D13808F · ISSN 0947-0867
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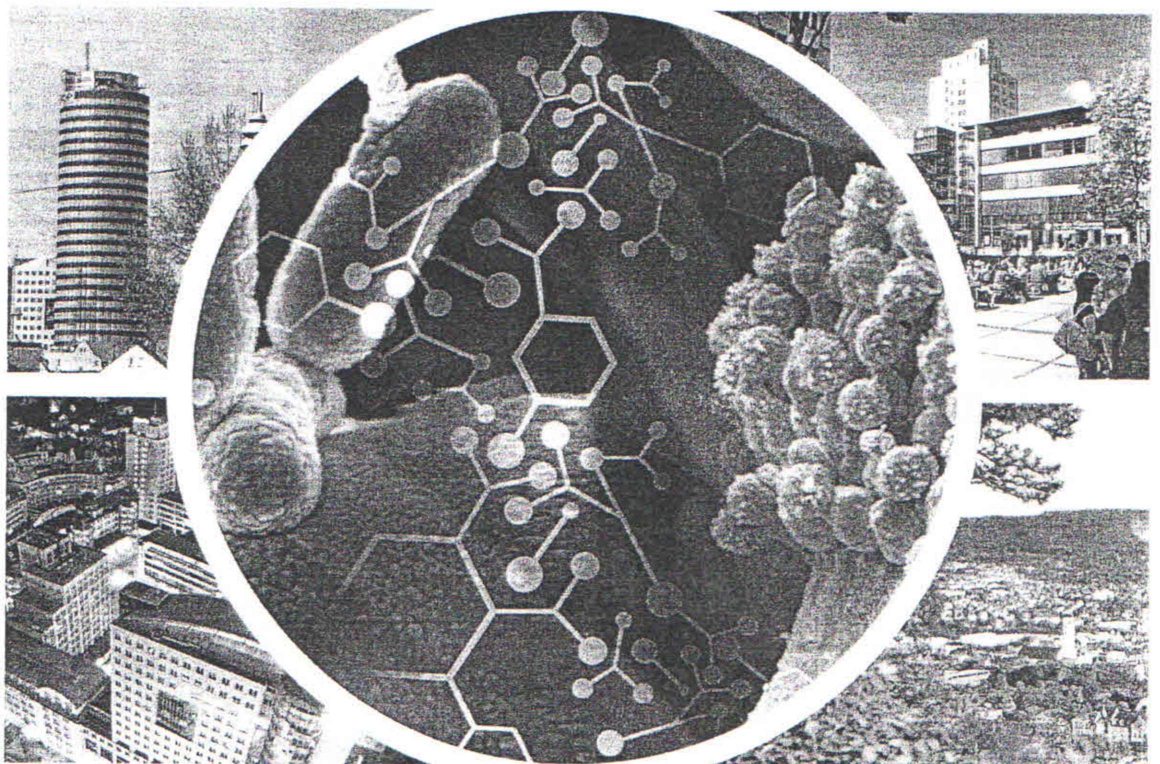
Das Magazin für Biowissenschaften



2016
Sonderausgabe

Tagungsband zur

VAAM-Jahrestagung 2016

13.–16. März in Jena

Conclusion: *T. kivui* can be adapted to grow on CO. CO oxidation leads to acetate and H_2 formation and is coupled to energy conservation.

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BEP15

Hydrogen production by *Escherichia coli* wild type and hydrogenase mutants upon formate and glycerol fermentation under different growth conditions

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One of the fermentation end products during glycerol and glucose fermentation in *Escherichia coli* is formate, which is decomposed by the formate hydrogen lyase (FHL) complex leading to H_2 production. FHL is formed by formate dehydrogenase H (FDH-H) and hydrogenase (Hyd). *Escherichia coli* possesses four [Ni-Fe]-hydrogenase (Hyd) enzymes, encoded by the *hya, hyb, hyc* and *hyf* operons. In this study H_2 production was investigated with the help of two redox Pt (sensitive to H_2) and Ti-Si electrodes. From the end of the log growth phase the drop of two redox Pt and Ti-Si electrodes form positive to low negative ((Pt) - 450± 0.05) values was detected in *E. coli* BW25113 wild type upon glycerol fermentation in peptone rich medium pointing out H_2 formation. *E. coli* BW25113 wild type growth and H_2 production were also observed in minmal salt growth medium at pHs 5.5 to 7.5. H_2 production was delyed and observed after 24 h growth, moreover, the bacterial growth and H_2 production reached their maximal values at 72 h at pHs 6.5 and 7.5. Our previous findings identified the conditions when formate alone or with glycerol had stimulatory effect on bacterial growth and H_2 production. The role of deleting the large subunits of each Hyd (1-4) enzymes for bacterial growth and H_2 production was evaluated. In this study role of formate alone or with glycerol on hydrogen production and growth were investigated in double *hyaBhybC* (lacking Hyd 1 and 2; triple *hyaBhybChycE* (lucking Hyds 1-3), and *selC* (lacking FDH-H) mutants were investigated upon 24 h bacterial growth. H_2 production was absent in *selC* and triple *hyaBhybChycE* mutants during log growth phase upon glycerol or formate alone or with glycerol fermentation at pH 6.5 and 7.5 and formate supplementation had no effect. H_2 evolution was delayed at pH 7.5, but observed and stimulated at pH 6.5 upon glycerol and formate utilization in double *hyaBhybC* mutant.
The results point out the ability of *E. coli* to grow and utilize glycerol in minimal salt medium, as well as confirmed the key role of Hyd-3 at both pH 6.5 and 7.5 as well as the role of Hyd-2 and Hyd-4 at pH 7.5 for H_2 production by *E. coli* during glycerol fermentation with formate supplementation.

BTP01

Alkaline protease as detergents and solvents compatible nanobiocatalyst via immobilization onto functionalized rattle-type magnetic core@mesoporous shell silica nanoparticles

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Alkaline protease from alkaliphilic *Bacillus* sp. NPST-AK15 was immobilized onto functionalized and non-functionalized rattle-type magnetic core@mesoporous shell silica (RT-MCMSS) nanoparticles by physical adsorption and covalent attachment. However, the covalent attachment approach was superior for NPST-AK15 protease immobilization onto the activated RT-MCMSS-NH_2 nanoparticles and was used for further studies. In comparison to free protease, the immobilized enzyme exhibited a shift in the optimal temperature and pH from 60 °C to 65 °C and pH 10.5 to 11.0, respectively. While free protease was completely inactivated after treatment for 1 h at 60 °C, the immobilized enzyme maintained 66.5 % of its initial activity at similar conditions. The immobilized protease showed higher V_{max}, k_{cat} and k_{cat}/K_m, than the soluble enzyme by about 1.3-, 1.3-, and 1.6-fold, respectively. In addition, the results revealed significant improvement of NPST-AK15 protease stability in variety of organic solvents, surfactants, and commercial laundry detergents, upon immobilization onto activated RT-MCMSS-NH_2 nanoparticles. Furthermore, the immobilized protease maintained significant catalytic efficiency for ten consecutive reaction cycles, and was separated easily from the reaction mixture using an external magnetic field. To the best of our knowledge this is the first report about protease immobilization onto rattle-type magnetic core@mesoporous shell silica nanoparticles. The results clearly suggest that the developed immobilized enzyme system is a promising nanobiocatalyst for various bioprocess and protease applications.

BTP02

Construction of a recombinant biocatalyst for the production of phenylacetic acids and phenylethanols from styrenes

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Question: Numerous soil bacteria have been reported to be able to metabolize styrene via the pathway of side-chain oxygenation. This pathway comprises a styrene monooxygenase (SMO), which oxidizes styrene to styrene oxide, a styrene oxide isomerase (SOI), which converts styrene oxide into phenylacetaldehyde, and a phenylacetaldehyde dehydrogenase (PAD). The latter enzyme enables the oxidation of the aldehyde to the central metabolite phenylacetic acid. Further enzymatic steps allow subsequently the degradation of the acid to intermediates of the tricarboxylic acid cycle [1,2]. In this study the construction of a recombinant biocatalyst under consideration of suitable SMOs, SOIs and PADs was intended because this pathway is of potential relevance for the biotechnological production of phenylacetic acids and similar compounds.
Methods: During our present study various enzymes of this pathway from different microorganisms (*Rhodococcus opacus* 1CP, *Sphingopyxis fribergensis* Kp5.2, *Variovorax paradoxus* EPS, *Pseudomonas fluorescens* ST) were investigated with respect to their applicability for the transformation of styrenes to phenylacetic acids. The most active and stable ones were combined to an enzyme cascade which was expressed in suitable *Escherichia coli* strains. Enzyme assays and whole cell biotransformation were used to evaluate these putative biocatalysts.
Results: Remarkably, the integration of a styrene monooxygenase and a styrene oxide isomerase in *E. coli* BL21(DE3) and T7Express resulted in the accumulation of phenylethanols instead of - as expected - phenylacetaldehydes. It can be assumed that most probably the phenylacetaldehydes are transformed immediately into the alcohols by unspecific alcohol dehydrogenases. But, additional transformation of a gene encoding a phenylacetaldehyde dehydrogenase resulted in the formation of significant amounts of phenylacetic acids. Both biotransformations are mainly affected by the activity of the SMO. During this study some SMOs were identified which convert styrene to styrene oxide with high relative activities and allow a stable transformation rate over a period of several days.
Conclusion: Both, phenylacetic acids and phenylethanols, are important chemicals for various industries and these biocatalysts reported herein offer environment-friendly alternatives to common chemical strategies in order to synthesize such compounds.

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BTP03

Optimisation of the bioleaching of REE from FP with chemoorgano-heterotrophic microorganisms

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Rare earth elements (REE) are used in mostly all new technologies and until now, there is nearly no recycling of REE containing end-of-life products [1]. Furthermore, only poor information is available regarding interactions of microorganisms with REE and there are almost no studies describing the bioleaching of REE. However, it can be assumed that microorganisms play an important role in the biogeochemistry of REE. This study investigates the potential of organic acid and metal binding molecules producing microbes to extract REE from technical waste.
During recycling of energy-saving bulbs fluorescent phosphor (FP) is collected as a distinct fraction. It contains about 10 % REE-oxides bound in the hardly water-soluble triband dyes as oxides, phosphates, aluminates and silicates [2]. Previous experiments showed, that the chemoorgano-heterotrophic, organic acid producing microorganisms *Yarrowia lipolytica, Komagataeibacter xylinus* and *Lactobacillus casei* as well as the mixed culture Kombucha are in principle suitable for the bioleaching of REE from FP. In this presentationthe solubilisation process is investigated regarding the leaching metabolites and optimised with respect to maximal REE release.