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Fate of the herbicides mecoprop, dichlorprop, and 2,4-D in aerobic and anaerobic sewage sludge as determined by laboratory batch studies and enantiomer-specific analysis

Christian Zipper[1], Christof Bolliger[1], Thomas Fleischmann[1], Marc J.-F. Suter[1], Werner Angst[1], Markus D. Müller[2] & Hans-Peter E. Kohler[1,*]

[1]*Swiss Federal Institute for Environmental Science and Technology (EAWAG) and Swiss Federal Institute of Technology (ETH), CH-8600 Dübendorf, Switzerland;* [2]*Swiss Federal Research Station, CH-8820 Wädenswil, Switzerland (*author for correspondence; e-mail: kohler@eawag.ch)*

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Abstract

Aerobic degradation experiments with the racemic mixtures of mecoprop and dichlorprop revealed that activated sludge collected from the aeration tank of a municipal waste water treatment plant degraded both enantiomers of mecoprop and dichlorprop within 7 days, albeit in an enantioselective manner; the (*S*) enantiomers were preferentially degraded. Mecoprop, dichlorprop, and 2,4-D were completely metabolized under aerobic conditions, as shown by the 86–98% elimination of dissolved organic carbon. Under anaerobic conditions, the concentration of 2,4-D decreased exponentially with a first-order reaction rate constant of 0.24 per day and without a lag-phase. After an incubation time of 17 days, 2,4-D was completely removed. 2,4-Dichlorophenol was the main metabolite of anaerobic 2,4-D degradation; only traces of 4-chlorophenol were detected. In contrast, the chiral phenoxypropionic acid herbicides mecoprop and dichlorprop persisted under anaerobic conditions during 49 days of incubation.

Introduction

(*RS*)-2-(4-chloro-2-methylphenoxy)propionic acid (mecoprop), (*RS*)-2-(2,4-dichlorophenoxy)propionic acid (dichlorprop), and 2,4-dichlorophenoxyacetic acid (2,4-D) (Figure 1) – together with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and (*RS*)-2-(2,4,5-trichlorophenoxy)propionic acid (fenoprop) – belong to the group of the phenoxyalkanoic acid herbicides. After World War II these herbicides came into widespread use for controlling broad-leaved weeds. In 1979 the U.S. Environmental Protection Agency banned the use of 2,4,5-T and fenoprop due to potential oncogenic, fetotoxic, and teratogenic risks associated with their use (Gintautas et al. 1992). Mecoprop, dichlorprop, and 2,4-D remained among the most commonly applied weedkillers (Gintautas et al. 1992; Worthing & Hance 1991). Mecoprop – in the form of the polyglycol diester – is the herbicidal ingredient of Preventol®B2, a root protectant mixed into building materials such as bitumenous sealings, insulators for flat roofs, and rubber seals (Anonymous 1995). It was shown that mecoprop concentrations in the runoff from flat roofs with Preventol®B2-treated bitumenous sealings reached 15 μg/L (Voegelin 1997) and, therefore, such roofs comprise yet another source of racemic mecoprop, which is most likely to enter municipal sewage treatment plants. Mecoprop and 2,4-D are "probable or transient leachers" (Fielding et al. 1992) with the potential to contaminate water resources and, therefore, it is not a surprise that residues of mecoprop, dichlorprop and 2,4-D were found in groundwater samples throughout the U.S. (Gintautas et al. 1992) and Europe (Fielding et al. 1992).

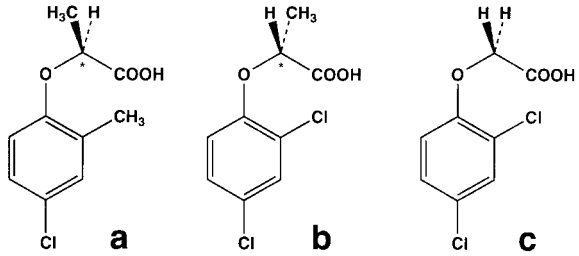

Figure 1. Structural formulas of (a) (*S*)-2-(4-chloro-2-methylphenoxy)propionic acid [(*S*)-mecoprop]; (b) (*R*)-2-(2,4-dichlorophenoxy)propionic acid [(*R*)-dichlorprop] and (c) 2,4-dichlorophenoxyacetic acid (2,4-D). The stereogenic centres of mecoprop and dichlorprop are marked by an asterisk (∗).

Mecoprop and dichlorprop are chiral molecules. Not only may the enantiomers of a chiral drug or pesticide exert different effects on the biological targets (Ariëns 1989), but also their biodegradation and environmental fate may differ (Kohler et al. 1997). For instance, when chiral (±)-1,2,3,4,5,6-α-hexachlorocyclohexane (±)-α-HCH), a byproduct of the production of technical mixtures of the insecticide γ-HCH (lindane), is incubated with anaerobic sewage sludge, the (+) enantiomer degrades three times faster than the (−) enantiomer (Buser & Müller 1995). *Rhodococcus rhodochrous* PB1 enantioselectively degrades chiral 3-phenylbutyric acid under aerobic conditions. Only the (*R*) enantiomer supports growth of strain PB1. The (*S*) enantiomer is transformed to a reactive intermediate that accumulates in the culture medium (Simoni et al. 1996). Ludwig et al. (Ludwig et al. 1992) showed that only (*R*)-dichlorprop is degraded by a mixed culture of marine microorganisms under aerobic laboratory conditions, a finding that is in agreement with analyses of North Sea water samples, in which an enrichment of (*S*)-dichlorprop was found. In contrast, (*S*)-dichlorprop disappears significantly faster from aerobic soil samples than the (*R*) enantiomer (Garrison et al. 1996). Enantioslective microbial degradation of mecoprop in soil (Buser & Müller 1997; Müller & Buser 1997), lake water (Buser & Müller 1998), groundwater and sediment suspension batches (Heron & Christensen 1992), and landfill leachates (Zipper et al. 1998) was reported and it was argued that measured changes in the enantiomeric ratio values of a chiral compound are strong indicators for in situ biological transformations (Zipper et al. 1998). Studies with pure cultures of bacteria able to grow with mecoprop as the sole carbon and energy source clearly showed that *Alcaligenes denitrificans* exlusively degrades the (*R*) enantiomer of the herbicide (Tett et al. 1994; Tett et al. 1997) while *Sphingomonas herbicidovorans* MH degrades both enantiomers of mecoprop to completion, however, in an enantioselective manner (Zipper et al. 1998; Zipper et al. 1996; Nickel et al. 1997).

Because mecoprop, dichlorprop, and 2,4-D are soluble in water, persistence of the compounds may increase the risk for contamination of groundwater and surface waters. In order to evaluate this risk, information is needed about the fate of these compounds in soil, groundwater, and sewage treatment. Studies about the fate of mecoprop in soils (Alexander & Aleem 1961; Karunen et al. 1978; Smith 1989), in municipal landfill leachates (Gintautas et al. 1992), and in aerobic aquifers (Heron & Christensen 1992) show that although mecoprop was degraded to some extent in top soil as well as in aerobic aquifers, it was not readily degraded in the landfill leachates and persisted for long periods of time in all these locations.

The purpose of our study was to evaluate the potential for degradation of mecoprop, dichlorprop, and 2,4-D in the aerobic as well as the anaerobic compartment of a sewage treatment plant with special emphasis on the stereochemistry of the compounds. Samples of aerobic and anerobic sewage sludge were taken from representative sewage treatment plants and were used for batch incubations in the laboratory. We were able to demonstrate that aerobic degradation of mecoprop and dichlorprop by sewage sludge is an enantioselective process – the (*S*) enantiomers are preferentially degraded. This might lead to input of non-racemic mecoprop into surface waters. Under anaerobic conditions, (*R*)-, (*S*)- and (*RS*)-mecoprop as well as (*R*), (*S*)- and (*RS*)-dichlorprop persisted until the end of the experiments, whereas 2,4-D was readily degraded.

Materials and methods

Reagents and chemicals

Pure (99%) (*RS*)- and (*R*)-mecoprop, (*RS*)- and (*R*)-dichlorprop, and 2,4-D were obtained from Riedel-de Haen (Seelze, Germany). (*RS*)-Dichlorprop-ring-$^{13}C_6$ (>98% ^{13}C) was bought from Cambridge Isotope Laboratories, Innerberg, Switzerland. The (*S*) enantiomers of mecoprop and dichlorprop are not commercially available. (*S*)-mecoprop was resolved from (*RS*)-mecoprop by the formation of diastereomeric salts as previously described (Zipper et al. 1996). (*S*)-dichlorprop was synthesized as described (Bolliger

1996). Anhydrous sodium sulfate, 2,4-dichlorophenol, 4-chlorophenol, dichloromethane, *n*-hexane and acetone were obtained from Fluka, Buchs, Switzerland. Sulfuric acid and sodium hydroxide were bought from Merck, Dietikon, Switzerland. Soluble starch was obtained from Difco Laboratories, Detroit, MI. 2,3,4,5,6-Pentafluorobenzylbromide (PFBBr) was obtained from Aldrich, Buchs, Switzerland.

Sewage sludge

The sewage sludge for the aerobic degradation experiments was taken from the aeration tank of the municipal waste water treatment plant Neugut, Dübendorf, Switzerland. The plant consists of mechanical and biological stages for the treatment of the waste water from 45 000 inhabitants and some industrial sites. The sludge contained approximately 2% dry matter and had a pH of 7.2. The sewage sludge for the anaerobic degradation experiments was taken from the anaerobic stabilizer of the municipal waste water treatment plant Zürich-Glatt, Switzerland. This plant consists of mechanical and biological treatment of waste water from 100 000 inhabitants and has a mesophilic anaerobic sludge stabilization. The anaerobic sewage sludge consisted of approximately 3% dry matter and had a pH of 7.8. Addition of starch and yeast to the sludge restarted gas production. Both plants are considered to be typical for many other installations in Switzerland. The plant Zürich-Glatt was previously studied in detail (Alder et al. 1990).

Batch incubations with mecoprop, dichlorprop and 2,4-D

Aerobic incubations were done according to the international standard (ISO 7827) for the evaluation of the ultimate aerobic biodegradability of organic compounds. The single test compound (*R*)-, (*S*)-, (*RS*)-mecoprop, (*R*)-, (*S*)-, (*RS*)-dichlorprop, or 2,4-D was the sole source of carbon and energy in the medium. The initial concentration of organic carbon from the test compounds was between 10 and 40 mg/L and the medium consisted of a phosphate buffer (pH 7.4) with additions of $MgSO_4$, $CaCl_2$ and $FeCl_3$. Sewage sludge was added to the medium to obtain 30 mg/L of suspended solids in the final mixture.

Anaerobic incubations of mecoprop, dichlorprop and 2,4-D were performed as follows. Approximately 200 g of undiluted digested sewage sludge was put into a 300-mL glass serum bottle and fortified with 100 μL of an acetone solution containing 450–500 μg of either i) (*R*)-mecoprop, (*R*)-dichlorprop and 2,4-D or ii) (*S*)-mecoprop, (*S*)- dichlorprop, and 2,4-D or iii) (*RS*)-mecoprop, (*RS*)-dichlorprop, and 2,4-D. Each series was run in triplicate. After thorough mixing, 1 g of soluble starch and 2.4 g of bakers' yeast dissolved in 10 mL of distilled water were added as nutrients. The bottles were tightly capped and incubated at 25 $\pm$ 1 °C for up to 49 days in the dark. Samples were taken at different time intervals, the first one after vigorous shaking immediately after all the additions were made. A control experiment with sterilized sewage sludge was carried out with (*RS*)-mecoprop, (*RS*)-dichlorprop and 2,4-D. For this experiment, the sludge was autoclaved (120 °C, 60 min) twice with an intervening period of 24 h and treated with 10 mL of a NaN_3 solution (5% wt/vol in water).

Storage and preparation of samples

Samples (10 mL) from aerobic incubations were filtered (0.45 μm, Nalgene cellulose acetate filters), collected in 25-mL teflon-stoppered glass vials and stored at -20 °C until analysis by HPLC.

Sewage sludge samples (20 g) from anaerobic incubation experiments were collected in 40-mL teflon-stoppered glass vials and stored at -20 °C. In order to prepare the samples for GC/MS analysis, they were thawed, spiked with 50 μL of the internal standard solution (0.3 μg (*RS*)-dichlorprop-ring-$^{13}C_6$), and acidified with H_2SO_4 (50% vol/vol) to a pH of 1. Then, 10 mL of dichloromethane was added and the samples were vigorously shaken. The dichloromethane layer was removed with a pasteur pipet after centrifugation (10 min, 1′000 χ g). The extraction was repeated once with 5 mL dichloromethane. The volume of the combined extracts was reduced to approximately 5 mL under a gentle flow of nitrogen at 30 °C. Two mL acetone and 2 drops of an aqueous K_2CO_3 solution (30% wt/vol) were added and the evaporation continued to a volume of approximately 1 mL. Twice, another 5 mL of acetone was added and evaporated to 1 mL in order to reduce the amount of dichloromethane. One mL acetone and 200 μL PFBBr solution (1% vol/vol in acetone) were added, the extract was vigorously shaken and then left to react overnight. Standard solutions were derivatized by adding 2 mL acetone, 2 drops of K_2CO_3 solution and 200 μL PFBBr solution. After reaction, 2 mL *n*-hexane and 4 mL H_2O were added to the samples and the standards, the vials were

vigorously shaken and the hexane extract was dried with approximately 5 mg anhydrous sodium sulfate. After filtration through a 0.5-μm filter (Millex LCR4, Millipore Corp., Bedford, MA, USA) the samples were stored in teflon-stoppered vials at 4 °C in the dark until analysis.

HPLC analysis

HPLC analyses were performed on a Gynkotek HPLC system with a M480G pump, a Gina 50T autosampler and a UVD340S photodiodearray detector (Gynkotek GmbH, Germering, Germany). The system was operated isocratically with an eluent consisting of 70% methanol and 30% NaH_2PO_4 (50 mM, pH 3.0) at a flow rate of 0.7 mL/min. 40 μL of the samples was injected and the eluting compounds were detected at a wavelength of 230 nm. Enantiomers of mecoprop and dichlorprop were separated on a Nucleodex-α-pmCD column (200 by 4.0 mm) with permethylated α-cyclodextrin as the stationary phase (Macherey-Nagel, Düren, Germany).

GC-MS analysis

A GC 8065 (Fisons Instruments, Manchester, GB) and a VG AutoSpecQ double focusing magnetic sector hybrid mass spectrometer (VG Analytical, Manchester, GB) were used. The (*R*) and (*S*) enantiomers of the 2,3,4,5,6-pentafluorobenzyl (PFB) esters of mecoprop and dichlorprop were separated on a 15-m glass column (0.25 mm i.d.) with an OV1701 polysiloxane phase containing 35% heptakis-[2,3-dimethyl-6-*t*-butyldimethylsilyl]-β-cyclodextrin (TBDMβ-CD) as the chiral selector (Müller et al. 1997). Samples were injected on-column at 60 °C, and the oven was temperature programmed as follows: 60 °C, 2-min isothermal, 20 °C/min to 186 °C, 2 °C/min to 200 °C, then 25 °C/min to 230 °C followed by an isothermal hold for 1 min at this temperature. Samples were analyzed by electron-ionization (EI+, 70 eV) with selected ion monitoring (SIM). The ion source temperature was kept at 250 °C. The mass spectrometric resolution was about 1000 at 8 kV source potential. Up to six ions were monitored simultaneously for the detection of the PFB-esters of (*R*)- and (*S*)-mecoprop (m/z 394.04), 2,4-D (m/z 399.97), (*R*)- and (*S*)-dichlorprop (m/z 413.98) 2,4-dichlorophenol (m/z 341.96), 4-chlorophenol (m/z 308.00) and (*R*)- and (*S*)-dichlorprop-ring-$^{13}C_6$ (m/z 420.01) and a lockmass of m/z 404.98 from pentafluorokerosene was used. Concentrations of the herbicides and the metabolites were determined by the internal standard method and were corrected for sample size and recovery.

Enantiomer-specific analysis of mecoprop and dichlorprop by HPLC and GC-MS

We developed two analytical methods for the separation of the (*R*) and the (*S*) enantiomers of mecoprop and dichlorprop in order to detect enantioselectivity, racemization, and enantiomerization in degradation experiments. Both methods are based on columns that contain modified cyclodextrins as chiral selectors in their stationary phases. An HPLC-method was developed for determining the fate of the enantiomers of mecoprop and dichlorprop in incubation mixtures with diluted aerobic sewage sludge, where the content of particulate material was low (30 mg/L) and the concentration of the analyte was relatively high (17 to 30 mM) at the start of the degradation experiments. After a filtration step the samples were, without further treatment, directly injected onto the column. The enantiomers of mecoprop and dichlorprop were well resolved on the Nucleodex-α-pmCD (permethylated α-CD) with enantiomeric resolutions of 2.7 and 2.5, respectively. The enantiomer resolution is defined as $(t_2 - t_1)/(w_1 + w_2)$, whereby t_1 and t_2 are the retention times of the earlier- and the later-eluting enantiomer, respectively, and w_1 and w_2 are the peak widths at half-height of the earlier- and the later-eluting enantiomer, respectively. The GC-MS method was developed for the detection of low concentrations of the herbicide enantiomers (11 μM at the start of the incubations) in undiluted digested sewage sludge (30 g/L of suspended solids). The PFB esters of mecoprop and dichlorprop were separated on the OV 1701-TBDM-β-CD glass column with an enantiomeric resolution of 0.9.

Results and discussion

Aerobic degradation studies

Degradation experiments with the racemic mixtures of mecoprop and dichlorprop revealed that aerobic sewage sludge was able to degrade both enantiomers of mecoprop and dichlorprop to completion, however, in an enantioselective manner (Figure 2a, b). The inoculum for these degradation experiments was diluted aerobic sewage sludge from a municipal wastewater treatment plant. At the end of such experiments, we

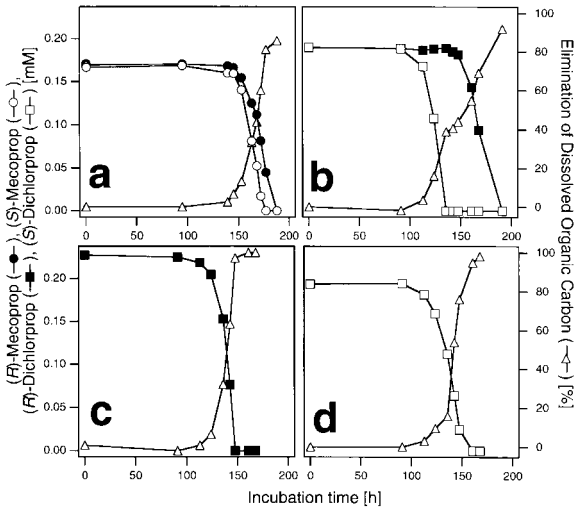

Figure 2. Aerobic degradation of (a) racemic mixture of mecoprop; (b) racemic mixture of dichlorprop; (c) (*R*)-dichlorprop and (d) (*S*)-dichlorprop by diluted sewage sludge from the aeration tank of a municipal waste water treatment plant at 25 °C. Concentrations [mM] (left-hand axis) and elimination of dissolved organic carbon [%] (right-hand axis) are plotted versus incubation time [h]. Note the faster degradation of the (*S*) enantiomers when sewage sludge was incubated with the racemic mixtures of mecoprop and dichlorprop (a, b).

Table 1. Lag-phase and degradation rate of (*R*)- and (*S*)-mecoprop, (*R*)- and (*S*)-dichlorprop and 2,4-D in aerobic sewage sludge from a municipal waste water treatment plant

Compound	Lag-phase[1] ± s [h] (n = 2)	Degradation rate[2] ± s [μmol/h/g of dry weight] (n = 2)
(*R*)-mecoprop[3]	146 ± 2	304 ± 88
(*R*)-mecoprop[4]	149 ± 2	176 ± 28
(*S*)-mecoprop[3]	143 ± 2	273 ± 47
(*S*)-mecoprop[4]	139 ± 6	174 ± 52
(*R*)-dichlorprop[3]	119 ± 2	352 ± 92
(*R*)-dichlorprop[4]	139 ± 15	213 ± 41
(*S*)-dichlorprop[3]	116 ± 5	188 ± 3
(*S*)-dichlorprop[4]	104 ± 3	225 ± 79
2,4-D	125 ± 14	241 ± 61

[1] The lag-phase was defined as the time interval from the start of the incubation to the time point at which the substrate concentration decreased to 95.
[2] The degradation rate was obtained by modeling the data to zero-order kinetics.
[3] Data from incubations with the single pure enantiomers.
[4] Data from incubations with the racemic mixture of both enantiomers.

observed a removal of dissolved organic carbon of 86–92% (Figure 2a, b). This indicates that aerobic degradation of (*RS*)-mecoprop and (*RS*)-dichlorprop was nearly complete. This view was substantiated by the fact that neither 4-chloro-2-methylphenol nor 2,4-dichlorophenol – proposed initial metabolites of aerobic degradation of mecoprop and dichlorprop, respectively (Garrison et al. 1996; Nickel et al. 1997; Smith 1985) – was detected by HPLC. Control experiments with sterilized sewage sludge did not show any degradation of the herbicides. Aerobic degradation appeared to be of zero-order and was modeled accordingly. Typically, degradation started after a lag-phase (104 to 149 h) and then commenced with rates of 174 to 225 μmol/h/g dry weight (Table 1). In all cases the (*S*) enantiomer was preferentially degraded. In contrast to the breakdown of (*S*)- and (*R*)-mecoprop during incubations with racemic mecoprop (Figure 2a), degradation of (*S*)- and (*R*)-dichlorprop was truly sequential when racemic dichlorprop was incubated with sewage sludge (Figure 2b); the concentration of (*R*)-dichlorprop remained at the initial value until (*S*)-dichlorprop was completely consumed (Figure 2b). Once degradation of (*R*)-dichlorprop commenced in incubations with the racemic mixture, it disappeared as fast as (*S*)-dichlorprop (Figure 2b, Table 1).

We also incubated aerobic sewage sludge with the single pure enantiomers of mecoprop and dichlorprop. During such incubations, we could never detect the (*R*) enantiomer when the (*S*) enantiomer was the substrate or the (*S*) enantiomer when the (*R*) enantiomer was the substrate (Figure 2c, d). Based on these results, we conclude that enantiomerization or racemisation did not occur in the course of these experiments. This is in contrast to observations, that mecoprop and dichlorprop racemized during degradation in aerobic soil (Buser & Müller 1997). Degradation rates for the (*R*) and (*S*) enantiomers in incubations with the single pure enantiomers were generally higher than the ones in incubations with the racemic mixtures (Table 1).

Additionally, we performed experiments to study the degradation potential of aerobic sewage sludge that was exposed to either (*R*)- or (*S*)-mecoprop prior to the degradation experiments. We incubated the sludge for 188 h with pure (*R*)-mecoprop and 176 h with pure (*S*)-mecoprop, divided each incubation mixture into two equal parts and exposed each part to pure (*R*)- and (*S*)-mecoprop for 20 h or more. Sewage sludge that had been pre-exposed to (*R*)-mecoprop was only able to consume the (*R*)-mecoprop, but not the *S*-mecoprop within 20 h (Figure 3a). Inversly, sewage sludge that had been pre-exposed to (*S*)-mecoprop specifically degraded (*S*)-mecoprop (Figure 3b). How-

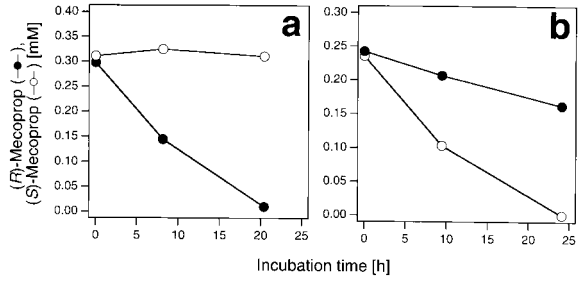

Figure 3. Aerobic degradation of (*R*)- and (*S*)-mecoprop by diluted sewage sludge from the aeration tank of a municipal waste water treatment plant which had been incubated with (a) (*R*)-mecoprop for 188 h and (b) (*S*)-mecoprop for 176 h prior to the degradation experiment.

ever, consumption of (*R*)-mecoprop was evident in this case. We could not observe enantiomerization or racemization in these experiments.

Enantioselectivity revealed itself in two ways during the degradation of mecoprop and dichlorprop by aerobic sewage sludge. First, such sludge preferentially degraded the (*S*) enantiomers of mecoprop and dichlorprop (Figure 2, Table 1). Second, aerobic sewage sludge that had been pre-exposed to (*R*)-mecoprop only consumed (*R*)-mecoprop, while sewage sludge that had been pre-exposed to (*S*)-mecoprop preferentially consumed (*S*)-mecoprop (Figure 3a, b). The sequential utilization of the two enantiomers of dichlorprop (Figure 2b) resembled sequential batch growth of bacteria on two different substrates, for example batch growth of *Escherichia coli* on a mixture of glucose and xylose (Standing et al. 1972). In such experiments, the concentration of xylose, the secondary substrate, remains at the initial value until glucose, the preferred substrate, is completely consumed. The authors concluded that xylose utilization is inhibited in the presence of glucose and that the xylose degrading enzymes are only induced after the depletion of glucose. Accordingly, one explanation of the sequential consumption of racemic dichlorprop is that (*S*)-dichlorprop, the preferred enantiomer, inhibits the degradation of (*R*)-dichlorprop, the secondary substrate. This view is substantiated by the fact that pure (*R*)-dichlorprop was completely degraded within 145 h (Figure 2c), whereas in incubations with the racemic mixture it took 190 h until (*R*)-dichlorprop vanished (Figure 2b). When achiral 2,4-D was incubated with aerobic sewage sludge, degradation typically started after a lag-phase of 125 h (Table 1) and was completed within 35 h. Removal of dissolved organic carbon was 97%. This shows that 2,4-D was completely degraded in such experiments.

Anaerobic degradation studies

Under anaerobic conditions, the concentration of 2,4-D decreased without an observable lag-phase and was, in a typical experiment, below the detection limit (5 nM) after 17 days (Figure 4). The degradation of 2,4-D could be described with the following equation

$$c = c_0 \mathrm{e}^{-kt} \qquad (1)$$

in which c [μM] is the concentration of 2,4-D at time t [h], c_0 [μM] is the initial concentration at t = 0, and k [h^{-1}] is the first-order reaction rate constant. We used a weighted exponential regression model to determine the first-order rate constant k (Figure 4) as 0.010 h^{-1} (0.24 d^{-1}) for the anaerobic degradation of 2,4-D. In sterilized sludge, the concentration of 2,4-D remained constant. This shows that the degradation of 2,4-D was biologically mediated. The proposed initial metabolites of anaerobic 2,4-D degradation, 2,4-dichlorophenol and 4-chlorophenol (Boyd & Shelton 1984; Mikkesell & Boyd 1985) could be detected during the course of the experiment. The concentration of 2,4-dichlorophenol increased with the concomitant decrease of 2,4-D, reached 7.9 μM after 9 days of incubation, and decreased to 5.9 μM at the end of the experiment (Figure 4). 4-Chlorophenol was present at very low concentrations during the whole experiment, and its concentration slightly increased at the end of the experiment to 0.4 μM. In contrast to other work (Boyd & Shelton 1984; Mikkesell & Boyd 1985), we did not find a rapid transformation of 2,4-dichlorophenol

The chiral phenoxypropionic acid herbicides mecoprop and dichlorprop persisted under anaerobic conditions during the 49 days, which the experiments lasted (Figure 5; shown are 17 d of incubation) irrespective whether the pure (*R*) enantiomers (Figure 5a), the pure (*S*) enantiomers (Figure 5b), or the racemic mixtures (Figure 5c) of mecoprop and dichlorprop were present. These findings cannot be attributed to a lack of biological activity in the sewage sludge, because 2,4-D was degraded in the same experiments (Figure 5) at rates that agree with literature data (Mikkesell & Boyd 1985). Furthermore, gas production was observed during the experiments and this strongly indicates that the sludge was biologically active. A pH of 7.3 was measured at the end of the

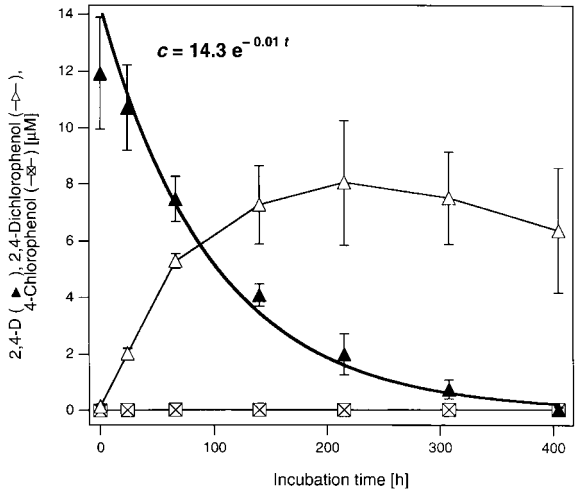

Figure 4. Degradation of 2,4-D, formation of 2,4-dichlorophenol, and formation of 4-chlorophenol during the anaerobic degradation of 2,4-D by undiluted digested sewage sludge from the anaerobic stabilizer of a municipal waste water treatment plant. Values are means of 9 replicates (2,4-D) and of triplicates (2,4-dichlorophenol and 4-chlorophenol). Standard deviations are indicated by the error bars. The solid line represents the best fit to an exponential model with a first-order reaction rate constant of 0.01 h^{-1} (0.24 d^{-1}).

experiment. This shows that the sludge was not inactivated by acidification. It rather seems that the methyl group at the 2-position of the alkanoic side chain – the only structural difference between dichlorprop and 2,4-D (Figure 1) – has a strong influence on the biodegradability of dichlorprop and mecoprop under anaerobic conditions. The enzymes which are responsible for the initial degradation of 2,4-D seem to attack the ether bond yielding 2,4-dichlorophenol, which was detected as the main metabolite. It is not surprising that structural differences in the proximity of the point of attack of the enzyme(s) may have a strong influence on the degradability.

Conclusions

Our experiments show that mecoprop and dichlorprop were enantioselectively degraded under aerobic conditions by activated sewage sludge. This implies that residues entering surface waters might be nonracemic mixtures although the original mixture at the top of the waste stream was racemic. The results of this study also led us to conclude that the degradation and the environmental fate of stereoisomers have to be studied with enantiomer-specific tools.

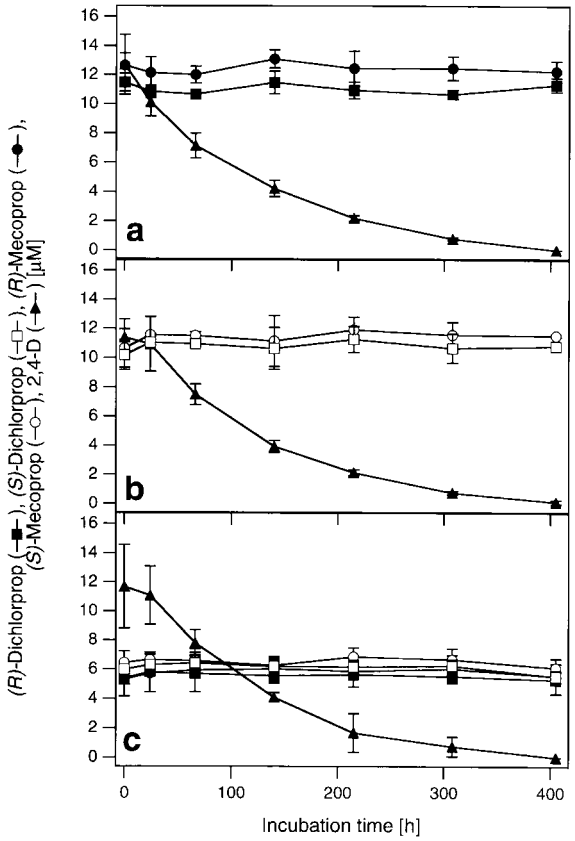

Figure 5. Anaerobic degradation experiments with undiluted digested sewage sludge from the anaerobic stabilizer of a municipal waste water treatment plant incubated with (a) (*R*)-mecoprop, (*R*)-dichlorprop and 2,4-D, (b) (*S*)-mecoprop, (*S*)-dichlorprop and 2,4-D, (c) (*RS*)-mecoprop, (*RS*)-dichlorprop and 2,4-D. Values are means of triplicates; standard deviations are indicated by the error bars.

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