

# Degradation of MCPA, 2,4-D, and Other Phenoxyalkanoic Acid Herbicides Using an Isolated Soil Bacterium

Allan E. Smith,\* Knud Mortensen, Andrew J. Aubin, and Margaret M. Molloy

Research Station, Agriculture Canada, 5000 Wascana Parkway, Box 440,  
Regina, Saskatchewan S4P 3A2, Canada

A soil bacterium was isolated capable of metabolizing the five phenoxyalkanoic acid herbicides 2,4-D, 2,4-DB, MCPA, MCPB, and 2,4,5-T in mineral solution with the individual herbicides as sole carbon source. The optimum temperature for breakdown was in the range 20–30 °C. In a 500-L aerated tank, the organism metabolized kilogram quantities of both 2,4-D and MCPA dimethylamine formulations to inorganic chloride. This bacterium retained activity for at least 18 months after storage in a frozen state at –70 °C. When applied to field plots, the organism is able to survive in the soil for at least 615 days and degrade both 2,4-D and MCPA significantly more rapidly than in soil from an untreated control plot.

## INTRODUCTION

Phenoxyalkanoic acid herbicides are used extensively throughout the world for the control of annual and perennial weeds, hardwood trees, and brush on cropland, rangeland, rights-of-ways, and industrial and private property. They are also used in lakes, ponds, and ditches for aquatic weed control (Smith, 1989). The most frequently used herbicides in this group are 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (2-methyl-4-chlorophenoxyacetic acid).

Since their introduction nearly 50 years ago, it has been established that the breakdown of phenoxy herbicides in soils is the result of microbiological mechanisms. The subject has been extensively reviewed (Freed and Montgomery, 1963; Audus, 1964; Loos, 1975; Bovey, 1980; Sandmann et al., 1988; Smith, 1989). Species belonging to such genera as *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bordetella*, *Flavobacterium*, *Pseudomonas*, and *Xanthobacter* have been isolated from soils and shown to degrade 2,4-D and MCPA in liquid nutrient media (Audus, 1964; Evans et al., 1971; Loos, 1975; Loos et al., 1979; Kilpi et al., 1980; Pieper et al., 1988; Kelly et al., 1989; Smith and Mortensen, 1991; Greer et al., 1992).

The use of microorganisms for the decontamination of pesticide waste is of importance (Kobayashi and Rittman, 1982; Bollag and Liu, 1990), and considerable efforts have been made to isolate organisms capable of metabolizing phenoxyalkanoic acids (Kelly et al., 1989; Oh and Tuovinen, 1991a,b; Smith and Mortensen, 1991; Greer et al., 1992). Recently, a soil bacterium, tentatively identified as *Pseudomonas testosteroni*, was isolated from a Saskatchewan field soil that had received annual treatments of 2,4-D formulations since 1947 (Smith and Mortensen, 1991). This organism, which would metabolize 2,4-D but not other phenoxyalkanoic acids, was able to completely degrade commercial 2,4-D amine formulations to inorganic chloride. Over a 75-day period approximately 3 kg of 2,4-D [acid equivalent (ae)] from amine formulations were completely and efficiently degraded in a plastic 500-L tank containing the organism and mineral salt solution (Smith and Mortensen, 1991).

This paper describes the isolation of a soil bacterium from field plots receiving annual applications of MCPA

amine formulations since 1953 and its ability to metabolize several phenoxyalkanoic acids. The large-scale breakdown of 2,4-D and MCPA amine formulations using the soil bacterium in an aerated tank of mineral salt solution is also discussed, as is its viability in field plots and activity following ultralow-temperature storage.

## MATERIALS AND METHODS

**Chemicals.** Analytical samples of 2,4-D, MCPA, 2,4-DB (2,4-dichlorophenoxybutyric acid), dichlorprop (2,4-dichlorophenoxypropionic acid), MCPB (2-methyl-4-chlorophenoxybutyric acid), mecoprop (2-methyl-4-chlorophenoxypropionic acid), and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) were obtained from the U.S. EPA (Research Triangle Park, NC). Both 2,4-dichlorophenol (2,4-DCP) and 2-methyl-4-chlorophenol (2,4-MCP) were purchased from Aldrich Chemical Co., Inc. (Milwaukee, WI). Commercial formulations of the dimethylamine salts of 2,4-D (Weedar 80, 475 g/L) and MCPA (MCPA Amine 500, 500 g/L) were obtained from Union Carbide Agricultural Products (Canada) Ltd. (Calgary, AB) and Interprovincial Cooperatives Ltd. (Saskatoon, SK), respectively. Chain-labeled 2,4-D (2,4-dichlorophenoxy[2-<sup>14</sup>C]-acetic acid) and carboxyl-labeled MCPA (2-methyl-4-chlorophenoxy[1-<sup>14</sup>C]acetic acid) were obtained from Amersham Canada Ltd. (Oakville, ON) as described (Smith et al., 1989).

**Isolation of Microorganism.** Soil sampled from a plot at Indian Head that had received annual treatments of 1.0 kg/ha MCPA dimethylamine formulation since 1953 (Smith et al., 1991) was used as an inoculum for a mineral salt medium enriched with MCPA acid (100 µg/mL). The medium contained the following (g/L): K<sub>2</sub>HPO<sub>4</sub> (1.17), KH<sub>2</sub>PO<sub>4</sub> (0.54), NH<sub>4</sub>NO<sub>3</sub> (0.38), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (0.04), MgSO<sub>4</sub>·7H<sub>2</sub>O (0.03), FeSO<sub>4</sub>·7H<sub>2</sub>O (0.007), and ZnSO<sub>4</sub> (0.004); the pH of the autoclaved solution was 6.9. The technique was the same as that used to isolate the previously described 2,4-D-degrading organism (Smith and Mortensen, 1991). The soil bacterium was finally grown on both nutrient agar (Difco, Bacto nutrient agar, 23 g/L) and agar medium prepared from the mineral salt medium containing MCPA acid (50 µg/mL) and Bacto agar (15 g/L). Single bacterial colonies were thus isolated. Inoculation of these organisms in 250 mL of the mineral salt medium containing MCPA (100 µg/mL), with incubation at 24 ± 1 °C and shaking (110 rpm) was carried out to confirm their ability to degrade MCPA. MCPA metabolism, as measured by loss of UV absorbance over the range 255–305 nm, was accompanied by an increase in bacterial cell numbers from (1.9 ± 0.6) × 10<sup>6</sup> organisms/mL (mean ± SD of three replicates) at the time of inoculation to (5.3 ± 3.1) × 10<sup>12</sup> cells/mL after 5 days. Similar studies also indicated that single-colony cultures of the soil bacterium capable of degrading MCPA were also capable of degrading 2,4-D as sole carbon source.

\* Author to whom correspondence should be addressed.

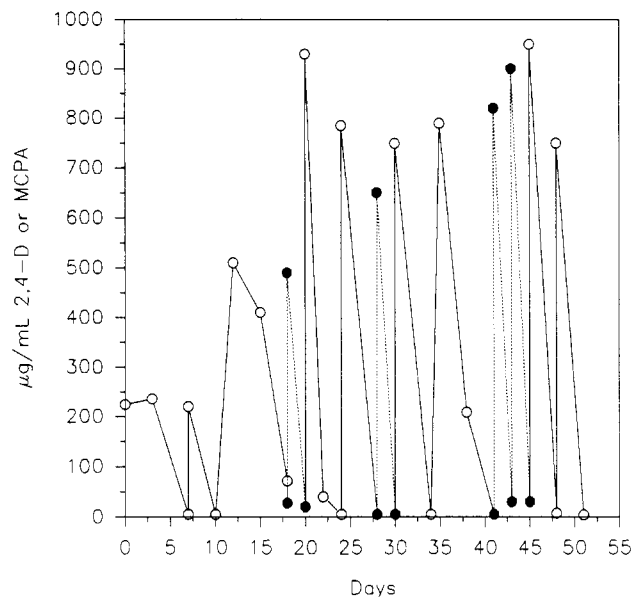
This soil bacterium was Gram-negative, rod-shaped, aerobic, oxidase negative, nonmotile, nonfermentative, nonfluorescent on King's B medium, and produced slightly raised small mucoid yellow colonies on yeast extract-dextrose-CaCO<sub>3</sub> agar, properties indicative of a Pseudomonadaceae (Bergey, 1979). According to two independent tests conducted at Agriculture Canada, Regina Research Station, and the Microbiology Section of the Regina General Hospital, using the API Rapid NFT Test (DMS Laboratories Inc., Dart Mills, NJ), the bacterium matched the characteristics of *Pseudomonas paucimobilis*. However, fatty acid profile analysis (Microbial ID, Inc., Newark, DE) was inconclusive but with indications that the organism could be a *Pseudomonas* spp. Since confirmatory taxonomic analysis was not within the scope of this study, the organism was tentatively assumed to be a *Pseudomonas* spp. In the text, the organism will be referred to as a soil bacterium.

**Activity of Soil Bacterium following Ultralow-Temperature Storage.** Cultures of the soil bacterium were stored in aqueous solutions containing 8% dimethyl sulfoxide (DMSO) or 15% glycerol at -70 °C in 2-mL vials. After 1, 3, 6, 12, and 18 months, vials were removed from the freezer and the organisms allowed to thaw at room temperature for 3 h. The contents were added directly to sterile mineral nutrient solutions (250 mL), containing 2,4-D or MCPA (100 µg/mL), which were then incubated at 25 ± 1 °C and subsequently analyzed by measuring UV absorbance to determine whether herbicide loss occurred with time. The viability of the soil bacterium was also tested by being plated on nutrient agar.

**Assessment of the Ability of the Soil Bacterium To Degrade Different Phenoxyalkanoic Acid Herbicides.** Individual herbicide solutions were prepared by dissolving 12.5 mg of 2,4-D, 2,4-DB, dichlorprop, MCPA, mecoprop, or 2,4,5-T in 250 mL of sterile mineral salt solution so that each solution contained 50 µg/mL of the respective herbicide as the sole carbon source. To this solution (10 mL) in sterile glass tubes (16 × 150 mm) was added mineral salt medium containing the soil bacterium (1 mL, ~9 × 10<sup>15</sup> cells). For control purposes 1 mL of sterile water was added to other tubes. There were 15 tubes for each herbicide treatment and 5 control samples. The tubes were covered with sterile plastic caps that allowed air exchange, mixed for 15 s on a vortex mixer, and incubated, without shaking, in the dark at 30 ± 1 °C. After 3, 7, 14, 21, and 28 days of incubation, the UV absorption remaining in each of three replicate tubes was determined spectrophotometrically by comparing the optical densities at the wavelength of maximum adsorption (Sironi et al., 1982) for each herbicide (280–285 nm) with those from one of the similarly incubated sterile control samples.

**Effects of Temperature on 2,4-D and MCPA Degradation.** To a portion (250 mL) of the autoclaved mineral salt medium (pH 7.0) were added 2,4-D (26 mg) and mineral salt medium (5 mL) containing ~10<sup>16</sup> bacterial cells, while a second portion was similarly treated with MCPA and the soil bacterium. Aliquots (10 mL) of the herbicide-enriched media were added to sterile glass tubes (16 × 150 mm) which were incubated in the dark at 10 ± 1, 15 ± 1, 20 ± 1, 25 ± 1, 30 ± 1, and 35 ± 1 °C. Three replicate tubes for each temperature and herbicide treatment were analyzed as above for loss of UV absorption after 5 min and then after 1, 2, 3, 4, 5, and 7 days.

**Aerated Tank Degrading System for MCPA Residues.** The degradation system has already been described in detail (Smith and Mortensen, 1991). The 500-L tank was placed outside during the first week of June 1991 and filled with unsterilized tap water (400 L) and the required amount of mineral nutrients added to give the same concentrations as in the mineral salt medium. Chloride analysis (see later) indicated the total chloride content of the nutrient solution to be approximately 25 µg/mL. Air for both aeration and mixing was passed through the system at a rate of 20 L/min as previously described (Smith and Mortensen, 1991). MCPA dimethylamine formulation was added to the tank to give a concentration of ~250 µg/mL based on MCPA acid equivalent, followed by inoculation with 100 mL of cell suspension (~2 × 10<sup>16</sup> cells) of the soil bacterium. The tank contents were monitored every 3 or 4 days for amounts of MCPA remaining, as well as for pH, chloride, and dimethylamine content. As the herbicide was degraded, more MCPA amine formulation was added to the tank. Additions of 2,4-D dimethylamine



**Figure 1.** Breakdown of repeated applications of MCPA (○) and 2,4-D (●) dimethylamine formulations in a sprayer tank system during June and July 1991. Additions of MCPA were made on days 0, 7, 10, 20, 24, 30, 35, 45, and 48 and of 2,4-D on days 18, 28, 41, and 43, respectively.

formulation were also made at regular intervals (cf. Figure 1). With time the nutrient solution in the spray tank became more acidic, and at a pH of ~6.0 degradation was reduced. When this occurred, approximately 100 mL of 50% aqueous sodium hydroxide was added to the tank to raise the pH to about 7.0. In general, the pH was maintained between 6.3 and 7.3. The experiment was continued until the last week of July 1991. The study was also repeated during the summer of 1992.

**Monitoring of 2,4-D, MCPA, Chloride, pH, and Dimethylamine.** At each sampling, approximately 50 mL of the tank contents was centrifuged to remove bacterial cell debris and the aqueous medium filtered under pressure through a disposable 0.45-µm hydrophilic nylon membrane syringe filter (Lida Manufacturing Corp., Bensenville, IL). The absorbance of the extract was measured over the range 255–305 nm using a Perkin-Elmer double-beam spectrophotometer with water as reference solution. Concentrations of 2,4-D and MCPA were estimated spectrophotometrically as described for 2,4-D (Smith and Mortensen, 1991). Using standards, it was demonstrated that the UV absorbance was proportional to 2,4-D and MCPA concentrations over the range 5–250 µg/mL. More concentrated solutions were diluted, with water, as necessary. For 2,4-D and MCPA concentrations <5 µg/mL, as well as for measurement of the degradation products 2,4-DCP and 2,4-MCP, which exhibit UV absorption maxima similar to those of the parent herbicides (Oh and Tuovinen, 1991a), aliquots of the centrifuged and filtered medium were subjected to direct high-performance liquid chromatography (HPLC).

The HPLC system consisted of a pump (Waters Model 510), an autosampler (Waters WISP Model 712B with 60-µL injection), a UV detector (Waters Model 490) operated at 280 nm, and a data system (Digital Pro 380). The Nova-Pak C<sub>18</sub> (150 mm × 4 mm) column was eluted with mobile phase [methanol-1% acetic acid in deionized water (50:50 v/v)] at a flow rate of 1.0 mL/min. Appropriate standards of the herbicides and the corresponding phenols were prepared in water. Under the operating conditions, the retention times for 2,4-D, 2,4-DCP, 2,4-MCP, and MCPA were 6.40, 8.13, 11.22, and 12.05 min, respectively. Amounts of the various compounds present in the aqueous extracts were calculated by comparing peak areas of the samples with those obtained from authentic standards of known concentrations.

Inorganic chloride was determined by titration using the Volhard method (APHA, 1955), and solution pH was monitored using a Check-Mate 90 portable pH meter (Corning Glass Works, Corning, NY).

**Table 1. Loss of UV Absorbance with Time from Mineral Salt Medium Containing Phenoxyalkanoic Acids (50  $\mu\text{g}/\text{mL}$ ) following Inoculation with the Soil Bacterium at  $30 \pm 1^\circ\text{C}$  Compared to Sterile Controls**

herbicide	% of $\text{abs}_{\text{max}}$ remaining compared to controls after				
	3 days	7 days	14 days	21 days	28 days
2,4-D	$20 \pm 2^a$	$0 \pm 0$	$-^b$	—	—
MCPA	$24 \pm 13$	$0 \pm 0$	—	—	—
2,4,5-T	$77 \pm 3$	$51 \pm 4$	$27 \pm 3$	$18 \pm 2$	$8 \pm 1$
2,4-DB	$53 \pm 3$	$9 \pm 3$	$0 \pm 0$	—	—
MCPB	$89 \pm 12$	$67 \pm 3$	$56 \pm 2$	$41 \pm 1$	$13 \pm 3$
dichlorprop	$100 \pm 2$	$100 \pm 2$	$100 \pm 2$	$100 \pm 2$	$100 \pm 2$
mecoprop	$100 \pm 2$	$100 \pm 2$	$100 \pm 2$	$100 \pm 2$	$100 \pm 2$

<sup>a</sup> Mean and standard deviations from three replicates. <sup>b</sup> Not determined.

Dimethylamine in the filtered solution was treated with ammoniacal cupric sulfate and carbon disulfide and analyzed colorimetrically as the yellow complex of cupric dithiocarbamate (absorption maximum at 440 nm) as previously described (Smith and Aubin, 1992).

**Viability Studies with the Soil Bacterium in Field Plots.** On August 7, 1991, after completion of the study described above, the entire contents of the tank were applied to a clay field plot (4 m  $\times$  20 m) at the Regina Research Station, whose characteristics have already been reported (Smith and Aubin, 1991), using a farm sprayer from which the jets had been removed. Several passes were necessary. After application, the plot was raked (with a garden rake) at right angles to the treatment direction. A control plot (4 m  $\times$  20 m) was marked out adjacent to the treatment area and similarly raked. At the time of application, the soil surface had 30% moisture.

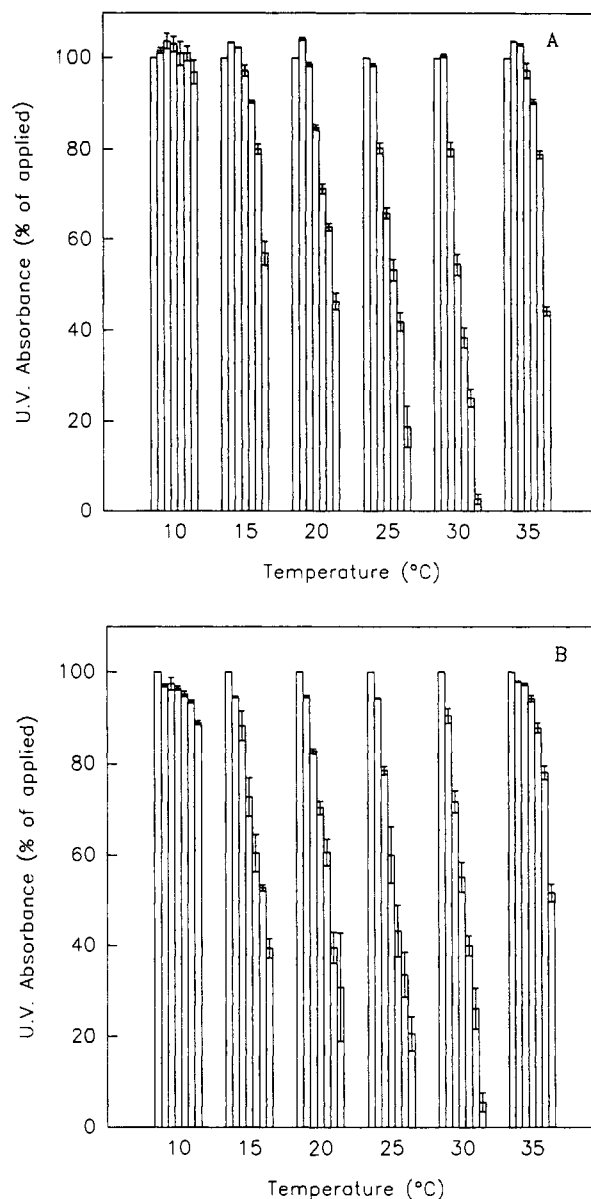
On October 8, 1992, following completion of the 1992 tank degradation studies with 2,4-D and MCPA, the contents were similarly applied to a second field plot with 28% topsoil moisture at the Research Station and incorporated as described.

In April 1993 the treated and control soils were tested for their ability to degrade 2,4-D and MCPA using the appropriately  $^{14}\text{C}$ -labeled herbicide. Soil samples (10) were randomly collected from the top 5 cm of the treated plot and pooled. The soils were then thoroughly mixed by passing five times through a sample splitter and finally sieved through a 2-mm screen. The laboratory degradation studies with the soil samples collected from the control and bacterium-treated plots were identical to those previously described (Smith et al., 1989). Soil aliquots (50 g) at 34% moisture were treated at a rate of 2  $\mu\text{g}/\text{g}$  with chain-labeled [ $^{14}\text{C}$ ]2,4-D and incubated in the dark at  $20 \pm 1^\circ\text{C}$ . Other soil samples were analogously treated with carboxyl-labeled [ $^{14}\text{C}$ ]MCPA and similarly incubated. Three replicate treatments were extracted and analyzed for [ $^{14}\text{C}$ ]2,4-D or [ $^{14}\text{C}$ ]MCPA remaining after 4 and 8 days, respectively, as described (Smith et al., 1989).

## RESULTS AND DISCUSSION

Incubation studies with the soil bacterium indicated that, in addition to 2,4-D and MCPA, it could metabolize other phenoxyalkanoic acids as sole carbon source. Typical results are indicated in Table 1. Thus, the phenoxyacetic acid 2,4,5-T and the phenoxybutyric acids 2,4-DB and MCPB were metabolized by the soil bacterium but at a slower rate than 2,4-D or MCPA. Both 2,4-DB and MCPB undergo biologically mediated  $\beta$ -oxidations that reduce the number of side-chain carbon atoms by two, converting them to 2,4-D and MCPA, respectively (Gutenmann et al., 1964; Smith, 1978; Smith and Hayden, 1981). Thus, it appears to be probable that the isolated soil bacterium is able to transform 2,4-DB to 2,4-D and MCPB to MCPA, which would then be further metabolized. There was no evidence of any breakdown of the phenoxypropionic acids dichlorprop or mecoprop (Table 1).

Unpublished data from this laboratory have indicated that another bacterium recently isolated from field plots receiving repeated annual 2,4-D treatments (Smith and



**Figure 2.** Loss of UV absorbance after 0, 1, 2, 3, 4, 5, and 7 days, respectively, from mineral salt medium at pH 7 containing 2,4-D (A) or MCPA (B) (100 mg/mL) following incubation with the soil bacterium at different temperatures. Each bar is the mean of three replicates, and the error bars denote standard deviation.

Mortensen, 1991) was able to degrade 2,4-D, and 2,4-DB to some extent but did not metabolize any other phenoxyalkanoic acids. Therefore, the observation that this bacterium was able to completely degrade a range of phenoxyalkanoic acid herbicides is of particular interest.

**Breakdown of 2,4-D and MCPA in nutrient solution at pH 7 was temperature dependent (Figure 2).** Under the experimental conditions, loss of UV absorbance over the 7-day period for both herbicides was  $<5\%$  at  $10^\circ\text{C}$  but rapidly increased with temperature to  $30^\circ\text{C}$ . At  $35^\circ\text{C}$  both rates of breakdown were slower and similar to those at  $15\text{--}20^\circ\text{C}$  (Figure 2). Optimum temperature for loss of 2,4-D was in the range  $25\text{--}30^\circ\text{C}$ , while for MCPA breakdown was good in the range  $20\text{--}30^\circ\text{C}$ .

Ultralow-temperature storage of the soil bacterium was successful in both the aqueous DMSO and glycerol systems with viability and the capability of degrading both 2,4-D and MCPA being retained for at least 18 months.

During the two study years, in the tank sprayer system, the soil bacterium metabolized MCPA and 2,4-D using

both herbicides as a carbon and energy source, so that with time there was complete loss of UV absorbance. The data for the 1991 study are summarized in Figure 1 and show that repeated additions of 2,4-D and MCPA amine formulations were continuously degraded. The breakdown was dependent on temperature and was slower during periods of cooler weather. Total breakdown of the herbicide was also confirmed by HPLC analysis. After 28 days, 610 g (ae) of MCPA and 110 g (ae) 2,4-D had been metabolized with measured release of 170 g of inorganic chloride (corrected for chloride initially determined in the mineral salt solution). The theoretical release was 150 g. By the end of the experiment, 660 g (ae) of 2,4-D and 1360 g (ae) of MCPA had been degraded with the release of  $420 \pm 20$  g (mean  $\pm$  SD of three replicates) of chloride. Calculation indicated that with complete metabolism of the two herbicides 450 g of chloride would have been released. Thus, both MCPA metabolism and 2,4-D metabolism were accompanied by complete release of chloride, confirming total herbicide breakdown.

The biological breakdown of phenoxyalkanoic acid herbicides in culture solution can result in formation of the corresponding phenols (Loos, 1975; Kilpi et al., 1980; Oh and Tuovinen, 1991a). On the basis of HPLC data, the breakdown of MCPA appeared to be accompanied by some formation of 2,4-MCP with maximum amounts detected on days 22 ( $\sim 90$   $\mu\text{g}/\text{mL}$ ) and 23 ( $\sim 50$   $\mu\text{g}/\text{mL}$ ). Phenol concentrations at all other sampling dates were less than 2  $\mu\text{g}/\text{mL}$ , suggesting no buildup of 2,4-MCP in the solution. Amounts of 2,4-DCP, formed from 2,4-D, were always  $<1$   $\mu\text{g}/\text{mL}$ .

Dimethylamine concentrations in the aerated tank were monitored during the 1992 study only. It was observed that during the experiment there were negligible losses of the volatile amine with time and that when the experiment was terminated, dimethylamine detected ( $98 \pm 8$  g) was, within experimental error, equivalent to that added as 2,4-D and MCPA dimethylamine salts. In the aqueous medium with a pH of  $\sim 7$ , 2,4-D and MCPA dimethylamine formulations would dissociate to form the respective phenoxyalkanoate anions ( $\text{p}K_a \sim 3$ ) and dimethylamine ( $\text{p}K_a = 2.93$ ). Dimethylamine is a gas (boiling point  $7^\circ\text{C}$ ) and a strong organic base with a high aqueous solubility; however, as there were no losses from the tank, it was assumed that the soil bacterium did not degrade the amine nor was dimethylamine volatilized from the 400-L reaction mixture by aeration over a 6-week period.

Following application of tank contents to field plots, it was assumed that the dimethylamine in the solution would be rapidly degraded by biological processes. Previous studies have indicated dimethylamine to be short-lived in the Regina clay over a range of soil concentrations (Smith and Aubin, 1992).

The results of the studies to investigate the ability of the soil bacterium to adapt and survive in field soils under Saskatchewan climatic conditions are summarized in Table 2. The losses of [ $^{14}\text{C}$ ]2,4-D and [ $^{14}\text{C}$ ]MCPA in the plot soils collected in April 1993 indicate that the rate of loss of each  $^{14}\text{C}$ -labeled herbicide is slowest in the control soil, significantly greater in the soil treated with the soil bacterium in August 1991, and even more rapid in the soil from the plots treated in October 1992. The rates of loss of [ $^{14}\text{C}$ ]2,4-D and [ $^{14}\text{C}$ ]MCPA in the control soils after the 4- and 8-day incubation, as observed from the amounts remaining, were not significantly different. A similar trend was noted for the two treatments. Thus, there is an indication under field conditions of significant herbicide degrading activity resulting in the degradation of both

**Table 2. Breakdown of [ $^{14}\text{C}$ ]2,4-D and [ $^{14}\text{C}$ ]MCPA after Incubation for 4 and 8 Days (at  $20^\circ\text{C}$  and 34% Moisture) in Soils Collected in April 1993 from Control Plots and from Plots Treated with the Soil Bacterium in August 1992 and October 1992**

plot treatment	applied [ $^{14}\text{C}$ ]2,4-D remaining <sup>a</sup> (%)		applied [ $^{14}\text{C}$ ]MCPA remaining <sup>a</sup> (%)	
	4 days	8 days	4 days	8 days
control	75 $\pm$ 1a	56 $\pm$ 5a	71 $\pm$ 2a	52 $\pm$ 2a
bacterium added Aug 1991	54 $\pm$ 8b	19 $\pm$ 8b	63 $\pm$ 3b	21 $\pm$ 3b
bacterium added Oct 1992	26 $\pm$ 4c	5 $\pm$ 1c	31 $\pm$ 6c	8 $\pm$ 2c

<sup>a</sup> Mean and standard deviation from three replicates. Means within a column followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple-range test.

2,4-D and MCPA after 615 days. During this period no 2,4-D or MCPA was added to the field plots, indicating the soil bacterium was probably able to exist on an alternative nutrient source.

The phenomenon of enhanced breakdown, where field and laboratory soils continually treated with a particular pesticide exhibit an increased ability to degrade the chemical as a result of a natural increase in populations of the pesticide-degrading organisms, is well established (Roeth, 1986; Racke and Coats, 1990). However, the introduction of pesticide-degrading organisms into soils as a means of reducing pesticide residues is different and requires that the microorganisms both survive and maintain their degrading capacity in that environment. In oligotrophic soil the introduced organisms will be subjected to the effects of numerous abiotic and biotic effects, which can result in loss of degrading capacity (Acea et al., 1988). It is considered that reduction of populations of bacteria introduced into soil is the result of their susceptibility to predation by indigenous populations, starvation, and possible antibiotic-producing or lytic microorganisms (Acea et al., 1988).

Both pure and mixed cultures of pesticide-degrading organisms have been isolated and added to soils, under laboratory conditions, for the accelerated biodegradation of such pesticides as dicamba (Krueger et al., 1989, 1991), parathion (Daughton and Hsieh, 1977), phenyl carbamates (Clark and Wright, 1970; McClure, 1972), 2,4-D (Greer and Shelton, 1992; Jacobsen and Pedersen, 1992), and 2,4,5-T (Chatterjee et al., 1982; Kilbane et al., 1983). Such cultures can maintain their degrading abilities in laboratory soils for periods of up to 10 weeks (Clark and Wright, 1970; McClure, 1972; Daughton and Hsieh, 1977; Greer and Shelton, 1992; Jacobsen and Pedersen, 1992) and perhaps longer. Parathion- and dicamba-degrading organisms introduced into soil of field plots retained their herbicide-degrading activity, in the absence of herbicide treatments, for up to 3 weeks (Barles et al., 1979; Krueger et al., 1991).

The isolated soil bacterium has the potential to completely degrade 2,4-D, 2,4-DB, MCPA, MCPB, and 2,4,5-T to inorganic chloride. The soil bacterium functions over a range of temperature and herbicide concentrations. The bacterium can be stored for at least 18 months in a frozen state. When applied to clay field plots, the soil bacterium is able to survive western Canadian conditions and, after 615 days, degrade both 2,4-D and MCPA more rapidly than untreated control soil. This would indicate that the bacterium has potential for the decontamination of phenoxyalkanoic acid wastes, by biological means in both soil and aquatic environments.

#### ACKNOWLEDGMENT

We thank D. Sealy for help with the enumeration studies.

## LITERATURE CITED

- Acea, M. J.; Moore, C. R.; Alexander, M. Survival and growth of bacteria introduced into soil. *Soil Biol. Biochem.* 1988, 20, 509-515.
- APHA. Chloride. In *Standard Methods for the Examination of Water, Sewage, and Industrial Wastes*, 10th ed.; American Public Health Association: New York, 1955; p 59.
- Audus, L. J. Herbicide behaviour in the soil. In *The Physiology and Biochemistry of Herbicides*; Audus, L. J., Ed.; Academic Press: London, 1964; pp 163-206.
- Barles, R. W.; Daughton, C. G.; Hsieh, D. P. H. Accelerated parathion degradation in soil inoculated with acclimated bacteria under field conditions. *Arch. Environ. Contam. Toxicol.* 1979, 8, 647-660.
- Bergey, D. H. Gram-negative aerobic rods and cocci. In *The Shorter Bergey's Manual of Determinative Bacteriology*, 8th ed.; Holt, J. G., Ed.; Williams & Wilkins: Baltimore, 1979; pp 77-98.
- Bollag, J.-M.; Liu, S.-Y. Biological transformation processes of pesticides. In *Pesticides in the Soil Environment: Processes, Impacts, and Modeling*; Cheng, H. H., Ed.; Soil Science Society of America: Madison, WI, 1990; pp 169-211.
- Bovey, R. W. In *The Science of 2,4,5-T and Associated Phenoxy Herbicides*; Bovey, R. W., Young A. L., Eds.; Wiley: New York, 1980; Chapter 9, pp 301-356.
- Chatterjee, D. K.; Kilbane, J. J.; Chakrabarty, A. M. Biodegradation of 2,4,5-trichlorophenoxyacetic acid in soil by a pure culture of *Pseudomonas cepacia*. *Appl. Environ. Microbiol.* 1982, 44, 514-516.
- Clark, C. G.; Wright, S. J. L. Detoxication of isopropyl *N*-phenylcarbamate IPC and isopropyl *N*-3-chlorophenylcarbamate CIPC in soil, and isolation of IPC-metabolizing bacteria. *Soil Biol. Biochem.* 1970, 2, 19-26.
- Daughton, C. G.; Hsieh, D. P. H. Accelerated parathion degradation in soil by inoculation with parathion-utilizing bacteria. *Bull. Environ. Contam. Toxicol.* 1977, 18, 48-56.
- Evans, W. C.; Smith, B. S. W.; Fernley, H. N.; Davies, J. I. Bacterial metabolism of 2,4-dichlorophenoxyacetate. *Biochem. J.* 1971, 122, 543-551.
- Freed, V. H.; Montgomery, M. L. The metabolism of herbicides by plants and soils. *Residue Rev.* 1963, 3, 1-18.
- Greer, L. E.; Shelton, D. R. Effect of inoculant strain and organic matter content on kinetics of 2,4-dichlorophenoxyacetic acid degradation in soil. *Appl. Environ. Microbiol.* 1992, 58, 1459-1465.
- Greer, L. E.; Robinson, J. A.; Shelton, D. R. Kinetic comparison of seven strains of 2,4-dichlorophenoxyacetic acid-degrading bacteria. *Appl. Environ. Microbiol.* 1992, 58, 1027-1030.
- Gutenmann, W. H.; Loos, M. A.; Alexander, M.; Lisk, D. J. Beta-oxidation of phenoxyalkanoic acids in soil. *Soil Sci. Soc. Am. Proc.* 1964, 28, 205-207.
- Jacobsen, C. S.; Pedersen, J. C. Growth and survival of *Pseudomonas cepacia* DBO1(pRO101) in soil amended with 2,4-dichlorophenoxyacetic acid. *Biodegradation* 1992, 2, 245-252.
- Kelly, M. P.; Hallberg, K. B.; Tuovinen, O. H. Biological degradation of 2,4-dichlorophenoxyacetic acid: Chloride mass balance in stirred tank reactors. *Appl. Environ. Microbiol.* 1989, 55, 2717-2719.
- Kilbane, J. J.; Chatterjee, D. K.; Chakrabarty, A. M. Detoxification of 2,4,5-trichlorophenoxyacetic acid from contaminated soil by *Pseudomonas cepacia*. *Appl. Environ. Microbiol.* 1983, 45, 1697-1700.
- Kilpi, S.; Backström, V.; Korhola, M. Degradation of 2-methyl-4-chlorophenoxyacetic acid (MCPA), 2,4-dichlorophenoxyacetic acid (2,4-D), benzoic acid and salicylic acid by *Pseudomonas* sp. HV3. *Microbiol. Lett.* 1980, 8, 177-182.
- Kobayashi, H.; Rittman, B. E. Microbial removal of hazardous organic compounds. *Environ. Sci. Technol.* 1982, 16, 170A-183A.
- Krueger, J. P.; Butz, R. G.; Atallah, Y. H.; Cork, D. J. Isolation and identification of microorganisms for the degradation of dicamba. *J. Agric. Food Chem.* 1989, 37, 534-538.
- Krueger, J. P.; Butz, R. G.; Cork, D. J. Use of dicamba-degrading microorganisms to protect dicamba susceptible plant species. *J. Agric. Food Chem.* 1991, 39, 1000-1003.
- Loos, M. A. Phenoxyalkanoic acids. In *Herbicides: Chemistry, Degradation, and Mode of Action*, 2nd ed.; Kearney, P. C., Kaufman, D. D., Eds.; Dekker: New York, 1975; Vol. 1, pp 1-128.
- Loos, M. A.; Schlosser, I. F.; Mapham, W. P. Phenoxy herbicide degradation in soils: Quantitation studies of 2,4-D- and MCPA-degrading microbial populations. *Soil Biol. Biochem.* 1979, 11, 377-385.
- McClure, G. W. Degradation of phenylcarbamates in soil by mixed suspension of IPC-adapted microorganisms. *J. Environ. Qual.* 1972, 1, 177-180.
- Oh, K.-H.; Tuovinen, O. H. Detection and identification of substituted phenols as intermediates of concurrent bacterial degradation of the phenoxy herbicides MCPP and 2,4-D. *FEMS Microbiol. Lett.* 1991a, 79, 141-146.
- Oh, K.-H.; Tuovinen, O. H. Bacterial degradation of phenoxy herbicide mixtures 2,4-D and MCPP. *Bull. Environ. Contam. Toxicol.* 1991b, 47, 222-229.
- Pieper, D. H.; Reineke, W.; Engesser, K.-H.; Knackmuss, H.-J. Metabolism of 2,4-dichlorophenoxyacetic acid, 4-chloro-2-methylphenoxyacetic acid and 2-methylphenoxyacetic acid by *Alcaligenes eutropus* JMP 134. *Arch. Microbiol.* 1988, 150, 95-102.
- Racke, K. D.; Coats, J. R., Eds. Enhanced biodegradation of pesticides in the environment. *ACS Symp. Ser.* 1990, No. 426.
- Roeth, F. W. Enhanced herbicide degradation with repeat application. *Rev. Weed Sci.* 1986, 2, 45-65.
- Sandmann, E. R. I. C.; Loos, M. A.; van Dyk, L. P. The microbial degradation of 2,4-dichlorophenoxyacetic acid in soil. *Rev. Environ. Contam. Toxicol.* 1988, 101, 1-53.
- Sirons, G. J.; Chau, A. S. Y.; Smith, A. E. The phenoxyalkyl acid herbicides (CPHs). In *Analysis of Pesticides in Water*; Chau, A. S. Y., Afgan, B. K., Eds.; CRC Press: Boca Raton, FL, 1982; Vol. II, pp 155-227.
- Smith, A. E. Relative persistence of di- and trichlorophenoxyalkanoic acid herbicides in Saskatchewan soils. *Weed Res.* 1978, 18, 275-279.
- Smith, A. E. Degradation, fate, and persistence of phenoxyalkanoic acid herbicides in soil. *Rev. Weed Sci.* 1989, 4, 1-24.
- Smith, A. E.; Aubin, A. J. Metabolites of [<sup>14</sup>C]-2,4-dichlorophenoxyacetic acid in Saskatchewan soils. *J. Agric. Food Chem.* 1991, 39, 2019-2021.
- Smith, A. E.; Aubin, A. J. Breakdown of [<sup>14</sup>C]dimethylamine in soils. *J. Agric. Food Chem.* 1992, 40, 2299-2301.
- Smith, A. E.; Hayden, B. J. Relative persistence of MCPA, MCPB and mecoprop in Saskatchewan soils, and the identification of MCPA in MCPB-treated soils. *Weed Res.* 1981, 21, 179-183.
- Smith, A. E.; Mortensen, K. Degradation of waste 2,4-D residues using a soil bacterium in a sprayer tank system. *Can. J. Soil Sci.* 1991, 71, 243-246.
- Smith, A. E.; Aubin, A. J.; Biederbeck, V. O. Effects of long-term 2,4-D and MCPA field applications on soil residues and their rates of breakdown. *J. Environ. Qual.* 1989, 18, 299-302.
- Smith, A. E.; Hume, L.; Lafond, G. P.; Biederbeck, V. O. A review of long-term 2,4-D and MCPA applications on wheat production and selected biochemical properties of a Black Chernozem. *Can. J. Soil Sci.* 1991, 71, 73-87.

Received for review August 12, 1993. Revised manuscript received November 22, 1993. Accepted November 29, 1993.\*

\* Abstract published in *Advance ACS Abstracts*, January 15, 1994.