Prediction of Dechlorination Pathways of Diverse Chlorinated Aromatic Pollutants Conducted by *Dehalococcoides ethenogenes* Strain 195

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Dechlorination of chlorinated aromatic pollutants (CAPs) has become a major issue in recent decades. This paper reported a theoretical indicator for proposing the reductive dechlorination pathways of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs) and chlorobenzenes transformed by *Dehalococcoides ethenogenes* strain 195. Quantum chemical calculations were carried out at the HF/6-31G(d) level for all related chlorinated aromatic pollutants and molecular total energy (E_T) of structuraly possible daughter products was adopted as the probe of the dechlorination reaction activity. E_T could indicate the main dechlorination daughter products of PCDD/Fs, PCBs and chlorobenzenes conducted by strain 195. The dechlorination reaction favoured the formation of daughter product having the lowest E_T. In addition, the energy gap (ΔE_T) between a structurally possible daughter product and the possible daughter product having the lowest E_T could be used to estimate the existence of multiple daughter products: the smaller the ΔE_T, the more possible to form multiple daughter products.

Key Words: Chlorinated aromatic pollutant, *Dehalococcoides ethenogenes* strain 195, Molecular total energy, Reductive dechlorination pathway, Theoretical indicator.

INTRODUCTION

Despite international efforts to control and regulate persistent chlorinated aromatic pollutants, past and ongoing releases have resulted in widespread contamination of soils and sediments[1]. It is of particular importance to develop effective technologies to remediate chlorinated aromatic pollutants contaminated sites. Several transformation-based technologies, including photolysis, photocatalysis, γ-radiolysis, biodegradation, incineration and thermal treatment, have been developed for treating these chemicals in different systems[2-9]. The use of microorganisms for *in situ* remediation is regarded as an alternative method to detoxify or remove chlorinated aromatic pollutants from the environment[1].

Reductive dechlorination is an important transformation process for chlorinated aromatic pollutants in the environment[1]. *Dehalococcoides* strains are highly specialized strictly anaerobic bacteria that are known to grow only by respiration using hydrogen as an electron donor and halogenated organic compounds as eletron acceptors[10]. Neither fermentation nor respiration with non-halogenated compounds has been observed with any of the described isolates[1,10-13]. *Dehalococcoides*

ethenogenes strain 195 was found to dechlorinate 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TCDD), 1,2,3,4,7,8-hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF), 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-PeCB), hexachlorobenzene and some of their daughter products[1,13]. Despite the above studies have provided insight information for the dechlorination of selected chlorinated aromatic pollutants, the dechlorination pathways by strain 195 for most chlorinated aromatic pollutants have not been clearly delineated in the literature yet.

In fact, experimental determination of dechlorination pathways is practically difficult for some chlorinated aromatic pollutants, such as polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs), many of which coelute on a GC/HPLC column. Furthermore, it is even impossible for some chlorinated aromatic pollutants due to the lack of pure standards, many of which might not have been synthesized or purified. Thus, it is necessary to develop a theoretical technique to propose the dechlorination pathways and products, which use certain molecular descriptors calculated directly from chemical structures of the pollutants to indicate the eliminating

activity of chlorines in different substituted positions. We had reported four rules of thumb for predicting the main reductive dechlorination pathways of PCDDs in the presence of zerovalent zinc based on the maximal Mulliken atomic charges on chlorine atoms ($Q_{Cl(n)}$)[14]. $Q_{Cl(n)}$ was also adopted as the probe of the dechlorination reaction activity of *Dehalococcoides* sp. strain CBDB1 on selected chlorinated aromatic pollutants, such as PCDDs, chlorobenzenes and chlorophenol anions[15]. However, the dechlorination pathways of chlorinated aromatic pollutants might differ under different environmental conditions. Studies had shown different dechlorination mechanisms for 1,2,3,4-TCDD conducted by strain 195[1] and strain CBDB1[11]. Thus, in addition to the Mulliken charge of the selected atom, other descriptors may offer alternative perspectives for predicting the dechlorination pathways of chlorinated aromatic pollutants in different systems[16].

In this work, we worked out *ab initio* Hartree-Fock calculations for all related chlorinated aromatic pollutants to grope for the theoretical indicator to validate the reported experimental dechlorination pathways of chlorinated aromatic pollutants conducted by strain 195 and to propose the dechlorination pathways of other chlorinated aromatic pollutants.

EXPERIMENTAL

The molecular modeling system HyperChem (Release 7.0, Hypercube Inc. 2002) was used to construct and view all molecular structures. Molecular geometry was optimized by using Hartree-Fock method at the HF/6-31G(d) level, which is reliable in computing structure descriptors of aromatic organic compounds[17]. The stability of molecular structures was confirmed by the vibrational frequency analysis. All calculations were carried out with the Gaussian 03 program[18] running on an Intel Core 2 Duo 3.00 GHz CPU computer equipped with 5.00 GB of internal memory and Microsoft Windows XP Professional operating system.

Hartree-Fock calculations were performed for all related chlorinated aromatic pollutants, resulting in useful structural descriptors of these compounds. These descriptors include the eigen value of the highest occupied/lowest unoccupied molecular orbital (E_{HOMO}/E_{LUMO}), the molecular total energy (E_T), the electronic spatial extent (R_e), the dipole moment (m), the Mulliken atomic charge on carbon and chlorine atoms ($Q_{C(n)}$ and $Q_{Cl(n)}$, n is the corbon atom number in the frame), the bond length between carbon and its substituted chlorine atoms (L_n). These descriptors were correlated with the reported experimental dechlorination pathways of chlorinated aromatic pollutants conducted by strain 195[1,13], respectively, to grope for theoretical indicator to validate the dechlorination pathways and to propose the dechlorination pathways of other chlorinated aromatic pollutants.

RESULTS AND DISCUSSION

Based on the the multivalidation of experimental dechlorination pathways of chlorinated aromatic pollutants conducted by strain 195[1,13], E_T was adopted as the theoretical indicator for the dechlorination reaction activity. The dechlorination reaction favoured the formation of daughter product having the lowest E_T. The E_T values for structurally possible dechlo-

rination products of chlorinated aromatic pollutants selected in this study are listed in Table-1.

TABLE-1
MOLECULAR TOTAL ENERGY FOR STRUCTURALLY
POSSIBLE DECHLORINATION PRODUCTS OF SELECTED
PCDD/Fs, PCBs AND CHLOROBENZENES

Compounds/products[a]	E_T	ΔE_T[b]
1,2,3,4-TCDD		
1,2,4-TrCDD	-1985.4716	0
1,2,3-TrCDD	-1985.4695	0.0020
1,2,4-TrCDD		
1,3-DCDD	-1526.5834	0
1,4-DCDD	-1526.5806	0.0028
1,2-DCDD	-1526.5789	0.0045
1,2,3,4,7,8-HxCDF		
1,3,4,7,8-PeCDF	-2828.4281	0
1,2,4,7,8-PeCDF	-2828.4275	0.0006
1,2,3,7,8-PeCDF	-2828.4245	0.0036
2,3,4,7,8-PeCDF	-2828.4225	0.0056
1,2,3,4,7-PeCDF	-2828.4211	0.0070
1,2,3,4,8-PeCDF	-2828.4207	0.0074
1,3,4,7,8-PeCDF		
1,3,7,8-TCDF	-2369.5393	0
1,3,4,7-TCDF	-2369.5371	0.0022
1,4,7,8-TCDF	-2369.5369	0.0024
1,3,4,8-TCDF	-2369.5368	0.0025
2,3,6,7-TCDF	-2369.5327	0.0066
1,2,4,7,8-PeCDF		
1,4,7,8-TCDF	-2369.5369	0
1,2,4,7-TCDF	-2369.5365	0.0004
2,3,6,8-TCDF	-2369.5365	0.0004
1,2,4,8-TCDF	-2369.5361	0.0008
1,2,7,8-TCDF	-2369.5347	0.0022
2,3,4,5,6-PeCB		
2,3,5,6-TCB	-2295.8259	0
2,3,4,6-TCB	-2295.8257	0.0002
2,3,4,5-TCB	-2295.8245	0.0014
2,3,4,6-TCB		
2,4,6-TrCB	-1836.9402	0
2,4,5-TrCB	-1836.9401	0.0002
2,3,6-TrCB	-1836.9353	0.0050
2,3,4-TrCB	-1836.9336	0.0066
PeCBz		
1,2,4,5-TCBz	-2066.2851	0
1,2,3,5-TCBz	-2066.2841	0.0010
1,2,3,4-TCBz	-2066.2792	0.0059
1,2,3,5-TCBz		
1,3,5-TrCBz	-1607.3979	0
1,2,4-TrCBz	-1607.3939	0.0041
1,2,3-TrCBz	-1607.3885	0.0094
1,2,3,4-TCBz		
1,2,4-TrCBz	-1607.3939	0
1,2,3-TrCBz	-1607.3885	0.0053
1,2,4-TrCBz		
1,4-DCBz	-1148.5018	0
1,3-DCBz	-1148.5015	0.0003
1,2-DCBz	-1148.4969	0.0049
1,2,3-TrCBz		
1,3-DCBz	-1148.5015	0
1,2-DCBz	-1148.4969	0.0046

a) Given in italic is the initial compounds and given in bold is the detected experimental dechlorination products conducted by *D. ethenogenes* strain 195; b) ΔE_T is the energy gap between this possible product and the possible product having the lowest E_T value; c) CB stands for chlorinated biphenyl and CBz stands for chlorobenzene

Validation of the dechlorination pathways of PCDDs:
Fennell *et al.*[1] reported that strain 195 could dechlorinate 1,2,3,4-TCDD to 1,2,4-trichlorodibenzo-*p*-dioxin (1,2,4-TrCDD) and subsequently, to 1,3-dichlorodibenzo-*p*-dioxin (1,3-DCDD) (Fig. 1).
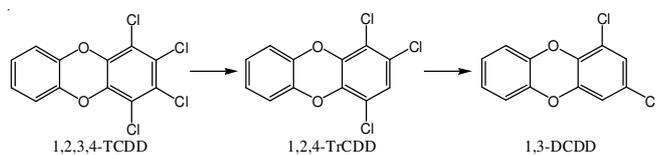

Fig. 1. Dechlorination pathway of 1,2,3,4-TCDD catalyzed by strain 195

As can be seen from the molecular structure, 1,2,3,4-TCDD structurally has two possible dechlorination products including 1,2,3- and 1,2,4-TrCDD, while 1,2,4-TrCDD structurally has three possible dechlorination products including 1,2-, 1,3- and 1,4-DCDD. Table-1 shows that both the two detected experimental dechlorination products (1,2,4-TrCDD and 1,3-DCDD) conducted by strain 195 have the lowest E_T values among the structurally possible dechlorination products. Thus, the dechlorination reaction of PCDDs conducted by strain 195 favoured the formation of daughter product having the lowest E_T.

Validation of the dechlorination pathways of PCBs:
Strain 195 could dechlorinated 2,3,4,5,6-pentachlorobiphenyl (2,3,4,5,6-PeCB) to 2,3,4,6-tetrachlorobiphenyl (2,3,4,6-TCB) and 2,3,5,6-TCB and subsequently, to 2,4,6-trichlorobiphenyl (2,4,6-TrCB)[1]. The dechlorination pathway is shown in Fig. 2.
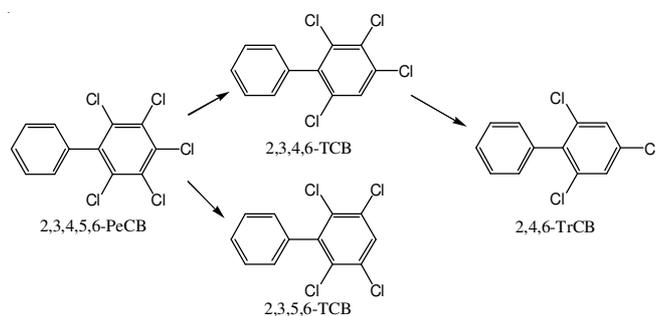

Fig. 2. Dechlorination pathway of 2,3,4,5,6-PeCB catalyzed by strain 195[1]

As can be seen from the molecular structure, 2,3,4,5,6-PeCB structurally has three possible dechlorination products including 2,3,4,5-, 2,3,4,6- and 2,3,5,6-TCB and 2,3,4,6-TCB structurally has four possible dechlorination products including 2,3,4-, 2,3,6-, 2,4,5- and 2,4,6-TrCB. As can be seen from Table-1, the detected experimental dechlorination products 2,3,5,6-TCB and 2,4,6-TrCB have the lowest E_T values among the structurally possible dechlorination products and as the other detected dechlorination product, 2,3,4,6-TCB has the next lowest E_T value.

Further inspection of the E_T values of the structurally possible dechlorination products found the energy gap (ΔE_T) between 2,3,4,6-TCB and 2,3,5,6-TCB was only 0.0002. It was likely that the ΔE_T is too small so that multiple dechlorination daughter products might be existed theoretically. Comparing the ΔE_T of the above PCDDs and PCBs, ΔE_T might be employed as the theoretical indocator for estimating whether a CAP exist multiple dechlorination products.

Validation of the dechlorination pathways of PCDFs:
Liu and Fennell[13] had investigated the dechlorination of 1,2,3,4,7,8- hexachlorodibenzofuran (1,2,3,4,7,8-HxCDF) by a mixed culture containing strain 195 and found 1,2,3,4,7,8-HxCDF was dechlorinated to 1,3,4,7,8-pentachlorodibenzofuran (1,3,4,7,8-PeCDF) and 1,2,4,7,8-PeCDF and further to 1,3,7,8-tetrachlorodibenzofurans (1,3,7,8-TCDF) and 1,2,4,8-TCDF. The dechlorination pathway is shown in Fig. 3.
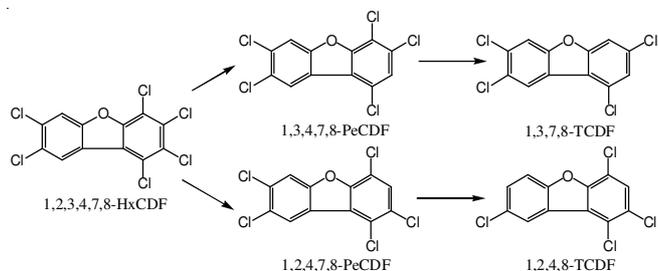

Fig. 3. Dechlorination pathway of 1,2,3,4,7,8-HxCDF catalyzed by strain 195[13]

As concluded from the molecular structure, 1,2,3,4,7,8-HxCDF structurally has six possible dechlorination products, while both 1,3,4,7,8- and 1,2,4,7,8-PeCDF structurally have five possible dechlorination products. As can be seen from Table-1: (1) the two detected experimental dechlorination products of 1,2,3,4,7,8-HxCDF have the lowest and the next lowest E_T value, respectively and the ΔE_T of these two products is only 0.0006, less than 10^{-3}. (2) 1,3,7,8-TCDF, the sole detected experimental dechlorination product of 1,3,4,7,8-PeCDF, has the lowest E_T value among the five structurally possible dechlorination products and the ΔE_T of 1,3,4,7-TCDF, the structurally possible dechlorination product having the next lowest E_T value, is as high as 0.0022, larger than 10^{-3}, which indicate small possibility to exist multiple dechlorination products.

However, 1,2,4,8-TCDF as the sole detected experimental dechlorination product of 1,2,4,7,8-PeCDF donot have the lowest E_T value among the five structurally possible dechlorination products and has a ΔE_T value of 0.0008. Among the possible dechlorination products of 1,2,4,7,8-PeCDF, the ΔE_T values of 4 products are $< 10^{-3}$, therefore, 1,2,4,7,8-PeCDF theoretially exists multiple dechlorination products. The experimentally undetected products are likely acumulated scarce and under detection limit of the instrument, so that only 1,2,4,8-TCDF was detected.

Validation of the dechlorination pathways of chlorobenzenes: Strain 195 was found to dechlorinate chlorobenzenes[1]. Some chlorobenzenes were successful transferred as sole eletron acceptor, while some others were needed to add tetrachloroetene to supply the dechlorination (Fig. 4).

For all the chlorobenzenes under studied, hexachlorobenzene (HxCBz), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TCBz) and 1,3,5-trichlorobenzene (1,3,5-TrCBz) structurally have sole possible dechlorination products, while all other chlorobenzenes having 3 to5 substituted chlorine atoms have 2 or more possible dechlorination products. As can be seen from Table- 1: (1) both 1,2,3,5-TCBz and 1,2,4-TrCBz structurally have three possible dechlorination products and experimentally detected two products, which have the lowest and the next lowest E_T value, respectively and the ΔE_T of these two products
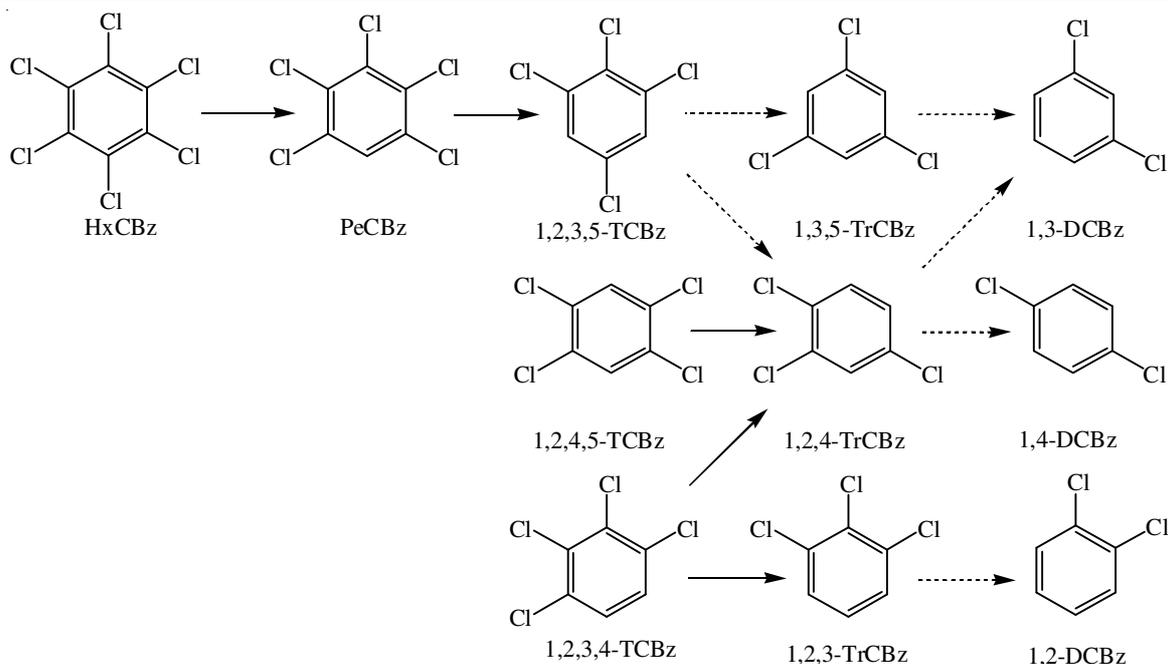
Fig. 4. Dechlorination pathway of chlorobenzenes catalyzed by strain 195[1]. Solid arrows indicate successful transfer with that chlorobenzene as sole electron acceptor; dashed arrows indicate observed dechlorination but the process did not occuur when this chlorobenzene was supplied as the sole electron acceptor

is < 0.005. (2) The ΔE_T of the two structurally possible dechlorination products of 1,2,3,4-TCBz is also closed to 0.005 and both the two products had been experimentally detected. (3) Though the experimentally detected products of pentachlorobenzene (PeCBz) and 1,2,3-TrCBz don't have the lowest E_T values, the ΔE_T of them are < 0.005. Therefore, $\Delta E_T < 0.005$ might be employed as the theoretical indicator to estimate a chlorobenzene molecule whether have multiple dechlorination products.

As can be seen in Fig. 4, each of 1,2,3,4-, 1,2,3,5-TCBz and 1,2,4-TrCBz has two experimental dechlorination products, respectively. Accoring to the literature data [1], the ratio of the two dechlorination products and the ΔE_T of the second product are listed in Table-2. From the table we can find the distribution of the two dechlorination products has relation with the ΔE_T of the second product that the ratio increases and the distribution of the two products tends to be more disequilibrium with the increasing of ΔE_T.

TABLE-2
RATIO OF DECHLORINATION PRODUCTS
OF SELECTED CHLOROBENZENES[1]

Chlorobenzenes	Main product	Second product	Main *vs.* second product	ΔE_T of the second product
1,2,3,4-TCBz	1,2,4-TrCBz	1,2,3-TrCBz	10:1	0.0053
1,2,3,5-TCBz	1,3,5-TrCBz	1,2,4-TrCBz	6:1	0.0041
1,2,4-TrCBz	1,4-DCBz	1,3-DCBz	2:1	0.0003

The chlorinated aromatic pollutants in Table-1 can be divided into two sets according to their structures: PCDD/Fs and PCBs have two benzene rings, while chlorobenzenes have only one benzene ring.

For PCDD/Fs and PCBs having two benzene rings, if the ΔE_T of a structurally possible dechlorination product (except

which having the lowest E_T) is < 0.001, this product might be formed by strain 195 and the mother PCDD/F or PCB molecule theoretically exists multiple dechlorination products. If the ΔE_T of a structurally possible dechlorination product (except which having the lowest E_T) is > 0.001, there has little possibility to form this product by strain 195. However, the critical value of ΔE_T for chlorobenzenes having only one benzene ring is about 0.005, which is about 5 times of ΔE_T for those having two benzene rings and is also larger than that calculated in B3LYP/6-31G(d) level[17].

For those chlorinated aromatic pollutants theoretically having multiple dechlorination products, the detected experimental dechlorination products have some uncertainty that some of theoretically products might not be detected experimentally and the detected product(s) might not have the lowest E_T. The experimental dechlorination products might be affected by some other factors and need further study to investigate the details.

In the theoretical study of Arulmozhiraja and Morita[19], they suggested that the electron attachment on the neutral PCDFs (in the gas phase) weakened the C-Cl bonds and the dechlorination was of particular interest to take place on the much-elongated C-Cl bonds of the anionic PCDFs, however, no experimental data was used to validate this hypothesis in the paper. We had considered the C-Cl bond length as an alternative probe, however, it didn't fit well with the current solution phase containing strain 195. Thus, beyond the above mentioned theoretical indicators, other descriptors such as electron affinity, electronegativity and chemical hardness indices offer alternative perspectives for predicting the dechlorination pathways of chlorinated aromatic pollutants in different systems[20].

Conclusion

Hartree-Fock calculations were carried out at the HF/6-31G(d) level for selected PCDD/Fs, PCBs and chlorobenzenes

and molecular total energy (E_T) of structurally possible daughter products was adopted as the probe of the dechlorination reaction activity. E_T could indicate the main dechlorination daughter products of PCDD/Fs, PCBs and chlorobenzenes conducted by *Dehalococcoides ethenogenes* strain 195. The dechlorination reaction favoured the formation of daughter product having the lowest E_T. In addition, the energy gap (ΔE_T) between a structuraly possible daughter product and the possible daughter product having the lowest E_T could be used to estimate the existence of a secondary daughter product: the smaller the ΔE_T, the more possible to form a secondary daughter product.

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