Degradation of difluorobenzenes by the wild strain *Labrys portucalensis*

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Introduction

Haloaromatic compounds have been produced industrially on a large scale for several decades, becoming environmental pollutants of soil, water and air. Fluorinated are among these, because of their useful properties, which make them suitable for a wide range of applications [1]. Whereas the degradation of chlorinated and brominated compounds has been studied extensively, fluoroaromatics have received less attention and fewer studies are available on their biodegradation. This study focuses on the biodegradation of difluorobenzenes (DFBs), which are commonly employed as chemical intermediates in various pharmaceutical and agricultural applications. Complete biodegradation of DFBs has not yet been reported to the best of our knowledge.

A previously isolated microbial strain (strain F11), identified as *Labrys portucalensis* [2], and with the capacity to degrade fluorobenzene (FB) as sole carbon and energy source, was tested for its capability to degrade 1,2-, 1,3- and 1,4-difluorobenzene.

Methods

Labrys portucalensis F11 was isolated from an industrially contaminated sediment soil in a mineral salts medium (MM) supplemented with FB as the sole source of carbon and energy, as previously described [3].

Cells of *L. portucalensis* F11 previously grown on FB were harvested by centrifugation (10000 rpm for 15 min at 4ºC), washed with MM and ressuspended in the same medium to a final OD (600 nm) of 0.05. The suspension was then used to inoculate sealed flasks supplemented with 0.5 mM of DFBs or FB (control of cells activity). The mixture was incubated at 25ºC on a rotary shaker (130 rpm). Degradation of DFBs (0.5, 1 and 2 mM) in co-metabolism with FB (1 mM) was also tested. All experiments were done in triplicate and controls without inoculum addition were also monitored. Samples were taken at regular intervals to determine growth and FB/DFBs degradation.

Growth was monitored by measuring the optical density at 600 nm. FB and DFBs consumption was analysed by gas chromatography [3]. FB and DFBs biodegradation was determined through fluoride release, by measuring the concentration of fluoride ions in the culture supernatant by potenciometry [4].

Results

DFBs biodegradation as a sole carbon and energy source

➢ 1,3-DFB was completely degraded and a stoichiometric liberation of fluoride was observed in 20 days (Fig. 1). *L. portucalensis* F11 was not able to defluorinate 1,2- and 1,4-DFB during the time course of the experiment. In the controls without inoculum addition there were no decrease in the DFBs concentration and no fluoride liberation (data not shown).

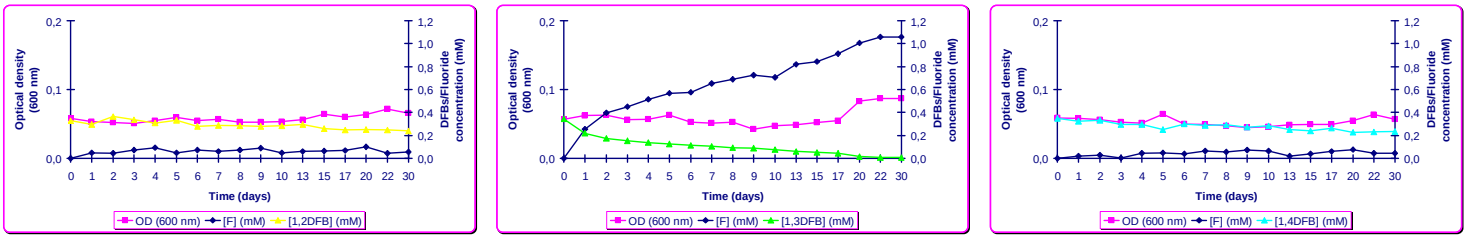

Figure 1. Optical density (OD 600 nm), DFBs concentration and fluoride release along time (days) of F11 cultures pregrown on FB and supplemented with 0.5 mM of 1,2-DFB, 1,3-DFB or 1,4-DFB. The experiment was conducted in triplicate.

DFBs biodegradation in co-metabolism with FB

The highest growth was observed in cultures fed with 0.5 mM of 1,3-DFB and 1 mM of FB (Fig. 2), with strain F11 being capable to degrade these compounds in less than two days. *L. portucalensis* F11 was also able to degrade 1 mM of 1,3-DFB and 1 mM of FB in 5 days, with a stoichiometric release of fluoride (Fig 2). Concerning 1,4-DFB, only 0.5 mM of this compound was degraded in co-metabolism with 1 mM of FB, in 7 days (Fig 2). For the other concentrations tested the degradation was not complete. *L Portucalensis* was not able to defluorinated 1,2-DFB in co-metabolism with FB during the time course of the experiment (data not shown).

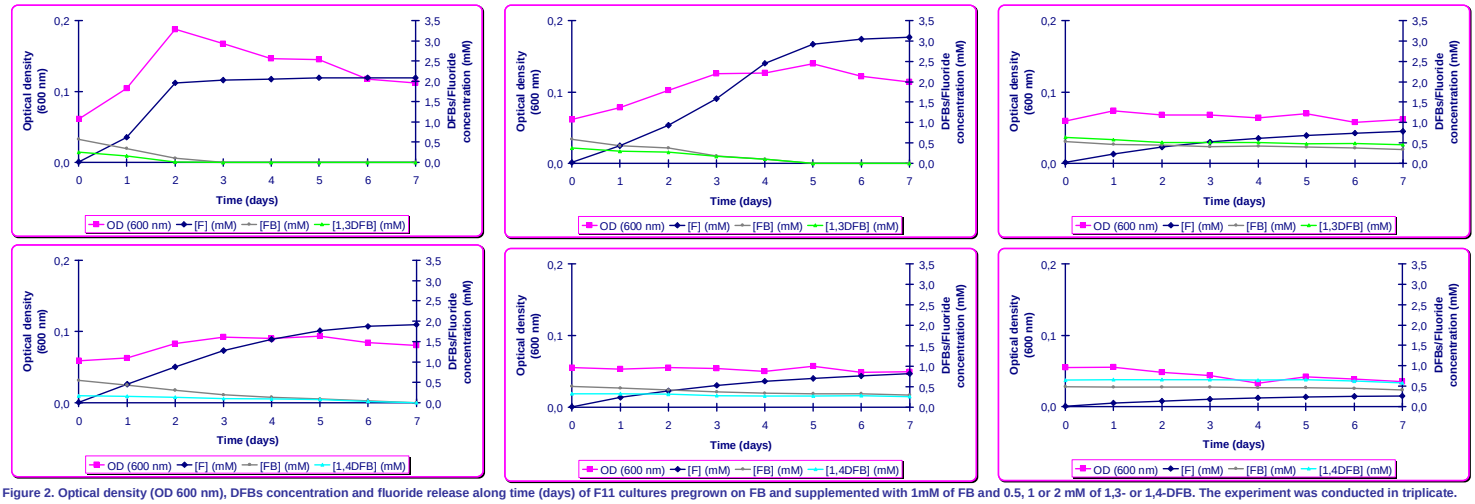

Figure 2. Optical density (OD 600 nm), DFBs concentration and fluoride release along time (days) of F11 cultures pregrown on FB and supplemented with 1mM of FB and 0.5, 1 or 2 mM of 1,3- or 1,4-DFB. The experiment was conducted in triplicate.

References

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