Biodegradation (2008) 19:463–480
DOI 10.1007/s10532-007-9155-1

REVIEW PAPER

Microbial degradation of chlorinated benzenes

Jim A. Field · Reyes Sierra-Alvarez

Received: 20 June 2007 / Accepted: 21 September 2007 / Published online: 5 October 2007

Abstract Chlorinated benzenes are important industrial intermediates and solvents. Their widespread use has resulted in broad distribution of these compounds in the environment. Chlorobenzenes (CBs) are subject to both aerobic and anaerobic metabolism. Under aerobic conditions, CBs with four or less chlorine groups are susceptible to oxidation by aerobic bacteria, including bacteria (*Burkholderia*, *Pseudomonas*, etc.) that grow on such compounds as the sole source of carbon and energy. Sound evidence for the mineralization of CBs has been provided based on stoichiometric release of chloride or mineralization of ^{14}C-labeled CBs to $^{14}CO_2$. The degradative attack of CBs by these strains is initiated with dioxygenases eventually yielding chlorocatechols as intermediates in a pathway leading to CO_2 and chloride. Higher CBs are readily reductively dehalogenated to lower chlorinated benzenes in anaerobic environments. Halorespiring bacteria from the genus *Dehalococcoides* are implicated in this conversion. Lower chlorinated benzenes are less readily converted, and mono-chlorinated benzene is recalcitrant to biotransformation under anaerobic conditions.

J. A. Field (✉) · R. Sierra-Alvarez
Department of Chemical and Environmental Engineering, University of Arizona, P.O. Box 210011, Tucson, AZ 85721, USA
e-mail: Jimfield@email.arizona.edu

Keywords Biotransformation · Chlorobenzenes · Dehalogenation · Dechlorination · Microbial degradation · Organohalogens

Abbreviations

CB	Chlorobenzene
DCB	Dichlorobenzene
TCB	Trichlorobenzene
TeCB	Tetrachlorobenzene
QCB	Pentachlorobenzene
HCB	Hexachlorobenzene
Dwt	Dry weight

Introduction

Chlorobenzenes (CBs) are isomeric chlorinated aromatic compounds having a benzene ring that is substituted with 1–6 chlorine atoms. Chlorobenzenes include CB, dichlorobenzene (DCB), trichlorobenzene (TCB), tetrachlorobenzene (TeCB), pentachlorobenzene (QCB), and hexachlorobenzene (HCB). Environmental contamination with CBs is widespread due to the importance of these chemicals as industrial intermediates, pesticides and solvents. The global cumulative production of HCB is ~100,000 tonnes and the peak annual worldwide emissions of HCB to the environment were estimated between 100 and 1,000 tonnes year^{-1} (Barber et al. 2005). CBs may

also be formed as by-products of microbial transformation of hexachlorocyclohexane in anoxic systems (Middeldorp et al. 1996; Phillips et al. 2005). With regard to the other CBs (1–5 chlorines), the environmental emissions in US have been reported to be 980 tonnes year^{-1} (Malcom et al. 2004).

The chemical properties of CBs vary depending on their degree of chlorination (MacLeod and Mackay 1999; Dolfing and Harrison 1992). CBs are more hydrophobic with increasing number of chlorine substituents. The maximum aqueous solubility ranges from 484 mg l^{-1} for CB down to 0.005 mg l^{-1} for HCB; whereas the logarithm of the octanol-water partition coefficient (Log P) ranges from 2.8 for CB to 5.5 for HCB. The vapor pressure of CB is high (1.58 kPa) whereas the values decrease downwards with increasing chlorine number to 0.0003 kPa for HCB. Based on these properties, lower chlorinated benzenes have a tendency to become volatilized, whereas higher chlorinated benzenes have a tendency to become sorbed on soil and sediment particles (Barber et al. 2005; MacLeod and Mackay 1999; Malcom et al. 2004; Williams et al. 2006). Chemical abiotic transformations such as hydrolysis or photolysis in soil and water are generally considered to be limited (Barber et al. 2005; Malcom et al. 2004). Although atmospheric emission of lower chlorinated is important, biodegradation can also be a significant fate in the subsurface where volatilization is limited. Biodegradation is considered to be the main mechanism of higher chlorinated benzene removal in sediments (Barber et al. 2005). The biodegradation of CBs is thermodynamically feasible under both aerobic and anaerobic conditions. Utilizing Gibbs free energy of formation data (Dolfing and Harrison 1992; Rittman and McCarty 2001), the standard change in Gibb's free energy ($\Delta G°'$) for the aerobic degradation of CBs can be calculated. The values range from –3,014.5 kJ mol^{-1} for CB to –2,130.9 5 kJ mol^{-1} for HCB. Under anaerobic conditions, the $\Delta G°'$ is also favorable for reductive dechlorination of CBs used as electron acceptors. The $\Delta G°'$ values range from –171.4 kJ mol^{-1} for HCB to –139.6 kJ mol^{-1} for CB when coupled to the oxidation of H_2 (Dolfing and Harrison 1992).

Only a few review articles are available that are specific for the biodegradation of CBs. Wang and Jones (1994a) reviewed the fate of CBs in soils, including a section summarizing previous research on the biodegradation of various CB compounds by bacterial strains and soil microorganisms. Van Agteren et al. (1998) wrote a chapter on the aerobic and anaerobic biodegradability of CBs in their *Handbook on Biodegradation and Biological treatment of Hazardous Organic Compounds*. Adrian and Görisch (2002) published a comprehensive review on the anaerobic biotransformation of CBs.

Biodegradation of chlorobenzenes

Aerobic degradation in the environment

Numerous studies have evaluated the biodegradability of CBs in either aerobic or anaerobic environments (Adrian and Görisch 2002; Van Agteren et al. 1998; Wang and Jones 1994a). Conflicting results have been reported with respect to the importance of biodegradation for the fate of CBs in aerobic soil. Two studies indicate that the major mechanism of CB loss is due to volatilization with biodegradation playing only a minor role. Wang and Jones (1994b) conducted experiments using a mixture of chlorinated benzenes containing 1,3-DCB, 1,2,3-TCB, 1,2,3,5-TeCB and QCB (144–287 μg ΣCBs kg^{-1}) and demonstrated that 74% of the chlorinated aromatics in sewage sludge-amended soil was lost by volatilization over 35 days, while 67% was lost from spiked soil over 20 days. Brahushi et al. (2002) observed that volatilization of ^{14}C-CB from aerated laboratory soil microcosms amended with different substrates was the main loss mechanism whereas mineralization was of minor importance However, other studies provide careful aerobic biodegradation measurements of chlorinated benzenes in contaminated soil incubated in enclosed vessels or microcosms (Marinucci and Bartha 1979; Schroll et al. 2004). Based on chloride release and increase in optical density (due to cell growth), it was determined that CB, 1,3-DCB, 1,4-DCB, 1,2,4-TCB, 1,2,3,4-TeCB, and 1,2,4,5-TeCB were biodegradable when incubated in soil slurries (Feidieker et al. 1994). In moist pristine soil, 1,2,3-TCB and 1,2,4-TCB were biologically mineralized at a rate of 0.012 and 0.052 nmol CO_2 g^{-1} soil dry weight day^{-1}, respectively (Marinucci and Bartha 1979). Radiolabeled 1,2,4-TCB was incubated aerobically in a microcosm with either agricultural soil or soil from an industrial site (Schroll et al. 2004). Very little mineralization of

radiolabeled 1,2,4-TCB was observed with the agricultural soil; however, the industrially impacted soil was responsible for 62% mineralization of [^{14}C]-TCB to $^{14}CO_2$ in 23 days. Nishino and coworkers were able to readily isolate indigenous CB degraders from chronically contaminated soils (Nishino et al. 1992, 1994). In a study examining soil and groundwater samples collected from four different sites with CB contamination histories of 6–30 years, the number of CB-degrading bacteria per gram soil–slurry increased with the CB concentration of a given site, and the highest counts of 5×10^6 CB-degraders g^{-1} were observed at the most contaminated site with 28 mg CB l^{-1} (Nishino et al. 1994). The natural microbial consortium in a soil-groundwater microcosm, which was prepared using material from a heavily contaminated site known to contain 25–150 mg CB l^{-1}, mineralized CB by 54% in 7 days (Nishino et al. 1992). In a similar study, eight different aerobic isolates capable of growth on CB as a sole source of carbon and energy were readily obtained from a contaminated aquifer (van der Meer et al. 1998). Groundwater microbes from another contaminated aquifer readily mineralized CB and 1,4-DCB based on O_2 uptake data (Dermietzel and Vieth 2002). The corresponding half-lives at 14°C for the degradation of CB ranged from 3 to 29 days. Experiments with [^{14}C]-1,4-DCB revealed that two-thirds of the compound was mineralized in the groundwater to $^{14}CO_2$ (Dermietzel and Vieth 2002). Evidence for the biodegradation of 1,2-DCB, 1,3-DCB, 1,4-DCB, and 1,2,4-TCB in the contaminated groundwater was also obtained by measurements of inorganic chloride release (Dermietzel and Vieth 2002). Finally, biodegradation of CB and 1,2,4-TCB was also reported in freshwater and estuarine surface waters (Bartholomew and Pfaender 1983).

Anaerobic degradation in the environment

Evidence for the anaerobic biotransformation of chlorinated benzenes has also been found in the natural environment. The best example comes from the comparison of historically archived sediments from 1972 with recently sampled and dated sediment core data from a large inland lake in Ketelmeer, the Netherlands (Beurskens et al. 1993). The Ketelmeer is the sedimentation basin of the inflowing Rhine River. HCB in the early 1970 layer of the recently sampled sediments was 80% lower than the HCB in archived sediments. Well-known anaerobic biotransformation products of HCB, 1,3,5-TCB and 1,3-DCB, were 2.1 and 5.7-fold higher in the 1970 layer compared to the archived sample, respectively. The maximum half-life of HCB in the sediments was estimated to be 7 years. To confirm that anaerobic dehalogenation was the dominant process for HCB transformation, laboratory microcosms of the sediment samples were shown to catalyze the biological dechlorination of HCB to 1,3,5-TCB and 1,3-DCB (Beurskens et al. 1993). Microcosms prepared from historically contaminated estuarine sediment were also shown to anaerobically dechlorinate HCB with a half-life of $\sim$1 year (Prytula and Pavlostathis 1996). Anaerobic microcosms prepared from a freshwater lake in Japan, dechlorinated HCB with a half-life of 63 days, and 1,2,4-TCB, 1,2,3-TCB, 1,4-DCB, and 1,3-DCB were observed as important biotransformation products (Susarla et al. 1997). Chlorinated benzenes were tested in sulfate-reducing river sediments from Japan (Masunaga et al. 1996). The half-life of HCB was only 27 days, and again, 1,2,4-TCB, 1,2,3-TCB, 1,4-DCB, and 1,3-DCB were observed as the main biotransformation products. The half-lives of QCB, TeCB and TCB were in the same order of magnitude as HCB; however, those of 1,4-DCB and 1,3-DCB were distinctly higher, corresponding to 385 and 433 days, respectively (Masunaga et al. 1996).

Monochlorinated CB is generally regarded as persistent in anaerobic environments. However, CB was shown to be degraded in situ at the fringe of the contaminant plume in an anoxic aquifer (with a long history of CB contamination) based on evidence from isotopic fractionation data and incorporation of [^{13}C]-labeled CB into long chain fatty acids of bacteria (Kaschl et al. 2005; Kastner et al. 2006).

Biodegradation in engineered systems

There are numerous examples in which engineered treatment systems have been utilized to degrade various congeners of chlorinated benzenes under aerobic, anaerobic as well as sequential anaerobic-aerobic conditions. Several research groups have explored the use of aerobic bioreactors to treat groundwater contaminated with lower chlorinated

benzenes (Nishino et al. 1994; Feidieker et al. 1995; Klecka et al. 1996; Alfreider et al. 2002; Lapertot et al. 2006). At one chlorinated solvents-contaminated site, two 135-l fixed-film pilot-scale bioreactors were operated to degrade CB present in the groundwater. One reactor was inoculated with a CB-degrading bacterium, *Pseudomonas* sp. strain JS150; while the other reactor was allowed to become colonized with the indigenous CB-degrading microorganisms from the site (Nishino et al. 1994). The reactors treated groundwater containing $\sim$1 mg l^{-1} CB and the removal efficiencies ranged from 87 to 95%. The introduced strain could not be recovered after 3 weeks, indicating that the indigenous microorganisms were responsible for CB degradation. A similar laboratory reactor was established and it confirmed the results of the pilot-study with hydraulic residence times as low as 30 min. A field-scale fluidized bed reactor (V = 0.8 m^3) supplied with granular activated carbon (GAC) as biofilm support was evaluated for the removal of CB in groundwater (Klecka et al. 1996). The bioreactor was inoculated with aerobic activated sludge. The reactor removed CB from an average ground water concentration of 145 mg l^{-1} to levels of 1 mg l^{-1} in the effluent. The average load of CB removed was 4.8 kg CB m^{-3} reactor day^{-1}. The elimination of dissolved oxygen accounted for 45% of the theoretical oxygen demand of CB, suggesting that at least 45% of the CB removed was due to biodegradation. The degradation of a mixture of CB and 1,2-DCB was evaluated in an aerobic bioreactor operated with sequenced pulses and continuously (Lapertot et al. 2006). The maximum conversion capacity of the aerobic bioreactor was 5.6 and 11.3 kg CB mixture m^{-3} reactor day^{-1}, for the sequenced pulse and continuous operation modes, respectively. An in situ bioreactor filled with aquifer sediments was used to aerobically degrade CB in anoxic groundwater by supplying oxygen via hydrogen peroxide (Vogt et al. 2004a). The reactor effectively removed 17.7 mg l^{-1} of CB with a supply of 29.2 mg l^{-1} of H_2O_2; however, with time higher H_2O_2 concentrations were required due to a shift in the bacterial population. Finally, the aerobic co-oxidation of CB-contaminated groundwater utilizing methane as the primary substrate was explored in a soil column (7.9 l) packed with 13.4 kg aquifer sediments and colonized with a natural mixed culture of methanotrophic bacteria (Jechorek et al. 2003). The column, which operated with a hydraulic retention time of 1.5 days, was highly effective in the removal of CB, reducing the influent concentration of 25–30 mg CB l^{-1} to 0.04 mg CB l^{-1} or less.

Degradation of mixtures of chlorinated benzenes has also been studied in slow sand filter columns under aerobic conditions (Zacharias et al. 1995; Bosma et al. 1996). In one study, contaminated water was filtered through 40 kg of sand placed in 32-l columns with a hydraulic retention time of 3.2 h. The sum of all CBs in the influent and effluent was 3.81 and 0.014 mg l^{-1}, respectively, corresponding to a removal efficiency of 99.6%. As evidence of biodegradation, the inorganic chloride concentration was shown to increase in the column. In another study, the removal of CBs during infiltration of water into sand dunes as part of a drinking water treatment scheme was evaluated with laboratory-scale columns (V = 0.6 or 5.7 l) filled with sand (Bosma et al. 1996). Under aerobic conditions, CB, 1,2-DCB, 1,3-DCB, 1,4-DCB, and 1,2,4-TCB were removed by >99, 90, 30, 90, and 40%, respectively.

Several studies considered the removal of CB in waste gases in biological trickling reactors (Oh and Bartha 1994; Mpanias and Baltzis 1998; Seignez et al. 2004; Mathur et al. 2006). In the first study, a laboratory biotrickle column of 1.57 l was packed with perlite and used to treat CB and 1,2-DCB vapors supplied at concentrations of 1.2 and 0.7 g m^{-3} air, respectively (Oh and Bartha 1994). The volumetric removal rates of the compounds in the trickling biofilter were 122.4 and 52.8 g m^{-3} reactor day^{-1} for CB and 1,2-DCB, respectively. Inorganic chloride accumulated in the liquid phase of the reactor and corresponded to 72% of the removal of CB and 1,2-DCB, indicating a high level of mineralization. The second study evaluated the removal of CB vapors in a 14.5-l laboratory trickle filter packed with ceramic saddles with liquid recirculation in counter flow to the gas (Mpanias and Baltzis 1998). Maximum volumetric removal rates of up to 1,500 g m^{-3} reactor day^{-1} were obtained. In the third study, a 40-l biotrickle reactor with cylindrical PVC as packing (Seignez et al. 2004) was used to treat mixtures of CB and 1,2-DCB. After 3 months of operation at steady-state, the reactor achieved maximum volumetric loading rates of 5,200 g m^{-3} reactor day^{-1} with removal efficiencies in the range of 95–99%. In the fourth study, CB was treated in a 2-l biotrickle filter with coal packing and inoculated with a mixed

culture from activated sludge (Mathur et al. 2006). The average CB elimination capacity of the column was 1,920 g m^{-3} reactor day^{-1}.

Also anaerobic bioreactor and bioremediation systems have been utilized to treat higher chlorinated benzenes. A large number of chlorinated benzene congeners were susceptible to biotransformation under anaerobic conditions in sediment or sand columns (Bosma et al. 1988, 1996; Van Der Meer et al. 1992). Laboratory columns packed with Rhine River sediments or sand were capable of transforming most congeners of chlorinated benzenes under methanogenic conditions by 90 to over 99% with the exception of CB (Bosma et al. 1988, 1996). CB was the main biotransformation product of DCBs, whereas 1,3- and/or 1,4-DCB were the main biotransformation products of TCBs, TeCBs, QCB and HCB. Similar experiments conducted under sulfate-reducing conditions revealed that 1,2,3-TCB, 1,2,4-TCB, 1,2,3,4-TeCB, QCB, and HCB were eliminated by more than 90% (Van Der Meer et al. 1992). None of the congeners were subject to biotransformation under denitrifying conditions (Bosma et al. 1996). The reductive dechlorination of HCB was observed to readily occur in anaerobic sewage sludge (Fathepure et al. 1988; Dionisi et al. 2006). HCB (50 mg l^{-1}) was completely converted within 3 weeks primarily to 1,3,5-TCB and to a lesser extent to 1,2,4-TCB and DCBs (Fathepure et al. 1988). 1,3,5-TCB (21.5 mg kg^{-1} dwt) present in municipal anaerobic digester sludge was removed by 53% when incubated anaerobically for 175 days with yeast extract as electron donor (Dionisi et al. 2006).

Several studies evaluated the bioremediation of chlorinated benzenes in anaerobic soil. Rosenbrock et al. (1997) investigated the bioremediation of HCB in soil microcosms incubated under anaerobic conditions and demonstrated 40% dechlorination of radiolabeled [^{36}Cl]-HCB (spiked at 30 mg kg^{-1} soil) to $^{36}Cl^-$ in 140 days. In some soils, the endogenous organic matter provided electron donor to support HCB dechlorination; whereas in other soils with low organic matter content, organic substrate addition was required. Ramanand et al. (1993) observed almost stoichiometric conversion of a mixture of chlorinated benzenes in spiked soil slurry consisting of HCB (8.3 mg l^{-1}), QCB (18.5 mg l^{-1}), and 1,2,4-TCB (206.9 mg l^{-1}) to CB (113.7 mg l^{-1}) after 142 days of incubation under methanogenic conditions with H_2 as electron donor. However, the lag period for HCB metabolism was about 2 months compared with little or no lag for the lower chlorinated benzenes. In another study, bioremediation of HCB in agricultural soil was achieved by flooding the soil in laboratory microcosms (Brahushi et al. 2004). After 20 weeks of incubation only 1% of applied [^{14}C]-HCB radiolabeled could be recovered in the extractable fraction. Products of reductive dechlorination, most notably 1,3,5-TCB, accounted for ~75% of the HCB removed from the soil.

The sequential anaerobic-aerobic treatment of HCB was evaluated in one study (Fathepure and Vogel 1991). A two-stage biological treatment scheme was tested for the biodegradation of HCB (0.075 mg l^{-1}) utilizing laboratory-scale anaerobic and aerobic biofilm reactors (each 0.25 l) operated in series, having a hydraulic retention times of 37.5 and 2.2 h, respectively (Fathepure and Vogel 1991). During the anaerobic stage, acetate was found to be the best electron-donating substrate, supporting 98.7% removal of HCB, which was recovered mostly as 1,2,3-TCB (60%) and 1,2-DCB (10%). Experiments with radiolabeled [^{14}C]-HCB revealed that HCB was mineralized by 23% to $^{14}CO_2$ during the sequential anaerobic-aerobic treatment and the total metabolism to both $^{14}CO_2$ and ^{14}C in non-volatile intermediates was 94%.

Microbiology and biochemistry of chlorobenzene biodegradation

Chlorobenzenes are subject to both aerobic and anaerobic metabolism. Under aerobic conditions, lower chlorinated benzene congeners can serve as a growth supporting substrates and in some cases become cometabolized. Bacteria utilizing CBs as a carbon and energy source have been isolated from 14 different genera (Table 1), indicating a high level of biodiversity. Aerobic cometabolism has been observed with additional genera of bacteria as well as fungi (see below). Under anaerobic conditions, higher chlorinated benzene congeners are subject to reductive dechlorination provided electron-donating substrates are available. Recent evidence also points to the use of higher chlorinated benzenes as electron acceptors supporting halorespiration. The biodiversity of halorespiring organisms implicated in the

Table 1 Aerobic bacterial strains capable of growing on chlorinated benzenes as a sole source of carbon and energy

Bacterial strain	Congener	References
Burkholderia sp. strain PS12	CB	Sander et al. (1991)
Burkholderia sp. strain PS14	CB	Sander et al. (1991)
Escherichia hermanii	CB	Kiernicka et al. (1999)
Hydrid strain WR1313	CB	Oltmanns et al. (1988)
Pseudomonas aeruginosa RHO1	CB	Brunsbach and Reineke (1994)
Pseudomonas putida GJ31	CB	Oldenhuis et al. (1989); Mars et al. (1997)
Pseudomonas sp. strain JS100	CB	Haigler et al. (1988)
Pseudomonas sp. strain JS150	CB	Haigler et al. (1992)
Pseudomonas sp. strain JS6	CB	Pettigrew et al. (1991)
Ralstonia sp. strain JS705	CB	van der Meer et al. (1998)
Rhodococcus phenolicus	CB	Rehfuss and Urban (2005)
Rhodococcus sp.	CB	Vogt et al. (2004a)
Pseudomonas sp.	CB	Vogt et al. (2004a)
Xanthobacter sp.	CB	Vogt et al. (2004a)
Paenibacillus sp.	CB	Vogt et al. (2004a)
Kocuria sp.	CB	Vogt et al. (2004a)
Stenotrophomonas sp.	CB	Vogt et al. (2004a)
Unidentified strain 1469	CB	Nishino et al. (1992)
Unidentified strain 1474	CB	Nishino et al. (1994)
Unidentified strain WR1306	CB	Reineke and Knackmuss (1984)
Planococcus sp strain ZD22	CB	Li et al. (2006)
Acidovorax facilis 13517	CB	Vogt et al. (2004a, b)
Cellulomonas turbata B529	CB	Vogt et al. (2004b)
Pseudomonas veronii 13547	CB	Vogt et al. (2004b)
Pseudomonas veronii B549	CB	Vogt et al. (2004b)
Paenibacillus polymyxa B550	CB	Vogt et al. (2004b)
Burkholderia sp. strain PS12	1,2-DCB	Sander et al. (1991)
Burkholderia sp. strain PS14	1,2-DCB	Sander et al. (1991), Rapp and Timmis (1999)
Pseudomonas sp. strain GJ60	1,2-DCB	Oldenhuis et al. (1989)
Pseudomonas sp. strain JS100	1,2-DCB	Haigler et al. (1988)
Pseudomonas sp. strain P5	1,2-DCB	Van Der Meer et al. (1987)
Acidovorax avenae	1,2-DCB	Monferran et al. (2005)
Alcaligenes sp. strain OBB65	1,3-DCB	Debont et al. (1986)
Burkholderia sp. strain PS12	1,3-DCB	Sander et al. (1991)
Burkholderia sp. strain PS14	1,3-DCB	Sander et al. (1991), Rapp and Timmis (1999)
Pseudomonas sp. strain P51	1,3-DCB	Van Der Meer et al. (1987)
Alcaligenes sp. R3	1,4-DCB	Oltmanns et al. (1988)
Burkholderia sp. PS12	1,4-DCB	Sander et al. (1991)
Burkholderia sp. PS14	1,4-DCB	Sander et al. (1991), Rapp and Timmis (1999)
Hydrid strain WR1323	1,4-DCB	Oltmanns et al. (1988)
Pseudomonas aeruginosa RHO1	1,4-DCB	Oltmanns et al. (1988), Brunsbach and Reineke (1994)
Pseudomonas sp. B1	1,4-DCB	Oltmanns et al. (1988)
Pseudomonas sp. JS150	1,4-DCB	Haigler et al. (1992)
Pseudomonas sp. JS6	1,4-DCB	Spain and Nishino (1987)
Pseudomonas sp. P51	1,4-DCB	Van Der Meer et al. (1987)

Table 1 continued

Bacterial strain	Congener	References
Sphingomonas (*Alcaligenes*) sp. strain A175	1,4-DCB	Schraa et al. (1986)
Unidentified strain 1474	1,4-DCB	Nishino et al. (1994)
Xanthobacter flavus 14p1	1,4-DCB	Sommer and Gorisch (1997)
Rhodococcus phenolicus	1,4-DCB	Rehfuss and Urban (2005)
Burkholderia sp. PS12	1,2,4-TCB	Sander et al. (1991)
Burkholderia sp. PS14	1,2,4-TCB	Sander et al. (1991)
		Rapp and Timmis (1999), Rapp (2001)
Pseudomonas sp. P51	1,2,4-TCB	Van Der Meer et al. (1987)
Pseudomonas chlororaphis RW71	1,2,3,4-TeCB	Potrawfke et al. (1998)
Burkholderia (*Pseudomonas*) sp. PS12	1,2,4,5-TeCB	Beil et al. (1997, 1998)
Burkholderia sp. strain PS14	1,2,4,5-TeCB	Sander et al. (1991), Rapp and Timmis (1999)

halorespiration of CBs is low. So far only members of the genera *Dehalobacter* and *Dehalococcoides* or closely related Chloroflexi have been identified (see below).

Aerobic bacterial cometabolism

Several examples of aerobic cometabolism of chlorinated benzenes are reported. CB-grown cells of the bacterium *Pseudomonas* sp. strain JS150 were able to oxidize 1,2-DCB and 1,3-DB which were otherwise not growth substrates (Haigler et al. 1992). The cometabolism of 1,2-DCB and 1,2,4-TCB by *Pseudomonas aeruginosa* strain RHO1, using CB and 1,4-DB as growth substrates, was reported (Brunsbach and Reineke 1994). The cometabolism of 1,2,3-TCB by the methane oxidizing bacterium, *Methylosinus trichosporium* OB3b, was studied by Sullivan and Chase (1996). Cells grown on methane under low copper conditions to stimulate soluble methane monooxygenase metabolized 1,2,3-TCB to 2,3,4- and 3,4,5-trichlorophenol when provided with formate as cosubstrate. Another methanotrophic strain, *Methylocystis* sp. GB 14, was found to cometabolize CB with methane-grown cells cultivated in copper-free medium (Jechorek et al. 2003). Under variable culture conditions, 80% of CB was eliminated by *Methylocystis* sp. GB 14, and chlorophenols were recovered as products accounting for 53% recovery of CB metabolized. Co-oxidation of CB by propane-grown cells of the bacterium, *Mycobacterium vaccae* strain JOB-5, has also been reported (Burback and Perry 1993). The main product from the conversion of the compound was 4-chlorophenol.

Finally, there are several reports describing the cometabolism of chlorinated benzene by *Pseudomonas putida* strains. Glutamate-grown cells of *P. putida* MST were shown to cometabolize CB to 3-chlorocatechol (Bestetti et al. 1992). Benzene-grown cells of *P. putida* cometabolized 1,2-DCB to 2,3-, 3,4-, and 2.6-dichlorophenols (Ballschmiter and Scholz 1981). A benzene-grown mixed culture from soil cometabolized 1,3,5-TCB to 2,4,6-trichlorophenol (Ballschmiter and Scholz 1981). In a similar study, almost all congeners of chlorinated benzenes ranging from mono- to tetra-CBs were oxidized to chlorophenols by a benzene-grown mixed culture from soil (Ballschmiter et al. 1977). Chlorocatechols were additionally recovered from experiments with the DCBs (Ballschmiter and Scholz 1980). In the experiments with benzene as the primary substrate, the results suggest the involvement of a monooxygenase in the cometabolism of CB. The initial formation of an epoxide is postulated. The epoxide is subsequently and rapidly converted to a chlorophenol. Additional reaction of the monooxygenase results in the conversion of the chlorophenol intermediates to chlorocatechols (Ballschmiter and Scholz 1980). The monooxygenase from *P. putida* has been isolated and it is responsible for the hydroxylation of chlorinated benzenes (Jones et al. 2001). Site-directed mutagenesis has been used to improve the activity and broaden the substrate specificity of the monooxygenase to include QCB and

HCB (Jones et al. 2001; Chen et al. 2002b). The pentachlorophenol (PCP)-degrading bacterium, *Sphingobium chlorophenolicum* ATCC 39723, was genetically engineered to include genes for a mutant form of the monooxygenase cytochrome P450 that can oxidize HCB to PCP (Yan et al. 2006). Glutamate-grown cells of the genetically engineered strain were able to cometabolize HCB and PCP was shown to be an intermediate.

Aerobic bacterial growth on chlorinated benzenes as sole carbon and energy source

There is convincing evidence that several chlorinated benzenes can be utilized as a sole source of carbon and energy. The first report of bacterial growth on a chlorinated benzene was that of an unidentified strain, WR1306, utilizing CB (Reineke and Knackmuss 1984). Since then, a wide variety of bacterial strains have been shown to utilize, CB, 1,2-DCB, 1,3-DCB, 1,4-DCB, 1,2,4-TCB, 1,2,4,5-TeCB, and 1,2,3,4-TeCB as growth substrates as outlined in Table 1. In most of these studies, sound evidence for the mineralization of the chlorinated benzene is provided. These include stoichiometric release of chloride (Reineke and Knackmuss 1984; Debont et al. 1986; Schraa et al. 1986; Spain and Nishino 1987; Haigler et al. 1988; Sander et al. 1991; Spiess et al. 1995; Sommer and Gorisch 1997; Potrawfke et al. 1998) or mineralization of ^{14}C-labeled chlorinated benzenes to $^{14}CO_2$ (Marinucci and Bartha 1979; Haigler et al. 1988; Nishino et al. 1992). The degradative attack of chlorinated benzenes by these strains is initiated with dioxygenases to produce chlorinated dihydrodiol intermediates that are subsequently rearomatized by dihydrodiol dehydroganeses, yielding chlorocatechols as intermediates (Spain and Nishino 1987; Haigler et al. 1988; Sander et al. 1991; Spiess et al. 1995; Beil et al. 1997, 1998; Mars et al. 1997; Potrawfke et al. 1998; van der Meer et al. 1998). A CB dioxygenase from *Burkholderia* sp. strain PS12 was cloned into *Escherichia coli*, which could express an active form of the enzyme (Beil et al. 1997). The heterologous recombinant CB dioxygenase converted 1,2,4,5-TeCB to an unstable tetrachlorodihydrodiol intermediate, which spontaneously rearomatizes with concomitant elimination of chloride to the corresponding 3,4,6-trichlorocatechol. In most other cases, the initial dioxygenation results in the formation of stable dihydrodiol intermediates with the same number of chlorine groups as the original substrate (Debont et al. 1986; Haigler et al. 1988; Sander et al. 1991; Spiess et al. 1995; Chartrain et al. 2000). For example, heterologous recombinant CB dioxygenase converted 1,2,4-TCB to the corresponding stable trichlorodihydrodiol (Beil et al. 1997). Theses *cis*-dihydrodiol intermediates are oxidized to the corresponding chlorocatechols by dihydrodiol dehydrogenases (Spain and Nishino 1987; Haigler et al. 1988; Sander et al. 1991; Spiess et al. 1995; Spiess and Gorisch 1996; Sommer and Gorisch 1997). The chlorocatechols are subsequently oxidized by either one of two types of chlorocatechol dioxygenases, causing either *ortho*-cleavage (catechol 1,2-dioxygenase) to chloromuconic acids (Schraa et al. 1986; Spain and Nishino 1987; Haigler et al. 1988; Spiess et al. 1995; Sander et al. 1991; Sommer and Gorisch 1997; Potrawfke et al. 1998) or *meta*-cleavage (catechol 2,3-dioxygenase) to 2-hydroxy-6-chlorocarbonyl muconic acid (acylchloride) (Klecka and Gibson 1981; Bartels et al. 1984; Pettigrew et al. 1991; Mars et al. 1997). Chloromuconic acids are metabolized further to intermediates of the Kreb's cycle as shown in Fig. 1. The formation of a reactive acylchloride by *meta*-cleavage usually results in inactivation of the catechol dioxygenase and eventually cell death (Klecka and Gibson 1981; Bartels et al. 1984). However, *P. putida* GJ31 was found to have a *meta*-cleavage enzyme that is resistant to suicide inhibition, and it converted chlorocatechol to 2-hydroxy-*cis*,*cis*-muconic acid, which was mineralized further (Mars et al. 1997) as shown in Fig. 1.

Degradation by fungi

The white-rot fungus, *Phanerochaete chrysosporium*, partially mineralizes radiolabeled mono- and DCBs to $^{14}CO_2$ by 12–28% (Yadav et al. 1995). The metabolism of CB by *P. chrysosporium* was very limited in low-nitrogen medium and greatly improved in high-nitrogen, indicating that ligninolytic enzymes were most likely not involved. Hexachlorobenzene was shown to be partially eliminated from soil by a *Lentinus* isolate (Matheus et al. 2000). Two white-rot fungi, *P. chrysosporium* and *Pleurotus pulmonarius*, were used to inoculate a contaminated soil that contained a mixture of chlorinated pollutants, including 17.4 and

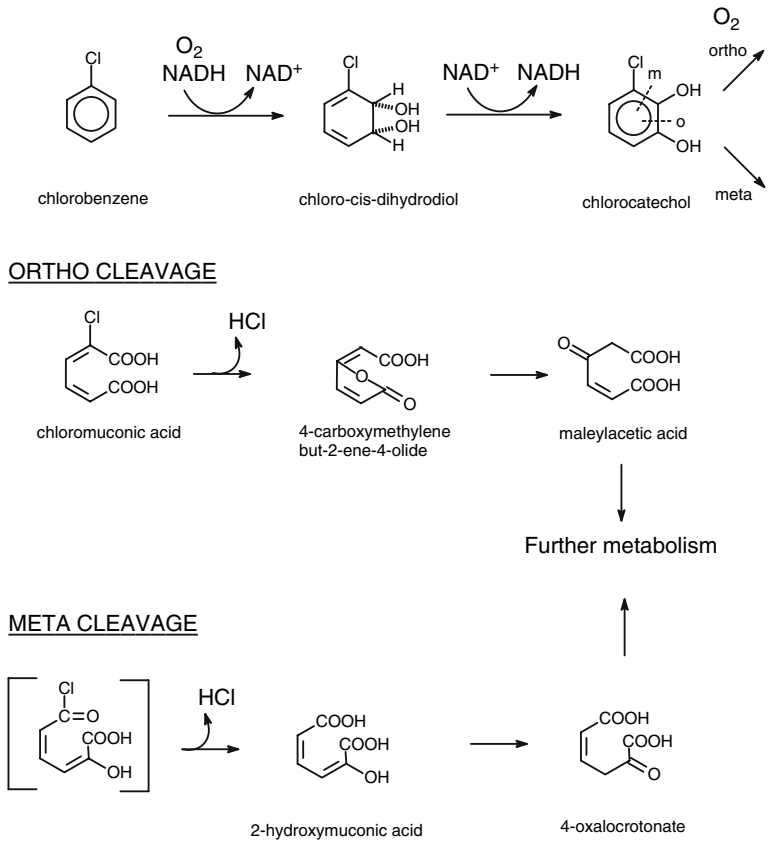

Fig. 1 Pathways of aerobic degradation of chlorobenzenes by strains utilizing the chlorinated compound as a growth substrate (Reineke and Knackmuss 1984; DeBont et al. 1986; Schraa et al. 1986; Spain and Nishino 1987; Haigler et al. 1988; Sander et al. 1991; Spiess et al. 1995; Mars et al. 1997; Potrawfke et al. 1998)

10.3 mg kg^{-1} of 1,2,3,4-TeCB and 1,2,4,5-TeCB, respectively (D'Annibale et al. 2005). After 30 days of incubation, the TeCB congeners were completely eliminated in soils inoculated with the white-rot fungi; whereas they were only eliminated by 50% with the indigenous soil population.

Anaerobic cometabolism

Reductive dechlorination of chlorinated benzenes under anaerobic conditions is a well established biotransformation process occurring either as a fortuitous cometabolic reaction or energy yielding halorespiration (Adrian and Gorisch 2002). The slow reductive biotransformation of 1,2,4-TCB to 1,2-DCB and CB in the presence of H_2 by *Staphylococcus epidermidis* isolated from the gastrointestinal tract of rats constitutes one of the first reported examples of chlorinated benzene cometabolism (Tsuchiya and Yamaha 1984). Ruptured cells of *S. epidermidis* were also capable of converting TCB when supplied with NADH as electron donor. Other example includes the conversion HCB to several TCB (1,3,5- and 1,2,4-) and DCB (1,2-, 1,3-, and 1,4-) isomers in anaerobic sewage sludge (Fathepure et al. 1988; Yuan et al. 1999). The main product recovered from the conversion was 1,3,5-TCB, accounting for almost 90% of HCB added (Fathepure et al. 1988).

Anaerobic metabolism by enrichment cultures

Several researchers have developed anaerobic enrichment cultures that dehalogenate chlorinated benzenes at considerably faster rates than the original inocula. Examples of anaerobic enrichment cultures and the isomers dechlorinated are shown in Table 2. The fact that organisms have become enriched by dehalogenating chlorinated benzenes suggests some kind of benefit is derived from the process (Holliger et al. 1992; Beurskens et al. 1994; Adrian et al. 2000a; Chen et al. 2002a). The evidence is supported by demonstrating that population growth is linked the

Table 2 Anaerobic enrichment cultures reductively dehalogenating chlorinated benzene compounds

Original inoculum	Congener(s)	Electron donor	Product(s)	References
River sediment	123-TCB	Lactate or H_2	13-DCB	Holliger et al. (1992)
Mixture of sediments	124-TCB	Lactate, glucose, ethanol, methanol, propionate, acetate, H_2	14-DCB, CB	Middeldorp et al. (1997)
Bioreactor	Mix 123-TCB and 124-TCB	Pyruvate or formate	DCBs	Adrian et al. (1998)
Bioreactor	Mix 123-TCB and 124-TCB	Pyruvate or H_2	DCBs	Adrian et al. (2000a)
PCB-enrichment culture (with DF-1)	QCB or HCB	Formate	1235-TeCB, 1,3,5-TCB	Wu et al. (2002)
Lake sediment	HCB	Lactate	QCB, 1235-TeCB, 1245-TeCB, 124-TCB, 135-TCB	Beurskens et al. (1994)
River sediments	HCB	Yeast extract	1,3,5-TCB	Chen et al. (2002a)
123-TCB enrichment from river sediments	HCB	Yeast extract	QCB, 1235-TeCB, 125-TCB, 124-TCB, 13DCB	Chang et al. (1998, 1997)
HCB enrichment from sediment	HCB	Surfactant (Tween 61)	135-TCB, 14-DCB, 13-DCB	Yeh and Pavlostathis (2005)

dechlorination of the chlorinated benzenes (Adrian et al. 2000a). In most studies the dechlorination patterns follow the thermodynamically most favorable reactions (Beurskens et al. 1994), as shown in Fig. 2. All the possible reductive dechlorination patterns have favorable standard reduction potentials ($E°'$) of 310–478 mV as an electron acceptor with H_2 as an electron donor (–414 mV) providing $\Delta G°'$ values of

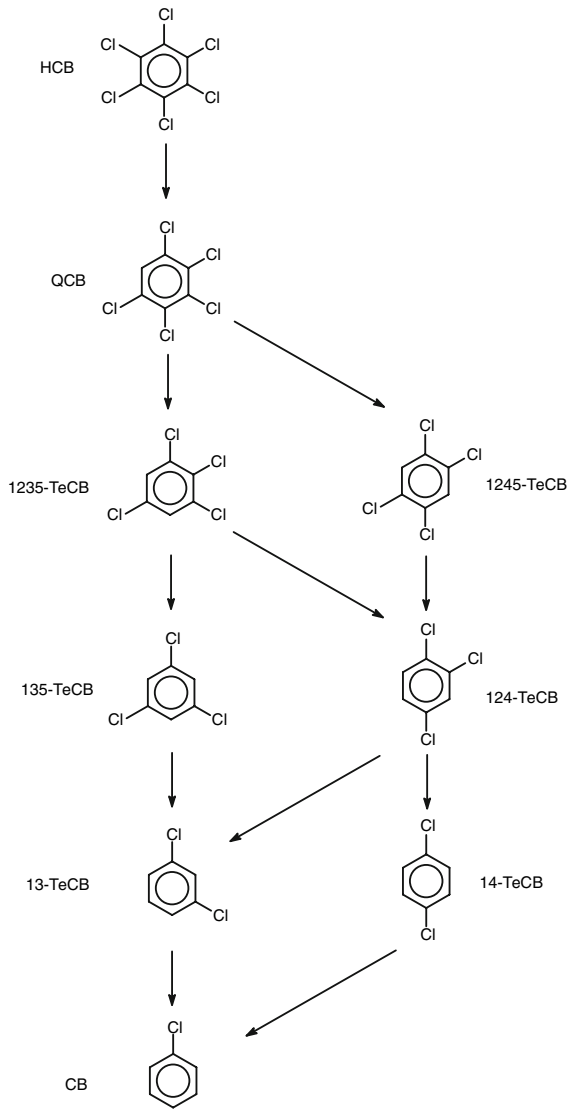

Fig. 2 Most common pathways of anaerobic reductive dechlorination of hexachlorobenzene by microbial enrichment cultures and environmental samples (Fathepure et al. 1988; Holliger et al. 1992; Beurskens et al. 1994; Middeldorp et al. 1997; Adrian et al. 1998; Chang et al. 1998; Adrian and Gorisch 2002; Chen et al. 2002a; Wu et al. 2002)

–139.6 to –171.4 kJ mol^{-1} (Dolfing and Harrison 1992). Nonetheless, in most studies a preferential dechlorination of doubly flanked chlorine over single flanked or unflanked chlorine is observed. These preferences coincide with the highest ΔG°' for dechlorination reactions double flanked chlorines; whereas reactions of the unflanked chlorines have the lowest ΔG°' values. The most frequently observed dechlorination pattern proceeds via 1,2,3,5-TeCB to 1,3,5-TCB or via 1,2,4,5-TeCB to 1,4,5-TCB. 1,3,5-TCB is dechlorinated further to 1,3-DCB and 1,4,5-TCB is dechlorinated further to 1,4-DCB or 1,3-DCB (Fig. 2). One study has observed an exception to the trend, in which single flanked chlorines are preferentially dechlorinated, resulting in a pathway to CB proceeding via 1,2,3,4-TeCB, 1,2,3-TCB, 1,2-DCB (Ramanand et al. 1993). Enrichment cultures have also been developed that can carry out unflanked chlorine dechlorinations (Bosma et al. 1988). These findings indicate that dechlorinating strains exist that can capitalize on the lower free energy change available in the unflanked chlorines.

The ability CB halorespiration to compete or coexist with sulfate reduction has been evaluated. From a bioenergetics stand point, haorespiration of CBs (E°' = 310–478 mV) should outcompete sulfate reduction (E°' = –217 mV). In most studies, the reductive dechlorination of CBs has been shown to proceed under sulfate reducing conditions (Chen et al. 2002a; Chang et al. 1998; Van der Meer et al. 1992; Masunaga et al. 1996). However, in another study, a highly enriched pyruvate-fed enrichment culture containing was not able to reductively dechlorinate TCBs in the presence of sulfate (Adrian et al. 1998).

In recent years, considerable progress has been made in identifying halorespiring organisms responsible for growth-linked dechlorination of chlorinated benzenes. A phylogenetic survey using 16S RNA genes and applied to an anaerobic TCB-transforming microbial community obtained from a fluidized bed reactor revealed the presence of the halorespiring bacterium, *Dehalobacter* sp (von Wintzingerode et al. 1999). The occurrence of halorespiring bacteria in the TCB-dechlorinating community was further confirmed by hybridization with molecular probes based on conserved regions of reductive dehalogenase genes (*PceA* and *CprA*) from known halorespiring bacteria (von Wintzingerode et al. 2001). These findings support the hypothesis that reductive dechlorination of TCB occurs via a respiratory pathway.

A pure culture capable of dechlorinating TeCB and TCB was isolated and characterized (Adrian et al. 2000b; Jayachandran et al. 2003). *Dehaloccocoides* strain CBDB1, a bacterium that is closely related to known PCE halorespiring bacterial strains, links its growth to the oxidation of hydrogen at the expense of respiring the chlorinated benzenes. The chlorinated benzene substrate range and products of *Dehaloccocoides* CBDB1 are shown in Table 3. The initial preference of dechlorination is doubly flanked chlorines, followed by single flanked chlorines. Chlorines that are not flanked at all are not dechlorinated by *Dehaloccocoides* CBDB1. Cell-free extracts prepared from this strain displayed dehalogenase activity towards many congeners of chlorinated benzenes,

Table 3 Range of chlorinated benzene congeners utilized by the halorespiring bacterium, *Dehaloccocoides* strain CBDB1, as terminal electron-acceptor and products from the dechlorination (Adrian et al. 2000b; Jayachandran et al. 2003; Griebler et al. 2004)

Chlorinated benzene congeners utilized	Transient intermediates	Final reduced products
123-TCB		13-DCB
124-TCB		13-DCB and 14-DCB
1234-TeCB	124-TCB	13-DCB and 14-DCB
1245-TeCB	124-TCB	13-DCB and 14-DCB
1235-TeCB		135-TCB
QCB	1245-TeCB, 1235-TeCB and 124-TCB	135-TCB, 13-DCB and 14-DCB
HCB	QCB, 1245-TeCB, 1235-TeCB and 124-TCB	135-TCB, 13-DCB and 14-DCB

Hydrogen is utilized as the electron donor

In the case of QCB, a highly enriched culture instead of a pure culture was utilized

Table 4 Microbial kinetics of the biodegradation of chlorinated benzenes

Substrate	Role	TEA	Culture	Growth rate (day^{-1})	Pseudo 1st order ($l\ mg^{-1}\ dwt\ day^{-1}$)	K_s ($mg\ l^{-1}$)	Specific activity ($mg\ g^{-1}\ dwt\ day^{-1}$)	Cell yield ($g\ dwt\ g^{-1}$)	References
Aerobic conditions									
CB	ED-growth		*Burkholderia* sp. PS12				1,227[a]		Sander et al. (1991)
CB	ED-growth		Strain WR1306	13.20					Reineke and Knackmuss (1984)
CB	ED-growth		*Pseudomonas* sp. JS100				1,458[a]		Haigler et al. (1988)
CB	ED-growth		*Pseudomonas* sp. JS6				1,319[a]		Spain and Nishino (1987)
CB	ED-growth		*Pseudomonas* sp. GJ13	5.54					Oldenhuis et al. (1989)
CB	ED-growth		*Sphingomonas* sp. A175				1,666[a]		Schraa et al. (1986)
CB	ED-growth		*Alcaligenes sp. OBB65*	1.11			1,140[a]		Debont et al. (1986)
CB	ED-growth		Enrichment culture				1,680	0.36	Lapertot et al. (2006)
12-DCB	ED-growth		Mixed culture CSTR	1.30		1.99		0.40	Naziruddin et al. (1995)
12-DCB	ED-growth		*Burkholderia* sp. PS12				733[a]		Sander et al. (1991)
12-DCB	ED-growth		*Pseudomonas* sp. JS100	3.02			2,459[a]		Haigler et al. (1988)
12-DCB	ED-growth		*Pseudomonas* sp. GJ60	3.02					Oldenhuis et al. (1989)
12-DCB	ED-growth		Enrichment culture				480		Lapertot et al. (2006)
13-DCB	ED-growth		*Burkholderia* sp. PS12				1,221[a]		Sander et al. (1991)
13-DCB	ED-growth		Mixed culture CSTR	1.58		3.74		0.41	Naziruddin et al. (1995)
13-DCB	ED-growth		*Alcaligenes sp. OBB65*				651[a]		de Bont et al. (1986)
13-DCB	ED-growth		*Sphingomonas* sp. A175				1,205[a]		Schraa et al. (1986)
14-DCB	ED-growth		Mixed culture CSTR	1.27		2.69		0.39	Naziruddin et al. (1995)
14-DCB	ED-growth		*Burkholderia* sp. PS12				554[a]		Sander et al. (1991)
14-DCB	ED-growth		*Pseudomonas* sp. JS6	3.33			1,026[a]		Spain and Nishino (1987)
14-DCB	ED-growth		*Sphingomonas* sp. A175	2.08			1,791[a]	0.95	Schraa et al. (1986)
14-DCB	ED-growth		*Alcaligenes sp. OBB65*	1.11			521[a]		de Bont et al. (1986)
14 DCB	ED-growth		*Xanthobacter flavus*	2.08			391[a]		Spiess et al. (1995)
123-TCB	Comet		*Methylosinus trichosporium* OB3b		0.0028				Sullivan and Chase (1996)
124-TCB	ED-growth		*Burkholderia sp.* PS14		18.48		9,723		Rapp (2001)
123-DCB	ED-growth		Mixed culture CSTR	1.51		3.28		0.43	Naziruddin et al. (1995)
124-TCB	ED-growth		*Burkholderia* sp. PS12	1.39				0.42	Sander et al. (1991)
124-TCB	ED-growth		*Burkholderia* sp. PS12				675[a]		Sander et al. (1991)
1245-TeCB	ED-growth		*Burkholderia* sp. PS14	2.08			520	0.33	Sander et al. (1991)

Table 4 continued

Substrate	Role	TEA	Culture	Growth rate (day^{-1})	Pseudo 1st order ($l\ mg^{-1}\ dwt\ day^{-1}$)	K_s ($mg\ l^{-1}$)	Specific activity ($mg\ g^{-1}\ dwt\ day^{-1}$)	Cell yield ($g\ dwt\ g^{-1}$)	References
Anaerobic conditions									
12-DCB	Comet	M	River sediment		5.8×10^{-8}		1.4×10^{-5}		Masunaga et al. (1996)
124-TCB	Comet	Fm	*Staphylococcus epidermidis*				1.2		Tsuchiya and Yamaha (1984)
123-TCB	Comet	M	River sediment		9.6×10^{-8}		3.7×10^{-5}		Masunaga et al. (1996)
123-TCB	EA-growth	HR	*Dehalococcoides* CBDB1				52,533		Jayachandran et al. (2003)
124-TCB	EA-growth	M	Enrichment culture				309		Adrian et al. (2000a)
1235-TeCB	Comet	M	Anaerobic sludge				9.5×10^{-3}		Fathepure et al. (1988)
1245-TeCB	Comet	M	River sediment		7.7×10^{-8}		3.2×10^{-5}		Masunaga et al. (1996)
1235-TeCB	Comet	M	River sediment		1.2×10^{-7}				Masunaga et al. (1996)
1234-TeCB	Comet (QCB)	HR	*Dehalococcoides* CBDB1				2,892,672		Jayachandran et al. (2003)
QCB	Comet	M	River sediment		1.3×10^{-7}		6.1×10^{-5}		Masunaga et al. (1996)
QCB	Comet (HCB)	HR	*Dehalococcoides* CBDB1				1,980,353		Jayachandran et al. (2003)
QCB	EA-growth	HR	*Dehalococcoides* CBDB1				238,075	0.060	Jayachandran et al. (2003)
HCB	Comet	M	Anaerobic sludge				1.6×10^{-1}		Fathepure et al. (1988)
HCB	Comet	M	Lake sediment		3.3×10^{-7}		1.3×10^{-4}		Susarla et al. (1997)
HCB	Comet	M	River sediment		8.2×10^{-8}				Masunaga et al. (1996)
HCB	EA-growth	HR	*Dehalococcoides* CBDB1				41,368	0.054	Jayachandran et al. (2003)
HCB	EA-growth	M	Enrichment culture				1.1×10^{-3}		Yuan et al. (1999)

[a] Activities calculated from O_2 uptake

TEA Terminal electron acceptor, *M* Methanogenesis, *Fm* Fermentation, *HR* Halorespiration

utilizing methyl viologen as an artificial electron donor (Holscher et al. 2003). Rates ranged from 0.3 to 355 nkat mg^{-1} protein for 1,2,4-TCB to 1,2,3,4-TeCB, respectively. The activity was associated with the membrane fraction of the cells and specific inhibitors indicated the involvement of corrinoid cofactors.

A pure culture of *Dehalococcoccoides ethenogens* strain 195, known for its ability to halorespire perchloroethene (PCE) to ethene, was shown to dehalogenate various highly chlorinated benzene congeners when PCE was also present as an electron acceptor to support growth (Fennell et al. 2004). The daughter products from the reductive dechlorination with either HCB or QCB were 1,2,3,5-TeCB, 1,2,4-TCB and 1,3,5-TCB. The daughter products from the dechlorination of 1,2,4,5-TeCB were 1,2,4-TCB, 1,4-DCB, and 1,3-DCB; while the dechlorination products of 1,2,3,4-TeCB were 1,2,4-TCB, 1,3,5-TCB, and 1,3-DCB. By comparison, the congener, 1,2,3,5-TeCB, was dehalogenated very slowly by *D. ethenogens* 195.

A highly enriched culture cultivated by growth-linked dehalogenation of the PCB congener, 2,3,4,5-tetrachlorobiphenyl, was shown to also dechlorinate HCB (Wu et al. 2002). The culture dechlorinated HCB to 1,3,5-TCB, via QCB and 1,2,3,5-TeCB. Using molecular ecology tools, the uncultured bacterium DF-1 was shown to be the responsible dehalogenator of HCB. Bacterium DF-1 had a closer relationship to known PCE- and TCE-dechlorinating strains of *Dehalococcoides* than to *Dehaloccocoides* sp. strain CBDB1 (only 89% base pair homology).

Microbial kinetics of chlorobenzene biodegradation

Microbial kinetic data for chlorinated benzene degradation are presented in Table 4. Aerobic growth rates on chlorinated benzenes as a sole source of carbon and energy is moderate to high ranging from 1.11 to 13.2 day^{-1}. These values correspond to doubling times of 1.3–15 h. The literature is in good agreement concerning the specific activity of aerobic bacteria growing on chlorinated benzenes, with the exception of one-value, the range is from 520 to 2,459 mg g^{-1} dwt day^{-1}. Likewise, there is good agreement on the cell yield from the aerobic growth on chlorinated benzenes with all but one value in the range of 0.33–0.43 g dwt g^{-1} chlorinated benzene consumed. Only one author, Naziruddin et al. (1995), has provided information on the half velocity constant, K_s, which indicates a value of a few mg l^{-1} for an aerobic mixed culture.

Less information is available on the biotransformation kinetics under anaerobic conditions. However, specific activities of chlorinated benzene biotransformation are highly dependent on the degree to which an anaerobic culture has been purified and enriched. The specific activities of chlorinated benzene dehalogenation in unadapted aquatic sediments ranged from 10^{-5} to 10^{-4} mg chlorinated benzene transformed g^{-1} dwt sediments day^{-1}. Rates in enrichment cultures or anaerobic reactor biofilms ranged from 10^{-2} to 10^{2} mg chlorinated benzene transformed g^{-1} dwt biomass day^{-1}. Rates in the halorespiring bacterium, *Dehalococcoides* sp. CBDB1, were 10^{4} to 10^{5} mg chlorinated benzene transformed g^{-1} dwt biomass d^{-1} for chlorinated benzenes used as the growth electron acceptor. The specific activities were even higher, up to 10^{6} mg chlorinated benzene transformed g^{-1} dwt biomass d^{-1}, for chlorinated benzenes when cometabolized with another electron acceptor (Jayachandran et al. 2003). Therefore, halorespiration of CBs is at least one order of magnitude faster than the oxidation of CB by pure cultures of aerobic bacteria. The cell yields of halorespiration averaged 0.057 g dwt biomass g^{-1} chlorinated benzene transformed.

Acknowledgment The authors are grateful to Eurochlor for their financial support.

References

Adrian L, Gorisch H (2002) Microbial transformation of chlorinated benzenes under anaerobic conditions. Res Microbiol 153:131–137

Adrian L, Manz W, Szewzyk U et al (1998) Physiological characterization of a bacterial consortium reductively dechlorinating 1,2,3- and 1,2,4-trichlorobenzene. Appl Environ Microbiol 64:496–503

Adrian L, Szewzyk U, Gorisch H (2000a) Bacterial growth based on reductive dechlorination of trichlorobenzenes. Biodegradation 11:73–81

Adrian L, Szewzyk U, Wecke J et al (2000b) Bacterial dehalorespiration with chlorinated benzenes. Nature 408:580–583

Alfreider A, Vogt C, Babel W (2002) Microbial diversity in an in situ reactor system treating monochlorobenzene contaminated groundwater as revealed by 16S ribosomal DNA analysis. Syst Appl Microbiol 25:232–240

Ballschmiter K, Scholz C (1980) Microbial degradation of chlorinated aromatic chemicals 6. formation of dichloro phenols and dichloro-benzocatechins from dichloro-benzene in micro molar solution by *Pseudomonas* spp. Chemosphere 9:457–468

Ballschmiter K, Scholz C (1981) Microbial-degradation of chlorinated arenes. 7. Initial steps in the degradation of chlorobenzene derivatives by *Pseudomonas putida*. Angew Chem Int Edit Engl 20:955–956

Ballschmiter K, Unglert C, Heinzmann P (1977) Microbiological degradation of aromatics. 4. formation of chlorophenols by microbial transformation of chlorobenzenes. Angew Chem Int Edit Engl 16:645–645

Barber JL, Sweetman AJ, van Wijk D, Jones KC (2005) Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. Sci Total Environ 349:1–44

Bartels I, Knackmuss HJ, Reineke W (1984) Suicide Inactivation of catechol 2 3 dioxygenase from *Pseudomonas putida* MT-2 by 3 halo catechols. Appl Environ Microbiol 47:500–505

Bartholomew GW, Pfaender FK (1983) Influence of spatial and temporal variations on organic pollutant bio degradation rates in an estuarine environment. Appl Environ Microbiol 45:103–109

Beil S, Happe B, Timmis KN et al (1997) Genetic and biochemical characterization of the broad spectrum chlorobenzene dioxygenase from *Burkholderia* sp. strain PS12—Dechlorination of 1,2,4,5-tetrachlorobenzene. Eur J Biochem 247:190–199

Beil S, Mason JR, Timmis KN et al (1998) Identification of chlorobenzene dioxygenase sequence elements involved in dechlorination of 1,2,4,5-tetrachlorobenzene. J Bacteriol 180:5520–5528

Bestetti G, Galli E, Leoni B et al (1992) Regioselective hydroxylation of chlorobenzene and chlorophenols by a *Pseudomonas putida*. Appl Microbiol Biotechnol 37:260–263

Beurskens JEM, Dekker CGC, Jonkhoff J et al (1993) Microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine River. Biogeochemical 19:61–81

Beurskens JEM, Dekker CGC, Vandenheuvel H et al (1994) Dechlorination of chlorinated benzenes by an anaerobic microbial consortium that selectively mediates the thermodynamic most favorable reactions. Environ Sci Technol 28:701–706

Bosma TNP, Ballemans EMW, Hoekstra NK et al (1996) Biotransformation of organics in soil columns and an infiltration area. Ground Water 34:49–56

Bosma TNP, Vandermeer JR, Schraa G et al (1988) Reductive dechlorination of all trichlorobenzene and dichlorobenzene isomers. FEMS Microbiol Ecol 53:223–229

Brahushi F, Dorfler U, Schroll R et al (2002) Environmental behavior of monochlorobenzene in an arable soil. Fresenius Environ Bull 11:599–604

Brahushi F, Dorfler U, Schroll R et al (2004) Stimulation of reductive dechlorination of hexachlorobenzene in soil by inducing the native microbial activity. Chemosphere 55:1477–1484

Brunsbach FR, Reineke W (1994) Degradation of chlorobenzenes in soil slurry by a specialized organism. Appl Microbiol Biotechnol 42:415–420

Burback BL, Perry JJ (1993) Biodegradation and biotransformation of groundwater pollutant mixtures by *Mycobacterium vaccae*. Appl Environ Microbiol 59:1025–1029

Chang BV, Chen YM, Yuan SY et al (1997) Reductive dechlorination of hexachlorobenzene by an anaerobic mixed culture. Water Air Soil Pollut 100:25–32

Chang BV, Su CJ, Yuan SY (1998) Microbial hexachlorobenzene dechlorination under three reducing conditions. Chemosphere 36:2721–2730

Chartrain M, Ikemoto N, Taylor et al (2000) Production of *cis*-1,2-dihydroxy-3-methylcyclohexa-3,5-diene (toluene-*cis*-glycol) by *Rhodococcus* sp MA 7249. J Biosci Bioeng 90:321–327

Chen IM, Chang BV, Yuan SY et al (2002a) Reductive dechlorination of hexachlorobenzene under various additions. Water Air Soil Pollut 139:61–74

Chen XH, Christopher A, Jones JP et al (2002b) Crystal structure of the F87W/Y96F/V247L mutant of cytochrome P-450cam with 1,3,5-trichlorobenzene bound and further protein engineering for the oxidation of pentachlorobenzene and hexachlorobenzene. J Biol Chem 277:37519–37526

D'Annibale A, Ricci M, Leonardi et al (2005) Degradation of aromatic hydrocarbons by white-rot fungi in a historically contaminated soil. Biotechnol Bioeng 90:723–731

Debont JAM, Vorage M, Hartmans S et al (1986) Microbial-degradation of 1,3-dichlorobenzene. Appl Environ Microbiol 52:677–680

Dermietzel J, Vieth A (2002) Chloroaromatics in groundwater: chances of bioremediation. Environ Geol 41:683–689

Dionisi D, Bertin L, Bornoroni L et al (2006) Removal of organic xenobiotics in activated sludges under aerobic conditions and anaerobic digestion of the adsorbed species. J Chem Technol Biotechnol 81:1496–1505

Dolfing J, Harrison KB (1992) Gibbs free energy of formation of halogenated aromatic compounds and their potential role as electron acceptors in anaerobic environments. Environ Sci Technol 26:2213–2218

Fathepure BZ, Tiedje JM, Boyd SA (1988) Reductive dechlorination of hexachlorobenzene to trichlorobenzenes and dichlorobenzenes in anaerobic sewage- sludge. Appl Environ Microbiol 54:327–330

Fathepure BZ, Vogel TM (1991) Complete Degradation of polychlorinated hydrocarbons by a 2- stage biofilm reactor. Appl Environ Microbiol 57:3418–3422

Feidieker D, Kampfer P, Dott W (1994) Microbiological and chemical evaluation of a site contaminated with chlorinated aromatic-compounds and hexachlorocyclohexanes. FEMS Microbiol Ecol 15:265–278

Feidieker D, Kampfer P, Dott W (1995) Field-scale investigations on the biodegradation of chlorinated aromatic-compounds and HCH in the subsurface environment. J Contam Hydrol 19:145–169

Fennell DE, Nijenhuis I, Wilson SF et al (2004) *Dehalococcoides ethenogenes* strain 195 reductively dechlorinates diverse chlorinated aromatic pollutants. Environ Sci Technol 38:2075–2081

Griebler C, Adrian L, Meckenstock RU et al (2004) Stable carbon isotope fractionation during aerobic and anaerobic transformation of trichlorobenzene. FEMS Microbiol Ecol 48:313–321

Haigler BE, Nishino SF, Spain JC (1988) Degradation of 1,2-dichlorobenzene by a *Pseudomonas* sp. Appl Environ Microbiol 54:294–301

Haigler BE, Pettigrew CA, Spain JC (1992) Biodegradation of mixtures of substituted benzenes by *Pseudomonas* sp strain-JS150. Appl Environ Microbiol 58:2237–2244

Holliger C, Schraa G, Stams AJM et al (1992) Enrichment and properties of an anaerobic mixed culture reductively dechlorinating 1,2,3-trichlorobenzene to 1,3- dichlorobenzene. Appl Environ Microbiol 58:1636–1644

Holscher T, Gorisch H, Adrian L (2003) Reductive dehalogenation of chlorobenzene congeners in cell extracts of *Dehalococcoides* sp strain CBDB1. Appl Environ Microbiol 69:2999–3001

Jayachandran G, Gorisch H, Adrian L (2003) Dehalorespiration with hexachlorobenzene and pentachlorobenzene by *Dehalococcoides* sp strain CBDB1. Arch Microbiol 180:411–416

Jechorek M, Wendlandt KD, Beck M (2003) Cometabolic degradation of chlorinated aromatic compounds. J Biotechnol 102:93–98

Jones JP, O'Hare EJ, Wong LL (2001) Oxidation of polychlorinated benzenes by genetically engineered CYP101 (cytochrome P450(cam)). Eur J Biochem 268:1460–1467

Kaschl A, Vogt C, Uhlig S et al (2005) Isotopic fractionation indicates anaerobic monochlorobenzene biodegradation. Environ Toxicol Chem 24:1315–1324

Kastner M, Fischer A, Nijenhuis L et al (2006) Assessment of microbial in situ activity in contaminated aquifers. Eng Life Sci 6:234–251

Kiernicka J, Seignez C, Peringer P (1999) *Escherichia hermanii*—A new bacterial strain for chlorobenzene degradation. Lett Appl Microbiol 28:27–30

Klecka GM, Gibson DT (1981) Inhibition of catechol 2 3 di oxygenase from *Pseudomonas putida* by 3 chloro catechol. Appl Environ Microbiol 41:1159–1165

Klecka GM, McDaniel SG, Wilson PS et al (1996) Field evaluation of a granular activated carbon fluid-bed bioreactor for treatment of chlorobenzene in groundwater. Environ Prog 15:93–107

Lapertot M, Seignez C, Ebrahimi S et al (2006) Enhancing production of adapted bacteria to degrade chlorinated aromatics. Ind Eng Chem Res 45:6778–6784

Li H, Liu YH, Luo N et al (2006) Biodegradation of benzene and its derivatives by a psychrotolerant and moderately haloalkaliphilic *Planococcus* sp strain ZD22. Res Microbiol 157:629–636

MacLeod M, Mackay D (1999) An assessment of the environmental fate and exposure of benzene and the chlorobenzenes in Canada. Chemosphere 38:1777–1796

Malcom HM, Howe PD, Dobson S (2004) Chlorobenzenes other than Hexachlorobenzene: Environmental Aspects. Concise International Chemical Assessment Document 60. World Health Organization, Geneva, p 36

Marinucci AC, Bartha R (1979) Biodegradation of 1,2,3-trichlorobenzene and 1,2,4- trichlorobenzene in soil and in liquid enrichment culture. Appl Environ Microbiol 38:811–817

Mars AE, Kasberg T, Kaschabek SR et al (1997) Microbial degradation of chloroaromatics: Use of the meta- cleavage pathway for mineralization of chlorobenzene. J Bacteriol 179:4530–4537

Masunaga S, Susarla S, Yonezawa Y (1996) Dechlorination of chlorobenzenes in anaerobic estuarine sediment. Water Sci Technol 33:173–180

Matheus DR, Bononi VLR, Machado KMG (2000) Biodegradation of hexachlorobenzene by basidiomycetes in soil contaminated with industrial residues. World J Microbiol Biotechnol 16:415–421

Mathur AK, Sundaramurthy J, Balomajumder C (2006) Kinetics of the removal of mono-chlorobenzene vapour from waste gases using a trickle bed air biofilter. J Hazard Mater 137:1560–1568

Middeldorp PJM, Jaspers M, Zehnder AJB, Schraa G (1996) Biotransformation of α-, β-, γ-, and δ-hexachlorocyclohexane under methanogenic conditions. Environ Sci Technol 30:2345–2349

Middeldorp PJM, deWolf J, Zehnder AJB et al (1997) Enrichment and properties of a 1,2,4-trichlorobenzene-dechlorinating methanogenic microbial consortium. Appl Environ Microbiol 63:1225–1229

Monferran MV, Echenique JR, Wunderlin DA (2005) Degradation of chlorobenzenes by a strain of *Acidovorax avenae* isolated from a polluted aquifer. Chemosphere 61:98–106

Mpanias CJ, Baltzis BC (1998) An experimental and modeling study on the removal of mono- chlorobenzene vapor in biotrickling filters. Biotechnol Bioeng 59:328–343

Naziruddin M, Grady CPL, Tabak HH (1995) Determination of biodegradation kinetics of volatile organic- compounds through the use of respirometry. Water Environ Res 67:151–158

Nishino SF, Spain JC, Belcher LA et al (1992) Chlorobenzene degradation by bacteria isolated from contaminated groundwater. Appl Environ Microbiol 58:1719–1726

Nishino SF, Spain JC, Pettigrew CA (1994) Biodegradation of chlorobenzene by indigenous bacteria. Environ Toxicol Chem 13:871–877

Oh YS, Bartha R (1994) Design and performance of a trickling air bio-filter for chlorobenzene and o-dichlorobenzene vapors. Appl Environ Microbiol 60:2717–2722

Oldenhuis R, Kuijk L, Lammers A et al (1989) Degradation of chlorinated and non-chlorinated aromatic solvents in soil suspensions by pure bacterial cultures. Appl Microbiol Biotechnol 30:211–217

Oltmanns RH, Rast HG, Reineke W (1988) Degradation of 1,4-dichlorobenzene by enriched and constructed bacteria. Appl Microbiol Biotechnol 28:609–616

Pettigrew CA, Haigler BE, Spain JC (1991) Simultaneous biodegradation of chlorobenzene and toluene by a *Pseudomonas* strain. Appl Environ Microbiol 57:157–162

Phillips TM, Seech AG, Lee H, Trevors JT (2005) Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. Biodegradation 16:363–392

Potrawfke T, Timmis KN, Wittich RM (1998) Degradation of 1,2,3,4-tetrachlorobenzene by *Pseudomonas chlororaphis* RW71. Appl Environ Microbiol 64:3798–3806

Prytula MT, Pavlostathis SG (1996) Effect of contaminant and organic matter bioavailability on the microbial dehalogenation of sediment-bound chlorobenzenes. Water Res 30:2669–2680

Ramanand K, Balba MT, Duffy J (1993) Reductive dehalogenation of chlorinated benzenes and toluenes under methanogenic conditions. Appl Environ Microbiol 59:3266–3272

Rapp P (2001) Multiphasic kinetics of transformation of 1,2,4-trichlorobenzene at nano- and micromolar concentrations by *Burkholderia* sp strain PS14. Appl Environ Microbiol 67:3496–3500

Rapp P, Timmis KN (1999) Degradation of chlorobenzenes at nanomolar concentrations by *Burkholderia* sp strain PS14 in liquid cultures and in soil. Appl Environ Microbiol 65:2547–2552

Rehfuss M, Urban J (2005) *Rhodococcus phenolicus* sp nov. a novel bioprocessor isolated actinomycete with the ability to degrade chlorobenzene, dichlorobenzene and phenol as sole carbon sources. Syst Appl Microbiol 28:695–701

Reineke W, Knackmuss HJ (1984) Microbial-metabolism of haloaromatics—isolation and properties of a chlorobenzene-degrading bacterium. Appl Environ Microbiol 47:395–402

Rittman BE, McCarty PL (2001) Environmental biotechnology: principles and applications. McGraw Hill, New York, p 754

Rosenbrock P, Martens R, Buscot F et al (1997) Initiation of [Cl-36]hexachlorobenzene dechlorination in three different soils under artificially induced anaerobic conditions. Appl Microbiol Biotechnol 48:115–120

Sander P, Wittich RM, Fortnagel P et al (1991) Degradation of 1,2,4-trichlorobenzene and 1,2,4,5- tetrachlorobenzene by *Pseudomonas* strains. Appl Environ Microbiol 57:1430–1440

Schraa G, Boone ML, Jetten MSM et al (1986) Degradation of 1,4-dichlorobenzene by *Alcaligenes* sp strain- A175. Appl Environ Microbiol 52:1374–1381

Schroll R, Brahushi F, Dorfler U et al (2004) Biomineralisation of 1,2,4-trichlorobenzene in soils by an adapted microbial population. Environ Poll 127:395–401

Seignez C, Adler N, Thoeni C et al (2004) Steady-state and transient-state performance of a biotrickling filter treating chlorobenzene-containing waste gas. Appl Microbiol Biotechnol 65:33–37

Sommer C, Gorisch H (1997) Enzymology of the degradation of (di)chlorobenzenes by *Xanthobacter flavus* 14p1. Arch Microbiol 167:384–391

Spain JC, Nishino SF (1987) Degradation of 1,4-dichlorobenzene by a *Pseudomonas* sp. Appl Environ Microbiol 53:1010–1019

Spiess E, Gorisch H (1996) Purification and characterization of chlorobenzene *cis*- dihydrodiol dehydrogenase from *Xanthobacter flavus* 14p1. Arch Microbiol 165:201–205

Spiess E, Sommer C, Gorisch H (1995) Degradation of 1,4-dichlorobenzene by *Xanthobacter flavus*-14p1. Appl Environ Microbiol 61:3884–3888

Sullivan JP, Chase HA (1996) 1,2,3-Trichlorobenzene transformation by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. Appl Microbiol Biotechnol 45:427–433

Susarla S, Yonezawa Y, Masunaga S (1997) Transformation kinetics and pathways of chlorophenols and hexachlorobenzene in fresh water lake sediment under anaerobic conditions. Environ Technol 18:903–911

Tsuchiya T, Yamaha T (1984) Reductive Dechlorination of 1,2,4-Trichlorobenzene by *Staphylococcus epidermidis* isolated from intestinal contents of rats. Agric Biol Chem 48:1545–1550

van Agteren MH, Keuning S, Janssen DB (1998) Handbook on biodegradation and biological treatment of hazardous organic compounds. Kluwer Academic Publishers, Dordrecht, 491 p

Van der Meer JR, Bosma TNP, De Bruin WP et al (1992) Versatility of soil column experiments to study biodegradation of halogenated compounds under environmental conditions. Biodegradation 3:265–284

Van der Meer JR, Roelofsen W, Schraa G et al (1987) Degradation of low concentrations of dichlorobenzenes and 1 2 4 trichlorobenzene by *Pseudomonas* sp strain P51 in non-sterile soil columns. FEMS Microbiol Ecol 45:333–342

Van der Meer JR, Werlen C, Nishino SF et al (1998) Evolution of a pathway for chlorobenzene metabolism leads to natural attenuation in contaminated groundwater. Appl Environ Microbiol 64:4185–4193

Vogt C, Alfreider A, Lorbeer H et al (2004a) Bioremediation of chlorobenzene-contaminated ground water in an in situ reactor mediated by hydrogen peroxide. J Contam Hydrol 68:121–141

Vogt C, Simon D, Alfreider A et al (2004b) Microbial degradation of chlorobenzene under oxygen-limited conditions leads to accumulation of 3-chlorocatechol. Environ Toxicol Chem 23:265–270

von Wintzingerode F, Schlotelburg C, Hauck R et al (2001) Development of primers for amplifying genes encoding CprA- and PceA-like reductive dehalogenases in anaerobic microbial consortia, dechlorinating trichlorobenzene and 1,2- dichloropropane. FEMS Microbiol Ecol 35:189–196

von Wintzingerode F, Selent B, Hegemann W et al (1999) Phylogenetic analysis of an anaerobic, trichlorobenzene transforming microbial consortium. Appl Environ Microbiol 65:283–286

Wang MJ, Jones KC (1994a) Behavior and fate of chlorobenzenes (CBs) introduced into soil- plant systems by sewage-sludge application—A review. Chemosphere 28:1325–1360

Wang MJ, Jones KC (1994b) Behavior and fate of chlorobenzenes in spiked and sewage sludge-amended soil. Environ Sci Technol 28:1843–1852

Williams M, Bosch S, Plewak D (2006) Toxicological Profile for Dichlorobenzenes. Agency for Toxic Substances and Disease Registry, Atlanta, p 404

Wu QZ, Milliken CE, Meier GP et al (2002) Dechlorination of chlorobenzenes by a culture containing bacterium DF-1, a PCB dechlorinating microorganism. Environ Sci Technol 36:3290–3294

Yadav JS, Wallace RE, Reddy CA (1995) Mineralization of monochlorobenzenes and dichlorobenzenes and simultaneous degradation of chloro-substituted and methyl-substituted benzenes by the white-rot fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 61:677–680

Yan DZ, Liu H, Zhou NY (2006) Conversion of *Sphingobium chlorophenolicum* ATCC 39723 to a hexachlorobenzene degrader by metabolic engineering. Appl Environ Microbiol 72:2283–2286

Yeh DH, Pavlostathis SG (2005) Anaerobic biodegradability of Tween surfactants used as a carbon source for the microbial reductive dechlorination of hexachlorobenzene. Water Sci Technol 52:343–349

Yuan SY, Su CJ, Chang BV (1999) Microbial dechlorination of hexachlorobenzene in anaerobic sewage sludge. Chemosphere 38:1015–1023

Zacharias B, Lang E, Hanert HH (1995) Biodegradation of chlorinated aromatic-hydrocarbons in slow sand filters simulating conditions in contaminated soil—pilot-study for in-situ cleaning of an industrial site. Water Res 29:1663–1671