ELSEVIER
International Biodeterioration & Biodegradation 59 (2007) 193–201
INTERNATIONAL BIODETERIORATION & BIODEGRADATION
www.elsevier.com/locate/ibiod

Effect of nutrient amendments and sterilization on mineralization and/or biodegradation of ^{14}C-labeled MCPP by soil bacteria under aerobic conditions

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Received 18 March 2005; received in revised form 1 June 2005; accepted 1 December 2006
Available online 31 January 2007

Abstract

Mineralization and/or degradation of the phenoxy herbicide mecoprop (MCPP) by a group of soil bacteria under the effects of nutrient amendments and sterilization were investigated. Five different species of *Pseudomonas* (*P. paucimobilis*, *P. aeruginosa*, *P. mallei*, *P. pseudomallei*, and *P. pickettii*) were isolated from sediments of Lake Mariut, a freshwater lake in south Alexandria, Egypt. MCPP mineralization and/or removal were tested by the selected *Pseudomonas* species as active and dead masses in minimal and nutrient-rich media supplemented with ^{14}C-MCPP at a final concentration of $10\,\mu g\,l^{-1}$ for 6 successive weeks. Results revealed significant variations in the removal percentages of MCPP by either mineralization or biodegradation. *Pseudomonas* spp. exhibited high selectivity toward MCPP. Considering the short duration of the experiment (45 days) *Pseudomonas* spp. investigated in this study provide an effective and selective potential for MCPP decontamination. As a general trend, all of the investigated species exhibited higher biodegradation and removal efficiency of MCPP (1.3–89.5%) compared to their mineralization abilities (0.10–9.28%) under the experimental conditions. Also the highest MCPP mineralization and degradation by the selected *Pseudomonas* spp. were achieved by their inactive (dead) followed by active-rich cultures (both were inoculated in nutrient-rich medium), confirming the positive effects of nutrient amendments and sterilization on MCPP decontamination. Efficiency of *Pseudomonas* spp. was positively correlated with time up to the 3rd week for biodegradation and up to the 6th week for mineralization, indicating high mineralization efficiency provided enough time. Finally, *Pseudomonas* spp. showed selective preferences among them toward MCPP with the highest mineralization efficiency achieved by *P. aeruginosa* (1SB) and *P. mallei* (2SA), while the highest biodegradation efficiency was achieved by *P. pickettii* (5SB) and *P. pseudomallei* (3S). They seemed very promising but require longer exposure and higher MCPP concentration to stimulate and enhance their metabolic and mineralization capabilities. Results of this study can be manipulated efficiently to select the most promising *Pseudomonas* species for decontaminating polluted systems providing the optimum degradation conditions.

Keywords: Biodegradation; Mecoprop; Mineralization; Nutrients; Sterilization

1. Introduction

Pesticides are persistent in the environment, where they often affect non-target organisms and disturb the quality of receiving aquatic ecosystems. Mecoprop (MCPP) [2-(2-methyl-4-chlorophenoxy) propionic acid] belongs to the group of phenoxyalkanoic acids. It is the most widely used herbicide for broadleaf weed control in cereal crops throughout the world (Tomlin, 1994). The phenoxyalkanoic herbicides are highly water-soluble acids ($pK_a = 3.11$) and have a low tendency to accumulate in organic matter. At pH 7 they are not sorbed to most soil types (Zipper et al., 1998a). MCPP is heat stable and resistant to reduction, hydrolysis, and atmospheric oxidation. At low temperatures, it crystallizes out of the solution and

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0964-8305/$ - see front matter
doi:10.1016/j.ibiod.2006.12.001

becomes unavailable for degradation while it has a very low adsorptive ability to soil particles, which makes it a persistent contaminant (Farm Chemical Handbook, 1984). MCPP is released into the environment as a result of its use, manufacture, formulation, transport, storage, and disposal. Runoff and seepage from treated fields may be a source of stream and groundwater pollution (Weast, 1983–84). It is moderately toxic and possibly carcinogenic to humans (Weast, 1983–84; IARC, 1987; Nitschke et al., 1999). Residues of phenoxyalkanoic herbicides are often found in subsurface and groundwater samples (Felding, 1995; Fielding et al., 1991; Gintautas et al., 1992). In European countries (Denmark, Germany, Great Britain, The Netherlands, Italy, Sweden) residues of (R, S)-mecoprop were found in drinking water in concentrations higher than the maximum allowable concentration for an individual pesticide, i.e., $100\,\mathrm{ng\,l^{-1}}$ (Zipper et al., 1998a).

Among the different remediation technologies for pesticide removal, enhanced biological degradation of these contaminants provides a potentially effective tool. The most important aspect to be considered in biological degradation of pesticides is the optimization. Optimization includes all factors that might in any way affect natural attenuation of the pesticide. Among these factors are indigenous microorganisms (their numbers and activity), concentration of the pesticide and its bioavailability, type of aquifer sediments, exposure history, temperature, pH, and oxygen availability.

MCPP degradation and/or mineralization in natural environments, mainly ground water and soils under the effect of some optimization factors, have been reported by many authors. MCPP degrades predominantly aerobically and the relative proportions of its two enantiomers R- and S- will change according to their biological behavior (Harrison et al., 1998; Johnson et al., 2000; Williams et al., 2003). Under aerobic conditions, degradation of MCPP was rapid (14 days) and lag phase was short, while under anaerobic (iron reducing, sulphate-reducing, methanogenic) conditions, degradation was inhibited and MCPP persisted. But R-MCPP degraded anaerobically under nitrate reducing conditions. Moreover, addition of nitrate to a dormant iron-reducing microcosm stimulated anaerobic degradation of R-MCPP after a lag period of 21 days (Harrison et al., 2003). Using activated sludge collected from the aeration tank of a municipal wastewater treatment plant, both enantiomers of MCPP and dichlorprop degraded completely under aerobic conditions within 7 days, albeit in an enantio-selective manner (Zipper et al., 1999).

Exposure history or repetition of the herbicide application results in the presence of acclimatized microbial communities dominated by specific degraders; this also enhances degradation capability. Tuxen et al. (2002) reported that up to 70% MCPP was mineralized and a linear correlation was observed between pre-exposure and amount of herbicide degraded within 50 days. An exponential correlation was also found between numbers of specific phenoxy acid degrading bacteria and the pre-exposure. Kosheleva et al. (2000) also stated that cultivation for a long time on phenanthrene produced mutants capable of growing on this substrate as the sole sources of carbon and energy. Ramanand et al. (1988) and Torang et al. (2003) reported other factors, including suitable temperature, increasing concentration of MCPP, in addition to long-term pre-exposure as optimizing factors lead to acclimatization, producing efficient degraders and enhanced degradation rates of the phenoxy acids. The increase in number, biomass, and activity of bacterial degraders under wet conditions and at 25 °C are attributed to the release of nutrients and organic matter (Lund and Goksoyer, 1980; West et al., 1986; Zelles et al., 1991). Degradation rate of MCPP is also a function of type of soils or sediments (Johnson et al., 2003), exposure time between MCPP and the degraders, and the depth at which MCPP pollution exists (Rugge et al., 2002).

Bioavailability of MCPP is very important and considered a limiting factor. It has been documented that aging has no effect on mineralization of MCPP in soil, aquifer sediment, or chalk, since it has low adsorption ability to sediments and thus has high bioavailabilty (Johannesen and Aamand, 2003; Kristensen et al., 2001).

Pseudomonas (Johannesen and Aamand, 2003), *Stenotrophomonas maltophilia* PM (Mai et al., 2001), *Alcaligenes* sp. CS1, *Alcaligenes denitrificans*, and *Ralstonia* sp. CS2 (Smejkal et al., 2001; Tett et al., 1997) were isolated from aquifers or agricultural soils contaminated with MCPP and other phenoxy acids and identified as highly efficient MCPP degraders that have the ability to grow using these herbicides as their sole carbon and energy sources. Hybridization analysis demonstrated that both *Alcaligenes* sp. CS1 and *Ralstonia* sp. CS2 harbored tfdA, tfdB, and tfdC genes on plasmids, and those tfd-like genes may be involved in the degradation of MCPP (Smejkal et al., 2001).

GC-MS analysis provided direct evidence for the biotransformation of MCPP to the transient metabolite 4-chloro-2-methylphenol (MCP). No NADPH-dependent activity was observed during this reaction. Pyruvate was verified as the second product derived from the aliphatic side chain of MCCP. MCP was subsequently transformed to a substituted catechol by an NADPH-dependent monooxygenase (Tett et al., 1997).

Limited mineralization of MCPP could result from the production of toxic metabolites or if insufficient energy is available to precede the complete oxidation (Mai et al., 2001). It was partially attributed to the very small percentage of active bacteria where a large part of the total bacterial population was inactive or uncultivable (Albrechtsen and Winding, 1992). Taiwo and Oso (1997) reported that after an initial rise, significant reductions in microbial populations, species diversity, and percentage of carbon, nitrogen, potassium, and pH were reported in loamy sand and sandy soils upon exposure to a mixture of herbicides for 8 weeks. Among other microbial species,