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Enhanced mineralisation of diuron using a cyclodextrin-based bioremediation technology

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Abstract

The phenylurea herbicide diuron [N-(3,4-dichlorophenyl)-N,N-dimethylurea] is widely used in a broad range of herbicide formulations, and consequently, it is frequently detected as a major soil and water contaminant in areas where there is extensive use. Diuron has the unfortunate combination of being strongly adsorbed by soil organic matter particles, and hence, slowly degraded in the environment due to its reduced bioavailability. N-phenylurea herbicides seem to be biodegraded in soil, but it must be kept in mind that this biotic or abiotic degradation could lead to accumulation of very toxic derived compounds, such as 3,4-dichloroaniline.

A research was conducted to find procedures that might result in an increase in the bioavailability of diuron in contaminated soils, through solubility enhancement. For our purpose we used a double system composed of hydroxypropyl-β-cyclodextrin (HPCD), which is capable of forming inclusion compounds in solution, and a two-members bacterial consortium formed by the diuron-degrading *Arthrobacter sulfonivorans* (*Arthrobacter* sp. N2) and the linuron-degrading *Variovorax soli* (*Variovorax* sp. SRS16), which will be able to achieve a complete biodegradation of diuron to CO_2. The cyclodextrin-based bioremediation technology here described shows for the first time an almost complete mineralisation of diuron in a soil system, as opposite to previous incomplete mineralization based on single or consortium bacterial degradation.

Keywords: Cyclodextrin, diuron, 3,4-dichloroaniline, soil contamination, biodegradation, bacterial consortium.

Introduction

Diuron is a biologically active pollutant present in soil, water and sediments. This substituted urea herbicide inhibits photosynthesis by preventing oxygen production[1] and

blocks the electron transfer at the level of photosystem II of photosynthetic micro-organisms and plants. Diuron has the unfortunate combination of being strongly adsorbed on soil organic matter particles, and hence, slowly degraded in the environment due to its reduced bioavailability.

Diuron is considered a Priority Hazardous Substance by the European Commission[2] since its degradation in soil leads to 3,4-dichloroaniline (3,4-DCA), a very toxic compound, which accumulates in the environment[3-6]. Consequently, diuron has been included in the European Commission's list of priority substances for European freshwater resources (Directive 2000/60/EC) and in the U.S. Contaminant Candidate List 3[7].

Biodegradation has been described as the primary mechanism for diuron dissipation in soils and waters[8], although dispersion of this compound in agriculture leads to pollution of the aquatic environment by soil leaching[9-11] and run-off[12].

In this work, some studies were conducted to find procedures that might result in an increase in the bioavailability of diuron in a contaminated soil, through solubility enhancement using biodegradable molecules. These molecules are cyclodextrins (CDs), which are cyclic oligosaccharides, containing 6 (α-CD), 7 (β-CD) or 8 (γ-CD) R-(1,4)-linked glucose units, formed from the enzymatic degradation of starch by bacteria. It is well-known that they are capable of forming inclusion compounds both in solution and in solid state with a variety of guest molecules, which are placed in their hydrophobic interior cavity[13]. A large number of papers describing the complexation of CDs with pesticides can be found in the literature. Most pesticide-CD complexes were aimed to improve their solubility in water[14-18]. However, no research has been reported with the aim of finding correlations between this increasing in solubility, desorption percentage from soil, and bioavailability by means of mineralising assays, confirming the complete

dissipation of the pesticides. But not only increasing of bioavailability will be enough to reach a significant soil diuron dissipation, since although several diuron-degrading bacteria have been isolated from different agricultural soils[3,8,19-20] and river waters[20] none of them has been identified as capable to reach a complete diuron mineralisation in the presence of soil. Sorensen et al.,[5] used a two member diuron-mineralising consortium which gave better results by combining the cooperative degradation capacities of two bacteria. In this work, we attempt to enhanced mineralisation of diuron using a cyclodextrin-based bioremediation technology involving a bacterial consortium, which resulted in an effective diuron mineralisation system thanks to the bioavailability increasing.

The development of an *in situ* and environmental friendly soil decontamination technique, which could give rise to a complete diuron mineralisation by means of increasing the bioavailability of the pollutant and employing specific chemical bacterium degraders, would involve an improvement from both, economical and environmental point of view.

Materials and Methods

Materials

Technical grade (98%) diuron [*N*-(3,4-dichlorophenyl)-*N*,*N*-dimethyl-urea] was provided by Presmar S.L. (Seville, Spain). Radiolabeled [ring-U-^{14}C]-diuron was purchased from Institute of Isotopes, Budapest, Hungary (specific activity 36 mCi mmol^{-1}, chemical purity 99.9% and radiochemical purity, 100%). The cyclodextrin hydroxypropyl-β-CD (HPCD) from Cyclolab, Budapest, Hungary, was selected because in previous tests (data not shown) it was demonstrated that this CD is not used as carbon

source by the bacteria tested, eliminating its use as a growth substrate by the strains studied.

A southwestern Spain loamy sandy soil with a pH of 8.7, 6.9% of $CaCO_3$, 1% of organic matter and a particle size distribution of 82.3% sand, 4.1% silt and 13.5% clay was selected for this study. The sample was taken from the superficial horizon (0-20 cm) and was air-dried for 24 h and sieved through 2 mm, in order to remove stones, plant materials, etc. The soil was analyzed for particle size distribution, measured by a Bouyoucos densimeter, organic matter, measured by $K_2Cr_2O_7$ oxidation, pH, determined in the 1:2.5 soil/water extract, and total carbonate content, measured by the manometric method[21].

The diuron-degrading organism *Arthrobacter* sp. N2[3] was purchased from the Institut Pasteur Collection. *Variovorax* sp. SRS16 was kindly provided by S.R. Sorensen[5]. The identification of phylogenetic neighbours was carried out by applying BLAST[22] to the GenBank sequence database and the EzTaxon database[23]. *Arthrobacter* sp. N2 showed a 99.567 pairwise similarity with *Arthrobacter sulfonivorans* (AF235091), while *Variovorax* sp. SRS16 yielded a 98.780 pairwise similarity with *Variovorax soli* (DQ432053). In this study we will refer to these strains as *A. sulfonivorans* and *V. soli*.

Methods

Diuron desorption studies from soil.

Prior to desorption studies triplicate batch adsorption experiments were performed by mixing 5 g of the soil with 10 mL of 0.01 M $Ca(NO_3)_2$ solution, containing various concentrations (5, 10 and 15 mg L^{-1}) of diuron, in 50 mL polypropylene centrifuge tubes. The samples were shaken for 24 h at 20 ± 1 °C. This time of reaction was chosen

from preliminary kinetic studies (not shown), which showed that adsorption had reached pseudoequilibrium. After shaking (on an orbital shaker), the suspensions were centrifuged, and the concentration of diuron in the supernatant was determined by using a Shimadzu HPLC equipped with UV detector. The difference in herbicide concentration between the initial and final equilibrium solutions was assumed to be due to sorption, and the amount of diuron retained by the adsorbent was calculated.

Desorption experiments were performed after adsorption equilibrium had been reached by removing half of the supernatant after centrifugation, replacing it by 5 mL of the extractant solution, allowing equilibration for an additional 24 h period, and after that, operating as in the adsorption experiment. This process was repeated twice. Desorption experiments were carried out using 0.01 M $Ca(NO_3)_2$ solution (named as $Ca(NO_3)_2$ solution) and the same solution plus HPCD with a final concentration of 50 mM (named as HPCD solution). The percentage of diuron desorbed with respect to that previously adsorbed during adsorption process (%D) was calculated for all the desorption experiments.

Inoculum preparation

After receiving both bacteria, ***A. sulfonivorans*** and ***V. soli*** were stored in criovials Microbank^{TM}, which are 2 mL microtubes containing an specific culture medium and 20 porous spheres of 3 mm diameter, and keept at -80 ºC. Before each experiment the criovials were thawed, and then ***A. sulfonivorans*** was grown in Luria-Bertani (LB) medium and ***V. soli*** was grown in a R2A medium[24]. The bacteria were harvested at the beginning of the stationary phase, and afterwards, washed twice in a sterile mineral salts (MS) solution[24] before initiation of the experiments. The initial cell densities of the bacteria in the degradation experiments were 10^7 CFU mL^{-1}. For diuron degradation,

the bacteria were grown in a MS medium supplemented with 40 mg L^{-1} diuron, as described by Sorensen et al.[24].

Mineralization and biodegradation experiments

Mineralisation of ^{14}C-labelled diuron in the soil was measured (in triplicate) through the evolution of $^{14}CO_2$ produced[25]. Soil was sterilized using an Autester-G, P-Selecta with 3 cycles at 121 ºC, inlet pressure 103 KPa, during 20 mins. The mineralization assays were carried out in respirometers: modified 250 mL Erlenmeyers into which 10 g soil together with 50 mL, of mineral salts medium (MMK) was placed. Acetone stock solution containing ^{14}C-labeled and unlabeled diuron was added to the soil to obtain a final concentration of 50 mg kg^{-1} and a radioactivity of approximately 900 Bq per flask. The flasks were inoculated with the specific bacterium or the consortium, prepared as described above, and were closed with Teflon-lined stoppers, and incubated at 20 ± 1 ºC. Non-inoculated soil sterile controls and non-innoculated soil sterile with HPBCD addition controls were also prepared and no mineralisation was detected. Production of $^{14}CO_2$ was measured as radioactivity appearing in the alkali trap of the biometer flasks, which contained 1 mL of 0.5 M NaOH. Periodically, the solution was removed from the trap and replaced with fresh alkali. The NaOH solution was mixed with 5 mL of liquid scintillation cocktail (Ready safe from PerkinElmer, Inc., USA) and the mixture kept in darkness for about 24 h for dissipation of chemiluminescence. Radioactivity was measured as it was described by Posada-Baquero et al.[26].

Biodegradation experiments were performed in parallel in the same way that mineralization ones, but in this case, only non-radiolabeled diuron was used, and the main metabolite 3,4-dichloroaniline and the parent compound were analyzed at different

195 time points by CG-MS. A gas chromatograph 6890N from Agilent Technologies
196 coupled to an Agilent Automass quadrupole mass spectrometer 5975A was used.
197 Injection was in splitless mode with the split valve closed for 48 s. Helium was
198 employed as gas carrier. A Hewlett-Packard DB 17 ms (30 cm × 0.25 i.d. × 0.17 μm
199 film thickness) capillary column was used. The temperature program for the
200 chromatographic run was as follows: injector temperature, 70 ºC (hold 0.5 min),
201 followed by a 100 ºC min^{-1} ramp to 300 ºC (hold 15 min). For mass spectrometric
202 detection, a potential of 70 eV was initially imposed in total ion scan or full scan (m/z
203 45-300). Then, acquisition was performed under time-scheduled selected ion monitoring
204 (SIM) using the following product ion-qualifers: 124, 187, 159, 97, 88 74 m/z being the
205 precursor ion 187 m/z for diuron quantification detected as intermediate 1,4-dichloro-2-
206 isocyanato benzene *(27).* In the case of the main metabolite (3,4-DCA) the precursor ion
207 was 162 m/z . The MS detector was kept at 200 °C. In order to avoid saturation and to
208 preserve it, the analyzer was switched off for 4 min during solvent elution and after the
209 last eluting analyte determination. The limit of quantification for the metabolite was 1
210 μg/g of soil. The organic solvent selected to carry out the extractions from aqueous
211 supernatant was hexane (1:1 ratio MMK medium:organic solvent, mL), and for
212 extractions from soil pellet dichloromethane was used (1:1 ratio soil:organic solvent).
213 Recoveries were 91-102%.
214 Both, mineralization and biodegradation experiments were incubated for 120 days.
215
216 *Cyclodextrin application on soil mineralisation and biodegradation*
217 A HPCD solution, with a concentration corresponding to 10 times the millimoles of
218 diuron previously added in soil degradation experiments flasks (50 mg kg^{-1}), was
219 employed to enhance the herbicide bioavailability and increase its biodegradation rate.

This solution was incorporated into either biodegradation and mineralisation experiments to determine the increase in bioavailability of the herbicide.

Model of mineralisation kinetics

Mineralisation data (expressed as the percentage [P] of the initial activity converted to $^{14}CO_2$ as a function of time [t]) were fitted to a first-order equation of the following form[28]:

$$P = P_{max}(1-e^{-kt}) \quad (1)$$

Nonlinear regression analysis (Sigmaplot v. 8.0) was used to estimate the parameters P_{max} (overall extent of ^{14}C mineralisation) and k (first-order mineralisation rate).

The parameters derived from this model accurately describe the mineralisation kinetics of nonsorbed diuron in systems under equilibrium (instantaneous sorption and/or desorption) and pseudoequilibrium (desorption rates much slower than degradation rates) conditions.

Results and Discussion

Diuron desorption experiments using 0.01M HPCD as extractant solution

The desorption percentages (%D) values obtained for the soil under study when the HPCD solution was employed as extractant in comparison to $Ca(NO_3)_2$ solution are shown in Table 1. In this soil for all diuron initial concentrations about 90% could be desorbed with HPCD solution. The results obtained indicate the high extracting power of HPCD towards the herbicide previously adsorbed on the soils in comparison to the percentages extracted with $Ca(NO_3)_2$ solution, due to the formation of water-soluble inclusion complexes between diuron and HPCD. Similar results have been obtained in

previous papers using HPCD and CDs as extractant solutions for the herbicide 2,4-D and norflurazon from soil[29,14-15,30-31]. In general, low-polarity pesticides have a high tendency to be adsorbed on soil surfaces, leading to their inactivation and low bioavailability and, sometimes, to soil contamination. If these pesticides are able to form inclusion complexes with CDs and, as a consequence, to increase their solubility, the application of CD solutions to soils containing a high concentration of pesticide residues adsorbed can increase their removal and pass to the soil solution where they become bioavailable.

Diuron mineralisation and biodegradation experiments in soil inoculated with A. sulfonivorans, V. soli and their bacterial consortium,

The principal product of diuron biodegradation, 3,4-DCA, exhibits a high toxicity and is also persistent in soil, water and groundwater. Diuron indirectly possesses a significant amount of toxicity and could be a potential poisoning herbicide contaminant of groundwater. Therefore, the ultimate objective of this work was to obtain a complete diuron mineralisation in a soil-water system. The chosen scenario was a loamy sandy soil from an agricultural site.

A two-member diuron-mineralising consortium, by combining the cooperative degradation capacities of the diuron-degrading bacteria, *A. sulfonivorans* and the linuron-mineralising bacteria *V. soli*, was used, in comparison to the individual bacteria.

The effects on the mineralisation of diuron in soil (50 mg kg^{-1}) after inoculation of *A. sulfonivorans* and *V. soli*, individually or in a co-culture, were determined (Fig. 1). The overall extent of ^{14}C mineralisation was estimated using a first-order production equation (1),[28] (Table 2). Inoculation with *A. sulfonivorans* resulted in a mineralisation of 6.86 %. The metabolite 3,4-DCA was detected in the parallel biodegradation

experiments only in the samples inoculated with this bacterium, and the amount was equivalent to 8.51 % of the initially added diuron. Inoculation with *V. soli* alone resulted in a diuron mineralisation in the soil of only the 5.22 %. Inoculation with the co-culture resulted in rapid diuron mineralisation, and an important mineralisation was observed (45.25 % of the added ^{14}C diuron was metabolised to $^{14}CO_2$ during the experiment, after 120 days) (Fig. 1), and no metabolite was determined after experiment (Table 2). The highest mineralisation rates values corresponded to the soil slurries inoculated with the bacterial consortium, k values of 5 and 6 times higher than those of *A. sulfonivorans* and *V. soli*, used separately (Table 2). The time necessary to reach mineralisation values over 5% (lag phase) was only of 12.53 days for systems inoculated with both selected bacteria, in comparison to 120 and 97 days for *A. sulfonivorans* and *V. soli*, respectively when grown alone.

In Table 2, the residual diuron measured at the end of the parallel biodegradation experiments is also shown, being remarkable that in the *A. sulfonivorans* inoculated system a 60.52 % of the diuron initially added was still present, confirming that about 40% of diuron is biodegraded, but not mineralised, as can be observed in the mineralisation experiments results (6.86 %), remaining in the form of the toxic metabolite, 3,4-DCA (8.51%) or other intermediate species. A similar result could be observed for the system inoculated with *V. soli*, but in this case, the percentage of diuron remaining at the end of the biodegradation experiment was lower (38.78%), from which only 5.22% was mineralised. However, the presence of the toxic metabolite was much lower (0.61%). Finally, the percentage of diuron measured after soil biodegradation experiment in the presence of the two-member bacterial consortium, was the lowest, only 21.73%, confirming again, the need of using the two bacteria to reduce drastically the real risk in a diuron contaminated soil. Moreover, there is an extremely

high increase in the percentage of diuron mineralisation, from about 5-6% for the individual bacteria to 45% when the consortium was used. In addition, the toxic metabolite was not detected. It is also important to highlight that the lag phase is reduced from 120-97 days (only one degrader) to 12 days in the presence of the consortium, which denotes that the cooperative diuron degradation of the consortium increase the mineralisation rate (0.28 $days^{-1}$), clearly turning out in an effective *in situ* bioremediation decontamination tool.

From these results it can be concluded that this type of chemicals (organochloride persistent compounds) will require a more complex and conscientious analysis about the best strategy to reach an optimal bioremediation of a contaminated soil, especially for those whose main metabolites are suspected of having unwanted effects on nontarget microorganism, like the scenario investigated in this work.

Diuron mineralisation and biodegradation experiments in the presence of a HPCD solution in soil inoculated with A. sulfonivorans and *V. soli* and their *bacterial consortium*

HPCD seems to be a good choice for being applied as decontamination technique in case of the herbicide diuron. Similar results were previously reported for naphthalene and phenanthrene by Badr et al.[32]. For this reason diuron mineralisation experiments employing soil slurries in the presence of HPCD solution were performed with the aim of enhancing diuron bioavailability and its subsequent dissipation, making that the potentially bioavailable herbicide fraction passes to the soil solution in a faster way, since, as demonstrated above, a higher desorption percentages of diuron from the soil studied were obtained with the HPCD solution employed as diuron extractant. The ability of soils to release (desorb) pollutants determines its susceptibility to suffer

321 microbial degradation, thereby influencing effectiveness of the bioremediation process.
322 The degradation of sorbed contaminants can presumably occur via microbially-
323 mediated desorption of contaminants and the development of a steep gradient between
324 solid phase and interfacial contaminant[33].

325 The use of CD solutions as enhancers in pollutant dissipation in soil has been
326 postulated as a promising *in situ* decontamination tool due to its capacity for pesticide
327 soil desorption. Previously, our group has reported numerous results using different
328 types of CDs solutions for enhancing soil desorption of the different herbicides with
329 CDs naturally originated and their derivates and a different aging periods[14,17,31] but, no
330 works correlating the CD solution desorption effect with microbial biodegradation
331 enhancing has been carried out yet.

332 In Figure 1, diuron mineralisation curves obtained from inoculated soil slurries in
333 the presence of HPCD in solution at a concentration equivalent of 10 times the amount
334 of diuron initially spiked are shown. In the case of inoculation with *A. sulfonivorans*
335 (Fig. 1a) mineralisation was very low (8.55%, Table 2) in spite of the fact that this
336 bacterium is a diuron degrader[3]. Then, the addition of the HPCD solution, which
337 provokes an slight increasing in herbicide bioavailability, provokes also a slight
338 increase of the main metabolite after degradation, as confirmed with the data obtained
339 for the 3,4-DCA analysis, 10.26 % (Table 2). When the HPCD solution was applied on
340 the soil slurries inoculated with *V. soli* (Fig. 1b) a clear increase in herbicide
341 bioavailability was translated into a higher percentage of diuron mineralised, reaching
342 an overall extent of ^{14}C mineralisation of 57.87 %. Finally, in Figure 1c the diuron
343 mineralisation curve, when the HPCD solution was added, shows a new significant
344 increase in herbicide mineralisation regarding to that observed in the absence of the CD
345 solution, reaching a maximum percentage of mineralisation of 98.67 % and the highest

346 value determined for mineralisation rate of 3.08 $days^{-1}$ (Table 2), demonstrating once
347 again, as the increasing in the amount of herbicide that passes to the soil solution is
348 unequivocally connected with the mineralisation rate, especially when diuron
349 mineralisation is managed by an effective bacterial consortium where cometabolism is
350 necessary. Likewise, in the presence of the HPCD solution, diuron biodegradation
351 assays showed up the almost disappearance of the diuron at the end of the experiment
352 when the bacterial consortium was applied, remaining only 6.85 % of the initial amount.
353
354 *Comparison of the cyclodextrin-based bioremediation with other diuron-degrading*
355 *technologies*
356 *A. sulfonivorans* (=*Arthrobacter* sp. N2) was isolated and characterised by
357 Widehem et al.[3] from a soil by diuron enrichment procedures. These authors observed
358 that this bacterium was capable to metabolise diuron to the degradation product 3,4-
359 DCA, and this metabolite was produced in stoichiometric amounts, but however, no
360 mineralisation was observed. On the other side, Sorensen et al.[24] isolated from a Danish
361 agricultural soil enriched with linuron a *Variovorax* sp. strain SRS16 (=*V. soli*). This
362 was a linuron-mineralizing bacterium able to use the herbicide as a carbon, nitrogen,
363 and energy source. Approximately 60 to 70% of ^{14}C-linuron was metabolized to $^{14}CO_2$
364 within 10 days with only low concentrations of 3,4-DCA detected. Satsuma *(34)*
365 isolated a *Variovorax* sp. strain RA8 from Japanese river sediments able to mineralize
366 70% of linuron and detected a trace amount of 3,4-DCA.
367 Further, Sorensen et al.[5] constructed a linuron- and diuron-mineralizing two-
368 member consortium by combining the cooperative degradation capacities of the diuron-
369 degrading organism *Arthrobacter globiformis* and the linuron-mineralizing organism *V.*
370 *soli*. Neither of the strains mineralized diuron alone in a mineral medium, but combined,

the two strains mineralized 31 to 62% of the added [*ring*-U-^{14}C]diuron to $^{14}CO_2$. These results are similar to those obtained in the present paper when using our two- member consortium. But this two-member consortium reached an almost complete mineralization of [ring-U-^{14}C]-diuron (98.67%) without traces of 3,4-DCA when HPCD was used to enhance diuron bioavailability. In conclusion and based on these results, the use of a HPCD solution at a very low concentration of only 10 times the diuron equimolar concentration in soil will act as an bioavailability enhancer, accelerating the pass of the diuron desorbing fraction from the soil particle surface to the soil solution, and improving microorganism accessibility to the herbicide, being necessary the use of a cometabolism to reach a complete mineralisation of the desorbing fraction avoiding the presence in the soil solution of its main toxic and also persistent metabolite 3,4-DCA.

The cyclodextrin-based bioremediation technology here described shows for the first time an almost complete mineralisation of diuron in a soil system, as opposite to previous incomplete mineralization (above 60%) based on single or consortium bacterial degradation.

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Table 1. Percentage of diuron desorbed from the soil

	Diuron initial concentrations ($mg\ L^{-1}$)	Extractant solutions (0.01 M)	
		$Ca(NO_3)_2$	HPCD
Soil	5	51.58 ± 0.66	94.64 ± 2.01
	10	84.01 ± 1.02	89.79 ± 3.33
	15	87.20 ± 2.55	100 ± 1.09

Table 2. First order diuron mineralisation kinetic parameters for *Arthrobacter sulfonivorans*, *Variovorax soli* and the two-strains consortium, in the absence or presence of cyclodextrin. Percentages of Diuron and 3,4-DCA at the end of the parallel biodegradation experiments.

Diuron degraders	**Lag phase (days)**	**Mineralisation rate ($days^{-1}$). K x 10^2**	**Overall extent of ^{14}C mineralisation (%)**	**Diuron (% at the end of experiment)**	**3,4-DCA (% at the end of experiment)**
A. Sulfonivorans	120.22 ± 0.99	5.32 ± 0.21	6.86 ± 0.84	60.52 ± 1.15	8.51 ± 0.55
V. Soli	97.41 ± 1.01	4.81 ± 0.52	5.22 ± 0.88	38.78 ± 0.99	0.61 ± 0.05
Bacterial consortium	12.53 ± 0.88	28.1 ± 1.1	45.25 ± 2.27	21.73 ± 0.91	0.00 ± 0.03
A. Sulfonivorans + HPCD solution	28.33 ± 1.03	6.42 ± 2.52	8.55 ± 0.56	22.81 ± 0.02	10.26 ± 0.02
V. Soli + HPCD solution	194.13 ± 2.25	45.6 ± 6.1	57.87 ± 1.21	24.34 ± 1.84	0.00 ± 0.01
Bacterial consortium + HPCD solution	7.62 ± 0.55	308 ± 9	98.67 ± 1.02	6.85 ± 0.44	0.00 ± 0.01

Figure 1

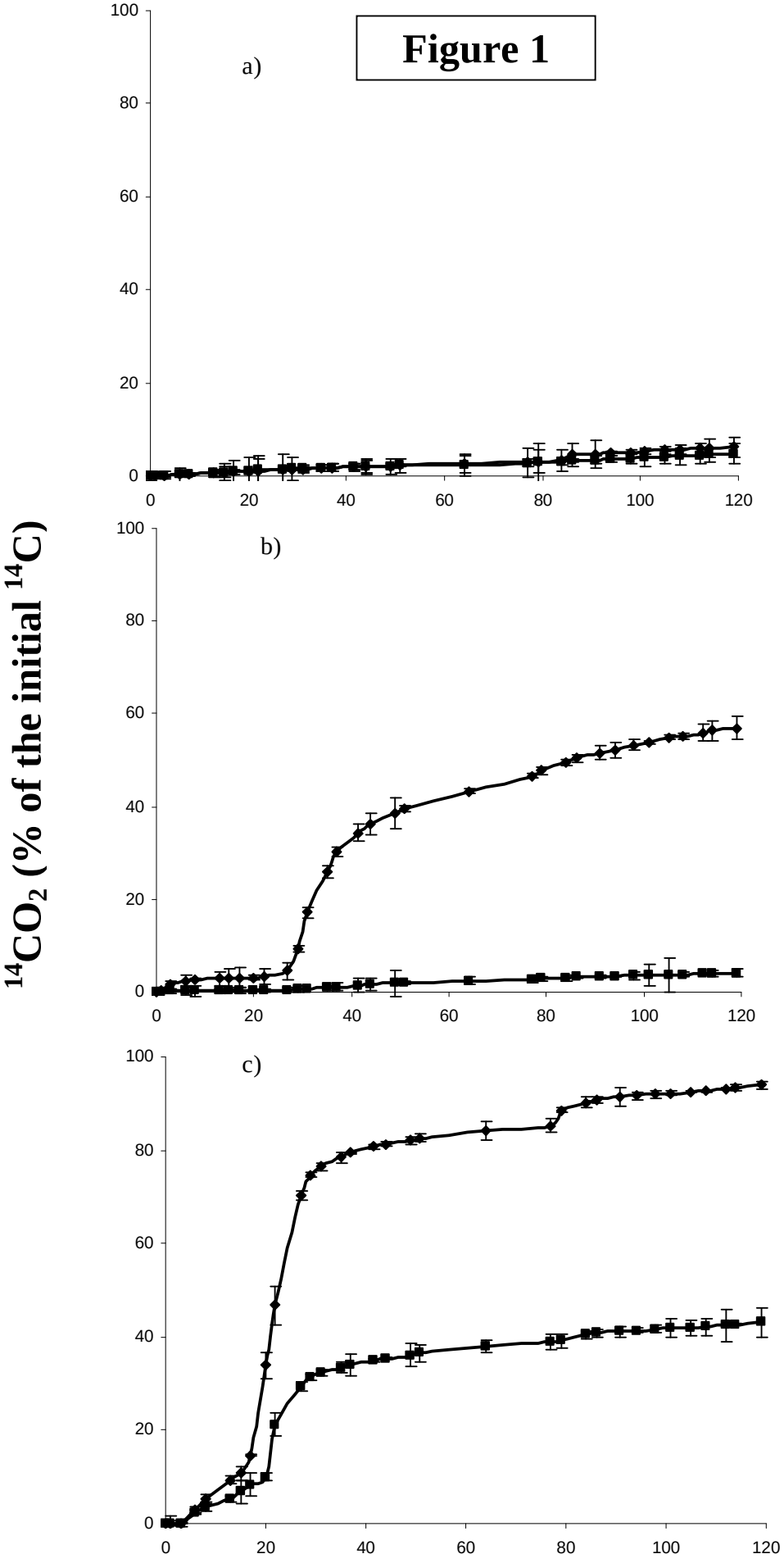

Figure Legend

Figure 1. Mineralisation of ^{14}C-labeled diuron in soil (50 mg kg^{-1}) and inoculated with a) *Arthrobacter sulfonivorans*; b). *Variovorax soli* and c) a bacterial consortium, in the presence (♦) and absence (■) of the HPCD solution.

TOC Graphic