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4-FLUOROCINNAMIC ACID BIODEGRADATION BY A *RHODOCOCCLUS* STRAIN

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Introduction

Fluorinated organic compounds

- ❖ Highly toxic to living organisms;
- ❖ Carcinogenic and mutagenic effects;
- ❖ Extremely persistent;



**Hazardous environmental
pollutants**



Introduction

Accumulation of fluorinated organic compounds in soils and water is a major environmental problem



BIODEGRADATION

Promising approach for the removal from environment

What is the potential for biodegradation from environmental compartments?



Introduction

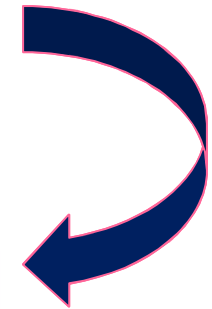
Chemical processes

- energy-intensive;
- expensive;
- can produce toxic byproducts;

Biodegradation

- cost effective
- allows total mineralization of the toxic

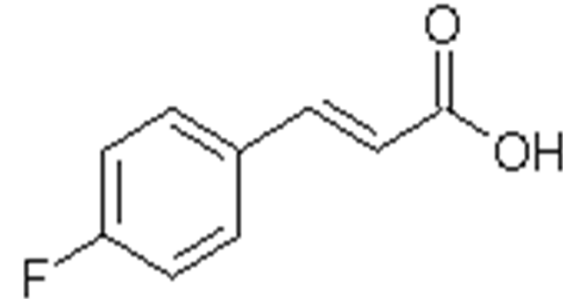
Emerging technology for the degradation of halogenated organic compounds



Introduction

4-Fluorocinnamic acid

- ❖ Intermediate in the synthesis of:
 - Agrochemicals;
 - Pharmaceuticals;
 - Fine chemicals;



4-FCA

- ❖ Scant information on biodegradation is available;

Biodegradation of this compound by a single strain not reported in the literature



Objective



Assessment of 4-FCA degradation by a pure bacterium

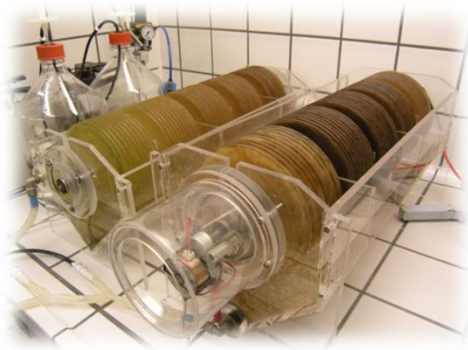


Experimental approach

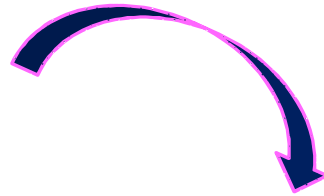
- ❖ Isolation of a 4-FCA degrading strain
- ❖ Classification of the 4-FCA degrading strain
- ❖ Degradation Assays
 - ❖ Biodegradation behaviour in the absence/presence of a conventional carbon source
 - ❖ Effect of the initial 4-FCA concentration on the degradation process



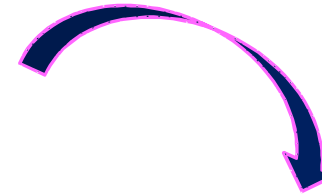
Isolation of a 4-FCA degrading strain



Recovery of strains from a Rotating Biological Contactor (RBC) fed with 2-fluorophenol (2-FP)



Isolation of microbial strains using the streak-plate procedure



Inoculation of the isolated strains with 4-FCA



Isolation of a 4-FCA degrading strain

Five microbial strains recovered from a RBC fed with 2- FP



Capacity to grow in batch cultures with 4-FCA supplied as the sole carbon and energy source at concentration of 0.5mM (cultures were incubated on an orbital shaker at 25°C)



Selection of a 4-FCA degrading strain



Classification of the 4-FCA degrading strain



The degrading strain
(**Strain S2**) was
classified by 16S
rRNA gene analysis



Strain S2 constitutes
a member of the
genus
Rhodococcus

One of the **most valuable** and **promising groups**
for use in the bioremediation processes



- ❖ metabolic versatility
- ❖ robustness
- ❖ persistence in adverse conditions



Degradation assays

Batch experiments were performed aerobically at 25 °C with constant shaking

- ❖ Biodegradation behaviour of 0.5mM of 4-FCA in the absence/presence of a conventional carbon source (acetate 20 mM)
- ❖ Effect of the initial 4-FCA concentration in the degradation process (in the presence of 20 mM acetate)



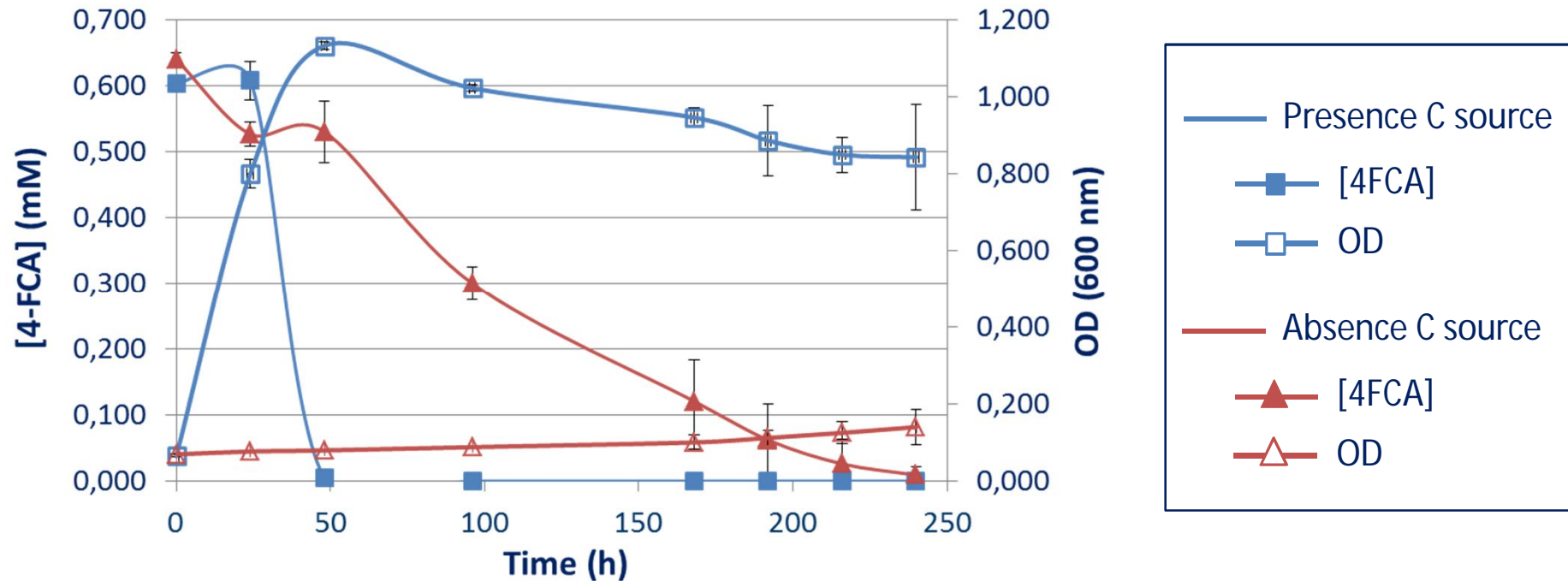
Monitored parameters:

- ❖ Growth of the strain
- ❖ Consumption of target compound
- ❖ Liberation of fluorine ion

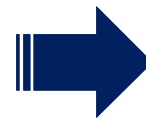


Results

Effect of the absence/presence of a conventional carbon source



- ❖ Complete mineralization of 4-FCA
- ❖ Carbon source enhances the rate of biodegradation

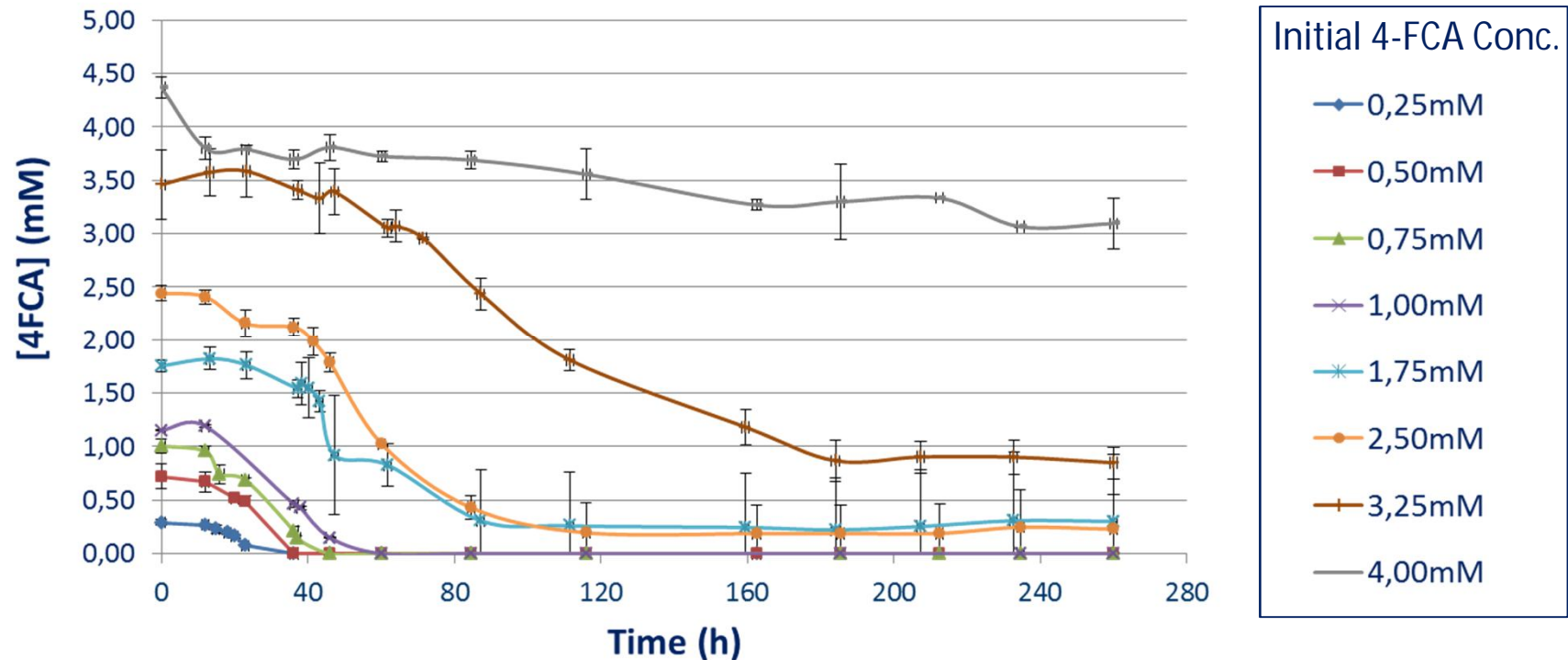


The additional substrate promotes the adaptation process towards 4-FCA degradation



Results

Effect of the initial 4-FCA concentration

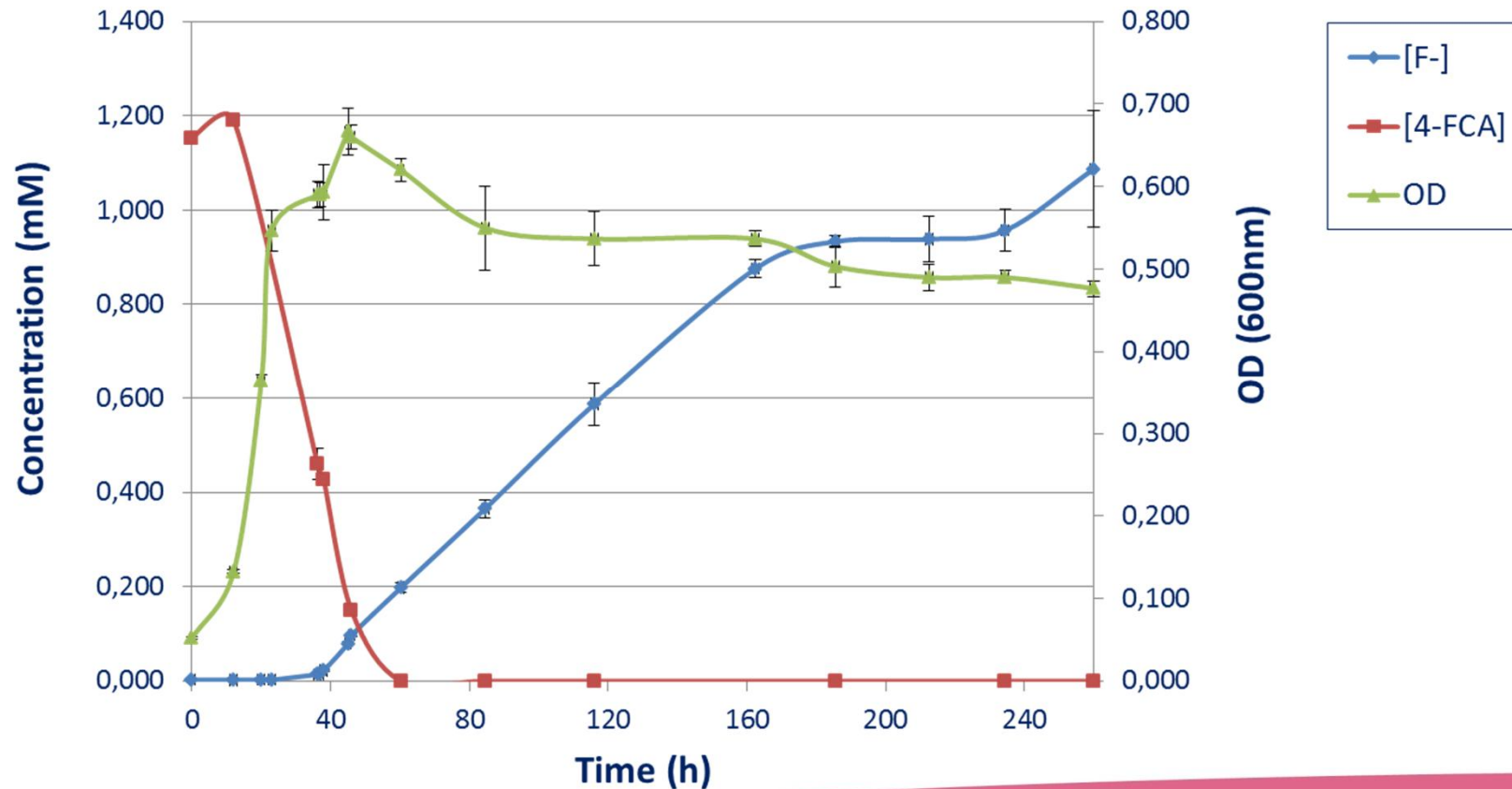


- ❖ The increase of the initial 4-FCA concentration leads to an increase of the lag phase
- ❖ Total degradation of 4-FCA up to 1 mM



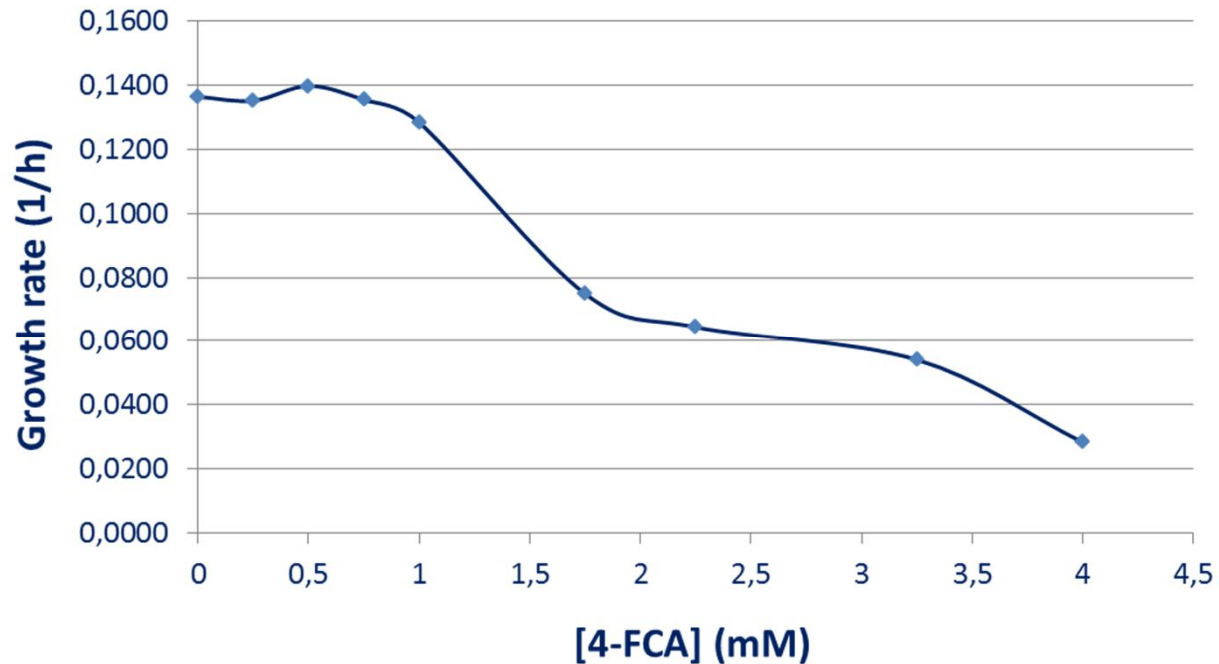
Results

Typical example of batch growth of strain S2 with a mixture of acetate and 4-FCA



Results

Growth kinetics of strain S2 on 4-FCA with a conventional C source



- ❖ An inhibitory effect with increasing 4-FCA concentration was observed at concentrations higher than 1mM



Conclusions

- ❖ Complete mineralization of 0.5 mM of 4-FCA as a sole carbon and energy source was achieved;
- ❖ The presence of conventional source of carbon improved the degradation rate of 4-FCA;
- ❖ Complete biodegradation of the target compound, in the presence of acetate, within a concentration range between 0 and 1 mM was observed;



Conclusions

Strain S2

- ❖ Adaptable strain : able to completely mineralize 4-FCA as a sole carbon and energy source or in the presence of a conventional carbon source
- ❖ Robust strain: able to degrade this recalcitrant and toxic compound



Candidate for use in biological treatment plants and bioremediation of contaminated sites containing 4-FCA



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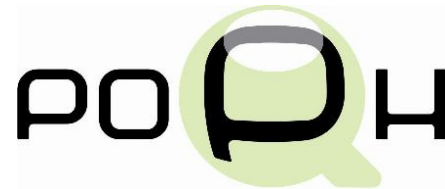
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FCT

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MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR



European Union
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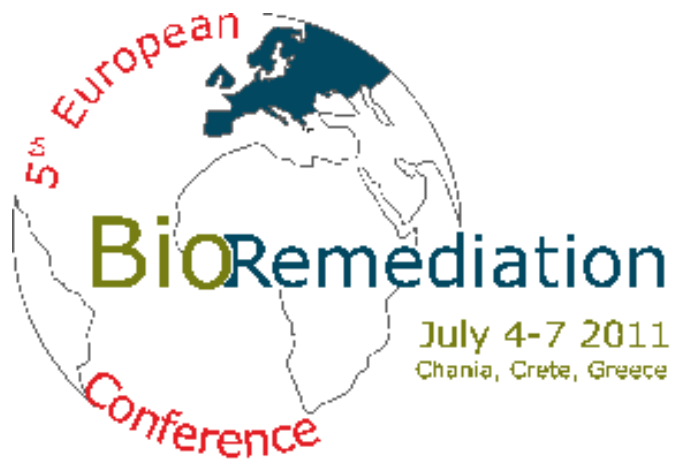
PROGRAMA OPERACIONAL **POTENCIAL HUMANO**



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Thank you for your attention!



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