

Characterization and engineering of *Pseudomonas putida* for aerobic n-butanol production



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(Alexandre Cruickshank)

CHARACTERIZATION AND ENGINEERING OF PSEUDOMONAS PUTIDA FOR AEROBIC N-BUTANOL PRODUCTION



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Shaker Verlag Mai 2012, 2012. Buch. Book Condition: Neu. 21x14.8x cm. Neuware - Recent technological and economical developments reflect the general need for sustainable and cost-effective biotechnological processes for the production of high-volume, low-value bulk chemicals, some of which can be applied as 2nd generation biofuels. The latter, such as n-butanol, benefit from cheap and/or unconventional raw materials and improved product characteristics. To become ecologically and economically competitive, n-butanol biosynthesis requires engineering to overcome the main limitations: product toxicity and relatively low volumetric productivity. This thesis is based on a comprehensive literature review of n-butanol fermentations - including key fermentation performance parameters. Best performing processes are visualized using the window of operation methodology. Focusing n-butanol fermentation development from large-scale, highly diluted batch fermentations to integrated processes, the identified limitations were investigated in the following chapters. Pseudomonas putida was chosen as an alternative host for n-butanol biosynthesis to overcome the primary limitation of current producers - insufficient product titers due to low n-butanol tolerance. Indeed, Pseudomonas strains increased tolerance when exposed to n-butanol. Decreased energy production hints to changes that are low-energy demanding. Glycerophospholipids that are involved in membrane (re-)adjustments to environmental stresses were investigated. Challenged with sub-lethal concentrations of n-butanol, membranes of different Pseudomonas strains responded with changing glycerophospholipid compositions. With glucose as the main industrial carbon source, its catabolization by Pseudomonas was investigated. The results indicate simultaneous use of three convergent peripheral pathways that might enable energy and redox cofactor homeostasis and can be important for n-butanol biosynthesis. In this thesis, the design, synthesis and characterization of new n-butanol producers based on solvent-tolerant Pseudomonas strains are presented. Using Escherichia coli as control, expression vectors based on the alkB promoter system were designed to investigate n-butanol biosynthesis with simultaneous analysis of expression and enzymatic activities. Optimized DNA sequences...

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