Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Biological treatment of propanil and 3,4-dichloroaniline: Kinetic and microbiological characterisation

G. Carvalho [a,b], R. Marques [a,b], A.R. Lopes [c], C. Faria [c], J.P. Noronha [a], A. Oehmen [a], O.C. Nunes [c], M.A.M. Reis [a,*]

[a] REQUIMTE/CQFB, Chemistry Department, FCT, Universidade Nova de Lisboa (UNL), 2829-516 Caparica, Portugal
[b] Instituto de Biologia Experimental e Tecnológica (IBET), Av. da República, 2780-157 Oeiras, Portugal
[c] LEPAE – Chemical Engineering Department, Faculty of Engineering, University of Porto, 4200-465 Porto, Portugal

ARTICLE INFO

Article history:
Received 10 April 2010
Received in revised form
23 July 2010
Accepted 8 August 2010
Available online 14 August 2010

Keywords:
Xenobiotics
Herbicides
Propanil
3,4-Dichloroaniline
Biodegradation rates
Microbial population analysis
DGGE

ABSTRACT

Propanil (3,4-dichloropropionanilide) is a widely used herbicide, applied worldwide in rice paddies. Propanil is primarily transformed in nature to 3,4-dichloroaniline (DCA), which is more slowly biodegradable. Both compounds have adverse health and ecotoxicity effects. This work investigated the microbial ecology and kinetics of propanil-degrading enrichments obtained from soil in a sequencing batch reactor (SBR) operated with different feeding strategies, aiming at the enhanced biological removal of propanil and DCA from contaminated waters.

During SBR operation with a dump feeding strategy, a high propanil concentration led to DCA accumulation, which was only fully degraded after 5 days, likely due to DCA inhibition. For this reason, the operational mode was changed to fed-batch operation with lower initial propanil concentrations, which resulted in faster propanil and DCA biodegradation. Thus a fed-batch operation seems more appropriate for the acclimatisation of an effective propanil- and DCA-degrading population.

The changes in performance were accompanied by a shift in the microbial population structure, as determined by DGGE of the 16S rRNA gene, particularly after a feed of DCA as the sole carbon source. Isolates obtained from the acclimatised population included members of the genera *Enterococcus* and *Rhodococcus*, as well as *Brevundimonas*, which displayed >90% propanil biodegradation efficiency.

1. Introduction

The intensive use of herbicides in today's agricultural practices is a matter of worldwide concern. Most of the compounds used to prevent undesirable grass and weed growth are xenobiotics, i.e. man-made and foreign to natural biological systems. Thus, the biological mechanisms necessary to fully biodegrade these molecules are often not readily available in nature, and they tend to accumulate intact or only partially transformed. Designed to have an effect on target organisms, most herbicides (and often also their metabolites) can represent a health and ecotoxicity hazard and should be removed from contaminated waters and soils.

Propanil, or 3,4-dichloropropionanilide, is a post-emergent contact herbicide, classified as an acylanilide. It is an extensively used herbicide worldwide, and it is often applied in the cultivation of rice to control the growth of broadleaf weeds (Silva et al., 2006; Primel et al., 2007; Marchesan et al., 2007).

* *Corresponding author*. Tel./fax: +351 212948385.
E-mail address: amr@dq.fct.unl.pt (M.A.M. Reis).
0043-1354/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2010.08.006

Propanil is applied to flooded rice paddies, thus contaminating large amounts of irrigation water, which can overflow from paddy fields or infiltrate through the soil and contaminate surface and ground water resources (Dabrowski et al., 2002). Propanil has been detected in concentrations between 0.1 and 3600 $\mu g\ L^{-1}$ in irrigation water (Primel et al., 2007), whereas the maximum allowed concentration for discharge into the aquatic environment is 0.1 $\mu g\ L^{-1}$ (Pesticides Framework Directive 2009/128/EC).

Propanil is primarily converted into 3,4-dichloroaniline (DCA) and propionate through enzymatic hydrolysis (Pothuluri et al., 1991), but it can also undergo chemical hydrolysis at pH below or above the 3–9 range, and photodegradation under direct sunlight with a half-life of 12 h (Dahchour et al., 1986). DCA is also a metabolite of the microbial transformation of other herbicides, such as diuron and linuron (Pothuluri et al., 1991; Livingston and Willacy, 1991; Widehem et al., 2002; Sørensen et al., 2008). In irrigation waters, DCA has been found at levels up to 568 $\mu g\ L^{-1}$ (Primel et al., 2007), and a similar concentration (470 $\mu g\ L^{-1}$) was observed in the Ebro river delta area (Santos et al., 2000). Indeed, DCA is known to have low biodegradability (Pothuluri et al., 1991) and to accumulate in the environment. DCA and, to a lower extent the propanil itself, have been shown to produce toxic effects on mammals and fish, and to affect the human immune system (Pothuluri et al., 1991; Salazar et al., 2008). Moreover, DCA can be converted into 3,3′,4,4′-tetrachloroazobenzene (TCAB), which is a known carcinogen and a potential genotoxin (Pothuluri et al., 1991). DCA biodegradation in soil seems to compete with binding and polymerisation reactions, which increase its recalcitrance in the environment (You and Bartha, 1982). Therefore, these compounds should be removed from irrigation waters before entering the natural aquatic systems and reaching water supply resources or accumulating in the soil.

Biological treatment can be an inexpensive and sustainable solution to remove propanil and DCA from contaminated waters and soils. The hydrolysis of propanil has been observed using pure cultures, including various species of *Pseudomonas* (Dahchour et al., 1986; Pothuluri et al., 1991; Zablotowicz et al., 2001) and some species of the fungus *Fusarium* (Lanzilotta and Pramer, 1970; Reichela et al., 1991). However, to date there is no report of a single culture able to totally mineralise propanil, although this has been achieved by a co-culture of *Pseudomonas putida* and *Streptococcus* (now *Enterococcus*) *avium* (Dahchour et al., 1986). You and Bartha (1982) identified a *P. putida* strain as able to mineralise DCA, but only as co-metabolism of its unchlorinated analogue, aniline. Since then, several other DCA-degrading bacteria have been isolated, such as *Pseudomonas* (now *Brevundimonas*) *diminuta* and *Paracoccus denitrificans*, which were isolated from soils contaminated with a propanil spill (Surovtseva et al., 1985; Bakhaeva et al., 2001), or *Variovorax* sp., *Delftia acidovorans* and *Arthrobacter* sp., isolated from cultures degrading linuron or diuron, herbicides with a chemical structure similar to propanil (Dejonghe et al., 2003; Breugelmans et al., 2007; Sørensen et al., 2008). In these studies, it was also found that complete herbicide biodegradation was better performed by bacterial consortia rather than by individual isolates. These studies suggest that the synergistic interactions occurring in mixed cultures enhance propanil mineralisation since different activity niches (e.g., a range of propanil or DCA concentrations) are naturally covered, which might not be the case with a consortium of only a few selected isolates. Such a mixed culture could be useful for *in situ* treatment of contaminated waters, bioaugmentation of industrial wastewater treatment plants or soil bioremediation. However, propanil biodegradation has not yet been studied using mixed culture enrichments and the best operational conditions for the enrichment of a propanil- and DCA-degrading consortium have not yet been elucidated.

In this study, different SBR operational modes were compared to enrich mixed microbial populations able to degrade propanil and DCA. The objective is to understand the conditions that enhance the selection of the appropriate communities and improve their performance. The efficiency of each process was assessed through a kinetic characterisation carried out with different propanil concentrations. Simultaneously, the structure and composition of the microbial community enriched in the SBR at different operating conditions was assessed through 16S rRNA gene-based denaturing gradient gel electrophoresis (DGGE).

2. Material and methods

2.1. Microbial enrichments

Microbial enrichments were initiated from a mixture of soil contaminated with several herbicides, including propanil, and soil from organic rice agriculture. Twenty grams of soil was added to 45 mL of mineral medium B (Barreiros et al., 2003), supplemented with 4 mM of $(NH_4)_2SO_4$ and 0.5 mM of propanil. The cultures were incubated at 30 °C with an agitation of 120 rpm. When propanil and DCA concentrations were very low, the culture was settled, decanted and 5 mL of the solids was used to inoculate the next culture medium. After eliminating the soil residues through this process, the culture was centrifuged at 8000 rpm during 15 min and resuspended in propanil-containing media (0.45 mM). This process was repeated for 60 days.

2.2. SBR operation

The pre-enriched mixed culture was used to inoculate a sequencing batch reactor (SBR), which was initially operated with dump feeding (5 min), followed by 24–96 h of aerated reaction, 1 h of settling and 0.25 h of decanting. During this stage, the biomass was fed mostly with a propanil concentration of 0.5 mM. Changes in the concentration fed or in the feeding frequency took place when batch tests were conducted (see Section 2.3) or when the culture failed in completely degrading the propanil and DCA. After 76 days, the SBR operational mode was changed to fed-batch, where the reactor was fed twice per 24 h-cycle with a propanil concentration of 0.15 mM in each feed. The second feeding was carried out 11 h after the beginning of the cycle, with 1 h of settling/decanting at the end of each cycle. The reactor was operated during 64 days with these conditions. While the hydraulic retention time (HRT) during the dump feed operational phase was variable