Evaluating a bioremediation tool for atrazine contaminated soils in open soil microcosms: The effectiveness of bioaugmentation and biostimulation approaches

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ABSTRACT

A previously developed potential cleanup tool for atrazine contaminated soils was evaluated in larger open soil microcosms for optimization under more realistic conditions, using a natural crop soil spiked with an atrazine commercial formulation (Atrazerba FL). The doses used were $20\times$ or $200\times$ higher than the recommended dose (RD) for an agricultural application, mimicking over-use or spill situations. *Pseudomonas* sp. strain ADP was used for bioaugmentation (around 10^7 or 10^8 viable cells g^{-1} of soil) and citrate for biostimulation (up to $4.8\,mg\,g^{-1}$ of soil). Bioremediation treatments providing fastest and higher atrazine biodegradation proved to differ according to the initial level of soil contamination. For $20\times$ RD of Atrazerba FL, a unique inoculation with *Pseudomonas* sp. ADP ($9\pm1\times10^7\,CFU\,g^{-1}$) resulted in rapid atrazine removal (99% of the initial $7.2\pm1.6\,\mu g\,g^{-1}$ after 8 d), independent of citrate. For $200\times$ RD, an inoculation with the atrazine-degrading bacteria ($8.5\pm0.5\times10^7\,CFU\,g^{-1}$) supplemented with citrate amendment ($2.4\,mg\,g^{-1}$) resulted in improved biodegradation (87%) compared with bioaugmentation alone (79%), even though $7.8\pm2.1\,\mu g$ of atrazine g^{-1} still remained in the soil after 1 wk. However, the same amount of inoculum, distributed over three successive inoculations and combined with citrate, increased *Pseudomonas* sp. ADP survival and atrazine biodegradation (to 98%, in 1 wk). We suggest that this bioremediation tool may be valuable for efficient removal of atrazine from contaminated field soils thus minimizing atrazine and its chlorinated derivatives from reaching water compartments.

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1. Introduction

The *s*-triazine herbicide atrazine has been extensively used worldwide and is relatively persistent in soil. Besides intensive application rates, accidental spillage or intentional disposal are major threats to the environment. Due to the high potential for reaching water compartments as a result of leaching, run-off and/ or drainage events (Seybold and Mersie, 1996; Fava et al., 2007), atrazine and its toxic chlorinated dealkylated metabolites are frequently detected in surface- and ground-waters (Cerejeira et al., 2003; Kalkhoff et al., 2003), resulting in concerns regarding their impact on human and ecosystems health (DeLorenzo et al., 2001; Oh et al., 2003; Hayes et al., 2006). These facts have promoted research on atrazine-degrading microorganims and on efficient bioremediation strategies for atrazine polluted environments aiming to reduce contamination to safe levels and to quickly

prevent the dispersion of this herbicide and of its chlorinated derivatives to non-agricultural environments (Mandelbaum et al., 1993, 1995; Newcombe and Crowley, 1999; Ralebitso et al., 2002; Wackett et al., 2002; Silva et al., 2004; Vibber et al., 2007; Biglione et al., 2008).

One of the best-characterized atrazine-degrading bacteria is *Pseudomonas* sp. strain ADP, which was isolated by Mandelbaum et al. (1995) from an herbicide spill site and uses atrazine as the sole nitrogen source by means of a catabolic pathway encoded in the plasmid pADP-1 (de Souza et al., 1998; Wackett et al., 2002). The breakdown of atrazine by *Pseudomonas* sp. ADP involves a first step of dechlorination to hydroxyatrazine and further metabolization of this metabolite to cyanuric acid and finally to carbon dioxide and ammonia (Wackett et al., 2002). Silva et al. (2004) presented evidence suggesting that a joint bioaugmentation (with a viable population of *Pseudomonas* sp. ADP) and biostimulation (with additional C source) approach may be effective for the cleanup of soil contaminated with high atrazine concentrations. Moreover, $C_s{:}N_{atz}$ (soluble carbon to atrazine nitrogen ratio) was found to be

a critical parameter determining the extent of atrazine mineralization by *Pseudomonas* sp. ADP and its increase to >40 by amending soil with organic acids was required for maximal mineralization (Silva et al., 2004).

The aim of the present study was to examine the efficacy of this potential bioremediation tool under more realistic conditions and at a larger scale, as part of a framework for rational bioremediation of atrazine-contaminated land. It is expectable that scale-dependent variables such as mass transport limitations and spatial heterogeneity, among others, may influence the effectiveness of field-scale bioremediation designs (Sturman et al., 1995), and information on bioremediation performance under ecologically relevant conditions may contribute for the implementation of effective atrazine cleanup protocols. Therefore, we transferred the bioaugmentation/biostimulation strategy previously examined based on mineralization assays carried out in small closed microcosms contaminated with pure atrazine (Silva et al., 2004), to larger open soil microcosms comprising a representative crop soil from Central Portugal spiked with an atrazine commercial formulation (Atrazerba FL). The doses used were 20× and 200× higher than the recommended dose (RD) for an agricultural application (hereafter designated as 20× RD and 200× RD), mimicking overuse, concentration "hotspots" that may arise from uneven applications and/or spill scenarios. The influences of inoculum density and application regime of the bioaugmentation agent *Pseudomonas* sp. ADP and of the soil amendment with citrate as additional carbon source were examined in order to optimize atrazine biodegradation in the open soil microcosms.

2. Materials and methods

2.1. Chemicals

^{14}C-ring-*UL*-Atrazine (purity 99%, specific activity 1.85 GBq mmol^{-1}) was purchased from American Radiolabeled Chemicals, Inc., atrazine (Pestanal, purity 99.1%) from Riedel-de-Haën, and trisodium citrate from Sigma Chemical Co. The formulated herbicide Atrazerba FL (500 g L^{-1} of atrazine as active ingredient, RD = 2 L ha^{-1}) was purchased from Sapec, Portugal.

2.2. Soil

A natural soil representative of a corn production field from Central Portugal (Escola Superior Agrária de Coimbra, Coimbra, Portugal) and having no history of atrazine applications was used. The soil was sieved (5 mm mesh) and stored in plastic bags at −20 °C until required. Prior to use in the experiments, soil was defrosted for at least 4 d at 4 °C (Pesaro et al., 2003). The main characteristics of this soil are summarized in Fig. 1, after analysis by Direcção Regional de Agricultura de Entre-Douro e Minho, Portu-

gal, for soil texture and cation exchange capacity, and by Agência Portuguesa do Ambiente, Portugal, for total nitrogen and organic matter content. Further characterization was as follows: pH (ISO, 2005), maximum water-holding capacity (WHC) (ISO, 1998), water-soluble organic carbon (Silva et al., 2004).

2.3. Preparation of the inoculum

A spontaneous rifampicin-resistant mutant of *Pseudomonas* sp. ADP which can transform atrazine with equal efficiency than the wild-type (García-González et al., 2003) was used and is hereafter designated as *P.* ADP. The cell suspension used as inoculum was prepared from a late-exponential culture of *P.* ADP grown at 30 °C in liquid atrazine medium (Mandelbaum et al., 1993) adapted as previously described (Silva et al., 2004). Briefly, trisodium citrate (10 g L^{-1}) was used as carbon source and the medium was buffered using 4-Morpholinepropanesulfonic acid (Sigma–Aldrich Co.) (pH 6.2, 0.1 M). In addition, atrazine (300 mg L^{-1}, as N source) was supplied from Atrazerba FL, unless otherwise indicated. Inoculum growth was monitored by measuring the concentration of CFU obtained by plating culture serial dilutions on agarized Lennox Broth (LB) or the culture optical density at 640 nm.

2.4. Mineralization experiments

Atrazine mineralization assays were carried out in EPA vials (40 mL, gastight TFE/Silicone septa, Sigma–Aldrich Co.) containing 5 g of soil (dry weight, dw) as previously described (Silva et al., 2004) with minor adaptations. Briefly, a mixture of ^{14}C-*UL*-ring-atrazine (stock solution in acetonitrile, 467.7 kBq mL^{-1}) plus non-labeled atrazine (from Atrazerba FL) was incorporated into the soil to give an activity of 0.65 kBq g^{-1} and an approximate dose of the commercial formulation of 40 L ha^{-1} (equivalent to 20× RD). For bioaugmentation treatments, the *P.* ADP cell suspension was added to the soil to give inoculum densities of around 10^7 or 10^8 CFU g^{-1} of soil. For biostimulation treatments, trisodium citrate was added to obtain a concentration of 2.4 mg g^{-1}. Soil moisture was brought up to 40% of the soil WHC, taking in account the total volume of liquid (atrazine mixture, inoculum, citrate solution, deionized water) added in each treatment. Vials were then stirred using a vortex apparatus and incubated at 25 °C. At different time intervals, the quantity of ^{14}CO$_2$ released was quantified as described before (Silva et al., 2004). The experiments were run in triplicate.

2.5. Biodegradation experiments in the open soil microcosms

The geometry of the open soil microcosms used in this study was adapted from Burrows and Edwards (2004) and is shown in Fig. 1. Briefly, glass cylinders (15 cm height × 4.5 cm internal diameter) contained 160 g dw of soil over a 2 cm high layer of glass
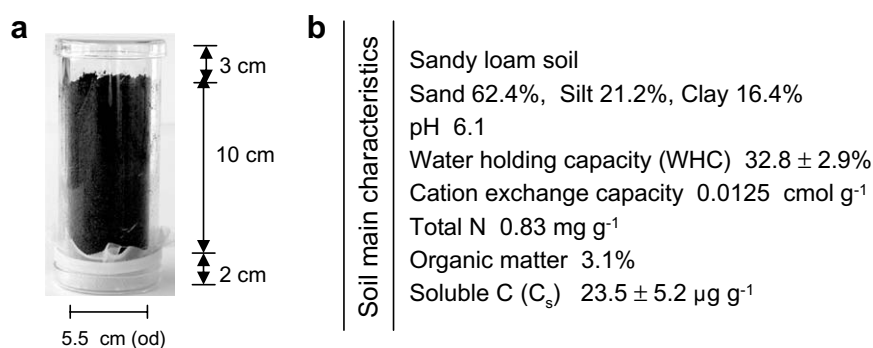

Fig. 1. (a) Geometry of the open soil microcosms and (b) characteristics of the soil used.

beads (2 mm diameter) supported by a fine Teflon mesh (to allow the collection of leachates in future experiments). Soil was spiked with aqueous suspensions of Atrazerba FL in order to obtain doses equivalent to 40 and 400 L ha^{-1}, and the whole soil volume was homogenized with a glass rod to promote incorporation of atrazine. Two sets of bioaugmentation treatments were performed, as follows: (i) one single inoculation with the *P.* ADP cell suspension to give approximate inoculum densities of 10^7 or 10^8 CFU g^{-1} at the beginning of the experiment, and (ii) three successive inoculations ($\sim 3.5 \times 10^7$ CFU g^{-1} each) at days 0, 2 and 4 (for 200× RD contaminated soils only). For biostimulation treatments, trisodium citrate was added to give 0.8 and 2.4 mg g^{-1} of soil when distributed. Non-inoculated and/or non-amended controls were also included in each set of experiments. Soil moisture was adjusted to 40% of the soil WHC as described above. Amended soils were again mixed thoroughly and gently packed into the glass cylinders. Microcosms were incubated at 25 °C in the dark (to avoid atrazine photodegradation) and weighted every day in order to replace the water lost by evaporation. Samples of soil were periodically collected and processed immediately or stored at −20 °C for microbiological or chemical analysis, respectively.

2.6. Microbiological analysis

To enumerate *P.* ADP viable cells, expressed as CFU g^{-1} of soil, serial dilutions in saline solution (0.9% v/v NaCl) of soil samples (1.2 ± 0.3 g) were spread plated onto LB agar supplemented with rifampicin (50 mg L^{-1}) and cycloheximide (100 mg L^{-1}). Plates were incubated at 30 °C and colonies counted after 72 h. The atrazine-degradation phenotype (Atr$^+$) of the colonies counted on this semi-selective medium was checked, by sub-culturing randomly picked colonies (around 100/plate) onto solid growth medium supplemented with 400 mg of atrazine L^{-1} (García-González et al., 2003); the Atr$^+$ colonies (surrounded by clear halos due to degradation of the precipitated atrazine) were counted after 72 h incubation at 30 °C and corresponded to 99 ± 1% of the total screened colonies.

2.7. Chemical analysis

For analysis of atrazine, soil samples were thawed at room temperature, dried at 40 °C, extracted with ethylacetate (3 × 10 mL) using a Liarre 60 ultrasonic apparatus (20 min; frequency 28–34 kHz) and centrifuged for 15 min at 2500 rpm. Analysis of the extracts was performed by GC–Electrospray Ionization (EI)–MS (Perkin–Elmer–Clarus 500). All extracts were injected in full scan mode to confirm the presence of each analyte and in single ion monitoring (SIM) for quantification purposes. External calibration was used for quantification. Surrogate atrazine D$_5$ was used to estimate recovery which ranged between 75% and 90%. The limit of quantification (LOQ) for atrazine was 25 ng g^{-1} of soil. For analysis of hydroxyatrazine, soil samples were thawed at room temperature, dried at 40 °C and extracted using soxhlet extraction (24 h) with a mixture of acetonitrile and methanol (1:4). The extracts were evaporated in a rapidvap N$_2$ evaporation system (Labconco 79100) and filtrated through a single use syringe filter (0.45 μm). The analysis of the extracts was performed by LC-EI-MS (Agilent 1100 series). The molecular ion was selected for quantitation in SIM mode. The LOQ for hydroxyatrazine was 1.25 ng g^{-1} of soil. Recovery ranged between 70% and 90%.

2.8. Reproductibility

Data reported are average values ± standard deviations from at least duplicate determinations from two or three independent bioremediation experiments carried out under identical conditions.

3. Results and discussion

3.1. Growth of P. ADP inoculum in medium supplemented with Atrazerba FL

P. ADP requires the presence of atrazine as sole N source in its growth medium, otherwise loss of bacterium degradative ability associated to instability of the catabolic plasmid pADP-1 may occur (de Souza et al., 1998). Prior to the bioremediation experiments, we examined the feasibility of supplementing inoculum growth medium with the commercial formulation under study that contains unknown formulating agents besides the active substance atrazine. The *P.* ADP population grew as well with 300 mg L^{-1} of atrazine supplied from Atrazerba FL as with the same concentration of pure atrazine (data not shown). Therefore, for the next bioremediation experiments, bacteria inoculum was obtained using the less expensive Atrazerba FL.

3.2. Atrazine mineralization by P. ADP in soil spiked with Atrazerba FL

The ability of *P.* ADP inoculum, either alone or combined with soil amendment with citrate trisodium to increase the ratio C$_s$:N$_{atz}$ in the soil (Silva et al., 2004), to mineralize atrazine in the natural soil spiked with a mixture of ^{14}C-ring-labeled atrazine plus atrazine from Atrazerba FL (20× RD), was examined (Fig. 2). The highest inoculum density tested (9.4 ± 0.6 × 10^7 CFU g^{-1} of soil) yielded rapid mineralization, with 68 ± 5% or 50 ± 3% of the initial labeled atrazine evolving as ^{14}CO$_2$ within 4 d, respectively in the presence or in the absence of citrate (2.4 mg g^{-1}) (Fig. 2). ^{14}CO$_2$ production continued evolving very slowly up to at least 8 d (Fig. 2, Table 1). Further increase of citrate supplementation (up to 4.8 mg g^{-1}) did not have a relevant effect on the total amount of ^{14}CO$_2$ produced (data not shown). Atrazine mineralization was significantly lower (36% or 26% after 5 d treatment, respectively in soil amended with citrate or non-amended) in the soils that were inoculated with 10× less quantity of viable cells of *P.* ADP (Fig. 2).

3.3. Biodegradation of atrazine and survival of P. ADP in the open soil microcosms

The bioaugmentation/biostimulation strategy was then examined at a larger scale in the open soil microcosms (Fig. 1a). In the first set of bioremediation experiments, soil spiked with 20×
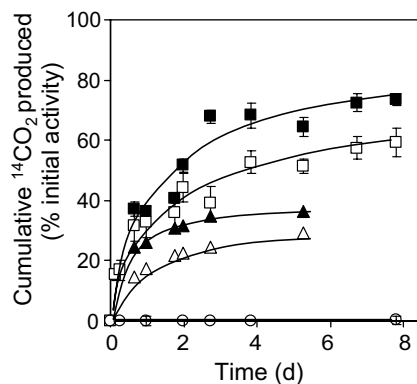
Fig. 2. Atrazine mineralization by *P.* ADP, at 25 °C, in soil freshly contaminated with ^{14}C-atrazine plus Atrazerba FL (20× RD), and inoculated with approximately 10^7 (△, ▲) or 10^8 (□, ■) CFU of *P.* ADP g^{-1} of soil combined with amendment with 2.4 mg of citrate g^{-1} (filled symbols) or without citrate (open symbols). The time-course evolution of ^{14}CO$_2$ release in the non-inoculated/non-amended control (○) is shown for comparison.

Table 1
Mineralization of atrazine in closed soil microcosms, and biodegradation of atrazine in larger open soil microcosms, contaminated with the indicated dosages of Atrazerba FL, after an 1 wk period of bioremediation treatments (consisting on bioaugmentation with $9 \pm 1 \times 10^7$ CFU of *P*. ADP g^{-1} of soil and biostimulation with the indicated concentrations of citrate).

Dose of Atrazerba FL	Mineralization (%)[a]		Biodegradation (%)[b] (atrazine concentration, μg g^{-1})[c]			
	Closed microcosms (scale: 5 g of soil)		Open microcosms (scale: 160 g of soil)			
	1 Inoculation		1 Inoculation		3 Inoculations	
	0 cit[d]	2.4 cit[d]	0 cit[d]	2.4 cit[d]	0.8 cit[d]	2.4 cit[d]
20× RD	55±3	70±1	99 (0.09±0.02)	99 (0.07±0.03)	nd	nd
200× RD	nd	nd	79 (13.3±5.7)	87 (7.8±2.1)	88 (7.2±2.0)	98 (1.6±0.4)

nd not determined.
[a] Percentage of the initial [^{14}C]-atrazine evolving as $^{14}CO_2$.
[b] Percentage of the initial atrazine removed from soil.
[c] Average residual concentration of atrazine measured in the soil.
[d] 0 cit, 0.8 cit or 2.4 cit, mean respectively non-amended soil or soil amended with 0.8 or 2.4 mg citrate g^{-1}.

RD of Atrazerba FL was exposed to unique inoculations with *P*. ADP differing 10-fold in density (Fig. 3). Consistent with previous observations (Silva et al., 2004), in the microcosms amended with citrate, the proliferation of the introduced bacterial cells was stimulated during the first 2 d following inoculation, allowing the survival of higher numbers of active cells of the bioaugmentation agent throughout the entire experiment, in comparison with the non-amended soil (Fig. 3a). Nevertheless, analysis of the atrazine remaining in the soil showed that all the bioremediation treatments resulted in the rapid removal of most of the initial atrazine during the first 2.5 d (Fig. 3b), even though slight differences on biodegradation extent were observed that may be relevant. Indeed, for the soil bioaugmented with the highest inoculum density ($9 \pm 1 \times 10^7$ CFU g^{-1}), the herbicide concentration was reduced by >97% from 7.2 ± 1.6 μg atrazine g^{-1} of soil to lower than 0.2 μg g^{-1} within at least 5 d, independent of soil amendment with citrate (Fig. 3b, Table 1). But, for the lowest inoculum density tested, atrazine levels declined to 1.0 ± 0.2 μg g^{-1} (corresponding to an 86% biodegradation) in the absence of citrate and to 0.3 ± 0.1 μg g^{-1} (96% biodegradation) when soil was amended with citrate, over a 5 d treatment period (Fig. 3b). In summary, for 20× RD of Atrazerba FL, that may be relevant for intensive uses at rates higher than the rec-

ommended field rate, bioaugmentation with around 9×10^7 CFU of *P*. ADP g^{-1} of soil seem to be useful to provide rapid (in 1 wk) atrazine removal from soil, and biostimulation with citrate should not be necessary in this particular situation (Table 1).

To further analyze the efficacy of this bioremediation strategy, we examined the performance of *P*. ADP under an extremer situation, using soil contaminated with a 10-fold higher dosage of Atrazerba FL (200× RD), that may be relevant for a hypothetical spill scenario. For this higher level of soil contamination (62.8 ± 6.5 μg g^{-1} of initial atrazine measured in the soil), atrazine biodegradation was relatively rapid over the first 4 d of treatment with one sole initial inoculum of *P*. ADP ($8.5 \pm 0.5 \times 10^7$ CFU g^{-1}) either combined or not with the addition of citrate (Fig. 4a). However, the atrazine concentrations measured in the soil after 1 wk of treatment were equal to 7.8 ± 2.1 or 13.3 ± 5.7 μg g^{-1} (Fig. 4a, Table 1), respectively in the presence or absence of citrate. Since these values are quite above the recommended field level of 1 ppm (equivalent to a standard field rate of 1 kg ha^{-1} distributed, for example, through a 5×5 cm soil column), an alternative strategy was examined, where the same amount of *P*. ADP viable cells was applied as three successive inoculations (3–3.5×10^7 CFU g^{-1} each) at 2 d intervals, in soil amended with citrate (Figs. 4a and 4b). This
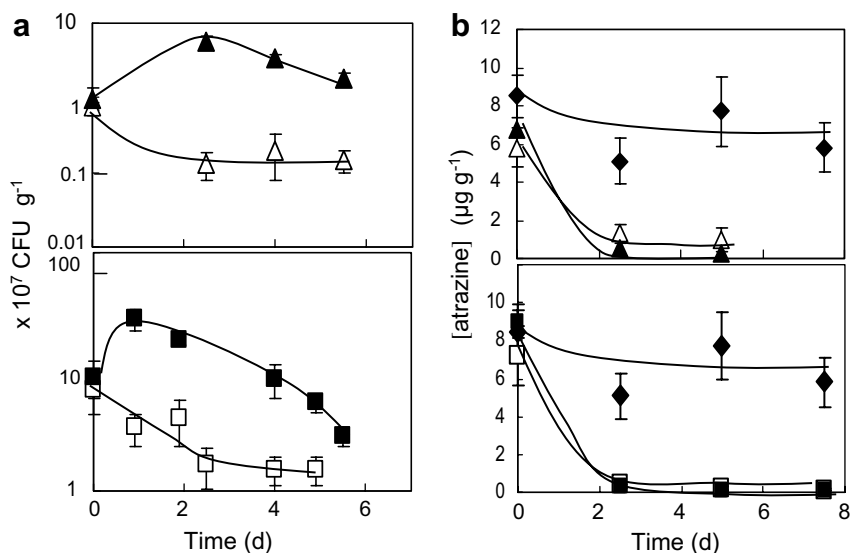
Fig. 3. (a) Evolution of the concentration of viable cells of *P*. ADP and (b) biodegradation of atrazine, during bioremediation treatments, at 25 °C, in open soil microcosms contaminated with 20× RD of Atrazerba FL. Treatments consisted on soil inoculation with approximately 9×10^6 (upper panels) or 9×10^7 (lower panels) CFU of *P*. ADP g^{-1} of soil combined with amendment with citrate at 2.4 mg g^{-1} (▲, ■) or without citrate (△, □). Evolution of atrazine concentration measured in the non-inoculated control soil (◆) is also shown for comparison.
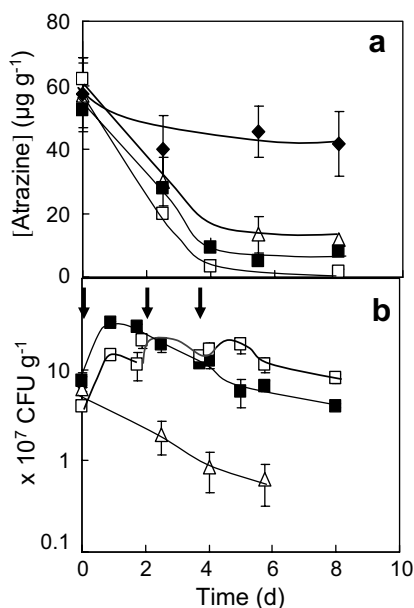
Fig. 4. (a) Biodegradation of atrazine and (b) concentration of viable cells of *P*. ADP, in open soil microcosms freshly contaminated with 200× RD of Atrazerba FL during one-wk bioremediation treatments, at 25 °C, where, one (△,■) or three successive inoculations with *P*. ADP at 2 d intervals (indicated by black arrows in b) (□), were used to amend soil together with citrate addition (2.4 mg g⁻¹) (■,□) or without citrate (△). Evolution of atrazine concentration measured in the non-inoculated control soil (◆) is also shown in (a).

regime of inoculation, combined with the addition of 2.4 mg citrate g^{-1}, was clearly more effective in promoting the removal of atrazine from the soil than the single inoculation plus citrate (Fig. 4a, Table 1) or the 3 inoculations plus soil amendment with a lower concentration of citrate (0.8 mg g^{-1}) (Table 1). Indeed, atrazine levels declined to only 1.6 ± 0.4 μg g^{-1} (98% biodegradation) over 1 wk treatment period (Fig. 4a, Table 1).

The advantage of repeated inoculations with atrazine-degrading bacteria of a soil contaminated with 110 μg atrazine g^{-1} was reported before by Newcombe and Crowley (1999), that measured 90% atrazine biodegradation over one month treatment period comprising 11 inoculations. In the present work, we provide additional evidence highlighting the importance of combining the successive soil inoculations with P. *ADP* with an adequate provision of citrate in order to achieve rapid (within 1 wk) removal of high concentrations of atrazine from contaminated soil. The speed, besides the extension, of the cleanup of soils heavily polluted with atrazine products due to accidental or intentional spill, is important in order to prevent contamination of other environmental compartments (e.g. surface- and ground-waters) with atrazine and its toxic chlorinated derivatives (Ralebitso et al., 2002; Wackett et al., 2002). Citrate addition may be critical presumably by helping *P*. ADP to overcome problems related to carbon limitation (Silva et al., 2004). We speculate that by combining the repeated inoculations with citrate addition, long-term survival of the bioaugmentation agent is efficiently extended. Indeed, a fresh batch of viable cells of *P*. ADP pre-grown in the presence of atrazine and consequently adapted to utilize rapidly this N source is introduced in the soil when the survival of the previous one start declining, while the addition of an adequate concentration of the organic acid may support *P*. ADP growth and degradative ability during at least 2 d before the next fresh batch of cells is added. Consistently, quantification of the *P*. ADP viable cells in the open soil microcosms amended with repeated inoculations plus citrate, indicated that higher levels of physiologically active cells of the introduced bacteria (>10^8 CFU g^{-1}) were maintained during longer periods of time

(e.g. for up to 8 d) (Fig. 4b), presumably enabling the rapid degradation of higher quantities of the herbicide, when compared with the other bioremediation treatments examined (Fig. 4a)

During the course of the bioremediation experiments in the open soil microcosms, we analysed the atrazine remaining in the soil. Apparently, for the soil contaminated with 20× RD of Atrazerba FL, there was almost complete degradation of atrazine in the open soil microcosms after 1 wk treatment, while the maximum% of labelled atrazine evolving as $^{14}CO_2$ in the mineralization assays indicated that mineralization was below completion (Table 1). These facts raise the question whether or not the herbicide is totally mineralized or only partially transformed in the open soil microcosms. Analysis of hydroxyatrazine did not show detectable accumulation of this metabolite during the bioremediation treatments examined (data not shown), suggesting that hydroxyatrazine formed from atrazine must have been further catabolized. Nevertheless, the rapid removal of most of the atrazine from the Atrazerba FL contaminated soils, even though complete mineralization is not proved, point to an important environmental impact of the herein examined bioremediation tool. Indeed, after the initial dechlorination step, the atrazine metabolites formed by *P*. ADP are considered to be non-herbicidal and relatively safe compared to atrazine or its chlorinated dealkhylated derivatives (Wackett et al., 2002; Ralebitso et al., 2002; Oh et al., 2003). Moreover, even though a fraction of atrazine may undergo bound residue formation and aging in the particular soil to be bioremediated, becoming essentially unavailable to microbial attack (Barriuso et al., 2004), this presumably deeply sequestered fraction is not likely to cause relevant injury to ecosystems (Alexander, 2000).

4. Conclusions

We provide evidence for the successful bioremediation of a natural soil spiked with an atrazine commercial formulation at doses mimicking over-use or spill situations, in open soil microcosms. The bioremediation treatment providing fastest and higher extent of atrazine removal differed according to the magnitude of soil pollution. The use of repeated inoculations with *P*. ADP combined with biostimulation with citrate seem to be necessary to provide rapid removal (e.g. in 1 wk) of high atrazine concentrations (e.g. around 62 μg g^{-1} of soil). On the contrary, for a more moderate level of soil contamination (~7 μg of atrazine g^{-1} of soil), bioaugmentation alone and using one single inoculation with *P*. ADP may be sufficient. Despite recommendations for controlling and managing the uses of this herbicide in several countries, it is likely that atrazine production and usage will continue worldwide. We anticipate that this may result in further contamination of soil and water compartments, one of the main environmental concerns regarding the use of atrazine-based products (Ralebitso et al., 2002; Wackett et al., 2002; Cerejeira et al., 2003; Kalkhoff et al., 2003). Even though several factors influencing bioremediation efficiency in the field still need optimization, we anticipate that the herein examined bioremediation tool may help to prevent atrazine and its toxic chlorinated derivatives from reaching freshwater compartments due to leaching and/or runoff from spill sites or concentration "hotspots" associated to uneven or intensive applications of atrazine formulations (Fava et al., 2007). It should be especially helpful for polluted soils where the activity of indigenous degraders is low or nonexistent or where other toxic contaminants that may be mixed with the target herbicide in a real field situation (Hayes et al., 2006) may affect microbial activity (DeLorenzo et al., 2001). Further optimization at larger scales (e.g. mesocosm and field scenarios) and using soils contaminated with mixed commercial formulations are foreseen. From a practical point of view, the feasibility of growing the inoculum of *P*. ADP on medium supplemented with Atrazerba FL as N source instead of pure atrazine (Silva et al., 2004), herein shown,

will contribute to improve the cost-effectiveness of the proposed cleanup treatments in scaled-up bioremediation of land areas.

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