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Energetics of Anaerobic Degradation Pathways of Chlorinated Aliphatic Compounds

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ABSTRACT

This minireview explores the energetics of the (anaerobic) oxidative and fermentative degradation of halogenated ethenes and ethanes. It is shown that these pathways are viable alternatives to the traditional routes that start with one or more reductive dechlorination steps. In contrast to reductive dehalogenation, oxidative and fermentative degradation pathways do not require an external source of reducing equivalents. This suggests that organisms that use these pathways are most likely to be found at high redox potentials, i.e, under conditions where competition for reducing equivalents is great.

Introduction

The first studies on the energetics of the anaerobic degradation of halogenated compounds concerned the reductive dehalogenation of 3-chlorobenzoate to benzoate [4, 10, 21] and of perchloroethylene to tri- and dichloroethylene [11–13, 19, 22]. The excitement about the fact that these reactions can sustain the growth of microorganisms combined with the observation that similar dechlorination reactions occur with a wide variety of other halogenated compounds has led to the general perception that the first steps in the anaerobic degradation of halogenated compounds involve reductive dehalogenation as depicted in Fig. 1 [7]. More recent observations, however, have shown that this is not necessarily the case. Bradley and Chapelle, for example, have presented evidence for the oxidative degradation of chlorinated ethenes via an as yet unknown mechanism [1], while Lorah and Olsen have reported that in a freshwater tidal wetland degradation of 1,1,2,2-tetrachloroethane involved substantial dichloroelimination (Fig. 2) to chlorinated ethenes, rather than only a "classical" reductive dehalogenation to lesser chlorinated ethanes [17]. This raises the question when each type of reductive dehalogenation will occur and when other mechanisms will prevail. The objective of this minireview is to present a thermodynamic framework to help answer these questions.

Anaerobic Degradation Processes

Anaerobic degradation processes can be conveniently depicted as an integrated series of decarboxylations and redox reactions [3, 25]. For a typical organic compound this amounts to the formation of CO_2 and H_2. Generally, the overall energetics of such a series of reactions is endergonic under standard conditions. Organisms can then only obtain

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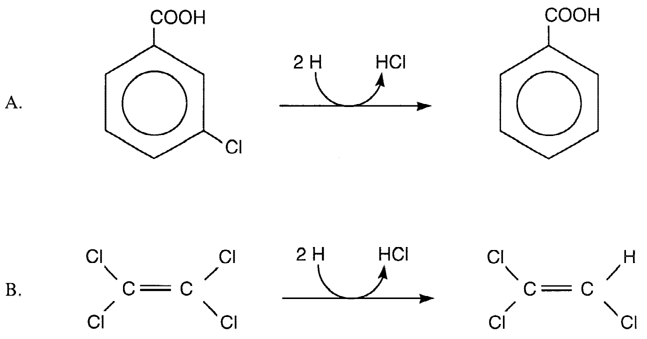

Fig. 1. Reductive dechlorination (hydrogenolysis) of (A) 3-chlorobenzoic acid to benzoic acid and (B) tetrachloroethylene to trichloroethylene.

energy for growth from these reactions if the resulting end product (H_2) is removed. Aerobes do this by oxidizing H_2 with O_2 to water. Anaerobes use other electron acceptors, but the general principle is the same, with the annotation that in methanogenic ecosystems complete mineralization is not brought about by one single organism, but rather by a series of organisms in a food chain—for example, [9] biocoenosis, where the acidogens and the acetogens rely on the activity of the methanogens for the removal of the reducing equivalents produced [3]. Aerobes, but also Fe(III)- and sulfate reducers, on the other hand, can be "complete oxidizers," i.e., organisms that mineralize an organic compound all the way to CO_2; such organisms remove reducing equivalents intracellularly by reducing O_2, Fe(III), or sulfate [2, 16, 24].

Degradation of Chlorinated Ethenes

Evaluating the energetics of the degradation of chlorinated compounds as a series of decarboxylations and redox reactions reveals an interesting characteristic of this group of compounds. Let us take chlorinated ethylenes as an example. Mineralization of ethene to H_2 and CO_2 is an endergonic reaction ($\Delta G^{o\prime} = 91.8$ kJ/mol) [8] that only becomes energetically favorable at low hydrogen concentrations. However, for chlorinated ethenes this picture is different: the presence of one or more chloro substituents makes mineralization to H_2, CO_2, and HCl an exergonic reaction (Table 1). This implies that, in practice, organisms that degrade chlorinated ethenes to H_2, CO_2, and HCl are energetically independent of—but not unaffected by—the activity of H_2-consuming organisms. Furthermore, this also implies that, theoretically, organisms can grow on the fermentative degradation of chlorinated ethylenes in a type of reaction in which the chloro substituent serves as an "internal" electron acceptor [5]. Table 1 gives a series of examples of such reactions. Chlorinated ethylenes can, for example, be fermented to ethene and CO_2 or to acetate. The exergonicity of such fermentations implies the potential existence of new types of dechlorinating bacteria that would not depend on the presence of external sources of reducing equivalents. Occurrence of such organisms would be most likely in environments where reducing equivalents are "expensive," i.e., at relatively low H_2 concentrations (high redox potentials).

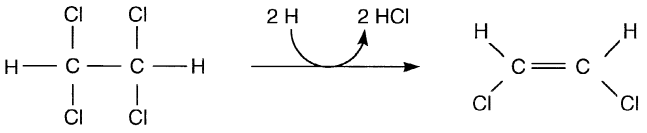

Fig. 2. Dichloroelimination of 1,1,2,2-tetrachloroethane to 1,2-dichloroethylene.

Thus far, however, the biodegradation pathway observed most often with polychlorinated ethylenes is a step-by-step reductive dechlorination, in a series that is generally, but not always, catalyzed by more than one organism and ultimately leads to the formation of ethene and ethane [20]. From an energetic point of view this is rather surprising, as the amount of energy that is available from complete mineralization of chlorinated ethylenes is significantly higher than the amount available from an individual dechlorination step. Apparently the availability of chlorinated compounds is not the factor that determines which reaction type comes to the fore in pollutant plumes and heavily loaded bioreactors. In these environments there is obviously a niche for specialized (H_2 consuming) dechlorinating organisms, especially if these organisms are able to outcompete other H_2 consumers, since the amount of energy available from the dechlorination reaction is more than the amount available from sulfate reduction or methanogenesis. Thus, under those redox conditions respiratory dechlorinators will prevail over chlorofermenters as long as their chlorinated substrate is not limiting. Under conditions where reducing equivalents are scarcer, e.g., under iron reducing conditions, it would (energetically) be more efficient for organisms to use the fermentative route, or, alternatively, the route in which the chlorinated ethylenes are completely mineralized. Figure 3 illustrates that at very low H_2 concentrations, complete mineralization would be even more favorable than fermentation. In addition to the availability of reducing equivalents as a

Table 1. Gibbs free energy values for the reductive dechlorination, the mineralization, and the fermentation of chlorinated ethylenes[a]

			$\Delta G^{\circ\prime}$	
			kJ/reaction	kJ/mol
Reductive dechlorination				
Tetrachloroethylene	+ H_2	→ Trichloroethylene + H^+ + Cl^-	−171.8	−171.8
Trichloroethylene	+ H_2	→ Dichloroethylene + H^+ + Cl^-	−166.1	−166.1
Dichloroethylene	+ H_2	→ Chloroethylene + H^+ + Cl^-	−144.8	−144.8
Chloroethylene	+ H_2	→ Ethene + H^+ + Cl^-	−154.5	−154.5
Mineralization				
Tetrachloroethylene	+ $4H_2O$	→ $2CO_2 + 4H^+ + 4Cl^- + 2H_2$	−545.3	−545.3
Trichloroethylene	+ $4H_2O$	→ $2CO_2 + 3H^+ + 3Cl^- + 3H_2$	−373.5	−373.5
Dichloroethylene	+ $4H_2O$	→ $2CO_2 + 2H^+ + 2Cl^- + 4H_2$	−207.4	−207.4
Chloroethylene	+ $4H_2O$	→ $2CO_2 + H^+ + Cl^- + 5H_2$	−62.7	−62.7
Ethene	+ $4H_2O$	→ $2CO_2 + 6H_2$	91.8	91.8
Fermentation to acetate				
4Tetrachloroethylene	+ $12H_2O$	→ $4CO_2$ + 2Acetate$^-$ + $18H^+ + 16Cl^-$	−2371.0	−592.8
4Trichloroethylene	+ $10H_2O$	→ $2CO_2$ + 3Acetate$^-$ + $15H^+ + 12Cl^-$	−1778.7	−444.7
4Dichloroethylene	+ $8H_2O$	→ 4Acetate$^-$ + $12H^+ + 8Cl^-$	−1209.5	−302.4
4Chloroethylene	+ $6H_2O$+ $2CO_2$	→ 5Acetate$^-$ + $9H^+ + 4Cl^-$	−725.4	−181.4
4Ethene	+ $4H_2O$+ $4CO_2$	→ 6Acetate$^-$ + $6H^+$	−202.4	−50.6
Fermentation to ethene				
6Tetrachloroethylene	+ $16H_2O$	→ $8CO_2$ + 2Ethene + $24H^+$+ $24Cl^-$	−3455.3	−575.9
6Trichloroethylene	+ $12H_2O$	→ $6CO_2$ + 3Ethene + $18H^+$+ $18Cl^-$	−2516.2	−419.4
6Dichloroethylene	+ $8H_2O$	→ $4CO_2$ + 4Ethene + $12H^+$+ $12Cl^-$	−1611.8	−268.6
6Chloroethylene	+ $4H_2O$	→ $2CO_2$ + 5Ethene + $6H^+$+ $6Cl^-$	−835.1	−139.2

[a] Calculated according to the relationship $\Delta G^{\circ\prime} = \Sigma\Delta G_f^\circ$ (products) $-\Sigma\Delta G_f^\circ$ (substrates). The ΔG_f° values were taken from Dolfing and Janssen [8] and from Thauer et al. [23]. The standard conditions were $T = 25°C$, pH = 7, H_2, CO_2 and (chlorinated) ethenes as gaseous compounds at a pressure of 1 atm (100 kPa), and Cl^- and acetate at aqueous concentrations of 1 M.

selective factor, the availability of the halogenated compound itself may also play a role. A search for anaerobes that degrade chlorinated ethylenes via "novel" pathways should thus specifically include environments where the availability of chlorinated compounds is an issue to the organisms that degrade these compounds; examples of such environments are the borders of contaminant plumes. In this context it will also be interesting to compare the ecophysiology of, e.g., *Dehalobacter restrictus* (an organism that dechlorinates tetrachloroethylene to dichloroethylene) [13] with that of *Dehalococcoides ethenogenes* [18,19], an organism that dechlorinates tetrachloroethylene all the way to ethylene. Based on the energy yield per mole of substrate it is tempting to predict that *D. ethenogenes* should outcompete *D. restrictus* for tetrachloroethylene, whereas in natural systems, where H_2 is usually the limiting factor, *D. restrictus* is expected to win the competition.

Degradation of Chlorinated Ethanes

A thermodynamic evaluation of the energetics of degradation routes of chlorinated ethanes yields results that are very similar to the evaluation of the energetics of the degradation routes of chlorinated ethenes given above. The presence of one or more chloro substituents makes chlorinated ethanes more oxidized than their unsubstituted forms. During the mineralization of chlorinated ethanes the chloro substituents serve as electron acceptor for the reducing equivalents generated in the hydrolytic oxidation of the carbon backbone of the molecule. The energetics of the formation of HCl are such that this partial reaction makes the overall mineralization of chlorinated ethanes exergonic even under standard conditions (H_2 partial pressure 1 atm) where the mineralization of ethane itself is endergonic. As an alternative to complete mineralization, fermentation of chlorinated ethanes is also conceivable and indeed energetically attractive. Table 2 shows theoretical examples of exergonic reactions in which chlorinated ethanes are fermented to ethane or acetate. (It should be noted though that certain of the fermentations described involve CO_2 reduction.) The amount of energy available per mole substrate is similar for conversions to either ethane or acetate. For polychlorinated ethanes these amounts are higher than the amount available

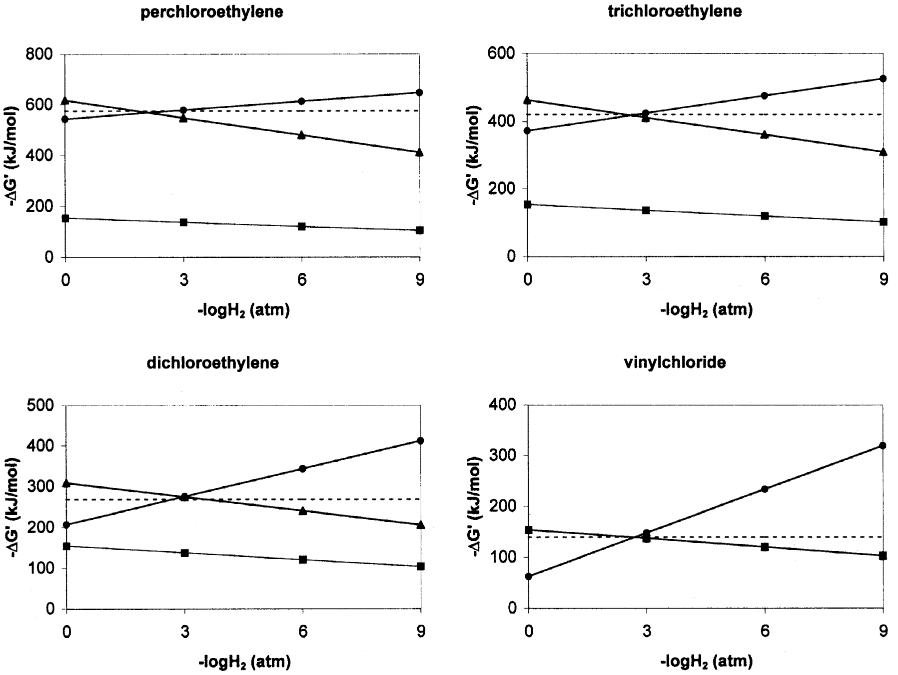

Fig. 3. Effect of the partial pressure of H_2 on the change in Gibbs free energy ($\Delta G'$) for various degradation reactions of chlorinated ethenes. ▲ complete dechlorination to ethene; ●, complete mineralization to CO_2, H_2, and HCl; --- , fermentation to acetate; ■ incomplete (single) dechlorination. Calculations were done as described previously [3, 23].

from a single dechlorination step. Thus, it is tempting to speculate that organisms that use a reductive dechlorination pathway are most likely to occur under conditions where reducing equivalents (electrons) are readily available. In addition to the alternatives to the classical reductive dechlorination sketched above, there is, however, another potential alternative: dichloroelimination. In this reaction (Fig. 2) two rather than one chlorine groups are removed from a chlorinated ethane under the formation of (chloro)ethene. The energy yield per mole of reducing equivalents (H_2) of a dichloroelimination of chlorinated ethanes (Table 3) is higher than for the corresponding classical reductive dechlorination (Table 2). There are indications that dichloroelimination is indeed the prevalent reaction in the anaerobic biodegradation of chlorinated ethanes. This is unfortunate, as dichloroelimination of 1,1,2,2-tetrachloroethane results in the formation of vinyl chloride [17]. Addition of easily available reducing equivalents may decrease the advantage of dichloro-eliminating organisms by making competition for reducing equivalents less important [6].

Fermentations

Bradley and Chapelle [1] have recently presented evidence indicating that monochloroethene is degraded via a fermentation reaction rather than via reductive dechlorination. Interestingly, this compound was not fermented to either ethene or acetate—the reactions presented in Table 1—but to equimolar amounts of both [1]. The consequence of this stoichiometry is that overall no CO_2 is produced or consumed.

Acetate as a Source of Reducing Equivalents

Hydrogen appears to be the most common source of reducing equivalents for the dechlorination of chlorinated aliphatics in the environment, but it is not the only one. Krumholz, for example, has recently described an organism that uses acetate rather than hydrogen (or formate) for the reductive dechlorination of tetra- and trichloroethylene [14, 15]. It is not yet clear how widely this trait is distributed in dechlorinating communities. However, the implications of the above discussion are not affected by this exception. The energy gain with the two reductants is different, but the conclusions (see below) remain the same.

Conclusions

The present paper may serve as a thermodynamic framework to guide a search for novel anaerobic dechlorination pathways. The main conclusions are that pathways other than the reductive dechlorination pathway that is presently observed most often in anaerobic environments indeed can be envisaged, and that these alternatives are, under certain conditions, energetically more favorable than reductive de-

Table 2. Gibbs free energy values for the reductive dechlorination, the mineralization, and the fermentation of chlorinated ethanes[a]

			$\Delta G^{\circ\prime}$	
			kJ/reaction	kJ/mol
Reductive dechlorination				
Hexachloroethane	+ H_2	$\rightarrow$ Pentachloroethane + H^+ + Cl^-	−186.5	−186.5
Pentachloroethane	+ H_2	$\rightarrow$ Tetrachloroethane + H^+ + Cl^-	−183.9	−183.9
Tetrachloroethane	+ H_2	$\rightarrow$ Trichloroethane + H^+ + Cl^-	−165.1	−165.1
Trichloroethane	+ H_2	$\rightarrow$ Dichloroethane + H^+ + Cl^-	−134.9	−134.9
Dichloroethane	+ H_2	$\rightarrow$ Chloroethane + H^+ + Cl^-	−173.7	−173.7
Chloroethylene	+ H_2	$\rightarrow$ Ethane + H^+ + Cl^-	−161.0	−161.0
Mineralization				
Hexachloroethane	+ $4H_2O$	$\rightarrow$ $2CO_2$ + $6H^+$ + $6Cl^-$ + H_2	−812.3	−812.3
Pentachloroethane	+ $4H_2O$	$\rightarrow$ $2CO_2$ + $5H^+$ + $5Cl^-$ + $2H_2$	−625.8	−625.8
Tetrachloroethane	+ $4H_2O$	$\rightarrow$ $2CO_2$ + $4H^+$ + $4Cl^-$ + $3H_2$	−441.9	−441.9
Trichloroethane	+ $4H_2O$	$\rightarrow$ $2CO_2$ + $3H^+$ + $3Cl^-$ + $4H_2$	−276.8	−276.8
Dichloroethane	+ $4H_2O$	$\rightarrow$ $2CO_2$ + $2H^+$ + $2Cl^-$ + $5H_2$	−141.9	−141.9
Chloroethane	+ $4H_2O$	$\rightarrow$ $2CO_2$ + H^+ + Cl^- + $6H_2$	31.8	31.8
Ethane	+ $4H_2O$	$\rightarrow$ $2CO_2$ + $7H_2$	192.8	192.8
Fermentation to acetate				
4Hexachloroethane	+ $14H_2O$	$\rightarrow$ $6CO_2$ + Acetate$^-$ + $25H^+$ + $24Cl^-$	−3,344.3	−836.1
4Pentachloroethane	+ $12H_2O$	$\rightarrow$ $4CO_2$ + 2Acetate$^-$ + $22H^+$ + $20Cl^-$	−2693.2	−673.3
4Tetrachloroethane	+ $10H_2O$	$\rightarrow$ $2CO_2$ + 3Acetate$^-$ + $19H^+$ + $16Cl^-$	−2052.6	−513.1
4Trichloroethane	+ $8H_2O$	$\rightarrow$ 4Acetate$^-$ + $16H^+$ + $12Cl^-$	−1487.2	−371.8
4Dichloroethane	+ $6H_2O$+ $2CO_2$	$\rightarrow$ 5Acetate$^-$ + $13H^+$ + $8Cl^-$	−1042.3	−260.6
4Chloroethane	+ $4H_2O$+ $4CO_2$	$\rightarrow$ 6Acetate$^-$ + $10H^+$ + $4Cl^-$	−442.5	−110.6
4Ethane	+ $2H_2O$+ $6CO_2$	$\rightarrow$ 7Acetate$^-$ + $7H^+$	106.4	26.6
Fermentation to ethane				
7Hexachloroethane	+ $24H_2O$	$\rightarrow$ $12CO_2$ + Ethane + $42H^+$ + $42Cl^-$	−5879.1	−839.9
7Pentachloroethane	+ $20H_2O$	$\rightarrow$ $10CO_2$ + 2Ethane + $35H^+$ + $35Cl^-$	−4766.3	−680.9
7Tetrachloroethane	+ $16H_2O$	$\rightarrow$ $8CO_2$ + 3Ethane + $28H^+$ + $28Cl^-$	−3671.8	−524.5
7Trichloroethane	+ $12H_2O$	$\rightarrow$ $6CO_2$ + 4Ethane + $21H^+$ + $21Cl^-$	−2709.0	−387.0
7Dichloroethane	+ $8H_2O$	$\rightarrow$ $4CO_2$ + 5Ethane + $14H^+$ + $14Cl^-$	−1957.1	−279.6
7Chloroethane	+ $4H_2O$	$\rightarrow$ $2CO_2$ + 6Ethane + $7H^+$ + $7Cl^-$	−934.0	−133.4

[a] Calculated according to the relationship $\Delta G^{\circ\prime} = \Sigma\Delta G_f^{\circ}$ (products) $-\Sigma\Delta G_f^{\circ}$ (substrates). The ΔG_f° values were taken from Dolfing and Janssen [8] and from Thauer et al. [23]. The standard conditions were T = 25 °C, pH = 7, H_2, CO_2 and (chlorinated) ethanes and ethenes as gaseous compounds at a pressure of 1 atm (100 kPa), and Cl^- and acetate at aqueous concentrations of 1 M.

chlorination. Conditions where those (hypothetical) alternatives are most likely to occur are to be found in environments with relatively high redox potentials and only limited availability of (fierce competition for) halogenated compounds. It cannot be emphasized enough that this treatise was rather single-minded: the emphasis was on thermodynamics and energetics without taking into consideration the limitations and constraints posed by the enzymatic machin-

Table 3. Gibbs free energy values for the dichloroelimination of chlorinated ethanes[a]

				$\Delta G^{\circ\prime}$ kJ/reaction
Hexachloroethane	+ H_2	$\rightarrow$ Tetrachloroethylene	+ $2H^+$ + $2Cl^-$	−267.0
Pentachloroethane	+ H_2	$\rightarrow$ Trichloroethylene	+ $2H^+$ + $2Cl^-$	−252.4
Tetrachloroethane	+ H_2	$\rightarrow$ Dichloroethylene	+ $2H^+$ + $2Cl^-$	−234.1
Trichlorethane	+ H_2	$\rightarrow$ Chloroethylene	+ $2H^+$ + $2Cl^-$	−214.2
Dichloroethane	+ H_2	$\rightarrow$ Ethylene	+ $2H^+$ + $2Cl^-$	−233.7

[a] Calculated according to the relationship $\Delta G^{\circ\prime} = \Sigma\Delta G_f^{\circ}$ (products) $-\Sigma\Delta G_f^{\circ}$ (substrates). The ΔG_f° values were taken from Dolfing and Janssen [8] and from Thauer et al. [23]. In case of multiple isomers average values were used. The standard conditions were T = 25 °C, pH = 7, H_2, chlorinated ethanes and ethenes as gaseous compounds at a pressure of 1 atm (100 kPa), and Cl^- at aqueous concentrations of 1 M.

ery in and of the cells that eventually have to do the actual work. It is even possible that these pathways are not the primary means of energy conservation by those organisms. Thus, the distribution and relative abundances of these organisms may be determined by their success in growing on other substrates, and they may not compete for use of chlorinated aliphatic compounds. Nevertheless, the above framework should help to identify environments and conditions where these hypothetical organisms are most likely to be found. Research on such organisms and subsequently on their ecology may help to better understand intrinsic bioremediation processes, and to find ways to enhance them.

Acknowledgments

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