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Reductive dechlorination pathways for substituted benzenes: a correlation with electronic properties

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Abstract

Electronic properties were correlated with observed reductive dechlorination pathways by unacclimated consortia for chlorinated phenols, dihydroxybenzenes, benzoic acids, and anilines. Molecular structures and properties were calculated using the semi-empirical Modified Neglect of Differential Overlap method at the Cornell Supercomputing Facility. Observed preferential positions for reductive dechlorination by unacclimated consortia correlate well with the largest negative value for the carbon-chlorine bond charge. Of 16 dechlorination pathways observed for unacclimated bacteria, the most negative carbon-chlorine bond charge correlated with 15 pathways.

This correlation between the observed dechlorination position and the parent compound's electronic properties is consistent with the observed reductive dechlorination of chlorophenols and chlorinated dihydroxybenzenes at the *ortho* position, and the *meta* dechlorination of chlorobenzoic acids. Net carbon-chlorine bond charges also correlate with the preferred dechlorination position for two of three known chloroaniline pathways, suggesting preferential removal of chlorines from the *ortho* position of chloroanilines.

Abbreviations: CA – chloroaniline, CBz – chlorobenzoic acid, CC – chlorocatechol, CP – chlorophenol, DCA – dichloroaniline, DCBz – dichlorobenzoic acid, DCC – dichlorocatechol, DCH – dichlorohydroquinone, DCP – dichlorophenol, DCR – dichlororesorcinol, PCP – pentachlorophenol, TCA – trichloroaniline, TCBz – trichlorobenzoic acid, TCC – trichlorocatechol, TCH – trichlorohydroquinone, TCP – trichlorophenol, TCR – trichlororesorcinol, TeCA – tetrachloroaniline, TeCBz – tetrachlorobenzoic acid, TeCC – tetrachlorocatechol, TeCH – tetrachlorohydroquinone, TeCP – tetrachlorophenol, TeCR – tetrachlororesorcinol

Introduction

The biodegradability, toxicity, and physical-chemical properties of chlorinated organic compounds vary with the position and number of chlorine atoms present on the aromatic ring. Reductive dechlorination has been observed for a wide range of halogenated organic compounds in anaerobic sediments, soils, sludges and treatment processes. It is a commonly observed anaerobic degradation process for chloroaromatic compounds and involves the removal of a chlorine from the aromatic ring and its replacement with hydrogen.

Understanding biodegradation pathways is important in understanding the fate of xenobiotic compounds in the environment, in developing bioremediation strategies for contaminated soils, or in designing wastewater treatment processes. However, acclimated consortia may yield different biotransformation pathways than unacclimated consortia. For example, Boyd & Shelton (1984) observed that sludges acclimated to 2-chlorophenol degrade 4-chlorophenol and 2,4-dichlorophenol but not 3-chlorophenol, and those acclimated to 3-chlorophenol are incapable of degrading 2-chlorophenol. Additionally, sludges acclimated to individual monochlorophenols produce different initial pentachlorophenol degradation products (Mikesell & Boyd 1986). Anaerobic sludge acclimated to 2-chlorophenol produces 2,3,4,5-tetrachlorophenol from pentachlorophenol by reductive dechlorination of the *ortho* chlorine. Sludge acclimated to 3-chlorophenol dechlorinates pentachlorophenol at the *meta* position to yield 2,3,4,6-tetrachlorophenol, and sludge acclimated to 4-chlorophenol yields 2,3,5,6-tetrachlorophenol by *para* dechlorination. In a similar study by Bryant et al. (1991), chlorophenol reductive dechlorination pathways were determined for organisms acclimated to either 2,4-dichlorophenol or 3,4-dichlorophenol. The 2,4-dichlorophenol-acclimated consortium produced *ortho* dechlorination products, while the 3,4-dichlorophenol-acclimated consortium produced *para* dechlorination products.

In this paper, known reductive dechlorination pathways for several groups of chlorinated aromatic compounds by unacclimated consortia are presented and a correlation is developed between the parent compound's anaerobic degradation pathway and its electronic properties. Predictions of reductive dechlorination pathways for compounds whose pathways have not been determined experimentally are made based upon the resulting correlation.

Observed reductive dechlorination pathways for unacclimated consortia are presented with model predictions in the Results and discussion.

Computational method

Semi-empirical methods are used in many quantum mechanical calculations. The MNDO (Modified Neglect of Differential Overlap) method is often used because of its success in providing reasonable enthalpies and geometries for many molecules (Goldblum 1987). MNDO is commonly used to determine electronic structure, chemical properties, and reactivity of molecular systems.

All semiempirical computations were performed using the standard MNDO method found in MOPAC versions 4.01 and 5.01 on the IBM supercomputer at the Cornell National Supercomputer Facility. The starting coordinates for the molecules were obtained from PC Model, a program for molecular modeling. Full optimization of all geometric variables including bond length, bond angle, and dihedral angle was specified for each structure. The MNDO program performs geometry optimizations on the starting molecular geometry with the BFGS (Broyden-Fletcher-Goldfarb-Shanno) optimizer.

Several criteria control the precision of the calculations and termination of execution. Self consistence is achieved when the change in energy for the molecule is less than 0.00001 kcal/mol on successive iterations. The heats of formation must differ by less than 0.002 kcal/mole on successive cycles, and the projected changes in the molecular geometry must be less than 0.0001 Angstroms. When the above tests are satisfied, execution of the program terminates.

Results and discussion

The sum of the carbon and chlorine charges obtained from the MNDO program was calculated for all carbon-chlorine bonds and correlated with reductive dechlorination pathways observed for unacclimated microbial consortia. These consortia include:

- fresh anaerobic digester sludge, anaerobic soils, or sediments,
- consortia that have not been exposed to a single or small number of chlorinated organic compounds for the purpose of enrichment, and

- consortia for which multiple reductive dechlorination pathways were not observed.

Many chlorophenols, chlorinated dihydroxybenzenes, chlorobenzoates, and chloroanilines exist in the environment as ionized species. However, because the mechanism of the reductive dechlorination process is still unclear and intermediates are unknown, the correlation was developed for the unionized molecule.

Chlorophenols

Observed reductive dechlorination pathways for chlorophenols are summarized in Table 1. Unacclimated microbial consortia preferentially dechlorinate chlorophenols at the *ortho* position. Reductive dechlorination of pentachlorophenol yields 2,3,4,5-tetrachlorophenol, which is further dechlorinated to yield 3,4,5-trichlorophenol. Similarly, sequential dechlorination of 2,4,6-TCP at the *ortho* positions yields 2,4-DCP and 4-CP. Preferential removal of chlorines at the *ortho* position has

Table 1. Summary of observed and predicted dechlorination pathways for chlorophenols.

Parent compound	Observed metabolite	Reference	Predicted metabolite[1]
PCP	2,3,4,5-TeCP	Woods et al. 1989	2,3,4,5-TeCP
PCP	3,4,5-TCP[2]	Mikesell & Boyd 1985	
2,3,5,6-TeCP			2,3,5-TCP
2,3,4,5-TeCP	3,4,5-TCP	Woods et al. 1989	3,4,5-TCP
2,3,4,6-TeCP	ND[3]		2,3,4-TCP
2,3,6-TCP	ND		2,3-DCP
2,3,5-TCP	ND		3,5-DCP
3,4,5-TCP	3,5-DCP	Mikesell & Boyd 1985	3,5-DCP
	3,4-DCP	Woods et al. 1989	
2,3,4-TCP	ND		3,4-DCP
2,4,5-TCP	3,4-DCP	Mikesell & Boyd 1985	3,4-DCP
2,4,6-TCP	2,4-DCP	Woods et al. 1989	2,4-DCP
2,4,6-TCP	4-CP[4]	Mikesell & Boyd 1985	
2,6-DCP	2-CP	Boyd & Shelton 1984	2-CP
	2-CP	Woods et al. 1989	
	2-CP	Hale et al. 1990	
2,3-DCP	3-CP	Boyd & Shelton 1984	3-CP
	3-CP	Woods et al. 1989	
	3-CP	Hale et al. 1990	
2,5-DCP	3-CP	Boyd & Shelton 1984	3-CP
	3-CP	Suflita & Miller 1985	
	3-CP	Gibson & Suflita 1986	
3,5-DCP	3-CP	Woods et al. 1989	3-CP
3,4-DCP	4-CP	Mikesell & Boyd 1985	3-CP
	4-CP	Woods et al. 1989	
	3-CP	Gibson & Suflita 1986	
2,4-DCP	4-CP	Boyd & Shelton 1984	4-CP
	4-CP	Suflita & Miller 1985	
	4-CP	Gibson & Suflita 1986	
	4-CP	Woods et al. 1989	
	4-CP	Kohring et al. 1989	
	4-CP	Hale et al. 1990	

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[1] Reductive dechlorination is assumed to occur at the position with the most negative carbon-chlorine bond charge.

[2] 2,3,4,5-TeCP was not observed, but is an assumed intermediate.

[3] Not determined.

[4] 24-DCP was not observed, but is an assumed intermediate.

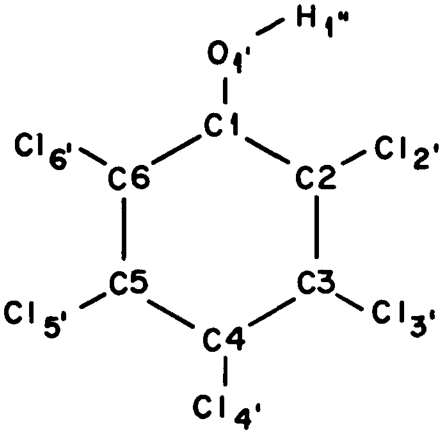

Fig. 1. Model position identification for pentachlorophenol.

also been observed for 2,4,5-TCP and 2,3-, 2,5-, and 2,6-DCP.

Net carbon-chlorine bond charges were calculated for all chlorophenol congeners and evaluated with respect to observed reductive dechlorination pathways. The charge pattern for PCP is presented in Table 2 and corresponds with the position numbering shown in Fig. 1. Although PCP appears to be a symmetrical molecule, MNDO calculations indicate that the hydrogen atom of the hydroxyl group is bent out of the plane of the benzene ring and toward one of the chlorine atoms yielding different charges for the two *ortho* chlorines. Evaluation of PCP's net carbon-chlorine bond charges indicates that the most negative value (−0.144) occurs at the position adjacent to the hydroxyl group (position 6). The observed reductive dechlorination pathway for PCP incorporates dechlorination at the *ortho* position (position 6) to yield 2,3,4,5-TeCP. Results for PCP suggest preferential dechlorination at the most negatively charged position (Table 1).

Comparison of the net carbon-chlorine charges indicates that for each chlorophenol possessing an *ortho* chlorine, the most negative charge occurs at an *ortho* position and *ortho* dechlorination is the observed dechlorination pathway (Tables 1 and 3). Observed reductive dechlorination pathways for compounds without an *ortho* chlorine were also evaluated with respect to bond charges. The observed pathway for 3,4,5-TCP by a consortium exposed to a group of nine chlorophenols (Woods et al. 1989) and unacclimated bacteria (Mikesell & Boyd 1985) involves dechlorination at the *meta* and *para* positions, respectively. The net carbonchlorine charges of 3,4,5-TCP indicate that the largest negative charge occurs at the *para* position, suggesting reductive dechlorination at the *para* position, consistent with Mikesell & Boyd's (1985) results with unacclimated bacteria. Similarly, the correlation suggests *para* dechlorination of 3,4-DCP to yield 3-CP. This is in contrast to the observed *meta* dechlorination of 3,4-dichlorophenol by Mikesell & Boyd (1985) and Woods et al. (1989), but consistent with the observed production of 3-CP by Gibson & Suflita (1986).

The observed reductive dechlorination pathways for eleven chlorophenols are summarized in Table 1. For two of these chlorophenols, 2,6- and 3,5-DCP, dechlorination is possible at only one position. Multiple products were observed for 3,4,5-TCP and 3,4-DCP. For the remaining seven chlo-

Table 2. Pentachlorophenol atomic charge pattern.

Carbon (Cn)[1]	Carbon charge	Atom (An')[1]	Atom charge	Atoms Cn-An'	Net charge[2]
C1	+0.180	O-1'	−0.214	1-1'	−0.035
C1		H-1''	+0.220	1'1''	+0.006
C2	−0.027	Cl-2'	−0.033	2-2'	−0.060
C3	+0.067	Cl-3'	−0.032	3-3'	+0.035
C4	−0.022	Cl-4'	−0.037	4-4'	−0.060
C5	+0.077	Cl-5'	−0.033	5-5'	+0.044
C6	−0.084	Cl-6'	−0.060	6-6'	−0.144

[1] Refer to Fig. 1; 'n' is the position on the ring beginning with the primary functional group.

[2] Sum of the carbon and atom charges at each position.

Table 3. Summary of carbon-chlorine bond charges for chlorophenols.[1]

Carbon	Atom	Net charge	Atom	Net charge	Atom	Net charge
Pentachlorophenol						
1	O	− 0.035				
C2	Cl	− 0.060				
C3	Cl	+ 0.035				
C4	Cl	− 0.060				
C5	Cl	+ 0.044				
C6	**Cl**	**− 0.144**				
Tetrachlorophenols						
	2,3,5,6-TeCP		*2,3,4,5-TeCP*		*2,3,4,6-TeCP*	
C1	O	− 0.047	O	− 0.071	O	− 0.040
C2	**Cl**	**− 0.148**	**Cl**	**− 0.146**	Cl	− 0.072
C3	Cl	+ 0.006	Cl	− 0.005	Cl	+ 0.028
C4	H	+ 0.038	Cl	− 0.063	Cl	− 0.093
C5	Cl	− 0.002	Cl	+ 0.029	H	+ 0.133
C6	Cl	− 0.065	H	+ 0.041	**Cl**	**− 0.174**
Trichlorophenols						
	2,3,6-TCP		*2,3,5-TCP*		*3,4,5-TCP*	
C1	O	− 0.054	O	− 0.083	O	− 0.105
C2	Cl	− 0.078	**Cl**	**− 0.151**	H	+ 0.039
C3	Cl	− 0.012	Cl	− 0.013	Cl	− 0.021
C4	H	+ 0.009	H	+ 0.040	**Cl**	**− 0.068**
C5	H	+ 0.103	Cl	− 0.044	Cl	− 0.014
C6	**Cl**	**− 0.182**	H	+ 0.038	H	− 0.036
	2,3,4-TCP		*2,4,5-TCP*		*2,4,6-TCP*	
C1	O	− 0.076	O	− 0.076	O	− 0.004
C2	**Cl**	**− 0.160**	**Cl**	**− 0.180**	Cl	− 0.106
C3	Cl	+ 0.018	H	+ 0.122	H	+ 0.122
C4	Cl	− 0.099	Cl	− 0.097	Cl	− 0.130
C5	H	+ 0.092	Cl	− 0.014	H	+ 0.131
C6	H	+ 0.011	H	+ 0.032	**Cl**	**− 0.187**
Dichlorophenols						
	2,6-DCP		*2,3-DCP*		*2,5-DCP*	
C1	O	− 0.060	O	− 0.093	O	− 0.092
C2	**Cl**	**− 0.195**	**Cl**	**− 0.165**	**Cl**	**− 0.188**
C3	H	+ 0.094	Cl	− 0.023	H	+ 0.090
C4	H	− 0.020	H	+ 0.005	H	+ 0.006
C5	H	+ 0.087	H	+ 0.058	Cl	− 0.055
C6	Cl	− 0.118	H	+ 0.006	H	+ 0.029
	3,5-DCP		*3,4-DCP*		*2,4-DCP*	
C1	O	− 0.118	O	− 0.112	O	− 0.085
C2	H	+ 0.037	H	− 0.049	**Cl**	**− 0.193**
C3	**Cl**	**− 0.063**	Cl	− 0.021	H	+ 0.117
C4	H	+ 0.037	**Cl**	**− 0.106**	Cl	− 0.139
C5	Cl	− 0.055	H	+ 0.077	H	+ 0.087
C6	H	− 0.039	H	+ 0.011	H	+ 0.002

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[1] Bold characters indicate the carbon-chloride bond with the most negative charge.

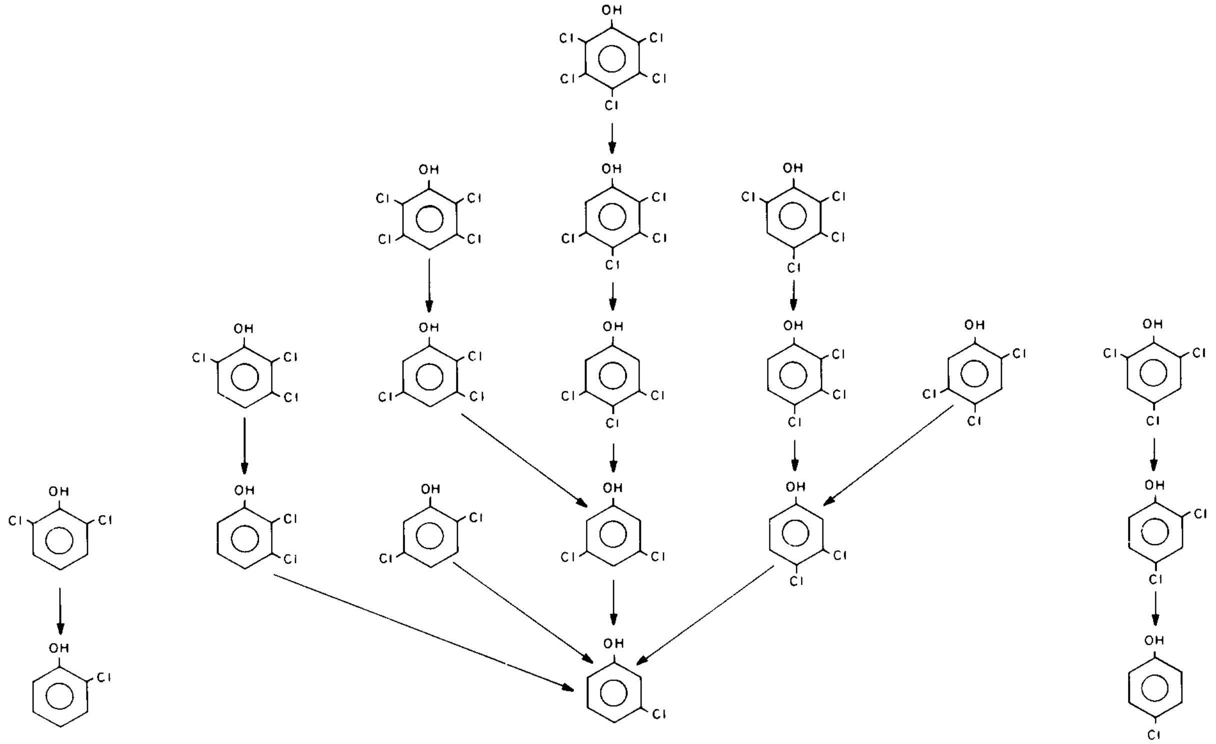

Fig. 2. Summary of chlorophenol degradation pathway predictions.

rophenols, the most negative carbon-chloride bond charge correlated perfectly with the observed position for reductive dechlorination.

Based on the correlation between charge patterns and reductive dechlorination pathways, predictions of reductive dechlorination pathways were made for several chlorophenols whose dechlorination pathways by unacclimated bacteria have not yet been determined experimentally. 2,3,4- and 2,3,5-trichlorophenol are expected to be dechlorinated at the *ortho* position. For unsymmetrical chlorophenol molecules possessing two *ortho* chlorines, charge patterns suggest that an *ortho* chlorine of 2,3,4,6-TeCP (position 6) is removed yielding 2,3,4-TCP (Table 3). Similarly, 2,3,6-TCP should be dechlorinated at the 6 position to yield 2,3-DCP. Thus, in comparing the two *ortho* chlorines of 2,3,4,6-TeCP and 2,3,6-TCP, the lone chlorine in the 6 position is more likely to be dechlorinated than the chlorine in the 2 position, between a hydroxyl group and a chlorine.

A summary of pathway predictions based on bond charges is presented in Fig. 2. Chlorinated phenols are initially dechlorinated at the *ortho* positions, followed by dechlorination at the *para* position. The model suggests that chlorophenols with two *ortho* chlorines are more likely to be reductively dechlorinated by the removal of the lone *ortho* chlorine. Because *meta* and *para* chlorines are less likely to be dechlorinated than *ortho* chlorines, 3,4,5-TCP, 3,4-DCP, and 3,5-DCP are expected to accumulate in anaerobic environments exposed to a variety of chlorophenols.

Chlorinated dihydroxybenzenes

To examine the correlation between carbon-chlorine bond charges and degradation pathways for chlorinated dihydroxybenzenes, the reductive dechlorination pathway for chlorocatechols were evaluated. An unacclimated methanogenic consortium converted tetrachloroveratrole (tetrachloro-1,2-dimethoxybenzene) to tetrachloroguaiacol followed by tetrachlorocatechol prior to reductive dechlorination (Woods et al. 1989). The subsequent appearance of 3,4,5-TCC and 4,5-DCC suggests that chlorocatechols are dechlorinated in a manner similar to that of the chlorophenols, sequential removal of the chlorines in the *ortho* positions.

Comparison of the net carbon-chlorine charges for TeCC indicates that the most negative charges are again adjacent to the hydroxyl groups (Table 4). Dechlorination of TeCC at the *ortho* position to yield 3,4,5-TCC is suggested by the net carbon-chlorine bond charges and is consistent with the observed degradation pathway. The second *ortho* chlorine should be removed from 3,4,5-TCC to produce 4,5-DCC. For 4,5-DCC, the chlorines in the 4 and 5 position have approximately equal charges, and removal of either chlorine yields the same product, 4-chlorocatechol. Thus, model predictions correlate well with the observed reductive dechlorination pathway for tetrachlorocatechol.

Because the most negative bond charge correlates with observed positions of reductive dechlorination for chlorophenols and chlorocatechols, these values were used to predict reductive dechlorination pathways for several other chlorinated

Table 4. Summary of carbon-chlorine bond charges for chlorinated dihydroxybenzenes.

Carbon	Atom	Net charge	Atom	Net charge	Atom	Net charge	Atom	Net charge
Chlorocatechols								
	TeCC		*3,4,5-TCC*		*4,5-DCC*		*3,4-DCC*	
C1	O	−0.082	O	−0.122	O	−0.128	O	−0.124
C2	O	−0.083	O	−0.186	O	−0.217	O	−0.100
C3	**Cl**	**−0.117**[1]	**Cl**	**−0.108**	H	+0.001	**Cl**	**−0.131**
C4	Cl	−0.013	Cl	−0.039	**Cl**	**−0.067**	Cl	−0.064
C5	Cl	−0.015	Cl	−0.039	Cl	−0.062	H	+0.052
C6	**Cl**	**−0.117**	H	+0.059	H	+0.053	H	−0.036
Chlororesorcinols								
	TeCR		*2,4,6-TCR*		*4,5,6-TCR*		*2,4,5-TCR*	
C1	O	+0.007	O	−0.007	O	−0.042	O	−0.032
C2	**Cl**	**−0.280**	Cl	−0.212	H	−0.014	**Cl**	**−0.281**
C3	O	+0.007	O	−0.002	O	−0.044	O	−0.010
C4	Cl	−0.122	**Cl**	**−0.230**	**Cl**	**−0.198**[2]	Cl	−0.121
C5	Cl	+0.069	H	+0.166	Cl	+0.067	Cl	+0.024
C6	Cl	−0.119	Cl	−0.151	**Cl**	**−0.201**[2]	H	−0.011
	2,4-DCR		*4,5-DCR*		*2,5-DCR*			
C1	O	−0.041	O	−0.075	O	−0.049		
C2	**Cl**	**−0.290**	H	−0.088	**Cl**	**−0.282**		
C3	O	−0.017	O	−0.051	O	−0.047		
C4	Cl	−0.159	**Cl**	**−0.203**	H	−0.014		
C5	H	+0.121	Cl	+0.011	Cl	−0.015		
C6	H	−0.039	H	−0.011	H	−0.016		
Chlorohydroquinones								
	TeCH		*2,3,6-TCH*					
C1	O	−0.090	O	−0.127				
C2	Cl	−0.023	Cl	−0.110				
C3	Cl	−0.023	Cl	−0.034				
C4	O	+0.090	O	−0.095				
C5	**Cl**	**−0.102**	H	+0.083				
C6	**Cl**	**−0.102**	**Cl**	**−0.140**				

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[1] Bold characters indicate the carbon-chlorine bond with the most negative value.

[2] Bond charges within 5% of the largest negative value are shown in bold type.

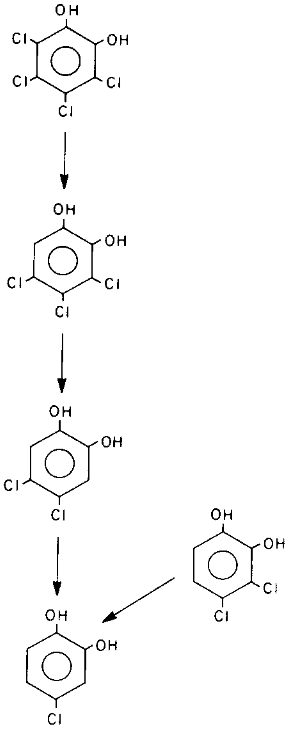

Fig. 3. Summary of chlorocatechol degradation pathway predictions.

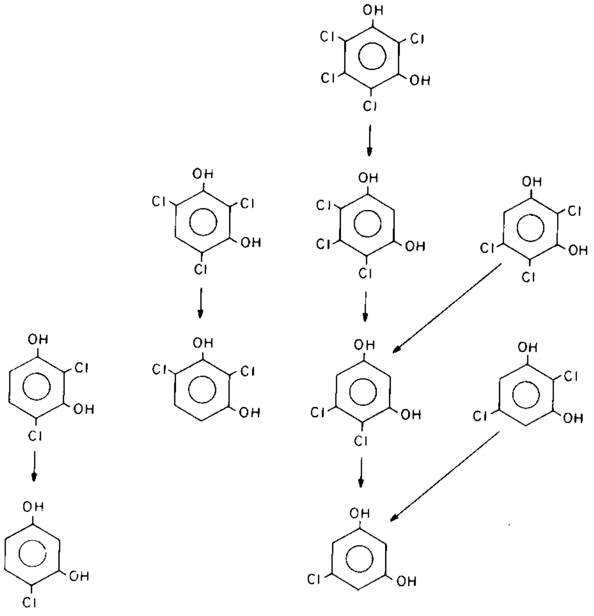

Fig. 4. Summary of chlororesorcinol degradation pathway predictions.

dihydroxybenzenes. 3,4-DCC is expected to be reductively dechlorinated at the *ortho* position to yield 4-CC (Fig. 3). For chlororesorcinols, the chlorine in the position between the two hydroxyl groups or in the position *ortho* to one hydroxyl group and *para* to the other possesses the most negative charge and is predicted to be most likely to be removed (Fig. 4).

Although it appears that the charges at each chlorinated position of tetrachlorohydroquinone should be equal since the molecule appears symmetrical, based upon the MNDO calculations the hydrogen atoms of the hydroxyl groups are bent out of the plane of the ring yielding different net charges (Table 4). Removal of any chlorine yields trichlorohydroquinone (Fig. 5). The model predicts that the lone chlorine of TCH will be dechlorinated to produce 2,3-DCH. This is similar to the tendency for removal of a lone chlorine from chlorophenols.

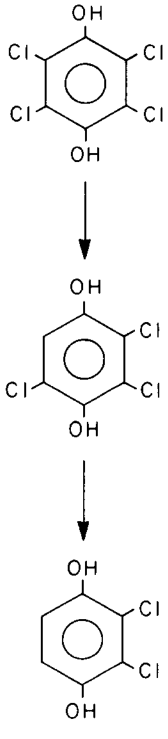

Fig. 5. Summary of chlorohydroquinone degradation pathway predictions.

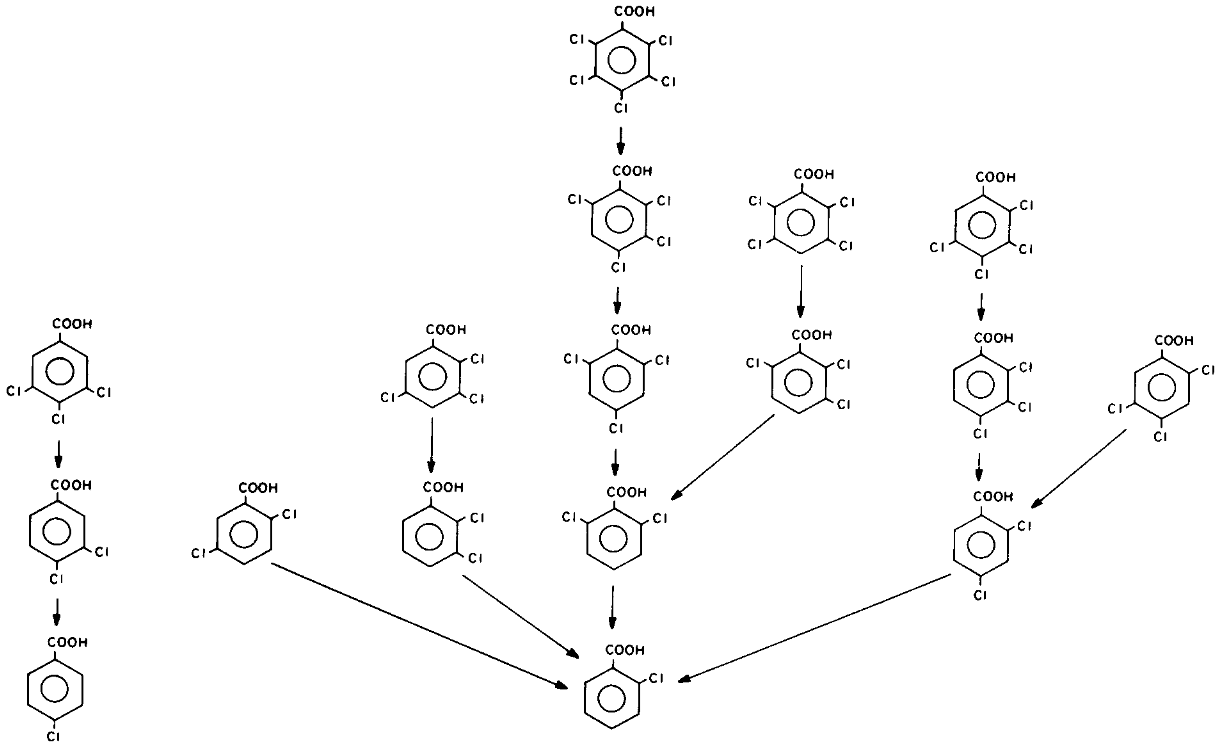

Fig. 6. Summary of chlorobenzoic acid degradation pathway predictions.

Chlorobenzoic acids

Chlorinated benzoic acids are preferentially dechlorinated by unacclimated microbial consortia at the position *meta* to the carboxyl groups. The reductive dechlorination of 2,3,6-trichlorobenzoate yields 2,6-dichlorobenzoate (Horowitz et al. 1983). Similarly, 2-chlorobenzoate is the observed metabolite from the reductive dechlorination of 2,5-dichlorobenzoate; and 4-chlorobenzoate is observed from the dechlorination of 3,4-dichlorobenzoate (Suflita et al. 1982).

The carbon-chlorine bond charges were evaluated for chlorobenzoic acids with known pathways (2,3,6-TCBz, 2,5-DCBz, and 3,4-DCBz). Values are presented in Table 5. The charge of the *meta* chlorine of 2,3,6-TCBz is -0.052, clearly a much larger negative value than either *ortho* chlorine. Similarly, the charges of the *meta* chlorines of 2,5-DCBz and 3,4-DCBz are 2 to 2.5 times the value of the charge for the *ortho* or *para* chlorines. Thus, the largest negative bond charge correlates well with the observed preference for chlorobenzoic acid dechlorination at the *meta* position.

The correlation was used to predict reductive dechlorination pathways for all chlorobenzoic acid congeners (Table 5, Fig. 6). The most negative carbon-chlorine net charges occurred at the positions *meta* to the carboxyl group for the chlorobenzoates possessing a *meta* chlorine. Those congeners containing two *meta* chlorines are expected to be dechlorinated at the chlorine position with an adjacent site containing a hydrogen. For example, 2,3,4,5-TeCBz is expected to produce 2,3,4-TCBz in contrast to 2,4,5-TCBz. 2,3,5-TCBz is expected to produce 2,3-DCBz in contrast to 2,5-DCBz.

Chlorobenzoic acids which do not possess a *meta* chlorine are expected to be preferentially dechlorinated at the *para* position. The correlation suggests reductive dechlorination of 2,4,6-TCBz to produce 2,6-DCBz and 2,4-DCBz to produce 2-chlorobenzoic acid. Because the model predicts

Table 5. Summary of carbon-chlorine bond charges for chlorobenzoic acids.

Carbon	Atom	Net charge	Atom	Net charge	Atom	Net charge
Pentachlorobenzoic Acid						
C1	C	+ 0.273				
C2	Cl	+ 0.022				
C3	Cl	− 0.006				
C4	Cl	+ 0.023				
C5	**Cl**	**− 0.009**				
C6	Cl	+ 0.025				
Tetrachlorobenzoic Acids						
	2,3,4,6-TeBz		*2,3,5,6-TeCBz*		*2,3,4,5-TeCBz*	
C1	C	+ 0.273	C	+ 0.273	C	+ 0.258
C2	Cl	+ 0.010	Cl	+ 0.014	Cl	+ 0.011
C3	**Cl**	**− 0.013**	**Cl**	**− 0.040**	Cl	− 0.017
C4	Cl	− 0.008	H	+ 0.113	Cl	+ 0.016
C5	H	+ 0.078	**Cl**	**− 0.040**	**Cl**	**− 0.041**
C6	Cl	− 0.008	Cl	+ 0.013	H	+ 0.117
Trichlorobenzoic Acids						
	3,4,5-TCBz			*2,3,5-TCBz*		*2,4,6-TCBz*
C1	C	+ 0.237	C	+ 0.252	C	+ 0.275
C2	H	+ 0.109	Cl	+ 0.005	Cl	− 0.024
C3	**Cl**	**− 0.057**	Cl	− 0.049	H	+ 0.079
C4	Cl	+ 0.012	H	+ 0.103	**Cl**	**− 0.044**
C5	Cl	**− 0.055**	**Cl**	**− 0.072**	H	+ 0.079
C6	H	+ 0.111	H	+ 0.111	Cl	− 0.025
Trichlorobenzoic Acids						
	2,3,6-TCBz			*2,3,4-TCBz*		*2,4,5-TCBz*
C1	C	+ 0.348	C	+ 0.255	C	+ 0.257
C2	Cl	− 0.001	Cl	− 0.003	Cl	− 0.025
C3	**Cl**	**− 0.052**	**Cl**	**− 0.025**	H	+ 0.077
C4	H	+ 0.077	Cl	− 0.020	Cl	− 0.019
C5	H	+ 0.056	H	+ 0.052	**Cl**	**− 0.049**
C6	Cl	− 0.027	H	+ 0.092	H	+ 0.109
Dichlorobenzoic Acids						
	3,4-DCBz		*2,5-DCBz*		*2,3-DCBz*	
C1	C	+ 0.245	C	+ 0.251	C	+ 0.326
C2	H	+ 0.137	Cl	− 0.034	Cl	− 0.009
C3	**Cl**	**− 0.068**	H	+ 0.045	**Cl**	**− 0.067**
C4	Cl	− 0.018	H	+ 0.081	H	+ 0.071
C5	H	+ 0.035	**Cl**	**− 0.091**	H	+ 0.023
C6	H	+ 0.053	H	+ 0.104	H	+ 0.076
	2,6-DCBz		*2,4-DCBz*			
C1	C	+ 0.438	C	+ 0.255		
C2	**Cl**	**− 0.132**	Cl	− 0.040		
C3	H	+ 0.048	H	+ 0.071		
C4	H	+ 0.048	**Cl**	**− 0.056**		
C5	H	+ 0.047	H	+ 0.047		
C6	Cl	− 0.040	H	+ 0.081		

ortho chlorines to be most resistant to removal, accumulation of 2,6-DCBz and 2-chlorobenzoic acid is expected in anaerobic environments exposed to a variety of chlorobenzoates.

Chloroanilines

Experimental results indicate that chlorinated anilines are preferentially dechlorinated by unacclimated bacteria at the positions *ortho* and *para* to the amine group (Kuhn & Suflita 1989). The observed dechlorination products of 2,3,4,5-TeCA are 2,3,5-TCA and 3,5-DCA. 3,4-DCA is converted to 3-CA (Kuhn & Suflita 1989; Struijs & Rogers 1989).

Consideration of the carbon-chlorine bond charges for 2,3,4,5-TeCA indicates that the most negative value is at the position *ortho* to the amine group to yield 3,4,5-TCA (Table 6). However, dechlorination for this compound was observed at the *para* position. 3,5-DCA was correctly predicted as the metabolic product of 2,3,5-TCA due to the dechlorination of the *ortho* chlorine. Removal of a *para* chlorine from 3,4-DCA was predicted, which is also consistent with the observed pathway. Therefore, the carbon-chlorine bond charges correlated with the position of dechlorination except in the case of 2,3,4,5-TeCA.

Although, reductive dechlorination has not been observed for those chloroanilines which do not contain chlorines in adjacent positions on the aromatic ring, reductive dechlorination pathways were predicted using the model. Unlike the chlorinated phenols, dihydroxybenzenes, or benzoates, examination of the charges (Table 6, Fig. 7) suggests the removal of chlorines from *ortho, meta,* and *para* positions. Dechlorination at the *ortho* position is predicted for 2,3,4,5-TeCA, 2,3,5-TCA, 2,3,4-TCA, 2,3-DCA, and 2,4-DCA. While 2,4,5-TCA and 2,5-DCA contain *ortho* chlorines, they are predicted to be dechlorinated at the *meta* position. When no *ortho* chlorines are present, the model predicts the removal of a *meta* chlorine from 3,4,5-TCA and a *para* chlorine from 3,4-DCA. Thus, an environment contaminated with a variety of chloroanilines would be expected to accumulate each of the three monochloroaniline isomers.

Table 6. Summary of bond charges for chloroanilines.

Carbon	Atom	Net charge	Atom	Net charge	Atom	Net charge	Atom	Net charge
	2,3,4,5-TeCA							
C1	N	− 0.134						
C2	**Cl**	**− 0.103**						
C3	Cl	+ 0.015						
C4	Cl	− 0.060						
C5	Cl	− 0.012						
C6	H	+ 0.009						
	2,3,5-TCA		*3,4,5-TCA*		*2,3,4-TCA*		*2,4,5-TCA*	
C1	N	− 0.144	N	− 0.309	N	− 0.141	N	− 0.320
C2	**Cl**	**− 0.105**	H	+ 0.156	**Cl**	**− 0.113**	Cl	+ 0.003
C3	Cl	− 0.026	**Cl**	**− 0.147**	Cl	+ 0.006	H	+ 0.053
C4	H	+ 0.044	Cl	− 0.011	Cl	− 0.098	Cl	− 0.057
C5	Cl	− 0.051	Cl	− 0.080	H	+ 0.086	**Cl**	**− 0.096**
C6	H	+ 0.009	H	+ 0.131	H	− 0.015	H	+ 0.140
	2,3-DCA		*3,4-DCA*		*2,4-DCA*		*2,5-DCA*	
C1	N	− 0.152	N	− 0.170	N	− 0.144	N	− 0.313
C2	**Cl**	**− 0.116**	H	+ 0.001	**Cl**	**− 0.147**	Cl	− 0.022
C3	Cl	− 0.038	Cl	− 0.037	H	+ 0.104	H	+ 0.043
C4	H	+ 0.011	**Cl**	**− 0.101**	Cl	− 0.134	H	+ 0.078
C5	H	+ 0.052	H	+ 0.071	H	+ 0.080	**Cl**	**− 0.173**
C6	H	− 0.020	H	− 0.015	H	− 0.025	H	+ 0.122

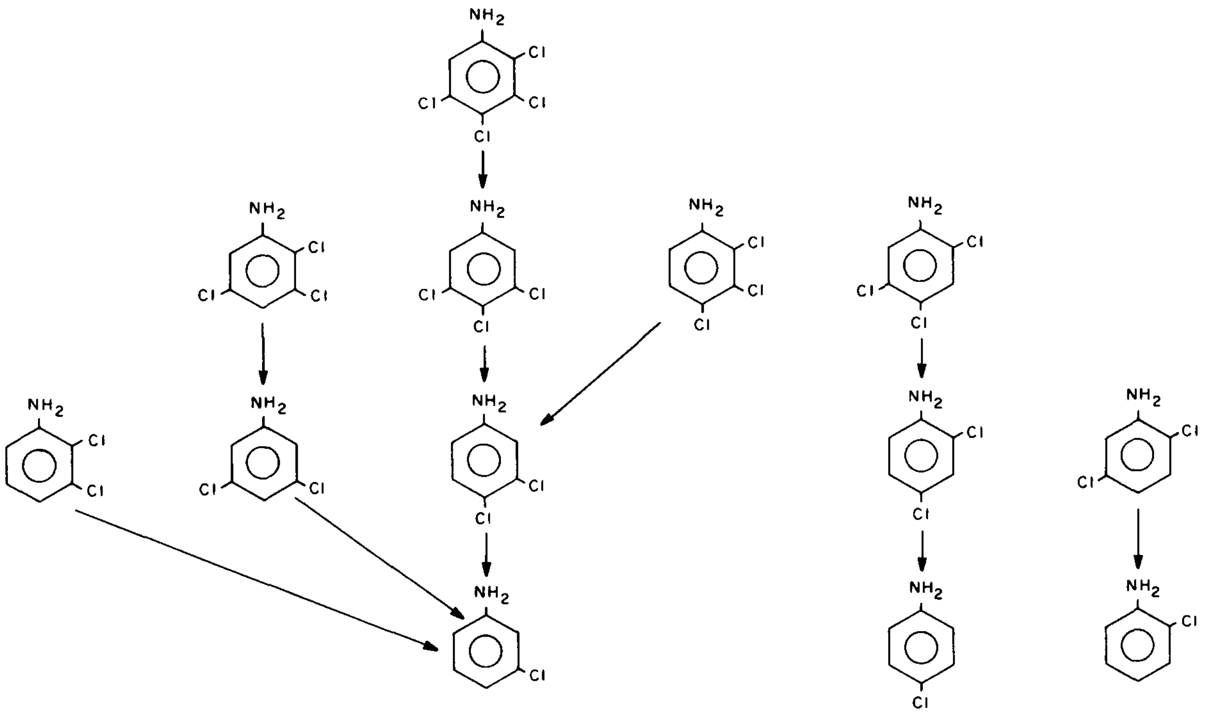

Fig. 7. Summary of chloroaniline degradation pathway predictions.

Conclusions

Although many factors including species distribution, enzyme interactions, steric hindrance, and thermodynamics are not taken into consideration, the carbon-chlorine bond charges correlate well with observed positions for reductive dechlorination reactions for a variety of chlorinated aromatic compounds. The model is based upon the assumption that the chlorine in the region (represented by the carbon-chlorine bond charge) with the largest negative charge is most likely to be reductively dechlorinated. Of 16 dechlorination pathways observed for unacclimated bacteria, the most negative carbon-chlorine bond charge correlated with 15 pathways.

The following is a summary of the correlation between carbon-chlorine bond charges and reductive dechlorination pathways.

Chlorophenols

- The model correctly predicts observed reductive dechlorination pathways for PCP, 2,3,4,5-TeCP, 2,4,5-TCP, 2,4,6-TCP, 2,3-DCP, 2,5-DCP, 2,4-DCP.
- For each chlorophenol possessing an *ortho* chlorine, the most negative carbon-chlorine bond charge occurs at an *ortho* position, suggesting preferential *ortho* dechlorination.
- Unsymmetrical chlorophenols possessing two *ortho* chlorines are expected to be dechlorinated at the *ortho* position with an adjacent site free of a chlorine.
- Once *ortho* chlorines are removed, the correlation suggests preferential removal of the *para* chlorine.

Chlorinated dihydroxybenzenes

- The correlation correctly predicts the observed reductive dechlorination pathway for tetrachlorocatechol, sequential removal of the chlorines in the two *ortho* positions to produce 3,4,5-TCC and 3,4-DCC.

- Based upon the model, chlorocatechols and chlorohydroquinones are also expected to be preferentially dechlorinated at the *ortho* positions.
- Chlororesorcinols are expected to be dechlorinated at the chlorine in a position between the two hydroxyl groups or *ortho* to one hydroxyl group and *para* to the other.
- The model predicts that for hydroquinones containing two chlorines *ortho* to one of the hydroxyl groups, the *ortho* chlorine with an adjacent site free of chlorine is most likely to be removed.

Chlorobenzoic acids

- The model correctly predicts all observed chlorobenzoic acid dechlorination pathways for unacclimated consortia.
- Carbon-chlorine bond charges suggest preferential dechlorination of chlorines in the *meta* positions.
- Those chlorobenzoic acids containing two *meta* chlorines are expected to be dechlorinated at the chlorine position with an adjacent site free of a chlorine.
- Chlorobenzoates which do not contain a *meta* chlorine would be expected to be preferentially dechlorinated at the *para* position.

Chloroanilines

- Chloroanilines have been observed to be dechlorinated at both the *ortho* and *para* positions.
- Carbon-chlorine bond charges correlate with the observed pathway for 2,3,5-TCA and 3,4-DCA, but does not correlate with the observed pathway for 2,3,4,5-TeCA.
- The model suggests that chlorines are removed from the *ortho, meta* and *para* positions of chloroanilines. However unlike the chlorophenols or chlorinated dihydroxybenzenes, *ortho* dechlorination is not exclusive but predominates for chloroanilines possessing an *ortho* chlorine.

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