# Biodegradation of Carbamazepine by the bacterial strain *Labrys portucalensis* F11

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## Introduction and Objectives

The occurrence of pharmaceuticals in the environment is a topic of concern. Most pharmaceuticals are not completely mineralized in the human body and are released to the sewage systems as the pharmaceutical itself and as their "biologically active" metabolites Wastewater treatment plants (WWTPs) are not designed to remove them and they are released into the environment. Despite generally found at low concentrations (ranging from $ngL^{-1}$ to $\mu gL^{-1}$), they are classified as persistent microcontaminants due to their continuous release. Carbamazepine (CBZ) is a widely used anticonvulsant which has been found in different environmental compartments and has been suggested as a molecular marker of contamination in surface water and groundwater.

In the present study, biodegradation of CBZ by strain *Labrys Portucaliensis* F11 was assessed. Transformation products (TPs) resulting from CBZ degradation were analysed.

## Methodology

- ✓ CBZ biodegradation assays were performed in minimal salts medium (MM) supplemented with 2.0, 4.0, 10.0, 20.0 and 40.0 $\mu$M of CBZ as a sole carbon and energy source and in the presence of acetate (5.9mM);
- ✓ CBZ was quantified by High Performance Liquid Chromatography (HPLC);
- ✓ Transformation products (TPs) were detected and identified by UPLCQTOF/MS/MS;
- ✓ Ecotoxicological effects of cultures containing CBZ and of cultures containing the TPs after microbial degradation were assessed using *Daphnia magna* (OCED Guideline 202) and *Lepidium sativum* (OECD Guideline 208).

## Results

### ✓ *Biodegradation assays*

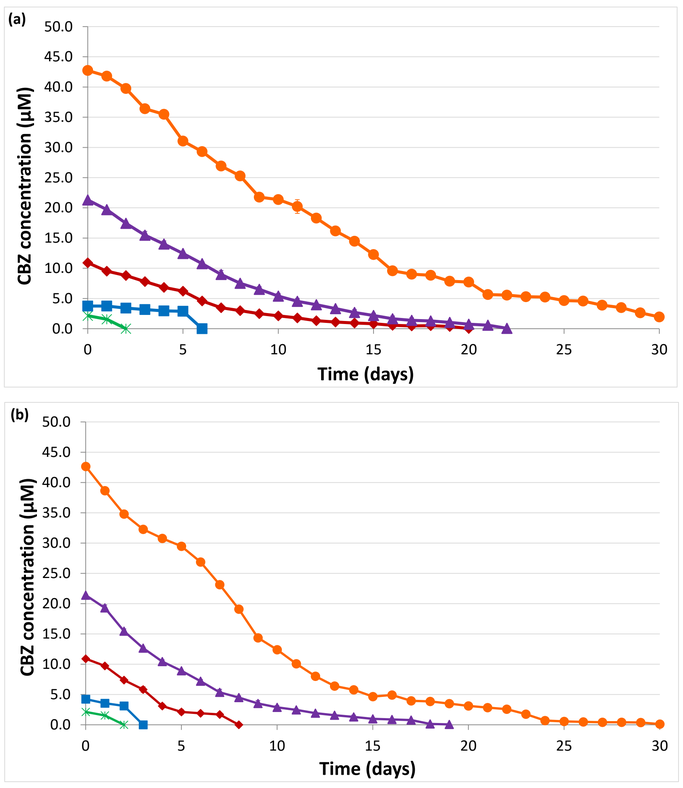


Figure 1: Carbamazepine (CBZ) degradation by *Labrys Portucalensis* F11 as a sole carbon and energy source (a)) and in the presence of acetate (b)) at different concentrations. (●) 40µM; (▲) 20µM; (◆) 10µM; (■) 4.0µM and (✖) 2.0µM.

- ✓ Strain F11 was able to degrade 95% of 40 µM of CBZ as sole carbon source during 30 days (Fig 1a);
- ✓ Supplementation with acetate (Fig 1b) slightly improved the degradation of CBZ.

### ✓ *Transformation products*

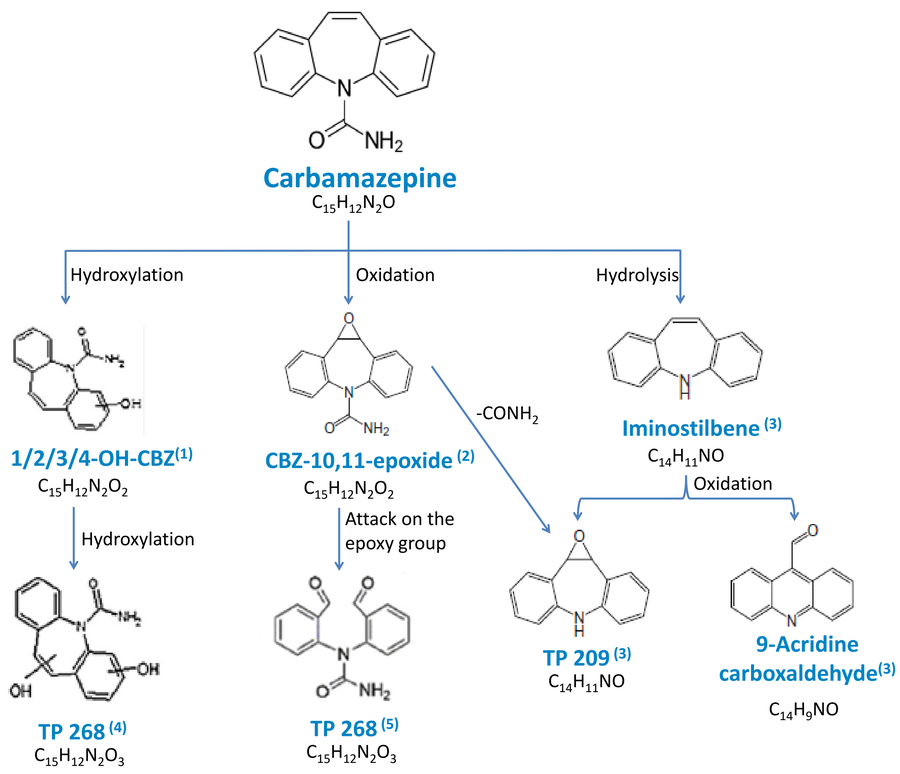


Figure 2: Proposed CBZ degradation intermediates produced by strain *Labrys portucalensis* F11. Degradation products also found by: (1) Bahlman A, et al., (2014). doi: 10.1016/j.watres.2014.03.022; (2) Seiwert B et al., (2015). doi: 10.1021/acs.est.5b02229; (3) Liu N et al., (2016). doi: 10.1016/j.cej.2016.03.040; (4) De Laurentiis E et al., (2012). doi: 10.1021/es3015887; (5) Zhu Z et al., (2016). doi: 10.1016/j.watres.2016.02.035.

### ✓ *Toxicological assays*

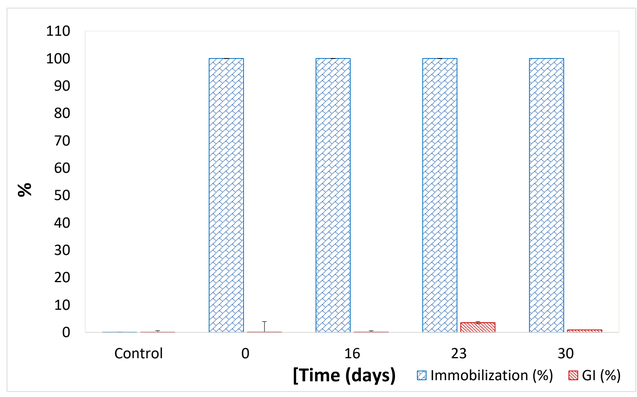


Figure 3: Effect of CBZ and respective transformation products during degradation of 40 µM of CBZ by *Labrys Portucalensis* F11 on *D. magna* (% immobilization, in blue) and *L. sativum* (% of root elongation inhibition, in red).

- ✓ For all the sampling times, *D. magna* response was similar, with complete immobilization at 48hr of exposition.
- ✓ Inhibition of *L. sativum* root elongation was practically neglectable.

## Highlights

- ✓ Supplementation with a second carbon source had a positive effect on CBZ degradation by strain *Labrys Portucalensis* F11;
- ✓ CBZ degradation by strain F11 proceeds mainly by oxidation, hydroxilation, hydrolysis and cleavage of the aromatic ring;
- ✓ CBZ TPs were detected until the end of the experiment time;
- ✓ CBZ and transformation products exhibited high toxicity on D. magna whereas inhibition of root elongation of L. sativum was practically neglectable.

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