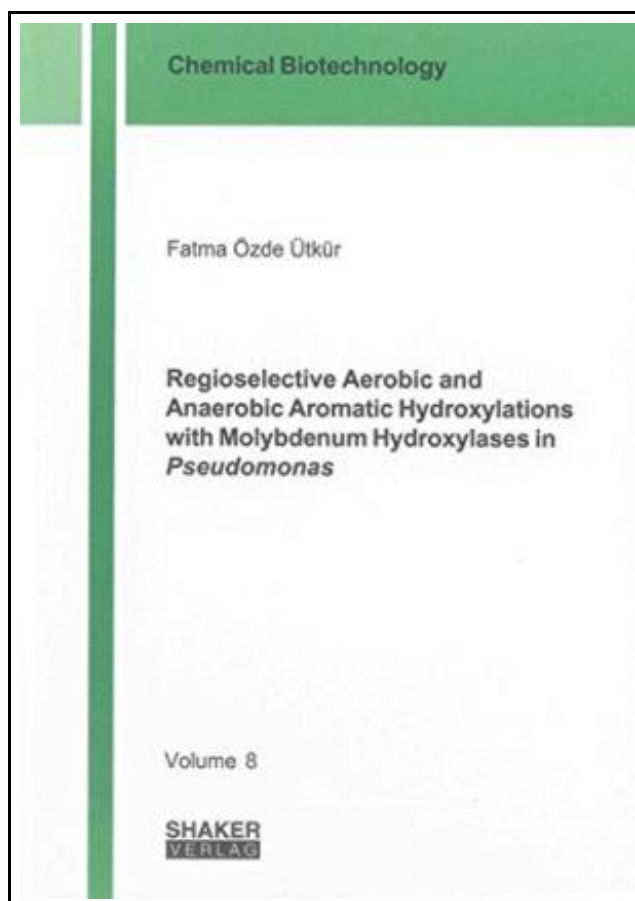


Regioselective Aerobic and Anaerobic Aromatic Hydroxylations with Molybdenum Hydroxylases in Pseudomonas



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REGIOSELECTIVE AEROBIC AND ANAEROBIC AROMATIC HYDROXYLATIONS WITH MOLYBDENUM HYDROXYLASES IN PSEUDOMONAS



Shaker Verlag Apr 2012, 2012. Buch. Book Condition: Neu. 21x14.8x cm. Neuware - Among many other interesting reactions, molybdenum (Mo) hydroxylases catalyze regioselective hydrocarbon oxyfunctionalizations, for which typically oxygenases are employed in synthetic applications. However, oxygenase-based processes are often limited by oxygen mass transfer, cofactor regeneration, and/or enzyme instability due to the formation of reactive oxygen species. As Mo-hydroxylases produce, rather than consume reducing equivalents during substrate hydroxylation and use water, rather than molecular oxygen as oxygen donor, these enzymes have a high potential for overcoming limitations encountered with oxygenases. The potential and feasibility of these enzymes for preparative applications was investigated in the frame of this thesis with quinoline 2-oxidoreductase (Qor) and quinaldine 4-oxidase (Qox) serving as model enzymes. Up-to-date, several Mo-hydroxylases have been described, but rarely applied on industrial scale. For specific quinaldine hydroxylation to 4-hydroxyquinaldine, different Qox-based biocatalysts, reaction conditions, and key process parameters have been evaluated. The use of 1-dodecanol as carrier solvent and Qox-containing *P. putida* KT2440 as biocatalyst enabled high productivities ($\sim 0.4 \text{ g l}^{-1} \text{ h}^{-1}$) in a 0.5-L bioreactor setup without active aeration. Further evaluation of the key process parameters showed that inhibition by 1-dodecanol and the product was the most critical factor affecting process performance. As a proof of concept, the Qox-based process was coupled to downstream processing, including supercritical carbon dioxide treatment for breaking the stable emulsion followed by liquid-liquid extraction and crystallization allowing the isolation of 138 mg product with high purity ($>99.9\%$). Furthermore, completely anaerobic quinoline hydroxylation was achieved with nitrate as electron acceptor using Qor-containing *P. putida* 86 expressing the nitrate reductase genes of *P. aeruginosa*. The achieved rate (7 U gCDW^{-1}) shows the remarkable potential of Mo-hydroxylase-containing whole cells for O_2 -independent C-H oxyfunctionalizations. In conclusion, the development of an efficient integrated process and the first...



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