Appl Microbiol Biotechnol (1998) 49: 624–630

ORIGINAL PAPER

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Rapid atrazine mineralisation in soil slurry and moist soil by inoculation of an atrazine-degrading *Pseudomonas* sp. strain

Received: 31 October 1997 / Received revision: 16 January 1998 / Accepted: 18 January 1998

Abstract The evaluation of pesticide-mineralising microorganisms to clean-up contaminated soils was studied with the widely applied and easily detectable compound atrazine, which is rapidly mineralised by several microorganisms including the *Pseudomonas* sp. strain Yaya 6. The rate of atrazine removal was proportional to the water content of the soil and the amount of bacteria added to the soil. In soil slurry, 6 mg atrazine kg soil^{-1} was eliminated within 1 day after application of 0.3 g dry weight inoculant biomass kg soil^{-1} and within 5 days when 0.003 g kg soil^{-1} was used. In partially saturated soil (60% of the maximal water-holding capacity) 15 mg atrazine kg soil^{-1} was eliminated within 2 days by 1 g biomass kg soil^{-1} and within 25 days when 0.01 g biomass kg soil^{-1} was used. In unsaturated soil, about 60% [U-*ring*-^{14}C]atrazine was converted to $^{14}CO_2$ within 14 days. Atrazine was very efficiently removed by the inoculant biomass, not only in soil that was freshly contaminated but also in soil aged with atrazine for up to 260 days. The bacteria exposed to atrazine in unsaturated sterile soil were still active after a starvation period of 240 days: 15 mg newly added atrazine kg soil^{-1} was eliminated within 5 days.

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Introduction

Atrazine [2-chloro-4-ethylamino-6-isopropylamino-1,3, 5-triazine] is one of the most frequently used herbicides world-wide. As a consequence of its broad application, the herbicide and its metabolites deethylatrazine and deisopropylatrazine are detected in all environmental compartments (Berg et al. 1995; Thurman et al. 1994), in particular in surface water and drainage networks as a result of run-off following the application period (Schottler et al. 1994), although the average half-life of the parent compound in agricultural soil was reported to be about 40 days (Seiler et al. 1992).

The metabolism of atrazine by soil microorganisms and enrichment cultures has been studied for many years (Cook 1987; Behki et al. 1993; Levanon 1993; Mills and Thurman 1994). Recently, bacterial isolates have been described that are able to mineralise atrazine efficiently under carbon-limiting conditions (Yanze-Kontchou and Gschwind 1994; Radosevich et al. 1995) or as a nitrogen source (Mandelbaum et al. 1993, 1995; Ernst and Rehm 1995). Masaphy et al. (1993) observed the degradation by fungal cultures and described the formation of a new metabolite. Other authors have reported some technical applications using atrazine-mineralising organisms to decontaminate groundwater (Stucki et al. 1994). Feakin et al. (1995) inoculated granular activated carbon with bacteria to study the potential removal of atrazine from slightly contaminated drinking water in the adsorption process.

The work was conducted as part of a technology development programme for bioremediation of pesticide-contaminated soils, either using a slurry reactor system or by inoculation of an unsaturated surface soil. Data are presented for atrazine degradation and mineralisation in a soil slurry and in a sterilised soil of various moisture contents, inoculated with the atrazine-mineralising *Pseudomonas* strain, which was isolated by Yanze-Kontchou and Gschwind (1994). The survival of the atrazine-degrading culture and the amount of active

biomass needed for rapid atrazine removal in soil are reported.

Materials and methods

Physical and chemical soil characteristics

The Stein soil, obtained from the Novartis agricultural research and development station at Stein, Switzerland, was a Swiss loam soil (FAO classification) and thus represents a well-balanced texture. It was characterised as follows: pH 7.8, content of organic matter 2.6%, clay 25%, silt 33%, sand 42%, and maximum water-holding capacity (WHC) 326 g water (kg soil dry weight)$^{-1}$.

The maximum WHC was determined by filling 200 g of dry soil into a glass cylinder (8 cm diameter) sealed at the bottom by a fine net. The soil column was saturated with water by putting it into a water bath (water level to the top of the soil level). The soil column was then placed onto a sand bed of 10 cm depth for 3 h. The difference in weight before and after soaking is defined as the maximum amount of water that the soil can retain. The soil dry weight was determined by drying at 105 °C over night (Alef 1994).

Adsorption and desorption of atrazine in soil slurry

Adsorption and desorption experiments were conducted in conical flasks containing 200 ml demineralised water and 100 g soil, which was sterilised by autoclaving. Atrazine solution (200 ml, containing 1, 2.5, 5 or 20 mg l^{-1} atrazine) was added to flasks containing 100 g Stein soil. The flasks were put on a rotary shaker (175 rpm) and atrazine was allowed to adsorb to the soil particles for 7 days at room temperature. To determine the extent of adsorption, samples were taken from the aqueous and solid phases. The aqueous phase was filtered (0.45-μm, polytetrafluoroethylene (PTFE) filter; BGB Analytik AG, Rothenfluh, Switzerland) and atrazine was directly determined by HPLC (high-pressure liquid chromatography) (Stucki et al. 1995). The quantification limit of the method was 0.01 mg l^{-1} atrazine. Atrazine from the solid phase was determined after extraction with acetonitrile (90%) for 2 h on a rotary shaker (175 rpm). The solvent solution was filtered and atrazine was quantified as described for the aqueous phase.

To study desorption of atrazine, soil slurry of the samples used for the adsorption studies was centrifuged; the supernatant was removed and replaced by demineralised water. Afterwards, the slurries were incubated for 7 days as done for the adsorption experiments, and atrazine was quantified in the aqueous and solid phases according to the same procedures as described for the adsorption experiments.

To simulate soil ageing conditions, soil slurries were incubated in the dark in unshaken bottles in the presence of different atrazine concentrations (approx. 20 mg l^{-1}, 5 mg l^{-1}, 2.5 mg l^{-1} or 1 mg l^{-1}) for 24, 40, 104 and 260 days. During the incubation period, the soil particles settled and were flooded with water. After the incubation periods, the supernatant was replaced by demineralised water and the soil was resuspended to determine the extent of desorption within 7 days as described above.

Comparative adsorption isotherm studies were carried out using granular activated carbon F 400 (Chemviron Carbon, Brussels, Belgium). Different amounts of pre-dried carbon (0.01, 0.03, 0.1, 0.3 and 1 g) were added to 1 l atrazine solution (18.2 mg l^{-1}) and the mixture was incubated on a rotary shaker (175 rpm) for 24 h at room temperature. Atrazine equilibrium concentrations in the aqueous phase were determined by HPLC.

The linear sorption partition coefficients K_d (mg kg^{-1}), as a measure of the degree of sorption or desorption, were calculated from the slope of plots of the aqueous equilibrium concentration against the amount of sorbed material per gram of soil.

Biodegradation in soil slurry

To investigate the biodegradation of atrazine in soil slurry, the herbicide was added to sterile soil and water as described for the adsorption experiments. After 7 days, the samples were centrifuged and the supernatant was removed and replaced by demineralised water and various amounts of a suspension of bacteria, quantified as dry weight. The total volume of liquid added corresponded to the volume of supernatant decanted. Afterwards, the slurries were put on a rotary shaker (175 rpm). Samples were taken after different incubation times and processed for atrazine determination in the soil (soil and aqueous phase for slurry samples) as described previously. All tests were done in triplicate.

Biodegradation of atrazine in aged soil was studied similarly. Instead of the replacement of water, the slurries aged for 7, 103 and 260 days were resuspended after centrifugation with different atrazine concentrations A–D (approx. 20 mg l^{-1}, 5 mg l^{-1}, 2.5 mg l^{-1} or 1 mg l^{-1}). The samples were then centrifuged and the aqueous phase was replaced by two different concentrations (0.01 g bacterial dry weight kg soil^{-1} or 1 g kg^{-1}) and incubated for 7 days on a rotary shaker (175 rpm), until atrazine was analysed in the aqueous and the soil phase. The tests were done in triplicate.

Biodegradation in moist soil

Experiments on unsaturated soil at different soil moisture contents, expressed as WHC(%), were conducted in closed 250-ml Duran-Schott flasks containing 100 g autoclaved soil. Water, atrazine solution or emulsion and biomass were mixed into the soil with a dough mixer (Hobart AG, Zurich, Switzerland). The liquids were added to the mixer at a rate of about 1 ml min^{-1}. The total volume of solution added (water, atrazine solution and biomass solution) contained the amount of water required for a given soil moisture. Tests done in triplicate were incubated for different times until the remaining atrazine had been extracted with acetonitrile for analysis.

Experiments with [U-ring^{14}C]atrazine

To confirm that the disappearance of atrazine is a result of microbial mineralisation, one experiment was repeated using [U-*ring*-^{14}C]atrazine. Stein soil was prepared at a WHC of 60% with a bacterial concentration of 1 g dry weight kg soil^{-1} and an atrazine concentration of 20 mg/kg soil containing a specific activity of 36.2 kBq kg soil^{-1}. Each Duran-Schott flask contained 50 g Stein soil and was tightly sealed by a PTFE membrane and cap. A polycarbonate tube containing 10 ml 1 M NaOH to trap CO_2 was placed in the top part of the flask. The samples were incubated unshaken at room temperature. At each sampling time, three flasks were sacrificed by the addition of 5 ml 5 M H_3PO_4 through the cap membrane. The aqueous volume added resulted in a WHC of 100%. The NaOH of the trap was removed 2 h after soil acidification. An aliquot of 10 ml was added to 10 ml Quicksave scintillation cocktail. A 1-g sample of acidified soil from the incubation flasks was thermally oxidised in a Heraeus termicon P oven (Zürich, Switzerland) at 800 °C. The off gas was directly led into 20 ml Oxysolve scintillation cocktail. Radioactivity was determined either in a Packard Tri-Carb 2700 or a Packard 2500TR scintillation counter (Packard Instrument Company, Meriden, Coon., USA), and was automatically corrected for quenching by an external radiation source.

Test organisms

The isolation and characterization of *Pseudomonas* sp. strain Yaya 6 (DSM strain 9399), able to mineralise atrazine as the sole carbon source, was described by Yanze-Kontchou and Gschwind (1994). The culture was grown aseptically on a diluted Gesaprim emulsion, a commercially available formulation of atrazine. The final medium contained 4 g l^{-1} active ingredient (atrazine) supplemented with

250 mg l^{-1} KH_2PO_4. It was fed at a rate of 700 ml day^{-1} to an aerated laboratory reactor (4 l) and then to a settling tank (2 l). The suspended solids concentration (atrazine-degrading bacteria) in the aerated tank was in the range of 4–6 g dry weight l^{-1}. Settled bacteria were recycled to the aeration tank at a rate of 650 ml h^{-1}. The pH was kept at 7.3 by the addition of 2 M NaOH.

Atrazine was completely degraded, as determined by HPLC and by analysis of dissolved organic carbon quantified on a TOC-Analyser 5050 (Shimadzu Corp., Kyoto, Japan). The effluent atrazine concentration was less than 1 mg l^{-1}, which yields an elimination efficiency of over 99.9%. Ammonia, derived from the nitrogen atoms of the side-chains and the ring of the *s*-triazine molecule, was analysed in the effluent with the Merck Reflectoquant test 16977 (E. Merck, Darmstadt, Germany). Its concentration was below 20 mg/l. The ammonia must have been further oxidised to nitrate (determined by the Merck Reflectoquant test 16971). The nitrate level in the effluent was in the range of 4500 ± 500 mg/l. The volumetric elimination rate of the reactor system was 2.8 g atrazine l^{-1} day^{-1}. Atrazine-degrading bacteria were harvested from the aeration tank by centrifugation. The cells were resuspended in tap water before use.

Chemicals

Technical-grade atrazine, analytical master standard (99.2%) and Gesaprim, a formulation containing 50% (w/w) atrazine were supplied by Novartis Crop Protection (Münchwilen AG, Switzerland). [U-*ring*-^{14}C]Atrazine, with a specific activity of 3.5 MBq mg^{-1} and a radioactive purity of 98.3%, was obtained from Novartis Crop Protection AG (Basle, Switzerland); acetonitrile (LiChrosolv) for chromatography from E. Merck (Darmstadt, Germany) and the scintillation cocktails Quicksafe A and Oxysolve C-400 from Zinsser Analytik (Frankfurt, Germany). Other chemicals were supplied by Fluka Chemie AG (Buchs SG, Switzerland).

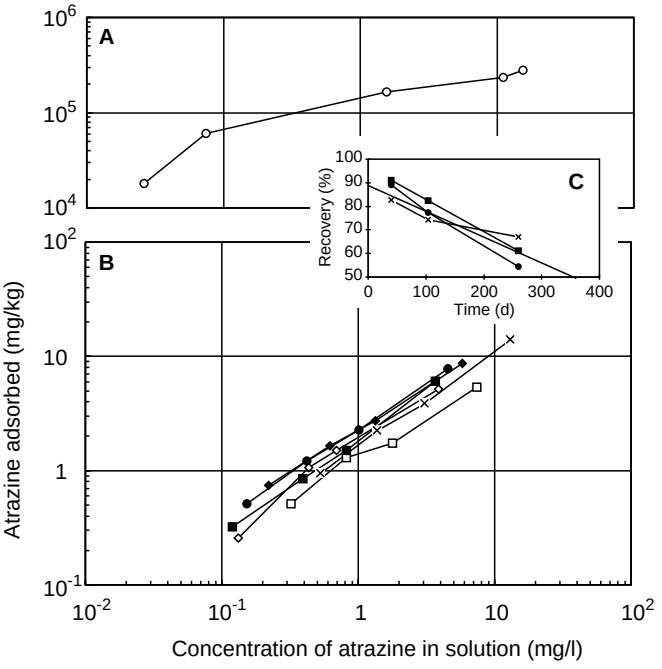

Fig. 1A–C Isotherms of atrazine adsorption to activated carbon and sterile Stein soil, and desorption isotherms from Stein soil after different atrazine exposure times. **A** Adsorption to activated carbon. **B** Adsorption to soil (x); desorption from soil after 7 (□), 24 (◆), 40 (●), 104 (■) and 260 days (◇). **C** Recovery of atrazine in slurry, after different exposure times, for 20 mg l^{-1} (■), 5 mg l^{-1} (●), 2.5 mg l^{-1} (×) atrazine. Linear trend of average values yielding an estimate for the abiotic half-life of atrazine

Results

Abiotic experiments in Stein soil

The physical atrazine adsorption and desorption properties were studied using soil slurry. The adsorption capacity of the Stein soil at aqueous equilibrium concentrations of 1 mg l^{-1} atrazine was 1.64 ± 0.21 mg kg^{-1} (Fig. 1B) with a linear sorption partition coefficient, $K_{d,}$ of 1.04 mg kg^{-1}. The period until the equilibrium concentration was determined was 7 days, although determination of aqueous-phase atrazine concentrations showed that most of the chemical must have been adsorbed after 2 days of incubation (data not shown). Sterilised soil was used to exclude any microbial degradation by indigenous microorganisms during the incubation time. Comparative adsorption isotherms using activated carbon yielded a 40 000-fold larger adsorption capacity of 130 g kg^{-1} for the same equilibrium concentration, thus indicating a low adsorption capacity of the Stein soil (Fig. 1A).

The desorption isotherms were determined after the 7-day adsorption period and also after incubation times of 24, 40 104 and 260 days with atrazine, to study the effect of soil ageing (Fig. 1B). The calculated linear sorption partition coefficients for the 7-, 24-, 40-, 104-, and 260-day soil aging time were 0.69, 1.43, 1.66, 1.61 and 1.27 mg kg^{-1} respectively. With the exception of the partition coefficient determined after 260 days, the values became larger with time and thus indicated a greater fixation of the herbicide to the soil with increasing incubation times. The overall recovery rates of atrazine in the adsorption desorption experiments were above 90% for the more briefly incubated samples. In samples exposed for 104 days and 260 days, the atrazine recovery from liquid and solid dropped to 75% and 63% respectively, yielding a half-life of about 1 year for the parent compound atrazine in sterile Stein soil slurry (Fig. 1C). We did not analyse for possible abiotic degradation products.

Biological degradation of atrazine in soil slurry

The experiments in soil slurry were designed to estimate the amount of biomass needed and the time required for removing atrazine from soil and aged soil in a slurry reactor. To start the experiment, atrazine was adsorbed to soil for 7 days, thus simulating a herbicide spill. Afterwards, the supernatant was replaced by a solution with various amounts of atrazine-mineralising biomass. Elimination of atrazine was measured in the aqueous solution (Fig. 2A) and in the soil fraction (Fig. 2B) after different incubation times. Atrazine levels fell within 1 day to below the quantification limit in both phases when 5 g and 0.3 g bacteria kg $soil^{-1}$ were used. With

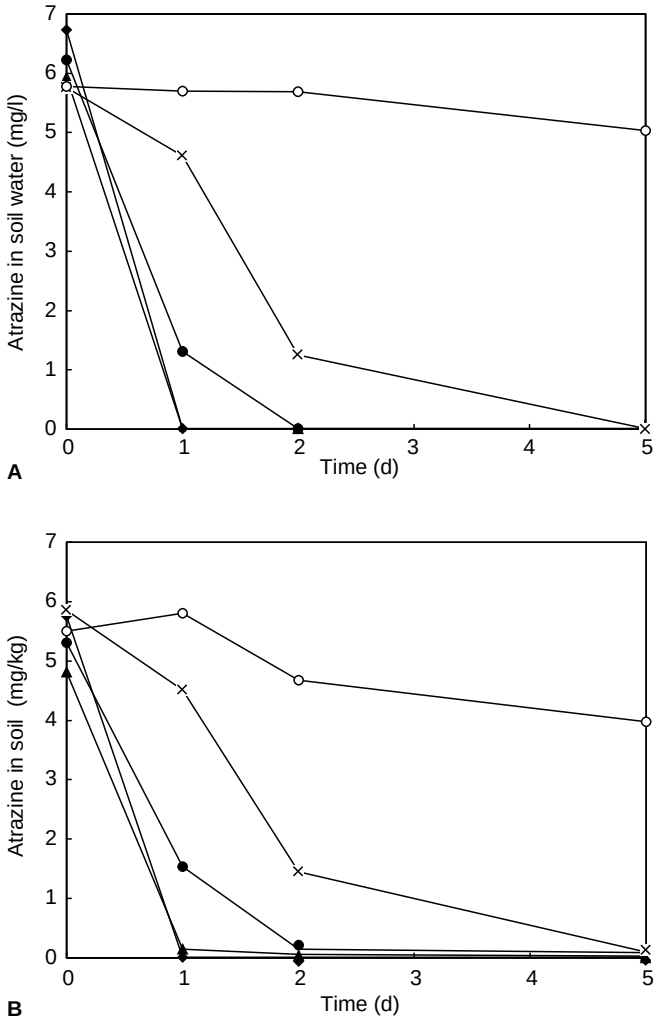

Fig. 2A, B Degradation of atrazine in soil slurry with different amounts of atrazine-mineralising biomass. **A** Atrazine concentration in aqueous phase of the slurry. **B** Atrazine concentration in solid phase of the slurry. Biomass (g dry weight kg soil^{-1}): 5 g (◆), 0.3 g (▲), 0.03 g (●), 0.003 g (×), 0 g (○)

only 3 mg bacteria kg soil^{-1}, atrazine was degraded within 5 days. No significant atrazine disappearance was recorded in non-inoculated soil slurry within the experimental period.

To check whether soil aging would reduce the bioavailability of atrazine in Stein soil and thus reduce the rate of atrazine removal, we kept different amounts of atrazine (Table 1, tests A–D) for extended periods of time in sterile soil slurry. After 103 or 260 days, the soil water was replaced by a suspension containing different amounts of *Pseudomonas* sp. One week after the addition of 0.01 g kg^{-1} or 1 g kg^{-1} atrazine-degrading bacteria to the aged slurry, the atrazine concentrations in the soil water dropped to below the quantification limit with both amounts of bacteria added (Table 1). Atrazine was still detectable in the solid phase, however, which is in contrast to the previous experiment with freshly contaminated soil, where the atrazine was removed to below the quantification limit in soil and soil water (Fig. 2A, B). The extent of biological atrazine removal from the soil phase was in the range 85%–90% in aged soils containing 5–6 mg atrazine and 63% and 85% in soils containing atrazine concentrations of below 1 mg/l at the time the bacteria were added. With the exception of test A (Table 1), the atrazine concentration decreased in the soil in the non-inoculated samples over the long incubation time, indicating a slow abiotic decrease of the chemical, as observed in the adsorption/desorption experiments described previously (Fig. 1C).

Degradation of atrazine in unsaturated soils

The next series of experiments was designed to estimate the degradation potential of *Pseudomonas* sp. in unsaturated soil systems. Slower degradation rates than those in soil slurry were expected because of the lower diffusion of the herbicide and the lower mobility of the microorganisms in this system.

Table 1 Biological removal of atrazine from soil slurry aged with atrazine. Soil was aged with solutions of different atrazine concentration (*A–D*; approx. 20 mg l^{-1}, 5 mg l^{-1}, 2.5 mg l^{-1} or 1 mg l^{-1}). After aging, the aqueous phase was replaced by water or a bacterial suspension. The samples were then incubated for 7 days until atrazine was determined. *ND* not determined

Aging for:	Concentration of atrazine						
	7 days (control), no biomass	104 days, no biomass	103 days, 0.01 g kg^{-1} biomass	103 days, 1 g kg^{-1} biomass	260 days, no biomass	260 days, 0.01 g kg^{-1} biomass	260 days, 1 g kg^{-1} biomass
Aqueous phase (mg l^{-1})							
A	7.38	3.68	< 0.01	< 0.01	3.89	< 0.01	< 0.01
B	1.78	0.82	ND	< 0.01	0.70	ND	< 0.01
C	0.82	0.39	< 0.01	ND	0.44	< 0.01	ND
D	0.32	0.12	< 0.01	< 0.01	0.13	< 0.01	< 0.01
Solid phase (mg kg^{-1})							
A	5.37	6.03	0.86	0.57	5.15	0.58	0.35
B	1.75	1.50	ND	0.26	1.50	ND	0.25
C	1.29	0.85	0.25	ND	1.06	0.24	ND
D	0.51	0.32	0.12	0.09	0.26	ND	0.04

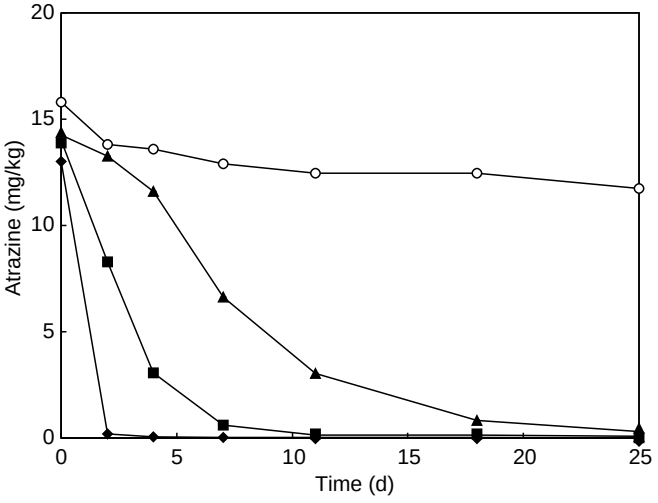

Fig. 3 Degradation of atrazine in moist soil (60% water-holding capacity, WHC) with different amounts of atrazine-mineralising biomass (g dry weight kg soil^{-1}): 1 g (◆), 0.1 g (■), 0.01 g (▲), 0 g (○)

The soil was autoclaved to differentiate abiotic conversions from those effected by the inoculant strain. In an initial experiment, atrazine-treated soil was mixed with different amounts of biomass at a soil moisture of 60% WHC. The rate of atrazine elimination was highest with the highest amount of biomass amended (Fig. 3). Even small quantities of bacteria efficiently degraded atrazine within 1 month (Fig. 3). During the degradation of atrazine using 0.1 g and 0.01 g biomass/kg of biomass, metabolites of the parent compounds appeared and disappeared again at the end of the experiment (data not shown). They were identified qualitatively by co-chromatography as deethyl- and deisopropylatrazine.

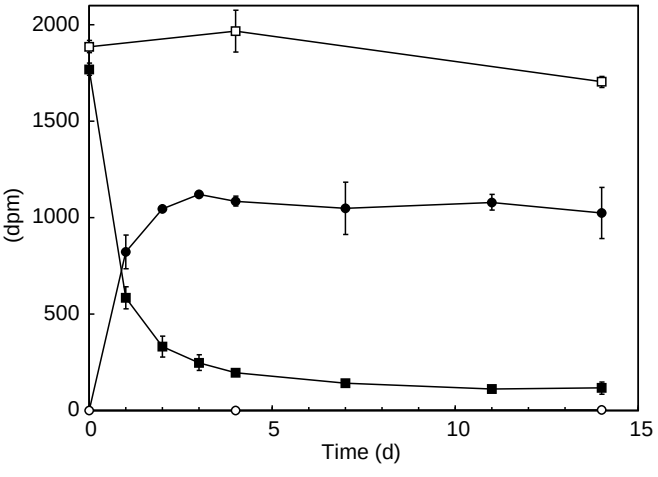

Fig. 4 Evolution of $^{14}CO_2$ (dpm) in soil at 60% WHC incubated with [U-*ring*-^{14}C]atrazine and 1 g kg^{-1} atrazine-mineralising bacteria (●) and in the non-inoculated control (○). Removal of the ^{14}C label (dpm) from soil in the same experiment with biomass (■) and in the non-inoculated control (□). *Bars* standard deviation of three tests

Other metabolites were not detected with the analytical method used.

The previous experiment was repeated using [U-*ring*-^{14}C]atrazine and a bacterial concentration of 1 g biomass/kg soil to prove that atrazine was effectively mineralised. The radioactivity (added as atrazine) disappeared from the soil at about the same rate as found for non-labelled atrazine (Fig. 4). After 4 days, approximately 90% of the radioactivity was removed, whereas in the non-inoculated soils almost all of the radioactivity remained in the soil over the 14-day incubation period. The disappearance of the radioactivity in the soils was accompanied by the formation of $^{14}CO_2$.

In an experiment with two different concentrations of atrazine-mineralising bacteria, the dependence of the degradation rate on the soil moisture was investigated. The higher the soil moisture, the faster was the atrazine degradation rate observed (Fig. 5A, B). At soil moistures of 10% and 20% WHC, the effect of enhanced biodegradation became almost insignificant, whatever the quantity of biomass amended. Under these conditions the herbicide remained detectable even after an incubation time of 250 days.

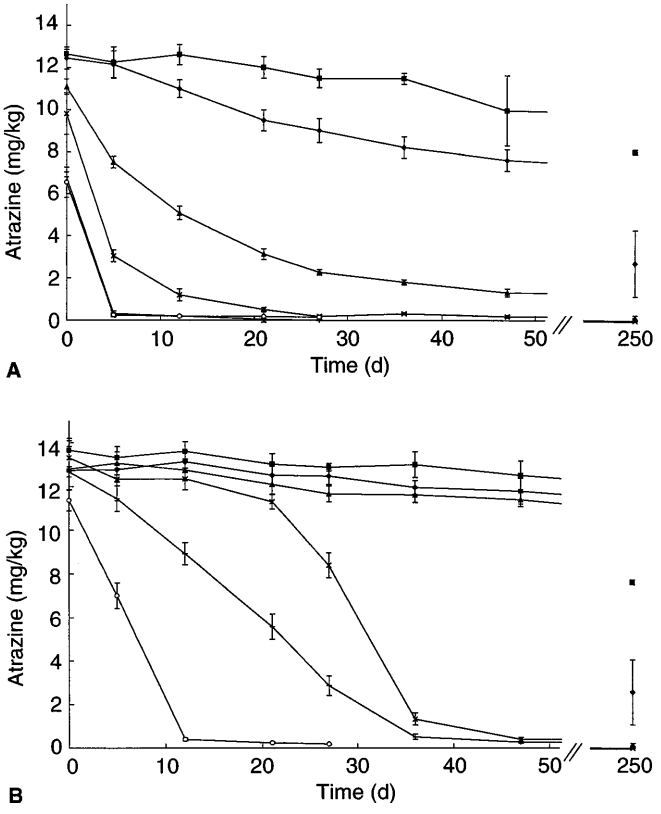

Fig. 5A, B Degradation of atrazine in unsaturated soils at different soil moistures (WHC) using: (**A**) 1 g dry weight of atrazine-mineralising biomass kg soil^{-1}; (**B**) 0.01 g dry weight of atrazine-mineralising *Pseudomonas* sp. kg soil^{-1}. WHC: 10% (◆), 20% (■), 30% (▲), 40% (×), 60% (+), 100% (○). *Bars* standard deviation of three tests

Conservation of the biological activity to degrade atrazine

A final experiment addressed the conservation of the atrazine degradability in unsaturated soil. The soils at a moisture of 60% WHC contained 15 mg kg^{-1} atrazine and 1 g kg^{-1} or 0.01 g kg^{-1} atrazine-mineralising culture (see previous experiment, Fig. 3). This atrazine was removed within 2 days with 1 g biomass kg^{-1} and after 25 days when 0.01 mg kg^{-1} was used (Fig. 6). After the test, the soils were kept for 240 days in the dark. They were not supplied with any added carbon source. After the long period without atrazine, this substrate was added again at the same concentration as before. In both biomass treatments the same removal rate was obtained, indicating that a similar number of bacteria must have survived (Fig. 6). The degradation rate observed corresponded to that of about 0.1 g kg^{-1} freshly inoculated biomass.

Discussion

Compared to the efficient adsorption of atrazine to activated carbon, the adsorption potential of this chemical in Stein soil was low, but it was very similar to that reported for other soils (Yanze-Kontchou and Gschwind 1995; Xing et al. 1996; Radosevich et al. 1997). With one exception, the distribution of atrazine in the aqueous and the soil phase shifted to the soil phase with the longer incubation times and thus confirmed for atrazine in Stein soil the effect of ageing described Hatzinger and Alexander (1995) for other chemicals and soils. The distribution coefficient of the chemicals after the 260-day incubation period was smaller than those determined at 24, 40 and 104 days, and is apparently contradictory. However, the extent of recovery in the 260-day-exposed samples dropped to between 55% and 67% (Fig. 1C). This result may indicate that some of the chemical was either abiotically decomposed, or atrazine was so tightly bound to the soil that it was not even extractable with the acetonitrile used for its determination in soil.

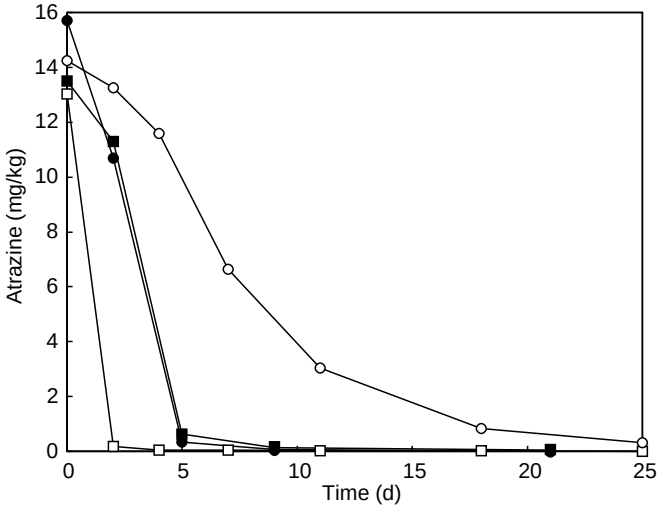

Fig. 6 Atrazine degradation in moist soil (60% WHC) at the beginning and after a starvation period of 240 days with 1 g bacterial biomass kg $soil^{-1}$: first amendment (□), after starvation (■); with 0.01 g biomass kg $soil^{-1}$: first amendment (○), after starvation (●)

The aging effect was also observed when bacteria were added to slurries exposed to atrazine for different times. Freshly contaminated soil slurry was purified in both aqueous and soil phase to below detection levels by the addition of the atrazine-mineralising bacteria. In the aged slurries, atrazine was still efficiently removed from the aqueous phase by the addition of *Pseudomonas* sp. Yaya 6 (Table 1). In the solid phase of the aged slurries, the degradation was incomplete, since atrazine was detectable even with high amounts of added biomass (Table 1). These results correspond well to those of Radosevich et al. (1997), who observed that the less mineralisation of atrazine occurred the longer the soil slurries were aged before amendment of atrazine-mineralising bacteria.

Pesticides spilled onto soil may remain for long periods in unsaturated zones, from where they may be leached into saturated soils and thus pose a long-term risk for contamination of the water table. To evaluate the potential of inoculant biomass that could be incorporated into soil by plough, we examined the rate and extent of atrazine degradation of different inoculant quantities at different soil moistures. Our results are in line with those reported by Fan and Scow (1993), who found decreasing biodegradation rates in soil for trichloroethene and toluene with decreasing soil moisture. In moist soils, as little biomass as 0.01 g kg^{-1} was required for an efficient atrazine removal, corresponding to an application rate of 20 kg ha^{-1} if the top 10 cm of the soils was to be treated.

Metabolites such as deethylatrazine and deisopropylatrazine appeared and disappeared in tests with low amounts of inoculant biomass. The same compounds have been observed when the same culture was used for ground water treatment (Stucki et al. 1995).

Eventually, some of the atrazine was mineralised (Fig. 4), which is clearly advantageous for the abiotic conversion and/or slow and incomplete removal by many indigenous soil microorganisms (Solomon et al. 1996; Willems et al. 1996). In addition, no external or additional carbon source had to be supplied to maintain catabolic activity as is needed for other atrazine degraders (Mandelbaum et al. 1993). Although the radioactivity remaining in the soil after biological incubation and acidification dropped to about 10% of the original level, only about 60% was recovered as $^{14}CO_2$ (Fig. 4). Therefore, about 30% of the radioactivity was missing in the mass balance. The mass balance was better with the control samples where no formation of $^{14}CO_2$ was observed. It is assumed that the choice of the scintillation cocktail to count $^{14}CO_2$ trapped in NaOH was not ideal because the large sample volume (10 ml) and the strong alkalinity (1 M) resulted in the formation of a gel, the consistency of which was temperature-dependent.

Alvey and Crowley (1996) investigated the survival and activity of an atrazine-mineralising consortium in rhizosphere soil of corn when evaluating the practical application potential of inoculant atrazine-mineralising cultures to clean up heavily contaminated agricultural soils. Other environments, such as contaminated wetlands, also could be augmented with atrazine degraders to remediate the water (Detenbeck et al. 1996). The *Pseudomonas* sp. Yaya 6 kept its ability to degrade atrazine for a long period of time without added food supply. Further experiments are needed, however, to evaluate the full potential of the strain, because the soil was initially autoclaved (added labile organic matter) and then kept under constant environmental conditions. Therefore, the results neither indicate whether the bacterial strain successfully competes with other soil microorganisms, nor show how it copes with changing conditions like temperature or extensive nutrient limitations.

If atrazine-mineralising bacteria were to be used for bioremediation, the questions about threshold concentrations remaining in soil and groundwater or the potential danger of enhanced biodegradation would also have to be addressed. The latter has been investigated by Wenk et al. (1997) recently, who found that atrazine lost its controlling activity towards atrazine-sensitive plants provided that the soil contained atrazine-degrading bacteria and that ideal conditions (humidity) prevailed in the soil.

Acknowledgements We thank the Corporate Safety and Environment Department of the former Ciba-Geigy AG, Basle, Switzerland, for the financial support, J. Allen from Novartis Crop Protection AG, Herbicides Research, CH-4332 Stein/AG, Switzerland, for supplying the soil material and J. Gonzalez-Valero and U. Plücken from Novartis Crop Protection AG, Basle, Switzerland, for technical assistance with the work with radio-labelled atrazine.

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