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Regiospecificity of Reductive Dechlorination of Chlorophenols in Mono- and Di-Chlorophenol Adapted Anoxic Sediments

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Abstract

The regiospecific potential for the reductive dechlorination of 2-, 3-, 4-, 2,3-, 2,4-, and 3,4-chlorophenols (CPs) was studied in mono- and di-CP(DCP) adapted sediment slurries(10% solids).

Freshwater sediments adapted to transform 2-CP dechlorinated all tested mono- and di-CPs except 4-CP without a lag period. Adaptation to 2-CP, thus, enhanced the onset of dechlorination of 3-CP and all *ortho*-substituted CPs tested. Sediment adapted to transform 3-CP dechlorinated all tested CPs, except 4-CP and 2,4-DCP, without a lag period. Sediments adapted to individual DCPs (2,3-, 2,4-, and 3,4-DCP) exhibited dechlorination (no lag phase) of 2-CP, 2,3-, 2,4-, and 3,4-DCP. Interestingly, *meta*-cleavage of 3,4-DCP in all tested adapted sediment occurred, while *para*-cleavage occurred in 3,4-DCP adapted sediment.

Sediments adapted to dechlorinate *ortho* and *meta*-chlorines exhibited a preference for *meta* following *ortho*-cleavage, but not for *para*-cleavage, while the preference for reductive dechlorination was *ortho* > *meta* > *para* for mono-CPs and *ortho* > *para* > *meta* for DCPs in unadapted freshwater anoxic sediments.

Key Words : Reductive dechlorination, Dichlorophenol(DCP), Regiospecificity, Adapted anoxic sediments

1. Introduction

As a consequence of rapid developments in the fields of industrial and agricultural chemicals, high quantities of organic compounds have been manufactured and their use has resulted in environmental contamination(Reineke 1984). Among these compounds, chlorinated aromatic compounds are well publicized as being deleterious to natural environments(Young 1984) and chlorinated phenols are produced on a scale of tons annually(Reineke 1984; Circelli 1978). For example, over 50,000 tons of pentachlorophenol (PCP) are produced annually worldwide (Steiert 1985). Because of their abundance and toxicity, some chlorophenols, including 2-CP, 2,4-DCP, 2,4,6-TCP, and PCP, are included in the U.S. E.P.A.'s list of priority pollutants (Keith 1979).

The reductive dechlorination of CPs in anaerobic ecosystems has been investigated using a variety of anoxic environmental sources, such as digesters, aquifers, and freshwater sediments and marine sediments(Madsen and Amand 1992; King 1988; Boyd and Shelton 1984; Hale et al. 1990; Kohring et al. 1989;

Capone et al. 1983; Chudoba et al. 1989; Hrudey et al. 1987). The anaerobic biotransformation of chlorophenols initially involves the reductive dechlorination (removal) of selected chlorine atoms and eventual total chlorine removal prior to cleavage of the aromatic nucleus ring. Chlorine removal generally results in less toxic forms of the organic contaminant.

The regiospecificity of reductive dechlorination has been investigated by several groups. For example, Hale et al.(1990) also investigated the cross-acclimation of chlorophenols by dichloro-phenoladapted freshwater sediments, and reported a wide range of substrate specificity. Zhang and Wiegel(1990) reported that the transformations of 2,4-DCP to 4-CP and subsequently to phenol and benzoate were catalyzed by different groups of anaerobic microorganisms. At present, it is generally accepted that at least two distinct groups of dechlorinating microorganisms exist in natural environments. To date, only two microorganisms have been isolated in pure culture capable of reductive dehalogenation : *Desulfomonile tiedjei* DCB-1 (Shelton and Tiedje 1984) and dehalogenating strain DCB-2 (Madsen and Light 1992). The activity by *D. tiedijei* exhibits preferentially to the removal of *meta* chlorine-substituents from a variety of halogenated aromatic substrates such as PCP, 2346- and 2356-TeCP, mono halogenated benzates etc., while strain DCB-2 preferentially removed *ortho* chlorines from chlorinated phenols and the *meta*-chlorine of 3,4-DCP.

The purpose of this study was to investigate the potential and regiospecificity of reductive dechlorination of mono- and diCPs in CP-adapted sediment slurries. Anoxic sediment slurries were initially adapted to specific chlorophenols by repeated amendment of the chlorophenol substrate.

2. Meterials and Methods

2-1. Sediment Collection and Preparation of Adapted Sediment.

Samples of anoxic sediment and site water were collected from a freshwater pond(Cherokee Trailer Park, Athens, GA, U.S.A). Mason jars were filled with surface sediment (approximately top 10cm), capped, and stored at 2-3℃ before processing. Prior to experimental setup, containers of sediment were placed in an anaerobic chamber (Coy Laboratory Products, Inc. Ann Arbor, Michigan, U.S.A) and sediment was passed through a 1-mm sieve to remove plant debris and large particles. Site water was purged with O_2-free N_2 gas for approximately 30 min and was subsequently used to dilute the sieved sediment to 10%(w/v) solids. Characteristics of the 10% sediment slurries are presented in Table 1 (Kong and Jones 1992). An aliquot of a concentrated aqueous stock solution (10,000 mg/L) of CPs was added to separate amber bottles containing approximately 600ml of the sediment slurry to yield a final CP concentration of 10 mg/L. Bottles were capped with a rubber stopper and incubated at 22-24℃ inside the anaerobic chamber. Additional aliquots of the CP-stock solutions were added when the parent CP concentration in the sediment slurries dropped to less than 1 mg/L. After a minimum of five amendments of the chlorophenol substrate, 30ml of the CP- adapted sediment slurries were transferred to amber serum vials(60 ml) which were capped with butyl rubber stoppers and aluminum crimp sealed. Anaerobic biotransformation experiments were then initiated by adding an aliquot of the anoxic chlorophenol stock solution to achieve a final chlorophenol concentration of 10 mg/L. All manipulations (distribution of sediment, CP-amendments, etc) were conducted inside the anaerobic chamber. All experiments were performed in duplicate and sediment slurries were incubated at room temperature(22-24℃). All chemicals(chlorophenols) were the highest purity and purchased from Aldrich Chemical Co. Inc.(Milwaukee, WI, U.S.A.).

2-2. Analytical Procedures

Quantitation of chlorophenols in sediment slurries was determined as follows : a subsample of the sediment slurry(0.5ml) was mixed with an equal volume of acetonitrile, vortexed for 20 sec, and centrifuged at 8,000 x g for 5 min (Eppendorf Centrifuge, model 5415C). The supernatant solution was collected, filtered (0.22㎛ GWSP filter, Millipore), and analyzed by HPLC. The chromatographic system consisted of a Waters C18μBondpak column(3.9 ×300mm), a Perkin-Elmer LC600 autosampler, a Waters 490 programmable multiwavelength detector operated at 280 nm, and a Waters 590 programmable HPLC pump. The mobile phase was methanol : water : acetic acid (60:38:2 v/v/v) delivered at a flow rate of 2ml/min. CP substrates and products were identified and quantitated by comparison to known standards.

3. Discussion

The times required for lag periods and complete biotransformation(T_{100}) of the parent chlorophenol substrate in unadapted and chlorophenol-adapted sediment slurries (10% solids) are summarized in Table 2. Among these tested CPs in unadapted sediment slurries, differences were observed in the lag periods before the onset of dechlorination, during which no significant loss of substrate or appearance of products was detected, and in the T_{100}. The preference of reductive dechlorination of CPs in freshwater sediments observed in this study was in the order of ortho > meta > para for the mono-CPs, while the order of ortho > para > meta for the DCPs.

The dechlorination profiles of adapted-sediment slurries were distinctly different from those of unadapted (fresh) sediment slurries. No lag periods were observed before the onset of dechlorination in tested adapted sediment slurries amended with the respective chlorophenol substrate. Complete loss of the amended respective chlorophenol in adapted sediment slurries occurred within 8 days.

3-1. Reductive Dechlorination of CPs in Mono-CP Adapted Sediments.

The profiles of reductive dechlorination of mono- and di-CPs in the 2-CP adapted sediment slurries are presented in Fig. 1. As illustrated in Fig. 1 and Table 2, complete dechlorination of 3-CP occurred near day 15; no appreciable lag period was noted prior to onset of dechlorination of 3-CP in 2-CP adapted sediment slurries. Sediment slurries adapted to 2-CP did not dechlorinate 4-CP after a total incubation period of 35 days(Fig. 1A and Table 2). Of the DCP isomers examined, 2,4- and 2,3-DCP were dechlorinated at a rate similar to that of dechlorination of the parent chlorophenol (2-CP) (Fig. 1B-C); in these samples, dechlorination initially occurred at the ortho-chlorine position. The dechlorination of 2,3-DCP was rapid and resulted in the transient fromation of 3-CP which was completely dechlorinated by day 15. Reductive dechlorination of 2,4-DCP was also rapid and resulted in the formation of 4-CP which persisted for the remainder of the incubation period (50 days total) (Fig. 1C). Thus, 4-CP was persistent both as a product 3,4-DCP was noted and only 2-3 mg/L of the product 4-CP was detected(Fig. 1D).

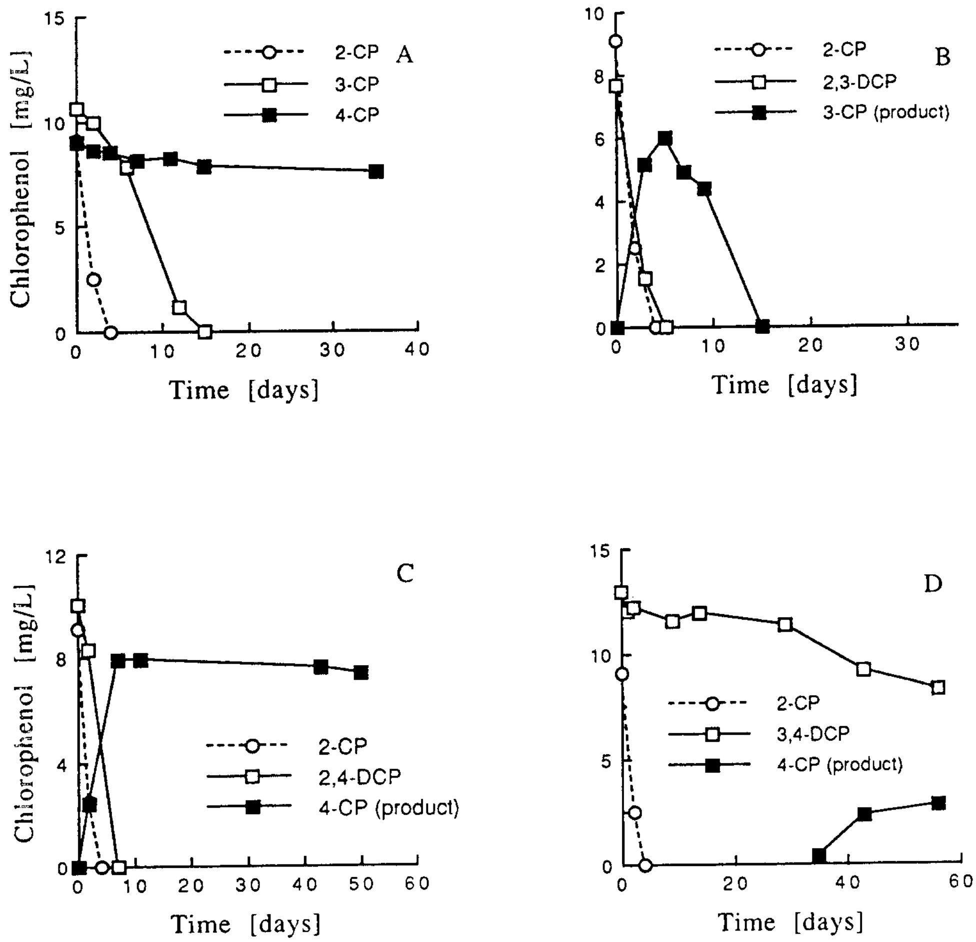

Figure 1. Reductive dechlorination of CPs in fresh water sediment slurries adapted to dechlorinate 2-CP and 2,3-DCP; C, dech lorination of 2-CP and 2,4-DCP; D. dechlorination of 2-CP and 3,4-DCP.

and as theparent compound in 2-CP adapted sediment. 3,4-DCP was also persistent in 2-CP adapted sediments. In contrast to the preferential para- cleavage of 3,4,-DCP in unadapted sediment slurries (Table 2), the slow but significant dechlorination of 3,4-DCP occurred only at the meta position in 2-CP adapted sediment slurries. After incubation for 55 days, a 35% reduction in the initial concentration of

The profiles of reductive dechlorination of CPs in the 3-CP adapted sediment slurries are presented in Fig. 2. Complete dechlorination of 2-CP occurrred within 4 days(Fig. 2A and Table 2), while dechlorination of 4-CP did not occur after a total incubation period of 30 days (Fig. 2A). Of the DCP isomers tested, 2,4-, and 3,4-DCP were dechlorinated whthout a lag period and complete loss of the parent isomer

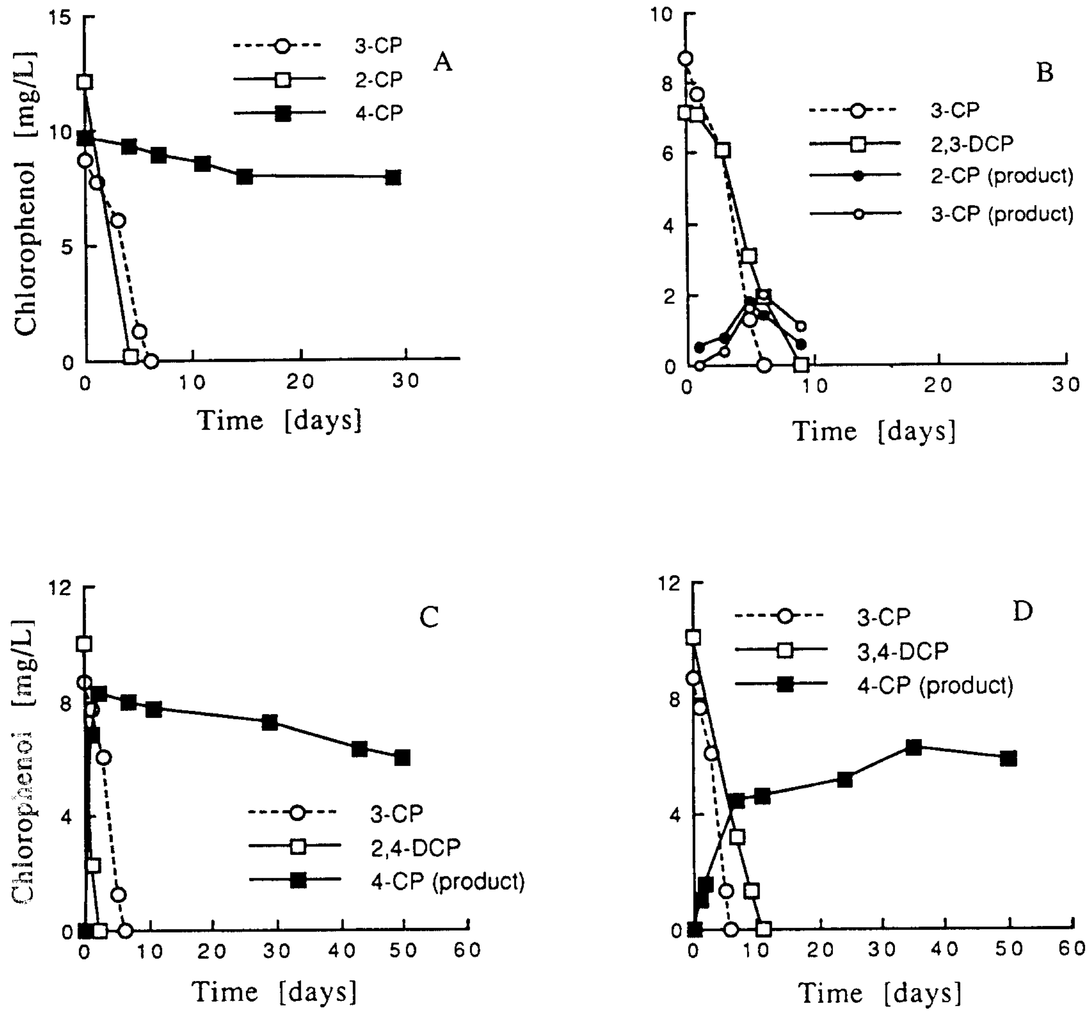

Figure 2. Reductive dechlorination of CPs in freshwater sediment slurries adapted to dechlorinate 3-CP; A, dechlorination of 2-, 3-, and 4-CP; B, dechlorination of 3-CP and 2,3-DCP; C, dechlorination of 3-CP and 2,4-DCP; D. dechlorination of 3-CP and 3,4- DCP.

occurred after 3 days and 11 days, respectively. 2,3-DCP was completely dechlorinated after 8 days incubation following a short (2 days) lag period ; both 2- and 3-CP appeared as transient dechlorination products which were subsequently dechlorinated by day 10 (Fig. 2B). 4-CP, the product of 2,4-DCP, was persistent and little or no appreciable dechlorination was noted after 50days of total incubation(Fig. 2C). 3,4-DCP was dechlorinated at the *meta* position to yield 4-CP which also persisted for the entire incubation period of 50 days (Fig. 2D).

3-2. Reductive Dechlorination of CPs in DCP Adapted Sediments.

The profiles of reductive dechlorination of CPs in 2,3-DCP adapted sediment slurries are presented in Fig. 3. Complete loss of the amended concentration of all DCPs in DCP

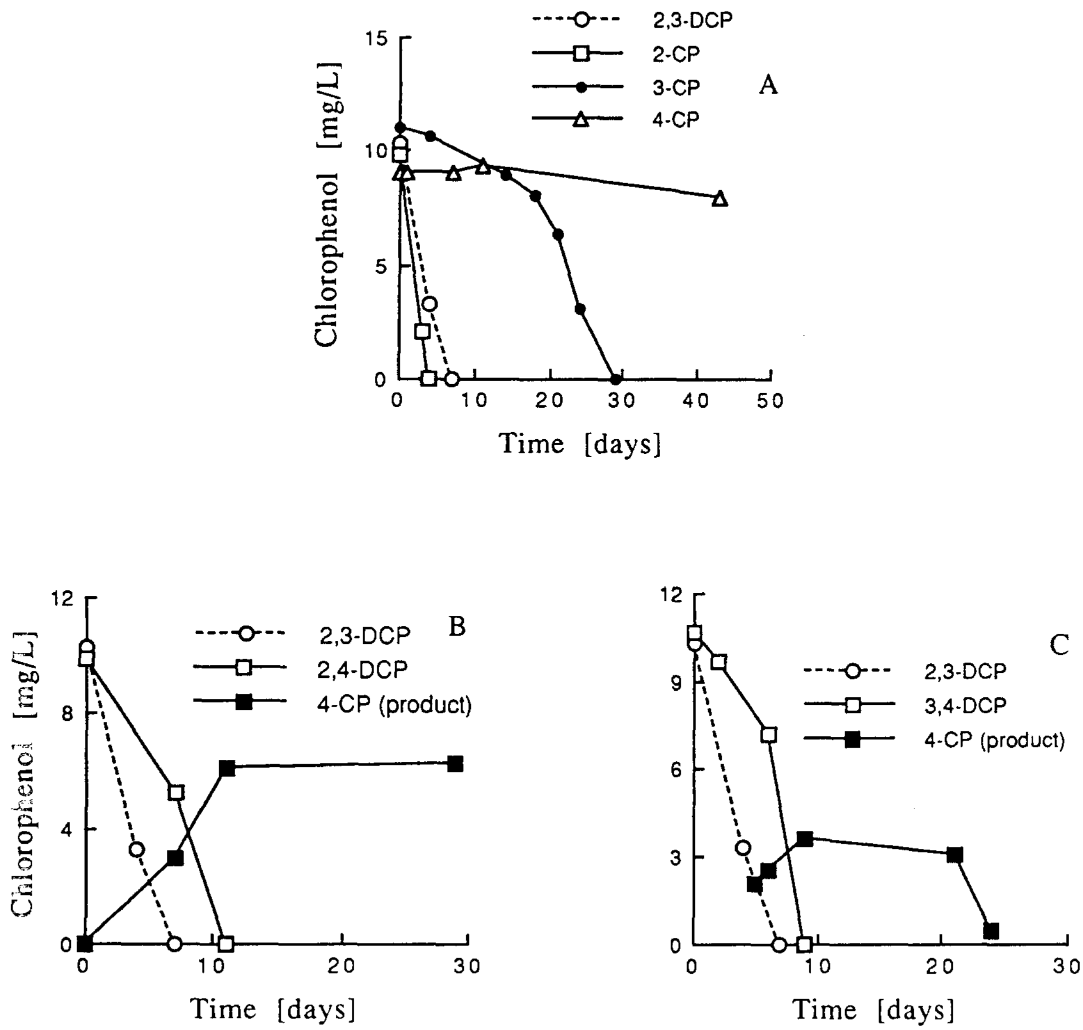

Figure 3. Reductive dechlorination of CPs in freshwater sediment slurries adapted to dechlorinate 2,3-DCP; A, dechlorination of 2-, 3-, 4-CP, and 2,3-DCP; B, dechlorination of 2,3- and 2,4-DCP; C, dechlorination of 2,3- and 3,4- DCP.

adapted sediment slurries occurred within 5 days with no appreciable lag periods. Reductive dechlorination of 2-CP was rapid and total dechlorination occurred within 5 days, which was slightly faster than dechlorination of 2,3-DCP (Fig. 3A). Reductive dechlorination in sediments amended with 3-CP was complete within 30 days and occurred at a slow rate compared to the dechlorination of the parent chlorophenol (2,3-DCP). In this case, the lag period before the onset of dechlorination was approximately 15 days. Dechlorination of 4-CP, however, was not observed in 2,3-DCP adapted sediments event after 45 days of incubation. Reductive dechlorination of 2,4- and 3,4-DCP occurred at slower rates compared to dechlorination of 2,3-DCP (parent compound). Approximately 12 and 9 days, respectively, were required for complete dechlorination of 2,4- and 3,4-DCP. In both cases, 4-CP appeared as the

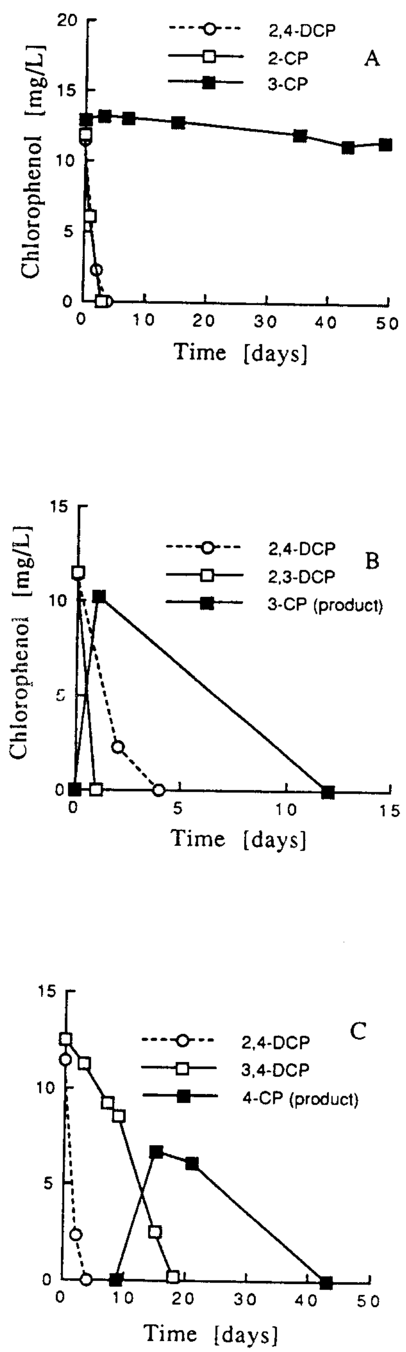

Figure 4. Reductive dechlorination of CPs in fresh-water sediment slurries adapted to dechlorinate 2,4-DCP; A, dechlorination of 2-, 3-, 4-CP, and 2,4-DCP; B, dechlorination of 2,3- and 2,4-DCP; C, dechlorination of 2,4- and 3,4- DCP.

transient dechlorinations (Table 2) suggesting that *ortho-* and *meta-* directed dechlorinations were preferred over the *para* dechlorination in 2,3-DCP adapted sediments. However, transient product of 2,4-DCP, 4-CP, was persistent during 30 days incubation, while that of 3,4-DCP was dechlorinated within 25 days(Fig. 3B, C).

The profiles of reductive dechlorination of 2-CP, 3-CP, 4-CP, 2,3-DCP, and 3,4-DCP in 2,4-DCP adapted sediment slurries are presented in Fig. 4.. Dechlorination of 2-CP and 2,3-DCP occurred rapidly and at a rate similar to the dechlorination of the parent chlorophenol (2,4-DCP). In both cases, dechlorination occurred at the *ortho*-chlorine position (Fig. 4A, B). 3-CP, which appeared as a transient product of the dechlorination of 2,3-DCP, was subsequently dechlorinated within an additional 15 days of incubation. However, 3-CP added to 2,4-DCP adapted sediments was not dechlorinated during the 50 day incubation period. The slow *meta*-directed dechlorination of 3,4-DCP was complete after approximately 18 days and occurred without an appreciable lag periods; 4-CP was produced as an intermediate dechlorination product and was subsequently dechlorinated within a total of 43 days (Fig. 4C).

The profiles of reductive dechlorination of 2-CP, 3-CP, 4-CP, 2,3-DCP, 2,4-DCP, and 3,4-DCP in 3,4-DCP adapted sediment slurries are presented in Fig.5. Sediment slurries rapidly (within 4 days) dechlorinated 2-and 3-CP at a rate similar to the dechlorination of 3,4-DCP. However, 4-CP added to 3,4-DCP adapted sediments was not transformed during the 45 day incubation period (Fig. 5A). The rate of dechlorination of 2,4-and 2,3-DCP was also similar to that of 3,4-DCP. 2-CP and low levels of 3-CP were produced as transient products of

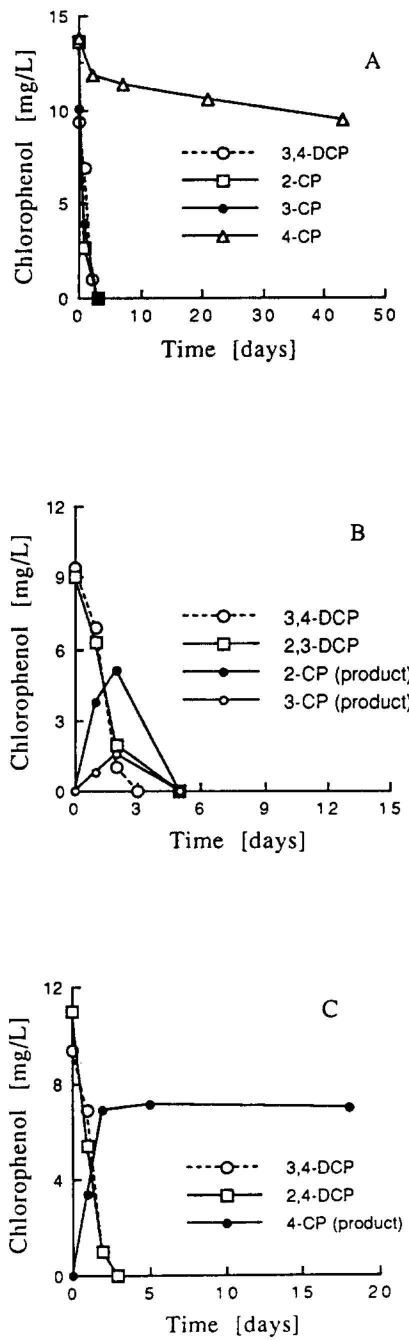

Figure 5. Reductive dechlorination of CPs in freshwater sediment slurries adapted to dechlorinate 3,4-DCP; A, dechlorination of 2-, 3-, 4-CP, and 3,4-DCP; B, dechlorination of 2,3- and 3,4-DCP; C, dechlorination of 2,4- and 3,4- DCP.

dechlorination of 2,3-DCP (Fig. 5B). Both products were completely transformed after a total incubation period of 5 days. 4-CP was produced as a product of dechlorination of 2,4-DCP and persistent during 20 days incubation periods.

Table 1. Chemical characteristics of Cherokee pond sediment slurry (10% w/v).

pH	TOC[a]	Sulfate [mg/L]	Total Metal [mg/L] Cu	Cr	Cd	Aqueous Metal [mg/L] Cu	Cr	Cd
7.0	16.0	0.8	5.77	4.71	<0.1	all less than 0.03		

[a]Total organic carbon [mg Carbon/g solid]

Table 2. Reductive Dechlorination of Mono-and Di-CPs in Unadapted and CP-Adapted Sediments

Substrate	Unadapted Sediment	Sediment Adapted to : 2-CP	3-CP	2,3-DCP	2,4-DCP	3,4-DCP
2-CP	12a (30)	0 (4)	0 (4)	0 (5)	0 (4)	0 (4)
3-CP	59 (70)	0 (15)	0 (6)	15 (30)	> 50	0 (4)
4-CP	70 (95)	> 35	> 30	> 45	-	> 45
2,3-DCP	10 (21)	0 (5)	2 (8)	0 (7)	0 (2)	0 (5)
2,4-DCP	8 (20)	0 (6)	0 (3)	0 (12)	0 (4)	0 (4)
3,4-DCP	14 (22)	> 55	0 (11)	0 (9)	0 (18)	0 (4)
	Initial Products					
2,3-DCP	3->2-CP	3-CP	2-& 3-CP	3-CP	3-CP	2->3-CP
2,4-DCP	4-CP	4-CP	4-CP	4-CP	4-CP	4-CP
3,4-DCP	3-CP	4-CP	4-CP	4-CP	4-CP	3-CP

[a]Lag periods [days]
Values in parenthesis represents the time required for total substrate disappearance [days]

4. Results or Conclusion

In anoxic sediments and sludges, it is generally accepted that the primary processes responsible for reductive dechlorination of chloro-aromatic compounds are catalyzed by biological organisms even though it has not always been evident that the dehalogenating activity was biologically mediated (Mohn and Tiedje 1992). Complete inhibition of reductive dechlorination reactions have been observed in sterilized (autoclaved) experimental microcosms which lends evidence that (Hale et al. 1990; Kong and Jones 1993) reductive dechlorination are catalyzed by biologically dependent reactions. Recently, Hale et al. (1991) provided additional evidence to support the biologically mediated reductive dechlorination process; the authors reported that the dehalogenating microbial community, as measured by most probable number (MPN) analyses, increased in anoxic sediment slurries following sequential additions of selected dichlorophenols. In addition, Mohn and Tiedje (1992) postulated that reductive dehalogenation activities are catalyzed by distinct microbial populations with different dehalogenating enzymes; they further stated that the specificities of the reactions may reside at the level of enzymes, organisms, or broad physiological microbial groups.

In our investigation, sediment slurries adapted to 2-CP dechlorinated most tested CPs without a lag period and at rates significantly greater than unadapted sediments. Some CPs, however, including 4-CP and 3,4-DCP were not dechlorinated in 2-CP adapted sediments, indicating specificity of the dechlorination reaction by the CP-adapted microbial population adapted to dechlorinate 2-CP exhibited an expected preference for *ortho*-dechlorination but also dechlorinated the *meta*-chlorine of 3-CP and 2,3-DCP at a moderate rate. However, this same adapted microbial population only exhibited a very slow rate of *meta*-dechlorination of 3,4-DCP after an appreciable lag period.

Sediment slurries adapted to dechlorinate 3-CP (*meta*-specificity) dechlorinated 2-CP, 2,3-, 2,4-, and 3,4-DCP without lag periods and at rates comparable to dechlorination of 3-CP. Dechlorination of 4-CP, however, was not observed during the incubation period. Based on these results, at least two distinct dechlorinationg activities were demonstrated. CPs containing an *ortho*-or *meta*-chlorine were preferentially dechlorinated at the *ortho* position, followed by *meta*-chlorine dechlorination. In the second case, CPs containing *para*-chlorine was persistent in sediments adapted to dechlorinate *ortho*-and *meta*-chlorines. In a separate study, Boyd and Shelton (Boyd and Shelton 1984) investigated CP dehalogenation using anaerobic sludge adapted to the individual monochlorophenol isomers. These investigations also reported two distinct dehalogenation activities, one specific for *ortho* and *para* chlorines and the other specific fort the *meta* and *para* chlorines. More recently, Genthner et al. (1989) reported that freshwater sediments adapted to dechlorinate 2-CP did not dechlorinate chlorophenols at the *meta* or *para* positions and sediments adapted tó meta chlorine removal dechlorinated *ortho* (2-CP) chlorines but not *para* chlorines (4-CP). Thus, the substrate specificities of the various monochlorophenol-adapted cultures may be dependent on to a) the source of the indigenous microbial population; b) environmental or physiological condition; or c) the chemical nature of the CP substrate.

The reductive dechlorination of the *ortho*-chlorine of mono-and di-CPs was rapid in all CP-adapted sediments. Reductive dechlorination of 3-CP, however, occurred slowly and only after an appreciable lag period in 2,3-DCP adapted sediment; dechlorination of 3-CP did not occur in 2,4-DCP adapted sediment after incubation for 50 days. Sediments adapted to 2-CP, however, readily dechlorinated the lonely *meta*-chlorine (3-CP). These results suggest that the microbial population adapted to dechlorinate the lonely *ortho*-chlorine (2-CP) has different dechlorinating activity than the population adapted to dechlorinate ortho-chlorine containing DCPs. Interestingly, sediments adapted to dechlorinate 3,4-DCP had the broadest range of dechlorination activity. In unadapted sediments, 3,4-DCP was initially transformed to 3-CP, resulting in *para*-chlorine removal. However, as mentioned above, dechlorination of 4-CP was not observed when 4-CP was amended to sediments adapted to dechlorinate 3,4-DCP (*para*-cleavage). These results illustrate the diversity of dechlorination activities in anoxic sediment environments.

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Mono-와 Di-Chlorophenol에 적응시킨 혐기성 저질의 탈염소 특성

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(1993년 10월 7일 접수)

자연호소의 혐기성 저질을 특정한 chlorophenol(CP)에 적응시킨 후 다른 구조를 가진 CP에 대한 탈염소 특성을 검토하였다.

CP에 노출되지 않는 혐기성 저질에서는 mono-CP의 경우 ortho > meta > para-염소의 순으로 di-CP의 경우는 ortho > para > meta-염소의 순서로 짧은 지체기를 거친 후 탈염소가 발생하였다.

Mono-CP 중 2-CP에 적응된 저질은 4-CP와 3, 4DCP를 제외하고, 3-CP에 적응시킨 저질은 4-CP를 제외한 모든 시험물질에 대하여 지체기 없이 탈염소 특성을 나타내었다.

DCP에 적응된 모든 저질은 2-CP, 2, 3-, 2, 4-, and 3, 4-DCP을 지체기 없이 탈염소시켰다. 또한 초기에 para-염소기를 탈염소 시키는 3, 4-DCP에 적응된 저질에서는 노출된 4-CP의 탈염소가 발생하지 않았다. 이 결과에서 볼 때 mono-와 di-CP를 탈염소시키는 혐기성 미생물의 종류가 다양함을 알 수 있다.