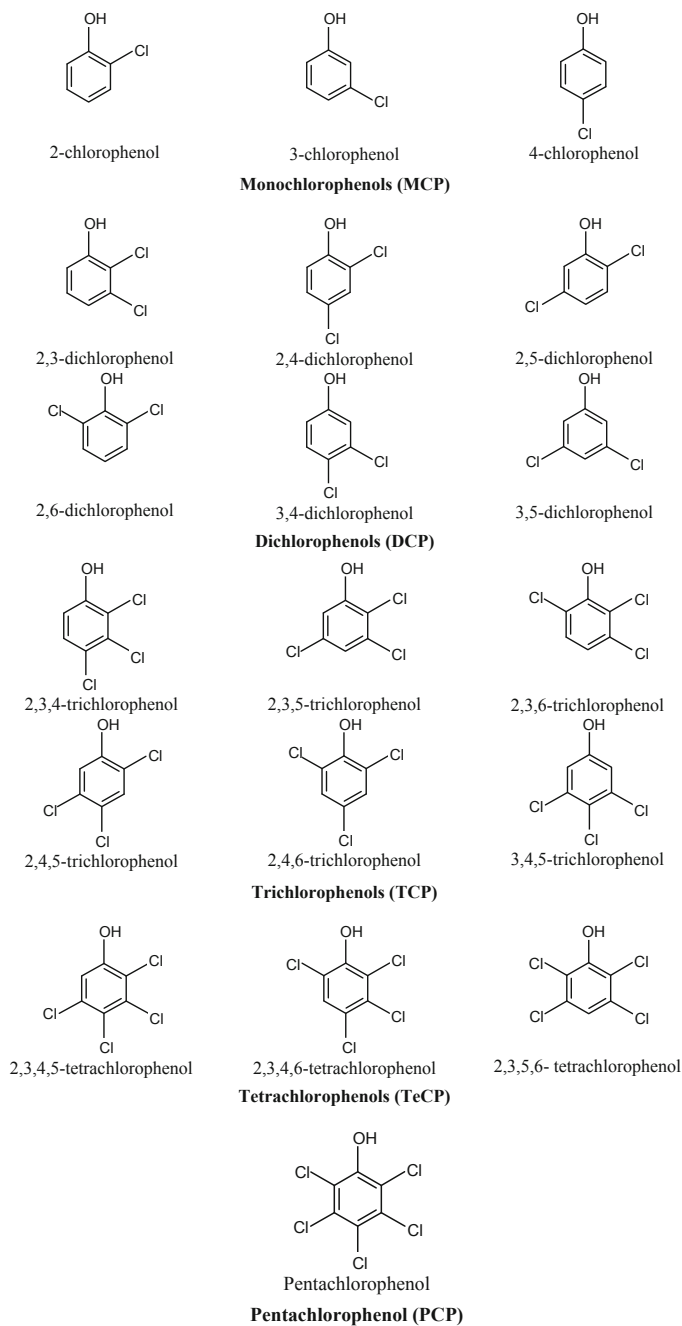


Fig. 1 Chemical structure of chlorophenols (Van Agteren et al. 1998)

3 Physical and Chemical Properties of Chlorophenols

The CPs consists of a benzene ring, OH group and atoms of chlorine. Together with the 19 main compounds, chloroderivatives of methyl- and ethyl-phenols are also considered as CPs. The whole group of CPs comprises tens of compounds, which are significantly differing from each other with respect to their molecular structures and consequently with their physical and chemical properties. With the exception of 2-chlorophenol, all CPs are solids, with melting temperature ranging between 33 and 191 °C. In general, these compounds are weakly dissolved in water, but strongly in organic solvents. Their water solubility decreases with increasing number of chlorine atoms in a molecule. They are weakly acidic and their acidity is slightly higher than that of phenols. In reactions with alkaline non-metals (sodium, potassium) in the aquatic environment, they yield salts highly soluble in water. The physical and chemical properties of selected chlorophenols are presented in Table 1 (Czaplicka 2004).

The fate and transport of a chemical compound in the natural environment strictly depend on the value of the dissociation constant (K_a) and the partition co-efficient ($K_{o/w}$) in the octanol-water system. The dissociation constant depends on the structure of the compound and on the number of chlorine atoms in the molecule. In the case of CPs, the dissociation constant of a compound increases (i.e. its pK_a — $\log K_a$ decreases) with increasing the number of chlorine atoms in the compound. Depending on the value of pK_a , CPs get dissociated totally or partially. Their octanol-water partition co-efficients ($K_{o/w}$) strongly increase with the number of chlorine atoms, and the water solubility (hydrophilicity) inversely decreases. Also, the degree of dissociation of chlorophenols increases (indicated as descending pK_a values) with increasing the number of chlorine atoms (Czaplicka 2004).

In addition to being produced commercially, small amounts of some CPs, especially the mono- and dichlorophenols, may be produced when wastewater or drinking water is disinfected with chlorine, if certain contaminants are present in the raw water. They are also produced during the bleaching of wood pulp with chlorine, when paper is being produced. Sorption, volatilization, degradation, and leaching are the primary processes governing their fate and transport in the environment (Ivanciuc et al. 2006).

It has also been reported that physicochemical properties of CPs, such as water solubility, Henry's law constant, organic carbon sorption coefficient, volatilization rate, and photolysis rate determine the transport processes of these compounds. Important factors, influencing these processes include organic matter content and clay content in soil, sediment, and water, as CPs are specially adsorbed to these soil constituents (Czaplicka 2004; Ivanciuc et al. 2006). Generally, as the number of chlorine molecules increases, there is a reduction in the vapor pressure, an increase in boiling point, and a reduction in water solubility of the CPs. Thus, increasing chlorination increases the tendency of these compounds to partition into sediments and lipids and to bioconcentrate (Ivanciuc et al. 2006).

Table 1 Physico-chemical properties of CPs (Czaplicka 2004)

No	Compound	Formula	Molecular weight	Boiling point (°C)	Melting point (°C)	Solubility at 20 °C (g ⁻¹)	pKa	Log <i>K_{ow}</i>
1	2-Chlorophenol	C ₆ H ₅ ClO	128.56	174.9	9.3	28	8.3–8.6	2.12–2.17
2	3-Chlorophenol	C ₆ H ₅ ClO	128.56	214	33–34	26	8.8–9.1	2.48–2.50
3	4-Chlorophenol	C ₆ H ₅ ClO	128.56	217–219	42–44	27	9.1–9.4	2.35–2.44
4	2,3-Dichlorophenol	C ₆ H ₄ Cl ₂ O	163.00	206	57–58	NA	6.4–7.8	3.15–3.19
5	2,4-Dichlorophenol	C ₆ H ₄ Cl ₂ O	163.00	210	45	4.50	7.5–8.1	2.75–3.30
6	2,5-Dichlorophenol	C ₆ H ₄ Cl ₂ O	163.00	211	58–59	NA	6.4–7.5	3.20–3.24
7	2,6-Dichlorophenol	C ₆ H ₄ Cl ₂ O	163.00	219	68	NA	6.7–7.8	2.57–2.86
8	3,4-Dichlorophenol	C ₆ H ₄ Cl ₂ O	163.00	253–254	65–68	NA	7.4–8.7	3.13–3.44
9	3,5-Dichlorophenol	C ₆ H ₄ Cl ₂ O	163.00	233	68	NA	6.9–8.3	2.57–3.56
10	2,3,4-Trichlorophenol	C ₆ H ₃ Cl ₃ O	197.45	Sublimes	77–84	0.22	6.5–7.7	3.49–4.07
11	2,3,5-Trichlorophenol	C ₆ H ₃ Cl ₃ O	197.45	248–255	57–62	0.22	6.8–7.4	3.84–4.56
12	2,3,6-Trichlorophenol	C ₆ H ₃ Cl ₃ O	197.45	246	58	NA	6.0–7.1	3.88
13	2,4,5-Trichlorophenol	C ₆ H ₃ Cl ₃ O	197.45	Sublimes	67–70	0.948	7.0–7.7	3.72–4.10
14	2,4,6-Trichlorophenol	C ₆ H ₃ Cl ₃ O	197.45	243–249	69	0.434	6.0–7.4	3.60–4.05
15	3,4,5-Trichlorophenol	C ₆ H ₃ Cl ₃ O	197.45	271–277	101	NA	7.7–7.8	4.01–4.39
16	2,3,4,5-Tetrachlorophenol	C ₆ H ₂ Cl ₄ O	231.89	Sublimes	116–117	0.166	6.2–7.0	4.21–5.16
17	2,3,4,6-Tetrachlorophenol	C ₆ H ₂ Cl ₄ O	231.89	150	70	0.183	5.3–6.6	4.10–4.81
18	2,3,5,6-Tetrachlorophenol	C ₆ H ₂ Cl ₄ O	231.89	188	114–116	0.100	5.2–5.5	3.88–4.92
19	PCP	C ₆ Cl ₅ OH	266.34	300	190	0.014	4.7–4.9	5.01–5.86

NA, not available

4 Distribution, Toxicity and Environmental Fate of Chlorophenols

Chlorophenols are introduced into the environment as effluent waste from the industrial processes, through their use as biocides or as by-products of other industrial operations, such as pulp bleaching with chlorine, water disinfection or even waste burning. Chlorophenols have also been used as general purpose disinfectants, and it has been observed that they can also appear as degradation products of other chlorinated xenobiotics. Many studies have shown that chlorophenols are persistent in water environments, but a few workers have investigated the fate and behavior of these chlorinated aromatics in soil (Lallai and Mura 2004a; Olaniran and Igbiosa 2011). The key source of water pollution by chlorinated phenols is the industrial waste discharge and the leaching of such pollutants from landfills (Zhang and Bennett 2005). Volatile chlorophenols, such mono- and dichlorophenols enter the atmosphere as vapors through volatilization (Ivanciuc et al. 2006). The primary non-point source pollution of chlorophenols comes from the application of pesticides that are made from chlorophenols and the chlorination of wastewater containing phenol (Czaplicka 2004). Monochlorophenols and 2,4-DCP are highly volatile compared to trichlorophenols and tetrachlorophenols, and their volatilization facilitates their diffusion into the atmosphere. However, trichlorophenols and tetrachlorophenols are slightly volatile. Only a small fraction (approximately 5 %) of CPs is emitted to the atmosphere (ATSDR 1999). These releases are primarily in vapor form and are primarily associated with CP production and its use in the manufacture of enduse products (ATSDR 1999). Chlorophenolic compounds are known to be resistant to biodegradation, which makes them persistent in the environment. Because of their lipophilicity, they can be transported through the cell membrane and hence bioaccumulate in the aquatic organisms (Pedroza et al. 2007).

The transformation of CPs in particular could lead to an increase in the toxicity of intermediate compounds or end products due to formation of electrophilic metabolites that may bind and damage DNA or gene products (Michałowicz and Duda 2007). The harmful influence of CPs and their derivatives on the eco-biota may lead to acute toxicity, histopathological changes, mutagenicity, and cancer. These serious health issues make it imperative not only to control CPs in the environment, but also to assess and understand their fate in the environment with a view to protecting the environment and public health.

Cleaning up of contaminated soils by biological processes may be an effective alternative to the chemical technologies in the case of chlorophenols pollution (Al-Thani et al. 2007; Dong et al. 2009). The presence of degraders capable of mineralizing chlorinated aromatic compounds in certain soils are justified by the fact that these compounds are produced naturally (Okpokwasili and Nweke 2005; Potgieter et al. 2009). As the chlorinated phenolic compounds have broad applications in industrial and medical uses, they have become the most common environmental contaminants, especially in the underground water (Breining et al. 2000). CPs get attached to soil and sediments at the bottom of lakes, rivers, or streams and

rapidly enter the body through the skin and the gastrointestinal tract. Owing to their high toxicity, carcinogenicity and wide distribution in industrial wastes they cause harm to human beings and marine organisms (Zhang et al. 2005). Therefore, CPs are of growing concern (Sittig 1981). Therefore, many studies were carried out regarding environmental fate of chlorinated phenols, including photochemical degradation and sequential aerobic and anaerobic degradation. However, bioremediation of CPs has been generally accepted by the public, because they lead to innocuous tailings, are non-invasive to environments and are also cost-effective compared to physiochemical approaches (Gallizia et al. 2003). Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds and the organic pollutants using them as the sole source of carbon and energy by microbes. Generally, chlorinated phenols are transformed via oxidative dechlorination (aerobic biodegradation) (Steinle et al. 1998), while in anaerobic conditions via reductive dechlorination (Annachhatre and Gheewala 1996).

CPs can also be emitted into the environment as waste from sawmills, pulp and paper mills, and due to the incineration of wood wastes (Jones 1983). Industrial effluents can contain all chlorophenols, but 2,4-DCP, 2,4,6-TrCP, and 2,3,4,5-TeCP are detected predominantly (Valo and Salkinoja-Salonen 1986). Some dichlorophenol isomers occur naturally and can be produced by a variety of microorganisms (Siuda 1980). Because of their aromatic structure, phenols are resistant to natural biodegradation due to the difficulty of cleaving of the benzene ring. However, several microorganisms can tolerate phenol and use it as a carbon and energy source (El-Naas et al. 2009). The biological degradation is accomplished through benzene ring cleavage mediated by the enzymes present in the microorganism.

5 Microorganisms to Degrade CPs

Depending on the microbial abilities to grow in specific conditions, organic material can be degraded aerobically or anaerobically (Wang et al. 2007a). A wide range of microorganisms have been shown to be capable of degrading the chloroaromatic compounds. Degradation of the CPs has been demonstrated in the laboratory conditions using both pure cultures and mixed cultures.

CPs degradation studies have been mostly carried out using isolated pure strains in *in vitro* conditions. Of the strains used, the most common genus is *Pseudomonas*, which has a remarkable potential to evolve entire catabolic sequences for specific degradation pathways. These organisms are ubiquitous in nature and have been found in large numbers in all major natural environments i.e. terrestrial, freshwater and marine. They are also known to have a remarkable nutritional versatility and are capable of utilizing a wide range of organic compounds as growth substrates. However, a major drawback in biological treatment is the inhibition of microorganisms at high substrate concentrations (Nuhoglu and Yalcin 2005).

The term Pseudomonad is used to describe strictly aerobic, Gram-negative, non-sporulating bacteria. They are oxidase positive with non-acid fast rods, which

are generally straight, but maybe slightly curved, 0.5–1 μm in diameter and 1.5–5 μm in length (Krieg and Holt 1984). They are generally motile with polar flagella and generally do not ferment carbohydrates and fix nitrogen and are also not photosynthesizing. The optimum growth temperature for most strains is 28 $^{\circ}\text{C}$, but many are capable of growing in the temperature range of 4–43 $^{\circ}\text{C}$. Members of the genus *Pseudomonas* are free-living organisms and occupy a dominant position in the biosphere in terms of variety of habitats and the number of species in a given habitat. *Pseudomonas* species are sub-divided on the basis of rRNA homology into five similarity groups. Group I is the largest, including scientifically validated relevant strains, such as *P. aeruginosa*, *P. fluorescense* and *P. putida*. Group I can be further divided into the fluorescent and non-fluorescent species. One of the most striking properties of members of this genus is their remarkable nutritional versatility. They play an important role in the carbon and nitrogen cycles. Organic compounds such as alcohols, aliphatic acids, amides, amines, amino acids, aromatic compounds, carbohydrates and hydrocarbons are all readily used by *Pseudomonas* species as growth substrates. Of these compounds, the aromatic compounds are particularly interesting due to the various pathways by which they are metabolized (Palleroni 1986). The ability of Pseudomonads to utilize a wide range of compounds makes them a vital component of any biodegradative system, particularly in the treatment of wastewaters derived from the chemical industries.

Although both aerobic and anaerobic microorganisms are able to degrade CPs, aerobic processes are generally preferred. Aerobic microorganisms are more efficient for degrading toxic compounds, because they grow faster and usually achieve complete mineralization of toxic organic compounds to inorganic compounds (CO_2 , H_2O) (Kim et al. 2002; Kargi and Eker 2004; Sahinkaya and Dilek 2007). For these reasons, there is a limited interest in the utilization of anaerobic bacteria for the degradation of CPs. However, several studies have also been carried out in the anaerobic conditions (Levén et al. 2006; Azbar et al. 2009; Bajaj et al. 2009; Hussain et al. 2010; Puyol et al. 2011; Muñoz et al. 2013).

A large number of CPs degrading bacteria have been isolated and characterized at the physiological and genetic level. The microorganisms used are usually aerobes, such as *Pseudomonas* sp., *Alcaligenes* sp., *Azotobacter* sp., *Rhodococcus* sp. and *Cryptococcus* sp. (Quan et al. 2004; Sahinkaya and Dilek 2007). Pure and mixed cultures of the *Pseudomonas* spp. are the most commonly utilized biomass for the biodegradation of CPs (Stoilova et al. 2007; Caliz et al. 2011). However, *Pseudomonas putida*, has been commonly used for the degradation of phenols due to its high removal efficiency (Hsieh et al. 2008; El-Naas et al. 2010). Responses of *P. putida* to chemical stresses have indicated that its cells could use diverse protective mechanisms for its survival in various extreme environments. These studies could help in synthesizing new bacterial strains with enhanced degradation capability and improved tolerance to toxic pollutants (Loh and Cao 2008).

Although the *Pseudomonas* genus has been widely used to treat chlorophenols, considerable attention has been recently paid to new and more efficient microorganisms for this purpose. *Ralstonia pickettii*, formerly known as *Burkholderia pickettii*, has been reported to have several advantages over other candidate strains

such as *P. putida*, which is only weakly pathogenic. However, no phytopathogenic or animal pathogenic incidents have been reported. *R. pickettii* strain LD1 can metabolize mono-chlorophenols, which poses a challenge as they are particularly formed during the chlorination of wastewaters (Ryan et al. 2007). This aerobic bacterium was found to be able to degrade 2 CP completely (initial concentration: 195 mg L⁻¹), 3 CP (initial concentration: 73.5 mg L⁻¹) and 4 CP (initial concentration: 97 mg L⁻¹) within 30, 30 and 40 h of incubation, respectively, under batch conditions. The rates, at which mono-chlorophenols were degraded, largely depended on the type of chlorophenol: 2 CP > 4 CP > 3 CP (when used as sole carbon sources) (Fava et al. 1995). Al-Zuhair and El-Naas (2012) isolated an indigenous mixed bacterial culture, identified mainly as *R. pickettii*, from oil sludge samples of a local petroleum refinery. Its efficiency for phenol removal was found to be comparable to that of a commercially available mixed culture consisting mainly of *P. putida* (Al-Zuhair and El-Naas 2012).

Fungi share a significant role in the recycling of aromatic compounds in the biosphere. They are capable of consuming a wide variety of carbon sources by enzymatic mechanisms, thus providing possibilities for metabolizing chlorophenols (Stoilova et al. 2007). The most abundant fungi in polluted environments are yeasts. Some yeasts, such as *Candida tropicalis*, *Fusarium flocciferum* and *Trichosporon cutaneum* are capable of utilizing phenols as the major carbon and energy source (Agarry et al. 2008). Moreover, there are studies which confirm the ability of strains from *Penicillium*, *Aspergillus*, *Graphium* and *Phanerochaete* to disintegrate chlorophenolic compounds (Stoilova et al. 2006). Rubilar et al. (2008) analyzed the degradation of CPs by white rot fungi, which are a group of organisms very suitable for the removal of chlorinated phenolic compounds (Rubilar et al. 2008). They are robust organisms which are tolerant to the presence of high concentrations of various pollutants, even with a low bioavailability and this ability is mainly conferred to their very powerful extracellular oxidative enzymatic system (Diez et al. 2012). The first study on the biodegradation of 4 CP by *Fusarium* sp. HJ01 was carried out by Li et al. (2011).

Recently, there has been more interest in evaluating the capabilities of some algae for chlorophenol biodegradation. While some algae have low tolerance to the acute toxicity of chlorophenols, cyanobacteria and eukaryotic microalgae (e.g., *Chlorella* sp., *Scenedesmus* sp., *Selenastrum capricornutum*, *Tetraselmis marina*, *Ochromonas danica*, *Lyngbya gracilis*, *Nostoc punctiforme*, *Oscillatoria animalis* and *Phormidium foveolamm*) are capable of biotransforming phenolic compounds (Lika and Papadakis 2009). Papazi and Kotzabasis (2007) reported that the degradation of phenolic compounds by microalgae seems to be mostly a bioenergetic process, which needs additional energy sources to be exogenously supplied as carbon and light (Papazi and Kotzabasis 2007). Klekner and Kosaric (1992) conducted a study on the degradation of binary phenolic mixtures by *Chlorella*, in which phenol might have induced the proper metabolic pathway of other phenol derivatives (Klekner and Kosaric 1992).

6 Biodegradation of CPs

Biodegradation is based on two processes: growth and co-metabolism. Organic pollutants are used by the microbes as the sole source of carbon and energy during the growth stage, resulting in a complete degradation (mineralization) of organic pollutants. Microbial degradation of chlorophenols has been reported by several researchers (Cortes et al. 2002; Murialdo et al. 2003; Crawford et al. 2007). Chlorophenols are subjected to both abiotic and biotic degradation and conversions (Cortes et al. 2002). Several studies have been carried out on the microbial degradation of chlorophenols in both water and sediments (Demnerova et al. 2005) as well as by sludge (Lallai and Mura 2004a). Even though chlorophenols are poorly biodegradable, several publications have indicated that the aerobic degradation of CPs is possible (Bergauer et al. 2005; Demnerova et al. 2005; Al-Khalid and El-Naas 2013, 2014). The aerobic degradation of chlorophenols by microorganisms requires the involvement of the enzymes i.e. oxygenases, to incorporate atmospheric oxygen into their substrates. The ring is dihydroxylated firstly by an oxygenase in the way that two hydroxyl groups are located either ortho or para to one another in the benzene ring (Pieper and Reineke 2000). Ring fission occurs subsequently, ingestion of dioxygen atoms into the aromatic nucleus. The vital step in the biodegradation of CPs is the removal of the chlorine substituents. For the catabolism of the lesser substituted phenols (mono- and dichlorophenols), dioxygenase from chlorophenol-degrading bacteria usually opens the dihydroxylated aromatic ring before dechlorination takes place (Farrell and Quilty 2002). However, highly substituted phenols and some of the chloro-substituents must be removed before ring cleavage, as the halogen atoms deactivate the aromatic nucleus to electrophilic attack by dioxygenases (Bergauer et al. 2005). Degradation of 4-chlorophenol is usually accomplished by the oxidation of the substrate to 4-chlorocatechol, followed by ortho cleavage of the aromatic ring (Farrell and Quilty, 2002; Park and Kim 2003). Some microorganisms attack 4-chlorocatechol by meta-cleavage, although it is less common. After ring cleavage, the chlorine atom is removed and the carbon skeleton is transformed into products that are assimilated into the central metabolism of the cell (Bae et al. 1997).

6.1 Biodegradation of CPs in Soil

Lallai and Mura (2004b) reported the biodegradation of 2-CP using microorganisms isolated from the forest soil (Lallai and Mura 2004b). Other studies reported that chlorinated phenols, such as 2-, 3-, and 4-CP; 2,4-, 2,6- and 3,4- DCP; 2,4,6-, and 2,4,5-TCP; and PCP, were incubated under aerobic conditions and degraded with a Canadian clay loam grassland soil (Baker and Mayfield 1980); whereas, 3,4,5-TCP and 2,3,4,5-TeCP were not degraded. Chlorophenolic compounds with ortho and para- substituted chlorines were reported to be totally degraded within a few days.

Kiyohara et al. (1989) reported that 26 out of 170 soil samples were found to degrade 2,4,6-TCP, indicating a wide distribution of CPs degraders in the environment (Kiyohara et al. 1989). High concentrations up to 5000 mg kg^{-1} of 2,4,6-TCP were easily degraded by CP free forest soil (Sanchez et al. 2004). It was also observed that soil microbial structure was not changed by doses of 2,4,6-TCP as high as 500 mg kg^{-1} . PCP at concentrations of 200 mg kg^{-1} was reported to be degraded by local microorganisms in unspoiled grassland soils at an average rate of $1.2 \text{ mg kg}^{-1} \text{ d}^{-1}$ (Mahmood et al. 2005). Molecular fingerprinting of the soil microbial population was utilized to demonstrate the enhancement of PCP-degrading strains of bacteria. Degradation of PCP was also observed in Japanese soils, where it had been used as an herbicide (Watanabe 1978). Reddy and Gold (2000) reported that five of ten wetland soil samples from five states in the USA caused PCP to be biodegraded, and about 75-100 % of the PCP was removed in 30 days (Reddy and Gold 2000).

6.2 Biodegradation of CPs in Sediments

Aerobic degradation of chlorinated phenols occurred in surface water samples, where radio labeled 3,4-DCP, 2,4,5-TCP and PCP were mineralized into CO_2 in two types of lake water samples (Larsson and Lemkemeier 1989). Surface water from an estuarine river degraded 4-CP (Hwang et al. 1986). Mississippi river channels, exposed for several weeks to PCP, developed microbial populations responsible for the biological mineralization of PCP to CO_2 . This degrading activity was attributed to microorganisms attached to rock or water plant surfaces (Pignatello et al. 1983). The biodegradation of 4-CP, 2,4-DCP, 2,5-DCP, 3,4-DCP and 2,4,5-TCP in pool sediments and 4-CP, 2,4-DCP and 2,5-DCP in methanogenic aquifer sediments was observed (Gibson and Suffita 1986). They also reported that phenols and lower chlorinated phenols were detected as intermediate compounds of CP degradation.

The bioconversion of 2-CP was also observed in Lake Michigan sediments (Becker et al. 1999). All isomers of mono-chlorinated phenol were degraded by river sediments of the upper Hudson River in New York State under methanogenic and sulfate reducing conditions (Haggbloom et al. 1993) and phenol, benzoate and 3-chlorobenzoate were observed as intermediates of the biotransformation. Susarla et al. (1997) measured the kinetics of anaerobic degradation of all 6 DCP isomers in lake sediments in Japan and determined the first-order rate constants for 3,4-DCP and 2,5-DCP. Monochlorophenol and phenol were invariably observed as intermediates in the degradation process. Three isomers of DCP, 2,3-DCP, 2,4-DCP and 2,6-DCP were anaerobically degraded with first-order rate constants of $0.017\text{--}0.033$ and $0.087\text{--}0.139 \text{ d}^{-1}$, respectively; for two different chemicals free pond sediments collected in the Southeastern U.S. Lake sediment samples from the Southeastern U.S. were also shown to anaerobically degrade 2,4-DCP with the formation of 4-CP as a main intermediate in the process (Kohring et al. 1989; Zhang and Wiegel 1990).

The first-order rate constants of PCP degradation ranged from 0.082 to 0.38 d⁻¹. Tetrachloro-1,4-hydroquinone was dehydroxylated and dechlorinated by a mixed culture derived from Baltimore harbor sediments to yield 2,3,5-TCP (Milliken et al. 2004). Anoxic estuarine sediments from Taiwan were found to degrade 2-CP, 3-CP, 4-CP, 2,5-DCP, 3,4-DCP, 3,5-DCP and PCP (Liu et al. 1996). PCP degradation was linked to sulfate reduction. Estuarine sediments from the lower Hudson River in New York were able to degrade 2-CP, 3-CP and 4-CP under sulfate-reducing conditions (Uotila et al. 1992). Estuarine sediments from the Chesapeake Bay biotransformed 2,4-DCP to 4-CP when incubated under methanogenic conditions (Warner et al. 2002). Similarly, 2,4-DCP and 3,4-DCP were dechlorinated by marine sediments to 4-CP and 3-CP, respectively (Boothe et al. 1997). Marine sediments collected off the coast of Sweden were observed to dechlorinate PCP, yielding 2,3,4,5-TeCP, 3,4,5-TCP and 3,5-DCP (Abrahamsson and Klick 1991).

6.3 Biodegradation of CPs by Sludge

Municipal anaerobic digester sludge has also been used to degrade chlorophenols. All monochlorinated isomers were degraded by anaerobically-digested primary sewage sludge (Boyd and Shelton 1984), and all dichlorophenols (except 3,4-DCP and 3,5-DCP) were degraded via monochlorophenol intermediates. Municipal sludge, from the anaerobic digester dechlorinated 2-CP to phenol. Further, evaluation of selective inhibitors demonstrated that the population responsible for the dechlorination reaction was associated with syntrophic acetogens (Basu et al. 2005). The ability of municipal anaerobic digester sludge to degrade 12 chlorinated phenolic compounds was tested and 10 of the compounds were found to be degraded (Loh and Wang 1998). However, the digested sludge culture, did not degrade PCP or 2,3,4,6-TeCP. In another study, all chlorinated phenol isomers were degraded by methanogenic sludge with first-order rate constants of 0.00046–0.161 d⁻¹ (Takeuchi et al. 2000). They reported that dechlorination of ortho-chlorines occurred at the fastest rate; whereas dechlorination of para-chlorines occurred at the slowest rate. First-order rate constants decreased with decreasing number of chlorine substituent.

7 Mechanisms of CPs Biodegradation

Under aerobic conditions, chlorinated aromatic compounds persist in the environment because chlorine atoms interfere with the action of many dioxygenase enzymes that normally initiate the degradation of aromatic rings. The interference is likely to be due to both steric and electronic effects, because chlorine atoms are both larger and more electron-withdrawing than hydrogen atoms. Despite these problems, microorganisms that can degrade chlorinated aromatic compounds, can often

be found at highly contaminated sites. Many of these microorganisms contain dehalogenase enzymes that catalyze the removal of chlorine atoms from the aromatic ring. Once the chlorine atoms are removed, the aromatic compound becomes susceptible to microbial degradation.

Chlorinated aromatic compounds are also degraded under anaerobic conditions. For example, the PCBs in the sediments of the Hudson River are gradually being converted to the lesser chlorinated congeners (Brown et al. 1987). The pathways of anaerobic degradation of chlorinated aromatic compounds are less extensive than that of pathway for aerobic degradation, however. Furthermore, mechanistic studies of an aromatic dehalogenase from an anaerobic microorganism have never been carried out. Chlorine substituents can be removed from aromatic rings in three fundamentally different ways as illustrated in Fig. 2. Hydrolytic dehalogenases replace chlorine substituents with hydroxyl groups that are derived from water; reductive dehalogenases replace chlorine substituents with hydrogen atoms; and oxygen-dependent dehalogenases replace chlorine substituents with hydroxyl groups whose oxygen atoms are derived from O_2 . Although only a limited number of dehalogenases have been studied, it is already evident that there is more than one way to accomplish each of three classes of reactions. The mechanisms used by enzymes in each of these three classes evidently show the diversity of mechanisms found in nature and the relationships between dehalogenase enzymes and other known enzymes (Copley 1997).

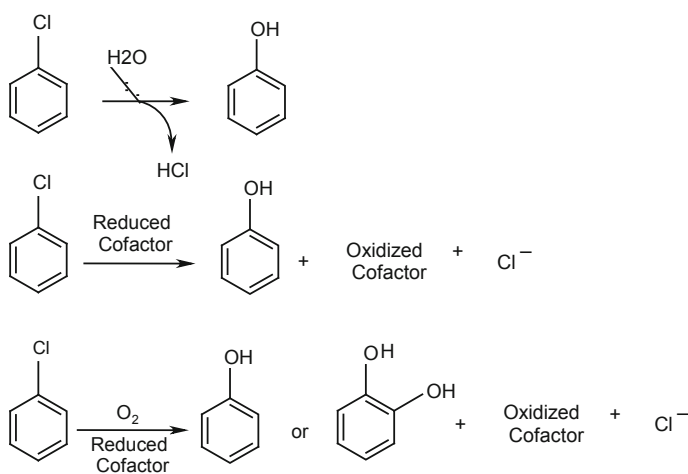


Fig. 2 Different processes for the removal of chlorine substituent from aromatic compounds (Copley 1997)

7.1 Aerobic Biodegradation of CPs

Aerobic degradation of phenol occurs via the formation of catechol. The bacterium, *Pseudomonas putida*, utilizes phenol through meta-oxidation and the yeast, *Trichosporon cutaneum*, operates through ortho oxidation (Yang and Humphrey 1975; Al-Khalid and El-Naas 2011). Haloaromatic compounds are known to be degraded through the formation of halocatechols as intermediates, which are subsequently cleaved by dioxygenases. This is followed by dehalogenation through the elimination of the hydrogen halide, with subsequent double bond formation on the aliphatic intermediate (Annachhatre and Gheewala 1996).

Removal of chlorine can be achieved in three fundamentally different ways in which the dehalogenases replace chlorine substituent with (a) hydroxyl groups derived from water, (b) hydrogen atoms, and (c) hydroxyl groups whose oxygen atoms are derived from O₂ (Copley 1997).

Research on the degradation of phenols and CPs in the aerobic soil environment revealed that phenol, o-chlorophenol, 2,4-DCP, 2,6-DCP and 2,4,6-TCP could be rapidly degraded as compared to PCP, 2,4,5-TCP, 3,4-DCP and m-chlorophenol, whereas 3,4,5-TCP and 2,3,4,5-TeCP persisted in the soil for over 160 days (Baker and Mayfield 1980). Chlorine attachment in the meta-position, toxicity to soil microorganisms and unavailability of compounds to microbes due to their adsorption on soil were some of the possible reasons for their reduced biodegradability. However, satisfactory degradation of phenol, monochlorophenols, dichlorophenols, trichlorophenols and PCP in a fine sandy loam soil was observed (Namkoong et al. 1988). Likewise, phenol and PCP were degraded easily in lake waters and fresh water streams, respectively (Pignatello et al. 1983; Annachhatre and Gheewala 1996).

The degradation of CPs by aerobic bacteria using two main strategies was reported by Solyanikova and Golovleva (2004). Lower chlorinated phenols are initially attacked by monooxygenases yielding chlorocatechols as the first intermediates (chlorocatechol pathway), which are subjected to ring cleavage prior to dechlorination. On the other hand, polychlorinated phenols are converted to chlorohydroquinones as the initial intermediates (hydroquinone pathway). Subsequent reactions gradually remove chlorines from the ring prior to its cleavage (Solyanikova and Golovleva 2004). The chlorocatechol pathway is illustrated for the chlorophenol, 2,4-DCP, as shown in Fig. 3.

The 2,4-DCP attack is initiated by a monooxygenases forming 3,5-dichlorocatechol (Finkel'shtein et al. 2000). The chlorocatechol is ortho cleaved, yielding 2,4-dichloromuconic acid (Tiedje et al. 1969). The first dechlorination takes place when a lactonizing enzyme converts the dichloromuconic acid to 2-chloro-4-carboxymethylenebut-2-enolide (Sharpee et al. 1973). The degradation of PCP is initiated by the formation of tetrachlorohydroquinone (TeCHQ) because of the hydroxylation at the para-position by either PCP-4-monooxygenase (Orser and Lange 1994) or cytochrome P-450 type enzyme (Uotila and Salkinoja-Salonen 1991; Uotila et al. 1992). In *Sphingomonas chlorophenolicum*

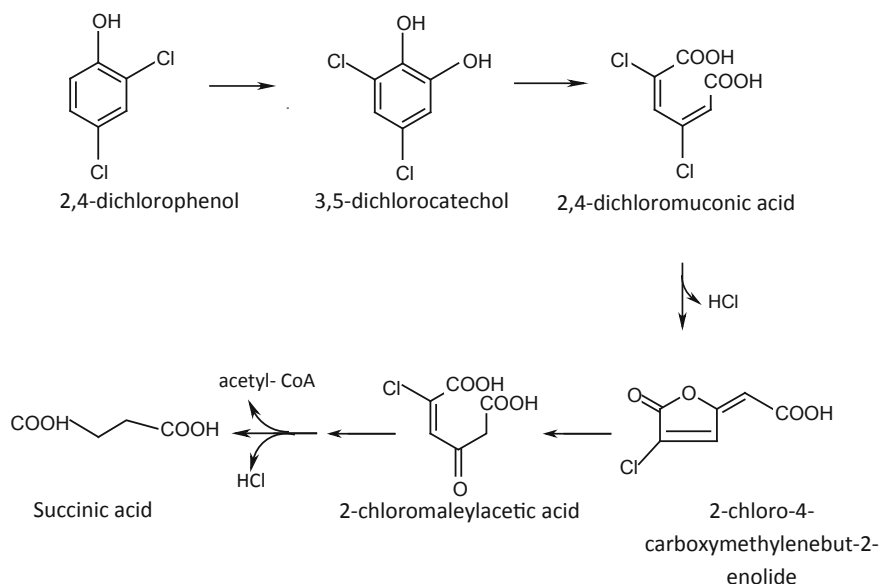


Fig. 3 Proposed pathway of the aerobic degradation of 2,4-dichlorophenol by bacteria (Field and Sierra-Alvarez 2008)

L-1, PCP-4-monooxygenase (PcpA) catalyzes the conversion of PCP to TeCHQ via the removal of chloride ions (Orser and Lange 1994). In the next step, TeCHQ is consecutively dehalogenated to 2,6-dichloro-1,4-hydroquinone (2,6-DCHQ) through a TeCHQ-reductive dehalogenase. Further degradation of 2,6-DCHQ happens via ring cleavage by the 2,6-DCHQ-1,2-dioxygenase, leading to the formation of 2-chloromaleylacetate, which is terminally degraded via the TCA cycle (Arora and Bae 2014, Fig. 4a).

In *Mycobacterium chlorophenolicum* PCP-1 and *Mycobacterium fortuitum* CG-2 (formerly *Rhodococcus* strains), PCP is hydroxylated to TeCHQ by a membrane bound cytochrome P-450 type enzyme (Uotila et al. 1992); then, TeCHQ goes through hydrolytic dehalogenation followed by reductive dehalogenation to produce dichloro-1,2,4-trihydroxybenzene (Apajalahti and Salkinoja-Salonen 1987) which yields BT after two successive reductive dehalogenation as represented in Fig. 4b (Arora and Bae 2014).

Different pathways of the aerobic degradation of chlorophenol have been reported. For example, the degradation of 4-CP by *Arthrobacter ureafaciens* strain CPR706 and *Arthrobacter chlorophenolicus* strain A6 is initiated by dechlorination, yielding 1,4-hydroquinone as the intermediate (Bae et al. 1997; Nordin et al. 2005). The proposed pathway involves the conversion of 4-CP to hydroquinone, to hydroxyquinol and then to maleylacetate (Nordin et al. 2005). A ring cleaving hydroxyquinol dioxygenase is a key enzyme of this pathway. When a gene coding for the dioxygenase is disrupted, *Arthrobacter chlorophenolicus* has a negligible

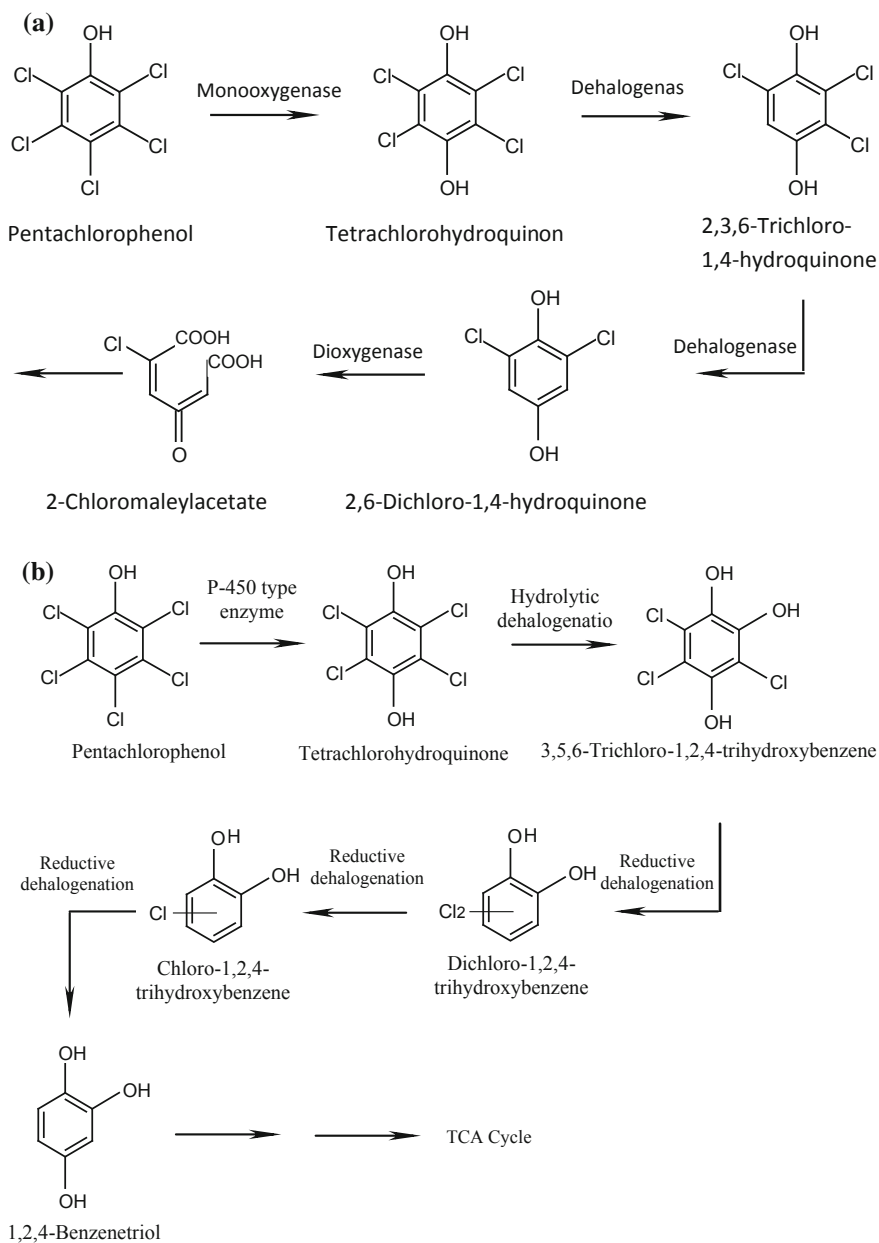


Fig. 4 **a** Proposed pathway of the aerobic degradation of pentachlorophenol by *Spingomonas* bacteria (Arora and Bae 2014). **b** Proposed pathway of the aerobic degradation of PCP by *Mycobacterium* bacteria (Arora and Bae 2014)

growth on 4-CP (Nordin et al. 2005). The monooxygenase from *Ralstonia eutropha* strain JMP134 (pJP4) catalyzes successive dechlorination reactions of 2,4,6-TCP to 2,6-dichlorohydroquinone and then to 6-chlorohydroxyquinol prior to ring cleavage by a hydroxyquinol dioxygenase (Matus et al. 2003).

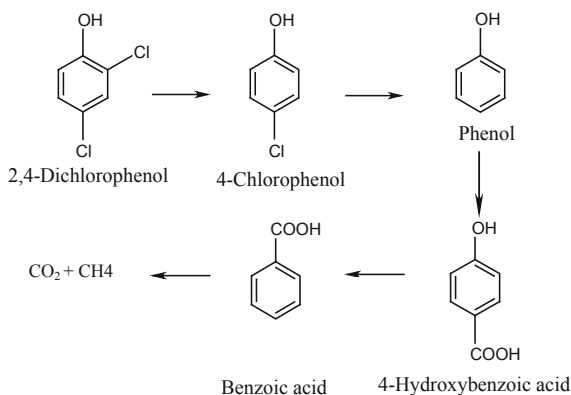
A unique property of the monooxygenase is that the second dechlorination reaction does not require O_2 , instead the dechlorination of 2,6-dichlorohydroquinone is catalyzed by a hydrolytic activity associated with the monooxygenase (Xun and Webster 2004). Some aerobic bacteria implicated in the rapid degradation of higher chlorinated phenols are known to convert CPs into methylated biotransformation products. *Mycobacterium* strains were known to O-methylate a variety of CPs (Haggbloom et al. 1988b). The preferred substrates of the methylation were hydroxyl groups doubly flanked by chlorine groups.

Chlorinated hydroquinones were readily O-methylated (Haggbloom et al. 1988a) accounting for the occurrence of methylated chlorohydroquinone intermediates during PCP biodegradation (Suzuki 1983). Direct methylation of PCP resulted in the formation of pentachloroanisole in soil (D'Angelo and Reddy 2000).

7.2 Anaerobic Biodegradation of CPs

In anaerobic environments, the biodegradation of chlorinated aromatic takes place through reductive dehalogenation (Horowitz et al. 1983) leading to the formation of less toxic and more biodegradable compounds (Gibson and Suflita 1986; Mohn and Kennedy 1992). Reductive dechlorination of 2,4-DCP was followed by carboxylation, ring fission and acetogenesis, and methanogenesis, which led to the complete mineralization of 2,4-DCP as illustrated in Fig. 5 (Zhang and Wiegel 1990). 2,4-DCP was also biodegraded to 4-CP in anaerobic sediments (Boyd and Shelton 1984).

Fig. 5 Anaerobic mineralization of 2,4-dichlorophenol (Zhang and Wiegel 1990)



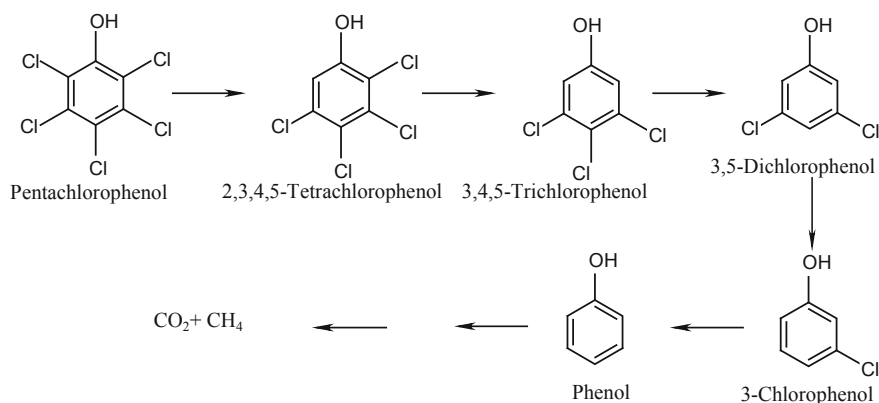


Fig. 6 Anaerobic dehalogenation and degradation of PCP (Arora and Bae 2014)

The reductive dehalogenation is a critical step for the anaerobic biodegradation of CPs, especially for poly-CPs. Several poly-CPs resist the aerobic bacterial attack and can be reductively dehalogenated into chlorinated phenols of less number of chlorine atoms which can be mineralized easily by anaerobic bacteria (Mikesell and Boyd 1986; Londry and Fedorak 1992, Fig. 6).

The presence of phenol-dehalogenating and phenol-degrading bacterial cultures together in a system enhanced the anaerobic mineralization process of PCP (Yang et al. 2009). In this process, a phenol-dehalogenating culture dehalogenates PCP to phenol under anaerobic conditions. The phenol is then further degraded by phenol-degrading bacterial culture under iron reducing or sulfate reducing conditions (Yang et al. 2009).

Suitable biodegradation of phenol, 2-CP, 2,4-DCP, 2,4,6-TCP and PCP under anaerobic conditions in the soil was observed by Smith and Novak (1987). The biodegradation rates followed the order: phenol = 2-CP > 2,4,6-TCP > 2,4-DCP. The PCP degradation rate was slower than that of phenol or 2-CP, but comparable to 2,4,6-TCP and 2,4-DCP. This suggests that increased chlorination of the aromatic ring does not necessarily have a direct correlation with resistance to biodegradation. Rates of degradation also varied with soil characteristics. Groundwater nitrate concentration was suggested as a possible reason for the difference in the degradation rate of chlorophenols (Annachhatre and Gheewala 1996).

7.3 Co-Metabolic Transformation of CPs

The first definitive report on the co-metabolic study appeared in the late 1950s (Kim and Hao 1999). A strain of *Pseudomonas methanica*, growing with methane (growth substrate) as a sole source of carbon and energy, was shown to oxidize other alkanes such as ethane and propane (non-growth substrate) to their

corresponding fatty acids. Since then, many other co-metabolizing cultures have been identified (Alvarez-Cohen and McCarty 1991). A large number of chlorinated solvents can be degraded under aerobic conditions by means of co-metabolic transformation reactions (Alvarez-Cohen and Speitel 2001). Co-metabolism of monochlorinated phenols with phenol has been shown to be an effective way for the biodegradation of these toxic compounds. The *Rhodococcus* species can co-oxidize aniline, phenol and their monochlorinated isomers in the presence of glucose or acetate (Janke et al. 1989; Dapaah and Hill 1992). The degradation of mixtures of monochlorophenols and phenol as substrates for free and immobilized cells of *Alcaligenes* sp. A7-2 was evaluated by Menke and Rehm (1992), and it was found that 3-CP could only be mineralized co-metabolically. It was also reported that *Acinetobacter* species could also transform monochlorophenols via co-metabolism (Kim and Hao 1999; Hao et al. 2002). *Pseudomonas putida* was also utilized to degrade phenol and monochlorinated phenols by means of cometabolic transformation (Loh and Wang 1998).

The difficulties of co-metabolism started with the description of the process (Semprini 1997). A general consensus refers to co-metabolism as the metabolism of a substrate not required for growth in which no apparent benefit is accrued by the metabolizing organism (Horvath 1972). Therefore, a growth substrate must be available at least periodically to support the cell growth. For example, 4-CP cannot serve as the sole carbon and energy source for the growth of *Pseudomonas putida*, but when phenol is present, they can be transformed together, with phenol as the growth substrate and 4-CP as the non-growth substrate. One main reason for such widely observed microbial co-metabolism is the lack of specificity of existing enzymes and co-factors. Therefore non-growth substrate with a similar structure to that of growth substrate could bind to enzymes and be transformed. Especially, oxygenase enzymes have shown non-specific activities towards non-growth substrates, such as chlorinated solvent (Kim and Hao 1999). The oxygenase reaction can oxidize the chlorinated compounds to intermediates that may be hydrolyzed spontaneously into carbon dioxide, chloride or other products that can be easily attacked by the microorganisms (Alvarez-Cohen and Speitel 2001).

The addition of conventional carbon source was also taken to augment the performance of co-metabolic degradation (Wang 1997). In spite of the rather extensive investigation of co-metabolic transformation, more studies (not only for qualitative perspective) were found of great practical significance, due to the complex nature of co-metabolism and its effectiveness as a treatment method of chlorophenols waste.

8 Factors Affecting the Biodegradation of CPs

Degradation rates are strongly influenced by a wide variety of environmental factors which affect microbial activity. The bacteria or their enzymes must be in contact with the pesticide, and the pesticide or its metabolites must be transported into the

cell to continue the degradation. Some bacteria display chemical responses to substrates, which may play an important role in the environment, while others grow in a filamentous form towards potential substrates (Fewson 1988). The environmental factors include presence of appropriate microorganisms, contact between the microbe and the substrate, pH, temperature and salinity, available water, oxygen tension and redox potential, nutrient availability, presence of alternative carbon substrates, light quality and intensity, binding to surfaces and alternative electron acceptor etc. However, molecule factors include chemical structure, molecular weight and functional groups concentration and toxicity, and solubility in water.

8.1 Effect of PH

The pH and alkalinity have important roles to play in the biological treatment of wastewater. Extreme pH values of the medium (less than 3 or greater than 9) as well as sudden changes in pH in which the microbe is present, can inhibit its growth (Agarry et al. 2008). In aerobic biological oxidation, pH in the range of 6.0–9.0 is suitable (Majumder and Gupta 2009). Therefore, laboratory studies on chlorophenols degradation are usually carried out at or near neutral pH values (pH 7.0). However, Alva and Peyton (2003) reported that a haloalkaliphilic bacterium, *Halomonas campisalis*, is able to thrive in both saline and alkaline environments, such as soda lakes. The bacterium degraded phenol and catechol both in alkaline (pH values of 8–11) and saline environments (0–150 g L⁻¹ NaCl). This is specifically significant for an industrial effluent of a petroleum refinery containing spent caustic, which is an alkaline and saline waste stream that contains phenol as well as other aromatic compounds (Alva and Peyton 2003).

Many investigators have noted that pH plays an important role in the adsorption of compounds with acidic functional groups on activated carbon and soil, because the neutral and ionic forms display very different adsorption properties (Kookana and Rogers 1995; Diez et al. 1999; Cea et al. 2007). However, CPs sorption in soil has been generally described assuming hydrophobic partitioning of the neutral species to soil organic matter (DiVincenzo and Sparks 2001). Diez et al. (1999) demonstrated that in a variable-charged soil, the adsorption of phenolic compounds from Kraft mill effluent increased with decreasing pH, possibly as a consequence of electrostatic repulsions between the organic compound and the resulting negative surface charge as the pH increases (Diez et al. 1999).

8.2 Effect of Temperature

Temperature is another important parameter that is known to have a significant effect on the growth and the biodegradation capabilities of microorganisms. Conventionally, organisms can be divided into psychophilic and psychrotrophic.

Psychrophiles are the organisms that have minimum, optimum and maximum growth temperatures of ≤ 0 , ≤ 15 , ≤ 20 °C, respectively. The corresponding temperatures for psychrotrophs are 0–5, > 15 and > 20 °C. It is evident that the most interesting organisms are often the psychrotrophs, since they are also active at temperatures above 20 °C (Li et al. 2010). Therefore, temperature-dependent performance of biological processes may be strongly influenced by their content of psychrotrophic bacteria. Most of the studies on CP biodegradation had been carried out in the temperature range 25–30 °C. Li et al. (2011) reported that there has been little focus on microorganisms that function at low temperature. Therefore, it is necessary to find a kind of organism that can adapt to the daily and seasonal fluctuations in the environmental temperatures, with a focus on cold-adapted microorganisms in environments where they are needed. The bacterial strain *P. putida* LY1, as a psychrotrophic microorganism, was shown to grow on phenol as the sole carbon and energy source and survive well in a wide range of temperatures (Li et al. 2011). On the other hand, only a few studies have focused on the biodegradation of phenols by thermophilic or hyperthermophilic microorganisms, although many phenolic effluents are very hot. Such microorganisms can be robust biocatalysts with potential applications (Christen et al. 2012). A strain of *P. putida* has been successfully used to degrade phenol at low temperature of 10 °C, while the bacterium *Bacillus stearothermophiles* was found to effectively degrade phenol at 50 °C (Agarry et al. 2008). Several studies on these aspects have been documented in this regard (Christen et al. 2012; Margesin et al. 2013).

Thus, temperature significantly affects critical process and design parameters, such as the critical dilution rate, which corresponds to the limit above which biomass washout occurs and, hence, failure of the biological treatment process (Onysko et al. 2000). According to Onysko et al. (2000), there is a lack of sufficient information about the change of microbial kinetics as a function of temperature. The authors attempted to model the temperature dependence of cell growth and phenol degradation kinetics of the psychrotrophic bacterium *P. putida* Q5 in both batch and continuous cultures in the temperature range of 10–25 °C (Onysko et al. 2000).

8.3 Effect of Substrate Concentration

High concentrations of chlorophenols are usually inhibitory for the microorganisms. However, pre-adaptation or acclimatization of microorganisms to chlorophenols improves their biodegradation capability and alleviates inhibition effects to some extent (Tobajas et al. 2012). Sometimes, due to substrate inhibition, combined processes have been used to treat highly loaded wastewaters, such as the sequential UV-biological degradation of a mixture of chlorophenols and sequential anaerobic-aerobic reactors (Armenante et al. 1999; Tamer et al. 2006; Majumder and Gupta 2007). Wang et al. (2007b) indicated that there is a little information on bacteria with a high tolerance to phenols and high metabolizing activity. Therefore,

there is still a need to isolate new phenols-degrading bacteria that can grow at elevated concentration of phenol (Wang et al. 2007b).

The ability of microbial communities to degrade pollutants is affected by the presence of naturally occurring carbon sources. In general, adaptation to variations in the concentration of nutrients, such as glucose, yeast extract and $(\text{NH}_4)_2\text{SO}_4$, enhances the ability to degrade phenols. However, it was observed that different CPs may give different responses to the presence of biogenic substrate (Sahinkaya and Dilek 2006a, b). The rate of degradation largely depends on the composition of the medium which affects the degradation efficiency. Shourian et al. (2009) found that phenol-containing minimal medium, supplemented with mannitol and casein, showed the highest degradation rate among all carbon and nitrogen nutrients tested. The highest inhibitory effects were observed with the addition of sucrose and glycerol. In another study, Stehlickova et al. (2009) observed higher biodegradation rate and more intensive growth during the cultivation in the presence of potassium humate (humic substance) than the cultivation without its addition. This effect of humic substances was also confirmed by Hahn et al. (2007) indicating a reduction in the 2,4 DCP toxicity to *P. putida* after oxidative incubation with humic substances.

9 Activities of Microbes in Soils Contaminated with CPs

Both indigenous and exogenous microorganisms are responsible for the degradation of pesticides in soils during the composting process. The presence of pesticides affected the population and activity of microbes in contaminated soil with composting. The interaction between contaminant and microbes also influences the effect of composting on soil remediation. Contaminant toxicity to microbes also affects the biodegradation of some pollutants. The undesirable properties of contaminants have adverse effects on the growth of microorganisms by interfering with their normal metabolic function. Studies on the dynamics of microbial community during composting of PCP-contaminated soils revealed that microbial abundance was inhibited by PCP stress (Ren et al. 2011). In a study carried out by (Scelza et al. 2008), the soil microflora was found very difficult to recover from its inhibitory state induced by the toxicity of PCP. Composting exhibited a strong effect on the removal of CP with a degradation potential of 85.85 % (decomposed from 212 to 30 mg kg^{-1}) in four summer months. After the second summer of composting, the biodegradation resulted in the concentration of chlorophenols to only 15 mg kg^{-1} (Valo and Salkinoja-Salonen 1986). Bacterial populations, which occurred in compost, manure and cornstalk-amended soils at a rate of 5 %, were increased significantly compared to non-amended soils, but these amendments had no effect on fungal or actinomycete populations (Moorman et al. 2001).

The addition of pollutant-degrading microorganisms can certainly improve the degradation progress, but a large number of microbial degraders do exist in the composting of contaminated soil. In the presence of *Rhodococcus chlorophenolicus* that can degrade several types of chlorophenols as amendment, the decomposition of chlorophenols was faster than that without the inoculation of this microorganism (Valo and Salkinoja-Salonen 1986). *P. chrysosporium*, known as a type of basidiomycete, exhibited high degradation ability for lignin, PCP, and others by secreting lignin peroxidase (LiP) and manganese peroxidase (MnP) (Martinez et al. 2005; Yu et al. 2011). Thus, *P. chrysosporium* inoculants could increase lignocelluloses biodegradability and ultimately improve the quality of compost products. Yu et al. (2011) showed that the inoculation of *P. chrysosporium* had a strong positive effect, both on the composting efficiency and the removal of PCP. This study also indicated that inoculation time influenced the removal of PCP in soil based on composting, because the inoculation on the fifteenth day of composting had a better effect on PCP removal than that in the beginning. Thus, the positive effect of microorganism addition on remediation of polluted soil with composting cannot be neglected. However, mineralization of pollutants in soil by microbes in composting may also pose ecotoxicological risks to the soil environment, if incomplete pollutant mineralization occurs (Laine and Jørgensen 1997). As suggested by Laine and Jørgensen (1997), safe, effective and fast composting methods with complete mineralization needed to be developed. White rot fungi have been shown to have a high capacity to remove various organic pollutants during composting. However, sometimes, white rot fungi also convert organic contaminants to harmful metabolites. For example, white rot fungi may transform CS into PCDD/Fs by peroxidase enzymes (Laine and Jørgensen 1997; Oberg and Rappe 1992).

The application of composting technology as a remediation strategy for contaminated soil requires an understanding of microbes involved in pollutant biodegradation and biotransformation. The knowledge about the effect of more types of pollutant-degrading microorganisms as amendments during composting of contaminated soil is still poor despite the availability of various degrading microorganisms. A number of microorganisms can grow on the chlorophenols compounds using them as a sole source of carbon and energy. A list of typical microbes that can degrade CPs has been prepared by Gaofeng et al. (2004) as reflected in Table 2.

As indicated above, *Pseudomonas* spp. can dechlorinate various CPs in suspension cultures. In fact, *Pseudomonas* spp. can also degrade many other aromatic compounds, such as other chlorinated aromatic compounds, nitrified aromatic compounds, aminophenols, and polycyclic aromatic hydrocarbon (An et al. 2001). *Desulfomonile tiedjei*, a strictly anaerobic Gram-negative sulfate-reducing bacterium, is the best-described dechlorinating anaerobic bacterium to date (Middeldorp et al. 1997).

Table 2 Common chlorophenols-degrading microbes (Gaofeng et al. 2004)

Chlorophenols	Microbes
2-chlorophenol	<i>Desulfovibrio dechloracetivorans</i> (ATCC700921), <i>Alcaligenes</i> sp., <i>Ralstonia</i> sp., <i>Azotobacter</i> sp., <i>Pseudomonas putida</i> , <i>Cystobacteri</i> sp., <i>Ps. cepacia</i>
3-chlorophenol	<i>Desulfomonile tiedjei</i>
4-chlorophenol	<i>Ps. putida</i> , <i>Comamonas testosteroni</i> JH5, <i>Ps. cepacia</i> , <i>Ralstonia eutropha</i> , <i>Alcaligenes</i> sp., <i>Azotobacter</i> sp., <i>Ralstonia</i> sp.
2,3-dichlorophenol	<i>Desulfitobacterium dehalogenans</i> (JW/IU-DC1), <i>Desulfomonile tiedjei</i>
2,4-dichlorophenol	<i>Desulfitobacterium dehalogenans</i> (JW/IU-DC1), <i>Desulfomonile tiedjei</i> , <i>Ralstonia</i> sp., <i>Clostridium</i> sp., <i>Burkholderia cepacia</i> , <i>Pseudomonas pickettii</i> (DTP0606)
2,5-dichlorophenol	<i>Desulfomonile tiedjei</i> , <i>Desulfovibrio dechloracetivorans</i>
2,6-dichlorophenol	<i>Desulfitobacterium dehalogenans</i> (JW/IU-DC1), <i>Mycobacterium chlophenolicum</i> , <i>Ps. cepacia</i> , <i>Azotobacter</i> sp., <i>Ps. pickettii</i> (DTP0606), <i>Desulfovibrio dechloracetivorans</i> , <i>Ralstonia</i> sp.
3,4-dichlorophenol	<i>Ps. pickettii</i> (DTPO602)
3,5-dichlorophenol	<i>Clostridium</i> sp., <i>Desulfomonile tiedjei</i>
2,3,4-trichlorophenol	<i>Desulfovibrio dechloracetivorans</i> (JW/IC-DC1), <i>Ps. pickettii</i> (DTPO602)
2,4,6-trichlorophenol	<i>Ps. pickettii</i> (DTPO602), <i>Azotobacter</i> sp., <i>Desulfitobacterium dehalogenans</i> (JW/IU-DC1), <i>Clostridium</i> sp., <i>Phanerochaete chrysosporium</i>
2,4,5-trichlorophenol	<i>Clostridium</i> sp., <i>Ps. pickettii</i> (DTPO602)
Tetrachlorophenols	<i>Ps. pickettii</i> (DTPO602), <i>Ralstonia</i> sp., <i>Arthrobacter</i> sp.
Pentachlorophenol	<i>Flavobacterium</i> sp., <i>Desulfomonile tiedjei</i> sp., <i>Clostridium</i> sp., <i>Rhodococcus chlorophenolicus</i> , <i>Desulfitobacterium frappier</i> (PCP-1), <i>Desulfitobacterium dehalogenans</i> (JW/IU-DC1), <i>Ps. cepacia</i> (AC110)

10 Involvements of Enzymes in Degradation of CPs

The enzymes in the microbial degradation of CPs are categorized mainly as the oxygenases and dioxygenases (Olaniran and Igbinsosa 2011). Based on the literature describing CP biodegradation pathways, anaerobic degradation of CP includes reductive dehalogenation as the first step with the favored replacement of chlorine by hydrogen from ortho- position. The aerobic degradation of CPs may occur either through the ortho-pathway or the meta- pathway, following their conversion to chlorocatechols (Solyanikova and Golovleva 2004; Majumder and Gupta 2007). Thus, catechol is a central intermediate in the aerobic degradation pathways of various aromatic compounds. Fava et al. (1995) suggested the chlorocatechol pathway in their investigation of aerobic degradation of different mono-chlorophenols by a *Pseudomonas pickettii* strain. However, it has been reported in the literature that aerobic biodegradation of mono and dichlorophenols follows mainly the ortho-cleavage pathway after the formation of chlorocatechol,

because the meta- cleavage produces toxic products from chlorocatechol (Lu et al. 1996; Solyanikova and Golovleva 2004).

Conversion of chlorocatechol as a rule does not proceed via meta- cleavage of the aromatic ring, as catechol dioxygenase enzyme is inactivated by the accumulation of an intermediate, which is toxic to cells. Meta-cleavage of chloroaromatics generally results in the formation of dead-end metabolites. In general, the ortho-cleavage pathway is required for the complete degradation of mono and di-chlorophenols. Only meta- cleavage pathways have been observed for chlorocatechols (Ferraroni et al. 2006). *P. putida* CP1 was added to a commercial mixed culture that degraded 2 CP. The augmented mixed culture degraded 2 CP using an ortho-cleavage pathway, whereas previous degradation of the mixed culture occurred using the meta-cleavage pathway.

Metabolic processes are governed by the action of enzymes, which are specific for each type of reaction. Thus microbial metabolism is a process of energy conversion sustained by specific reactions and provides the ultimate source of energy (Agarry et al. 2008). The biodegradation process requires the presence of molecular oxygen to initiate an enzymatic attack on the aromatic rings. A typical pathway for metabolizing phenols is to hydroxylate the ring by the enzyme phenol hydroxylase, form catechol and then open the ring through ortho- (also termed β -ketoadipate pathway) or meta-oxidation (Jiang et al. 2006; Lika and Papadakis 2009). Phenol hydroxylase represents the first enzyme in the metabolic pathway of phenol degradation. Both ortho-pathway and meta-pathway are distinguishable by evaluating their characteristic enzyme activities. In the ortho-pathway, the aromatic ring is cleaved by the enzyme catechol 1, 2 dioxygenase (C12O). In the meta-pathway, the ring is cleaved by the enzyme catechol 2, 3 dioxygenase (C23O). Therefore, the ring is first opened and then degraded (Agarry et al. 2008). The ring cleavage occurs in two different directions and this difference in cleavage site is used to classify catechol dioxygenases in two groups: the intradiol (such as C12O) and extradiol (such as C23O) cleaving enzymes (Cai et al. 2007). The genes of ring cleavage dioxygenases may serve as good targets for monitoring the biodegrading biomass, and also provide a rapid method for monitoring the microbial community during the treatment process (Heinaru et al. 2005).

The use of enzymes in industrial processes is usually linked to a reduced consumption of energy as well as chemicals and thus beneficial for the environment (Demarche et al. 2012). In biodegradation processes, free enzymes often undergo deactivation as a result of sudden changes in the surrounding environment, such as pH, temperature, and chemical and hydrodynamic shocks. These faults can be overcome by immobilization. Enzyme immobilization could enhance its stability against sudden changes in environmental factors: extend lifetime, increase reusability and easy handling. A few studies for the removal of phenolic pollutants by enzymatic treatment have been reported in recent years (Demarche et al. 2012; Fodil et al. 2012; Mohidem and Mat 2012; Lee et al. 2013). These studies have focused on offering novel, practical and inexpensive immobilization methods, utilizing various enzymes, such as laccases, peroxidases, tyrosinases and dioxygenases.

11 Future Prospective

In spite of the considerable amount of research on the biodegradation of CP's, there is still a need for more effective approaches for theoretical and experimental investigations. The pathways and the degradation mechanisms should be evaluated through mathematical modeling to predict the effect of different factors on the degradation process. In recent years, researchers have mainly focused on the isolation and activities of the CPs-degrading bacteria in liquid media, and hence most of the reported findings are mainly limited to laboratory scale experiments. However, the results observed in the laboratory often differ from those observed in the field or in treatment processes in practice. Therefore, it is essential to carry out a large-scale direct study to evaluate the efficiency of CPs-degrading microbes in *in situ* conditions. Thus, innovative, effective and economical biodegradation methods should be proposed and evaluated at the laboratory scale and then applied to large scale to test their effectiveness.

12 Conclusion

The production and usage of large amounts of CPs have led to the entry of many contaminants into the environment. CPs are considered harmful to human health due to their potential carcinogenic and toxic effects. Although some of CPs are resistant to degradation and therefore persistent in the environment, microorganisms exposed to CPs have developed the ability to degrade some of them. Bacteria of different species have been reported to degrade CPs. Thus, such biological degradation can be exploited to remediate environmental pollution problems. In this chapter, some of the achievements in degrading of CPs by microorganisms have been reviewed. The degradation mechanism and pathways of two CPs; 2,4-dichlorophenol and Pentachlorophenol have been also discussed.

Different studies have revealed that CPs can be degraded aerobically or anaerobically, depending on the microbial abilities to grow in the particular environment. Aerobic microorganisms are more efficient for degrading CPs, because they grow fast and achieve complete mineralization of toxic organic compounds to inorganic compounds i.e. CO_2 and H_2O . For these reasons, there is a limited interest in the utilization of anaerobic bacteria for the degradation of CPs. Both pure and mixed cultures of the microorganisms have been utilized for the degradation of CP. *Pseudomonas putida* is one which is commonly used for the degradation due to their high removal efficiency. In fact, *P. putida* cells have been known to use diverse protective mechanisms for survival in various extreme environments.

Degradation rates are affected by a wide range of environmental factors which affect microbial action. For degradation to continue, bacteria, or their enzymes must be in contact with the CPs, and the CPs or its metabolites must be transported into the cell. Some bacteria display chemical responses to substrates, which may play an

important role in the environmental remediation. The environmental factors that regulate the degradation process include appropriate microorganisms, contact between the microbe and the substrate, pH, temperature, salinity, available water, oxygen, redox potential, nutrient availability, presence of alternative carbon substrates, light quality and intensity, binding to surfaces and alternative electron acceptor. In addition, molecular factors such as chemical structure, molecular weight and functional groups concentration and toxicity, and solubility in water can play a key role in the degradation process.

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References

- Abrahamsson, K., & Klick, S. (1991). Degradation of halogenated phenols in anoxic marine sediments. *Marine Pollution Bulletin*, 22, 227–233.
- Agarry, S. E., Durojaiye, A. O., & Solomon, B. O. (2008). Microbial degradation of phenols: A review. *International Journal of Environment and Pollution*, 32, 12–28.
- Aislabie, J., & Lloyd-Jones, G. (1995). A review of bacterial-degradation of pesticides. *Australian Journal of Soil Research*, 33, 925–942.
- Al-Khalid, T., & El-Naas, M. (2013). Transient behavior in biodegradation of 2, 4 dichlorophenol: Is it a starvation effect? *International Journal of Chemical Engineering and Applications*, 4, 365–368.
- Al-Khalid, T., & El-Naas, M. (2014). Biodegradation of phenol and 2, 4 dichlorophenol: The role of glucose in biomass acclimatization. *International Journal of Engineering Research and Technology*, 3, 1579–1586.
- Al-Khalid, T., & El-Naas, M. H. (2011). Aerobic biodegradation of phenols: A comprehensive review. *Critical Reviews in Environmental Science and Technology*, 42, 1631–1690.
- Al-Thani, R. F., Abd-El-Haleem, A. M., & Al-Shammri, A. (2007). Isolation, biochemical and molecular characterization of 2-chlorophenol-degrading *Bacillus* isolates. *African Journal of Biotechnology*, 6, 2675–2681.
- Al-Zuhair, S., & El-Naas, M. H. (2012). Phenol biodegradation by *Ralstonia pickettii* extracted from petroleum refinery oil sludge. *Chemical Engineering Communications*, 199, 1194–1204.
- Alva, V., & Peyton, B. (2003). Phenol and catechol biodegradation by the haloalkaliphile *Halomonas campisalis*: Influence of pH and salinity. *Environmental Science and Technology*, 37, 4397–4402.
- Alvarez-Cohen, L., & McCarty, P. L. (1991). A cometabolic biotransformation model for halogenated aliphatic compounds exhibiting product toxicity. *Environmental Science and Technology*, 25, 1381–1387.
- Alvarez-Cohen, L., & Speitel, J. (2001). Kinetics of aerobic cometabolism of chlorinated solvents. *Biodegradation*, 12, 105–126.
- An, H.-R., Park, H.-J., & Kim, E.-S. (2001). Cloning and expression of thermophilic catechol 1,2-dioxygenase gene (catA) from *Streptomyces setonii*. *FEMS Microbiology Letters*, 195, 17–22.
- Annachhatre, A. P., & Gheewala, S. H. (1996). Biodegradation of chlorinated phenolic compounds. *Biotechnology Advances*, 14, 35–56.

- Antizar-Ladislao, B., & Galil, N. (2004). Absorption of phenol and chlorophenols by acclimated residential biomass under bioremediation conditions in study aquifer. *Water Research*, 38, 267–276.
- Apajalahti, J. H. A., & Salkinoja-Salonen, M. S. (1987). Complete dechlorination of tetrachloro-hydroquinone by cell-extracts of pentachlorophenol-induced *Rhodococcus chlorophenolicus*. *Journal of Bacteriology*, 169, 5125–5130.
- Araya, M. & Lakhi, A. (2004). Response to consecutive nematicide applications using the same product in musa AAACv. *Grande naine* originated from *in vitro* propagative material and cultivated in virgin soil. *Nematologia Brasileira* 28, 55.
- Armenante, P. M., Kafkewitz, D., Lewandowski, G. A., & Jou, C.-J. (1999). Anaerobic–Aerobic treatment of halogenated phenolic compounds. *Water Research*, 33, 681–692.
- Arora, P., & Bae, H. (2014). Bacterial degradation of chlorophenols and their derivatives. *Microbial Cell Factories*, 13, 31.
- ATSDR (1999). Toxicological profile for chlorophenols. In Services, U.D. o. H.a.H. (ed.). Atlanta, GA.
- Azbar, N., Tutuk, F., & Keskin, T. (2009). Biodegradation performance of an anaerobic hybrid reactor treating olive mill effluent under various organic loading rates. *International Biodeterioration and Biodegradation*, 63, 690–698.
- Bae, H. S., Rhee, S. K., Cho, Y. G., Hong, J. K., & Lee, S. T. (1997). Two different pathways (a chlorocatechol and a hydroquinone pathway) for the 4-chlorophenol degradation in two isolated bacterial strains. *Journal of Microbiology and Biotechnology*, 7, 237–241.
- Bajaj, M., Gallert, C., & Winter, J. (2009). Treatment of phenolic wastewater in an anaerobic fixed bed reactor (AFBR)—Recovery after shock loading. *Journal of Hazardous Materials*, 162, 1330–1339.
- Baker, M. D., & Mayfield, C. J. (1980). Microbial and non-biological decomposition of chlorophenols and phenol in soil. *Water, Air, and Soil Pollution*, 13, 411–424.
- Basu, S. K., Oleszkiewicz, J. A., & Sparling, R. (2005). Effect of sulfidogenic and methanogenic inhibitors on reductive dehalogenation of 2-chlorophenol. *Environmental Technology*, 26, 1383–1391.
- Becker, J. G., Stahl, D. A., & Rittmann, B. E. (1999). Reductive dehalogenation and conversion of 2-chlorophenol to 3-chlorobenzoate in a methanogenic sediment community: Implications for predicting the of chlorinated pollutants. *Applied and Environment Microbiology*, 65, 5169–5172.
- Bergauer, P., Fonteyne, P. A., Nolard, N., Schinner, F., & Margesin, R. (2005). Biodegradation of phenol and phenol-related compounds by psychrophilic and cold-tolerant alpine yeasts. *Chemosphere*, 59, 909–918.
- Boothe, D. D. H., Rogers, J. E., & Wiegel, J. (1997). Reductive dechlorination of chlorophenols in slurries of low- organic-carbon marine sediments and subsurface soils. *Applied Microbiology and Biotechnology*, 47, 742–748.
- Boyd, S. A., & Shelton, D. R. (1984). Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. *Applied and Environment Microbiology*, 47, 272–277.
- Breining, S., Schilts, E., & Fuchs, G. (2000). Genes involved in metabolism of phenol in bacterium *Thauera aromatica*. *Journal of Bacteriology*, 182, 5849–5863.
- Brown, J. F., Bedard, D. L., Brennan, M. J., Camahan, J. C., Feng, H., & Wagner, R. E. (1987). Polychlorinated biphenyl dechlorination in aquatic sediments. *Science*, 236, 709–712.
- Cai, W., Li, J., & Zhang, Z. (2007). The characteristics and mechanisms of phenol biodegradation by *Fusarium* sp. *Journal of Hazardous Materials*, 148, 38–42.
- Caliz, J., Vila, X., Martí, E., Sierra, J., Nordgren, J., Lindgren, P.-E., et al. (2011). The microbiota of an unpolluted calcareous soil faces up chlorophenols: Evidences of resistant strains with potential for bioremediation. *Chemosphere*, 83, 104–116.
- Cea, M., Seaman, J. C., Jara, A., Fuentes, B., Mora, M. L., & Diez, M. C. (2007). Adsorption behaviour of 2,4-dichlorophenol and pentachlorophenol in an allophanic soil. *Chemosphere*, 67, 1354–1360.

- Christen, P., Vega, A., Casalot, L., Simon, G., & Auria, R. (2012). Kinetics of aerobic phenol biodegradation by the acidophilic and hyperthermophilic archaeon *Sulfolobus solfataricus* 98/2. *Biochemical Engineering Journal*, 62, 56–61.
- Copley, S. D. (1997). Diverse mechanistic approaches to difficult chemical transformations: Microbial dehalogenation of chlorinated aromatic compounds. *Chemistry and Biology*, 4, 169–174.
- Cortes, D., Garrios-Gonzalez, J., & Tomasini, A. (2002). Pentachlorophenol tolerance and removal by *Rhizopus nigricans* in solid-state culture. *Process Biochemistry*, 37, 881–884.
- Crawford, R. L., Jung, C. M., & Strap, J. L. (2007). The recent evolution of pentachlorophenol (PCP)-4-monooxygenase (PcpB) and associated pathways for bacterial degradation of PCP. *Biodegradation*, 18, 525–539.
- Czaplicka, M. (2004). Sources and transformations of chlorophenols in the natural environment. *Science of the Total Environment*, 322, 21–39.
- D'Angelo, E. M., & Reddy, K. R. (2000). Aerobic and anaerobic transformations of pentachlorophenol in wetland soils. *Soil Science Society of America Journal*, 64, 933–943.
- Dapaah, S. Y., & Hill, G. A. (1992). Biodegradation of chlorophenol mixtures by *Pseudomonas putida*. *Biotechnology and Bioengineering*, 40(11), 1353–1358.
- Demarche, P., Junghanns, C., Nair, R. R., & Agathos, S. N. (2012). Harnessing the power of enzymes for environmental stewardship. *Biotechnology Advances*, 30, 933–953.
- Demnerova, K., Mackova, M., Spevakova, V., Beranova, K., Kochankova, L., Lovecka, P., et al. (2005). Two approaches to biological decontamination of groundwater and soil polluted by aromatics-characterization of microbial populations. *International Microbiology*, 8, 205–211.
- Diez, M. C., Gallardo, F., Tortella, G., Rubilar, O., Navia, R., & Bornhardt, C. (2012). Chlorophenol degradation in soil columns inoculated with *Anthracophyllum discolor* immobilized on wheat grains. *Journal of Environmental Management*, 95(Suppl), S83–S87.
- Diez, M. C., Mora, M. L., & Videla, S. (1999). Adsorption of phenolic compounds and color from bleached Kraft mill effluent using allophanic compounds. *Water Research*, 33, 125–130.
- DiVincenzo, J., & Sparks, D. L. (2001). Sorption of the neutral and charges forms of pentachlorophenol on soil: evidence for different mechanisms. *Archives of Environmental Contamination and Toxicology*, 40, 445–450.
- Dong, X., Hong, Q., He, L., Jiang, X., & Li, S. (2009). Characterization of phenol-degrading bacterial strains isolated from natural soil. *International Biodeterioration and Biodegradation*, 63, 365–370.
- El-Naas, M. H., Al-Muhtaseb, S. A., & Makhlof, S. (2009). Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. *Journal of Hazardous Materials*, 164, 720–725.
- El-Naas, M. H., Al-Zuhair, S., & Makhlof, S. (2010). Continuous biodegradation of phenol in a spouted bed bioreactor (SBBR). *Chemical Engineering Journal*, 160, 565–570.
- Esteve-Núñez, A., Caballero, A., & Ramos, J. L. (2001). Biological degradation of 2,4,6-trinitrotoluene. *Microbiology and Molecular Biology Reviews*, 65, 335–352.
- Farrell, A., & Quilty, B. (2002). The enhancement of 2-chlorophenol degradation by a mixed microbial community when augmented with *Pseudomonas putida* CP1. *Water Research*, 36, 2443–2450.
- Fava, F., Armenante, P. M., & Kafkewitz, D. (1995). Aerobic degradation and dechlorination of 2 = chlorophenol, 3-chlorophenol and 4-chlorophenol by a *Pseudomonas pickettii* strain. *Letters in Applied Microbiology*, 21, 307–312.
- Ferraroni, M., Kolomytseva, M. P., Solyanikova, I. P., Scozzafava, A., Golovleva, L. A., & Briganti, F. (2006). Crystal structure of 3-chlorocatechol 1,2-dioxygenase key enzyme of a new modified ortho-pathway from the gram-positive *Rhodococcus opacus* 1CP grown on 2-chlorophenol. *Journal of Molecular Biology*, 360, 788–799.
- Fewson, C. A. (1988). Biodegradation of xenobiotic and other persistent compounds: The causes of recalcitrance. *Trends in Biotechnology*, 6, 148–153.

- Field, J. A., & Sierra-Alvarez, R. (2008). Microbial transformation and degradation of polychlorinated biphenyls. *Environmental Pollution*, 155, 1–12.
- Finkel'shtein, Z. I., Baskunov, B. P., Golovlev, E. L., Moiseeva, O. V., Vervoort, J., Rietjens, I., et al. (2000). Dependence of the conversion of chlorophenols by rhodococci on the number and position of chlorine atoms in the aromatic ring. *Microbiology*, 69, 40–70.
- Fodil, D., Jaouadi, B., Badis, A., Nadia, Z. J., Ferradji, F. Z., Bejar, S., et al. (2012). A thermostable humic acid peroxidase from *Streptomyces* sp. strain AH4: Purification and biochemical characterization. *Bioresource Technology*, 111, 383–390.
- Gallizia, I., McClean, S., & Banat, I. (2003). Bacterial biodegradation of phenol and 2,4-dichlorophenol. *Journal of Chemical Technology and Biotechnology*, 78, 959–963.
- Gaofeng, W., Hong, X., & Mei, J. (2004). Biodegradation of chlorophenols: A review. *Chemical Journal on Internet*, 6, 1–67.
- Gibson, S. A., & Suflita, J. M. (1986). Extrapolation of biodegradation results to groundwater aquifers: Reductive dehalogenation of aromatic compounds. *Applied and Environment Microbiology*, 52, 681–688.
- Hagblom, M. M., Apajalahti, J. H., & Salkinoja-Salonen, M. S. (1988a). O-methylation of chlorinated para-hydroquinones by *Rhodococcus chlorophenolicus*. *Applied and Environment Microbiology*, 54, 1818–1824.
- Hagblom, M. M., Nohynek, L. J., & Salkinoja Salonen, M. S. (1988b). Degradation and o-methylation of chlorinated phenolic-compounds by *Rhodococcus* and *Mycobacterium* strains. *Applied and Environment Microbiology*, 54, 3043–3052.
- Hagblom, M. M., Rivera, M. D., & Young, L. Y. (1993). Influence of alternative electron-acceptors on the anaerobic biodegradability of chlorinated phenols and benzoic-acids. *Applied and Environment Microbiology*, 59, 1162–1167.
- Hahn, D., Cozzolino, A., Piccolo, A., & Armenante, P. M. (2007). Reduction of 2,4-dichlorophenol toxicity to *Pseudomonas putida* after oxidative incubation with humic substances and a biomimetic catalyst. *Ecotoxicol Environ Safety*, 66, 335–342.
- Hale, D. D., Reineke, W., & Wiegel, J. (1994). Chlorophenol degradation. In G. R. Chaudhry (Ed.), *Biological degradation and bioremediation of toxic chemicals* (pp. 74–91). London, UK: Chapman and Hall.
- Hao, O. J., Kim, M. H., Seagren, E. A., & Kim, H. (2002). Kinetics of phenol and chlorophenol utilization by *Acinetobacter* species. *Chemosphere*, 46, 797–807.
- Heinaru, E., Merimaa, M., Viggor, S., Lehiste, M., Leito, I., Truu, J., et al. (2005). Biodegradation efficiency of functionally important populations selected for bioaugmentation in phenol- and oil-polluted area. *FEMS Microbiology Ecology*, 51, 363–373.
- Horowitz, A., Suflita, J. M., & Tiedje, J. M. (1983). Reductive dehalogenation of halobenzoates by anaerobic lake sediment microorganisms. *Applied and Environment Microbiology*, 45, 1459–1465.
- Horvath, R. S. (1972). Microbial co-metabolism and the degradation of organic compounds in nature. *Bacteriological Reviews*, 36, 146–155.
- Hsieh, F.-M., Huang, C., Lin, T.-F., Chen, Y.-M., & Lin, J.-C. (2008). Study of sodium tripolyphosphate-crosslinked chitosan beads entrapped with *Pseudomonas putida* for phenol degradation. *Process Biochemistry*, 43, 83–92.
- Hussain, A., Kumar, P., & Mehrotra, I. (2010). Nitrogen biotransformation in anaerobic treatment of phenolic wastewater. *Desalination*, 250, 35–41.
- Hwang, H. M., Hodson, R. E., & Lee, R. F. (1986). Degradation of phenol and chlorophenols by sunlight and microbes in estuarine water. *Environmental Science and Technology*, 20, 1002–1007.
- Iranzo, M., Sain-Pardo, I., Boluda, R., Sanchez, J., & Mor-meneo, S. (2001). The use of microorganisms in environmental remediation. *Annals of Microbiology*, 51, 135–143.
- Ivanciuc, T., Ivanciuc, O., & Douglas, J. K. (2006). Prediction of environmental properties for chlorophenols with posetic quantitative super-structure/property relationships (QSSPR). *International Journal of Molecular Sciences*, 7, 358–374.

- Janke, D., Ihn, W., & Tresselt, D. (1989). Critical steps in degradation of chloroaromatics by *Rhodococci*. IV. Detailed kinetics of substrate removal and product formation by resting preadapted cells. *Journal of Basic Microbiology*, 29, 1–10.
- Jensen, J. (1996). Chlorophenols in the terrestrial environment. *Reviews of Environmental Contamination and Toxicology*, 146, 211–223.
- Jiang, H.-L., Tay, T.-L., Maszenan, A. M., & Tay, J.-H. (2006). Physiological traits of bacterial strains isolated from phenol-degrading aerobic granules. *FEMS Microbiology Ecology*, 57, 182–191.
- Jones, P. A. (1983). *Chlorophenols and their impurities in the Canadian environment*. Environmental Protection Service: Environment Canada.
- Kargi, F., & Eker, S. (2004). Toxicity and batch biodegradation kinetics of 2,4 dichlorophenol by pure *Pseudomonas putida* culture. *Enzyme and Microbial Technology*, 35, 424–428.
- Kim, J.-H., Oh, K.-K., Lee, S.-T., Kim, S.-W., & Hong, S.-I. (2002). Biodegradation of phenol and chlorophenols with defined mixed culture in shake-flasks and a packed bed reactor. *Process Biochemistry*, 37, 1367–1373.
- Kim, M. H., & Hao, O. J. (1999). Cometabolic degradation of chlorophenols by *Acinetobacter* species. *Water Research*, 33, 562–574.
- Kiyohara, H., Takizawa, N., Uchiyama, T., Ikarugi, H., & Nagao, K. (1989). Degradability of polychlorinated phenols by bacterial populations in soil. *Journal of Fermentation and Bioengineering*, 67, 339–344.
- Klekner, V., & Kosaric, N. (1992). Degradation of phenolic mixtures by *chlorella*. *Environmental Technology*, 13, 503–506.
- Kohring, G. W., Rogers, J. E., & Wiegel, J. (1989). Anaerobic biodegradation of 2,4-dichlorophenol in fresh-water lake-sediments at different temperatures. *Applied and Environmental Microbiology*, 55, 348–353.
- Kookana, R. S., & Rogers, S. L. (1995). Effects of pulp mill effluent disposal on soil. *Reviews of Environmental Contamination and Toxicology*, 142, 13–64.
- Krieg, N. R., & Holt, J. C. (Eds.). (1984). *Bergey's Manual of Systematic Bacteriology* (1st ed., Vol. 1). Baltimore: Williams and Wilkins.
- Laine, M. M., & Jørgensen, K. S. (1997). Effective and safe composting of chlorophenol-contaminated soil in pilot scale. *Environmental Science and Technology*, 31, 371–378.
- Lallai, A., & Mura, G. (2004a). Biodegradation of 2-chlorophenol in forest soil: effect of inoculation with aerobic sewage sludge. *Environmental Toxicology and Chemistry*, 23, 325–330.
- Lallai, A., & Mura, G. (2004b). Biodegradation of 2-chlorophenol in forest soil: effect of inoculation with aerobic sewage sludge. *Environmental Toxicology and Chemistry*, 23(2), 325–330.
- Larsson, P., & Lemkemeier, K. (1989). Microbial mineralization of chlorinated phenols and biphenyls in sediment-water systems from humic and clear-water lakes. *Water Research*, 23, 1081–1085.
- Lee, S. H., Lee, S. H., Ryu, S. J., Kang, C. S., Suma, Y., & Kim, H. S. (2013). Effective biochemical decomposition of chlorinated aromatic hydrocarbons with a biocatalyst immobilized on a natural enzyme support. *Bioresource Technology*, 141, 89–96.
- Levé, L., Nyberg, K., Korkea-aho, L., & Schnürer, A. (2006). Phenols in anaerobic digestion processes and inhibition of ammonia oxidising bacteria (AOB) in soil. *Science of the Total Environment*, 364, 229–238.
- Li, J., Cai, W., & Zhu, L. (2011). The characteristics and enzyme activities of 4-chlorophenol biodegradation by *Fusarium* sp. *Bioresource Technology*, 102, 2985–2989.
- Li, Y., Li, J., Wang, C., & Wang, P. (2010). Growth kinetics and phenol biodegradation of psychrotrophic *Pseudomonas putida* LY1. *Bioresource Technology*, 101, 6740–6744.
- Lika, K., & Papadakis, I. A. (2009). Modeling the biodegradation of phenolic compounds by microalgae. *Journal of Sea Research*, 62, 135–146.

- Liu, S.-M., Kuo, C.-E., & Hsu, T.-B. (1996). Reductive dechlorination of chlorophenols and pentachlorophenol in anoxic estuarine sediments. *Chemosphere*, 32, 1287–1300.
- Loh, K.-C., & Cao, B. (2008). Paradigm in biodegradation using *Pseudomonas putida*—A review of proteomics studies. *Enzyme and Microbial Technology*, 43, 1–12.
- Loh, K.-C., & Wang, S.-J. (1998). Enhancement of biodegradation of phenol and a non-growth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. *Biodegradation*, 8, 329–338.
- Londry, K. L., & Fedorak, P. M. (1992). Benzoic acid intermediates in the anaerobic biodegradation of phenols. *Canadian Journal of Microbiology*, 38, 1–11.
- Lu, C.-J., Lee, C.-M., & Huang, C.-Z. (1996). Biodegradation of chlorophenols by immobilized pure-culture microorganisms. *Water Science and Technology*, 34, 67–72.
- Mahmood, S., Paton, G. I., & Prosser, J. I. (2005). Cultivation-independent in situ molecular analysis of bacteria involved in degradation of pentachlorophenol in soil. *Environmental Microbiology*, 7, 1349–1360.
- Majumder, P. S., & Gupta, S. K. (2007). Removal of chlorophenols in sequential anaerobic–aerobic reactors. *Bioresource Technology*, 98, 118–129.
- Majumder, P. S., & Gupta, S. K. (2009). Effect of influent pH and alkalinity on the removal of chlorophenols in sequential anaerobic–aerobic reactors. *Bioresource Technology*, 100, 1881–1883.
- Margesin, R., Moertelmaier, C., & Mair, J. (2013). Low-temperature biodegradation of petroleum hydrocarbons (n-alkanes, phenol, anthracene,) by four actinobacterial strains. *International Biodeterioration and Biodegradation*, 84, 185–191.
- Martinez, A. T., Speranza, M., Ruiz-Duenas, F. J., Ferreira, P., Camarero, S., Guillen, F., et al. (2005). Biodegradation of lignocellulosics: Microbial, chemical, and enzymatic aspects of the fungal attack of lignin. *International Microbiology*, 8, 195–204.
- Matus, V., Sanchez, M. A., Martinez, M., & Gonzalez, B. (2003). Efficient degradation of 2,4,6-trichlorophenol requires a set of catabolic genes related to tcp genes from *Ralstonia eutropha* JMP134 (pJP4). *Applied and Environment Microbiology*, 69, 7108–7115.
- Menke, B., & Rehm, H.-J. (1992). Degradation of mixtures of monochlorophenols and phenol as substrates for free and immobilized cells of *Alcaligenes* sp. A 7–2. *Applied Microbiology and Biotechnology*, 37, 655–661.
- Michałowicz, J., & Duda, W. (2007). Phenols—sources and toxicity. *Polish Journal of Environmental Studies*, 16, 347–362.
- Middeldorp, P., De Wolf, J., Zehnder, A., & Schraa, G. (1997). Enrichment and properties of a 1,2,4-trichlorobenzene-dechlorinating methanogenic microbial consortium. *Applied and Environment Microbiology*, 63, 1225–1229.
- Mikesell, M. D., & Boyd, S. A. (1986). Complete reductive dechlorination and mineralization of pentachlorophenol by anaerobic microorganisms. *Applied and Environment Microbiology*, 52, 861–865.
- Milliken, C. E., Meier, G. P., Sowers, K. R., & May, H. D. (2004). Chlorophenol production by anaerobic microorganisms: Transformation of a biogenic chlorinated hydroquinone metabolite. *Applied and Environment Microbiology*, 70, 2494–2496.
- Mohidem, N. A., & Mat, H. B. (2012). The catalytic activity enhancement and biodegradation potential of free laccase and novel sol–gel laccase in non-conventional solvents. *Bioresource technology*, 114, 472–477.
- Mohn, W. M., & Kennedy, K. J. (1992). Reductive dehalogenation of chlorophenols by *Desulfomonile tieckii* DCB-1. *Applied and Environment Microbiology*, 58, 1367–1370.
- Moorman, T. B., Cowan, J. K., Arthur, E. L., & Coats, J. R. (2001). Organic amendments to enhance herbicide biodegradation in contaminated soils. *Biology and Fertility of Soils*, 33, 541–545.
- Murialdo, S. E., Fenoglio, R., Haure, P. M., & Gonzalez, J. F. (2003). Degradation of phenol and chlorophenols by mixed and pure cultures. *Water SA*, 29, 457–463.

- Muñoz, R., Souza, T. S. O., Glittmann, L., Pérez, R., & Quijano, G. (2013). Biological anoxic treatment of O₂-free VOC emissions from the petrochemical industry: A proof of concept study. *Journal of Hazardous Materials*, 260, 442–450.
- Namkoong, W., Loehr, R. C., & Malina, J. F. (1988). Kinetics of phenolic compounds removal in soil. *Hazardous Waste and Hazardous Materials*, 5, 321–328.
- Nordin, K., Unell, M., & Jansson, J. K. (2005). Novel 4-chlorophenol degradation gene cluster and degradation route via hydroxyquinol in *Arthrobacter chlorophenolicus* A6. *Applied and Environment Microbiology*, 71, 6538–6544.
- Nuhoglu, A., & Yalcin, B. (2005). Modelling of phenol removal in a batch reactor. *Process Biochemistry*, 40, 1233–1239.
- Oberg, L. G., & Rappe, C. (1992). Biochemical formation of PCDD/Fs from chlorophenols. *Chemosphere*, 25, 49–52.
- Okpokwasili, G. C., & Nweke, C. O. (2005). Microbial growth and substrate utilization kinetics. *African Journal of Biotechnology*, 5, 305–307.
- Olaniran, A. O., & Igbinosa, E. O. (2011). Chlorophenols and other related derivatives of environmental concern: Properties, distribution and microbial degradation processes. *Chemosphere*, 83, 1297–1306.
- Onysko, K. A., Budman, H. M., & Robinson, C. W. (2000). Effect of temperature on the inhibition kinetics of phenol biodegradation by *Pseudomonas putida* Q5. *Biotechnology and Bioengineering*, 70, 291–299.
- Orser, C. S., & Lange, C. C. (1994). Molecular analysis of pentachlorophenol degradation. *Biodegradation*, 5, 277–288.
- Palleroni, N. J. (1986). Taxonomy of the *Pseudomonads*. In J. R. Sokatch (Ed.), *The bacteria* (Vol. X, pp. 3–25). The biology of *Pseudomonads*. New York: Academic Press.
- Pandey, G., Paul, D., & Jain, R. K. (2003). Branching of o-nitrobenzoate degradation pathway in *Arthrobacter protophormiae* RKJ100: Identification of new intermediates. *FEMS Microbiology Letters*, 229, 231–236.
- Papazi, A., & Kotzabasis, K. (2007). Bioenergetic strategy of microalgae for the biodegradation of phenolic compounds—Exogenously supplied energy and carbon sources adjust the level of biodegradation. *Journal of Biotechnology*, 129, 706–716.
- Park, H.-J., & Kim, E.-S. (2003). An inducible Streptomyces gene cluster involved in aromatic compound metabolism. *FEMS Microbiology Letters*, 226, 151–157.
- Pedroza, A. M., Mosqueda, R., Alonso-Vante, N., & Rodríguez-Vázquez, R. (2007). Sequential treatment via and UV/RuxSey to reduce contaminants in waste water resulting from the bleaching process during paper production. *Chemosphere*, 67, 793–801.
- Pieper, D., & Reineke, W. (2000). Engineering bacteria for bioremediation. *Current Opinion in Biotechnology*, 11, 262–270.
- Pignatello, J. J., Martinson, M. M., Steiert, J. G., Carlson, R. E., & Crawford, R. L. (1983). Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. *Applied and Environment Microbiology*, 46(5), 1024–1031.
- Potgieter, J. H., Bada, S. O., & Potgieter-Vermaak, S. S. (2009). Adsorption removal of various phenol from waste by South African coal fly ash. *Water SA*, 35, 110–112.
- Puyol, D., Mohedano, A. F., Rodriguez, J. J., & Sanz, J. L. (2011). Effect of 2,4,6-trichlorophenol on the microbial activity of adapted anaerobic granular sludge bioaugmented with *Desulfito bacterium* strains. *New Biotechnology*, 29, 79–89.
- Qiu, X., Zhong, Q., Li, M., Bai, W., & Li, B. (2007). Biodegradation of p-nitrophenol by methyl parathion-degrading *Ochrobactrum* sp. B2. *International Biodeterioration and Biodegradation*, 59, 297–301.
- Quan, X., Shi, H., Zhang, Y., Wang, J., & Qian, Y. (2004). Biodegradation of 2,4-dichlorophenol and phenol in an airlift inner-loop bioreactor immobilized with *Achromobacter* sp. *Separation and Purification Technology*, 34, 97–103.
- Reddy, G. V. B., & Gold, M. H. (2000). Degradation of pentachlorophenol by *Phanerochaete chrysosporium*: Intermediates and reactions involved. *Microbiology*, 146, 405–413.

- Ren, M., Song, Y., Xiao, S., Zeng, P., & Peng, J. (2011). Treatment of berberine hydrochloride wastewater by using pulse electro-coagulation process with Fe electrode. *Chemical Engineering Journal*, 169, 84–90.
- Rubilar, O., Diez, M. C., & Gianfreda, L. (2008). Transformation of chlorinated phenolic compounds by white rot fungi. *Critical Reviews in Environmental Science and Technology*, 38, 227–268.
- Ryan, M. P., Pembroke, J. T., & Adley, C. C. (2007). *Ralstonia pickettii* in environmental biotechnology: Potential and applications. *Journal of Applied Microbiology*, 103, 754–764.
- Sahinkaya, E., & Dilek, F. B. (2006a). Biodegradation of 4-CP and 2,4-DCP mixture in a rotating biological contactor (RBC). *Biochemical Engineering Journal*, 31, 141–147.
- Sahinkaya, E., & Dilek, F. B. (2006b). Effect of biogenic substrate concentration on the performance of sequencing batch reactor treating 4-CP and 2,4-DCP mixtures. *Journal of Hazardous Materials*, 128, 258–264.
- Sahinkaya, E., & Dilek, F. B. (2007). Biodegradation kinetics of 2,4-dichlorophenol by acclimated mixed cultures. *Journal of Biotechnology*, 127, 716–726.
- Sanchez, M. A., Vasquez, M., & Gonzalez, B. (2004). A previously unexposed forest soil microbial community degrades high levels of the pollutant 2,4,6-trichlorophenol. *Applied and Environment Microbiology*, 70, 7567–7570.
- Scelza, R., Rao, M. A., & Gianfreda, L. (2008). Response of an agricultural soil to pentachlorophenol (PCP) contamination and the addition of compost or dissolved organic matter. *Soil Biology and Biochemistry*, 40, 2162–2169.
- Semprini, L. (1997). Strategies for the aerobic co-metabolism of chlorinated solvents. *Current Opinion in Biotechnology*, 8, 296–308.
- Sharpee, K. W., Duxbury, J. M., & Alexande, M. (1973). 2,4-Dichlorophenoxyacetate metabolism by *Arthrobacter* sp.: Accumulation of a chlorobutenolide. *Journal of Applied Microbiology*, 26, 445–447.
- Shourian, M., Noghabi, K. A., Zahiri, H. S., Bagheri, T., Karballaei, G., Mollaei, M., et al. (2009). Efficient phenol degradation by a newly characterized *Pseudomonas* sp. SA01 isolated from pharmaceutical wastewaters. *Desalination*, 246, 577–594.
- Singh, B., & Walker, A. (2006). Microbial degradation of organophosphorus compounds. *Microbiology Reviews*, 30, 428–471.
- Sittig, M. (1981). Handbook of toxic and hazardous chemicals.
- Siuda, J. F. (1980). Natural production of organohalogenes. In: *Water chlorination: Environmental impact and health effects*, vol 3 (pp. 63–72). Science Publishers Inc.
- Smith, J. A., & Novak, J. T. (1987). Biodegradation of chlorinated phenols in subsurface soils. *Water, Air, and Soil Pollution*, 33, 29–42.
- Solyanikova, I. P., & Golovleva, L. A. (2004). Bacterial degradation of chlorophenols: Pathways, biochemica and genetic aspects. *Journal of Environmental Science and Health*, B39, 333–351.
- Stehlickova, L., Svab, M., Wimmerova, L., & Kozler, J. (2009). Intensification of phenol biodegradation by humic substances. *International Biodeterioration and Biodegradation*, 63, 923–927.
- Steinle, P., Stucki, G., Stettler, R., & Hanselmann, W. (1998). Aerobic mineralization of 2,6-Dichlorophenol by *Ralstonia* sp. strain RK1. *Applied and Environment Microbiology*, 64, 2566–2571.
- Stoilova, I., Krastanov, A., Stanchev, V., Daniel, D., Gerginova, M., & Alexieva, Z. (2006). Biodegradation of high amounts of phenol, catechol, 2,4-dichlorophenol and 2,6-dimethoxyphenol by *Aspergillus awamori* cells. *Enzyme and Microbial Technology*, 39, 1036–1041.
- Stoilova, I., Krastanov, A., Yanakieva, I., Kratchanova, M., & Yemendjiev, H. (2007). Biodegradation of mixed phenolic compounds by *Aspergillus awamori* NRRL 3112. *International Biodeterioration and Biodegradation*, 60, 342–346.
- Susarla, S., Yonezawa, Y., Nakanishi, J., & Masunaga, S. (1997). Anaerobic transformation kinetics and pathways of chlorophenols in fresh water lake sediment. *Water Science and Technology*, 36, 99–105.

- Suzuki, T. (1983). Methylation and hydroxylation of pentachlorophenol by mycobacterium-sp isolated from soil. *Journal of Pesticide Science*, 8, 419–428.
- Takeuchi, R., Suwa, Y., Yamagishi, T., & Yonezawa, Y. (2000). Anaerobic transformation of chlorophenols in methanogenic sludge unexposed to chlorophenols. *Chemosphere*, 41, 1457–1462.
- Tamer, E., Hamid, Z., Aly, A. M., Ossama, E. T., Bo, M., & Benoit, G. (2006). Sequential UV–biological degradation of chlorophenols. *Chemosphere*, 63, 277–284.
- Tiedje, J. M., Duxbury, J. M., Alexande, M., & Dawson, J. E. (1969). 2,4-D metabolism: Pathway of degradation of chlorocatechols by *Arthrobacter* sp. *Journal of Agricultural and Food Chemistry*, 17, 1021–1026.
- Tobajas, M., Monsalvo, V. M., Mohedano, A. F., & Rodriguez, J. J. (2012). Enhancement of cometabolic biodegradation of 4-chlorophenol induced with phenol and glucose as carbon sources by *Comamonas testosteroni*. *Journal of Environmental Management*, 95(Suppl), 116–121.
- Uotila, J., Kitunen, V., Saastamoinen, T., Coote, T., Haggblom, M., & Salkinoja-Salonen, M. (1992). Characterization of aromatic dehalogenases of *Mycobacterium fortuitum* CG-2. *Journal of Bacteriology*, 174, 5669–5675.
- Uotila, J. S., & Salkinoja-Salonen, M. S. (1991). Dechlorination of pentachlorophenol by membrane boundenzymes of *Rhodococcus chlorophenolicus* PCP-I. *Biodegradation*, 2, 25–31.
- Valo, R. J., & Salkinoja-Salonen, M. S. (1986). Bioreclamation of chlorophenol-contaminated soil by composting. *Applied Microbiology and Biotechnology*, 25, 68–75.
- Van Agteren, M. H., Keuning, S., & Janssen, D. B. (1998). *Handbook on biodegradation and biological treatment of hazardous organic compounds*. London: Kluwer Academic Publishers.
- Vischetti, C., Casucci, C., Perucci, P. (2002). *Relationship between changes of soil microbial biomass content and imazamox and benfluralin degradation*. Springer Verlag.
- Wang, S. J. (1997). Influence of conventional carbon supplements on biodegradation of phenol and cometabolite, 4-chlorophenol (4-CP). ME thesis, National University of Singapore.
- Wang, S.-G., Liu, X.-W., Zhang, H.-Y., Gong, W.-X., Sun, X.-F., & Gao, B.-Y. (2007a). Aerobic granulation for 2,4-dichlorophenol biodegradation in a sequencing batch reactor. *Chemosphere*, 69, 769–775.
- Wang, Y., Tian, Y., Han, B., H-b, Zhao, J-n, Bi, & B-l, Cai. (2007b). Biodegradation of phenol by free and immobilized *Acinetobacter* sp. strain PD12. *Journal of Environmental Science*, 19, 222–225.
- Warner, K. A., Gilmour, C. C., & Capone, D. G. (2002). Reductive dechlorination of 2,4-dichlorophenol and related microbial processes under limiting and non-limiting sulfate concentration in anaerobic mid-Chesapeake Bay sediments. *FEMS Microbiology Ecology*, 40, 159–165.
- Watanabe, I. (1978). Pentachlorophenol (PCP) decomposing activity of field soils treated annually with PCP. *Soil Biology and Biochemistry*, 10, 71–75.
- Weber, R., Gaus, C., & Tysklind, M. (2008). Dioxin- and POP-contaminated sites—contemporary and future relevance and challenges: Overview on background aims and scope of the series. *Environmental Science and Pollution Research*, 15, 363–393.
- Xun, L. Y., & Webster, C. M. (2004). A monooxygenase catalyzes sequential dechlorinations of 2,4,6-trichlorophenol by oxidative and hydrolytic reactions. *Journal of Biological Chemistry*, 279, 6696–6700.
- Yang, R. D., & Humphrey, A. E. (1975). Dynamic and steady state studies of phenol biodegradation in pure and mixed cultures. *Biotechnology and Bioengineering*, 17, 1211–1235.
- Yang, S., Shibata, A., Yoshida, N., & Katayama, A. (2009). Anaerobic mineralization of pentachlorophenol (PCP) by combining PCP-dechlorinating and phenol-degrading cultures. *Biotechnology and Bioengineering*, 102, 81–90.
- Yu, Z., Zeng, G.-M., Chen, Y.-N., Zhang, J.-C., Yu, Y., Li, H., et al. (2011). Effects of inoculation with *Phanerochaete chrysosporium* on remediation of pentachlorophenol-contaminated soil waste by composting. *Process Biochemistry*, 46, 1285–1291.

- Zhang, C., & Bennett, G. N. (2005). Biodegradation of xenobiotic by anaerobic bacteria. *Applied Microbiology and Biotechnology*, 67(5), 600–618.
- Zhang, G., Gao, Y., Zhang, Y., & Gu, P. (2005). Removal of fluoride from drinking water by a membrane coagulation reactor (MCR). *Desalination*, 177, 143–155.
- Zhang, X., & Wiegel, J. (1990). Sequential anaerobic degradation of 2,4- dichlorophenol in freshwater sediments. *Applied and Environment Microbiology*, 56, 1119–1127.

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