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PRODUCTION OF SUBSTITUTED PHENYLACETIC ACIDS BY STYRENE-DEGRADING BACTERIA

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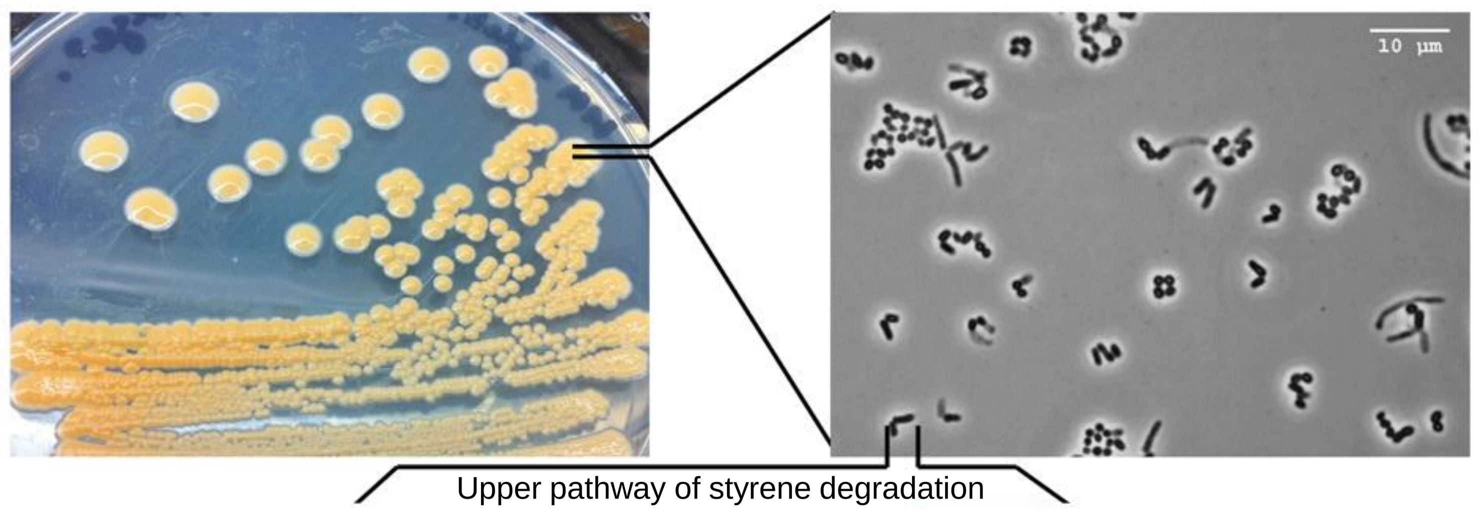
Introduction

A number of soil bacteria is able to metabolize styrene via initial side-chain oxygenation into the central metabolite phenylacetic acid and harbors corresponding genes [1]. This pathway of styrene degradation is of potential biotechnological relevance for the production of especially phenylacetic acids. These aromatic acids are important for various industries. In this study we report on the establishment of a process using native cells of ***Pseudomonas fluorescens*** **ST** for the co-metabolic production of **4-chlorophenylacetic acid**. Therefore, cultivation and inducing conditions were optimized and biotransformation strategies were developed to accumulate the product to high concentrations.

The styrene-degrading pathway



Phenylacetic acid occurs only as temporary intermediate, but are substituted products obtainable?

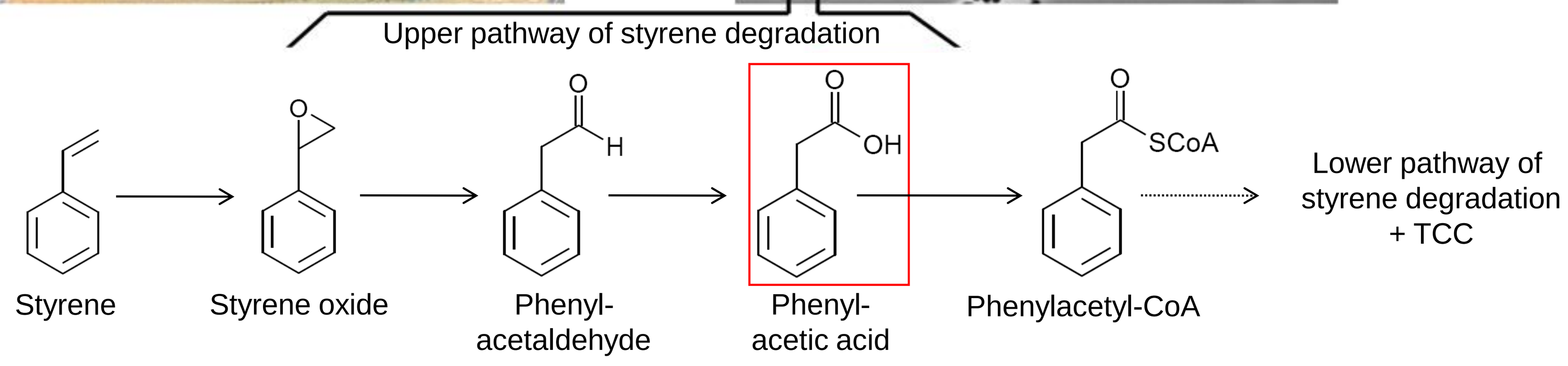


Fig. 1: Side-chain oxygenation of styrene by styrene-degrading bacteria (*Rhodococcus opacus* 1CP, *Sphingopyxis fribergensis* Kp5.2, *Gordonia* sp. CWB2 and *Pseudomonas fluorescens* ST) led to an **accumulation of very small amounts of phenylacetic acid**. But, the **acid is also metabolized** which avoids higher product concentrations.

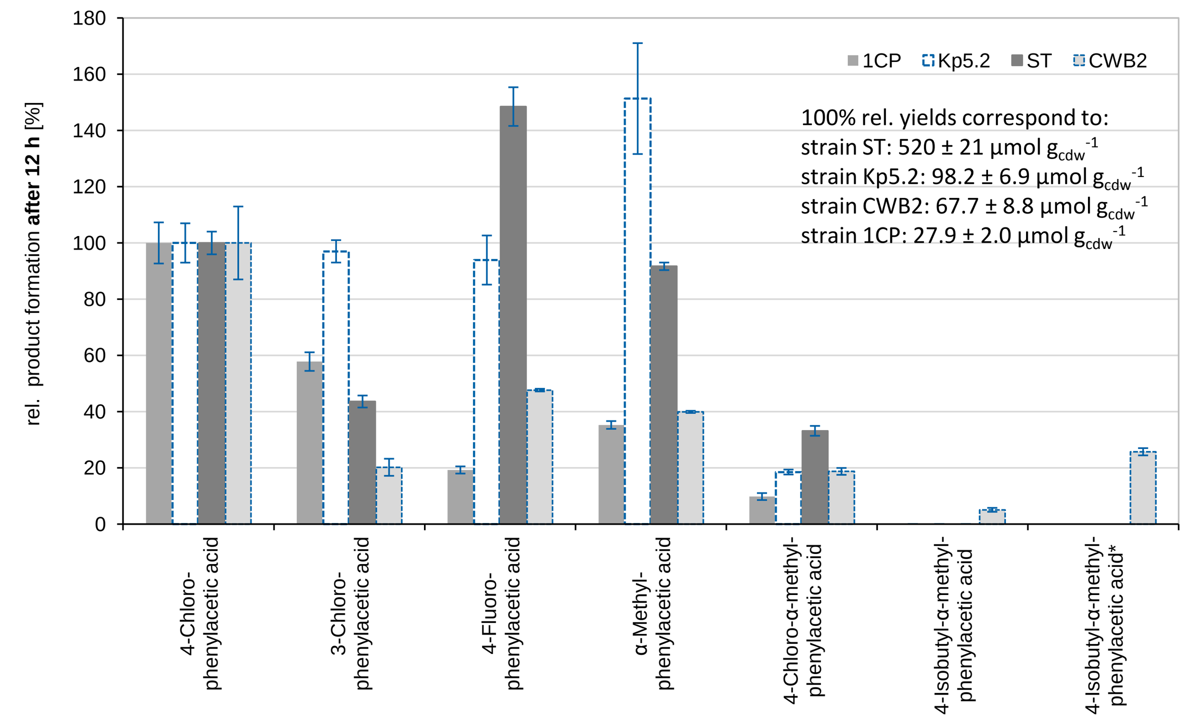


Fig. 2: Accumulation of substituted phenylacetic acids in the culture medium after addition of substituted styrenes via vaporizer to four styrene-degrading strains (* direct addition to the medium).

Substituted, especially halogenated phenylacetic acids are co-metabolically produced and enriched! Highest transformation rates were gained with *Pseudomonas fluorescens* ST.

Establishment of a process applying cells of *Pseudomonas fluorescens* ST

Upscaled production of styrene-induced biomass in the fermenter (OD_{600} of 13; 5 L of biomass) for biotechnological application + first biotransformation of 4-chlorostyrene:

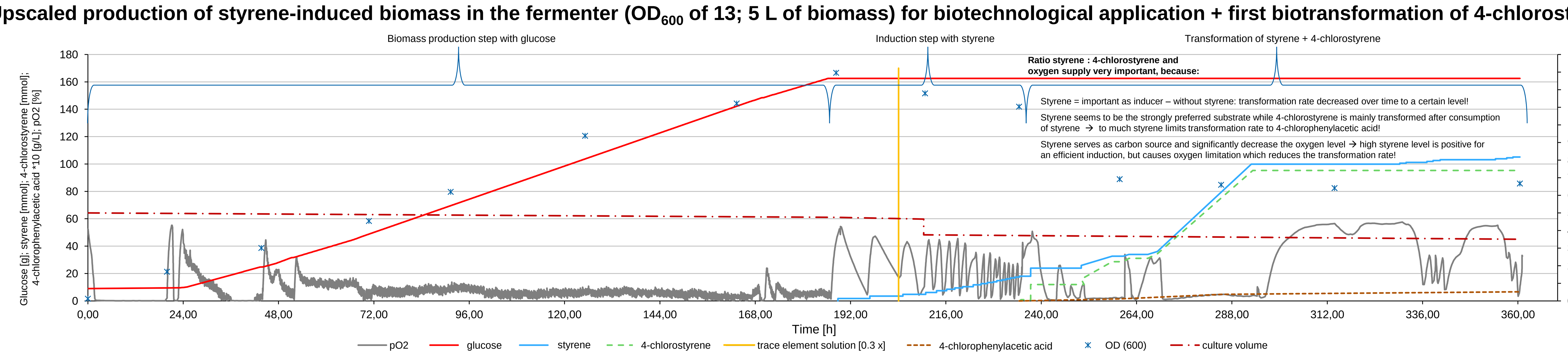


Fig. 3: Cultivation of *Pseudomonas fluorescens* ST in a 5-L fermenter and investigation of the influence of styrene and 4-chlorostyrene on the induction, transformation and oxygen supply.

Optimization of the transformation step with respect to pH, medium, feeding rate of the co-substrate styrene, OD_{600}, optimal volume/ oxygen limitation – selected results:

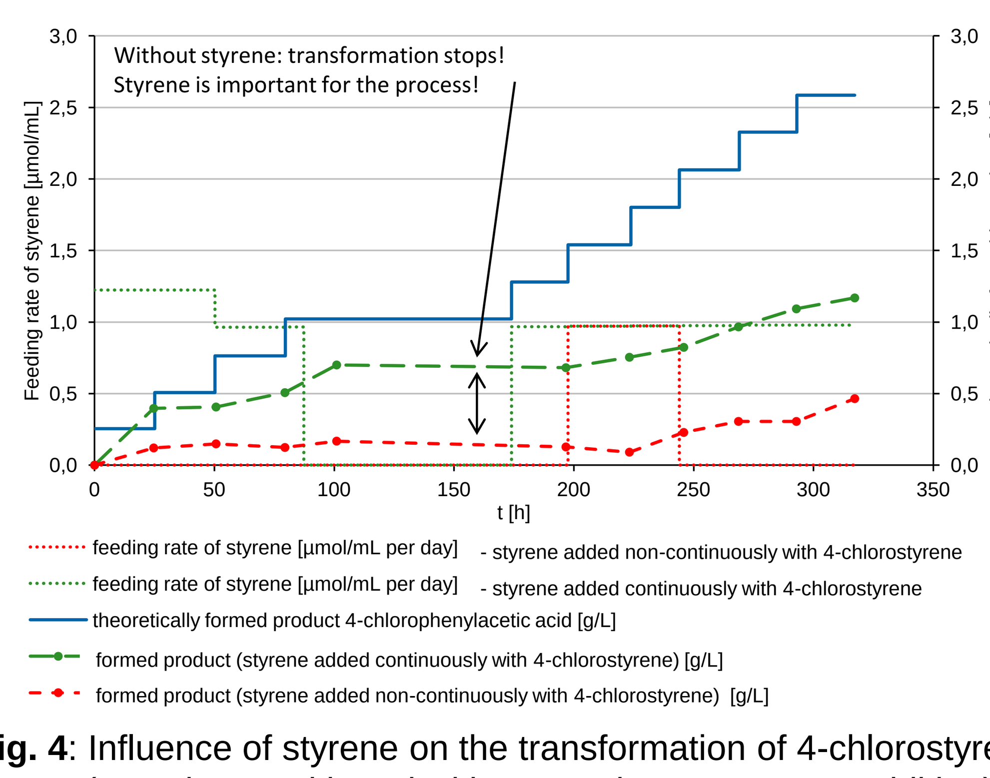


Fig. 4: Influence of styrene on the transformation of 4-chlorostyrene (experiment with and without continuous styrene addition).

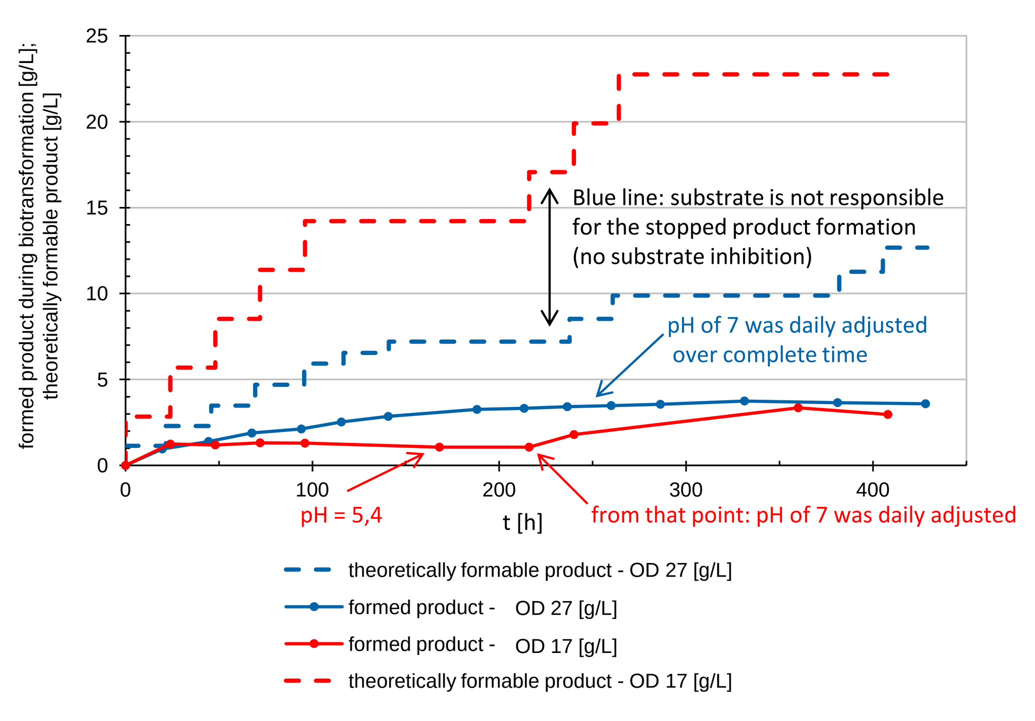


Fig. 5: Influence of the pH and the substrate on the transformation of 4-chlorostyrene.

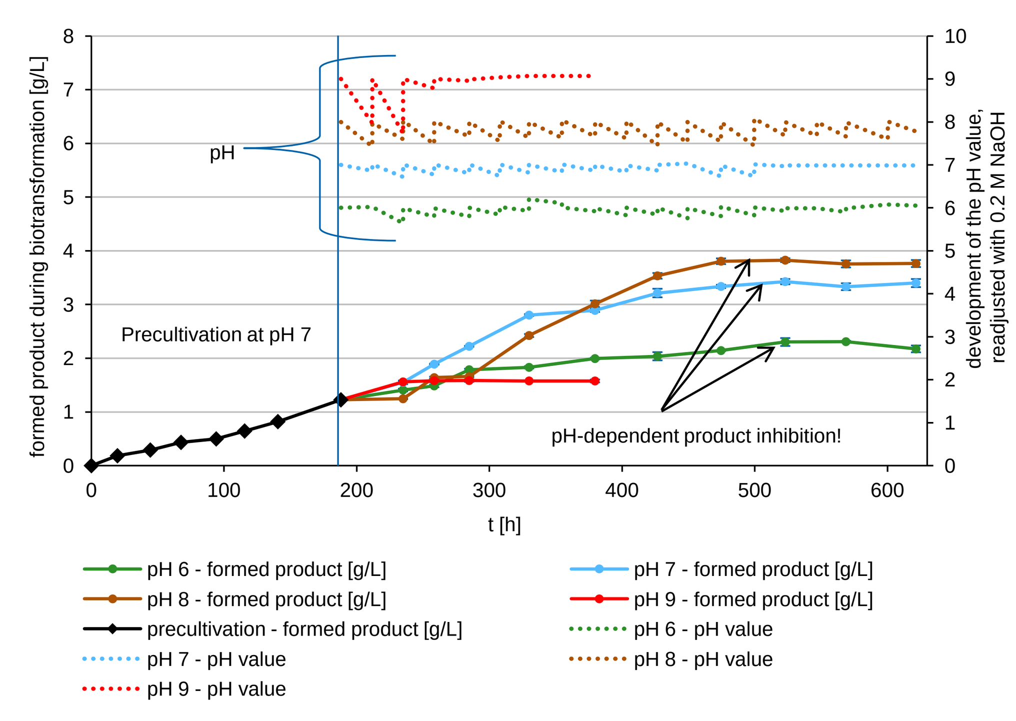


Fig. 6: Influence of the pH on the product formation of 4-chlorophenylacetic acid.

Conclusion

A promising strategy to produce substituted phenylacetic acids applying *Pseudomonas fluorescens* ST was developed. Optimal conditions for the cultivation were revealed. Nearly 4 g/L 4-chlorophenylacetic acid were produced during these first experiments. But, the formed product also inhibits the transformation, most likely on transcription level. Further substrates shall be tested in future experiments with respect to reachable product concentrations.

[1] O'Leary, N. D., K. E. O´Connor, A. D. W. Dobson. 2002. FEMS Microbiol Rev **26**:403-417.

Publications on that topic:
M. Oelschlägel, S. R. Kaschabek, J. Zimmerling, M. Schlömann, D. Tischler (2015) Co-metabolic formation of substituted phenylacetic acids by styrene-degrading bacteria. *Biotechnology Reports* 6:20-26.
M. Oelschlägel, J. Zimmerling, D. Tischler, M. Schlömann (2013) Biotechnologische Herstellung von substituierten und unsubstituierten Phenylessigsäuren und Ketonen aus entsprechenden Styrolen und bicyclischen aromatischen Kohlenwasserstoffen unter Verwendung von Enzymen des mikrobiellen Styrolabbaus. Patent 00426P0073DEWO (applied).

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