Production of 4-fluorocatechol from fluorobenzene by the wild strain *Labrys portucalensis*

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Introduction

Microbial production of catechols has been subject of investigation because of the advantages it may pose in comparison with organic synthesis. 4-Fluorocatechol (4-FC) is formed as an intermediate during fluorobenzene (FB) degradation by cell suspensions of *Labrys portucalensis* strain F11. The metabolism of this intermediate proceeds through *ortho* cleavage by a (fluoro)catechol 1,2-dioxygenase [1]. A strategy to possibly accumulate 4-FC by cells of strain F11 consist in the inhibition of catechol 1,2-dioxygenase by the addition of chemical inhibitors.

The aim of this work was to evaluate the effect of the addition of three different inhibitors on FB degradation and on 4-FC accumulation.

Methods

Cells of strain F11 pregrown on FB

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Harvested by centrifugation

↓

Washed and ressuspended in phosphate buffer

↓

2.5 mM FB
+
1 mM ascorbic acid

+

- $AgNO_3$ 0.5 mM / 1 mM
- $CuSO_4$ 0.5 mM / 1 mM
- $FeSO_4$ 0.5 mM / 1 mM
- - (control)

↓

Sampling:

- 4-FC formation/accumulation (LC-MS analysis)
- fluoride release (potenciometry)

Results

• LC-MS analysis showed higher accumulation of 4-FC, by cells induced with FB, when incubated in the presence of FB and $CuSO_4$ (Fig. 1).

• The level of 4-FC accumulation was lower when $AgNO_3$ was present (Fig. 1), and Ag^+ showed a stronger inhibiting effect on FB degradation (Fig. 2).

• $FeSO_4$ had no effect on either FB degradation or on 4-FC accumulation (Figs. 1 and 2).

• Induced cells of strain F11, in which ring-cleavage dioxygenase is inhibited, may be used for catechol accumulation, and further optimisation may require cloning and recombinant overexpression of the dioxygenase gene, a work which is ongoing.

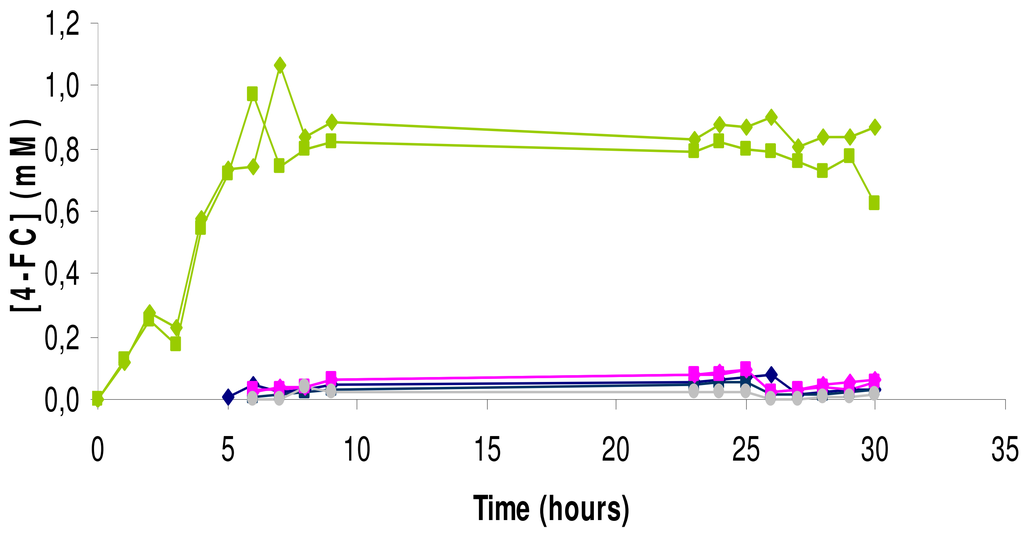

Figure 1. 4-FC accumulation during FB degradation by *Labrys portucalensis* strain F11 resting cells suspended in KH2PO4-Na2HPO4 buffer with 1 mM ascorbic acid, 2.5 mM FB and: 1 mM AgNO3 (), 0.5 mM AgNO3 (),1mM CuSO4 (), 0.5 mM CuSO4 (), 1 mM FeSO4 (), 0.5 mM FeSO4 () and control without inhibitor ().

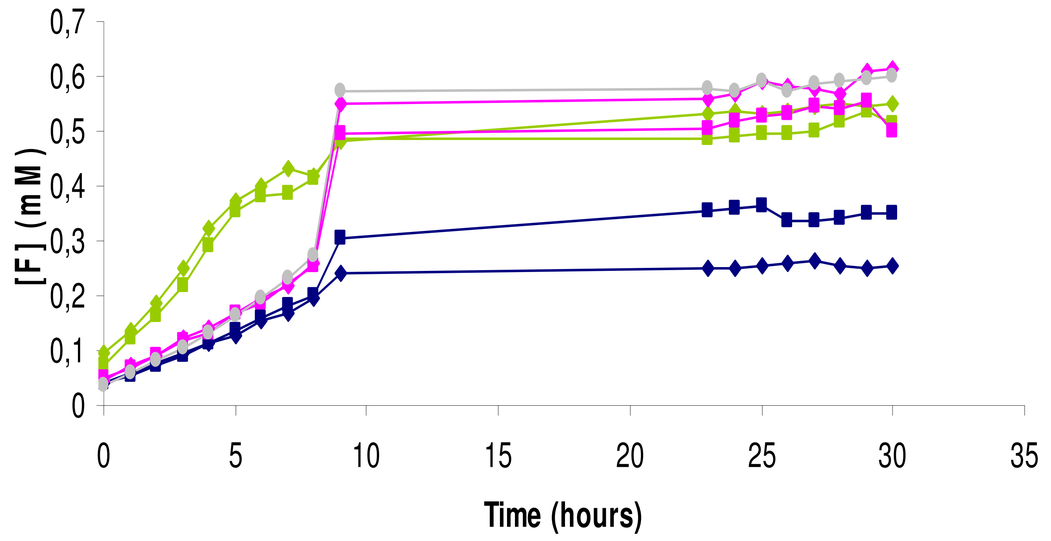

Figure 2. Fluoride release during FB degradation by *Labrys portucalensis* strain F11 resting cells suspended in KH2PO4-Na2HPO4 buffer with 1 mM ascorbic acid, 2.5 mM FB and: 1 mM AgNO3 (), 0.5 mM AgNO3 (),1mM CuSO4 (), 0.5 mM CuSO4 (), 1 mM FeSO4 (), 0.5 mM FeSO4 () and control without inhibitor ().

Acknowledgements

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References

[1] M. F. Carvalho, M. I. M. Ferreira, I. S. Moreira, P. M. L. Castro, D. B. Janssen, *Appl. Environ. Microbiol.*, 2006, 72, 7413.