

## Application of microbial hot spots enhances pesticide degradation in soils

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Received 3 August 2006; received in revised form 12 December 2006; accepted 15 December 2006

Available online 8 February 2007

### Abstract

Through transfer of an active, isoproturon degrading microbial community, pesticide mineralization could be successfully enhanced in various soils under laboratory and outdoor conditions. The microbes, extracted from a soil having high native ability to mineralize this chemical, were established on expanded clay particles and distributed to various soils in the form of microbial “hot spots”. Both, diffusion controlled isoproturon mass flow towards these “hot spots” ( $6 \mu\text{g d}^{-1}$ ) as well as microbial ability to mineralize the herbicide (approximately  $5 \mu\text{g d}^{-1}$ ) were identified as the main processes enabling a multiple augmentation of the native isoproturon mineralization even in soils with heavy metal contamination. Soil pH-value appears to exert an important effect on the sustainability of this process. © 2006 Elsevier Ltd. All rights reserved.

**Keywords:** <sup>14</sup>C-isoproturon; Enhanced mineralization; Diffusion; Microbial community; Lysimeter

### 1. Introduction

Pesticide leaching to groundwater (Johnson et al., 2001) represents a severe problem especially in cases where groundwater is a drinking water source due to the very low potential for microbial pesticide degradation of such surface and groundwater sources (Mouvet et al., 1997; Johnson et al., 2000). Several pesticides, e.g., the phenylurea herbicide isoproturon (IPU) already can be found in groundwater and some reports (e.g. Johnson et al., 2001) have documented isoproturon concentrations exceeding the critical approved values for drinking water ( $0.1 \mu\text{g l}^{-1}$ ). Furthermore, we have also determined isoproturon presence in the leaching water below 2-meter deep outdoor lysimeters (Dörfler et al., 2006) at concentrations 40 to 50-fold

above the approved European threshold for drinking water ( $0.1 \mu\text{g l}^{-1}$ ).

In addition, radical changes in soil's capacity to mineralize isoproturon following hot and dry climate conditions have been also observed (Levy et al., 2006). If such climatic changes, perhaps resulting from global temperature fluctuations (ECCP, 2003), are able to exert long-term effects on specific soil functions, development of strategies for mitigating these negative effects on both environment and human health is necessary and essential. In order to reduce the risk of groundwater contamination by chemical residues, enhancing degradation of pesticide residues in top soils represents therefore a positive step towards addressing this problem.

One approach for enhancing contaminant degradation involves application of microorganisms via soil inoculum as describes by Schroll et al. (2004), whereby successful *in situ* decontamination of 1,2,4-trichlorobenzene (TCB) contaminated soils was demonstrated. Further, a lysimeter

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study by Schroll and Kühn (2004) identified a “Calcaric Regosol” soil able to mineralize the herbicide isoproturon intensely under actual environmental field conditions. We therefore used the following strategy in the present study: (1) inoculation another soil in a lysimeter study with a small aliquot of “Calcaric Regosol” soil to determine whether increased IPU mineralization can be achieved under outdoor conditions; (2) analysis of this augmentation using other soils within a laboratory setting; (3) isolating microbial community in liquid cultures. The observed isoproturon mineralization by this microbial community has remained stable for the past 3 years. Despite application of microbiological approaches described by Sørensen et al. (2001), among others, we have not been able thus far to isolate the key organisms of this community; this work is still under progress; (4) developing a very effective approach for application of this microbial community to agricultural soils to obtain increased IPU mineralization.

To enable a direct comparison of IPU mineralization results in all soils, experiments were conducted at a soil water potential of  $-15$  kPa and bulk density of  $1.3 \text{ g cm}^{-3}$ , according to a recent report by Schroll et al. (2006) on the quantification effects of soil moisture on the aerobic microbial mineralization of selected pesticides in different soils.

## 2. Materials and methods

### 2.1. Soils

Six soils (Table 1), representing typical but different agricultural soils of southern Germany and Switzerland, were used for the experiments. A complete soil characterization has already been published elsewhere (Schroll et al., 2006), except for both “Urbic Anthrosol” soils. Nevertheless, some main soil characteristics are summarized in Table 1. “Urbic Anthrosol HM” soil was artificially contaminated with heavy metals ( $7 \text{ mg kg}^{-1}$  Cd,  $342 \text{ mg kg}^{-1}$  Cu,  $1800 \text{ mg kg}^{-1}$  Zn) 5 years prior to this study. The solid density of the soils was determined according to DIN 18124:1997-07.

### 2.2. Pesticide

$^{14}\text{C}$ -ring-labeled-isoproturon [3-(4-isopropylphenyl)-1,1-dimethylurea] (IPU) with a specific radioactivity of  $3.96 \text{ MBq mg}^{-1}$  was used as a representative pesticide for phenylurea-herbicides. Some IPU characteristics have been previously described by Schroll and Kühn (2004). The radioactive IPU purity was  $>98\%$ .  $^{14}\text{C}$ -IPU was mixed with the commercial available product Arelon to a final isoproturon concentration of  $500 \text{ mg ml}^{-1}$ , according to guidelines provided by the pesticide producer Agrevo (Frankfurt, Hoechst, Germany), and resulting in a final specific radioactivity of  $686 \text{ Bq } \mu\text{g}^{-1}$  (IPU standard 1 for soil experiments). For liquid culture experiments a separate Arelon stock solution (IPU standard 2) was prepared to a final concentration of  $10 \text{ mg ml}^{-1}$  and specific radioactivity of  $5.5 \text{ Bq } \mu\text{g}^{-1}$ .

### 2.3. Transfer of a microbial community

As previously reported by Schroll and Kühn (2004) and Schroll et al. (2006), the “Calcaric Regosol” soil showed a high indigenous ability for mineralizing isoproturon. We therefore translated this microbial ability into an approach for accelerating the *in situ* degradation of isoproturon in other agricultural soils. Two different methods were used for delivery of isoproturon degrading microbial community present in “Calcaric Regosol” soil to other soils showing low IPU mineralization rates:

1. Soil inoculum from “Calcaric Regosol” soil was done according to Schroll et al. (2004) for the compound TCB. In this recent study a 5% inoculum containing adapted TCB degrading microbes was added to another soil to enhance its TCB mineralization ability.
2. A specific isoproturon mineralizing microbial community was isolated from “Calcaric Regosol” soil and established on expanded clay particles.

The effects of these approaches were tested by laboratory and field lysimeter experiments.

Table 1  
Main characteristics of the Ap-horizon of all soils

	Calcaric Regosol	Humic Cambisol	Mollic Gleysol	Aric Anthrosol	Urbic Anthrosol	Urbic Anthrosol HM
<i>Soil characteristics</i>						
$<2 \mu\text{m}$ clay%	33	11	22	13	14	13
2–63 $\mu\text{m}$ silt%	34	19	60	19	52	49
63–2000 $\mu\text{m}$ sand%	33	70	18	68	34	38
pH $\text{CaCl}_2$	7.2	6.9	5.4	6.7	6.7	6.4
Organic C%	2.70	1.30	1.50	0.99	1.49	1.64
Total N%	0.27	0.10	0.17	0.10	0.16	0.13
$\text{CaCO}_3\%$	5.1	0.5	$<0.2$	$<0.2$	$<0.2$	$<0.2$
Solid density $\text{g cm}^{-3}$	2.47	2.64	2.53	2.47	n.d.	n.d.
Isoproturon sorption $K_d$ -value $\text{cm}^3 \text{ g}^{-1}$	1.83	0.86	1.06	0.71	n.d.	n.d.

### 2.3.1. Lysimeter experiment

A test system as per our previous report (Schroll and Kühn, 2004) was used for studying the IPU behavior under field conditions.

An aliquot of “Calcaric Regosol” soil (mixture of the uppermost 5 cm of “Calcaric Regosol” soil) was transferred to the uppermost centimeter of “Mollic Gleysol” soil to achieve a final soil inoculum concentration of 5%. Application of isoproturon to the lysimeter and the experimental paradigm are both described in detail elsewhere (Schroll and Kühn, 2004). Specific soil humidity (Ruth and Munch, 2005) and temperature sensors for recording the environmental parameters for microbial degradation processes were placed at 1 cm depth where the highest pesticide concentration was identified by  $^{14}\text{C}$ -analysis.

### 2.3.2. Liquid cultures

The bacterial community of “Calcaric Regosol” soil was cultivated in mineral salt (MS) medium (Sørensen et al., 2001) with IPU, dissolved in the formulation Arelon. For liquid cultures, 5 g of this soil (dry weight equivalent; equilibrated for 10 days at 20 °C and  $-15$  kPa) were incubated in 100 ml flasks with 25 ml of sterile MS-medium (culture “A”).  $^{14}\text{C}$ -labeled IPU was added to the flask from an Arelon stock solution (IPU standard 2) to reach a concentration of  $25 \text{ mg l}^{-1}$ . Incubation flasks were linked to the laboratory system (see below) to measure volatile  $^{14}\text{C}$ -compounds and  $^{14}\text{CO}_2$ . Air was drawn through a  $0.2 \mu\text{m}$  sterile filter (Midisart 2000, Sartorius AG, Göttingen) before entering the incubation flask. We confirmed the sterility of this system by incubating already sterilized medium for 2 weeks. Liquid cultures were then incubated up to 39 days on an orbital shaker (3005, GFL, Burgwedel) at 100 rpm in the dark at 20 °C.

After incubating the cultures for 4 weeks, 1 ml of soil suspension culture (“A”) was transferred to 24 ml of fresh IPU containing MS-medium (culture “B”). Two gram of sterile expanded clay particles with a diameter of 2–4 mm (approx. pH 7; total N:  $3\text{--}8 \text{ mg l}^{-1}$ ;  $\text{P}_2\text{O}_5$ :  $5\text{--}10 \text{ mg l}^{-1}$ ;  $\text{K}_2\text{O}$ :  $100\text{--}120 \text{ mg l}^{-1}$ ; pore volume: was  $>80\%$ ; Datasheet Seramis, Masterfoods GmbH, Mogendorf, Germany) consisting of fired clay material with Illit-Muskovit and Quarz as dominating minerals were then added to liquid culture “B”, and the microorganisms were allowed to grow on the artificial surfaces. Expanded clay was used as it is often applied as the conventional material for immobilizing microorganisms in bioreactor studies (Chua and Chen, 1995; Chang et al., 2002). After incubating culture “B” 4 weeks, the expanded clay was bisected to obtain replicates and was then transferred to 25 ml fresh IPU containing MS medium (culture “C”), respectively. 1 g of sterile expanded clay particles was added to liquid culture “C” to double the growth surface. The last incubation step (culture “B” incubation to culture “C”) was repeated for at least 10 cycles, whereupon an aliquot of the expanded clay particles was sampled to scrutinise the IPU mineralization ability of the microbes. The remaining major part of the clay

particles was then used in soil and liquid culture experiments.

### 2.3.3. Laboratory biodegradation experiments

We studied the mineralization of  $^{14}\text{C}$ -labeled IPU under laboratory conditions as previously described (Schroll et al., 2004). Soils (50 g, dry weight equivalent, each) were incubated in 100 ml double-wall flasks in the dark at  $20 \text{ °C} \pm 1 \text{ °C}$ . Humidified air ( $1.01 \text{ h}^{-1}$ ) was drawn via a pump through the system at three intervals per week. After passing through the flasks, the air was trapped in four subsequent absorption tubes, the first two of which were filled with ethyleneglycolmonomethylether to fix volatile  $^{14}\text{C}$ -substances, and the following two were filled with 0.1 M NaOH solution to fix  $^{14}\text{CO}_2$  from mineralization processes separately.

Soil (46.5 g, dry weight equivalent) was equilibrated at soil water content equivalent to 80% of the respective optimum for mineralizing chemicals (Schroll et al., 2006) for 2 weeks at  $20 \text{ °C} \pm 1 \text{ °C}$ . Isoproturon (230  $\mu\text{g}$ , IPU standard 1) was applied dropwise with a Hamilton syringe to 3.5 g of soil previously dried ( $105 \text{ °C}$ , 24 h) and mixed thoroughly. These soil aliquots were added to equilibrated soils, mixed well with a spatula and transferred to incubation flasks. Water was added to reach a final soil water potential of  $-15$  kPa and the soils were then compacted with a spatula to a volume equivalent to a soil density of  $1.3 \text{ g cm}^{-3}$ . This allowed us to study the mineralization of isoproturon in different soils at their respective optimum (Schroll et al., 2006). After 46 days, experiments were terminated and extractable and non-extractable  $^{14}\text{C}$ -labeled pesticide residues were quantified as previously described by Schroll and Kühn (2004).

### 2.3.4. Application of the microbial community via soil inoculum and clay particles

In soil inoculum studies, 5% of equilibrated “Calcaric Regosol” soil was mixed with other soil material prior to pesticide application (see Section 2.3.3). When microbes were applied via clay particles, 1.5 g of wet clay particles (=20 clay particles, equivalent to approx. 200 mg dry weight) were transferred after 10 consecutive incubation cycles (see Section 2.3.2) from the liquid cultures to soils (50 g, dry weight equivalent), and then the particles were mixed with the soil subsequent to pesticide application (see Section 2.3.3). At termination of experiments, expanded clay particles and soil material were separated and extracted, whereby extractable and non-extractable  $^{14}\text{C}$ -labeled residues were quantified and identified as described previously (Schroll and Kühn, 2004).

### 2.3.5. Cell counting

To determine the number of colony forming units (CFU), serial 10-fold dilutions of liquid cultures were prepared in  $1 \times \text{PBS}$ -buffer ( $10 \times \text{PBS}$  with  $12 \text{ g l}^{-1} \text{ NaH}_2\text{PO}_4$ ,  $14.2 \text{ g l}^{-1} \text{ Na}_2\text{HPO}_4$ ,  $75.97 \text{ g l}^{-1} \text{ NaCl}$ , sterilized before use at  $121 \text{ °C}$ , 20 min) and spread (0.1 ml) over mineral salt

agar (MS-medium plus 15 g l<sup>-1</sup> Agar-Agar, Euler, Frankfurt, Germany) or R2A-agar (Merck, Darmstadt, Germany). The CFU of expanded clay was determined by crushing and mixing 0.2–0.4 g of expanded clay in 1 ml 1×PBS before diluting and spreading the sample on MS- or R2A-agar plates.

### 2.3.6. Determination of $K_d$ -value

The  $K_d$  value for isoproturon in soils was determined according to the OECD guideline 106 (OECD, 2000).

### 2.3.7. Determination of isoproturon diffusion in soil material

We used a diffusion tube experiment as described by Schaefer et al. (1995) to measure the isoproturon diffusion coefficient in “Humic Cambisol” soil. Briefly, 0.1 mg g<sup>-1</sup> dry soil sodium azide was added to inhibit microbial IPU degradation. We observed no <sup>14</sup>C<sub>2</sub>O presence in NaOH-traps during the experiments, indicating an effective inhibition of microbial mineralization of <sup>14</sup>C-isoproturon. One half (2.8 cm) of each tube (material: polypropylene, diameter: 29.1 mm) was packed stepwise with 24 g soil (1.3 g cm<sup>-3</sup>; -15 kPa) lacking isoproturon and 24 g soil with 4.37 ppm <sup>14</sup>C-labeled isoproturon was subsequently packed to the second half (2.8 cm) of the tube. After 14 days the soil was extracted and sectioned into 2 mm slices. Soil samples were analyzed for isoproturon concentration and resultant concentration profiles were fitted to a diffusion model described by Gillham et al. (1984) to determine the apparent diffusion coefficient. A <sup>14</sup>C-mass balance was established at the end of the experiment.

### 2.3.8. Prediction of isoproturon diffusion in soil material

We attempted to predict the diffusion of isoproturon from soil material to expanded clay particles containing IPU-degrading microbes. Our calculations were based on Fick's second law (Crank, 1970):

$$\frac{\partial C}{\partial t} = \nabla \cdot (D \nabla C) \quad (1)$$

where  $C$  is pesticide concentration,  $t$  the time,  $\nabla$  the Nabla-operator and  $D$  is the diffusion coefficient. The value of  $D$  in our experiments was determined experimentally (see Section 2.3.7).

Since Eq. (1) is rather complex to solve, especially within the context of three-dimensional space, and since we were interested in the order of magnitude of diffusive mass transport alone, we chose the model of a semi-infinite region internally bounded by a single spherical clay particle. The analytical solution here is provided by Crank (1970):

$$\frac{C - C_0}{C_1 - C_0} = \frac{a}{r} \cdot \operatorname{erfc} \frac{r - a}{2\sqrt{Dt}} \quad (2)$$

where  $C_0$  is the initial pesticide concentration in soil,  $C_1$  the constant pesticide concentration at the particle surface (whose value we assumed at zero),  $\operatorname{erfc}$  the complementary

error function,  $a$  the particle radius and  $r$  is the radial position coordinate.

This model corresponds with our experimental design and is appropriate for finite and short diffusion times only. Mass balance provides the IPU mass ( $M$ ) that has diffused into the clay particle at a given time:

$$\begin{aligned} M &= \int_V C_0 \cdot \frac{a}{r} \cdot \operatorname{erfc} \frac{r - a}{2\sqrt{Dt}} dV \\ &= 4\pi \cdot C_0 \cdot a \cdot \int_a^\infty r \cdot \operatorname{erfc} \frac{r - a}{2\sqrt{Dt}} dr \end{aligned} \quad (3)$$

where  $\int_V [\dots] dV$  denotes a volumetric integral.

This rather simple formula discounts overlapping influence of multiple clay particles on isoproturon diffusion, such that the resulting overestimation of isoproturon diffusion to a clay particle is again small for short diffusion times. In effect, we obtained the product of resulting IPU mass and number of clay particles (=20) in our experiments.

## 3. Results

The observed mass balances for <sup>14</sup>C-labeled isoproturon in all experiments were 98% ± 3.5%. Further, we found a significant variation of IPU mineralization in the investigated soils under both environmental (Schroll and Kühn, 2004) and laboratory (Table 2) conditions.

### 3.1. Enhanced herbicide mineralization by soil inoculum in a lysimeter experiment

The average soil temperature in top soil was 22 °C and fluctuated between 17 °C and 27 °C. The average soil humidity was 61.4% of the maximum water holding capacity (mWHC) and fluctuated between 15.8% and 85.9%.

Isoproturon mineralization in soil “Calcaric Regosol”, the source for soil inoculum, was 44% (Fig. 1). In the non-inoculated soil “Mollic Gleysol” we found that 22.6% of applied <sup>14</sup>C-isoproturon was degraded to <sup>14</sup>C<sub>2</sub>O, whereas in inoculated soil “Mollic Gleysol” 49.2% mineralization within 46 days could be demonstrated.

### 3.2. Laboratory biodegradation experiments with non-inoculated soil material

The high cumulative isoproturon mineralization in “Calcaric Regosol” (Schroll and Kühn, 2004) and “Aric Anthrosol” soils (Table 2) was characterized by a lag-phase of approximately 4 days after application and a following very rapid rise which continued for 22 days. In contrast, herbicide mineralization in soils “Mollic Gleysol” (Schroll and Kühn, 2004), “Humic Cambisol” and “Urbic Anthrosol” (Table 2) occurred with nearly constant and very low mineralization rates. These biodegradation experiments demonstrated two different soil groups showing

Table 2

Mineralization (MIN), methanol extractable residues (MER), non-extractable residues (NER) and mass balance (MBL) of the applied  $^{14}\text{C}$ -isoproturon in native soils “Calcaric Regosol”, “Mollic Gleysol”, “Humic Cambisol”, “Aric Anthrosol”, “Urbic Anthrosol” and “Urbic Anthrosol” contaminated with heavy metals (HM) and after transfer of microorganisms via soil inoculum or clay particles at the end of the biodegradation experiments after 46 days ( $n = 4$ ; values given are: means  $\pm$  SD)

Soil treatment	$^{14}\text{C}$ in % of applied $^{14}\text{C}$			
	MIN	MER	NER	MBL
Calcaric Regosol	43.7 $\pm$ 0.6	5.4 $\pm$ 1.0	48.6 $\pm$ 1.7	97.7 $\pm$ 1.5
Mollic Gleysol/control	13.1 $\pm$ 0.9	14.8 $\pm$ 1.6	68.5 $\pm$ 0.9	96.4 $\pm$ 1.4
Mollic Gleysol + soil inoculum	28.9 $\pm$ 1.1	4.1 $\pm$ 0.5	67.1 $\pm$ 1.4	100.1 $\pm$ 1.9
Mollic Gleysol + clay particles	25.8 $\pm$ 0.2	9.2 $\pm$ 0.9	69.7 $\pm$ 0.9	104.7 $\pm$ 0.4
Humic Cambisol/control	21.5 $\pm$ 2.1	11.5 $\pm$ 0.8	60.1 $\pm$ 0.5	93.1 $\pm$ 0.4
Humic Cambisol + clay particles	53.2 $\pm$ 4.2	3.2 $\pm$ 0.2	33.2 $\pm$ 1.8	89.6 $\pm$ 6.1
Aric Anthrosol/control	36.8 $\pm$ 1.2	2.8 $\pm$ 0.3	57.6 $\pm$ 0.4	97.2 $\pm$ 1.3
Aric Anthrosol + soil inoculum	41.1 $\pm$ 3.7	3.0 $\pm$ 0.3	54.0 $\pm$ 1.3	98.1 $\pm$ 2.6
Urbic Anthrosol/control	15.2 $\pm$ 1.4	37.0 $\pm$ 5.5	64.3 $\pm$ 6.2	116.5 $\pm$ 9.6
Urbic Anthrosol + clay particles	51.1 $\pm$ 18.2	4.3 $\pm$ 0.2	44.8 $\pm$ 2.6	100.1 $\pm$ 17.4
Urbic Anthrosol HM/control	2.3 $\pm$ 0.5	74.6 $\pm$ 3.7	18.4 $\pm$ 0.5	95.3 $\pm$ 3.7
Urbic Anthrosol HM + clay particles	23.9 $\pm$ 6.8	44.3 $\pm$ 12.9	41.4 $\pm$ 2.7	109.7 $\pm$ 6.2

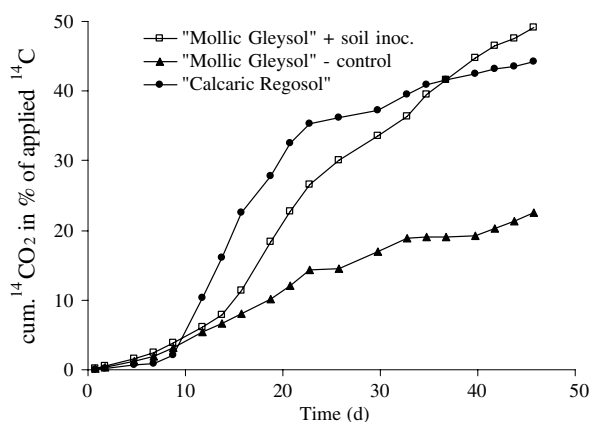


Fig. 1. Mineralization of  $^{14}\text{C}$ -isoproturon in native soil Mollic Gleysol (control), in “Mollic Gleysol” inoculated with soil “Calcaric Regosol” and in native “Calcaric Regosol” in a lysimeter experiment.

markedly different capabilities for mineralizing isoproturon.

### 3.3. Enhanced herbicide mineralization by soil inoculum in laboratory experiments

We observed that transferring soil inoculum (5%) had a high significant effect on IPU mineralization in soils (Table 2).

### 3.4. Enhanced herbicide mineralization by transfer of expanded clay particles covered with microorganisms

After 10 consecutive incubation cycles (see Section 2.3.2), a microbial community with stable IPU mineralization rates established itself on clay particles characterized by a cell density of  $5.5 \times 10^7 \text{ CFU g}^{-1} \pm 2.8 \times 10^7 \text{ CFU g}^{-1}$ . Within a time course of 20 days, we could observe a daily maximum isoproturon mineralization rate of  $7.5\% \pm 1.4\%$  and a cumulative mineralization of  $58.7\% \pm 3.8\%$ ; these characteristics have remained stable for the past 3 years.

Following application of microbe-covered clay particles we found a striking rise in isoproturon mineralization for “Humic Cambisol” and “Urbic Anthrosol” soils of 2.47 and 3.36-fold, respectively (Table 2), and in the heavy metal-contaminated “Urbic Anthrosol HM” soil, mineralization was increased over 10-fold (from 2.3% to 23.9%). Control experiments utilizing sterilized clay particles found no increased isoproturon mineralization. Taken together, these data strongly suggest a critical role of microbes applied on enhanced mineralization.

### 3.5. Comparing enhanced herbicide mineralization in liquid cultures and soil “Urbic Anthrosol”

Isoproturon mineralization was determined in a microbe liquid culture paradigm, as well as in “Urbic Anthrosol” soil following application of  $8.2 \times 10^7 \text{ CFU} \pm 4.2 \times 10^7 \text{ CFU}$  via 1.5 g of microbe-covered expanded clay particles. No significant differences were found between the pattern of mineralization dynamics and the total amount of mineralized herbicide in both experiments (Fig. 2 and Table 2). The mean observed isoproturon mineralization during the first 20 days of testing was about 42% (Fig. 2), and which reflected a mineralization rate of approximately  $5 \mu\text{g d}^{-1}$  for both experiments.

### 3.6. Quality and quantity of $^{14}\text{C}$ -residues in expanded clay particles

We determined the quality and quantity of pesticide residues at completion of experiments. Whereas the amount of extractable  $^{14}\text{C}$  from clay particles was negligible ( $<0.01\%$  of applied  $^{14}\text{C}$ ) we were able to extract between 3.0% and 44.3% of  $^{14}\text{C}$ -residues from inoculated soils (Table 2). However, the total  $^{14}\text{C}$ -residue concentration (calculated on isoproturon equivalents) on clay particles was as much as 10-fold higher compared to total  $^{14}\text{C}$ -residues in soils having  $^{14}\text{C}$ -concentrations of 10.8–18.8 and

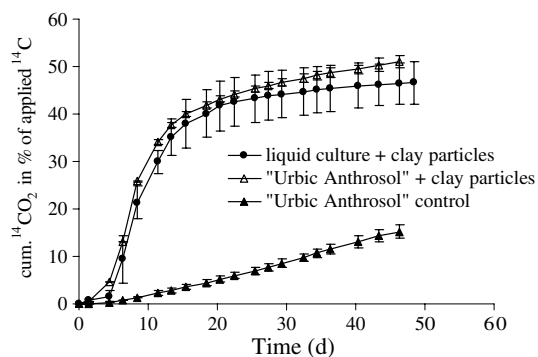


Fig. 2. Mineralization of  $^{14}\text{C}$ -isoproturon in liquid culture of the microbial community and in soil "Urbic Anthrosol" with and without biofilm inoculum; bars indicate standard deviation ( $n = 4$ ).

$1.7\text{--}3.0 \mu\text{g g}^{-1}$  determined in clay particles and in soil material, respectively. In contrast, control experiments with sterilized expanded clay particles (see Section 3.4) showed negligible  $^{14}\text{C}$ -residue levels.

### 3.7. Diffusion of isoproturon in soils

The IPU diffusion coefficient in "Humic Cambisol" soil was  $2.8 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$  ( $\text{SD} = 0.2 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ ,  $n = 6$ ), and the maximum possible IPU diffusive massflow into the expanded clay particles during the experiment was therefore calculated to  $6 \mu\text{g d}^{-1}$ .

## 4. Discussion

The primary goal of our study was to develop an approach for enhancing pesticide degradation *in situ*, not only in an optimized laboratory setting but in field conditions as well. Secondly, we attempted to identify the principle physical-chemical mechanisms underlying our experimental system, even though the key organisms of the microbial community still remain unidentified.

The concept of transferring specific microorganisms to soils for remediation processes was born several years ago (see Assaf and Turco, 1994; Cullington and Walker, 1999). The significant differences in isoproturon mineralization (e.g. Schroll and Kühn, 2004) at optimal soil moisture content (Schroll et al., 2006) have clearly shown that these differences may be explained by the varying abilities of the microbial communities to mineralize the chemical. Thus, our strategy was to extract these microbes from soils with a high native ability for IPU degradation and introduce them in soils with reduced native isoproturon degrading activity. Three different central processes may be relevant for successful degradation of chemicals in soils via the transfer of microorganisms: (i) applied microbes establish and disperse in the new soil, resulting in high degradation of the chemical; (ii) transfer of genetic information (e.g., via plasmids) from donor cells (i.e. the introduced active microorganisms) to acceptor cells in the native microbial population occurs, enabling an enhanced and more efficient

degradation in the host soil; (iii) chemical mass transfer from contaminated soil towards chemical-degrading microbes ensures effective pesticide degradation; the driving force behind this process is the pesticide concentration gradient (Bosma et al., 1997) between degrading microbes ("low" pesticide concentration) and contaminated soil material ("high" pesticide concentration). Microbial dispersion (i) and transfer of genetic information (ii) are slow-acting processes, which may be evident in the presence of a particular selection pressure, e.g., a high specific concentration of a chemical destined for degradation (e.g. Neilson et al., 1994). It is therefore quite unlikely that these two processes are able to explain the very rapid isoproturon mineralization in our experiments.

We propose that isoproturon mass transfer from isoproturon "contaminated" soil to IPU-degrading microbes established on clay particles is the most likely principal process occurring in our experiments. Several indicators support this assertion:

- (i) The  $^{14}\text{C}$ -concentration in clay particles is over 10-fold higher than that found in the surrounding soil particles. However, we could not extract  $^{14}\text{C}$ -isoproturon from clay particles, suggesting a microbe-induced metabolism of isoproturon. This degradation process occurs principally in the microbe-covered clay particles.
- (ii) Isoproturon mineralization was increased 10-fold in heavy metal-contaminated soil. Thus, isoproturon was promptly and intensely mineralized by microbes on clay particles after the start of experiments, when the inhibitory effect of heavy metals is not as pronounced.
- (iii) Maximum pesticide mineralization could be achieved (Table 2) in nearly all soils following microbe transfer by clay particles. Moreover, it is noteworthy that the pattern and extent of pesticide mineralization for liquid cultures and "Urbic Anthrosol" soil biodegradation experiment were identical (Fig. 2, Table 2), in which equal microbe amounts were applied. We can assume that isoproturon mineralization in liquid cultures is solely controlled by the intrinsic degradation ability of the applied microbes. By way of similarity between both experiments on isoproturon mineralization dynamics we conclude that those microbes applied to the soils played a decisive role in the IPU mineralization process as well. What is the underlying reason for this effect? The Arelon substrate supply (isoproturon plus formulation additives) of microbes was different in both experiments whereas a homogeneous distribution of herbicide may be expected in liquid cultures, a herbicide concentration gradient is established in soils, ranging from a "high" chemical concentration in soil which is placed in a certain distance from the clay particles and a "low" concentration nearest the clay particles. As observed by Bosma et al. (1997), "mass transfer – and not the intrinsic

microbial activity – is in most cases the critical factor in bioremediation”. We therefore measured pesticide diffusion in “Humic Cambisol” soil and used that information to calculate the mass transfer in isoproturon biodegradation experiments with “Humic Cambisol” soil. Our results indicated that both mass transfer rates ( $6 \mu\text{g d}^{-1}$ ; Table 1) and biodegradation rates (approx.  $5 \mu\text{g d}^{-1}$  in the first 20 days; Fig. 2) are within the same order of magnitude, thus allowing us to conclude that high mass transfer rates enable the high isoproturon mineralization rates observed in our studies.

The lesser enhancement of IPU mineralization found in the inoculated “Mollic Gleysol” soil can be explained by the low pH value of this soil. Liquid culture experiments carried out at a pH of 5.5 showed lesser IPU mineralization (approx. 12% after 46 days) than when done at pH 7.2 (58.7% within 20 days). These findings are in accordance with those of Walker et al. (2001), who reported a reduced IPU degradation associated with lowered soil pH value.

## 5. Conclusion

Application of IPU-degrading microbes to soils results in increased, intense herbicide mineralization. Several indications suggest that pesticide diffusion towards the degrading microbes and their high IPU degradation capability are the main processes underlying such rapid herbicide mineralization in soils. Enhancement of mineralization by this microbe inoculation approach appears to be limited in the case of isoproturon however by low soil pH, such that additional studies addressing the sustainability of this method are required. Our approach is nevertheless useful for contaminated soils, given that diffusion of contaminants towards the applied microbes demonstrating specific mineralization ability takes place. Our findings also account for our previous results (Schroll et al., 2004) in which increased mineralization of 1,2,4-trichlorobenzene, a chemical with higher mobility in soils than isoproturon, could be demonstrated when soils were inoculated with a specific microbial community.

As reported by Gonod et al. (2003) an uneven distribution of microbes with high potential for mineralizing a specific pesticide in native soils are organized into centimeter sized hot spots. Taken together, we can here conclude that the process of substrate diffusion towards chemical-degrading microbes bears critical importance for degradation of chemicals and bioremediation in native soils.

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