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# Biofilms as living catalysts for fine chemical synthesis: analysis, process design and scale-up

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## Summary

Biofilms are resilient to a wide variety of environmental stresses. This inherited robustness has been exploited mainly for bioremediation. This thesis addressed the key challenges for the use of biofilms as biocatalysts on biological as well as on technical level. On biological level the question how do catalytic biofilms react to the biologically challenging compound styrene was studied. On technical level, crucial process parameters such as substratum for biofilm growth and oxygen mass transfer have been targeted.

To address the biological question, a reliable toolbox based on a modified flow-cell coupled to confocal microscopy and a GFP-marker system was established. Through a time-resolved, non-invasive and quantitative approach, *Pseudomonas* sp. strain VLB120ΔC biofilm development and its response to the toxic solvent styrene was investigated. Although *Pseudomonas* cells experience severe membrane damage during styrene treatment, they are able to adapt to the toxic conditions and recover. The solvent styrene did not affect the growth rate and overall biofilm structural integrity. Compared to control experiments with planktonic cells, the *Pseudomonas* biofilm adapted much better to toxic concentrations of styrene, as nearly 65% of biofilm cells were not permeabilized (viable), compared to only 7% in analogous planktonic cultures.

For the synthesis of target product (*S*)-styrene oxide on a process level, a unique solid support membrane aerated biofilm reactor was designed, constructed and scaled-up utilizing *Pseudomonas* sp. strain VLB120ΔC growing in a biofilm as biocatalyst. In the optimized system a sintered stainless steel membrane was used as a growth surface and efficient oxygen transfer unit, while a highly stable expanded PTFE (ePTFE) membrane showed the best performance regarding *in situ* substrate delivery and product extraction. With these modifications, the scalability of the system was demonstrated and the solid support membrane aerated biofilm reactor was scaled-up by a factor of 12 with respect to an aqueous phase volume. In the scaled up system, a productivity of 24 g $L_{aq}^{-1}$ $day^{-1}$ could be achieved with an excellent product on biomass yield of 23 $g_{product}$ $g_{biomass}^{-1}$. In total, 46 g of (*S*)-styrene oxide was produced in this system and was subsequently isolated by vacuum distillation (purity: 99%; *ee*: > 99%). The achieved productivity is in a similar range to the small scale biofilm reactor verifying the success of the scale-up. Finally, to test the compatibility of this reactor concept, an equally challenging reaction for limonene

hydroxylation was tested. (*S*)-perillyl alcohol was synthesized at the rate of 7 g $L_{aq}^{-1}$ $day^{-1}$ using *Pseudomonas* sp. strain VLB120 harboring cytochrome P450 monooxygenase. A stable catalytic activity of more than 32 days was achieved before the system was terminated actively.

These results set a new status and open new possibilities for future developments in the field of productive catalytic biofilms.