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International Biodeterioration & Biodegradation 55 (2005) 109–113

INTERNATIONAL BIODETERIORATION & BIODEGRADATION

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Comparative biodegradation studies of pre-emergence broadleaf and grass herbicides in aqueous medium

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Received 18 August 2003; received in revised form 6 July 2004; accepted 30 August 2004

Abstract

The biodegradation of the herbicides hexazinone, metribuzin, metobromuron and propachlor was evaluated in liquid cultures of an adapted and a non-adapted mixed microbial culture obtained from a wastewater treatment plant. All herbicides were poorly degraded after 50 days ($<50\%$). The adaptation of microbial culture prior to the biodegradation test shortened only the lag-time in degradation of hexazinone. The level of biodegradation correlated with the log K_{ow} values of the molecules tested ($R^2 = 0.970$). Metobromuron at $100\,mg\,L^{-1}$ inhibited the metabolism of aniline by the mixed culture.

Keywords: Hexazinone; Metribuzin; Metobromuron; Propachlor; Water pollutants

1. Introduction

Photosynthesis-inhibitor herbicides such as triazinone and phenylurea types and cell division inhibitors such as acid amide types are widely used as pre-emergence broadleaf and grass herbicides (DeFelice, 1999). Biodegradation of triazinone, phenylurea and acid amide-type herbicides is usually investigated in soil microcosms, or in cultures of individual microbial strains isolated from soils. Garcia-Valcarcel and Tadeo (1999) found that the degradation rates of hexazinone and simazine increased in a sandy loam soil with increased moisture levels. Bordjiba et al. (2001) isolated and assayed 53 fungal species for their ability to remove metribuzin from a liquid culture. Fungi were isolated from soil samples corresponding to pesticide-contaminated soil and uncontaminated soil in the vicinity of Annaba (Algeria). Only *Botrytis cinerea* from uncontaminated soil and *Sordaria superba* and *Absidia fusca* from pesticide-contaminated soil removed more than 50% of the metribuzin after 5 days. El-Fantroussi (2000) showed that an enrichment culture isolated from soil treated with linuron for more than 10 years degraded methoxy-methyl urea herbicides very efficiently, but dimethyl urea herbicides such as diuron, chlorotoluron and isoproturon were not transformed.

In most pesticide-contaminated agrochemical facilities, herbicides are found in combination with other widely used agricultural chemicals, and remediation strategies must take into account of the presence of multiple contaminants. The ability of the atrazine-mineralising culture to degrade other s-triazines in a liquid culture was evaluated by Grigg et al. (1997), who demonstrated depletion of cyanazine and simazine added to a liquid culture, either alone or combined with atrazine, after 6 days. However, the cyanazine was transformed to persistent metabolite(s). In this same experiment, metribuzin was resistant to biodegradation. In field experiments, Walker and Welch (1992) evaluated the effect of repeated application of eight herbicides on their persistence. They found that the rates of loss of

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doi:10.1016/j.ibiod.2004.08.004

metribuzin, metolachlor and isoproturon were unaffected by up to three previous soil treatments of the same herbicide. Martin et al. (1999) examined propachlor metabolism by *Acinetobacter* strain BEM2. They identified two degradation pathways. Stamper and Tuovinen (1998) found that alachlor, metolachlor and propachlor are transformed to glutathione-acetanilide conjugates. When Galassi et al. (1996) tested the biodegradation of acetanilide herbicide alachlor under different experimental conditions, they found that biodegradation depended on concentration. Biodegradation was most efficient at very low concentrations. They detected several breakdown products of alachlor, including 2,6-diethylaniline (DEA) and 2′,6′-diethyl-*N*-2-hydroxy(methoxymethyl) acetanilide. The latter has a high mobility in soil and is a precursor of mutagenic compounds in mammalian metabolism.

Misuse of herbicides leads to serious water quality problems that impair the use of water for crop and animal production and human consumption. The most important properties influencing herbicide mobility in soil are: solubility and partition coefficient (PC) between the fraction bound to soil particles and the one dissolved in soil water. Herbicides with smaller PC values are more likely to be leached than those with larger PC values (Bicki, 1988). PC Values are not available for all herbicides. However, as a rough estimate for PC, the logarithm of the partition coefficient organic carbon–water (log K_{oc}.) can be used. The smaller the log K_{oc}, the more likely the herbicide will leach with water, thus causing surface and underground water pollution (Rao et al., 2002).

The purpose of our study was to evaluate the biodegradation of three photosynthesis-inhibitors (hexazinone, metobromuron and metribuzin) and a cell division inhibitor (propachlor) by a mixed microbial population obtained from a wastewater treatment plant. Water solubility of the selected herbicides ranges from 330 to 3300 mg L^{-1} at 20 °C, and their log K_{oc} values range is 1.3–2.7, making them potential water pollutants (Table 1).

2. Materials and methods

2.1. Test chemicals

Hexazinone 99%, metribuzin 99%, propachlor 99% and a reference substance—aniline 99.5% were obtained from Riedel-de Häen, metobromuron 99.8% from Ciba Geigy Ltd., dichloromethane from Riedel-de Häen, and naphthalene and sodium hydroxide from Kemika, Zagreb.

2.2. Preparation of bacterial inoculum

Samples of raw sewage were collected from the ŠTUDA wastewater treatment plant, Domžale, Slovenia. The samples were decanted and filtered. Into five 5-L containers, 2-L portions of filtrate were transferred and to each 1 L peptone–glucose medium, composed of 1.5 g glucose, 1.5 g K_2HPO_4 and 1.95 g peptone dissolved in distilled water, was added. These cultures were incubated with constant aeration in a Combi Cold Rac Laboratory Refrigerator 5201 set at 25 °C. Each day, one-third of the culture was replaced with fresh peptone–glucose medium. One culture was without addition, and was grown until it reached a dry mass of 5.88 mg mL^{-1}. Four herbicide-adapted cultures were prepared by adding 1 mg hexazinone, metribuzin, metobromuron or propachlor L^{-1} on the second, seventh and 14th day of incubation and the cultures were allowed to grow until the dry mass reached 5.61, 5.41, 5.39 and 5.32 mg mL^{-1}, respectively. Growth was evaluated by periodic sampling for dry weight measurements. Culture was terminated after the first drop in dry weight was detected, which occurred after 15 days.

2.3. Biodegradation tests

The biodegradation experiment was set up using a modified biodegradability MITI test (I) OECD 301 C. This test is both standardised and suitable for the determination of aerobic biodegradability in an aqueous medium (Commission of the European Communities,

Table 1
Selected herbicides and their properties

Common name	IUPAC name	log K_{ow}[a]	log K_{oc}[b]	Solubility in water[a] (mg L^{-1}) at 20 °C
Hexazinone	3-Cyclohexyl-6-dimethylamino-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione	1.85	1.30–1.43	3300
Metribuzin	4-Amino-6-tert-butyl-3-methylthio-1,2,4-triazin-5(4H)-one	1.70	1.81–2.72	1050
Metobromuron	3-(4-bromophenyl)-1-methoxy-1-methylurea	2.38	2.67	330
Propachlor	2-Chloro-*N*-isopropylacetanilide	2.18	1.62–2.3	700

log K_{ow}—logarithm of partition coefficient octanol/water. log K_{oc}—logarithm of partition coefficient organic carbon–water.
[a]Data obtained from Interactive PhysProp Demo.
[b]Data obtained from Hazardous Substances Data Bank (2001).