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# Specificity of Reductive Dehalogenation of Substituted *ortho*-Chlorophenols by *Desulfitobacterium dehalogenans* JW/IU-DC1

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**Resting cells of *Desulfitobacterium dehalogenans* JW/IU-DC1 grown with pyruvate and 3-chloro-4-hydroxyphenylacetate (3-Cl-4-OHPA) as the electron acceptor and inducer of dehalogenation reductively *ortho*-dehalogenate pentachlorophenol (PCP); tetrachlorophenols (TeCPs); the trichlorophenols 2,3,4-TCP, 2,3,6-TCP, and 2,4,6-TCP; the dichlorophenols 2,3-DCP, 2,4-DCP, and 2,6-DCP; 2,6-dichloro-4-R-phenols, where R is -H, -F, -Cl, -$NO_2$, -$CO_2^-$, or -$COOCH_3$; 2-chloro-4-R-phenols (2-Cl-4-RPs, where R is -H, -F, -Cl, -Br, -$NO_2$, -$CO_2^-$, -$CH_2CO_2^-$, or -$COOCH_3$); and bromophenols (2-BrP, 2,6-DBrP, and 2-Br-4ClP). Monochlorophenols, the dichlorophenols 2,5-DCP, 3,4-DCP, and 3,5-DCP, the trichlorophenols 2,3,5-TCP, 2,4,5-TCP, and 3,4,5-TCP, and the fluorinated analog of 3-Cl-4-OHPA, 3-F-4-OHPA ("2-F-4-$CH_2CO_2^-$ P"), are not dehalogenated. A chlorine substituent in position 3 (*meta*), 4 (*para*), or 6 (second *ortho*) of the phenolic moiety facilitates *ortho* dehalogenation in position 2. Chlorine in the 5 (second *meta*) position has a negative effect on the dehalogenation rate or even prevents dechlorination in the 2 position. In general, 2,6-DCl-4-RPs are dechlorinated faster than the corresponding 2-Cl-4-RPs with the same substituent R in the 4 position. The highest dechlorination rate, however, was found for dechlorination of 2,3-DCP, with a maximal observed first-order rate constant of 19.4 $h^{-1}$ g (dry weight) of biomass$^{-1}$. There is no strong linear correlation between the logarithm of pseudo-first-order rate constants for the dehalogenation of 2,6-DCl-4-RPs and 2-Cl-4-RPs and electronic (Hammet $\sigma_m$), hydrophobic ($\pi$), and stearic ($E_s$) constants of the substituent R. The substrate specificity and induction pattern found for dehalogenation with the pure culture of *D. dehalogenans* and the original 2,4-DCP-enrichment, derived from a methanogenic sediment, were similar, suggesting that the conditions used led to only one type of dechlorinating organism.**

Chlorophenols are widespread toxic compounds that are included in the U.S. Environmental Protection Agency list of priority pollutants. Mineralization of chlorophenols in methanogenic environments often starts with reductive dechlorination to phenol and ends with formation of methane and carbon dioxide (1, 6, 7, 17, 18, 20–23). Regioselectivity of reductive dehalogenation in methanogenic sediments depends on the source of the microbial community and adaptation conditions (2, 8, 23).

Isolated organisms capable of reductively dehalogenating halophenols display broad substrate specificity. The sulfate-reducing *Desulfomonile tiedjei* DCB-1, obtained from a 3-chlorobenzoate-enriched community derived from sewage sludge (16), *meta*-dehalogenated a variety of substituted halobenzoates (5, 11) and chlorophenols (14). The clostridium-like dechlorinating bacterium DCB-2, isolated from a 2,4,6-trichlorophenol (TCP)-adapted enrichment culture derived from sewage sludge fermentation in Denmark, *ortho*-dehalogenated pentachlorophenol (PCP), 2,4,5-TCP, 2,4,6-TCP, and 2,4-dichlorophenol (DCP) and *meta*-dehalogenated 3,5-DCP (12, 13). It did not dehalogenate monochlorophenols, 3,4-DCP, or 3,4,5-TCP. Dietrich and Winter assumed that a spirillum in their coculture was responsible for dechlorination of 2-chlorophenol (CP) (4). However, the organism could not be isolated in pure culture.

Using 2,4-DCP as electron acceptor, we obtained from freshwater sediments (10) an enrichment that reductively *ortho*-dehalogenated 2,4-DCP (3). An anaerobic bacterium, *Desulfitobacterium dehalogenans*, capable of reductive *ortho* dechlorination of 2,4-DCP and 3-chloro-4-hydroxyphenylacetate (3-Cl-4-OHPA) was isolated from the enrichment when 3-Cl-4-OHPA was used as the electron acceptor (19). This organism grows optimally at pH 7.5 and at 38°C on pyruvate as the carbon source in the presence of yeast extract. It can use various alternative electron acceptors including sulfur, sulfoxy anions (except sulfate), nitrate, and fumarate, and it can use $H_2$ or pyruvate as the electron donor (19).

Here we present a study on substrate specificity and induction of the activity for reductive *ortho* dehalogenation of substituted halophenols by *D. dehalogenans.*

## MATERIALS AND METHODS

**Cultures and media.** The sediment-free enrichment (3) was cultivated anaerobically either in a mineral medium as described previously (19) with yeast extract (0.5%) as a carbon source or in autoclaved (121°C for 40 min) spent enrichment culture medium adjusted to pH 8.3 with sterile anaerobic 2 N NaOH. The spent culture medium was obtained by centrifugation (with anaerobic centrifuge tubes) of the enrichment culture grown in 0.5% yeast extract medium. The pH was kept within 8.0 to 8.5 by adjustment with anaerobic sterile 2 N NaOH. *D. dehalogenans* JW/IU-DC1 was grown in the base medium described previously (19) supplemented as indicated in the text at 37°C and pH 7.5.

**Dehalogenation in growing enrichment cultures.** Duplicate cultures of the 2,4-DCP-transforming consortium were grown in Hungate tubes containing 10 ml of 0.5 or 1% yeast extract-containing medium and various chloroaromatic compounds (30 ppm or 0.2 mM) in the presence and absence of 0.2 mM 2,4-DCP as the inducer. The enrichment which had previously transformed 30 ppm of 2,4-DCP was used as the inoculum (1 or 2%). The cultures were incubated at 31 or 37°C and frequently titrated to pH 8.5 with sterile anaerobic 2 N NaOH. The longest incubation time was 10 days.

**Dehalogenation by *D. dehalogenans* JW/IU-DC1.** To determine the substrate specificity of reductive dehalogenation by the purified culture, *D. dehalogenans* JW/IU-DC1 was grown in the base medium containing 0.1% yeast extract, 20 mM pyruvate, and 10 mM 3-Cl-4-OHPA as the inducer (purified-culture medium). After 3-C1-4-OHPA was dehalogenated, the culture was titrated back to pH 7.5, dispensed into Hungate tubes, and supplemented with halophenolic compounds. Concentrations of halophenols are indicated in the text and varied

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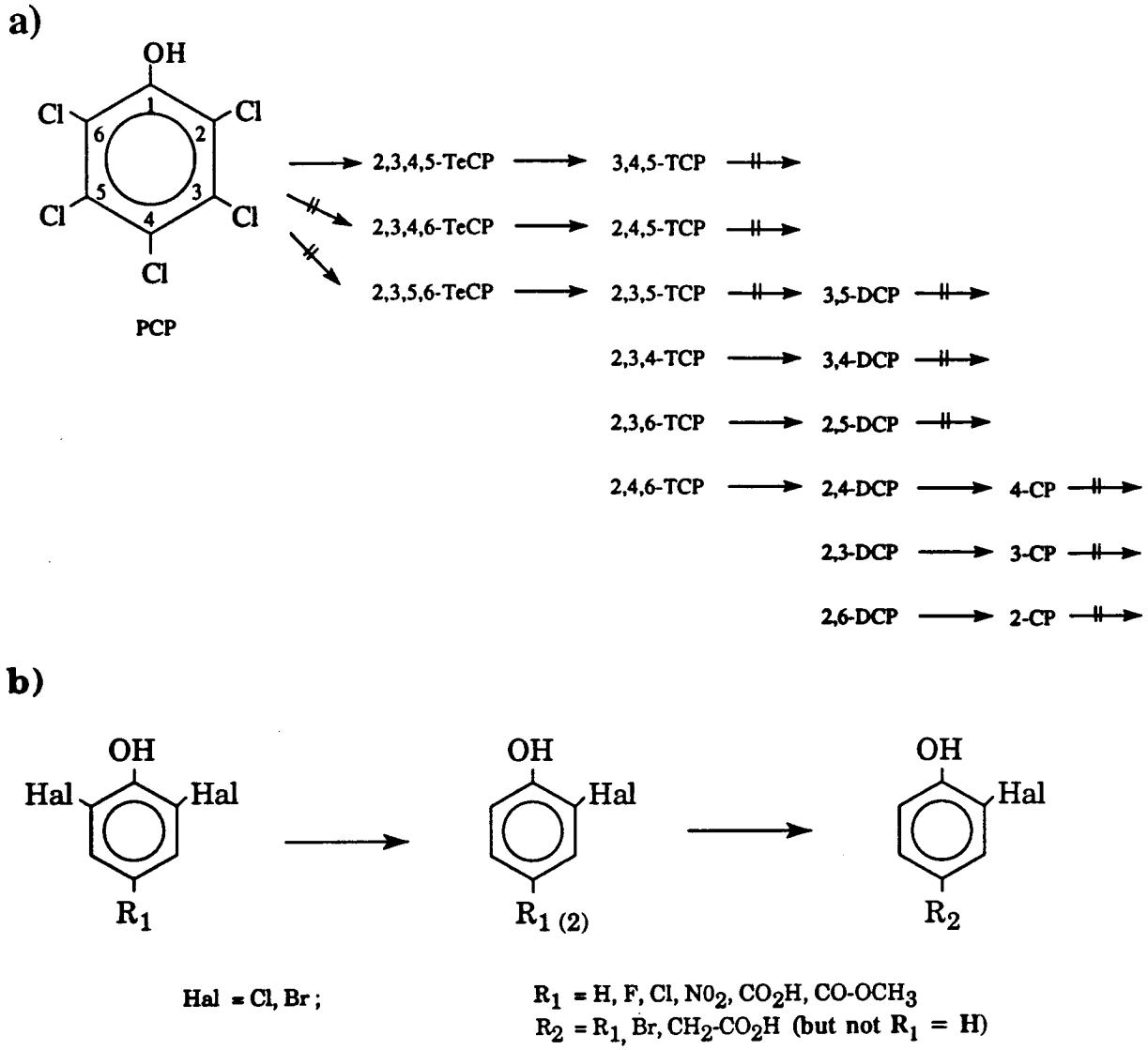


FIG. 1. General scheme of reductive *ortho* dechlorination with *D. dehalogenans* cultures grown in 0.1% yeast extract medium containing 20 mM pyruvate and 10 mM 3-Cl-4-OHPA as the inducer. (a) PCP and its possible dehalogenation products. (b) 2,6-DCl-4RP and 2-Cl-4RPs. Note that only a few compounds with Hal = Br were available for testing.

from 0.1 mM for PCP to 5 mM for 3-Cl-4-OHPA. The cultures were incubated at 37°C and pH 7.5. *D. dehalogenans*, grown in the base medium (19) supplemented with 0.1% yeast extract, 20 mM pyruvate, and usually 50 ppm of halophenol, was used to determine which halogenated aromatic compound induced its own dechlorination. The precultures for inoculation (10%, vol/vol) were grown in the same medium but supplemented with 5 mM thiosulfate as the electron acceptor instead of the halophenols. Control cultures were supplemented with chloramphenicol (100 μg/ml) prior to the addition of halophenols.

**Analytical procedures.** The various aromatic compounds were quantified by high-performance liquid chromatography (HPLC pump no. 2350, gradient programmer no. 2360, and detector no. UA-5 with a 280-nm filter [Isco, Inc.]; integrator no. SP4600 [Spectra-Physics]; and HPLC autosampler no. 738 [Alcott Chromatography] with the 150-mm reversed-phase column hypersil ODS-C18, 5 μm, [Altech; no. 9876]). The mobile phase consisted of methanol, water, and 0.5% acetic acid. The methanol/water ratio varied from 70:30 for PCP to 5:95 for 3-chloro-4-hydroxymandelate (3-Cl-4-OHM). Pseudo-first-order dechlorination rate constants were determined from the time course of substrate disappearance, using three to six points in the linear portions of graphs that related the logarithm of substrate concentration to time (data omitted). Compounds were identified by chromatography of standards at two different mobile-phase compositions. The chlorinated compounds were purchased from Aldrich, except for the tetrachlorophenols (TeCPs) and TCPs (Ultra Scientific, Kingstown, R.I.), 2,6-dichloro-4-nitrophenol [2,6-DCl-4-($NO_2$)P] (Pfaltz and Bauer Inc., Waterbury, Conn.), and

TABLE 1. Observed maximum dehalogenation rates and rate constants of selected chlorophenols by resting cells of *D. dehalogenans* induced by 3-Cl-4-OHPA[a]

| Chlorophenol | Dehalogenation rate (mmol $h^{-1}$ g [dry wt] of biomass$^{-1}$) | Pseudo-first-order rate constant ($h^{-1}$ g [dry wt] of biomass$^{-1}$) |
|---|---|---|
| 2,3-DCP | 5.8 | 19.4 |
| 2,4-DCP | 0.36 | 0.36 |
| 2,6-DCP | 2.8 | 2.8 |
| 2,4,6-TCP | 0.59 | 0.59 |
| PCP | 0.049 | 0.49 |

[a] Cultures of *D. dehalogenans* grown previously in 0.1% yeast extract medium containing 20 mM pyruvate and 10 mM 3-Cl-4-OHPA as an electron acceptor and inducer were supplemented with 1 mM 2,4-DCP, 1 mM 2,6-DCP, 1 mM 2,4,6-TCP, 0.3 mM 2,3-DCP, or 0.1 mM PCP and incubated at 37°C and pH 7.5. 2-CP, 2,5-DCP, 2,3,5-TCP, and 2,4,5-TCP were not dechlorinated.

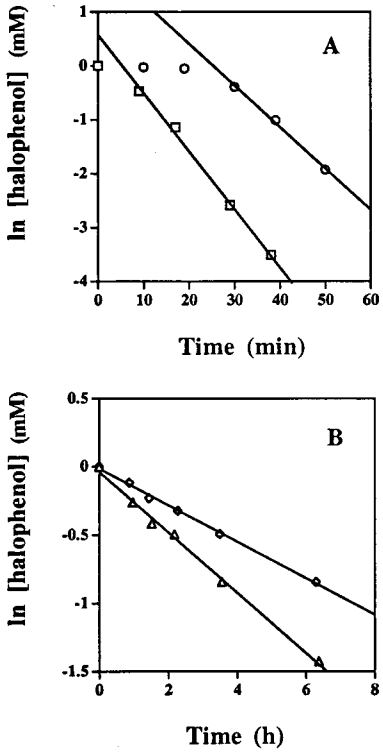


FIG. 2. Time course of the dehalogenation of 2,6-DCl-4-($CO_2CH_3$)P (○), 2,6-DCl-4-FP (□), 2,4-DCP (◇), and 2,4,6-TCP (△) by *D. dehalogenans* grown in 0.1% yeast extract medium containing 20 mM pyruvate and 10 mM 3-Cl-4-OHPA as the inducer at 37°C and pH 7.5.

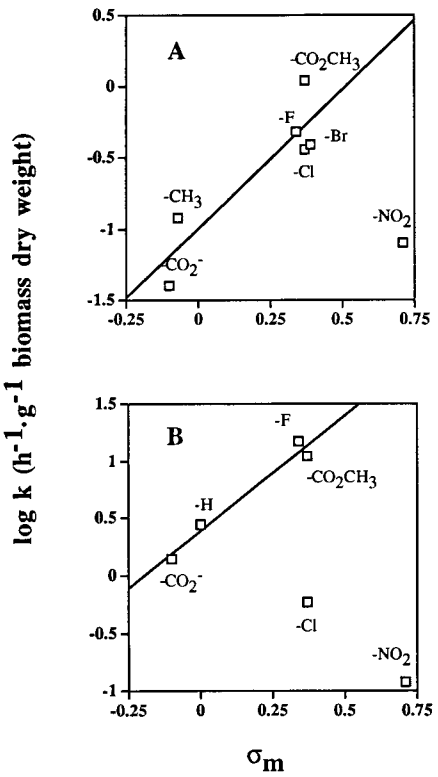


FIG. 3. Relationship between the logarithm of pseudo-first-order rate constants of the dechlorination of 2-Cl-4-RPs (A) and 2,6-DCl-4-RPs (B) and $\sigma_m$ values of the substituent R in the 4 (*para*) position of the phenolic moiety. The lines suggest possible trends but no firm relationships.

2-chloro-4-nitrophenol [2-Cl-4-($NO_2$)P] (ICN Biomedicals Inc., Costa Mesa, Calif.).

## RESULTS AND DISCUSSION

**Stereoselectivity of aryl reductive dehalogenation in *D. dehalogenans* cultures grown with 3-Cl-4-OHPA.** *D. dehalogenans* cultures grown in 0.1% yeast extract medium containing 20 mM pyruvate and 10 mM 3-Cl-4-OHPA [2-Cl-4-($CH_2CO_2^-$)P] as the inducer reductively *ortho*-dechlorinated a wide range of chlorophenols (Fig. 1). The 3-Cl-4-OHPA was chosen because of its solubility, since it is the second-fastest compound dechlorinated and is tolerated at concentrations above 20 mM, thus leading to relatively high densities of the cultures. 4-Substituted dichlorophenols of the type 2,6-DCl-4-RP, where R is -H, -F, -Cl, -$NO_2$, -$CO_2^-$, or -$COOCH_3$, were dechlorinated to the corresponding 2-Cl-4-RPs, and the chlorophenols of the type 2-Cl-4-RP, where R is -H, -F, -Cl, -Br, -$NO_2$, -$CO_2^-$, -$CH_2CO_2^-$, or -$COOCH_3$ were dechlorinated to the corresponding 4-RPs (Fig. 1b). The brominated phenols 2,6-DBrP and 2-BrP were transformed to phenol, and 2-Br-4-CP was transformed to 4-CP. The dechlorination of all tested halophenolic compounds followed pseudo-first-order kinetics with lag phases of 30 min or shorter (Fig. 2; Table 1), indicating that at substrate concentrations up to 1 mM no inhibition occurred under the conditions used. Monochlorophenols, the dichlorophenols 2,5-DCP, 3,4-DCP, and 3,5-DCP, the trichlorophenols 2,3,5-TCP, 2,4,5-TCP, and 3,4,5-TCP, and 3-F-4-OHPA (2F-4-acetyl P), the fluorinated analog of 3-Cl-4-OHPA, were not dehalogenated after 2 days of incubation under the conditions used. Thus, the organism is capable of reductive *ortho* dehalogenation of chlorophenols and *ortho*-bromophenols but not of *ortho*-fluorophenols.

None of the tested halogenated compounds were dehalogenated in sterile noninoculated 0.5% yeast extract medium at 37°C and pH 8.5 within 10 days; this indicated that dehalogenation was of microbial origin. However, 1-bromo-2-naphthol, 5-bromo-2,4-dihydroxybenzoate, and 4-bromoresorcinol (0.2 mM each) were completely dehalogenated under the control conditions within 60 h, indicating that a nonmicrobial dehalogenation was occurring.

**Induction of dehalogenation activity in cultures grown in the absence of chlorophenols but with thiosulfate as the electron acceptor.** *D. dehalogenans* grown with 20 mM pyruvate and 5 mM thiosulfate, when inoculated (10%, vol/vol) into fresh medium containing 20 mM pyruvate and 0.3 mM chlorophenols, completely *ortho*-dehalogenated 3-Cl-4-OHPA, 2,4,6-TCP, 2,4-DCP, 2,3-DCP, 2-Br-4-CP, 2-Br-4-$CH_3$-P, and 2-Cl-4-RP, where R is F, Br, $CO_3^-$, $CH_3$, and $COOCH_3$. These compounds, however, were not dehalogenated in thiosulfate-grown subcultures supplemented with chloramphenicol (100 μg/ml) prior to the addition of the halogenated compounds. Therefore, we conclude that the dehalogenation activity is inducible. Compounds which were dehalogenated by dehalogenation-induced cultures, i.e., cultures grown in the presence of 2,4-DCP or 3-Cl-4-OHPA, but were not dehalogenated in thiosulfate-grown cultures, i.e., cultures not induced for dechlorination, included PCP, 2,3,4,5-TeCP, 2,3,4,6-TeCP, 2,3,5,6-TeCP, 2,3,4-TCP, 2,3,6-TCP, 2,6-DCP, 2,6-DCl-4-FP, 2,6-DCl-(4-$CO_2^-$)P, 2,6-DBrP, and 2-BrP. Thus, generally most 2-Cl(or Br)-4-RP compounds were able to induce the dechlorination activity whereas most 2,6-DCl-4-RP compounds were not.

TABLE 2. Pseudo-first-order rate constants of reductive *ortho* dehalogenation of selected 2-chloro-4-R-phenols and 2,6-dichloro-4-R-phenols by *D. dehalogenans*[a]

| R | Descriptor[b] | | | Rate constant ($h^{-1}$ g [dry wt] of biomass$^{-1}$) for: | | |
|---|---|---|---|---|---|---|
| | $\sigma_m$ | $\pi$ | $E_s$ | 2-Cl-4-RP | 2,6-DCl-4-RP | 2-Br-4-RP |
| -H | 0 | 0 | 0 | ND[c] | 2.8 | >3[d] |
| -F | 0.34 | 0.14 | −0.46 | 0.48 | 15 | NT[e] |
| -Cl | 0.37 | 0.71 | −0.97 | 0.36 | 0.59 | 0.29 |
| -Br | 0.39 | 0.86 | −1.16 | 0.39 | NT | NT |
| $-CH_3$ | −0.07 | 0.56 | −1.24 | 0.12 | NT | >3[d] |
| $-NO_2$ | 0.71 | −0.28 | 2.52 (*L*)[f] | 0.08 | 0.12 | NT |
| $-CO_2^-$ | −0.1 | −4.36 | | 0.04 | 1.4 | NT |
| $-CO_2CH_3$ | 0.37 | −0.01 | | 1.1 | 11.1 | NT |
| $-CH_2CO_2^-$ | | | | 3.9 | NT | NT |

[a] *D. dehalogenans* was grown with 20 mM pyruvate and 10 mM 3-Cl-4-OHPA. After 3-Cl-4-OHPA was dechlorinated, the cultures were partitioned, supplemented with substrates (1 mM), and incubated at 37°C and pH 7.5.

[b] The values of hydrophobic ($\pi$), electronic ($\sigma_m$), and stearic ($E_s$) constants for the substituent R in the 4 position of the phenolic moiety were taken from Hansch and Leo (9).

[c] ND, no dehalogenation.

[d] 2-BrP and 2-Br-4-MeP (1 mM) were completely dechlorinated within 1 h; therefore, no rate was determined.

[e] NT, not tested.

[f] *L*, maximum dimension for stearic effect.

The capability to dechlorinate both 2,4-DCP and 2,6-DCP was similarly induced by various 2-Cl-4-RP, where R is Cl, F, Br, $CH_3$, and $CO_2H$. Thus, there was no indication that 2,4-DCP and 2,6-DCP were dechlorinated by different enzyme systems (detailed data not shown). This, however, must be verified with the purified enzyme(s). Furthermore, a comparison of the substrate specificity and the induction pattern of the purified culture and the original enrichment culture after heat treatment (three times for 15 min at 80°C and intermittently cultured at pH 8.0 to 8.5 with 100% $H_2$ as the headspace gas) showed no differences (data not shown). This finding indicates that *D. dehalogenans* might be the major pasteurization-resistant *ortho*-dehalogenating anaerobe in the Sandy Creek Nature Park lake sediment.

**Effect of substituents on the rate of dehalogenation.** *D. dehalogenans* is able to remove halogen substituents in the *ortho* position only to a phenylhydroxyl group and thus is very specific in its dehalogenation spectrum. On the other hand, the organism is able to dehalogenate compounds with various substituents in other positions, especially in the 4 (*para* to the OH group) position, leading to a wide spectrum of halogenated compounds such as halogenated nitrophenols, hydroxybenzoates, phenylacetic acids, and methylphenols; the various ring substituents, however, exert different effects on the dehalogenation activity.

**(i) 2-Fluoro and 2-bromo substituents.** *D. dehalogenans* is not capable of dehalogenation of fluoro compounds (see above). The fluorine substituent has a higher minus I effect (lowering the electron density of the $\pi$-electron system) but a lower plus M effect (extending the aryl $\pi$-electron system) than chlorine, and hence it has a lower tendency to be removed as an anion through reductive dehalogenation. In contrast, the larger bromo substituent has a higher tendency to be removed as an anion than does the chloro substituent. Thus, it is not surprising that some of the brominated compounds were spontaneously debrominated under the conditions used and also that 2-BrP, the only monohalogenated and *para*-unsubstituted compound, was dehalogenated whereas 2-CP was not.

**(ii) 3-, 4-, 5-, or 6-chloro substituents.** The rate of dechlorination of dichlorophenols by *D. dehalogenans* cultures grown previously in the purified-culture medium decreased in the order 2,3-DCP > 2,6-DCP > 2,4-DCP, with no measurable dechlorination for 2-CP, 2,5-DCP, 2,3,5-TCP, and 2,4,5-TCP (Table 1), indicating that chlorine substituents in the 3 (*meta*), 4 (*para*), and 6 (second *ortho*) positions facilitate the dechlorination of the *ortho* (2-position) chlorine whereas the chlorine in the 5 (second *meta*) position has a negative effect on the dechlorination rate or even prevents the *ortho* dechlorination. The trichlorophenols 2,3,4-TCP, 2,3,6-TCP, and 2,4,6-TCP (0.25 mM each) were completely dechlorinated within less than 2 days. The tetrachlorophenol 2,3,4,6-TeCP (0.2 mM) was

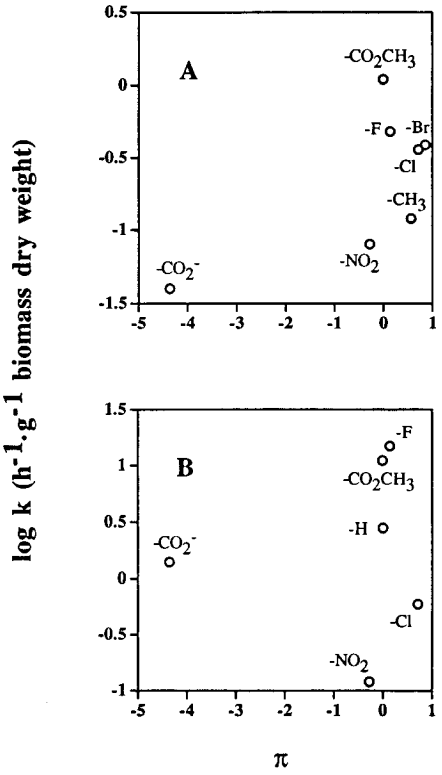


FIG. 4. Relationship between the logarithm of pseudo-first-order rate constants of the dechlorination of 2-Cl-4-RPs (A) and 2,6-DCl-4-RPs (B) and $\pi$ values of the substituent R in the 4 (*para*) position of the phenolic moiety.

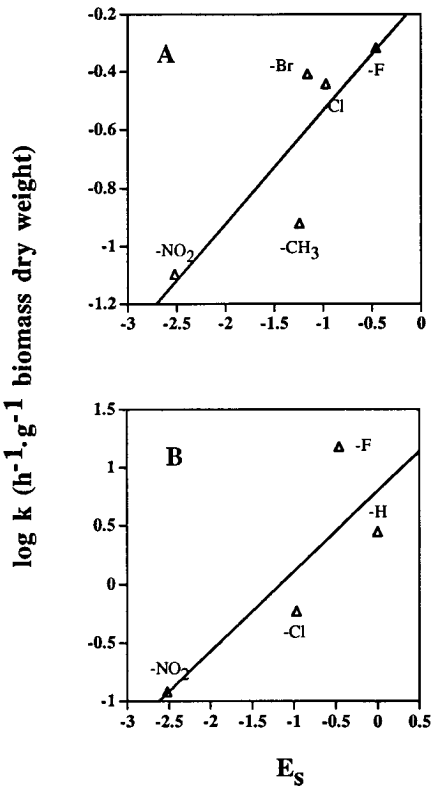


FIG. 5. Relationship between the logarithm of pseudo-first-order rate constants of the dechlorination of 2-Cl-4-RPs (A) and 2,6-DCl-4-RPs (B) and $E_s$ values of the substituent R in the 4 (*para*) position of the phenolic moiety. The lines suggest possible trends but no firm relationships.

completely dechlorinated after 3 h of incubation in the presence of 2.5% ethanol (the only form of 2,3,4,6-TeCP available to us was a stock solution in ethanol), whereas the conversion of PCP, 2,3,4,5-TeCP, and 2,3,5,6-TeCP (0.2 mM each) was 31, 23, and 67%, respectively, within 6 h, displaying again the negative effect of the *meta*-substituted chlorine in the 5 position on the dechlorination rate. The occurrence of noticeable dehalogenation of PCP and 5-substituted TeCPs (compare with 2,5-DCP, 2,3,5-TCP, and 2,4,5-TCP, which are not dehalogenated) indicates that a cumulative effect of chlorines at positions 3, 4, and 6 overcomes, at least partially, the inhibitory effect of the chlorine in the 5 position.

**(iii) Substitution in the 4 position.** 2,6-DCl-4-RPs were dechlorinated faster than were the corresponding 2-Cl-4-RPs (Table 2). Thus, chlorine in the 6 position enhances the *ortho* dechlorination. Unfortunately, the compounds of the type 6-F-2-Cl-4-RP were not available to elucidate this stimulation further. 2,6-DCl-4-RPs with the smallest (R represent -F) and the largest (R represents $-COOCH_3$) substituents in the 4 position were dehalogenated at similar rates and faster than other tested 2,6-DCl-4-RPs, indicating that the difference in the observed rates could not be attributed to only the size of the substituent R.

For the dehalogenation of 2-Cl-4-RPs, the lowest rates were obtained with the most electron-donating (R represents $-CH_3$, and $-CO_2^-$) and the most electron-accepting (R represents $-NO_2$) groups, indicating that electronic effects alone cannot account for the observed rates. Interestingly, the dechlorination rates of 2-Cl-4-RPs with a hydrophobic substituent R such as -F, -Cl, -Br, $-CH_3$, or $-NO_2$, fell between the dechlorination rates of 3-chloro-4-hydroxybenzoate (R represents $-CO_2^-$) and 3-Cl-4-OHPA (R represents $-CH_2CO_2^-$), i.e., the compounds with the most hydrophilic substituent R. Thus, there is no simple relationship between the hydrophobicity of the substituent R and the dechlorination rate.

Furthermore, we found no strong linear correlation between the logarithms of pseudo-first-order dechlorination rate constants and available descriptors of the substituent R such as the electronic (Hammet $\sigma_m$), stearic ($E_s$), and hydrophobic ($\pi$) constants (Table 2; Fig. 3 to 5), although for $\sigma_m$ (the $NO_2$ groups behaved differently) and $E_s$, trends can be observed. Thus, to develop quantitative structure-activity relationships, QSARs, the dehalogenation rate constants should be related to a combination of descriptors rather than to differential descriptors. This approach was successfully used by Peijnenburg et al. (15), who developed quantitative structure-activity relationships for predicting potential aryl reductive dehalogenation rate constants in anoxic sediments, relating the pseudo-first-order disappearance rate constants of 45 compounds to four molecular descriptors. Although such quantitative structure-activity relationships are helpful for predicting potential dehalogenation rates of structurally different chlorinated phenolic compounds in sediments, they do not provide essential information about the reaction mechanism of reductive dehalogenation, because various factors (e.g., substrate diffusion and the existence of isoenzymes) may contribute to the observed dehalogenation rates. Therefore, a possible structure-activity relationship must be reevaluated with a purified dehalogenase when available.

In conclusion, the observed dechlorination was highly specific for the *ortho* position and thus differs significantly from the mainly *meta*-aryl dehalogenation observed with the gram-type-negative sulfate reducer *Desulfomonile tiedjei* isolated from sewage sludge. The substrate specificity also differs from the one observed by Madson and Licht (13) and Dietrich and Winter (4). Our organism could not dehalogenate monochlorophenols such as 2-chlorophenol without any substitution in the 4 (*para*) position but did so when the *para* position was substituted regardless of whether the substituent was an $\pi$-electron-increasing or -decreasing substituent (Fig. 1). This led to a wide range of substrates for dechlorination, such as chlorinated *p*-hydroxy phenylacetate, *p*-hydroxy benzoates, *p*-nitrophenols, and *p*-cresols, all containing the halogen to be removed in the *ortho* position to a phenolic hydroxyl group. Therefore, we believe that this organism could be useful in bioremediation of sediments contaminated with a variety of chlorinated aromatic compounds.

## ACKNOWLEDGMENT

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## ADDENDUM IN PROOF

If high cell densities of cultures grown with 10 mM pyruvate and 1.5 mM 3-Cl-4-OHPA and incubation times of 4 to 8 days and 50 ppm of chlorophenols are used, *D. dehalogenans* can slowly *ortho* dehalogenate 2-CP faster than 2,3,5-TCP and faster than 2,4,5-TCP 2,5-DCP (Zhang and Wiegel).

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