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Bacterial Degradation of Phenoxy Herbicide Mixtures 2,4-D and MCPP

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The phenoxy herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2-(2-methyl-4-chlorophenoxy)propionic acid (MCPP) have auxin-like growth-regulating properties and are extensively used for the control of broad-leaf angiosperm weeds. The microbiological degradation of 2,4-D by pure and mixed cultures has been examined in a number of studies, recently reviewed by Sandmann et al. (1988) and Sinton et al. (1986). Pure cultures capable of using 2,4-D for carbon and energy include several species in the genera *Arthrobacter, Pseudomonas, Xanthobacter, and Alcaligenes* (Fisher et al. 1978; Kilpi et al. 1980; Don and Pemberton et al. 1981; Pieper et al. 1988; Sandmann and Loos 1988; Ditzelmuller et al. 1989). However, only few studies have been published on the bacterial degradation of MCPP (Kilpi 1980; Smith and Hayden 1981; Lindholm et al. 1982). Mixed cultures of bacteria enriched from soil samples were demonstrated to degrade MCPP, but in pure culture none of the isolates were able to utilize MCPP as the sole source of carbon and energy (Lappin et al. 1985). With the use of soil samples amended with ring-labelled [^{14}C]-MCPP, Smith (1985) detected 2-methyl-4-chlorophenol (2,4-MCP) as a degradation product of MCPP metabolism.

We have previously evaluated the concurrent microbiological degradation of 2,4-D and MCPP in stirred tank reactors (Oh and Tuovinen 1991). For the present paper, we examined the utilization of the two substrates by three mixed cultures that had a previously history of growth with the respective single phenoxy herbicide.

MATERIALS AND METHODS

Three mixed bacterial cultures, designated as S1, S2, and S3, were used in this study. These cultures were originally derived from soil samples that had a previous history of phenoxy herbicide treatment. After the initial enrichment, the mixed cultures were maintained separately on 2,4-D and MCPP. The cultures were maintained in liquid media which contained mineral salts (Oh and Tuovinen 1990)

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supplemented with mixtures of 2,4-D and MCPP (0.5-1.0 g/liter each) as the sole carbon and energy sources. Cultures were grown in shake flasks (156 rpm) at 22°C. Growth was monitored by optical density at 550 nm and by protein determination (Lowry et al. 1951).

The phenoxy compounds were analyzed both by reverse phase HPLC and by UV-spectrometry. For HPLC analysis, a Phenomenex ODS column (150 mm x 4.6 mm, particle size 5 μm) was eluted with a mobile phase which contained 40%(vol/vol) acetonitrile and 60%(vol/vol) phosphate buffer (6 g K_2HPO_4 and 3 ml concentrated H_3PO_4 per liter). The flow rate of the mobile phase was 1.8 ml/min. The analytical methodology has been previously described in detail (Oh and Tuovinen 1991). Inorganic chloride concentration was determined by a coulometric method. Technical and analytical grade 2,4-D and MCPP were obtained from Dow Chemical Co. (Midland, MI); analytical grade 2,4-DCP from Sigma Co. (St. Louis, MO); and HPLC-grade acetonitrile and water from J.T. Baker Chemical Co. (Phillipsburg, NJ).

RESULTS AND DISCUSSION

In initial experiments, the degradation of 2,4-D and MCPP was studied with three test cultures previously grown with the respective single substrate. Cultures previously grown with 2,4-D completely degraded this substrate but could not degrade MCPP. Cultures initially enriched with MCPP were able to partially degrade this substrate whereas 2,4-D was degraded to completion.

The concurrent degradation of these substrates is shown in Figure 1. As also in single substrate experiments, cultures previously grown only with 2,4-D could not degrade MCPP in the mixed substrate media. Although neither 2,4-D nor MCPP was completely degraded in these experiments, 2,4-D was degraded faster and more quantitatively than MCPP.

Figure 2 shows the effect of inoculum size on the herbicide utilization. An increase in the inoculum reduced the lag period but partial degradation of the phenoxy substrates persisted.

The effect of pH on the concurrent degradation of 2,4-D and MCPP by culture S1 is shown in Figure 3. In pH-adjusted cultures, the degradation of 2,4-D proceeded to completion. Only about 40% of 2,4-D was utilized when pH adjustments were not made. The respective culture turbidity data reflected similar differences in the extent of growth (Figure 3). MCPP was only partially utilized with and without the pH control. Without pH-adjustment, the pH values decreased to pH 3.5-3.7 range. We have previously measured as low values as pH 3.4 toward the end of the incubation in the test cultures growing with 1 g of 2,4-D per liter (Oh and Tuovinen 1990).

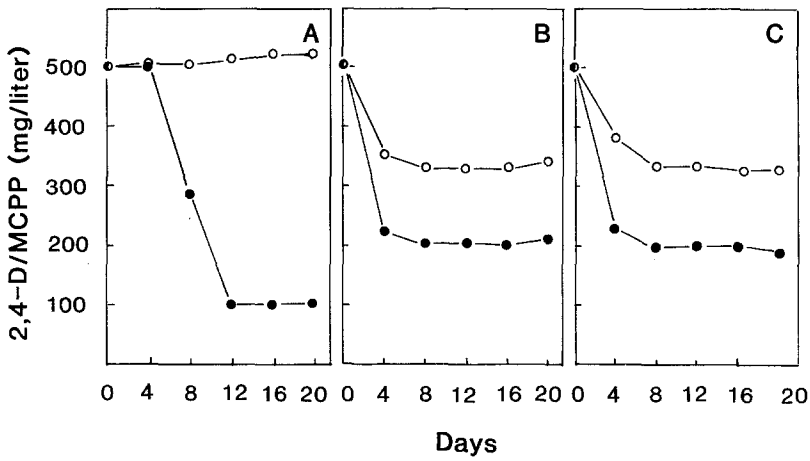

Figure 1. Concurrent degradation of 500 mg.liter each of 2,4-D (●) and MCPP (○) by culture S1 which had been initially enriched with either 2,4-D (A) or MCPP (B). A mixture of these two subcultures was used in experiment C.

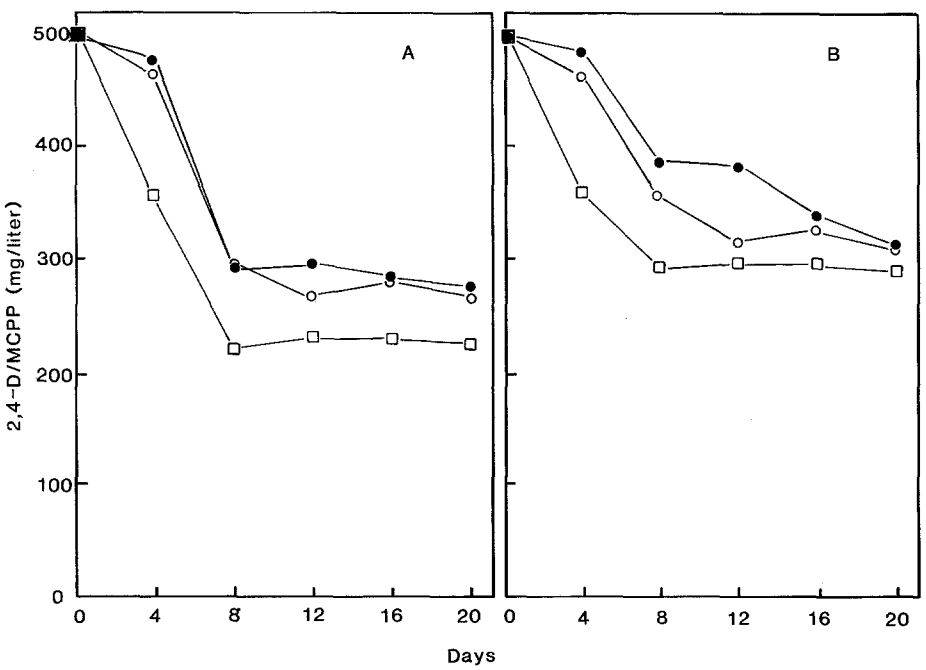

Figure 2. Concurrent degradation of 500 mg/liter each of 2,4-D (A) and MCPP (B) at initial cell densities equivalent to 10% (●), 20% (○), and 40% (□) inocula from fully grown culture S1 which had a cell density of approximately 8.5×10^7 cells/ml. Before inoculation, the cells were removed from spent culture media by centrifugation.

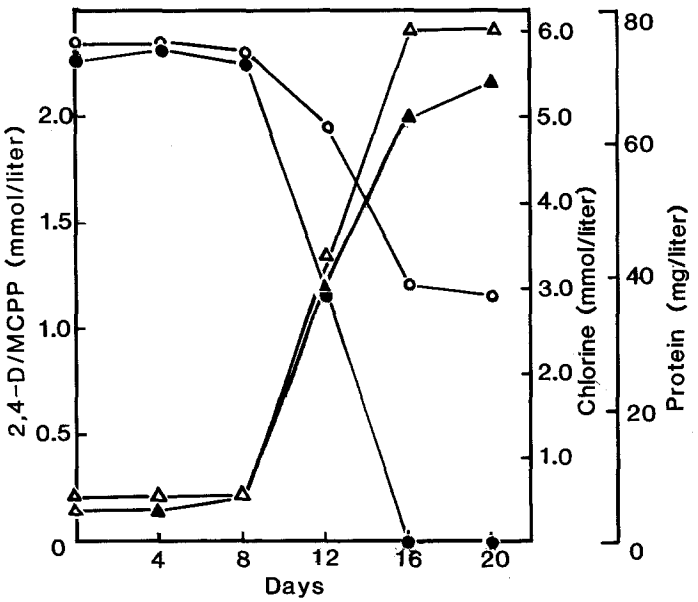

Figure 3. Concurrent degradation of 500 mg/liter each of 2,4-D (●) and MCPP (○) by culture S1 and the associated changes in protein (▲) and inorganic chloride (Δ) concentrations. Culture S1 was adjusted to pH 7.4 every four days.

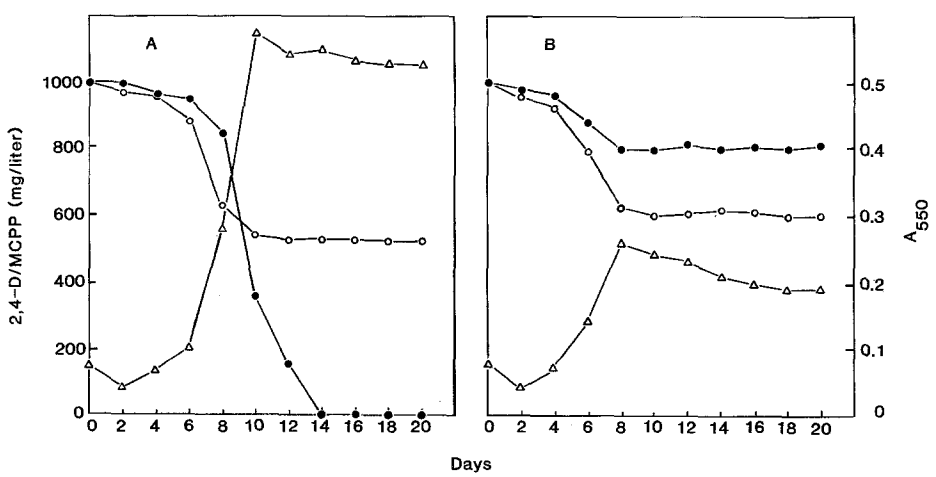

Figure 4. Growth of test culture S1, measured as cell density (Δ), and the concurrent degradation of 1 g/liter each of 2,4-D (●) and MCPP (○). The experiments were performed with (A) and without (B) a pH-adjustment to 7.4 with NaOH.

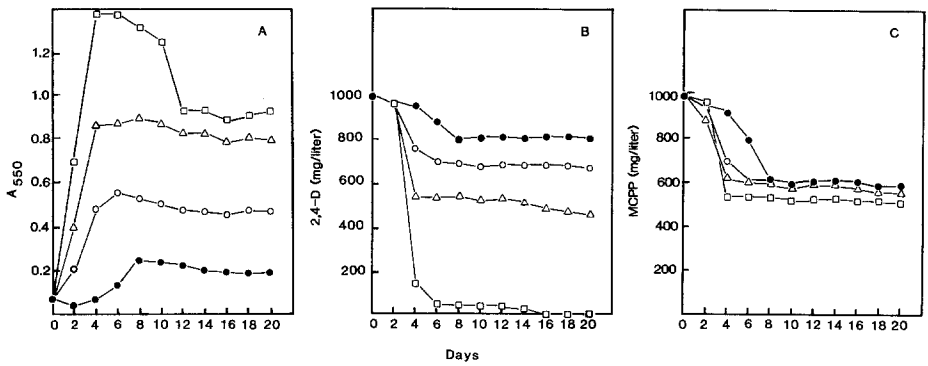

Figure 5. Growth of culture S1 (A) and the concurrent degradation of 1 g/liter each of 2,4-D (B) and MCPP (C) in the presence of 0 mg (●), 200 mg (○), 500 mg (Δ), and 1000 mg (□) of yeast extract per liter.

Changes in biomass and inorganic chloride concentration upon degradation of 2,4-D and MCPP with pH adjustment are shown in Figure 4 for culture S1. Complete degradation of 2,4-D was achieved in this experiment within 16 days during which time only about 50% of the co-substrate, MCPP, was degraded. The concentration of inorganic chloride as a measure of dechlorination was in good agreement with the residual substrate concentration, based on molar ratios of 2Cl per 2,4-D and 1Cl per MCPP. The inorganic chloride concentration was within 94.7% agreement of the value calculated from the 2,4-D and MCPP degradation data.

Yeast extract was tested as an additional source of nutrients. Increasing concentrations of yeast extract resulted in higher growth yields, based on the turbidity measurement, and complete degradation of 2,4-D (Figure 5). However, MCPP degradation was essentially uninfluenced by the addition of yeast extract.

The HPLC chromatograms typically displayed 2,4-DCP as an intermediate of herbicide degradation (Figure 6). Our previous work has demonstrated that 2,4-DCP is a metabolite associated with 2,4-D degradation (Oh and Tuovinen 1990). The HPLC chromatograms did not display other additional peaks for the presence of intermediates such as 2,4-MCP which was previously detected in MCPP culture media (Oh and Tuovinen 1991).

UV-spectrometry of the authentic standards showed a maximum peaks of absorption of 283 nm for 2,4-D, 279 nm for MCPP, and 282 nm for a mixture of 2,4-D and MCPP. In culture media, the wavelength of maximum absorption shifted from 282 nm to 279 nm toward the end of

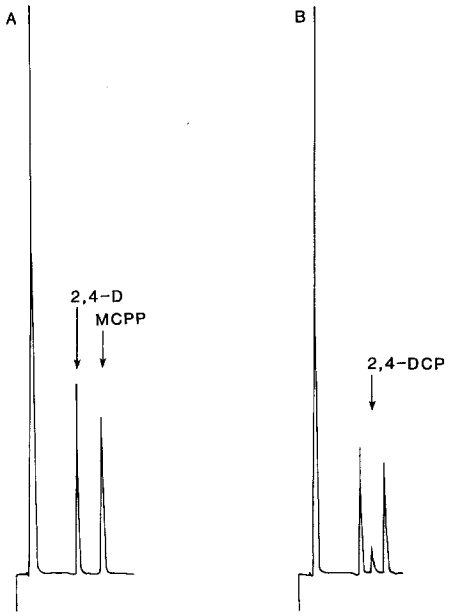

Figure 6. HPLC chromatograms of culture S3 filtrates initially (A) and after 16 days of incubation (B). The retention times for 2,4-D, 2,4-DCP, and MCPP are 3.23, 3.80, and 4.51 min, respectively.

incubation (Figure 7) when 2,4-D was completely degraded while residual MCPP persisted. 2,4-DCP (A_{max}) was not detected in the spectra.

Simultaneous degradation of 2,4-D and MCPP is of particular interest in biological waste treatment of fertilizer formulations and other materials containing these herbicides. The results of this study show that MCPP is more persistent than 2,4-D to bacterial degradation. Based on dechlorination and spectral data, partial degradation of MCPP occurred even when the test cultures were initially enriched with MCPP. Conditions that favored the complete degradation of 2,4-D were not conductive to the complete degradation of MCPP by the same culture. The incomplete degradation of MCPP may be a result of several factors, including the following: (i) the test cultures were sensitive to intermediates of MCPP degradation; (ii) the cultures displayed stereospecificity for one isomer in the racemic mixture (about 50% each) of MCPP; and (iii) the degradation of MCPP proceeds via a pathway that does not yield metabolically useful products for intermediate carbon or energy metabolism. Because similar incomplete degradation also occurred in a single substrate experiments. It can be concluded that 2,4-D neither inhibited nor stimulated the degradation of MCPP. These questions warrant further work on resolving intermediates and on elucidating

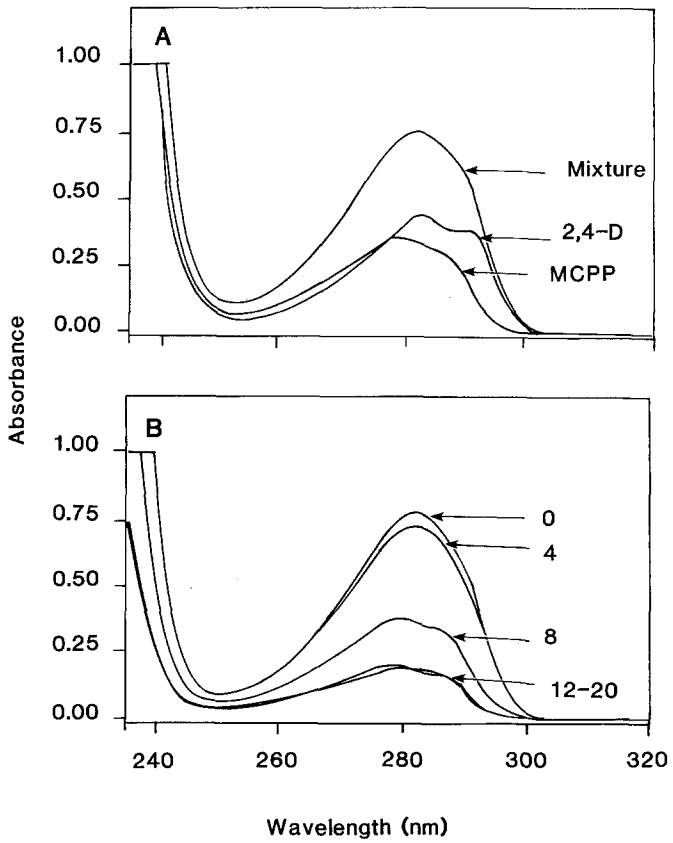

Figure 7. UV spectral scans of (A) standard solutions containing 50 mg of 2,4-D or MCPP and their mixture; and (B) supernatants of culture S2 samples. The length of incubation preceding the scan is indicated in days.

degradative pathway(s) with other experimental approaches such as enzyme assays and isotope techniques.

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