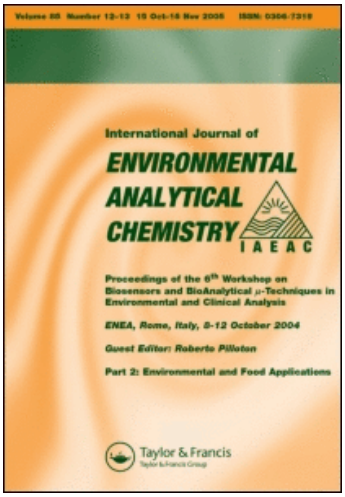

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DEGRADATION OF THE HERBICIDE BROMOXYNIL IN BATCH CULTURES UNDER GROUNDWATER CONDITIONS

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The biological degradation of bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) under simulated groundwater conditions was investigated. The influence of aerobic and anaerobic conditions on the degradation rate was examined in batch cultures at 8°C during 32 days. The cultures consisted of 400 ml groundwater plus 2000 ml salt basal medium. Final bromoxynil concentration was 1 mg/l. Incubation was carried out with and without 100 mg/l acetate added as carbon source. Dissolved organic carbon (DOC), oxygen, nitrate and sulphate, bacterial number (CFU), enzymatic hydrolysis of fluorescein-di-acetate (FDA), and bromoxynil concentration was estimated along the test. Only anaerobic, nitrate reducing conditions caused biodegradation of bromoxynil after 17 - 21 days. The addition of acetate delayed this process, although the complete degradation after 32 days in both tests amounted to 99%. In spite of a high bioactivity, no degradation of bromoxynil could be found under aerobic conditions within 32 days.

KEY WORDS: Herbicides, bromoxynil, biodegradation, groundwater, bankfiltration, batch cultures.

INTRODUCTION

Bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) as salts and esters are used in combination with other herbicides like ioxynil and mecoprop for weed control in cereal crops. Additionally bromoxynil is known as an effective metabolite of the herbicide bromofenoxim. Since the application of atrazine is prohibited in Germany, bromoxynil shows increasing importance as a substitute for this pesticide. The recommended application rates are about 50 kg/km^2 for bromoxynil and 150 – 200 kg/km^2 for bromofenoxim[1].

Bromoxynil esters imply a potential risk for aquatic species. Earlier investigations have shown the sensitvity of green algae and other microorganisms towards bromoxynil[5,6], and its toxicity for water organisms like *Daphnia sp.* and fishes[4]. Some investigations have shown the hydrolysis of bromoxynil-octanoate and bromoxynil-butyrate in surface water within a few hours[2,3] but a half-live for bromoxynil-phenol of 9 – 17 days[4].

Photolytic destruction of the herbicide in water has been described depending on the amount of fulvic acids[7,8]. Anyhow the photodegradation of bromoxynil is much slower than the photolysis of the herbicide bromofenoxim[3].

Biodegradation of bromoxynil is assumed as the most important process of dissipation[9]. Enzymatic degradation by purified pentachlorophenol hydrolase and by cells of *Flavobacterium sp.* has been described[10]. There are indications that cyanide, bromide and di-bromo-hydroquinone are the first products in the metabolism of this substance. Other degradation products are 3,5-dibrom-4-hydroxybenzamide or 3,5-dibromo-4-hydroxybenzoic acid and ammonium[11,12].

Relatively few studies have been conducted on the interaction of bromoxynil and the bioactivity of the soil microflora. The half-live of bromoxynil in soil generally amounts to 2 weeks[13,14]. Temperature and moisture appear to be a major factor influencing the microbial breakdown of bromoxynil in soil under laboratory conditions[14].

We assume that the concentration of oxygen, the availability of alternative terminal electron acceptors, and the content of degradable organic carbon is important for the rate of biodegradation of herbicides both in top soil and aquifer. Concentration of these compounds can change distinctively during bankfiltration, artificial groundwater recharge and underground passage of water. The influence of different redox conditions in subsoil on biodegradation of contaminants is already shown by other authors[15,16].

These investigations focus on possible interactions between the herbicide bromoxynil, soil, water and microorganisms during these infiltration processes. In situ investigations on the biodegradation of pesticides in the subsurface are prohibited in Germany because of the potential risk of groundwater contamination. In these experiments batch cultures were incubated simulating groundwater conditions. The effects of electron acceptors and acetate on degradation were determined.

EXPERIMENTAL

Batch cultures

Salt basal medium

Solution a 21.75 g K_2HPO_4, 8.5 g KH_2PO_4, 44.6 g Na_2HPO_4 * 12 H_2O, 1.7 g NH_4Cl, 1000 ml deionised water, pH 7.2

Solution b 22.5 g $MgSO_4$ * 7 H_2O, 1000 ml deionised water

Solution c 27.5 g $CaCl_2$, 1000 ml deionised water

Solution d 0.25 g $FeCl_3$, 1000 ml deionised water

3 ml of sterilized solution a, b, c and d were added with sterile deionised water to 1000 ml.

To 2 I of this basal medium 400 ml groundwater from a water catchment area with artificial groundwater recharge[17] were added as inoculum. Bromoxynil was added to give a final concentration of 1 mg/l. The cultures were incubated in the dark for 32 days at 8°C (in situ temperature) under aerobic and anaerobic conditions, with and without 100 mg/l acetate.

For anaerobic incubation 25 mg/l nitrate was added as an alternative electron acceptor and the bottles were closed with paraffin.

The following analyses were carried out every second or third day.

Analysis of bromoxynil

Solid phase extraction

100 ml samples were acidified with 200 µl hydrochloric acid. 2 µl fenoprop (Sigma, ethylene-glycol-monoethylester) was used as internal standard. Each sample was

preconcentrated on bakerbond SPE 18 cartridges (1 g). After washing with 5 ml deionised water the herbicide was eluted twice with 2 ml methanol (Merck, for gas chromatography). The eluat was concentrated under nitrogen flow at 30°C to a volume of 50 - 100 µl. These residues were brought to 1 ml methanol.

Gas chromatography

- Hewlett Packard Series II 5890 gas chromatograph with ^{63}Ni ECD,
- Fused silica capillary column (DB5, 30 m* 0.25 mm ID*1.0 µm film),
- Carrier gas (He) at 0.9 bar,
- Temperature program: 50°C for 1 min, from 50°C to 270°C with 10°C/min, and 270°C for 17 min,
- Detector 320°C,
- Cold injection starting temperature: 50°C for 45 sec, from 50°C to 270°C with 12°C/min, and 270°C for 2 min and 30 sec,
- Injection volume 5 µl.

Determination of DOC, NO_3^-, $SO_4^=$

10 ml of each sample was filtered through a 0.45 µm membrane filter and the dissolved organic carbon was determined with a Shimadzu TOC-5000.

Nitrate and sulphate were measured with an ion chromatograph Dionex Series 4500i according to the "Deutsche Einheitsverfahren".

Bacteria counts

Colony forming units (CFU) were determined on oligotrophic agar and on agar with bromoxynil after an incubation of 14 days at 20°C.

Oligotrophic agar[18]
1 mg peptone (beef), 0.1 mg glucose, 0.1 mg K_2HPO_4, 0.02 g $FeSO_4$* 7 H_2O, 15 g agar, 1000 ml tape water, pH 7.2

Bromoxynil agar
Like the oligotrophic agar but with 1 mg/l bromoxynil added after cooling the sterilised agar to 55°C.

Determination of bioactivity (esterase)

The hydrolysis of fluorescein-di-acetate (FDA) by esterase was determined with modifications as an indicator of bioactivity according to Schnürer and Rosswall {19} and Obst {20}. 20 µl FDA-solution (20 mg/10 ml acetone, stored at -18°C) were added to 3 ml sample and 0.5 ml buffer (Merck, HEPES-Na, pH adjusted to 7.5). After incubation (sterile conditions, 90 min at 20°C, darkness) the fluorescein production was measured immediately on a Perkin-Elmer fluorescence spectrometer LC 50 at 480 nm (exication) and 505 nm (emission).

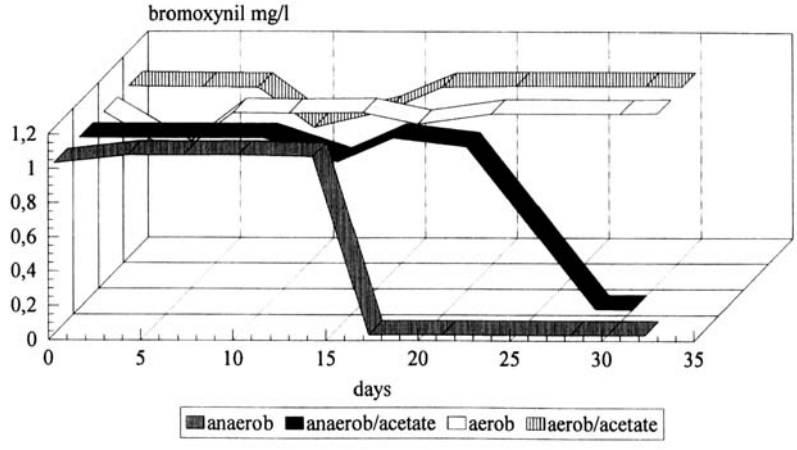

Figure 1 Degradation of bromoxynil in different batch cultures.

RESULTS

Biodegradation of bromoxynil was observed only under anaerobic conditions (Figure 1). After 32 days 99,4% (with acetate) or even 99,9% (without acetate) of the herbicide were degraded. No removal of bromoxynil could be found in sterile control tests. In anaerobic cultures without an additional source of carbon, the maximum degradation rate occurred after 15 days. In cultures with acetate added, bromoxynil degradation was delayed (Figure 1). In these cases, the degradation of bromoxynil started after 17 days. This result seems to imply that the microorganisms first used acetate as a readily degradable compound before they decomposed bromoxynil.

The concentration of sulphate was not affected by degradation. Only nitrate was used as terminal electron acceptor in the anaerobic cultures. In cultures without acetate the nitrate concentration decreased from 26 mg/l to 14 mg/l after 32 days. In culture media spiked with acetate the removal of nitrate was much faster. No nitrate could be detected after 17 days (Figure 2).

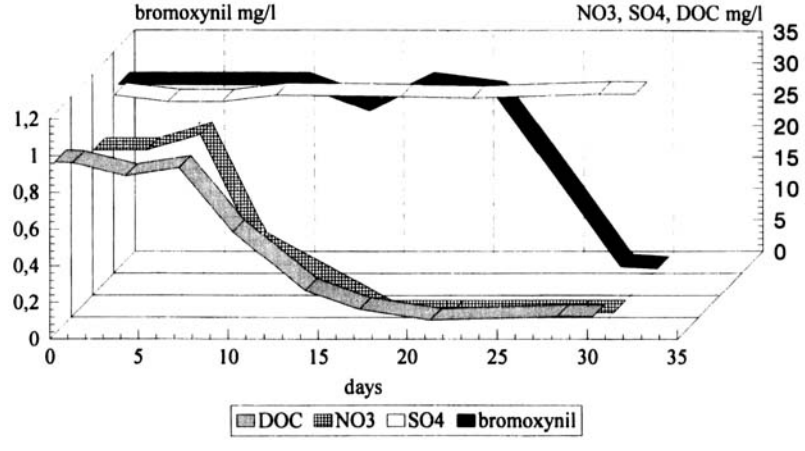

Figure 2 Removal of DOC and nitrate in an anaerobic culture with acetate.

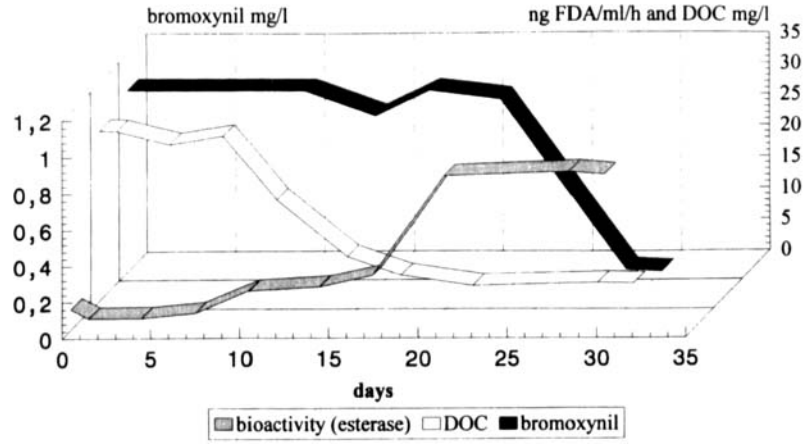

Figure 3 Bioactivity (FDA hydrolysis) and bromoxynil degradation in an anaerobic culture with acetate.

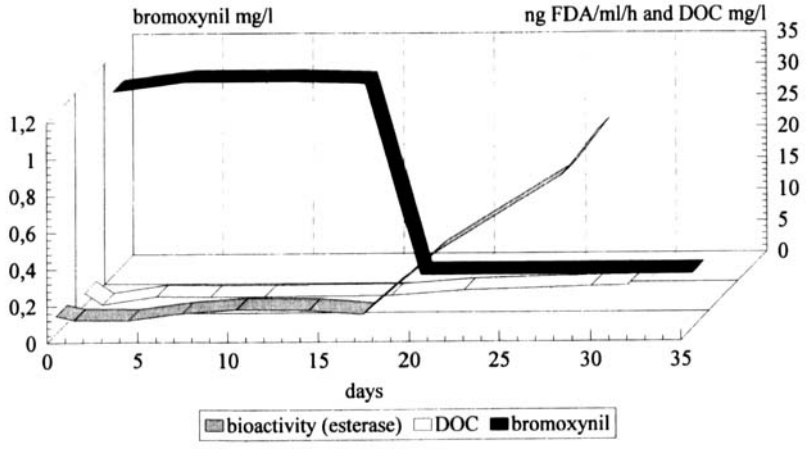

Figure 4 Bioactivity and bromoxynil degradation in an anaerobic culture without acetate.

The bioactivity – determined by the hydrolysis of FDA - increased with decreasing dissolved organic carbon (Figure 3). This increase of microbial activity corresponded to the degradation of bromoxynil (Figure 4). The persistent DOC level in different cultures was 1.1 – 3.5 mg/l after about 14 – 17 days. This concentration did not change during the incubation.

In spite of a relative high bioactivity, no biodegradation could be found with aerobic culturing within 32 days (Figure 4). Under aerobic conditions the hydrolysis of FDA and the DOC removal increased faster than under anaerobic conditions (Figures 5 and 3) but without any change of the bromoxynil concentration.

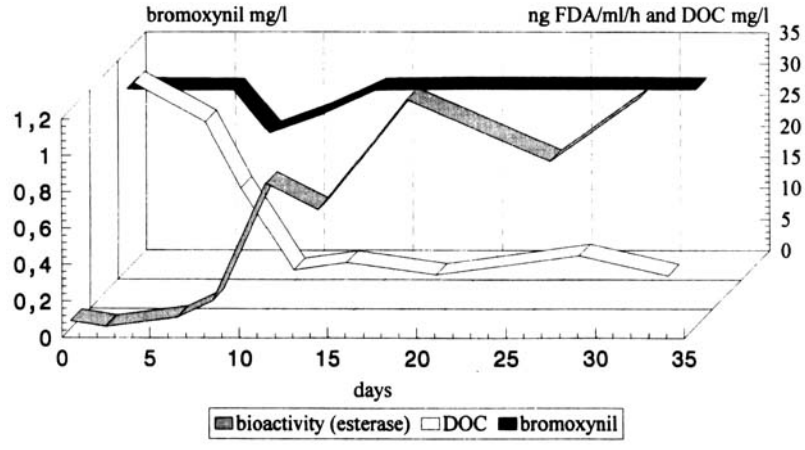

Figure 5 Bioactivity and concentration bromoxynil in an aerobic culture with acetate.

DISCUSSION

Biodegradation of bromoxynil was only observed under anaerobic, nitrate reducing conditions. The final degradation rate of bromoxynil after 32 days was greater than 99%. The results indicate that acetate as a readily degradable source of carbon delays the decomposition of bromoxynil. Only after the removal of acetate, which was accompanied by the consumption of nitrate, the microflora decomposed bromoxynil. No reduction of sulphate could be observed. Under aerobic conditions no degradation of bromoxynil was found within 32 days.

The interpretation of these data with respect to in situ groundwater conditions is difficult. The results indicate that bromoxynil could be persistent in aerobic groundwater for more than 4 weeks. Biodegradation of bromoxynil may appear in anaerobic groundwater or during bankfiltration with nitrate reducing conditions. The succession of redox changes should be considered as an important factor influencing the fate of bromoxynil during infiltration and in groundwater.

Biodegradation tests with batch cultures should only be taken as a first step for the simulation of real field conditions. Therefore further studies are necessary to confirm these results with the help of larger scale pilot plants and field investigations.

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