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Biodegradation of oxadiazon by a soil isolated *Pseudomonas fluorescens* strain CG5: Implementation in an herbicide removal reactor and modelling

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ABSTRACT

An oxadiazon-degrading bacterial, *Pseudomonas* strain CG5, was isolated from an agricultural contaminated soil. This strain CG5 was able to grow on 10 mg of oxadiazon per l, yielding 5.18 ± 0.2 mg of protein biomass mol^{-1}. GC-MS analyses of the metabolites from oxadiazon catabolism revealed its dehalogenation and degradation to form non-toxic end-products, cells were then immobilized by adsorption on a ceramic support to be used as biocatalysts in herbicide removal biofilm-reactor processes. Seventy-two per cent of the oxadiazon was removed, and the maximum specific substrate uptake rate was $10.63 \pm 0.5\ \mu g\ h^{-1}\ mg^{-1}$ prot. A new mathematical model was developed to interpret and predict the behaviour of the bacteria and pollutants in a biofilm-reactor system, to consider biofilm structural and morphological properties.

1. Introduction

Oxadiazon (ODZ) (5-terc-butyl-3-(2,4-dichloro-5-isopropoxi-phenyl)-1,3,4-oxadiazol-2-ona) is an active pre-emergence herbicide against a wide range of annual dicotyledons, and a post-emergence measure against annual broad-leaved weeds. The degradation of an herbicide after application affects the environment. Once the herbicide enters the soil, it is subject to various degradation processes including biodegradation, chemical degradation and photodegradation (Lin et al., 2000; Rhône-Poulenc AG Company, 1989; Whitaley et al., 2001; Harris et al., 1997). The widespread use of nutrients and agrochemicals in greenhouse nurseries and in agricultural production has led to losses of chemicals into surface water. Studies have been made of the degradation of ODZ (Lin et al., 2000; Ying and Williams, 1999) in both soil and water. Carboxylic acid, phenol, dealkylated derivatives and polar products have been identified as metabolites from soil ODZ degradation (Ying and Williams, 1999).

Immobilized microorganisms are used for the decontamination of wastewater in fixed/film treatment plants with systems such as trickling filters, rotating biological contactors, or fluidised beds (Costerton et al., 1995). In all of which, the bacteria responsible for biodegradation are present in a

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microbial biofilm. During the complex process of adhesion, the phenotypes of bacterial cells are altered in response to the proximity of a surface (Lawrence et al., 1991). Physiological cooperativity is a major factor in shaping the structure and in establishing the eventual juxtapositions that make mature biofilms very efficient microbial communities adhering to surfaces (Lawrence et al., 1991; Heipieper et al., 1991). Previous studies demonstrated that the tolerance of various bacterial species to different organic compounds (Chakrabarty et al., 1999; Martin et al., 2000; Heipieper and de Bont, 1994; Miller, 1972) could be raised by cell immobilization. Here we study the viability and degradative capacity of *P. fluorescens* strain CG5 in the design of a process of biological ODZ decontamination. The control and understanding of processes catalysed by biofilms are important from an industrial as well as from an ecological perspective (Panikov, 1995). Mathematical models represent one end of a spectrum of activities designed to investigate natural phenomena. They can simplify systems to uncover relationships, which yield a consistent pattern when compared with in situ behaviour (Wimpenny, 1999).

2. Materials and methods

2.1. Bacterial isolation and initial characterization

Soil samples were obtained from agricultural fields in Madrid (Spain) that had been exposed to ODZ, up to 12 g per Kg of soil, for 36 months. These samples showed an ODZ concentration of 1.6 mg per Kg of soil. ODZ-degrading bacteria were isolated by enrichment culture (Martin et al., 2000) by using 0.006 mM ODZ as the carbon source. One of the isolated bacteria, designated CG5, was selected for further analysis of substrate specificity and biochemical reactions (Api20NE kit, bioMérieux S.A. Marcy l'Etoile, France). Taxonomic confirmation of *Pseudomonas* was performed as described previously (Heipieper and de Bont, 1994), and *Pseudomonas*-specific PCR primers Ps-for and Ps-rev (Isogen Bioscience BV) were used in the test. DNA was prepared with the Kristal kit (DNA extraction kit; Cambridge Molecular Technologies, Cambridge, UK).

2.2. Chemicals

Oxadiazon (99% purity) was purchased from Labor. Dr. Ehrenstorfer-Schäfer-Bgm. (Augsburg, Germany). All the chemical compounds were of the highest purity commercially available.

2.3. Media and growth conditions

The cells were grown aerobically at 30°C in MB medium. We sterilized the carbon sources separately and added them to give 5 mM benzoate (BA), 5 mM 4-hydroxybenzoate (4HBA), 5 mM 3,4-dihydroxybenzoate (3,4DHBA), 5 mM 2,5-dihydroxybenzoate (2,5DHBA), 0.5 mM propachlor (PCH), 0.4 mM alachlor (ACH), 0.01 mM simazine (SZ), 3 mM glycerol, and 0.006–0.7 mM ODZ. The ODZ was solved in 10 mM ethanol.

2.4. Degradation of oxadiazon

The oxadiazon degradation by *P. fluorescens* CG5 was determined in batch cultures in triplicate experiments, as previously reported (Heipieper and de Bont, 1994). Aliquots of the cultures from ODZ growth experiments were filtered though a 0.2 μm filter and analyzed by gas chromatography (GC) as described below. Mineralization of uniformly ring-labelled [^{14}C]-BA (Sigma Chemical, St. Louis, Mo.) by *P. fluorescens* CG5 was determined in MB medium with 5 mM unlabeled BA and supplemented with 2μCi of [ring-U-^{14}C]-BA. This medium was inoculated with 10^6 ODZ-grown cells from early logarithmic-phase culture. Cultures were grown at 30 °C during 24 h. $^{14}CO_2$ from mineralization was trapped in a vial with 1 ml of 1 N NaOH, and radioactivity was measured by scintillation counting with a Hewlett Packard spectrometer model 2500TR.

2.5. Characterization of the ODZ degradative intermediates

We identified intermediates by non-growing cell experiments as described previously (Sanchez et al., 2005). The gas chromatography-mass spectrometry (GC-MS) analyses were done with a Hewlett-Packard model 5890 Series II gas chromatograph equipped with a VA-5 capillary column (30 m, 0.25 mm i.d.), programmed from 80 to 290 °C (15 °C/min), and connected to an HP-5989A quadrupole mass detector. Solid-phase extractions were done with BOND ELUT C-18 cartridges (Varian), and acetone was used as the eluent. Strain CG5 cells were grown in MB medium with 0.03 mM ODZ, these cultures were centrifuged at 10,000*g* for 10 min at 4 °C, and the pellets were washed twice with 10 mM phosphate buffer (pH 7.2) and resuspended in the same buffer. ODZ (0.03 mM) was added to the cell suspensions and incubated at 30 °C. Samples were taken at 3 and 24 h, and identification of intermediates was performed by GC-MS analyses. These samples were centrifuged at 10,000*g* for 10 min at 4 °C, and the pellets frozen and thawed twice used for preparing the cytosolic fractions. Those were resuspended in 500 μl acetone, centrifuged at 3000*g* for 5 min at 4 °C, and the supernatants used as cytosolic fractions. Metabolites were identified by comparing their electron impact—MS spectra with those of standard samples and by coelution in GC.

2.6. Analytical methods

ODZ was measured by GC analysis. The GC analyses of the solid-phase extracts were done with a KONIK model HRGC 4000A gas chromatograph equipped with a DB-5 capillary column (15 m, 0.25 mm i.d.), programmed from 200 to 255 °C (15 °C min^{-1}) and an ECD detector. Acetone was used as eluent and the injection volume was 1 μl in splitless (50 s) with nitrogen as carrier gas (1 ml min^{-1}) and make-up (70 ml min^{-1}). The limit of detection was established in 1 μg l^{-1}. ODZ was identified by coelution with the standard in the GC analysis.

2.7. Cell immobilization

We used one ceramic support for cell immobilization: granular sepiolite (3–5 mm ∅) (Tolsa SA, Spain), using the pure culture of CG5 strain grown on 0.03 mM ODZ as the cell source, harvesting cells at the exponential phase for use as the inoculum in column reactors, pilot-scale reactor (BRL), and field-scale reactor. The immobilization method has been previously described (Sanchez et al., 2005).

2.8. Column reactor experiments

Column bioreactors built up as 10 cm-columns of 2 cm diameter were filled with immobilized bacteria on the ceramic support tested. The experiments were performed at room temperature (20±2 °C) by circulation of a synthetic wastewater composed of MB medium, 65 mM ethanol and 0.03 mM ODZ. The column reactors were operated with a cycle length of 150 days at a flow of 60 ml min^{-1}. Samples were taken periodically to monitor the state of the chlorinated herbicide and microorganisms.

2.9. Pilot-scale reactor (BRL) experiments

The internal diameter of the reactor glass column was 8 cm, the total height was 38 cm, filled with 500 g of granular sepiolite, which provided a total of 120,000 m^2 of surface for biofilm growth, and liquid samples were distributed over the packing material through a micro-sprinkler. Dissolved oxygen, pH and temperature were monitored each minute by specific sensors connected to a BIOCONTROLLER ADI 1030 (APPLIKON). The experiments were performed at room temperature (20±2 °C) by circulation of a synthetic wastewater composed of MB medium, 10 mM ethanol and different concentrations of ODZ (0.03–0.7 mM). The circulation flow was of 50 ml min^{-1}, and the reactor was operated with a cycle length of 150 days.

2.10. Laser-scanning confocal microscopy analysis (LSCM)

The viability of the immobilized cells was analyzed by LSCM analysis as previously reported (Heipieper and de Bont, 1994). A Bio-Rad MRC 1024 confocal laser-scanning microscope was set up with the standard configuration. Immobilized cell samples from the surface and from inside the reactor were incubated with two fluorescent dyes, 10 mg ml^{-1} SYTO-13 and 5 mg ml^{-1} propidium iodide (PI) (Molecular Probes, Europe BV). Viability data were measured in triplicate. The area measured was 0.23 mm^2, with an approximate number of cells of 0.2×10^6.

3. Results and discussion

3.1. Isolation, identification and characterization of strain CG5

We isolated a new bacterial strain from an herbicide-contaminated soil, for its ability to grow on ODZ as carbon source by enrichment culture. This isolated strain, designated CG5, is a Gram-negative, oxidase positive, obligatorily aerobic bacterium, unable to reduce nitrate to nitrite or to grow at 40 °C. The biochemical analysis (API 20NE) showed that strain CG5 has a 0.975 similarity to *Pseudomonas fluorescens*. To determine more precisely the taxonomic identity of the organism, purified DNAs from *Burkholderia cepacia* ATCC 17759, *P. anguilliseptica* ATCC 33600, and the isolated CG5 were checked by PCR analysis using the specific probes Ps for (20-mer; 5′- GGTCTGAGAGGATGATCAGT-3′) and Ps rev (18-mer; 5′- TTAGCTCCACCTCGCGGC- 3′). The analyses revealed that *P. anguilliseptica* and strain CG5 tested positive by this protocol, so strain CG5 was considered a *P. fluorescens*.

The isolated strain was tested for its capacity to degrade different organic compounds such as: BA, 4HBA, 3,4-DHBA, 2,5-DHBA, PCH, ACH, Sz, and ODZ. Strain CG5 grew on BA, 4HBA, 2,5-DHBA, PCH, ACH, and ODZ; neither 3,4-DHBA nor Sz were used as growth substrates. Many aromatic compounds such as phenol or benzene are toxic to different organisms, and some have recently been found to be estrogenic (Lobo et al., 2002), so it is important to know whether the aromatic part of ODZ is degraded by CG5. To determine this, we studied mineralization of [ring-U-^{14}C]-BA in batch cultures. The cells produced $^{14}CO_2$ from [ring-U-^{14}C]-BA at a specific rate of $0.11(\pm 0.02) \times 10^{-2}$ (μmol mg^{-1} protein) h^{-1}, showing that BA could be mineralised by *P. fluorescens* strain CG5. The rate of substrate utilization by CG5 cells was estimated to be 0.65±0.03 mmol BA h^{-1}.

3.2. ODZ metabolism by P. fluorescens strain CG5

P. fluorescens strain CG5 was grown in liquid minimal MB medium containing 0.03 mM ODZ as the sole C source. After 5 days of growth at 30 °C, the culture reached a final A_{680} of 0.26, and a growth yield of 1.62±0.03 mg of protein mol^{-1}. This may be due to the use of supersaturated solutions of ODZ, which exceed the water solubility of 0.7±0.01 mg l^{-1}. Above this concentration ODZ forms microprecipitates, and resolubilisation may limit biodegradation. One of the main problems in studying the microbial degradation of xenobiotics is their high hydrophobicity and low bioavailavility. To enhance bioavailavility, ethanol was used to dissolve the herbicide, which increased its solubility (100 g l^{-1}, 20 °C). In preliminary experiments, ethanol 10 mM was assayed as sole carbon source for the strain CG5. After 3 days of growth at 30 °C, the culture reached an optical density at 680 nm of 0.38, and the molar growth yield was 1.84±0.07 mg protein mol^{-1} with a mean generation time of 3.1 h during the early exponential phase. The enhancement of ODZ bioavailavility was observed when strain CG5 was grown in minimal MB medium with ODZ (0.03 mM) and ethanol (10 mM) as carbon source (Fig. 1). The culture reached a final A_{680} of 0.8, and after 60 h of incubation, 86% of the ODZ had been removed. Strain CG5 showed a growth yield of 5.18±0.2 mg of protein mol^{-1}, and the mean generation time during growth on 0.03 mM ODZ at 30 °C was 4.5 h during the early exponential phase. ODZ was removed at a specific rate of 20±0.86 μg (mg of protein)$^{-1}$ h^{-1}. These results suggest that CG5 first metabolises the ethanol with the consequent increase of the biomass, and then catabolizes the ODZ. Figure 1(a) shows the rapid disappearance of the ODZ in the first hours of incubation, coincident

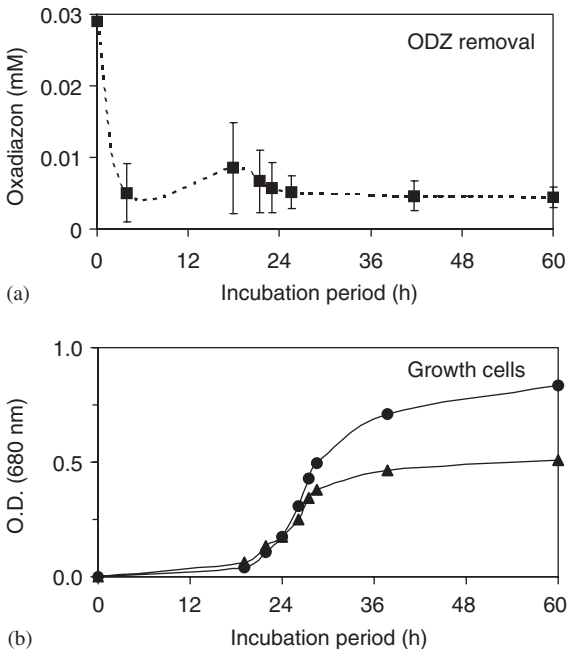

Fig. 1 – (a) Utilization of ODZ (■) by *P. fluorescens* strain CG5 cultured at 30 °C in minimal MB medium with 0.03 mM ODZ and 10 mM ethanol as carbon source. Herbicide was measured by HPLC analysis as described in Materials and Methods. (b) Growth curves for *P. fluorescens* strain CG5 cultured at 30 °C in minimal MB medium with: (●) 0.03 mM ODZ and 10 mM ethanol, and (▲) 0.03 mM ODZ, as carbon sources. Values given as means and s.d. of triplicate samples.

with the lag phase, possibly due to the high hydrophobicity of ODZ. We observed this phenomena with other hydrophobic compounds, such as Propachlor (Martin et al., 2000) and Simazine (Sanchez et al., 2005). These compounds were solubilized in the cellular membrane, according to their K_{ow} values, and then metabolized by the cell.

3.3. *Identification of metabolites*

Since the above metabolic test data had demonstrated the ability of *P. fluorescens* strain CG5 to grow on ODZ (0.03 mM) and ethanol (10 mM) as carbon source, attempts were then made to identify the corresponding metabolites in the ODZ catabolic pathway. The metabolites produced by CG5 during ODZ and ethanol degradation were indole, trimethyl benzene, benzoic acid and acetic acid detected by GC-MS analysis. The most interesting result was the disappearance of chlorinated substitute intermediates, which would mean an important decrease in toxicity.

Samples taken at 3 h showed that in the early exponential phase ODZ disappeared during cell incubation, while indole (Rt = 18.118 min) with a molecular ion at m/z 117 and the fragment ions: *m/z* 90 [M-CNH, $C_7H_6]^+$, and 57 [M-C_5H, $C_3NH_7]^+$ had accumulated in the culture medium. This intermediate was estimated at only 7% of the initial ODZ added to the medium. Acetic acid (Rt = 5.23 min) was also identified in the samples, a result of ethanol catabolism. When the culture reached the exponential phase (12 h), the medium was analysed by GC-MS, and benzoic acid (Rt = 16.12 min) with a molecular ion at *m/z* 122 and the ions at *m/z* 105 [M-OH, $C_6H_5CO]^+$, 77 [M-CO_2H, $C_6H_5]^+$, and 51 $[C_4H_4]^+$, was identified as an intermediate from ODZ catabolism. When the cytosolic fraction was analyzed, another low molecular weight aromatic compound was identified: 1,2,4 trimethylbenzene (Rt = 6.7 min, *m/z* 120 $[M]^+$, 105 [M-CH_3, $C_8H_9]^+$). ODZ was also found in this cytosolic fraction, and in addition to the molecular ion peak at *m/z* 344, oxadiazon had the following fragment ions: m/z 302 [M-$C_3H_6]^+$, m/z 258 [302–$CO_2]^+$, *m/z* 202 [258–$C_4H_8]^+$, and the base peak at *m/z* 175.

The degradation of ODZ by *P. fluorescens* strain CG5 implies its dehalogenation and the formation of intermediates in the form of low molecular weight aromatic compounds. Our results in accordance with those of Ambrosi et al. (1977), confirmed the dehalogenation and degradation of the ODZ and the formation of less toxic compounds. Moreover, they suggested that strain CG5 was able to metabolize monocyclic aromatic compounds and lead to their complete degradation.

3.4. *Column bioreactors*

Granular sepiolite column (GSR) reactor experiments were performed by recirculation of a synthetic solution containing 0.03 mM ODZ and 10 mM ethanol, at a working flow of 60 ml min^{-1}. As a control, during the start-up period, we studied the abiotic ODZ sorption at different contact times, obtaining a 35% ODZ sorption at 2 h. The reactor reached the steady state after 12 h, with a 62% ODZ sorption.

To study ODZ biodegradation by *P. fluorescens* strain CG5 in cell systems, we inoculated the reactor after steady state sorption with 4×10^6 cells g^{-1} of support, and analyzed the biofilm formation. Once cell adhesion was reached, the analysis was followed by a determination of cell viability. Table 1 shows the data of viability rate during the operation cycle. Cell viability increased during the first 13 days, after which it began to stabilize. These data suggest that at the beginning of biofilm formation a large number of living cells become attached to the granular sepiolite, while cells

Table 1 – Viability evolution of immobilized biomass in the column-reactors

	Granular sepiolite reactor[a]		
	13	60	100
Live cells[b]	50.6±9.05	52.5±10.2	118±16.2
Dead cells[b]	0.31±0.05	4.4±0.97	8.9±1.05
Viability rate[c]	0.99	0.92	0.93
Biofilm thickness (μm)	50±4.14	60±4.97	60±4.85

Values are given as means and s.d. of triplicate samples.
[a] Data obtained at 13, 60, and 100 days of reactor operation.
[b] Data expressed as fluorescence intensity.
[c] Viability rate defined as: number live cells/total number cells.

adhering to the biofilm and thickening biomass are also being removed from the film as a result of the hydrodynamics in the reactor and the growth rate of the biomass. The steady-state biofilm was reached after about 2 months of continuous culture, and the maximal thickness reached was 60 μm. The ODZ kinetic parameters (Table 2) were determined from a steady-state biofilm, and the substrate utilization rate reached a maximum of $2.04 \pm 0.06\ mg\ h^{-1}$. About 98% of the initial ODZ was removed from the wastewater.

Table 2 – Reactor data at the steady-state biofilm

	Column G S R[a]	BRL[b]
Ceramic support (g)	10	500
Flow ($ml\ min^{-1}$)	60	50
HRT(min)	0.16	1
Oxadiazon removal (%)[c]	98.6 ± 1.6	72.1 ± 0.7
Maximum specific substrate uptake rate ($\mu g\ h^{-1}$)[c]	2.04 ± 0.06	10.63 ± 0.5
ODZ removal rate ($mg\ l^{-1}\ h^{-1}$)[c] at		
0.03 mM		7 ± 0.4
0.06 mM		12 ± 0.6
0.15 mM		28 ± 1.4
0.7 mM		80 ± 3.5

[a] Granular sepiolite reactor.
[b] Pilot-scale reactor.
[c] Values given as means and s.d. of triplicate samples.

3.5. *BRL reactor experiments*

Initially the ceramic support was placed in the reactor for test by continuous recirculation of synthetic wastewater through the column, with a flow of $50\ ml\ min^{-1}$. The bioreactor was fed with increasing ODZ concentrations (0.03–0.7 mM, solved in 10 mM ethanol), and operated under aerobic conditions during 150 days. To determine abiotic ODZ sorption by granular sepiolite, synthetic water containing 0.03 mM ODZ was passed. During the start-up period, we recorded 41% ODZ sorption by the ceramic support, and after 16 h the reactor reached the steady state. On comparing these results with those of the GSR column, we found that the adsorption of ODZ is lower and that it took 4 h longer to reach the steady state. This may be due to the larger size of the BRL since the amount of sepiolite (50 times more) means that in the inner zones of the reactor the sepiolite offers less surface for adsorption of the herbicide, and in consequence the time to reach the steady state is longer.

To study the biological degradation of ODZ by immobilized CG5 cells, the reactor was inoculated with 3.8×10^6 cells g^{-1} of support. This inoculum rapidly produced a biofilm (Fig. 2), which effectively removed ODZ. When designing a reactor, the concentration of toxic compounds in the waste stream to be treated, and the concentration of the pollutant in the output of the reactor, have a maximum level specified by environmental legislation. With this in mind, we tested increased ODZ concentrations to be treated in the BRL. The data in Table 2 give the details of reactor performance and the kinetics of ODZ degradation by *P. fluorescens* CG5 in the biofilm, and showed that the rate of ODZ utilization rose as

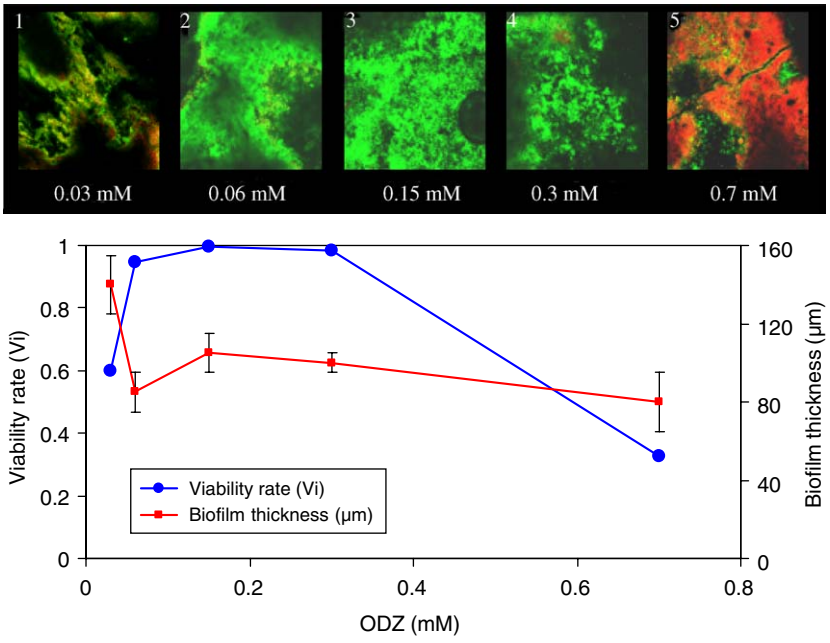

Fig. 2 – (a) LSCM images (1–5) of the development of biofilm on sepiolite by *Pseudomonas fluorescens* strain CG5, when the BRL reactor was fed with increased ODZ concentrations: (1) 0.03 mM, 34 days; (2) 0.06 mM, 92 days; (3) 0.15 mM, 95 days; (4) 0.3 mM, 105 days; (5) 0.7 mM, 124 days. Viability rate (Vi) and biofilm thickness at the different herbicide concentrations used during the operation time. (b) Data of viability rate (Vi) and biofilm thickness from the field-scale reactor installed in an OPN operating during 150 days.

the concentration was increased. It is important to underline the fact that ODZ concentrations higher than 0.15 mM were toxic for planktonic CG5 cells. Once the biofilm was formed, sessile bacteria adhered to the ceramic surface adapted to use these high herbicide concentrations (0.15–0.7 mM), allowing their degradative capacity to increase. Very low degrading activity was detected when O_2 was not supplied to the system, and samples of the effluent were analysed by HPLC. Monocyclic aromatic compounds such as benzoic acid were detected at the very low concentration of $0.07 \pm 0.0008\,\mu M$.

Immobilized cell samples from the operating reactor were analysed by LSCM when BRL was fed with increasing concentrations of the herbicide (Fig. 2). The analysis of the early events in biofilm formation by *P. fluorescens* CG5 showed that within 60 min, a monolayer of bacteria formed on the ceramic surface, the bacteria continued to move, and by 6–12 h, this monolayer almost completely covered the ceramic support and was punctuated by microcolonies which became more numerous. Viable cells increased during the first 30 days of the process and consecutive additions of ODZ promoted the formation of the maximum depth of the biofilm (130 μm). An increase in viability rate was observed when 0.03–0.15 mM ODZ were added (Fig. 2). The mature biofilm formation sustains a maximum of the cell viability, and as a consequence the rate of ODZ removal and the substrate utilization is raised (Table 2).

The development of the biofilm changed when the applied ODZ concentration was 0.3–0.7 mM, and the biofilm thickness decreased (100–80 μm). The viability rate fell to 0.4 while the biofilm thickness remained at 80 μm, although dead cells in the biofilm amounted to 80%. In spite of this, the ODZ removal rate was $80 \pm 3.5\,mg\,l^{-1}\,h^{-1}$ during the 2 days after ODZ application, possibly due to the adsorption of the ODZ into the cellular membrane; 5 days later this decreased to $6 \pm 0.2\,mg\,l^{-1}\,h^{-1}$. In spite of the greater number of cells destroyed by the toxicity of the concentrations of the herbicide, the removal rate of the ODZ was high for several days.

3.6. Mathematical models

One of the remaining challenges in the design of biofilm reactors is related to the control of biofilm thickness and formation, but knowledge of biofilm structure has progressed in the past decade. The prediction of biofilm structure and thickness is of primary importance in designing biofilm reactors because hydrodynamics and mass transfer are relevant parameters in the operation of full-scale reactors (Nicolella et al., 2000). To understand the dynamics of microbial growth in the biofilm we made use of a mathematical model to simplify systems to uncover relationships yielding a consistent pattern when compared with in situ behaviour (Wimpenny, 1999).

A simple model can describe the biofilm thickness. Let $x(t)$ be the number of living cells and $y(t)$ the number of dead cells on an arbitrary point of the material (sepiolite), then

$$dx/dt = \mu_x(t)x(t) - dx^2(t) - D(t)x(t), \quad (1)$$

$$dy/dt = dx^2(t) - D(t)x(t).$$

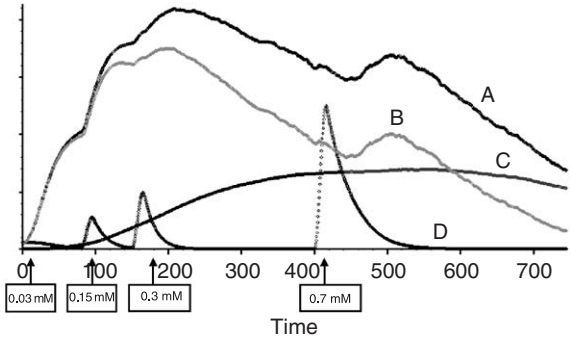

Fig. 3 – Simulation of biofilm evolution considering extra inputs of substrate. A: biofilm thickness. B: live cells, C: dead cells, and D: oxadiazon inputs.

The term $D(t)$ is a random variable representing the intensity of the (local) sloughing of the biofilm due to fluid-mechanical and migration processes.

The biofilm thickness is given by $c_1x(t)+c_2y(t)$, where c_1 and c_2 are constants proportional to the respective volumes of a living cell and a dead cell.

Like an experimental model, a mathematical model is an analogue of reality. It takes characteristic properties of the system and translates them into mathematical equations. Each equation defines relationships within the model according to the perceived behaviour of the natural phenomena (Wimpenny, 1999). We tested increased ODZ concentrations to be treated in the pilot-scale reactor. We illustrate this model with a simulation (Fig. 3) where the (four) extra inputs of substrate are those of a real experiment. The addition of substrate in the bioreactor determines the growth rate of the bacteria and the thickness of the biofilm. This growth rate decreases when the concentration of substrate approaches the toxic level ($100\,mg\,l^{-1}$ in this case). As a consequence, the thickness of the biofilm shows also a decreasing trend, reproducing the experimental behaviour.

4. Conclusions

A serious problem in the biodegradation of some halogenated organic compounds such as ODZ is the very low solubility of these compounds in the aqueous phase. The resulting decreased bioavailavility is partly responsible for the recalcitrance of the herbicides, but soil adapted degrading bacteria can in fact degrade these substrates and are physiologically able to take up and assimilate the pollutant compounds.

The immobilization of these microorganisms entails a biofilm formation, which increases the stability and operativity of the process. We found granular sepiolite a suitable support since its sorption of substrates and high contact surface contribute to the removal of the contaminant from wastewater and its later biodegradation.

The mathematical models presented in this work provide useful insights into the interpretation of experimental data as well as suggestions for further experiments. Our current work

aims to determine the detailed (local space-time) distribution of cells inside the biofilm.

Biofilm reactors can be useful when large volumes of water with low xenobiotic concentrations have to be treated, and the biomass retention is the main requirement in the process.

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