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Production of microparticles of molinate degrading biocatalysts using the spray drying technique

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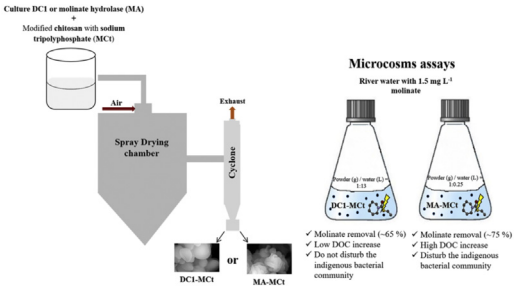
HIGHLIGHTS

- Microencapsulation of mixed culture DC1 cells and molinate hydrolase using spray drier was successful.
- The molinate degrading activity of the microencapsulated biocatalysts was maintained after 6 months storage.
- Biocatalyst microparticles with modified chitosan were better than those with calcium alginate.
- Biocatalyst microparticles with modified chitosan removed environmental concentrations of molinate from river water.
- DC1 microparticles did not disturb the river indigenous bacterial community.

GRAPHICAL ABSTRACT



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ABSTRACT

Previous studies demonstrated the capability of mixed culture DC1 to mineralize the thiocarbamate herbicide molinate through the activity of molinate hydrolase (MolA). Because liquid suspensions are not compatible with long-term storage and are not easy to handle when bioremediation strategies are envisaged, in this study spray drying was evaluated as a cost-effective method to store and transport these molinate biocatalysts. Microparticles of mixed culture DC1 (DC1) and of cell free crude extracts containing MolA (MA) were obtained without any carrier polymer, and with calcium alginate (CA) or modified chitosan (MCt) as immobilizing agents. All the DC1 microparticles showed high molinate degrading activity upon storage for 6 months, or after 9 additions of ~0.4 mM molinate over 1 month. The DC1-MCt microparticles were those with the highest survival rate and lowest heterogeneity. For MA microparticles, only MA-MCt degraded molinate. However, its V_{max} was only 1.4% of that of the fresh cell free extract (non spray dried). The feasibility of using the DC1-MCt and MA-MCt microparticles in bioaugmentation processes was assessed in river water microcosms, using mass (g):volume (L) ratios of 1:13 and 1:0.25, respectively. Both type of microparticles removed ~65–75% of the initial 1.5 mg L^{-1} molinate,

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after 7 days of incubation. However, only DC1-MCt microparticles were able to degrade this environmental concentration of molinate without disturbing the native bacterial community. These results suggest that spray drying can be successfully used to produce DC1-MCt microparticles to remediate molinate polluted sites through a bioaugmentation strategy.

1. Introduction

Molinate is a thiocarbamate herbicide used for rice crop protection. Despite its benefits in rice production, it is an environmental pollutant (Nunes et al., 2013). In fact, molinate disperses and reacts in the environment originating diverse oxidation products, which can be more toxic than the parent compound (Cochran et al., 1997). Thus, treatment of molinate contaminated sites is important. Bioremediation, which involves the metabolic potential of microorganisms to clear-out the environment, emerged as a promising alternative to traditional physicochemical methods (Watanabe, 2001). Bioremediation relies on the utilization of organisms capable of degrading the target compounds under real conditions. Whenever the indigenous microbiota does not harbour efficient degrading organisms, bioaugmentation, i.e. inoculation with previously isolated degrading organisms, can be carried out (Mrozik and Piotrowska-Seget, 2010; Thompson et al., 2005). One of the critical factors of bioaugmentation is the survival and/or the maintenance of the degrading activity of the exogenous organisms (Petrich et al., 1998; Thompson et al., 2005). Alternatively, enzymes able to transform the pollutant into innocuous compounds may be used (Hsu et al., 2013; Pizzul et al., 2009; Scott et al., 2010; Sutherland et al., 2004), overcoming some of the bioaugmentation limitations, such as the inability of inoculated organisms to compete with the native microbiota, or to degrade *in situ* contaminants at low concentrations (Petrich et al., 1998; Thompson et al., 2005).

Several microorganisms were described as capable of molinate transformation (Nunes et al., 2013). Nevertheless, up to now, only mixed culture DC mineralizes molinate without the accumulation of degradation products (Barreiros et al., 2003, 2008; Correia et al., 2006). In culture DC, molinate is primarily cleaved by *Gulosibacter molinativorax* ON4^T (Manaia et al., 2004), through the activity of the molinate hydrolase (MolA) (Duarte et al., 2011). This enzyme catalyses the cleavage of the thioester bond of molinate, releasing ethanethiol and azepane-1-carboxylate (ACA) (Barreiros et al., 2008; Leite et al., 2015). While ACA serves as energy and carbon sources for all the five members of culture DC, including *G. molinativorax* ON4^T, ethanethiol is only used by *Pseudomonas chlororaphis* ON1 and *Stenotrophomonas maltophilia* ON2 (Barreiros et al., 2008). Hence, efficient molinate mineralization can be achieved either by culture DC or when *P. chlororaphis* ON1 is grown together with *G. molinativorax* ON4^T. This two-membered mixed culture was designated DC1 (Barreiros et al., 2012). In fact, culture DC and DC1 were shown to be efficient on the removal of molinate from contaminated rice paddy floodwater (Barreiros et al., 2011, 2012; Castro et al., 2005) or soil (Lopes et al., 2013). In contrast, the feasibility of using MolA in the decontamination of molinate polluted sites was never reported.

The utilization of cells and/or enzymes for *in situ* bioaugmentation implies the cultivation and processing of large volumes of cells immediately before their utilization as inocula. The transport of a high volume of cells/enzymes in liquid media into the contaminated fields is highly costly. In addition, when stored in liquid formulations (i.e., suspensions/solutions), cells and/or enzymes, lose activity after a short period. Indeed, liquid formulations are not easily to handle and have lower storage stability than a powder product (Cassidy et al., 1996; de Vos et al., 2010; Namaldi et al., 2006). There are different available solutions to obtain powder formulations, such as freeze drying and spray drying (de Vos et al., 2010). Freeze drying has been the method of choice to preserve pharmaceuticals, food and microorganisms; however it involves high costs due to the need of deep freezing followed by water sublimation at low pressure (de Vos et al., 2010). In contrast, spray drying produces a dry powder of microparticles from a suspension or solution by rapidly drying it with hot air or other gas. It has been extensively applied in industrial settings as it is a clean technology (avoids the utilization of organic solvents), rapid, reproducible, low cost and can be easily scaled-up, when compared with other drying techniques (Desai and Park, 2005; Pu et al., 2011; Schafroth et al., 2012). Spray drying allows not only the removal of water from the product of interest (e.g. cells, enzymes, drugs) but also its immobilization through encapsulation (Boza et al., 2004; de Vos et al., 2010; Estevinho et al., 2013; Namaldi et al., 2006; Rattes and Oliveira, 2007). The immobilization of biocatalysts allows longer operating lifetimes due to enhanced stability or survival. Because of the presence of the encapsulating agent (carrier), biocatalysts are protected against toxic chemicals, denaturant agents or predators. In addition, encapsulation provides suitable micro environmental conditions, allowing, for instance, the inclusion of nutrients and carbon sources in the immediate cell environment (Bayat et al., 2015; Nedovic et al., 2011; Petrich et al., 1998). Microencapsulation of biocatalysts have been widely used in agrochemical, food, and pharmaceutical industrial processes (de Vos et al., 2010; Estevinho et al., 2014b; Pu et al., 2011; Rajam et al., 2012; Rattes and Oliveira, 2007) and are now increasingly being used in biotechnology processes for biochemical conversion and bioremediation (Bayat et al., 2015; Hsu et al., 2013; Krajewska, 2004; Krasnan et al., 2016; Sakkos et al., 2016).

The aim of the present study was to assess the feasibility of using spray drying as a cost-effective method to store and transport molinate biocatalysts. For that, we compared the survival rate and the molinate degrading activity of microparticles produced by spray drying of mixed culture DC1 in the absence and in the presence of biopolymers (calcium-alginate and modified chitosan) as encapsulating agents. In addition, the activity of MolA in cell free crude cell extracts encapsulated with the same biopolymers was determined. Finally, the feasibility of using the modified chitosan encapsulated biocatalysts to remove molinate from surface water was assessed *ex situ*, using microcosms.

2. Materials and methods

2.1. Reagents

Molinate (S-ethyl azepane-1-carbothioate) was obtained from Herbex, produtos químicos (Portugal). Cycloate was obtained from Riedel de Haën, (Germany). Sodium alginate (alginic acid, sodium salt) and sodium tripolyphosphate were from Aldrich (USA), and the acetic acid (glacial) and calcium chloride were from Merck