DECHEMA
FORSCHUNGSINSTITUT
Stiftung bürgerlichen Rechts

Industrial Biotechnology

Research Activities 2017

Preface

DECHEMA-Forschungsinstitut
Interdisciplinary Research for Sustainable Technologies

The DECHEMA-Forschungsinstitut (DFI) stands at the forefront of interdisciplinary research for sustainable materials, processes and products for the industrialized society. It is a scientific research center where chemists, engineers and biotechnologists jointly work on creating novel concepts and innovative interdisciplinary solutions based on materials science, chemical engineering and biotechnology.

The institute has a staff of approx. 80 who are involved in

- Basic and preindustrial **research** in Chemical Engineering, Biotechnology, Environmental Technology, and Materials Sciences
- **Teaching activities** at German universities in the fields mentioned above
- **Continuing professional development** courses for participants from industry and universities
- Development of solutions to **industrial problems**
- **Scientific support** for DECHEMA working parties and conferences

The structure of the institute is undoubtedly unique in Germany: based on the competencies of five academic research groups:

- High Temperature Materials
- Corrosion
- Electrochemistry
- Chemical Technology
- Industrial Biotechnology

These groups, together with additional service units, strive for novel ideas and scientific concepts to target the needs of our industrialized society.
It focuses on three main areas of research, covering the whole spectrum from fundamental aspects to application:

- Energy Efficiency
  - Fuel Cells
  - Metal-Air-Batteries and other energy storage systems
  - Photocatalytic Systems
- Conservation of Resources
  - Innovative Corrosion Protection Systems
  - Recycling of precious metals
  - Water Treatment

- Biotech for Chemical Production
  Utilization of Renewable Resources
  Biotechnological Production Routes for Chemical Products

Driven by the needs of HiTech industries in the fields of biotechnology, materials, and chemical engineering and other industrial areas including energy conversion, automotive and aircraft technologies, the research activities at the DECHEMA-Forschungsinstitut cover the whole spectrum from fundamental aspects to application.

These activities reflect the institute's commitment to bridging the gap between academia and industry in the scientific and technological fields represented by DECHEMA.

Fields of expertise at the DECHEMA-Forschungsinstitut are:

- High temperature materials
- Corrosion protection in extremely aggressive environments
- Development of novel coating systems
- Advanced investigation methods for high temperature corrosion
- Nanoparticle-based coatings
- Modification of anodic oxide layers
- High resolution methods for corrosion investigations
- Microbially influenced corrosion
- Redox-flow batteries
- Metal-air energy storage systems
- Fuel cells
- Reaction engineering
- Photocatalysis
- Functional surfaces
- Molecular electrochemistry
- Electrochemical water treatment
- Bioelectrochemistry
- Bioprocess development
- Enzymatic catalysis and microbial syntheses of fine chemicals
- Metabolic engineering of microorganisms for industrial production

Every year, we publish five *Research Activities* brochures, each presenting one research group.

For more information about the DECHEMA-Forschungsinstitut, please visit: www.dechema-dfi.de

Contents

Industrial Biotechnology Group - Overview

We focus on the synthesis of flavor and fragrance compounds, functional ingredients and other chemicals, which can be neither produced chemically nor harvested from their natural sources in sustainable ways. Special interest is in terpenoids as well as fatty acid and amino acid derivatives. Here, industrial biotechnology offers great opportunities to produce the desired target compounds by using wild type and engineered microbes or enzymes in a safe and environmentally friendly bioprocess under defined and reproducible conditions.

To develop microbial production hosts, we combine metabolic pathway engineering tools and microbiological screening and selection systems. Key challenges for industrial bioprocesses are product toxicity and low conversion yields. We investigate the mechanisms of microbial solvent tolerance to rationally design robust production strains. Investigating transcriptome, proteome and metabolome data leads to a deeper understanding of the molecular mechanisms in the producing cells. This knowledge is employed to develop more efficient production strains and process regimes exhibiting higher product titers and productivities. The biological part of our research is complemented by process engineering approaches already at an early stage of bioprocess development. We design new *in situ* product recovery systems based on adsorption, membrane separation or organic solvent extraction to fully harness the metabolic potential of the engineered production strains. Our research activities converge to tailored bioprocesses on laboratory or pilot scale as a proof-of-concept for novel biotechnological production strategies.

Integrated chemical-biotechnological production

- "Biologisation" of chemical production processes
- Combination of bio-, electro-, and chemo-catalysis
- Microbial electrosynthesis

In the 21st century the chemical industry will play a major role in the development of new materials, substances and processes.

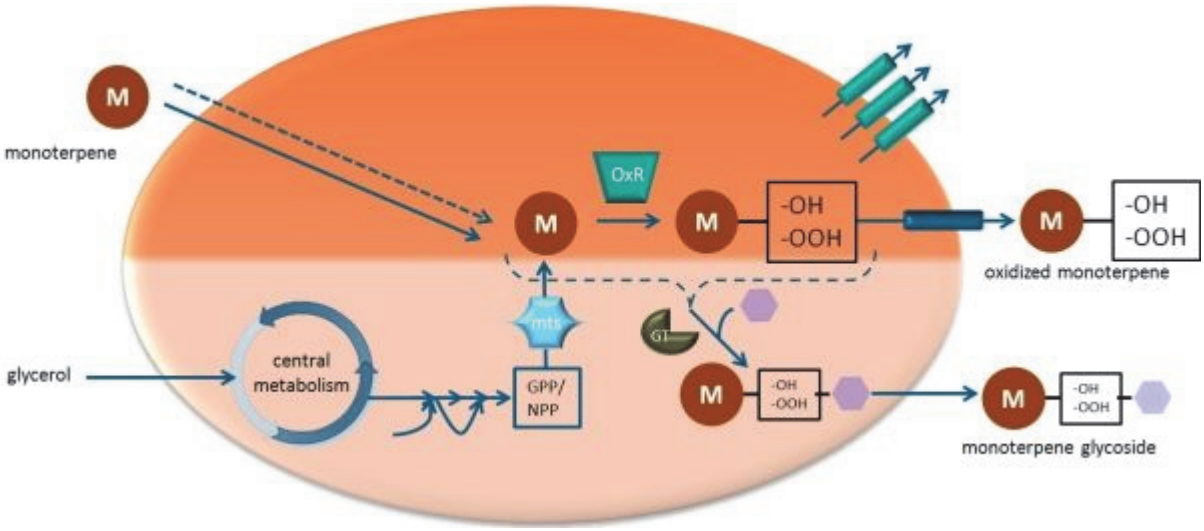
In the future an increase in efficiency and flexibility of the existing production routes is necessary to reduce material and energy consumption. The integration of biotechnological and chemical reaction steps allows novel processes with improved performances and will address the challenges of climate change and clean energy.

By combining different disciplines such as bio-, electro- and chemo-catalysis, molecular biology and process engineering novel production routes for the pharmaceutical, cosmetics and chemical industry, agro-chemicals as well as the food area will be developed. The chemo-, regio-and enantioselectivity of enzymatic reactions enables novel synthesis routes that are currently difficult to achieve by chemical processes. The combination of chemo and biocatalysis is a key technology for the efficient use of renewable resources and the development of environmentally friendly and resources-efficient production processes. For this purpose, molecular biology is used to develop enzymes and microorganisms with tailored properties.

The combination of electrochemistry and enzyme reactions to electroenzymatic processes offers a variety of possibilities for advanced production systems. As a novel and highly energy efficient process microbial electrosynthesis is investigated. In microbial electrosynthesis electrons are transferred between electrodes and microorganisms. The electrical energy can be used directly in the metabolism of microorganisms to produce valuable compounds.

Research Projects 2017

BioProMo - Biotechnological production of Monoterpenoids



Period: 01.04.2016 - 31.03.2019

Partners: Universität Wageningen (Wageningen, NL)
Centre de Recerca en AgriGenomica (Barcelona, ES)
Symrise AG (Holzminden, DE)

Funder: BMEL über Fachagentur Nachwachsende Rohstoffe (FNR)

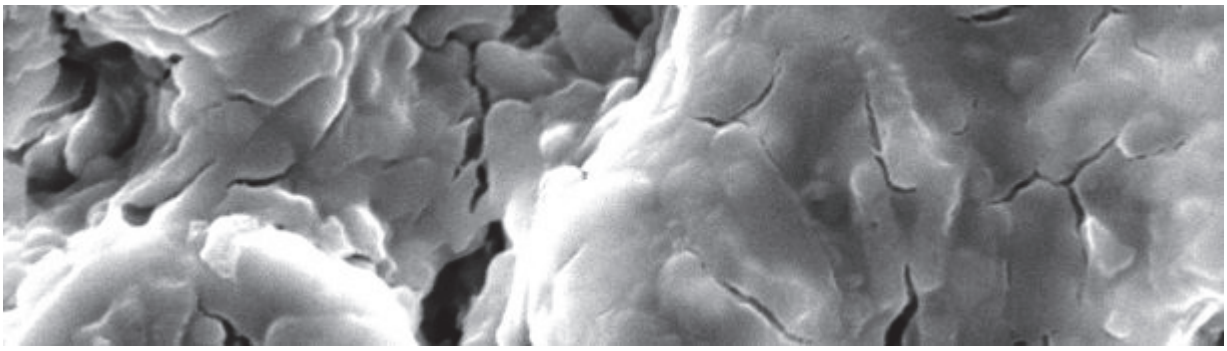
Many different monoterpenoids are valuable flavor and fragrance compounds, functional ingredients in cosmetics and potential agrochemicals. The BioProMo consortium will create an industrial biotechnology complement to fossil-resources-based chemical processes for industrial monoterpenoid production. A sustainable and competitive platform technology based on the solvent resistant microbe *Pseudomonas putida* will be established by combining biotechnological methods such as functional genomics, metabolic engineering, synthetic biology and bioprocess engineering.
Key aspects addressed by BioProMo are the increase of resilience in a monoterpenoid tolerant *P. putida* strain at the level of efflux pumps, the mitigation of monoterpenoid toxicity by producing them as glycosides and the usage of metabolic switches, shunt pathways and enzyme coupling to create a resilient and efficient production host.
The microbial platform aimed at will create two novel production routes: a) a whole-cell biocatalysis to selectively oxyfunctionalize a monoterpene hydrocarbon, a cheap by-product of the food processing industry (short –term goal) and b) a self-regulated

de novo production circuit starting from the renewable raw material glycerol, a by-product of biodiesel production (mid-term goal). The project unites the complementary know-how and expertise of European research groups from three different countries to accomplish the goal of establishing a microbial production platform for monoterpenoids. The industrial partner of BioProMo will not only advise the research project from its market-oriented viewpoint but also actively participate in research and development to transfer respective processes into application.

Gefördert durch:

aufgrund eines Beschlusses
des Deutschen Bundestages

Combining innovative biofilm monitoring with microbial electrosynthesis for resource-efficient production of hydroxylated base chemicals



Period: 01.11.2014 - 31.10.2017

Partner: Kurt-Schwabe Institut für Mess- und Sensortechnik e.V. Meinsberg

Funder: AiF

Biofilms are consortia of microorganisms which form a matrix of extra polymeric substances. These biofilms could have a higher resistance to antibiotics in the medical field, and in addition, lead to biocorrosion in industrial plants. Apart from the listed negative examples biofilms can play an important role in continuous bioprocesses for cell retention and long-term stability of the bio-catalyst (productive biofilms). In systems in which whole cells are the catalysts artificial immobilization techniques are often used. This can affect the long-term activity of the microorganisms and cause additional efforts and costs. Biofilms provide "natural" immobilisats of microorganisms.

In this context the "fitness" of biofilms is important to measure. Through this, efficient processes can be developed based on the catalytic biofilms. No suitable monitoring concept for the online measurement of biofilm formation is established yet. The aim is therefore to develop online sensors which provide the information at any time about the current state of a biofilm. Here the electrochemical impedance spectroscopy (EIS) is used, which was already tested in some application. As an example biofilms are investigated, which are used for microbial electrosynthesis.

Supported by:

Federal Ministry
for Economic Affairs
and Energy

on the basis of a decision
by the German Bundestag

Das IGF-Vorhaben Nr. 18450 BG der Forschungsvereinigung DECHEMA e.V., Theodor-Heuss-Allee 25, 60486 Frankfurt am Main wurde über die AiF im Rahmen des Programms zur Förderung der industriellen Gemeinschaftsforschung (IGF) vom Bundesministerium für Wirtschaft und Energie aufgrund eines Beschlusses des Deutschen Bundestages gefördert.

NIESEL – Deep eutectic solvents as reaction media for biocatalytic processes

Period: 01.04.2015 - 31.03.2018

Partners: Autodisplay Biotech GmbH
Phytowelt Green Technologies GmbH,
Westfälische Wilhelms Universität Münster

Funder: BMBF

The biocatalytic transformation of substrates that are sparingly soluble in aqueous media is challenging in terms of reaction and process design. On the one hand, suitable solvents are necessary to deliver water-insoluble substrates to the respective target enzyme. On the other hand, solvent-resistant enzymes or microorganisms are required exhibiting good activity in organic solvents. The existing concepts to overcome such limitations comprise two-phase systems, supercritical fluids or the application of ionic liquids. However, from an ecological as well as from an economical perspective some of these solutions are sometimes inacceptable.

In this project the application of deep eutectic solvents (DES; also called NADES in the case of natural deep eutectic solvents) is investigated in terms of dissolution or extraction of sparingly water-soluble substrates. DESs are a novel class of solvents generated by mixing two solids, upon which a depression of the freezing point is caused by the interaction of the initial compounds on the molecular level (hydrogen bond). In contrast to classical ionic liquids, DESs are associated with less toxicity, better storability and higher biodegradability. Moreover, DESs are non-volatile and might be an interesting alternative to conventional solvents when hardly soluble substrates are present in a biocatalytic reaction.

Within the framework of this project different DESs will be synthesized, characterized by different methods and eventually applied in various biotransformations. The results obtained with DES-based biotransformations will be compared to already established systems such as organic solvent or ionic liquids.

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MIKE – Methanation of CO_2 from biogas by microbial electrosynthesis

Period:	01.09.2016 - 31.08.2019
Partners:	Infraserv GmbH & Co. Höchst KG, Ifn FTZ GmbH, Provadis School of International Management and Technology
Funder:	BMBF

Commercial produced biogas contains app. 60% of methane, 35% of CO_2 and 5% of steam, nitrogen and further impurities. To feed in the biogas into the existing natural gas grid it has to be cleaned from all impurities at the moment in a cost-intensive way. In line with the funding initiative "CO_2Plus – Stoffliche Nutzung von CO_2 zur Verbreiterung der Rohstoffbasis" from the Federal Ministry of Education and Research the project "MIKE" is going to use microbial electrosynthesis to increase the methane output of an industrial biogas plant and therefore to reduce the cleaning effort.

In microbial electrosynthesis electroactive microorganisms take up electrons from a cathode for the reduction of CO_2 to different chemical products, e.g. methane. In contrast to other processes, the microbial electrosynthesis has very high cathodic electron efficiency (› 80%). As electron source current can be used from renewable energies or from excess current.

The main goals of the project are as follows:

1. Development of a robust biocatalyst for the microbial electrosynthesis of CH_4 from impure CO_2 of an industrial biogas plant.
2. Engineering and construction of a MES-prototype and testing on the lab scale.
3. Integration of the MES pilot plant into one of the biggest industrial biogas plants at the industrial park Höchst (Frankfurt, Germany).
4. Operation of the MES-prototype under real industrial conditions over several months.
5. Evaluation of the developed processes by economic calculations and CO_2 foot-print.

Due to the project “MIKE”, not only the methane yield of an industrial biogas plant can be increased, but also excess current can be stored as methane.

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Methylobacterium extorquens - a microbial cell factory for production of chemicals from methanol

Methanol represents an attractive, very flexible feedstock for industrial biotechnology. It is efficiently synthesized via syngas from cheap natural gas and other fossil resources, but can also be derived from renewables such as wood or biogas. As a biotech feedstock, methanol does not interfere with the use of arable land for food and nutrition, and being a liquid it is easier to supply and control in bioprocesses than gaseous substrates such as syngas, methane, CO_2 or H_2. The host organism of our methylotrophic cell factory is *Methylobacterium extorquens*, which assimilates methanol via the serine cycle, whereas the ethylmalonyl-CoA pathway (EMCP) is necessary for glyoxylate regeneration during growth on methanol. Intermediates of the EMCP include acetoacetyl-CoA and dicarboxylic acid CoA esters usually not found in primary metabolism of conventional host strains and represent starting intermediates for the synthesis of e.g. dicarboxylic acids and terpenoids.

Chiramet – Chiral building blocks produced from the biomass conversion product methanol

Period: 01.07.2017 - 31.06.2020

Partners: Westfälische Wilhelms-Universität (Münster, DE)
Chiracon GmbH (Luckenwalde, DE)
Insilico Biotechnology AG (Stuttgart, DE)
Max-Planck-Institut für terrestrische Mikrobiologie (Marburg, DE)

Funder: BMBF

Chiral compounds are important building blocks for the synthesis of fine chemicals, especially pharmaceuticals. Goal of the Chiramet project is the development of biotechnological processes for production of different chiral compounds. Instead of sugar, the potential biomass conversion product methanol is used as carbon source, which is consistent with our efforts towards the biologisation of chemistry.

An increasing share of future methanol is expected to be produced from renewables or sustainable sources, by chemical conversion of biogas, wood, or solid municipal waste or by chemical recycling of carbon dioxide with renewable energy sources. The microorganism used in Chiramet is Methylobacterium extorquens, which is able to grow with methanol as sole carbon source. In order to secrete certain chiral substances, thioesterases catalyzing the hydrolysis of primary metabolism intermediates are expressed. In cooperation with the project partners, repective enzymes are identified and optimized and metabolic optimizations will be guided by in silico models. Furthermore lab scale bioprocesses and downstream procedures will be developed.

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Microbial Electrosynthesis – evaluating the usage of electric energy for future microbial production processes

Period: 01.03.2013 - 28.02.2018

Partner: Electrochemistry Group at DFI

Funder: BMBF

Microbial Electrosynthesis is a new interdisciplinary research field for the production of biofuels and basic chemicals from oxidized substrates such as carbon dioxide. Electrons supplied by a cathode enable the reductive synthesis of these substrates catalyzed by electroactive microorganisms. These organisms are able to interact directly with the cathode or utilize electrochemically reduced media components such as redox mediators or hydrogen. To date the biological mechanisms for the electron uptake are poorly understood.Therefore, our research at the DFI focuses on the interaction of electroactive microorganisms with cathodes including biofilm formation and cell immobilization on different electrode materials. Different electron uptake strategies such as the direct transfer are being compared to the indirect, mainly mediator-based electron transfer mechanisms concerning production efficiency.

Bioelectrochemical reactor designs are developed and optimized to enhance microbial growth and production of chemicals helping to improve our electroactive microorganisms. A broad spectrum of molecular and microbial as well as electrochemical methods (e.g. impedance spectroscopy, cyclic voltammetry) are used to characterize the systems. The research groups Induratrial biotechnology and Electrochemistry are working on this tandem project at the DFI.

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PseudoProKo - *Pseudomonas putida* – a platform for liquid hydrocarbon production

Period: 01.03.15 - 25.02.2018

Funder: BMEL über Fachagentur Nachwachsende Rohstoffe (FNR)

Considering the depletion of fossil fuels, it is essential to further develop the efficient utilization of renewable resources. In bioindustrial processes, microbial production platforms can be used to generate a diverse array of valuable chemicals from simple substrates. In this project we aim at the microbial conversion of domestic plant oil into higher value lipids such as fatty aldehydes, fatty alcohols, and alkanes/alkenes.
Most studies focus on a *de novo* approach via the fatty acid biosynthesis by fermentation of simple substrates such as glucose. In our work, an auto-displayed lipase hydrolyzes triglycerides from rapeseed oil to glycerol and free fatty acids (mainly unsaturated C16 and C18). While glycerol serves as carbon and energy source, the fatty acids are further converted intracellularly. A reduction to **fatty aldehydes** is achieved by introduction of a carboxylic acid reductase. Alternatively, a plant α-dioxygenase generates n-1 fatty aldehydes. The fatty aldehydes are further reduced to **fatty alcohols** by a variety of endogenous *E. coli* reductases. The overexpression or deletion of respective genes can increase or limit the alcohol formation. In a second step the fatty aldehydes can be converted to n-1 **alkanes/alkenes** by cyanobacterial aldehyde deformylating oxygenases.

Gefördert durch:

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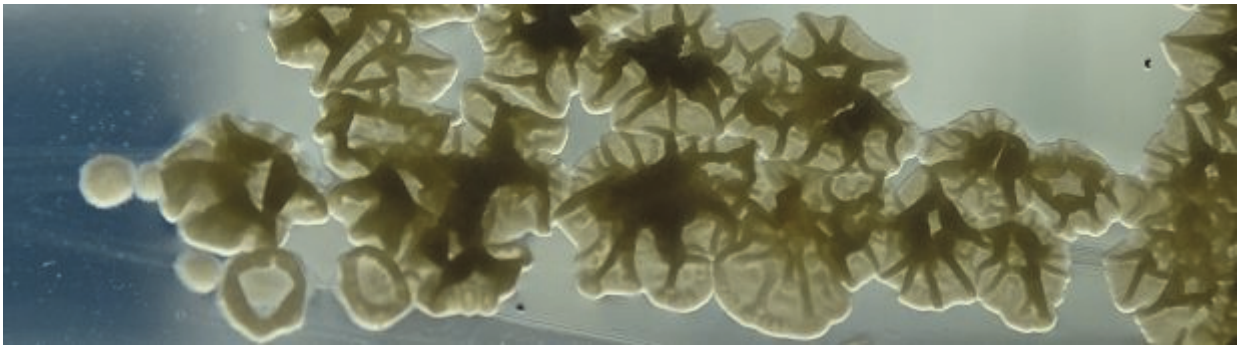
für Ernährung
und Landwirtschaft

aufgrund eines Beschlusses
des Deutschen Bundestages

"New to nature" terpenes for liquid fuels from renewable raw materials



Period: 01.09.2014 - 31.12.2017

Funder: BMEL über Fachagentur Nachwachsende Rohstoffe (FNR)

The substance class of terpenoid hydrocarbons is discussed as a resource for molecules able to substitute petrochemistry-based liquid fuels. Research done mainly by U.S. research groups and companies has already laid the foundation for microbial de novo synthesis of various terpenes including potential fuel molecules. However, it will not be possible to use a pure terpene directly in existing internal combustion engines. Instead, a mixture of various hydrocarbons is needed to obtain an engine fuel, which is compatible with the existing infrastructure.

With this research project, an extension of the product range of microbial terpene production processes is explored. During the project, the technological foundation for the synthesis of "new to nature" terpenes with bacterial enzymes will be laid. This aspect provides the substance diversity required for biotechnological production of "drop-in" fuels in a mid- and long-term perspective. Moreover, the variety of uncommon terpenoid structures might be also applied as aroma and pharma compounds.

Gefördert durch:

Bundesministerium
für Ernährung
und Landwirtschaft

aufgrund eines Beschlusses
des Deutschen Bundestages

Selected publications

J. Mi, H. Schewe, M. Buchhaupt, D. Holtmann, J. Schrader

Efficient hydroxylation of 1,8-cineole with monoterpenoid-resistant recombinant *Pseudomonas putida* GS1

World Journal of Microbiology and Biotechnology (2016) DOI: 10.1007/s11274-016-2071-y

In this work, monoterpenoid hydroxylation with *Pseudomonas putida* GS1 and KT2440 were investigated as host strains, and the cytochrome P450 monooxygenase CYP176A1 (P450cin) and its native redox partner cindoxin (CinC) from *Citrobacter braakii* were introduced in *P. putida* to catalyze the stereoselective hydroxylation of 1,8-cineole to (1*R*)-6-hydroxy-1,8-cineole. Growth experiments in the presence of 1,8-cineole confirmed pseudomonads' superior resilience compared to *E. coli*. Whole-cell *P. putida* harboring P450cin with and without CinC were capable of hydroxylating 1,8-cineole, whereas coexpression of CinC has been shown to accelerate this bioconversion. Under the same conditions, *P. putida* GS1 produced more than twice the amount of heterologous P450cin and bioconversion product than *P. putida* KT2440. A concentration of 1.1 ± 0.1 g/L (1*R*)-6-hydroxy-1,8-cineole was obtained within 55 h in shake flasks and 13.3 ± 1.9 g/L in 89 h in a bioreactor, the latter of which corresponds to a yield YP/S of 79 %. To the authors' knowledge, this is the highest product titer for a P450 based whole-cell monoterpene oxyfunctionalization reported so far. These results show that solvent-tolerant *P. putida* GS1 can be used as a highly efficient recombinant whole-cell biocatalyst for a P450 monooxygenase-based valorization of monoterpenoids.

A. Tosstorff, C. Kroner, D. J. Oppermann, F. Hollmann, D. Holtmann

Towards electroenzymatic processes involving old yellow enzymes and mediated cofactor regeneration

Engineering in Life Science (2016) DOI: 10.1002/elsc.201600158

Old yellow enzymes (OYEs) are able to catalyze asymmetric C = C reductions. A mediated electro enzymatic process to regenerate the NADPH in combination with an OYE was investigated. Due to the fact that the overall process was affected by a broad set of parameters a DoE approach was chosen to identify suitable process conditions. Process conditions with high productivities of up to 2.27 mM/h in combination with approx. 90% electron transfer efficiency were identified.

J. Mi, A. Sydow, F. Schempp, D. Becher, H. Schewe, J. Schrader, M. Buchhaupt
Investigation of plasmid-induced growth defect in *Pseudomonas putida*
Journal of Biotechnology 231 (2016) 167-173

Genetic engineering in bacteria mainly relies on the use of plasmids. But despite their pervasive use for physiological studies as well as for the design and optimization of industrially used production strains, only limited information about plasmid induced growth defects is available for different replicons and organisms. Here, we present the identification and characterization of such a phenomenon for Pseudomonas putida transformants carrying the pBBR1-derived plasmid pMiS1. We identified the kanamycin resistance gene and the transcription factor encoding rhaR gene to be causal for the growth defect in P. putida. In contrast, this effect was not observed in Escherichia coli. The plasmid-induced growth defect was eliminated after introduction of a mutation in the plasmid-encoded rep gene, thus enabling construction of the non-toxic variant pMiS4. GFP reporters construct analyses and qPCR experiments revealed a distinctly lowered plasmid copy number for pMiS4, which is probably the reason for alleviation of the growth defect by this mutation. Our work expands the knowledge about plasmid-induced growth defects and provides a useful low-copy pBBR1 replicon variant.

F. Sonntag, C. Kroner, P. Lubuta, R. Peyraud, A. Horst, M. Buchhaupt, J. Schrader
Engineering *Methylobacterium extorquens* for *de novo* synthesis of the sesquiterpenoid -humulene from methanol
Metabolic Engineering 32 (2015) 82-94

Over the last 10 to 15 years, metabolic engineering of microbes has become a versatile tool for high-level de novo synthesis of terpenoids, with the sesquiterpenoids armopha-1,4-diene, farnesene and artemisinic acid as prime examples. However, almost all cell factory approaches towards terpenoids to date have been based on sugar as the raw material, which is mainly used as a food resource and subject to high price volatilities. In this study we present de novo synthesis of the sesquiterpenoid -humulene from the abundantly available non-food carbon source methanol by metabolically engineered *Methylobacterium extorquens* AM1. Expression of -humulene synthase from *Zingiber zerumbet* in combination with farnesyl pyrophosphate (FPP) synthase from *Saccharomyces cerevisiae* led to concentrations of up to 18 mg/L -humulene. Introduction of a prokaryotic mevalonate pathway from *Myxococcus xanthus* in combination with ribosome binding site optimization of -humulene and FPP synthases increased product concentra-

tion 3-fold. This value was additionally raised by 30% using a carotenoid synthesis deficient mutant strain. Final product concentrations of up to 1.65 g/L were obtained in methanol limited fed-batch cultivations, which is the highest titer of de novo synthesized -humulene reported to date. This study demonstrates the potential of *M. extorquens* as a future platform strain for the production of high-value terpenoids from the alternative carbon source methanol.

F. Sonntag, J.E. Müller, P. Kiefer, J.A. Vorholt , J.Schrader, M. Buchhaupt

High-level production of ethylmalonyl-CoA pathway-derived dicarboxylic acids by *Methylobacterium extorquens* under cobalt-deficient conditions and by polyhydroxybutyrate negative strains

Applied Microbiology and Biotechnology 99/8 (2015) 3407-3419

Bio-based production of dicarboxylic acids is an emerging research field with remarkable progress during the last decades. The recently established synthesis of the ethylmalonyl-CoA pathway (EMCP)-derived dicarboxylic acids, mesaconic acid and (2*S*)-methylsuccinic acid, from the alternative carbon source methanol (Sonntag et al., Appl Microbiol Biotechnol 98:4533–4544, 2014) gave a proof of concept for the sustainable production of hitherto biotechnologically inaccessible monomers. In this study, substantial optimizations of the process by different approaches are presented. Abolishment of mesaconic and (2*S*)-methylsuccinic acid reuptake from culture supernatant and a productivity increase were achieved by 30-fold decreased sodium ion availability in culture medium. Undesired flux from EMCP into polyhydroxybutyrate (PHB) cycle was hindered by the knockout of polyhydroxyalkanoate synthase *phaC* which was concomitant with 5-fold increased product concentrations. However, frequently occurring suppressors of strain Δ*phaC* lost their beneficial properties probably due to redirected channeling of acetyl-CoA. Pool sizes of the product precursors were increased by exploiting the presence of two cobalt-dependent mutases in the EMCP: Fine-tuned growth-limiting cobalt concentrations led to 16-fold accumulation of mesaconyl- and (2*S*)-methylsuccinyl-CoA which in turn resulted in 6-fold increased concentrations of mesaconic and (2*S*)-methylsuccinic acids, with a combined titer of 0.65 g/l, representing a yield of 0.17 g/g methanol. This work represents an important step toward an industrially relevant production of ethylmalonyl-CoA pathway-derived dicarboxylic acids and the generation of a stable PHB synthesis negative *Methylobacterium extorquens* strain.

T. Krieg, F. Enzmann, D. Sell, J. Schrader, D. Holtmann

Simulation of the current generation of a microbial fuel cell in a laboratory wastewater treatment plant

Applied Energy 195 (2017) 942-949

Microbial fuel cells (MFCs) are devices generating electrical current from a wide range of organic substrates by using bacterial metabolism. Integrations of MFCs into wastewater treatment plants seem to be the most likely application of this technology. Due to the fact that the current flow in a MFC is fundamentally produced by the metabolic activity of microorganisms, it would be desirable to elucidate the capacity of the microbial systems to optimize the energy extraction processes in MFCs. In this study, the correlation between the parameters X_{BH} (active heterotrophic biomass) and X_{BA} (active autotrophic biomass) from the established activated sludge model number 1 (ASM1) and the measured current flow in MFCs was investigated for the first time. The simulation protocol based on ASM1 shows a good congruence between measured and simulated effluent values for the wastewater treatment plant. Comparisons between the measured current densities and the simulated concentrations of active biomass showed linear correlations at substrate pulses and at different residence times of the substrate. Therefore, it can be concluded that the model parameter X_{BH} and X_{BA} of the ASM1 can be used to estimate the current output of a MFC in wastewater treatment plants. The identified correlations can be used to optimize operating conditions and to generate high current outputs of the MFCs based on simulations.

A.E.W. Horst, S. Bormann, J. Meyer*, M. Steinhagen, R. Ludwig, A. Drews, M. Ansorge-Schumacher, D. Holtmann*

Electro-enzymatic hydroxylation of ethylbenzene by the evolved unspecific peroxygenase of *Agrocybe aegerita*

Journal of Molecular Catalysis B: Enzymatic (2016) DOI: 10.1002/elsc.201600158

The unspecific peroxygenase from the fungus *Agrocybe aegerita* (*Aae*UPO) is an up-and-coming biocatalyst that is able to perform specific oxyfunctionalizations of various substrates. Due to inactivation at excess concentrations of its co-substrate H2O2, *Aae*UPO's technical application is still limited. This study aims to promote catalyst efficiency *via* electrochemical *in situ* supply of H2O2, using an evolved variant of *Aae*UPO on the example of ethylbenzene hydroxylation. Total turnover numbers of up to 400,000 molproduct molAaeUPO–1 and space-time-yields of up to 25 g L–1 d–1 were achieved in

the electro-enzymatic system. These numbers are in the upper range of published data. The presented system stands out by its very high atom economy. Thus, combining electrochemistry and biocatalysis is one step closer towards the first application of peroxygenases in an industrial process.

A. Sydow, T. Krieg, R. Ulber, D. Holtmann

Growth medium and electrolyte – how to combine the different requirements on the reaction solution in bioelectrochemical systems using *Cupriavidus necator*

Engineering in Life Science (2017) DOI: 10.1002/elsc.201600252

Microbial electrosynthesis is a relatively new research field where microbial carbon dioxide fixation based on the energy supplied by a cathode is investigated. Reaction media used in such bioelectrochemical systems have to fulfill requirements of classical biotechnology as well as electrochemistry. The design and characterization of a medium that enables fast electroautotrophic growth of *Cupriavidus necator* in microbial electrosynthesis was investigated in detail. The identified chloride-free medium mainly consists of low buffer concentration and is supplied with trace elements. Biotechnologically relevant parameters, such as high specific growth rates and short lag phases, were determined for growth characterization. Fast growth under all conditions tested, i.e. heterotrophic, autotrophic and electroautotrophic was achieved. The lag phase was shortened by increasing the $FeSO_4$ concentration. Additionally, electrochemical robustness of the reaction media was proven. Under reductive conditions, no deposits on electrodes or precipitations in the media were observed and no detectable hydrogen peroxide evolved. In the bioelectrochemical system, no lag phase occurred and specific growth rate of *C. necator* was 0.09 h^{-1}. Using this medium shortens seed train drastically and enables fast electrobiotechnological production processes based on *C. necator*.

F. W. Ströhle, E. Kranen, J. Schrader, R. Maas, D. Holtmann

A simplified process design for P450 driven hydroxylation based on surface displayed enzymes Biotechnology and Bioengineering (2015)

Biotechnology and Bioengineering (2015) DOI: 10.1002/bit.25885

New production routes for fine and bulk chemicals are important to establish further sustainable processes in industry. Besides the identification of new biocatalysts and new production routes the optimization of existing processes in regard to an improved utilization of the catalysts are needed. In this paper we describe the successful expression of P450BM3 on the surface of *E. coli* cells with the Autodisplay system. The successful hydroxylation of palmitic acid by using surface-displayed P450BM3 was shown. Besides optimization of surface protein expression, several cofactor regeneration systems were compared and evaluated. Afterwards, the development of a suitable process for the biocatalytic hydroxylation of fatty acids based on the re-use of the catalysts after a simple centrifugation was investigated. It was shown that the catalyst can be used for several times without any loss in activity. By using surface-displayed P450s in combination with an enzymatic cofactor regeneration system a total turnover number of up to 54,700 could be reached, to the knowledge of the authors the highest value reported for a P450 monooxygenase to date. Further optimizations of the described reaction system can have an enormous impact on the process design for more sustainable bioprocesses.

For further information, also to completed projects or about the DECHEMA-Forschungsinstitut, please visit: www.dechema-dfi.de

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Preface

DECHEMA-Forschungsinstitut
Interdisciplinary Research for Sustainable Technologies

The DECHEMA-Forschungsinstitut (DFI) stands at the forefront of interdisciplinary research for sustainable materials, processes and products for the industrialized society. It is a scientific research center where chemists, engineers and biotechnologists jointly work on creating novel concepts and innovative interdisciplinary solutions based on materials science, chemical engineering and biotechnology.

The institute has a staff of approx. 80 who are involved in

- Basic and preindustrial **research** in Chemical Engineering, Biotechnology, Environmental Technology, and Materials Sciences
- **Teaching activities** at German universities in the fields mentioned above
- **Continuing professional development** courses for participants from industry and universities
- Development of solutions to **industrial problems**
- **Scientific support** for DECHEMA working parties and conferences

The structure of the institute is undoubtedly unique in Germany: based on the competencies of five academic research groups:

- High Temperature Materials
- Corrosion
- Electrochemistry
- Chemical Technology
- Industrial Biotechnology

These groups, together with additional service units, strive for novel ideas and scientific concepts to target the needs of our industrialized society.
It focuses on three main areas of research, covering the whole spectrum from fundamental aspects to application:

- Energy Efficiency
  - Fuel Cells
  - Metal-Air-Batteries and other energy storage systems
  - Photocatalytic Systems
- Conservation of Resources
  - Innovative Corrosion Protection Systems
  - Recycling of precious metals
  - Water Treatment

- Biotech for Chemical Production
  Utilization of Renewable Resources
  Biotechnological Production Routes for Chemical Products

Driven by the needs of HiTech industries in the fields of biotechnology, materials, and chemical engineering and other industrial areas including energy conversion, automotive and aircraft technologies, the research activities at the DECHEMA-Forschungsinstitut cover the whole spectrum from fundamental aspects to application.

These activities reflect the institute's commitment to bridging the gap between academia and industry in the scientific and technological fields represented by DECHEMA.

Fields of expertise at the DECHEMA-Forschungsinstitut are:

- High temperature materials
- Corrosion protection in extremely aggressive environments
- Development of novel coating systems
- Advanced investigation methods for high temperature corrosion
- Nanoparticle-based coatings
- Modification of anodic oxide layers
- High resolution methods for corrosion investigations
- Microbially influenced corrosion
- Redox-flow batteries
- Metal-air energy storage systems
- Fuel cells
- Reaction engineering
- Photocatalysis
- Functional surfaces
- Molecular electrochemistry
- Electrochemical water treatment
- Bioelectrochemistry
- Bioprocess development
- Enzymatic catalysis and microbial syntheses of fine chemicals
- Metabolic engineering of microorganisms for industrial production

Every year, we publish five *Research Activities* brochures, each presenting one research group.

For more information about the DECHEMA-Forschungsinstitut, please visit: www.dechema-dfi.de

Contents

Industrial Biotechnology Group - Overview

We focus on the synthesis of flavor and fragrance compounds, functional ingredients and other chemicals, which can be neither produced chemically nor harvested from their natural sources in sustainable ways. Special interest is in terpenoids as well as fatty acid and amino acid derivatives. Here, industrial biotechnology offers great opportunities to produce the desired target compounds by using wild type and engineered microbes or enzymes in a safe and environmentally friendly bioprocess under defined and reproducible conditions.

To develop microbial production hosts, we combine metabolic pathway engineering tools and microbiological screening and selection systems. Key challenges for industrial bioprocesses are product toxicity and low conversion yields. We investigate the mechanisms of microbial solvent tolerance to rationally design robust production strains. Investigating transcriptome, proteome and metabolome data leads to a deeper understanding of the molecular mechanisms in the producing cells. This knowledge is employed to develop more efficient production strains and process regimes exhibiting higher product titers and productivities. The biological part of our research is complemented by process engineering approaches already at an early stage of bioprocess development. We design new *in situ* product recovery systems based on adsorption, membrane separation or organic solvent extraction to fully harness the metabolic potential of the engineered production strains. Our research activities converge to tailored bioprocesses on laboratory or pilot scale as a proof-of-concept for novel biotechnological production strategies.

Integrated chemical-biotechnological production

- "Biologisation" of chemical production processes
- Combination of bio-, electro-, and chemo-catalysis
- Microbial electrosynthesis

In the 21st century the chemical industry will play a major role in the development of new materials, substances and processes.

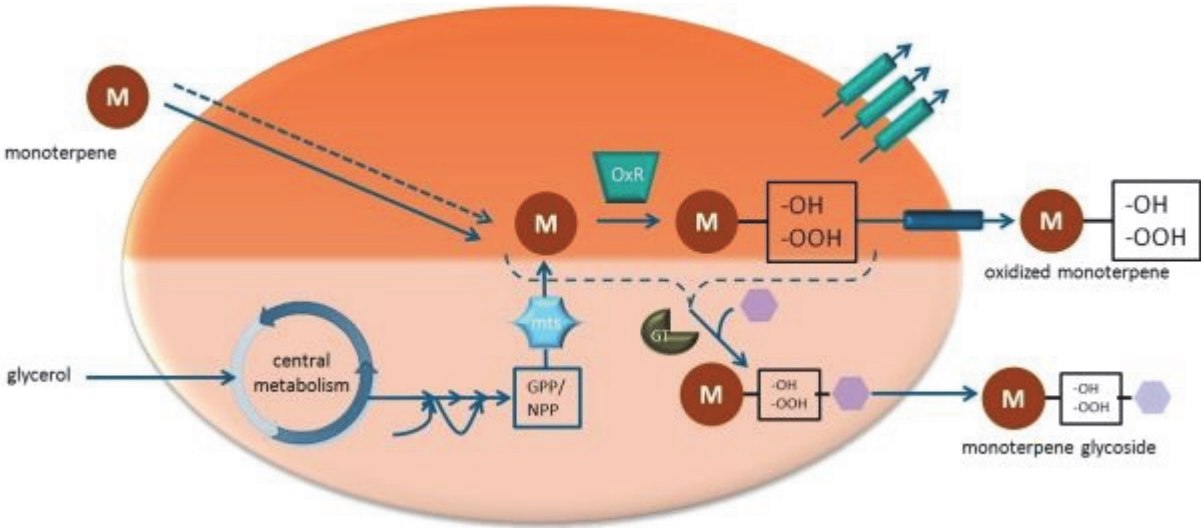
In the future an increase in efficiency and flexibility of the existing production routes is necessary to reduce material and energy consumption. The integration of biotechnological and chemical reaction steps allows novel processes with improved performances and will address the challenges of climate change and clean energy.

By combining different disciplines such as bio-, electro- and chemo-catalysis, molecular biology and process engineering novel production routes for the pharmaceutical, cosmetics and chemical industry, agro-chemicals as well as the food area will be developed. The chemo-, regio-and enantioselectivity of enzymatic reactions enables novel synthesis routes that are currently difficult to achieve by chemical processes. The combination of chemo and biocatalysis is a key technology for the efficient use of renewable resources and the development of environmentally friendly and resources-efficient production processes. For this purpose, molecular biology is used to develop enzymes and microorganisms with tailored properties.

The combination of electrochemistry and enzyme reactions to electroenzymatic processes offers a variety of possibilities for advanced production systems. As a novel and highly energy efficient process microbial electrosynthesis is investigated. In microbial electrosynthesis electrons are transferred between electrodes and microorganisms. The electrical energy can be used directly in the metabolism of microorganisms to produce valuable compounds.

Research Projects 2017

BioProMo - Biotechnological production of Monoterpenoids



Period: 01.04.2016 - 31.03.2019

Partners: Universität Wageningen (Wageningen, NL)
Centre de Recerca en AgriGenomica (Barcelona, ES)
Symrise AG (Holzminden, DE)

Funder: BMEL über Fachagentur Nachwachsende Rohstoffe (FNR)

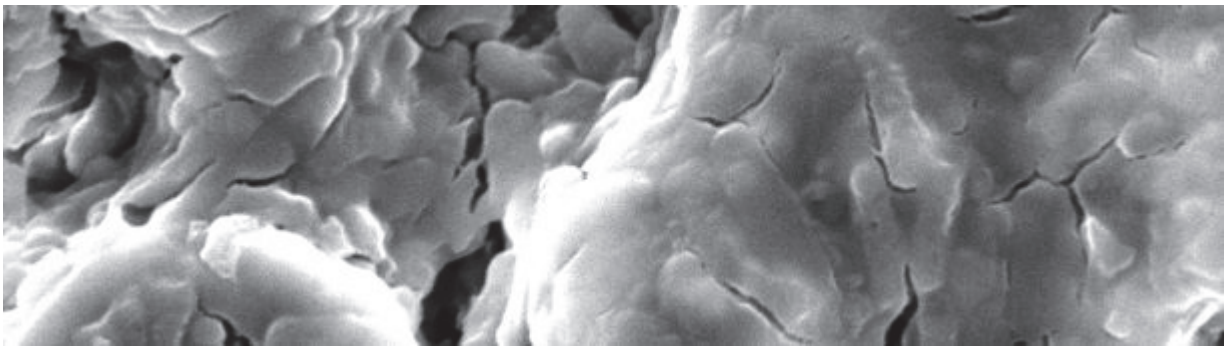
Many different monoterpenoids are valuable flavor and fragrance compounds, functional ingredients in cosmetics and potential agrochemicals. The BioProMo consortium will create an industrial biotechnology complement to fossil-resources-based chemical processes for industrial monoterpenoid production. A sustainable and competitive platform technology based on the solvent resistant microbe *Pseudomonas putida* will be established by combining biotechnological methods such as functional genomics, metabolic engineering, synthetic biology and bioprocess engineering.
Key aspects addressed by BioProMo are the increase of resilience in a monoterpenoid tolerant *P. putida* strain at the level of efflux pumps, the mitigation of monoterpenoid toxicity by producing them as glycosides and the usage of metabolic switches, shunt pathways and enzyme coupling to create a resilient and efficient production host.
The microbial platform aimed at will create two novel production routes: a) a whole-cell biocatalysis to selectively oxyfunctionalize a monoterpene hydrocarbon, a cheap by-product of the food processing industry (short –term goal) and b) a self-regulated

de novo production circuit starting from the renewable raw material glycerol, a by-product of biodiesel production (mid-term goal). The project unites the complementary know-how and expertise of European research groups from three different countries to accomplish the goal of establishing a microbial production platform for monoterpenoids. The industrial partner of BioProMo will not only advise the research project from its market-oriented viewpoint but also actively participate in research and development to transfer respective processes into application.

Gefördert durch:

aufgrund eines Beschlusses
des Deutschen Bundestages

Combining innovative biofilm monitoring with microbial electrosynthesis for resource-efficient production of hydroxylated base chemicals



Period: 01.11.2014 - 31.10.2017

Partner: Kurt-Schwabe Institut für Mess- und Sensortechnik e.V. Meinsberg

Funder: AiF

Biofilms are consortia of microorganisms which form a matrix of extra polymeric substances. These biofilms could have a higher resistance to antibiotics in the medical field, and in addition, lead to biocorrosion in industrial plants. Apart from the listed negative examples biofilms can play an important role in continuous bioprocesses for cell retention and long-term stability of the bio-catalyst (productive biofilms). In systems in which whole cells are the catalysts artificial immobilization techniques are often used. This can affect the long-term activity of the microorganisms and cause additional efforts and costs. Biofilms provide "natural" immobilisats of microorganisms.

In this context the "fitness" of biofilms is important to measure. Through this, efficient processes can be developed based on the catalytic biofilms. No suitable monitoring concept for the online measurement of biofilm formation is established yet. The aim is therefore to develop online sensors which provide the information at any time about the current state of a biofilm. Here the electrochemical impedance spectroscopy (EIS) is used, which was already tested in some application. As an example biofilms are investigated, which are used for microbial electrosynthesis.

Supported by:

Federal Ministry
for Economic Affairs
and Energy

on the basis of a decision
by the German Bundestag

Das IGF-Vorhaben Nr. 18450 BG der Forschungsvereinigung DECHEMA e.V., Theodor-Heuss-Allee 25, 60486 Frankfurt am Main wurde über die AiF im Rahmen des Programms zur Förderung der industriellen Gemeinschaftsforschung (IGF) vom Bundesministerium für Wirtschaft und Energie aufgrund eines Beschlusses des Deutschen Bundestages gefördert.

NIESEL – Deep eutectic solvents as reaction media for biocatalytic processes

Period: 01.04.2015 - 31.03.2018

Partners: Autodisplay Biotech GmbH
Phytowelt Green Technologies GmbH,
Westfälische Wilhelms Universität Münster

Funder: BMBF

The biocatalytic transformation of substrates that are sparingly soluble in aqueous media is challenging in terms of reaction and process design. On the one hand, suitable solvents are necessary to deliver water-insoluble substrates to the respective target enzyme. On the other hand, solvent-resistant enzymes or microorganisms are required exhibiting good activity in organic solvents. The existing concepts to overcome such limitations comprise two-phase systems, supercritical fluids or the application of ionic liquids. However, from an ecological as well as from an economical perspective some of these solutions are sometimes inacceptable.

In this project the application of deep eutectic solvents (DES; also called NADES in the case of natural deep eutectic solvents) is investigated in terms of dissolution or extraction of sparingly water-soluble substrates. DESs are a novel class of solvents generated by mixing two solids, upon which a depression of the freezing point is caused by the interaction of the initial compounds on the molecular level (hydrogen bond). In contrast to classical ionic liquids, DESs are associated with less toxicity, better storability and higher biodegradability. Moreover, DESs are non-volatile and might be an interesting alternative to conventional solvents when hardly soluble substrates are present in a biocatalytic reaction.

Within the framework of this project different DESs will be synthesized, characterized by different methods and eventually applied in various biotransformations. The results obtained with DES-based biotransformations will be compared to already established systems such as organic solvent or ionic liquids.

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of Education
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MIKE – Methanation of CO_2 from biogas by microbial electrosynthesis

Period:	01.09.2016 - 31.08.2019
Partners:	Infraserv GmbH & Co. Höchst KG, Ifn FTZ GmbH, Provadis School of International Management and Technology
Funder:	BMBF

Commercial produced biogas contains app. 60% of methane, 35% of CO_2 and 5% of steam, nitrogen and further impurities. To feed in the biogas into the existing natural gas grid it has to be cleaned from all impurities at the moment in a cost-intensive way. In line with the funding initiative "CO_2Plus – Stoffliche Nutzung von CO_2 zur Verbreiterung der Rohstoffbasis" from the Federal Ministry of Education and Research the project "MIKE" is going to use microbial electrosynthesis to increase the methane output of an industrial biogas plant and therefore to reduce the cleaning effort.

In microbial electrosynthesis electroactive microorganisms take up electrons from a cathode for the reduction of CO_2 to different chemical products, e.g. methane. In contrast to other processes, the microbial electrosynthesis has very high cathodic electron efficiency (› 80%). As electron source current can be used from renewable energies or from excess current.

The main goals of the project are as follows:

1. Development of a robust biocatalyst for the microbial electrosynthesis of CH_4 from impure CO_2 of an industrial biogas plant.
2. Engineering and construction of a MES-prototype and testing on the lab scale.
3. Integration of the MES pilot plant into one of the biggest industrial biogas plants at the industrial park Höchst (Frankfurt, Germany).
4. Operation of the MES-prototype under real industrial conditions over several months.
5. Evaluation of the developed processes by economic calculations and CO_2 foot-print.

Due to the project “MIKE”, not only the methane yield of an industrial biogas plant can be increased, but also excess current can be stored as methane.

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Methylobacterium extorquens - a microbial cell factory for production of chemicals from methanol

Methanol represents an attractive, very flexible feedstock for industrial biotechnology. It is efficiently synthesized via syngas from cheap natural gas and other fossil resources, but can also be derived from renewables such as wood or biogas. As a biotech feedstock, methanol does not interfere with the use of arable land for food and nutrition, and being a liquid it is easier to supply and control in bioprocesses than gaseous substrates such as syngas, methane, CO_2 or H_2. The host organism of our methylotrophic cell factory is *Methylobacterium extorquens*, which assimilates methanol via the serine cycle, whereas the ethylmalonyl-CoA pathway (EMCP) is necessary for glyoxylate regeneration during growth on methanol. Intermediates of the EMCP include acetoacetyl-CoA and dicarboxylic acid CoA esters usually not found in primary metabolism of conventional host strains and represent starting intermediates for the synthesis of e.g. dicarboxylic acids and terpenoids.

Chiramet – Chiral building blocks produced from the biomass conversion product methanol

Period: 01.07.2017 - 31.06.2020

Partners: Westfälische Wilhelms-Universität (Münster, DE)
Chiracon GmbH (Luckenwalde, DE)
Insilico Biotechnology AG (Stuttgart, DE)
Max-Planck-Institut für terrestrische Mikrobiologie (Marburg, DE)

Funder: BMBF

Chiral compounds are important building blocks for the synthesis of fine chemicals, especially pharmaceuticals. Goal of the Chiramet project is the development of biotechnological processes for production of different chiral compounds. Instead of sugar, the potential biomass conversion product methanol is used as carbon source, which is consistent with our efforts towards the biologisation of chemistry.

An increasing share of future methanol is expected to be produced from renewables or sustainable sources, by chemical conversion of biogas, wood, or solid municipal waste or by chemical recycling of carbon dioxide with renewable energy sources. The microorganism used in Chiramet is Methylobacterium extorquens, which is able to grow with methanol as sole carbon source. In order to secrete certain chiral substances, thioesterases catalyzing the hydrolysis of primary metabolism intermediates are expressed. In cooperation with the project partners, repective enzymes are identified and optimized and metabolic optimizations will be guided by in silico models. Furthermore lab scale bioprocesses and downstream procedures will be developed.

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Microbial Electrosynthesis – evaluating the usage of electric energy for future microbial production processes

Period: 01.03.2013 - 28.02.2018

Partner: Electrochemistry Group at DFI

Funder: BMBF

Microbial Electrosynthesis is a new interdisciplinary research field for the production of biofuels and basic chemicals from oxidized substrates such as carbon dioxide. Electrons supplied by a cathode enable the reductive synthesis of these substrates catalyzed by electroactive microorganisms. These organisms are able to interact directly with the cathode or utilize electrochemically reduced media components such as redox mediators or hydrogen. To date the biological mechanisms for the electron uptake are poorly understood.Therefore, our research at the DFI focuses on the interaction of electroactive microorganisms with cathodes including biofilm formation and cell immobilization on different electrode materials. Different electron uptake strategies such as the direct transfer are being compared to the indirect, mainly mediator-based electron transfer mechanisms concerning production efficiency.

Bioelectrochemical reactor designs are developed and optimized to enhance microbial growth and production of chemicals helping to improve our electroactive microorganisms. A broad spectrum of molecular and microbial as well as electrochemical methods (e.g. impedance spectroscopy, cyclic voltammetry) are used to characterize the systems. The research groups Induratrial biotechnology and Electrochemistry are working on this tandem project at the DFI.

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PseudoProKo - *Pseudomonas putida* – a platform for liquid hydrocarbon production

Period: 01.03.15 - 25.02.2018

Funder: BMEL über Fachagentur Nachwachsende Rohstoffe (FNR)

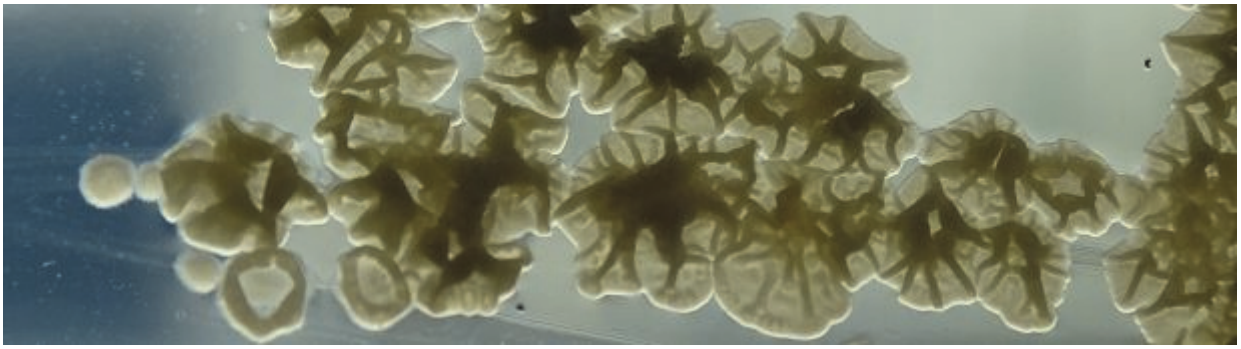
Considering the depletion of fossil fuels, it is essential to further develop the efficient utilization of renewable resources. In bioindustrial processes, microbial production platforms can be used to generate a diverse array of valuable chemicals from simple substrates. In this project we aim at the microbial conversion of domestic plant oil into higher value lipids such as fatty aldehydes, fatty alcohols, and alkanes/alkenes.
Most studies focus on a *de novo* approach via the fatty acid biosynthesis by fermentation of simple substrates such as glucose. In our work, an auto-displayed lipase hydrolyzes triglycerides from rapeseed oil to glycerol and free fatty acids (mainly unsaturated C16 and C18). While glycerol serves as carbon and energy source, the fatty acids are further converted intracellularly. A reduction to **fatty aldehydes** is achieved by introduction of a carboxylic acid reductase. Alternatively, a plant α-dioxygenase generates n-1 fatty aldehydes. The fatty aldehydes are further reduced to **fatty alcohols** by a variety of endogenous *E. coli* reductases. The overexpression or deletion of respective genes can increase or limit the alcohol formation. In a second step the fatty aldehydes can be converted to n-1 **alkanes/alkenes** by cyanobacterial aldehyde deformylating oxygenases.

Gefördert durch:

Bundesministerium
für Ernährung
und Landwirtschaft

aufgrund eines Beschlusses
des Deutschen Bundestages

"New to nature" terpenes for liquid fuels from renewable raw materials



Period: 01.09.2014 - 31.12.2017

Funder: BMEL über Fachagentur Nachwachsende Rohstoffe (FNR)

The substance class of terpenoid hydrocarbons is discussed as a resource for molecules able to substitute petrochemistry-based liquid fuels. Research done mainly by U.S. research groups and companies has already laid the foundation for microbial de novo synthesis of various terpenes including potential fuel molecules. However, it will not be possible to use a pure terpene directly in existing internal combustion engines. Instead, a mixture of various hydrocarbons is needed to obtain an engine fuel, which is compatible with the existing infrastructure.

With this research project, an extension of the product range of microbial terpene production processes is explored. During the project, the technological foundation for the synthesis of "new to nature" terpenes with bacterial enzymes will be laid. This aspect provides the substance diversity required for biotechnological production of "drop-in" fuels in a mid- and long-term perspective. Moreover, the variety of uncommon terpenoid structures might be also applied as aroma and pharma compounds.

Gefördert durch:

Bundesministerium
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aufgrund eines Beschlusses
des Deutschen Bundestages

Selected publications

J. Mi, H. Schewe, M. Buchhaupt, D. Holtmann, J. Schrader

Efficient hydroxylation of 1,8-cineole with monoterpenoid-resistant recombinant *Pseudomonas putida* GS1

World Journal of Microbiology and Biotechnology (2016) DOI: 10.1007/s11274-016-2071-y

In this work, monoterpenoid hydroxylation with *Pseudomonas putida* GS1 and KT2440 were investigated as host strains, and the cytochrome P450 monooxygenase CYP176A1 (P450cin) and its native redox partner cindoxin (CinC) from *Citrobacter braakii* were introduced in *P. putida* to catalyze the stereoselective hydroxylation of 1,8-cineole to (1*R*)-6-hydroxy-1,8-cineole. Growth experiments in the presence of 1,8-cineole confirmed pseudomonads' superior resilience compared to *E. coli*. Whole-cell *P. putida* harboring P450cin with and without CinC were capable of hydroxylating 1,8-cineole, whereas coexpression of CinC has been shown to accelerate this bioconversion. Under the same conditions, *P. putida* GS1 produced more than twice the amount of heterologous P450cin and bioconversion product than *P. putida* KT2440. A concentration of 1.1 ± 0.1 g/L (1*R*)-6-hydroxy-1,8-cineole was obtained within 55 h in shake flasks and 13.3 ± 1.9 g/L in 89 h in a bioreactor, the latter of which corresponds to a yield YP/S of 79 %. To the authors' knowledge, this is the highest product titer for a P450 based whole-cell monoterpene oxyfunctionalization reported so far. These results show that solvent-tolerant *P. putida* GS1 can be used as a highly efficient recombinant whole-cell biocatalyst for a P450 monooxygenase-based valorization of monoterpenoids.

A. Tosstorff, C. Kroner, D. J. Oppermann, F. Hollmann, D. Holtmann

Towards electroenzymatic processes involving old yellow enzymes and mediated cofactor regeneration

Engineering in Life Science (2016) DOI: 10.1002/elsc.201600158

Old yellow enzymes (OYEs) are able to catalyze asymmetric C = C reductions. A mediated electro enzymatic process to regenerate the NADPH in combination with an OYE was investigated. Due to the fact that the overall process was affected by a broad set of parameters a DoE approach was chosen to identify suitable process conditions. Process conditions with high productivities of up to 2.27 mM/h in combination with approx. 90% electron transfer efficiency were identified.

J. Mi, A. Sydow, F. Schempp, D. Becher, H. Schewe, J. Schrader, M. Buchhaupt
Investigation of plasmid-induced growth defect in *Pseudomonas putida*
Journal of Biotechnology 231 (2016) 167-173

Genetic engineering in bacteria mainly relies on the use of plasmids. But despite their pervasive use for physiological studies as well as for the design and optimization of industrially used production strains, only limited information about plasmid induced growth defects is available for different replicons and organisms. Here, we present the identification and characterization of such a phenomenon for Pseudomonas putida transformants carrying the pBBR1-derived plasmid pMiS1. We identified the kanamycin resistance gene and the transcription factor encoding rhaR gene to be causal for the growth defect in P. putida. In contrast, this effect was not observed in Escherichia coli. The plasmid-induced growth defect was eliminated after introduction of a mutation in the plasmid-encoded rep gene, thus enabling construction of the non-toxic variant pMiS4. GFP reporters construct analyses and qPCR experiments revealed a distinctly lowered plasmid copy number for pMiS4, which is probably the reason for alleviation of the growth defect by this mutation. Our work expands the knowledge about plasmid-induced growth defects and provides a useful low-copy pBBR1 replicon variant.

F. Sonntag, C. Kroner, P. Lubuta, R. Peyraud, A. Horst, M. Buchhaupt, J. Schrader
Engineering *Methylobacterium extorquens* for *de novo* synthesis of the sesquiterpenoid -humulene from methanol
Metabolic Engineering 32 (2015) 82-94

Over the last 10 to 15 years, metabolic engineering of microbes has become a versatile tool for high-level de novo synthesis of terpenoids, with the sesquiterpenoids armopha-1,4-diene, farnesene and artemisinic acid as prime examples. However, almost all cell factory approaches towards terpenoids to date have been based on sugar as the raw material, which is mainly used as a food resource and subject to high price volatilities. In this study we present de novo synthesis of the sesquiterpenoid -humulene from the abundantly available non-food carbon source methanol by metabolically engineered *Methylobacterium extorquens* AM1. Expression of -humulene synthase from *Zingiber zerumbet* in combination with farnesyl pyrophosphate (FPP) synthase from *Saccharomyces cerevisiae* led to concentrations of up to 18 mg/L -humulene. Introduction of a prokaryotic mevalonate pathway from *Myxococcus xanthus* in combination with ribosome binding site optimization of -humulene and FPP synthases increased product concentra-

tion 3-fold. This value was additionally raised by 30% using a carotenoid synthesis deficient mutant strain. Final product concentrations of up to 1.65 g/L were obtained in methanol limited fed-batch cultivations, which is the highest titer of de novo synthesized -humulene reported to date. This study demonstrates the potential of *M. extorquens* as a future platform strain for the production of high-value terpenoids from the alternative carbon source methanol.

F. Sonntag, J.E. Müller, P. Kiefer, J.A. Vorholt , J.Schrader, M. Buchhaupt

High-level production of ethylmalonyl-CoA pathway-derived dicarboxylic acids by *Methylobacterium extorquens* under cobalt-deficient conditions and by polyhydroxybutyrate negative strains

Applied Microbiology and Biotechnology 99/8 (2015) 3407-3419

Bio-based production of dicarboxylic acids is an emerging research field with remarkable progress during the last decades. The recently established synthesis of the ethylmalonyl-CoA pathway (EMCP)-derived dicarboxylic acids, mesaconic acid and (2*S*)-methylsuccinic acid, from the alternative carbon source methanol (Sonntag et al., Appl Microbiol Biotechnol 98:4533–4544, 2014) gave a proof of concept for the sustainable production of hitherto biotechnologically inaccessible monomers. In this study, substantial optimizations of the process by different approaches are presented. Abolishment of mesaconic and (2*S*)-methylsuccinic acid reuptake from culture supernatant and a productivity increase were achieved by 30-fold decreased sodium ion availability in culture medium. Undesired flux from EMCP into polyhydroxybutyrate (PHB) cycle was hindered by the knockout of polyhydroxyalkanoate synthase *phaC* which was concomitant with 5-fold increased product concentrations. However, frequently occurring suppressors of strain Δ*phaC* lost their beneficial properties probably due to redirected channeling of acetyl-CoA. Pool sizes of the product precursors were increased by exploiting the presence of two cobalt-dependent mutases in the EMCP: Fine-tuned growth-limiting cobalt concentrations led to 16-fold accumulation of mesaconyl- and (2*S*)-methylsuccinyl-CoA which in turn resulted in 6-fold increased concentrations of mesaconic and (2*S*)-methylsuccinic acids, with a combined titer of 0.65 g/l, representing a yield of 0.17 g/g methanol. This work represents an important step toward an industrially relevant production of ethylmalonyl-CoA pathway-derived dicarboxylic acids and the generation of a stable PHB synthesis negative *Methylobacterium extorquens* strain.

T. Krieg, F. Enzmann, D. Sell, J. Schrader, D. Holtmann

Simulation of the current generation of a microbial fuel cell in a laboratory wastewater treatment plant

Applied Energy 195 (2017) 942-949

Microbial fuel cells (MFCs) are devices generating electrical current from a wide range of organic substrates by using bacterial metabolism. Integrations of MFCs into wastewater treatment plants seem to be the most likely application of this technology. Due to the fact that the current flow in a MFC is fundamentally produced by the metabolic activity of microorganisms, it would be desirable to elucidate the capacity of the microbial systems to optimize the energy extraction processes in MFCs. In this study, the correlation between the parameters X_{BH} (active heterotrophic biomass) and X_{BA} (active autotrophic biomass) from the established activated sludge model number 1 (ASM1) and the measured current flow in MFCs was investigated for the first time. The simulation protocol based on ASM1 shows a good congruence between measured and simulated effluent values for the wastewater treatment plant. Comparisons between the measured current densities and the simulated concentrations of active biomass showed linear correlations at substrate pulses and at different residence times of the substrate. Therefore, it can be concluded that the model parameter X_{BH} and X_{BA} of the ASM1 can be used to estimate the current output of a MFC in wastewater treatment plants. The identified correlations can be used to optimize operating conditions and to generate high current outputs of the MFCs based on simulations.

A.E.W. Horst, S. Bormann, J. Meyer*, M. Steinhagen, R. Ludwig, A. Drews, M. Ansorge-Schumacher, D. Holtmann*

Electro-enzymatic hydroxylation of ethylbenzene by the evolved unspecific peroxygenase of *Agrocybe aegerita*

Journal of Molecular Catalysis B: Enzymatic (2016) DOI: 10.1002/elsc.201600158

The unspecific peroxygenase from the fungus *Agrocybe aegerita* (*Aae*UPO) is an up-and-coming biocatalyst that is able to perform specific oxyfunctionalizations of various substrates. Due to inactivation at excess concentrations of its co-substrate H2O2, *Aae*UPO's technical application is still limited. This study aims to promote catalyst efficiency *via* electrochemical *in situ* supply of H2O2, using an evolved variant of *Aae*UPO on the example of ethylbenzene hydroxylation. Total turnover numbers of up to 400,000 molproduct molAaeUPO–1 and space-time-yields of up to 25 g L–1 d–1 were achieved in

the electro-enzymatic system. These numbers are in the upper range of published data. The presented system stands out by its very high atom economy. Thus, combining electrochemistry and biocatalysis is one step closer towards the first application of peroxygenases in an industrial process.

A. Sydow, T. Krieg, R. Ulber, D. Holtmann

Growth medium and electrolyte – how to combine the different requirements on the reaction solution in bioelectrochemical systems using *Cupriavidus necator*

Engineering in Life Science (2017) DOI: 10.1002/elsc.201600252

Microbial electrosynthesis is a relatively new research field where microbial carbon dioxide fixation based on the energy supplied by a cathode is investigated. Reaction media used in such bioelectrochemical systems have to fulfill requirements of classical biotechnology as well as electrochemistry. The design and characterization of a medium that enables fast electroautotrophic growth of *Cupriavidus necator* in microbial electrosynthesis was investigated in detail. The identified chloride-free medium mainly consists of low buffer concentration and is supplied with trace elements. Biotechnologically relevant parameters, such as high specific growth rates and short lag phases, were determined for growth characterization. Fast growth under all conditions tested, i.e. heterotrophic, autotrophic and electroautotrophic was achieved. The lag phase was shortened by increasing the $FeSO_4$ concentration. Additionally, electrochemical robustness of the reaction media was proven. Under reductive conditions, no deposits on electrodes or precipitations in the media were observed and no detectable hydrogen peroxide evolved. In the bioelectrochemical system, no lag phase occurred and specific growth rate of *C. necator* was 0.09 h^{-1}. Using this medium shortens seed train drastically and enables fast electrobiotechnological production processes based on *C. necator*.

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A simplified process design for P450 driven hydroxylation based on surface displayed enzymes Biotechnology and Bioengineering (2015)

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New production routes for fine and bulk chemicals are important to establish further sustainable processes in industry. Besides the identification of new biocatalysts and new production routes the optimization of existing processes in regard to an improved utilization of the catalysts are needed. In this paper we describe the successful expression of P450BM3 on the surface of *E. coli* cells with the Autodisplay system. The successful hydroxylation of palmitic acid by using surface-displayed P450BM3 was shown. Besides optimization of surface protein expression, several cofactor regeneration systems were compared and evaluated. Afterwards, the development of a suitable process for the biocatalytic hydroxylation of fatty acids based on the re-use of the catalysts after a simple centrifugation was investigated. It was shown that the catalyst can be used for several times without any loss in activity. By using surface-displayed P450s in combination with an enzymatic cofactor regeneration system a total turnover number of up to 54,700 could be reached, to the knowledge of the authors the highest value reported for a P450 monooxygenase to date. Further optimizations of the described reaction system can have an enormous impact on the process design for more sustainable bioprocesses.

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