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CHAPTER 14. THEORETICAL APPROACH TO CHLORDECONE BIODEGRADATION

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Introduction

Nowadays, the sanitary, economic and social crisis caused by chlordecone (CLD) in the French West Indies (FWI) may be considered as essentially contained thanks to the management options that have been taken by the authorities to avoid the population dietary exposure (see chapters 3, 5.4 and 6). A final solution to the problem would consist however to eliminate the source of CLD responsible for the diffuse pollution of all the FWI environmental compartments and related food resources and so to destroy the stock of CLD still presents in the soils. One of the cheapest and most environmental friendly destruction methods corresponds to microbial degradation. Such a mode of destruction seems to be particularly appropriate in the case of FWI since it can be often implemented *in situ*, using techniques (e.g. watering; addition of nutrients, labile organic matter, microorganisms) which are fairly easy to incorporate into the existing agricultural practices taking into account that pollution is estimated to cover some 19,000 ha of arable lands according to Le Déaut and Procaccia (2009). Until now however there is no evidence of natural attenuation in the environments impacted by chlordecone and so of the possibility to stimulate the rate of the process. For instance, in 1989, Huggett stated with respect to the James River pollution by CLD that 13 years of observations did not demonstrate CLD degradation. In the same way, 20 years later Cabidoche et al. (2009) concluded the same about the fate of CLD in FWI soils using a simple water-leaching model to simulate soil CLD content. Old and more recent research consisting of incubating fresh water sediments or FWI soils in the presence of freshly spiked CLD under controlled conditions in the laboratory for short (1 month) or longer term (7 months) and following CLD concentration over time and/or the production of CO_2 seem to confirm in 1st analysis that the natural microbial populations present in these ecosystems are unable or at least have very poor capacity to attack CLD both under aerobic than more reduced redox conditions (Skaar et al. 1981, Portier and Meyers 1982; Gambrell et al. 1984; Fernandez-Bayo et al. 2013a). Similar results were obtained with 103 strains of aerobic fungi isolated from FWI soils (Merlin et al. 2014). The reason usually advocated to explain this apparent absence of degradation in the environment is the peculiar chemical structure of CLD

(bishomocubane "cage" structure with a high steric hindrance caused by the 10 chlorine atoms bound to the cage, Fig. 14.1), coupled to its low aqueous solubility (3 mg/l at 20°C)[1] and high hydrophobicity (Log $Kow_{20°C,\ pH\ 7}$ = 4.5)[1] that would make it refractory to degradation. Indeed, such characteristics indicate intuitively that CLD must be poorly available to microorganisms that necessarily thrive in water and that the access of their enzymes to the CLD carbon skeleton to open the cage will not be easy. CLD is also known to be toxic to microorganisms (e.g. Orndorff and Colwell 1980b) and was even patented as an antimicrobial agent against Gram-positive bacteria and dermatophytic fungi in 1969 (US patent 3,448,194).

In this chapter through a thermodynamic approach, we will demonstrate that there is no energetic reason why the structure of CLD should not be amenable to microbial degradation and we will propose some possible reasons for the apparent absence of CLD degradation in FWI and what could be done to reverse the situation.

Gibbs free energy of potential reactions of CLD transformations and reduction potential of CLD/CLD-$Cl_{10-n}H_n$ couples

Through the action of microorganisms in the environment CLD could - hypothetically - be fully mineralized or just partially transformed (Fig. 14.1). The ultimate degradation could proceed under different redox conditions (aerobiosis, iron reduction, denitrification, sulfate reduction, methanogenesis, etc) depending on the main electron acceptors (O_2, Fe^{3+}, NO_3^-, SO_4^{2-}, CO_2, others) present in the environmental compartments (soil, surface water, groundwater, freshwater or marine sediments) impacted by CLD. The CLD partial transformations that can be contemplated correspond (1) to the oxidation or reduction of its ketone group to form the corresponding lactone or alcohol, (2) its sequential dechlorination (removal of 1, 2, 3, 4,…10 chlorine atoms) that can generate up to 484[2] different partially dechlorinated intermediate products until the fully dechlorinated congener (Dolfing et al. 2012) or (3) its transformation by fermentation (Fig 14.1).

Among the different thermodynamic functions, the change in Gibbs free energy or ΔG associated with a chemical reaction is the one that allows predicting its direction. A negative value indicates that the reaction is exergonic and should occur spontaneously while a positive value indicates that it is endergonic and cannot proceed spontaneously under given conditions. It must be remembered however that even when a reaction is exergonic this does not necessarily imply that it will occur at an observable rate. The way to calculate the ΔG of the reactions is described in most biochemical or thermochemistry textbooks. The reader may also refer to Dolfing 2003 and Dolfing et al. 2012. The calculation of the $\Delta G°'$ for all the hypothetic reactions of CLD microbial transformation requires the knowledge of the $\Delta G_f°$ (Gibbs free energy of formation) of CLD and related CLD derivatives (e.g. dechlorination products) in their aqueous state[3]. Since these values were not available in the literature they had to be estimated by *ab initio quantum* calculations using the G3(MP2)/B3LYP method implemented in Gaussian 03 software.

[1] http://sitem.herts.ac.uk/aeru/iupac/Reports/1293.htm, Kow : octanol water partition coefficient

[2] Including 92 mesomeric compounds and 196 pairs of enantiomers

[3] (°) superscript indicates that ΔG calculations are done under standard conditions which means that the concentration of all aqueous reactants and products is 1 M or a partial pressure of 1 atm for gaseous compounds. (°') superscript means that ΔG calculations are done under standard conditions except for pH equal to 7. In both cases the temperature is equal to 298.15 K.

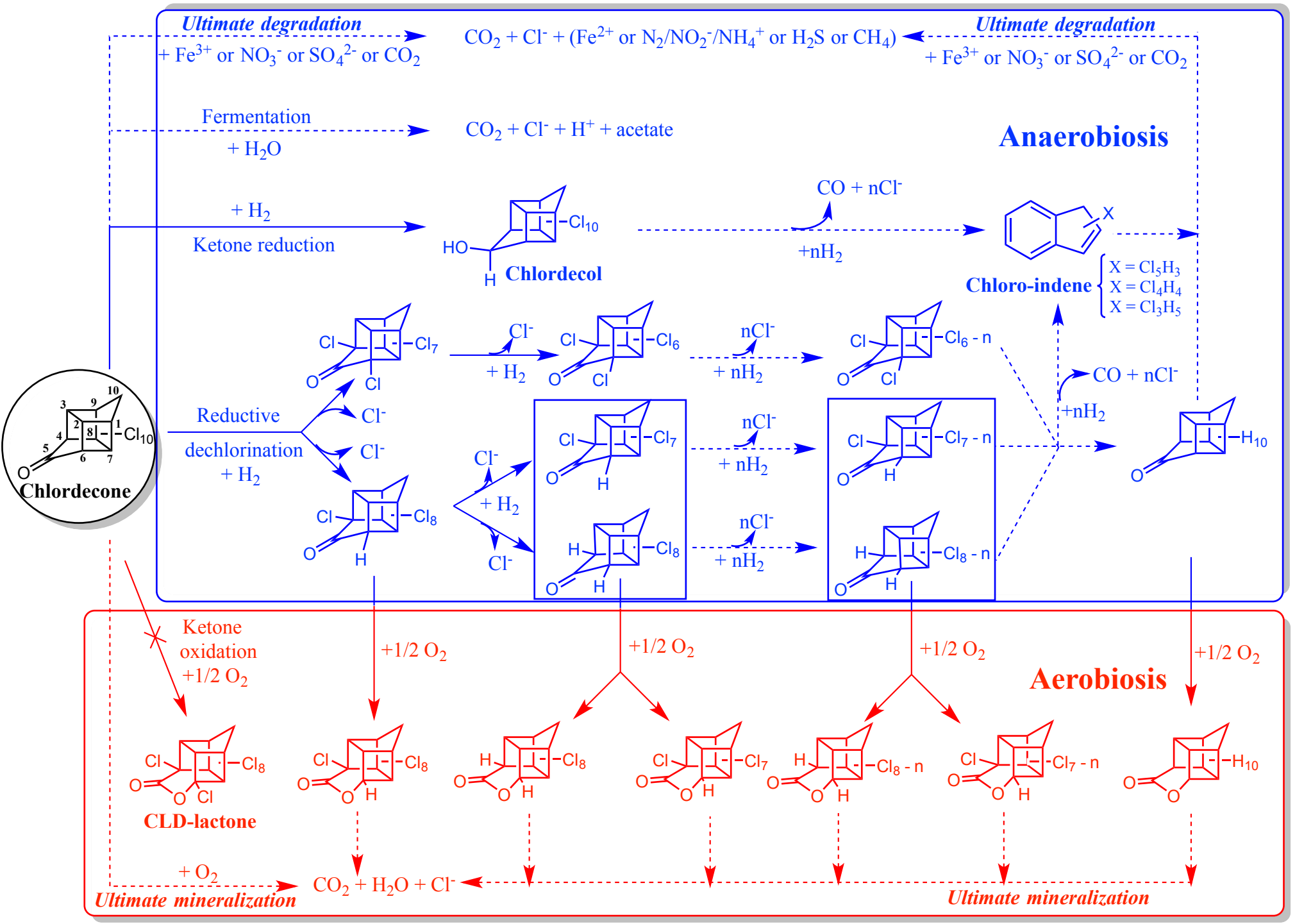


Figure 14.1 Different hypothetical and/or experimental pathways of CLD transformation.

The tabulated $\Delta G_f°$ have been reported previously (Dolfing et al. 2012).

The $\Delta G°'$ values calculated from these $\Delta G_f°$ for the hypothetic reactions mentioned above are presented in Table 14.1. They show that all these reactions are extremely favorable ($\Delta G°'$ very negative) and that even the dechlorination of a single chlorine atom or the oxidation or reduction of the CLD ketone function should liberate enough energy to allow the synthesis of ATP and therefore microbial growth.

The standard conditions used for $\Delta G°'$ calculations being far away from those found in the environment (reactants and products are generally not present at a concentration of 1 M or 1 atm!) it was necessary to evaluate whether the reactions would remain favorable under more realistic conditions. This was possible for some of the reactions and environmental compartments (groundwater, soil water solution) for which the requested *in situ* concentrations were available. The resulting *in situ* ΔG showed that the reactions that could be tested (CLD aerobic mineralization and removal of 1 Cl) are as favorable or even more favorable than under standard conditions (Table 14.1), a situation that should be similar for the other reactions (Dolfing et al. 2012).

$\Delta G°'$ calculations show also that CLD dechlorination is thermodynamically more favorable than the formation of chlordecol resulting from the reduction of the CLD ketone group. This suggests that in natural environments where the reducing equivalents (e.g. H_2) required by the two reactions are present in limited amounts, both reactions will compete for them and that the dechlorination should proceed preferentially to chlordecol formation. When the reducing equivalents are not limiting, it is not impossible however that some microorganisms may be able to dechlorinate CLD and simultaneously reduce its ketone group in order to maximize energy recovery. A last option that must be considered is that in some ecosystems, the microorganisms present may have only the metabolic capacity to reduce CLD into chlordecol.

CLD dechlorination being a series of oxidation-reduction reactions, it is interesting to know the reduction potential ($E°'$) corresponding to the redox couples formed by CLD and its dechlorinated products that can be schematized as follows: $C_{10}Cl_nH_{(10-n)}O$ / $C_{10}Cl_{(n-1)}H_{(11-n)}O$ with n = 1 to 10 (e.g. $C_{10}Cl_{10}O/C_{10}Cl_9HO$; $C_{10}Cl_9HO/C_{10}Cl_8H_2O$….$C_{10}ClH_9O/C_{10}H_{10}O$). The $E°'$ of the resulting couples can be calculated from the $\Delta G°'$ of the dechlorination reaction with H^+/H_2 couple as electron donor (i.e. $C_{10}Cl_nH_{(10-n)}O + H_2 \rightarrow C_{10}Cl_{(n-1)}H_{(11-n)}O + H^+ + Cl^-$) using the Nernst Equation ($\Delta G°' = - n F \Delta E°'$ see Dolfing, 2003)[4]. Starting in 1984 with the isolation of *Desulfomonile tiedjei*, the first dechlorinating bacterium, a new group of microorganisms with the capacity to "respire" halogenated organic compounds, i.e. to use them as terminal electron acceptors through their dehalogenation coupled to energy conservation, was discovered (Mohn and Tiedje 1992). Nowadays, a large range of alkyl (perchlorethylene, tetrachloromethane, hexachloroethane, etc) and aryl (chlorobenzoates, chlorophenols, chlorobenzenes, dioxins, PCB, etc) halogenated compounds are known to be "respired" by a wide diversity of bacteria (e.g. Maphosa et al. 2010). As can be seen in Fig. 14.2, the reduction potentials of CLD and of its partially dechlorinated intermediates indicate that they correspond to electron acceptors comparable to the other organochlorine compounds and as strong as NO_3^-. Indeed, the $E°'$ of the CLD/monohydroCLD, monohydroCLD/dihydroCLD and CLD/decahydroCLD couples range from +322 to +442 mV against +363 mV for the NO_3^-/NH_4^+ and +431 mV for the NO_3^-/NO_2^-

[4] In the Nernst equation $\Delta E°' = E°'$(electron acceptor couple) – $E°'$(electron donor couple) = $E°'$ [$C_{10}Cl_nH_{(10-n)}O$ / $C_{10}Cl_{(n-1)}H_{(11-n)}O$] – $E°'$ (H^+/H_2); n = number of electrons transferred in the reaction = 2; F = Faraday constant = 0.096485 kJ/mV; $E°'(H^+/H_2)$ = redox potential of H^+/H_2 couple = -414 mV (Thauer et al. 1977). After rearrangement this gives that $E°'$[$C_{10}Cl_nH_{(10-n)}O$ / $C_{10}Cl_{(n-1)}H_{(11-n)}O$] in mV = ($-\Delta G°'$ / n F) + $E°'(H^+/H_2)$ = ($-\Delta G°'/0.193$) – 414

Table 14.1 Gibbs free energy of potential reactions of transformation of CLD and some of its degradation products under standard (pH 7) and *in situ* conditions compared to that of ATP synthesis

Reaction	$\Delta G°'$	ΔG *in situ*
Mineralization or ultimate degradation	kJ/mol CLD	
Aerobic conditions		
$C_{10}Cl_{10}O + 15\ H_2O + 7\ O_2 \rightarrow 10\ HCO_3^- + 20\ H^+ + 10\ Cl^-$	-4443	-5344[gw]
Iron(III) reducing conditions		
$C_{10}Cl_{10}O + 29\ H_2O + 28\ Fe^{3+} \rightarrow 10\ HCO_3^- + 48\ H^+ + 10\ Cl^- + 28\ Fe^{2+}$	-4204	-
Nitrate reducing conditions 1		
$5\ C_{10}Cl_{10}O + 61\ H_2O + 28\ NO_3^- \rightarrow 50\ HCO_3^- + 72\ H^+ + 50\ Cl^- + 14\ N_2$	-4146	-
Nitrate reducing conditions 2		
$C_{10}Cl_{10}O + 15\ H_2O + 14\ NO_3^- \rightarrow 10\ HCO_3^- + 20\ H^+ + 10\ Cl^- + 14\ NO_2^-$	-3291	-
Sulfate reducing conditions		
$2\ C_{10}Cl_{10}O + 7\ SO_4^{2-} + 30\ H_2O \rightarrow 20\ HCO_3^- + 33\ H^+ + 20\ Cl^- + 7\ HS^-$	-1541	-
Methanogenic conditions		
$2\ C_{10}Cl_{10}O + 37\ H_2O \rightarrow 13\ HCO_3^- + 33\ H^+ + 20\ Cl^- + 7\ CH_4$	-1483	-
Dechlorination		
$CLD + H_2 \rightarrow monohydroCLD + H^+ + Cl^-$	[-160 ; -155]	[-160 ; -138][gw] [-164 ; -142][sw]
$monohydroCLD + H_2 \rightarrow dihydroCLD + H^+ + Cl^-$	[-165 ; -142]	
$CLD + 10\ H_2 \rightarrow decahydroCLD + 10\ H^+ + 10\ Cl^-$ **	-1448 (-145)	
Reduction of CLD ketone group		
$CLD + H_2 \rightarrow$ chlordecol	-70	-
Oxidation of CLD ketone group		
$CLD + ½\ O_2 \rightarrow$ CLD-lactone	-148	-
Fermentation		
$2\ C_{10}Cl_{10}O + 30\ H_2O \rightarrow 6\ HCO_3^- + 33\ H^+ + 20\ Cl^- + 7\ CH_3COO^-$	-1375	-
ATP synthesis from ADP and AMP	kJ/reaction	
$ADP + PO_4^{3-} + H^+ \rightarrow ATP + H_2O$	+30.5	~ +70***
$AMP + 2\ PO_4^{3-} + 2\ H^+ \rightarrow ATP + 2\ H_2O$	+61	-

"gw" and "sw" superscripts stand for "groundwater" and "soil water" respectively. Concentrations and temperature used for the groundwater in situ ΔG calculations correspond to average values found in Martinique aquifers (Gourcy et al. 2009; Gourcy personal communication): 4.97 µg CLD/L, 0.22 µg (8-monohydroCLD)/L, 44.6 mg Cl^-/L, 102 mg HCO_3^-/L, 2.7 mg O_2/L, pH 6.8, 28°C. Concentrations used for the soil water in situ ΔG calculation correspond to the average values measured in the leaching water of a lysimeter implemented in an andosol of Guadeloupe containing 5.4 mg CLD/kg soil dry weight and considered as representative of the soil solution: 2.2 µg CLD/L, 43 mg Cl^-/L, pH 7.2 (Cabidoche personal communication). The concentration of monohydroCLD was estimated from the average 8-monohydroCLD/CLD mass ratio value found in the FWI dry soils (19.7‰, Devault et al. 2016) that also have an average temperature of 25°C within the first 30 cm (Christophe Mouvet, BRGM, personal communication). The $\Delta G_f°$ used for the calculations for all the compounds were taken from Thauer et al. 1979 and Stumm and Morgan 1996 except CLD and its derivatives that were taken from Dolfing et al. 2012. The H_2 concentration used for the calculation of the groundwater and soil in situ dechlorination ΔG covers the range of concentrations (0.01 to 70 nM equivalent to a partial pressure of 0.001 to 10 Pa) usually found at steady state in natural environments poised by O_2, Fe^{3+}, Mn^{4+}, NO_3^-, SO_4^{2-} and CO_2 according to Lovley and Goodwin (1988), Conrad (1996) and Heimann et al. (2009). **Value within brackets correspond to kJ/mol Cl removed.*Amount of energy required in vivo for the synthesis of 1 mol ATP taking into account that the efficiency of energy conservation is not 100% (see Schink, 1997).*

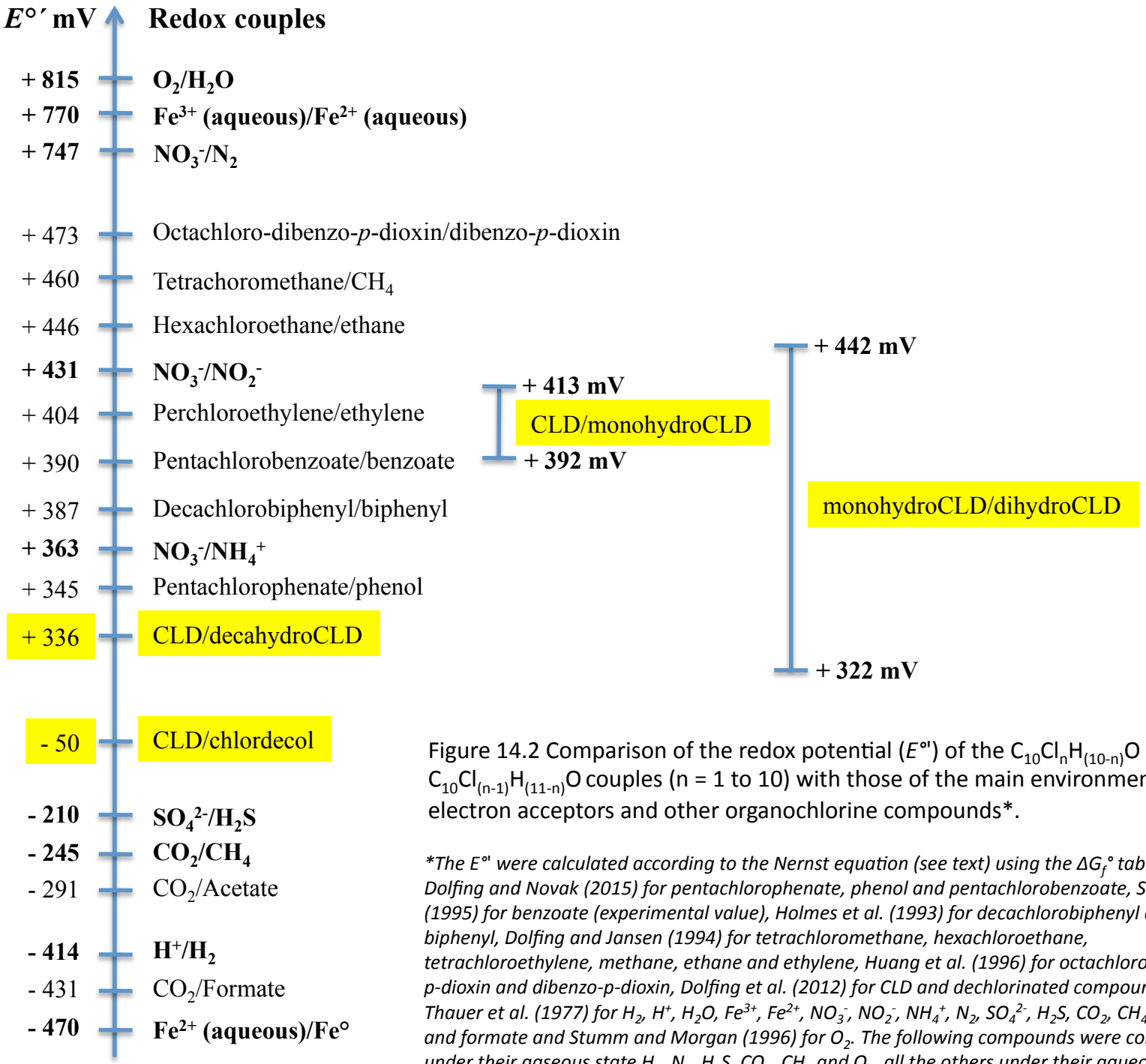


Figure 14.2 Comparison of the redox potential ($E°'$) of the $C_{10}Cl_nH_{(10-n)}O$ / $C_{10}Cl_{(n-1)}H_{(11-n)}O$ couples (n = 1 to 10) with those of the main environmental electron acceptors and other organochlorine compounds*.

**The E°' were calculated according to the Nernst equation (see text) using the $\Delta G_f°$ tabulated by Dolfing and Novak (2015) for pentachlorophenate, phenol and pentachlorobenzoate, Shock (1995) for benzoate (experimental value), Holmes et al. (1993) for decachlorobiphenyl and biphenyl, Dolfing and Jansen (1994) for tetrachloromethane, hexachloroethane, tetrachloroethylene, methane, ethane and ethylene, Huang et al. (1996) for octachloro-dibenzo-p-dioxin and dibenzo-p-dioxin, Dolfing et al. (2012) for CLD and dechlorinated compound, Thauer et al. (1977) for H_2, H^+, H_2O, Fe^{3+}, Fe^{2+}, NO_3^-, NO_2^-, NH_4^+, N_2, SO_4^{2-}, H_2S, CO_2, CH_4, acetate and formate and Stumm and Morgan (1996) for O_2. The following compounds were considered under their gaseous state H_2, N_2, H_2S, CO_2, CH_4 and O_2, all the others under their aqueous state.*

couples. The amount of energy that can be recovered per chlorine atom removed in the case of CLD ($\Delta G°'_{reaction}$ = -142 to -165 kJ/mole Cl, Table 14.1) is also well within the range of energy that can be recovered for the dechlorination of the above mentioned organochlorine compounds ($\Delta G°'_{reaction}$ = -130 to -180 kJ/mole Cl, Dolfing 2003). All this shows that in spite of its peculiar cage structure, CLD is thermodynamically very similar to other organochlorine compounds and should behave like them.

Experimental confirmation of CLD susceptibility to microbial transformations

The previous considerations show that there are apparently no thermodynamic impediments to several chemical transformations of CLD that could be mediated by microorganisms. Three of the papers published after the poisoning of the workers of the Life Science CLD production site in the city of Hopewell, Virginia, USA, in 1975 and the associated pollution of the James River clearly confirmed that CLD is at least susceptible to dechlorination under the action of microorganisms or some of their coenzymes. For instance, Orndorff and Colwell (1980a) demonstrated that a pure culture of a *Pseudomonas aeruginosa* strain isolated from the water of a lagoon used for the storage of a CLD contaminated sewage sludge at Hopewell was able to convert 30% of the initial CLD (5 mg/l) into a mixture of 8-monohydroCLD[5] and 2,8-dihydroCLD after one week of incubation in the dark under aerobic conditions at 25°C and in the presence of peptone, acetone (solvent for CLD stock solution) and yeast extract. This bacterial strain was however unable to use CLD as sole carbon and energy source or to modify further its carbon skeleton. In fact its action on CLD was apparently limited to the removal of 2 chlorine atoms under conditions of cometabolism. Two years before the work of Orndorff and Colwell (1980a), Schrauzer and Katz (1978) had demonstrated *in vitro* that CLD was highly susceptible to dechlorination in presence of vitamin B_{12s} under reducing conditions. Vitamin B_{12s} is a cobalt transition metal coenzyme very common among prokaryotes and known since a long time to be involved in non specific fortuitous reductive dechlorination of chlorine atoms bound to alkyl (aliphatic compounds) or aryl (aromatic compounds) carbons (Mohn and Tiedje 1992). Depending on the experimental conditions, vitamin B_{12s} revealed the capacity to remove up to 4 chlorine atoms from the CLD bishomocubane "cage" but more interestingly to induce the opening of the "cage" resulting in the formation of indene compounds of formula $C_9Cl_{8-n}H_n$ (with n = 3-5, see Fig. 14.1 for structure). The last article in the aforesaid series of 3 papers showed *in vivo* that a culture of the methanogenic *Archaea*, *Methanosarcina thermophila*, could convert 86% of the initial CLD into products which gave on thin layer chromatography (TLC) a profile identical to the one obtained with vitamin B_{12s} and that two transition metal complexes of this *Archaea* containing Co and Ni respectively were involved in the process since they gave on TLC the same profile of CLD transformation products as the whole cells (Jablonski et al. 1996). The previous old reports are complemented by the recent work of Belghit et al. (2015) who showed that CLD could easily loose up to 5 chlorine atoms when put into contact with a micrometric elemental iron powder in an aqueous phase at room temperature in the dark. Although this reaction was not biologically mediated, it confirms that CLD is not chemically inert but susceptible to chemical transformations under mild conditions compatible with biochemical reactions. Therefore it is not surprising that again recently Devault et al. (2016) could demonstrate indirectly that the 8-monohydroCLD detected in the soils of Martinique and

[5] carbon numbering according to IUPAC nomenclature throughout the chapter – see Fig. 14.1 & Dolfing et al. 2012

which had been considered for a long time to be an impurity formed during the synthesis of CLD and brought into the soils as an accompanying product during the spread of the CLD pesticide commercial formulations, was in fact a dechlorination product of CLD although the biotic or abiotic nature of the dechlorination process in the soils could not be established.

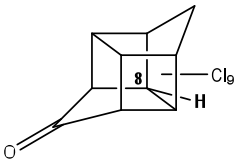
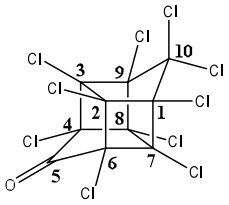
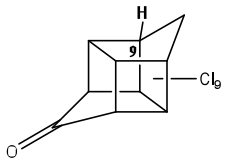
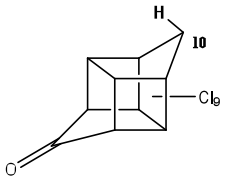
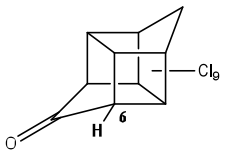
The formation of chlordecol and chlordecol dechlorinated products were also recorded by Orndorff and Colwell (1980a) and Schrauzer and Katz (1978). Since the reactions leading to the formation of the CLD alcohol products described by Schrauzer and Katz (1978) were performed in the presence of methanol, it cannot be totally excluded that these products may correspond in this case to analytical artefacts formed in the injector of the gas chromatograph used for the analysis as this was observed by Soine et al. (1983). The biological formation of chlordecol from CLD has however been unambiguously demonstrated in the liver of humans and some other mammals (gerbil, pig, rabbit) where it is catalyzed by a specific aldo-keto reductase (Molowa et al. 1986).

Limits of the thermodynamic approach

In principle thermodynamics should also help to rationalize degradation pathways. In the case of dechlorination for instance it has been often observed that the dechlorination products that are formed result from the reactions that liberate the most energy (e.g. Dolfing 2003). For CLD, this would imply that among the 4 possible monohydroCLD isomers the formation of 8-monohydroCLD should be favored over the others and that in turn this compound should be preferentially dechlorinated into 4,8-dihydroCLD (Table 14.2).

While 8-monohydroCLD was actually found as the sole monohydroCLD formed by CLD photolysis (Alley et al. 1974) or aerobic microbial attack by *P. aeruginosa* (Orndorff and Colwell 1980a), it was only a minor product in the reactions performed with vitamin B_{12s} and Fe° where it was accompanied by another major monohydroCLD isomer, 10-monohydroCLD in the case of vitamin B_{12s} (Katz 1978) and 9 or 10-monohydroCLD (absolute identification impossible with the analytical tools used) in the case of Fe° (Belghit et al. 2015). Incongruencies with the thermodynamic predictions, are also evident at the level of the dihydroCLD isomers formed by photolysis (2,8-dihydroCLD; Wilson and Zehr 1979) or by vitamin B_{12s} (*cis*-8,10-dihydroCLD; Katz 1978; Schrauzer and Katz 1978) which were not the first to be expected to be formed from 8- or 10-monohydroCLD (see Table 14.2). In the cases discussed above the observed inconsistencies may result from the fact that the differences between the $\Delta G°'$ of the reactions of formation of the different dechlorination isomers are most often between 0.4 to 7.3 kJ/reaction which is below the accuracy (within 4-8 kJ/mol) that can be achieved in the estimation of the $\Delta G_f°$ values (Dolfing et al. 2012), which causes considerable incertitude in their use to discriminate among pathways. The discrepancy may also come from the fact that all calculations were done using $\Delta G_f°$ of the carbonyl forms of CLD and of its dechlorinated products while all these compounds are known to convert easily into diols in presence of water and in polar solvents such as acetone and acetonitrile (Wilson and Zehr 1979) which could be therefore their main form in some of the above mentioned experiments. The diols could be even deprotonated in some of the experiments with vitamin B_{12s} performed at pH 9.6 (Schrauzer and Katz 1978). In any case, it is important to keep in mind that a thermodynamic approach based on the energy of the initial and final products does not take into account the kinetics of the reactions and the activation energy (barrier) that has to be overcome for a reaction to proceed. For identical initial and final energetic states (i.e. same $\Delta G_{reaction}$) it is indeed the reaction with the lower activation energy that will be favored over the others. The calculation of the activation

Table 14.2 $\Delta G°'$ of the different reactions of CLD dechlorination up to the removal of 2 chlorine atoms (compounds in red correspond to those whose formation is thermodynamically most favorable; compounds in yellow were detected after CLD photolysis; compounds in green including 8-monohydroCLD were detected after reaction with B_{12S})[a]

Original compound	monohydroCLD	$\Delta G°'$ kJ/mol	dihydroCLD	$\Delta G°'$ kJ/mol
Chlordecone	**8-monohydroCLD***	**-159.7**	**4,8-dihydroCLD***	**-161.1**
			6,8-dihydroCLD*	-160.4
			2,8-dihydroCLD*	-159.5
			8,9-dihydroCLD*	-158.6
			cis-8,10-dihydroCLD*	-157.6
			trans-8,10-dihydroCLD*	-156.8
			1,8-dihydroCLD*	-155.3
			7,8-dihydroCLD	-153.7
			3,8-dihydroCLD	-142.1
	9-monohydroCLD	-159.3	**6,9-dihydroCLD**	**-160.2**
			4,9-dihydroCLD	-159.3
			8,9-dihydroCLD*	-159.0
			9,10-dihydroCLD*	-156.3
			7,9-dihydroCLD*	-155.7
			1,9-dihydroCLD	-155.4
	10-monohydroCLD	-158.4	**6,10-dihydroCLD***	**-161.1**
			cis-8,10-dihydroCLD*	-158.9
			*trans*-8,10-dihydroCLD*	-158.1
			9,10-dihydroCLD*	-157.2
			10,10-dihydroCLD	-150.1
	6-monohydroCLD	-155.5	**6,7-dihydroCLD***	**-165.3**
			6,8-dihydroCLD*	-164.6
			6,10-dihydroCLD*	-164.0
			6,9-dihydroCLD	-164.0
			4,6-dihydroCLD	-163.9
			1,6-dihydroCLD	-163.1

[a] *$\Delta G°'$ values were calculated according to the reactions $C_{10}Cl_nH_{(10-n)}O + H_2 => C_{10}Cl_{(n-1)}H_{(11-n)}O + Cl^- + H^+$. Compounds with an asterisk have an enantiomer. Carbon numbering have been selected to show direct affiliation to parent compounds and do not follow necessarily the standard IUPAC numbering rule which gives the highest number to the carbon bearing a hydrogen*

energy is theoretically possible for each isomers but it would require knowing the mechanism involved in the reactions, which is presently not the case for CLD and its derivatives.

The limits of the thermodynamic approach appear also when considering the formation of the CLD-lactone that could be the first intermediate to be formed by direct reaction of O_2 with the keto group of CLD during a potential aerobic degradation of the insecticide and which is thermodynamically favorable according to our calculations (Fig. 14.1; Table 14.1). *In vivo*, the oxidation of carbonyl groups to lactones are catalyzed by Flavin-dependent-Bayer-Villiger monoxygenases that are active even when the carbonyl group belongs to polycyclic structures (e.g. adamantanone) which are energetically as constrained as CLD (Selifonov 1992). Since 1976, moreover, Metha et al. have shown that a lactone could be formed from 1,4-bishomocubanone, i.e. the fully dechlorinated CLD, by reaction with the strong oxidant ceric ion. To our knowledge, such an oxidation however, does not seem to occur when the carbons adjacent to the carbonyl group bear a chlorine atom. Indeed, the migrating ability of the alkyl group to form the lactone is decreased if an electron-withdrawing substituent such as Cl is placed to the adjacent alkyl group (Grein et al. 2006). This suggests that lactone formation from CLD will probably be possible only after the removal of at least one of the Cl atoms carried by the carbons 4 and 6 (Fig. 14.1).

Possible reasons for the apparent absence of CLD natural attenuation in FWI environments

Both the thermodynamic approach used here as well as the old and recent experimental results discussed in the previous sections clearly show that the chemical structure of CLD should not be refractory to a microbial attack and so should not be the reason *per se* why its degradation has not been observed so far - apparently at least - in the FWI environments. Other reasons that may be advocated to explain this are:

1. The absence of autochthonous microorganisms in FWI environments with the capacity to attack CLD, although such organisms may exist somewhere else in the world and this in spite the fact that 4 decades elapsed since CLD was used for the first time in FWI, which would have allowed for a long enrichment process.
2. Inadequate environmental conditions to permit the expression of the catabolic capacities of the CLD-degrading autochthonous microorganisms that could be present.
3. Trapping of CLD within the clay-organic complex of FWI soils making it inaccessible to the potential degrading autochthonous microorganisms or to their enzymes, a process that could become more prominent with soil aging, making CLD less and less bio-available with time and which is well known to reduce in soils the rate and extent of biodegradation of organic compounds even one as readily biodegradable as citrate (Chenu and Stotzky 2002).

The extremely high propensity of CLD to partition form water to organic matter and so the importance of the third point is well exemplified by the experiments of Cimetiere et al. (2014) who showed that in less than 5 min, 85% of CLD at 1 mg/l in water disappeared from solution after the addition of 0.5 g/l of inactivated sewage sludge. High experimental Koc[6] values (580 to

[6] compound soil water partition coefficient normalized to the soil organic content

16522 ml/g) reported in the literature for CLD on clay materials (Iyengar et al. 1983) and FWI andosol and nitisol (Fernandez-Bayo et al. 2013b) further support such a possibility.

The recent finding by Devault et al. (2016) that 8-monohydroCLD was apparently naturally formed in FWI soils suggests however that the second point certainly warrants further scrutiny. Although thermodynamics indicates that in principle CLD should be mineralizable under a wide range of redox conditions, it is now well established that polyhalogenated compounds will be much more readily attacked anaerobically rather than aerobically while the contrary is observed for less halogenated compounds (e.g. Vogel et al. 1987; Field et al. 1995; Holliger et al. 2003; Löffler et al. 2003). This is probably due to the oxidized nature of the chloro substituents which hinder their electrophilic attack by oxygenases (Löffler et al. 2003). Under anaerobic conditions, the main mechanism involved in organochlorine attack corresponds to reductive dechlorination, which results in the replacement of a chlorine atom by hydrogen. As mentioned previously, in this scheme, organochlorine compounds play the role of electron acceptors instead of electron donors. This means also that their dechlorination will require the presence of electron donors, usually molecular hydrogen, generated during fermentative processes of readily biodegradable organic compounds performed by non-dehalogenating microorganisms. According to the redox scale presented in Figure 14.2, O_2 ($E°'$ O_2/H_2O = +815 mV) is a so strong electron acceptor that under aerobiosis, the reducing equivalents will be used so efficiently for O_2 reduction by aerobes that they will be unavailable for a reductive dechlorination process.

In FWI volcanic islands, banana crop and its associated CLD spreading was performed on 3 main types of volcanic soils, namely, andosol, nitisol and ferralsol all known to be particularly well draining (Dorel et al. 2000; Vidal-Torrado and Cooper 2008) and therefore to be well aerated. This characteristic is appropriate for banana trees whose roots do not develop in poorly aerated conditions (Aguilar et al. 2003) but will necessarily prevent the occurrence of CLD reductive dechlorination according to the previous comments. Such a restriction should be even stronger in the case of andosols whose organic matter is known to be extremely poorly biodegradable (e.g. Chevallier et al. 2010) and so probably unable to play efficiently the role of electron donor required by a reductive dechlorination process. Therefore, it is not surprising that a Guadeloupean andosol incubated up to 7 months under aerobic conditions showed an extremely low capacity to mineralize or even just to partially transform freshly spiked ^{14}C-CLD (Fernandez-Bayo et al. 2013a).

A similar situation is expected to occur in the ground waters of Martinique that have been reported to contain in average 2.7±0.9 mg dissolved O_2/l which is indicative of oxic conditions and an amount of organic carbon (1.4±0.6 mg/l) probably too low to be an adequate source of electron donors for the removal of chloro substituents (values from Gourcy et al. 2009; Gourcy personal communication).

Adequate conditions for CLD reductive dechlorination may exist however in FWI ecosystems dominated by electron acceptors weaker than CLD like sulfates ($E°'$ SO_4^{2-}/H_2S = -210 mV) or CO_2 ($E°'$ CO_2/CH_4 = -245 mV; methanogenic conditions). Such ecosystems correspond to the sediments of coastal marshes, mangroves, lake and seashore bottoms that can be the receptacle of CLD from banana plantations and where the flooding regime favors the development of anaerobic conditions as confirmed by very negative measured redox potentials (e.g. $E_{Ag/AgCl}$ between -50 and – 350 mV at 0.2 and 0.8 m depth and pH 6.2 to 7.4 in Guadeloupean swamps and mangroves, Imbert and Delbé 2006). It is well known also that anoxic microniches may form in the interior of soil aggregates allowing the functioning of

anaerobic processes in otherwise macroscopically oxic soils (e.g. Sexstone et al. 1985). N_2O emissions from andosols and nitisols under forest cover or used for the cultivation of bananas in Costa Rica and Panama, with climatic conditions similar to FWI, and that were clearly mediated by denitrification (an anaerobic process) provide circumstantial evidence that such anoxic microniches may occur in FWI soils in absence of flooding (Veldkamp and Keller 1997; Corre et al. 2014)[7]. Therefore, anoxic microniches could well be at the origin of the increased 8-monohydroCLD/CLD ratios observed in the soils from Martinique compared to the ratios found in the CLD commercial formulations spread on these soils (Devault et al. 2016).

Concluding remarks and perspectives

The information discussed in this chapter shows that in principle there should be no thermodynamic impediment to a wide range of biologically mediated chemical transformations of CLD and that Nature has already evolved some of the essential building blocks (e.g. corrinoids, other transition metal complexes) that are necessary for microorganisms to perform at least its dechlorination. In order to achieve an extended biological transformation of CLD structure 4 initial conditions seems however to be required:

1. anaerobic conditions
2. presence of an electron donor and of an additional carbon source (they can be the same)
3. presence of microorganisms with CLD dehalogenating activity
4. bioavailability of CLD

Due to the aforesaid characteristics of the FWI soils impacted by CLD, direct human action will be at least necessary to fulfill the first two conditions.

In order to obtain anoxic conditions two strategies may be followed. The first one consists of reducing the oxygen diffusion from the atmosphere to the soil. This should be achievable by soil compaction in order to reduce the soil porosity at the surface and so to decrease physically the capacity of atmospheric gases to freely move into the soil structure. Oxygen transfer from the atmosphere to the soil could be further reduced by increasing the soil humidity through watering - if possible at least until field capacity - in order to fill the soil pores with water and take advantage of the low solubility of oxygen into water. The second strategy, which may be combined with the first one, consists of increasing the oxygen consumption by soil microorganisms above the soil aeration capacity. This can be achieved through the addition of easily biodegradable organic matter that could be chosen among organic residues available on the islands and whose characteristics are compatible with the soil needs[8]. Particularly, the selected organic matter should allow maintaining an adequate equilibrium between the main nutrients C ad N in order to avoid any microbial disequilibrium that would prevent a possible CLD biodegradation. The organic matter addition will also provide the electron donors required

[7] In the work by Corre et al. (2014) denitrification was proven by monitoring of $^{15}N_2O$ emission upon addition of $^{15}NO_3^-$ or $^{15}NH_4^+$ to the soil. In the work by Veldkamp and Keller (1997), denitrification was likely because N_2O peaks occurred at WFPS (Water Filled Pore Space) ≥ 70%, a value widely accepted as the threshold between nitrification/denitrification as the dominant source of N_2O. Note: andosol and nitisol appear as andisol and inceptisol respectively in the USDA soil taxonomy used by these last authors.

[8] e.g. sugarcane vinasse for a quick effect combined with biosolids from wastewater treatment plants to maintain anoxic conditions for a longer period but not sugarcane bagasse that is too bulky and would promote soil aeration or compost that is a stabilized organic matter and so necessarily a poor electron donor

for a dechlorination process. Data from the literature and practical experience confirm that both approaches can indeed result in the formation of anoxic conditions sometimes clearly evidenced by a substantial decrease of the soil redox potential (e.g. Dorel 1993; Stepniewski et al. 1994; de Cockborne et al. 1999). In terms of CLD bioavailability, a test of the ISCR (In Situ Chemical Reduction) process performed by BRGM (French geological survey office) consisting of adding a mixture of micrometric Fe° and plant organic matter (crushed dried alfalfa) to the soil to promote dechlorination has shown at the lab scale that 90 to 95% of the CLD present in historically polluted FWI ferralsols and nitisols could be removed by such a process (Dictor et al. 2011). This indicates that despite of its high Koc and so its tight binding to the soil matrix, most of the CLD of these 2 types of soils was accessible to the added chemical agents, which should be also the case for biological agents. The efficiency of the ISCR process was much less when applied to andosols (44% removal; Dictor et al. 2011) revealing a lower bioavailability of CLD in this type of soil compared to the others. The CLD removed by the ISCR process could correspond however to the CLD fraction that is mobile in such a soil and whose removal could be sufficient to avoid the contamination of crops and percolating waters but probably not of the livestocks since the digestive tracts of birds and mammals seem to be very efficient in extracting CLD from soil particles independently of the soil type (see chapters 3.3 and 3.4).

Although anaerobic conditions are nowadays widely recognized as almost indispensable for achieving the primary attack on polychlorinated compounds, it is also recognized that their full mineralization will usually not occur under such conditions and that their degradation will result in the accumulation of less chlorinated compounds (e.g. Mohn and Tiedje 1992; Field et al., 1995) that could be more toxic than the parent compound as observed in some cases (e.g. vinyl chloride resulting from tetra- and tri-chlorethylene dechlorination; Rosner et al. 1997). Such a risk does not appear however to be significant in the case of CLD. Indeed, the only two comparative toxicity studies that exist in the literature and that were performed with the few CLD congeners existing as pure compounds have shown that CLD alcohol toxicity ≥ CLD > 8-monohydroCLD >>> 2,8-dihydroCLD for rat liver mitochondria (Soileau and Moreland 1983) and mysid shrimps (Carver and Griffith 1979). This suggests that the toxicity of CLD congeners for biological targets decreases with decreasing degree of chlorination. Another good news is that usually the partially dechlorinated compounds formed under anaerobic conditions have been observed to be more accessible to aerobic microorganisms than the original parent compounds and to degrade with fast kinetics aerobically (Guiot et al., 1994; Field et al., 1995). As suggested by Orndorff and Colwell back in 1980a, a succession of anaerobic and aerobic conditions could thus be optimal to achieve the ultimate mineralization of CLD as this has been observed for other polychlorinated pesticides such as DDT (Beunink and Rehm 1988), methoxychlor (Fogel et al. 1982) or 2,3,6-trichlorobenzoic acid (Gerritse and Gottschal 1992). The establishment of aerobic conditions after an anaerobic phase could be obtained by a tilling of the soil. This would also allow de-compacting the soil when compaction was used to create anoxic conditions since this practice could otherwise negatively impact future crops due to possible root anoxia and difficulty for the roots to colonize a compacted environment (e.g. Dorel 1993). The present management of banana plantations in FWI consists to destroy every 4 to 5 years the banana plants by direct injection of glyphosate at the base of the pseudo-stem followed by a one-year fallow and subsequent plantation of pest-free banana plants produced by tissue culture (Chabrier and Quénéhervé 2003). Such a management results in a more rational harvest since all banana plants will be productive at the same time and allows a good control of nematodes and also indirectly of the black weevil populations that could otherwise cause a significant banana yield

reduction. In terms of soil remediation the existence of the one year fallow period for those polluted lands that are still used for banana production is excellent since it would allow sufficient time to perform all the operations that could be necessary (e.g. soil compaction, watering, amendment with labile organic matter and source of microorganisms, re-aeration by tilling) to achieve CLD destruction without the need to stop the agronomic use of otherwise productive parcels with the associated economic loss. Hopefully, the length of the fallow would be long enough also to allow the soil to rest for sufficient time after the remediation activities in order to be ready for the next five years banana crop cycle.

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Dedication

This chapter is dedicated to the memory of Dr. Gerhard N. Schrauzer who passed away in September 2014. His pioneering work on chlordecone degradation by vitamin B_{12S} remains an inspiration for those currently working on this subject.

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