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# Key Technologies in the Bioeconomy

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# Abstract Book

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## Session 1 – Bioeconomy & Circular Economy – Strategies for Waste Utilization



## Session 2 – Bioeconomy meets Hydrogen Economy



## Session 3 – Regional and Global Effects of the Bioeconomy

## Session 4 – Hybrid Technologies for the Bioeconomy



## Session 5 – When Bio is Better – New Products and Solutions for and from the Bioeconomy

## Poster

# Session 1

## Bioeconomy & Circular Economy – Strategies for Waste Utilization

S1T1

# The carbon revolution: scaling circularity to replace fossil oil

Michael Köpke

*LanzaTech, United States*

The accelerating climate crisis combined with rapid population growth poses some of the most urgent challenges to humankind, all linked to the unabated release and accumulation of CO2 and waste across the biosphere. By harnessing our capacity to partner with biology, we can begin to take advantage of the abundance of available CO2 and waste carbon streams to transform the way the world creates and uses carbon-based materials.

LanzaTech has pioneered and commercialized a gas fermentation process for conversion of an array of carbon oxide containing gas streams (e.g. emissions from heavy industry, gasified agricultural or municipal waste, or from direct CO2 capture) leveraging carbon-fixing chemolithoautotrophic microorganisms and enable carbon-negative biomanufacturing of a wide range of fuels, chemicals, proteins and materials. Today, LanzaTech is operating several commercial plants and produced over 50 million gallons of ethanol while mitigating over 200,000 tons of CO2. LanzaTech has also developed technology to convert ethanol to jet fuel and scaled direct production of further chemical building blocks such as acetone and isopropanol through genetic engineering.

Only a decade ago, most carbon-fixing chemolithoautotrophic microorganisms were poorly understood, considered to be genetically inaccessible and mass-transfer of gases was perceived as a major scale-up hurdle. Advancements in Synthetic Biology, Process Engineering, Automation, AI and Modelling have enabled development and scale up of highly efficient production strains for carbon oxide conversion into a range of products that today rely exclusively on fresh fossil feedstocks and results in large amounts of CO2 emissions during manufacturing. In contrast, the LanzaTech process captures more CO2 than it emits, effectively taking CO2 out of the atmosphere and fixing it into the product.

S1T2

# 3rd Generation Biorefinery - Production of basic chemicals by utilization of biogenic residues via entrained flow gasification with coupled gas fermentation

**Sebastian Fendt[1];** Leuter, Philipp[1]; Dossow, Marcel[1]; Spliethoff, Hartmut[1]

[1] *Technical University of Munich, Germany*

Within the framework of the projects ReGasFerm“ and „GOLD“, innovative process options utilizing biogenic residues and contaminated crops are examined. The concept integrates thermochemical and biotechnological process units to produce C2-C6 compounds in a biorefinery concept. More specifically, the concept incorporates two core technologies: the thermochemical conversion of biomass via entrained flow gasification and a downstream fermentative processing of the produced synthesis gas using microorganisms. Acetogenic bacteria strains metabolize synthesis gas and CO2 as carbon source to generate alcohols such as ethanol, butanol, 2,3-butandiol and hexanol. Feedstock such as green cuttings, residual leaves and contaminated crop are thus used in an efficient way. The application of an autothermal entrained flow gasification ensures a efficient utilization of the difficult accessible carbon content including lignin. Synthesis gas impurities can be minimized through high gasification temperatures, but can still cause significant problems in the fermentation process. Thus, selective and robust purification of the synthesis gas is crucial for the process to prevent degradation or inhibition of the utilized microorganisms.

The aim of our ongoing efforts is the proof-of-concept of the entire process chain for the utilization of residual and contaminated feedstock as a competitive technology to the reference process based on the catalytic utilization of synthesis gas. In parallel to experimental investigations with focus on the gasification and gas cleaning steps, techno-economic analysis and process simulations using AspenPlus for gasification, gas purification and gas fermentation are applied. These activities aim at an optimized concept for (decentralized) biorefineries and an estimation of a feasible plant size.

For the future, coupling thermochemical, biotechnological and electrochemical processes is seen as a highly promising way to address some of the most crucial challenges in a future bioeconomy. In the International Future Lab REDEFINE H2E, international researchers will join our efforts to advance our basic understanding.“

S1T3

# Systems and synthetic biology for carbon recycling

Esteban Marcellin

*The University of Queensland, Australia*

The current environmental crisis threatens our way of life. This year, the United Nations published the Sustainable Development Goals, highlighting 2022 as critical to fulfilling the UN Sustainable Development Goals (SDGs). The report suggests the need for accelerated action on modern renewable energy to ensure affordable, reliable and sustainable energy. Gas fermentation using acetogens offers numerous sustainable advantages for the biological production of liquid fuels and chemicals. Acetogens play a key role in the global carbon cycle, capturing an estimated 20% of $CO_2$ on Earth. Their ability to fix carbon makes acetogens perfectly suited for greenhouse gas valorization and carbon recycling. Gas fermentation is also agnostic to contaminants, scalable and economically viable even for small gas streams. As such gas fermentation has positioned itself as a viable alternative for the biological production of chemicals and fuels from recycled carbon with a lower environmental impact than other approaches. Acetogens are amongst the most promising organisms capable of utilizing CO, $CO_2/H_2$ as the sole carbon source and are used by Lanzatech at their commercial gas fermentation facilities around the globe. The current Lanzatech commercial facilities produce ethanol. However, expanding the product spectrum of acetogens is crucial for the widespread adoption of the technology as many aspects of acteogens are poorly understood. We are developing comprehensive systems and synthetic biology toolboxes to create a large-scale systems-level quantification of acetogen genotype-phenotype relationships. To this end, we are using mathematical models to guide the improvement of acetogens through a better understanding C1 metabolism.

S1T4

# Cell factories for methanol- or methylamine-based production of amines

Volker F. Wendisch

*University of Bielefeld, Germany*

Nitrogen is essential for all forms of life with amino acids and derived amines fulfilling diverse functions. There is a growing demand for food and feed amino acids, such as L-glutamate and L-lysine, as well as for specialty amino acids for dedicated applications [1]. Their sustainable production will have to be based on substrates that do not have competing uses as food or feed. Valorization of sidestreams from agri- and aqua-culture as substrates has focused on production of biofuels and carboxylic acids, neglecting the nitrogen present in these sidestreams [2]. As in these biorefineries, valorization of reduced forms of carbon dioxide, such as methanol, is mostly discussed in a carbon-centric way. Methylamine is produced commercially from methanol and ammonium. I will discuss metabolic engineering of bacteria for the production of amino acids and amines from methanol as a carbon source [3] and of the fermentative routes for bioproduction of N-methylated amino acids by reductive methylamination of 2-oxoacids using methylamine [4].

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S1T5

# Methanol-based production of glycolic acid using engineered Methylorubrum extorquens

Jonathan Thomas Fabarius[1]; Dietz, Katharina[1]; Sagstetter, Carina[1]; Speck, Melanie[1]

[1] *Fraunhofer IGB, Germany*

The rise of interest in replacing fossil-based production routes for chemicals led to the concept of circular production harnessing CO2. Particularly, liquid C1 fermentation substrates, i.e. methanol or formic acid, gained attention. These substrates are efficiently utilized by methylotrophic microbes and provide advantages in comparison to gas fermentation in terms of mass transfer within the aqueous phase. Moreover, methanol can be directly produced using industrial synthesis gas or even green synthesis gas, a mixture of CO2 and CO with green hydrogen. Consequently, the microbial utilization of CO2-based methanol paves the road to close the carbon cycle in industrial biotechnological production. We grasp the coupling of chemical methanol synthesis from synthesis gas with fermentation as a new process concept termed “Power-to-X-to-Y”.

Here we present an example of the Power-to-X-to-Y concept within the framework of the Fraunhofer EVOBIO-Demo project. In particular, the methanol-based production of the industrial-relevant chemical glycolic acid using proprietary engineered strains of Methylorubrum extorquens is demonstrated. Initially, metabolic modelling was applied to evaluate the methylotrophic production of glycolic acid using M. extorquens. Flux Balance Analysis and Elementary Mode analysis predicted increased potential of this strain to convert the serine cycle intermediate glyoxylate to the target product. A first producer strain was obtained by overexpression screening of various heterologous glyoxylate reductases. It is shown that functional glyoxylate reductase overexpression leads to significant titers of the organic acid, even using methanol derived from synthesis gas. In addition, it was found that the successful glycolic acid formation is accompanied with overproduction of lactic acid opening the door to a CO2-derived poly(lactate-co-glycolate) route. Next, metabolic modelling and literature investigations suggested that overexpression of ethylmalonyl-CoA mutase (ECM) of the ethylmalonyl-CoA pathway is beneficial to increase the glyoxylate pool for precursor supply. Finally, it was evaluated in vivo if ECM overexpression unlocks efficient product formation.

# Session 2

## Bioeconomy meets Hydrogen Economy

S2T1

# Biotechnology in this decade of action

Ludo Diels

*VITO, Belgium*

The Green Deal of the EC sets the strategy and boundary conditions to reach a climate neutral and circular economy by 2050.

Our societal system uses chemicals and materials based on inorganics and organics. The last ones do have a very high carbon content nowadays mostly based on fossil-carbon. In order to develop a sustainable economy it will be necessary to stay away from fossil-carbon and to move to the so called 'renewable carbon' which means carbon from CO2, biomass or recycling. Energy should come from renewable sources all generating green electrons and the intermediate H2.

The bioeconomy generates biomass as a carbon neutral source and provides processes with high specificity operational at low temperatures. At the level of resource the limitation is availability and processes are quite diluted.

The availability of biomass can be solved if i) only very limited use of biomass as energy source is regulated. This means biomass residues and wastes that are too difficult to be reused or recycled, and ii) if the biobased chemicals/materials will enter a recycling system. Recycling can only be done at the expense of some waste which easily is between 20 and 25% (residues that can be used as energy source and go in an end of life system). The recycling asks for 20-25% addition of virgin material. By adding every year new virgin biomass in the recycle system slowly fossil-based materials will be phased out and on a long term the carbon-based material cycle will run on bio-based carbon (and of course also CO2-based carbon).

Bio-based developments of chemicals and materials will become successful if they are outstanding in their performance and safety. They will create impact at the climate level if they not only base on a climate neutral process, but on a system of carbon sinks.

S2T2

# Carbon dioxide fixation via production of succinic acid from glycerol in engineered Saccharomyces cerevisiae

Elke Nevoigt[1]; Malubhoy, Zahabiya[1]

[1] *Jacobs University Bremen, Germany*

The microbial production of succinic acid (SA) from renewable carbon sources via the reverse TCA (rTCA) pathway is a process potentially accompanied by net-fixation of carbon dioxide (CO2). Among carbon sources, glycerol is particularly attractive since it allows a nearly twofold higher CO2-fixation yield compared to sugars. The current study aimed at improving the flux into the rTCA pathway accompanied by a higher CO2-fixation and SA yield. By changing the design of the expression cassettes for the rTCA pathway, overexpressing PYC2, and adding CaCO3 to the batch fermentations, an SA yield on glycerol of 0.63 Cmol Cmol-1 was achieved (i.e. 47.1% of the theoretical maximum). The modifications in this 2nd-generation SA producer improved the maximum biomass-specific glycerol consumption rate by a factor of nearly four compared to the isogenic baseline strain solely equipped with the dihydroxyacetone (DHA) pathway for glycerol catabolism. Cultivation conditions which directly or indirectly increased the concentration of bicarbonate, led to an accumulation of malate in addition to the predominant product SA (ca. 0.1 Cmol Cmol 1 at the time point when SA yield was highest). Off-gas analysis in controlled bioreactors with CO2-enriched gas-phase indicated that CO2 was fixed during the SA production phase. The data strongly suggest that a major part of dicarboxylic acids in our 2nd-generation SA-producer was formed via the rTCA pathway enabling a net fixation of CO2. The greatly increased capacity of the rTCA pathway obviously allowed successful competition with other pathways for the common precursor pyruvate. The overexpression of PYC2 and the increased availability of bicarbonate, the co-substrate for the PYC reaction, further strengthened this capacity. The achievements are encouraging to invest in future efforts establishing a process for SA production from (crude) glycerol and CO2.

S2T3

# Sustainable mobility with Clariant's sunliquid® technology: First commercial sunliquid® cellulosic ethanol plant in Podari, Romania

Christian Librera[1]; Jacobsen, Nikolas[1]

[1] *Clariant AG, Germany*

In today's growing efforts to limit global warming, action to reduce greenhouse gas emissions, especially in the transport sector, is inevitable. Cellulosic ethanol, an advanced biofuel, presents a low-emission, carbon-neutral solution. In many countries around the world legislation already recognizes advanced biofuels to play an important role in decarbonizing the transport sector.

Clariant's sunliquid® process is a highly innovative and sustainable technology to produce cellulosic ethanol from agricultural residues such as cereal straw, corn stover, or sugarcane bagasse. The cellulosic ethanol produced can be used as a drop-in solution for fuel blending and offers further downstream application opportunities into sustainable aviation fuel and bio-based chemicals.

Since 2012, Clariant has been operating its pre-commercial sunliquid® plant in Straubing, Germany and has started production at its first full-scale commercial cellulosic ethanol plant in southwestern Romania in June 2022. The flagship plant will process approx. 250,000 tons of straw to produce approx. 50,000 tons of cellulosic ethanol per annum and represents an important step for the commercial deployment of the sunliquid® technology, supporting Clariant's licensing business strategy.

The process is energy self-sufficient as it requires no fossil-based energy sources. It obtains its energy from the combustion of residual flows, mainly lignin, that remains after extracting the cellulose from the lignocellulose. As a result, the emission profile is considerably better than for conventional bioethanol production. The bioethanol produced by the sunliquid® technology process helps decarbonize the transport sector by providing up to 95% CO2 savings compared to fossil fuel, and by as much as 120% if carbon capture is considered and used as part of the production process.

Clariant licenses its sunliquid® technology platform globally.

S2T4

# Engineering Corynebacterium glutamicum for the production of itaconic acid from acetate

Marc Schmollack[1]; Siebert, Daniel[1]; Blombach, Bastian[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

Industrial biotechnology is an important pillar of the circular and sustainable economy with the aim of converting biogenic resources into chemicals and fuels. The established production host C. glutamicum is ideally suited for the conversion of the non-food substrate acetate, as it exhibits high substrate uptake rates and tolerates high concentrations of it (Kiefer et al., 2021). Here, we engineered C. glutamicum for efficient conversion of acetate to itaconic acid - an unsaturated dicarboxylic acid of industrial and medical interest. To introduce itaconic acid production, an optimized version of the cis-aconitate decarboxylase was expressed from a pEKEx2 vector (Otten et al., 2015) in C. glutamicum ATCC13032. The generated strains were cultured in CGXII minimal medium supplemented with 20 g acetate L-1 under nitrogen limitation (C:N ratio of 40:1). After 72 h, a final titer of 0.32 ± 0.09 g itaconic acid L-1 was detected in the culture supernatant (YP/S of 8 ± 2 mmol mol-1). Reduction of isocitrate dehydrogenase activity, deletion of the global regulator of acetate metabolism RamB, and deletion of glutamate dehydrogenase increased the final titers to 3.43 ± 0.59 g of itaconic acid L-1 (YP/S of 81 ± 9 mmol mol-1). Final titers reached with C. glutamicum ΔramB Δgdh IDHR453C (pEKEx2-malEcadopt) were further increased to 5.01 ± 0.67 g of itaconic acid L-1 (YP/S of 116 ± 15 mmol mol-1) by lowering the cultivation temperature from 30 °C to 25 °C. These values correspond to 34% of the theoretical maximum and are the highest yields and titers of itaconic acid produced from acetate in shaking-flask cultures reported so far.

S2T5

# Using insect transcriptome and metatranscriptome to find genes coding for biomass degradation enzymes

Martin Langer

*BRAIN Biotech AG, Germany*

From many stakeholders in politics and industry, biotechnology is considered to be a key technology that can make a significant contribution to transforming the current economy into a circular bioeconomy. BRAIN Biotech supports that position and over the last 15 years has established a broad range of biotechnological solutions with have the potential to upcycle industrial side streams.

How exactly does it work? BRAIN together with its industry partners found the most creative microorganisms managing that job very well. Some of these microorganisms can use CO2 from industrial point sources to convert it into renewable building blocks, others are able to recover valuable metals from e-waste or help to recycle lithium-ion batteries – biotechnology with the use of these microorganisms can help to promote a sustainable economy.

Where do we stand in that transformation process? Where will we find ourselves in 10 years from now? A potential path forward but also current challenges will be discussed. Visions to get to a circular economy in place through transforming industrial processes will be shared.

S2T6

# Electrifying Synthesis

Tobias Gärtner

*ESy-Labs GmbH, Germany*

Electrosynthesis and in detail the technology ESy-Screening, provided by ESy-Labs, will be the future key technology for the production of high value fine and specialty chemicals as well as pharmaceutical compounds and the recycling of inorganic waste streams or battery materials. The creation of value with electrosynthesis is performed through the application of electricity instead of the use of expensive, toxic and stoichiometric reagents. These outstanding advantages position electrosynthesis as a game changer in chemical conversion and helps to reduce or completely avoid chemical waste, the application of critical raw materials, and safety issues. Especially the diverse combination of organic as well as inorganic electrosynthesis, accounts also for the future combination with biotechnology at ESy-Labs.

In combination with ESy-Screening, Design of experiment (DoE) is a powerful statistical tool in establishing improved chemical processes. An optimization and scale-up of the electrochemical reduction of L-cystine to L-cysteine is presented. Subsequent to an electrode screening of 17 metals and alloys in divided batch-type cells, the lead electrode was selected for a systematic optimization in flow cells with a geometric cathodic surface area ranging from 10 to 100 cm2.

Furthermore, scale-up to industrial scale is an essential bridge to technical aplication. Here, the development of ESy-Zinc for the recycling of zinc containing industrial waste will be presented.

# Session 3

## Regional and Global Effects of the Bioeconomy

S3T1

# Bioeconomy International: A BMBF instrument to strengthen global cooperation in Bioeconomy

Veronika Jablonowski

*Project Management Jülich, Germany*

Realizing the bioeconomy as a sustainable bio-based economy requires not only national and European but also international initiatives. Global cooperation is needed to achieve the objectives set out for establishing the bioeconomy. This is the purpose of the BMBF-Bioeconomy International funding activity. Funding will be provided for research and development projects in close cooperation with relevant foreign partners on core issues of the bioeconomy in order to strengthen international collaborations and to establish active, sustainable partnerships.

S3T2

# Future.Bioeconomy.Bavaria

Klaus-Peter Potthast

*Bavarian Ministry of Economic Affairs, Regional Development and Energy, Germany*

The Bavarian Bioeconomy Strategy Future.Bioeconomy.Bavaria addresses all relevant players: society, administration and politics, agriculture and forestry, industry and science. We want to actively develop the transformation with the implementation of fifty measures. The success of the bioeconomy critically depends on society's acceptance of these measures. For this reason, we must integrate all citizens and provide education about the benefits of the bioeconomy, e.g. by establishing targeted educational offers and fostering an intensive public discourse. At the same time, policy-makers and administration are to initiate necessary changes for the amendment of laws and ordinances and take on a role model function with respect to usage and consideration for climate and environmentally friendly products. Agriculture and forestry as producers of renewable resources are thereby strengthened offering business and industry an opportunity to become a driver of innovation within the bioeconomy. Science and research form the basis for new insights and for a science-based bioeconomy. Interdisciplinary cooperation and improved communication promote the transfer of new insights for practical application.

Consequently, the strategy was developed as an open and constructive process with the integration of all relevant players. We want to express our heartfelt gratitude to all workshop participants, all surveyed experts, the involved ministries and the clusters and especially the Bioeconomy Council Bavaria with its outstanding level of expertise.

Future.Bioeconomy.Bavaria is the guiding principle for the actions we will take in the future.

S3T3

# Regional Ecosystem, Global Effect? – Scaling up the Biobased Economy in Straubing, Bavaria

Ann-Kathrin Wagner

*BioCampus Straubing GmbH, Germany*

Straubing, branding as the region of renewable resources, is considered the center of the bioeconomy in Bavaria. More than 50 players from agriculture and forestry, industry, the public sector, science and research along bioeconomic value chains are active here. Corporate actors that can be assigned to the bioeconomy are mainly working in the fields of industrial biotechnology, renewable fuels, food and feed industry, and the primary sectors of agriculture and forestry.

In this presentation, from a public business development point of view, Straubing serves as an example of how regional, geo-economical characteristics and political decisions can be harnessed to develop an ecosystem for the biobased economy. The first steps of this development were taken almost two decades ago, making Straubing one of the first bioeconomy hubs in Germany.

Public and private investments and activities implemented along the bioeconomy's innovation and value chains are contributing to the regional ecosystem and can also have a significant effect on the economic transformation towards a more sustainable, biobased economy beyond the region. This effect can both result from a transfer of lessons learnt to other emerging bioeconomy regions and from products, processes and knowledge developed in the region itself. Important future building blocks that will drive this development include the continued strengthening of excellent education and research, investing in accessible scaling and demonstration infrastructure as well as focusing on start-ups, spin-offs and supporting regional SMEs in their quest towards sustainability.

As an example of the latest activities, infrastructure investments within the Straubing ecosystem such as the BioCampus MultiPilot, an open-access, multi-purpose demonstration plant for industrial biotech process upscaling are presented. Attention will also be put on the challenges faced and an outlook into the biobased future which might be rooted in regional hubs but has to function globally is given.

S3T4

# Regional Transformation as the Key to a Sustainable Bioeconomy: the Rheinische Revier as a Model Case

Sandra Venghaus[1]; Siekmann, Florian[1]

*BioSC Jülich/RWTH Aachen, Germany*

Following the decision of the German government to phase-out coal power by 2038, in the coming years the lignite mining region the Rheinische Revier, Europe's largest interconnected lignite mining area, will undergo significant transformative change. To mitigate the impacts of structural change, the objective is to turn the region into a model region for a sustainable bioeconomy. This process creates a unique opportunity for society as it will provide rare insights into the way such transformation processes work. In addition, valuable knowledge for the future will be gained.

To guide the success of this transformation, as part of the BioSC-project Transform2Bio, a comprehensive monitoring system was developed that captures crucial aspects related to the transition and considers regional specificities along with stakeholder perspectives. Without respective indicator systems as a reliable source of region-specific information, decision-makers lack a central component for making forward-looking and comprehensible decisions and developing associated policy options. The monitoring system developed for this study combines elements of national sustainability strategies and links these with the key principles of a sustainable bioeconomy.

To enhance comparability between similar transformation initiatives, the established Shared Socioeconomic Pathways (SSPs) and related narratives serve as a basis for the subsequent quantification of regional transformation pathways (RTPs). The monitoring system rooted in the sustainability strategy ensures data availability and increases the legitimacy of potential decisions based upon it. The connection to the SSPs strengthens transparency and allows researchers and policy-makers to relate to the underlying main assumptions.

S3T5

# The Circular Economy Towards the Sustainability: The Case for the Automotive Sector

Alcides Leao[1]; Cesarino, Ivana[2]; Jawaid, Mohammad[3]

[1] UNESP - School of Agricultural Sciences, Brazil; [2] São Paulo State University UNESP, Brazil; [3] INTROP, Malaysia

The bioeconomy together with the circular economy are the most recent strategies toward sustainable development. In this context, many industrial sectors are considering the design of new products based on this concept. New materials and designs have been considered aiming to enhance the sustainability of derived products. Most of the automotive corporations are focused nowadays in reducing their demand for energy and promoting renewable raw materials, cycling products under the concept of cradle to cradle. Vehicle manufacture, use, and disposal are very intense in terms of materials and supply demands, and in most cases rely on non-renewable resources. Therefore, the use of the circular economy approach can help to reduce the automotive industry impact over the environment. This paper discusses the historical aspects of the bioeconomy and circular economy, including biofuels, natural polymers, and the use of renewable resources. The concept of balancing energy and materials can be a key aspect in the decision for the future of our mobility: electrical and hybrid cars, internal combustion, renewable fuel, low carbon footprint, etc. In this scenario the bioeconomy and the circular economy act together, although at different levels, aiming to improve the sustainable mobility in production, use and end of life vehicles (recycling and final disposal). Finally, the circular bioeconomy means the replacement of non-biogenic resources, or fossil-based, by renewable materials, including in this case the natural polymers, biopolymers, biomasses, renewable energy and green feedstocks, which will result in a better transition to a global bioeconomy.

S3T6

# The role of Bioeconomy in promoting Bioenergy for combating climate change in the built environment: challenges and opportunities in sub-Saharan Africa

Dan Duah

*Kwame Nkrumah University of Science and Technology, Ghana*

Sub-Saharan Africa is expected to be among the most severely affected continents by climate change in the coming decades. The construction industry, as one of the continent's largest employers, is considered very resource intensive and contributes significantly to greenhouse gas (GHG) emissions. As a result, increasing the use of renewable resources in construction, particularly bioenergy and low carbon construction materials, could make the built environment more sustainable and a part of the bioeconomy. Nonetheless, there has been little attention paid to the potential of bioenergy as a by-product of bioeconomy for combating GHG emissions associated with construction activities. The aim of this keynote address is to present the current state of bioeconomy adoption in Sub-Saharan Africa, as well as to interrogate the role of bioeconomy in promoting bioenergy for combating climate change in the built environment. Bioeconomies face enormous challenges, ranging from policy and regulation-related, infrastructural-related, technology-related, and institutional-related issues to the development of new business models and the production of new biomaterials and bioenergy in a sustainable and cost-effective manner for the built environment. However, opportunities exist in bioeconomies for the production and use of modern bioenergy to help reduce GHG emissions, promote energy security, diversify energy resources, and contribute to a sustainable built environment. The main drivers and enabling environment for promoting bioeconomy adoption for climate action within the built environment are proposed. This keynote address will contribute to the bioeconomy discourse by assisting in the achievement of SDGs 7, 11 and 12. Additionally, it will help policymakers in sub-Saharan Africa to develop and implement effective policies to guide the implementation of bioeconomy in the built environment.

**Keywords**

Bioeconomy; Bioenergy; Built environment; Construction; Climate change; Enabling environment

S3T7

# From lab to market - examples of bioeconomy concept evaluation

Andreas Rudi

*Karlsruher Institut für Technologie, Germany*

The bioeconomy is based on the valorization of biomass. Since thousands of years biomass has been and is still extensively used as a fuel to produce heat. In the near future, biomass shall substitute fossil carbon as a renewable source of valuable chemical compounds for sectors such as the chemical industry in various applications. Bioeconomy concepts such as the biorefinery integrate processes to convert biomass into valuable products. Its implementation follows development stages from the laboratory to the scale-up. In order to master the Valley of Death, concepts must be evaluated and assessed by applying advanced interdisciplinary methods. Classical methods such as the economic analysis are combined with life cycle assessment, stakeholder analysis and comprehensive process simulation as well as mathematical programming models to obtain insights into its complex performance. Only in the case of a sufficient overall performance innovative biomass valorization concepts thrive. Several examples exist that started from a basic idea and grew through research to become first-of-its-kind concepts. This talk will provide a few examples of successfully implemented bioeconomy concepts and aggregate success factors for the implementation of concepts to come.

# Session 4

## Hybrid Technologies for the Bioeconomy

S4T1

# The role of (bio)catalysis for chemical production in a sustainable society

John Woodley

*Technical University of Denmark, Denmark*

The green transition includes the development of new synthetic routes for the production of chemicals (from pharmaceuticals to commodities). One interesting route is using catalysis, which has the potential to drive new sustainable production processes. In particular the opportunity is opened for the use of effective chemical processes based on renewable (and potentially sustainable) raw materials. Catalysts can be classified into several categories and the use of heterogeneous catalysts, fermentative growing cells and biocatalysts (non-growing cells or isolated enzymes) each have different roles to play [1,2]. Nevertheless some technical hurdles still need to be overcome, not least in the development of new processes where a combination of technologies are required and therefore multidisciplinary approaches become essential. In this lecture I will outline some of the options, metrics used for assessment of new bioprocess [3] and discuss the role of different technologies.

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S4T2

# Engineering methane-converting platform organisms for a future bioeconomy

**Lars Lauterbach[1];** Zill, Domenic[1]; Lettau, Elisabeth[1]; Lorent, Christian[2]; Seifert, Franziska[3]; Singh, Praveen K.[1]

*[1] RWTH Aachen University, Germany; [2] Technische Universität Berlin, Germany; [3] Martin-Luther-Universität Halle-Wittenberg, Germany*

A desired bioeconomy is a prerequisite for a carbon-neutral or even slightly carbon-negative society. While the advanced production processes of industrial biotechnology are based on glucose from starch and sucrose, the challenge of land use and competition with the food industry are coming into focus. This is especially true for the economic production of low-cost bulk chemicals or biofuels. As the most reduced form of carbon, methane is not only a potent climate gas, but can also serve as an excellent energy and carbon source for methane-based fermentations.

However, in recent decades, various research groups have failed to produce the crucial enzymes for methane conversion in industrial relevant platform organisms [1]. We demonstrated for the first time the heterologous production of catalytically active soluble methane monooxygenase (sMMO) from the marine Methylomonas methanica MC09 in Escherichia coli [2,3]. Key to this was the co-synthesis of the chaperonin GroES/EL, which bears great similarity to one of the proteins within the sMMO operon. For comprehensive characterization by biochemical and spectroscopic techniques, we purified the sMMO by affinity chromatography. Iron determination, electron paramagnetic resonance spectroscopy, photometric assays, and immunoblotting in native gel revealed the incorporation of the non-heme di-iron center and the formation of homodimers of the active sMMO [2,3].

Future development of methane-converting platform organisms and their biotechnological applications are discussed.

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S4T3

# Hydrogen as a reductant for cleaner biocatalysis in chemical synthesis

Kylie Vincent

*University of Oxford, United Kingdom*

S4T4

# Teaching an Old Dog New Tricks - Engineering E. coli for New-to-Nature Metabolism

Markus Jeschek

*University of Regensburg, Germany*

Substantial recent progress in biocatalysis, metabolic engineering and the reading and writing of DNA open up a plethora of new possibilities to construct microbes for sustainable bioprocesses. While we are only seeing the onset of these technological developments, it is not presumptuous to predict that they will critically shape the inevitably required transition towards a circular, bio-based economy. In my talk, I will share some of our previous and planned contributions to this exciting endeavor.

To this end, my group develops enzymes with catalytic features not found amongst natural biological systems. For instance, our efforts on the assembly and directed evolution of artificial metalloenzymes in the periplasm of the bacterium Escherichia coli are targeted towards expanding the available biocatalytic repertoire with transition-metal catalyzed reactions[1-3]. Most notably, we have recently developed artificial, ruthenium-containing enzymes for olefin metathesis, a metal-catalyzed reaction mechanism that features scission and regeneration of carbon-carbon double bonds using olefins as starting material[2,3]. Relying on high-throughput screening and metabolic selection, we systematically engineer such hybrid biocatalysts for a variety of non-natural reactions by directed evolution. Additionally, we capitalize on the obtained data to develop in silico models that facilitate the forward design of new enzyme variants with user-defined properties while drastically reducing the associated experimental screening effort[3]. Furthermore, we develop molecular tools and computational models to rationally integrate such “new parts” into higher-order systems such as metabolic pathways and production strains[4,5].

The overarching goal of our work is the development of a versatile molecular and methodological toolbox for the streamlined metabolic engineering of microbial cell factories with new-to-nature capabilities.

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S4T5

# Bringing resarch to the field: A biosensor test strip for determining plant nutrient needs

Alaa Alsheikh Oughli[1]; Vöpel, Tobias[1]; Plumeré, Nicolas[2]; Monteiro Moretti, Debora[1]

[1] *LiveSen, Germany;* [2] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

We have developed a novel biosensor platform for the detection of plant nutrients, like nitrate and phosphate directly on the field.

From a small drop of plant sap we can determine the nutrient concentration with with laboratory precision.

With our sensor we determine what the plants need and guide fertilizer application. In combination with remote sensing we can provide spatially resolved fertilizer application maps.

Our system has the potential to reduce the fertilizer input thereby reducing the overall greenhouse gas emissions of fertilization by up to 20%.

Here I will present the biotechnological basis of our system and the transfer activities to make our technology available to farmers.

S4T6

# A Precision Compost Strategy will increase global food production and mitigate climate change

Susanne Schmidt

*The University of Queensland, Australia*

The bioeconomy requires sustainable bioproduction systems aligned with the circular economy and the Sustainable Development Goals. Compost represents an important input for bioproduction, but the use of diverse compost types causes uncertain outcomes, and compost use remains at the fringes of modern agriculture. We performed a global meta-analysis with over 2,000 observations to determine whether a Precision Compost Strategy (PCS) that aligns suitable composts and application methods with target crops and growth environments can advance sustainable bioproduction. Eleven key predictors of compost (carbon-to-nutrient ratios, pH, electric conductivity), management (nitrogen supply) and biophysical settings (crop, soil texture, soil organic carbon, pH, temperature, rainfall) determined 80% of the effects on crop yield, SOC, and nitrous oxide emissions. The benefits of a PCS are more pronounced in drier and warmer climates and soils with acidic pH and sandy or clay texture, achieving up to 40% higher yields than conventional practices. We estimate that a global PCS can increase the production of major cereal crops by 96.3 Tg annually, which is 4% of current global production. It also has the technological potential to restore 19.5 Pg carbon in cropland topsoil (0-20cm), equivalent to 26.5% of current topsoil carbon stocks. Together, this points to a central role of PCS in the bioeconomy enabled by sustainable high-production systems and contributing to climate change mitigation.

# Session 5

## When Bio is Better – New Products and Solutions for and from the Bioeconomy

S5T1

# Artificial Photosynthesis - The Rheticus project

Thomas Haas

*Evonik Operations GmbH, Germany*

Climate change is one of the most discussed topics in our society at the moment. It is a common understanding, that $CO_2$ emissions contribute significantly to global warming. Therefore many different approaches are needed to limit or even reduce these emissions. In order to meet this trend, we have to find solutions, efficiently using our resources and at the same time reducing $CO_2$ emissions. In our present world the natural photosynthesis is a crucial factor, generating compounds from $CO_2$ and sunlight and therefore reducing $CO_2$ in our atmosphere. The Rheticus project mimics the natural photosynthesis designing an artificial photosynthesis system.

The energy efficiency and water consumption of the natural photosynthesis are limiting factors. Artificial photosynthesis improves this energy efficiency and water consumption.

Our concept of an artificial photosynthesis is based on the combination of electrolysis and biocatalysis. By this means bulk chemicals as well as specialties are accessible. Therefore this concept represents an additional interesting power to chemicals approach to preserve our current living standard in a sustainable manner.

S5T2

# VerBioChem – employing metathesis to produce biobased building blocks for the chemical industry from renewable sources

Andreas Kohl

*Verbio Vereinigte Bioenergie AG, Germany*

The Chemcial industry has a growing interest and demand for Chemicals based on bio or renewable soruces with a low CO2 footprint. VERBIO Vereinigte BioEnergie AG in cooperation with its subsidiary XiMo Hungary kft, has developed a process and a unique new catalyst system to produce methyl-9-decenoate (9DAME), 1-decene and C18 diacids derivatives from renewable rapeseed methyl ester by olefin metathesis / ethenolysis, commercializing a new platform for renewable specialty chemicals.

The new process VerBioChem, provides access to functionalized and unfunctionalized medium chain C10 alpha olefins in an environmentally friendly and economically attractive way from readily available renewable rapeseed methyl ester. Furthermore, the metathesis platform can be used to produce a number of useful C18 diacids e.g. Dimethyl-9-octadecenedioate (9ODDAME) or Dimethyl octadecanediaote by homocross-metathesis as new biobased building blocks for the chemical and especially the polymer industry.

Verbio will construct a first-of-its-kind commercial scale ethenolysis and catalyst production plant in Germany and Hungary respectively. In a first step, Verbio will invest in a ethenolysis plant with a nominal capacity 50-60 ktpa of products. The investment will provide Verbio's customers access to biobased specialty chemicals with a low CO2 footprint at commercial scale by 2024/25. The catalyst production plant of XiMo Hungary kft will have a capacity of 10-12 tpa and will provide access to Schrock type metathesis catalysts for Verbio as well as for external customers.

S5T3

# Biobased Polyamids from Waste

Paul Stockmann

*Fraunhofer BioCat, Germany*

Commercial polyamides that are partly or fully derived from renewable resources are one of the few examples for biopolymers that can compete with their fossil-based counterparts in the group of engineering plastics. A good example is PA11, which is produced from castor beans after several processing steps and subsequent chemical conversions. PA11 contains eleven methylene groups between the name-giving amide groups, whereas the fossil-based PA6 and PA12 contain six and 12 methylene groups, respectively. In all cases, these hydrocarbon backbones are linear, bearing no substituents or ring structures, thus offering only minor differences in their fundamental properties and, consequently, applications. To further extend the structural variety of polyamides and explore new sustainable raw materials for their precursors, the utilization of industrial side streams has become a focus of research. Monoterpenes are side products that can be isolated during industrial wood processing or juice production. They are very well suited as precursors for bio-based polyamides with novel structures and properties. The Fraunhofer IGB Straubing branch has investigated several polyamide monomers derived from monoterpenes, resulting in the development of the 100% bio-based polyamides Caramid-S® and Caramid-R®. These polyamides are both accessible from the monoterpene 3-carene that is converted to the monomers 3S-caranlactam and 3R-caranlactam in a scalable four-step synthesis. Both polyamides show a high glass transition temperature (Tg) of over 110 °C. While Caramid-S® is opaque and semi-crystalline and melts at a temperature as high as 290 °C, Caramid-R® is transparent and amorphous. The natural chirality of 3-carene is maintained during the polymer synthesis, which leads to chiral polyamides that can potentially be utilized for growth control of microorganisms. Another distinction to commercial bio-based polyamides is the possibility to produce Caramid-S® and Caramid-R® by anionic ring-opening polymerization, potentially leading to cast polyamides with outstanding molecular weight and mechanical properties.

S5T4

# Circular Economy in the Automotive Industry – the BMW approach

Markus Seidl

*BMW, Germany*

S5T5

# The transition of a chemical company to be green - the I am green case

Ana Carolina Saad Reitas

*Braskem, Brazil*

Braskem's strategy to achieve carbon neutrality by 2050 and the successful case of a bio-based process development – "I'm green" the first plastic from a renewable source produced on an industrial scale in the world, a polyethylene that uses sugarcane as its raw material.

S5T6

# Approaching Circularity with Wood, Fibrillated Lignocelluloses and Biomass Residuals

Orlando Rojas

*University of British Columbia, Canada*

I introduce three emblematic cases associated with our recent work that highlight the great possibilities of circularity in the bioeconomy based on forest biomass and residuals. First, I discuss a processing route that transforms low-value wood (residual, damaged, decayed, disposed or fractured) into lightweight and strong structural materials. The process involves delignification, combined with partial dissolution and regeneration, to expose cellulose fibrils originally present in the cell walls. The latter form strong hydrogen bonding networks at interphases, leading to a 'healed' wood with a mechanical strength that exceeds that of typical metals and commercial laminated wood. Moreover, recyclability as well as excellent resistance against organic solvents are demonstrated, providing a promising valorization and sustainability pathway for low-value wood [1]. Following similar approaches, I next discuss an option for valorization of biomass, in this case, blueberries pruning residuals and food waste and losses, sourced from agro-forestry operations that can be used to produce added-value products, including platform chemicals and value-added materials [2,3]. Along such examples, I briefly show the premise of new routes for the production of fibrillated cellulose [4,5]. Finally, I given an example of a facile strategy to synthesize all-green SUPs based on chitin nanofibers . The latter are demonstrated for their facile recyclability and biodegradability in natural environments, addressing the limitations of circularity and end of life of non-renewable products [6]. Given the low-cost of the raw materials, their natural micro-structural design and self-adhesion, this presentations show fully sustainable alternatives to products based on nonrenewable carbon.

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S5T7

# Hydrocarbon-producing cytochrome P450 enzymes: mechanisms and selectivities

Leticia Zanphorlin Murakami

*CNPEM, Brazil*

Alka(e)nes are excellent hydrocarbon candidates that can be directly used as drop-in biofuels, as they have similar properties to those of current petroleum-based fuels, being energy-dense biofuels with no oxygen in their composition. The usage of drop-in biofuels can decarbonize the aviation and marine transport sectors, in which electrification is a challenging alternative. Moreover, alkenes (olefins) also represent an important industrially building block to produce ethylene, propylene, normal butylene, and isobutylene, which have an end-use in plastics, artificial rubber, solvents, and resins. After the discovery of the first P450 CYP152 OleTJE in Rude et al., (2011), reported with its unique property of decarboxylating fatty acids (FA), by using hydrogen peroxide as a cofactor and producing 1-alkenes as the main product, the scientific and technological interest in this family of enzymes vastly increased. Aiming at exploring new decarboxylase representatives within CYP152 members, phylogenetic analyses were performed, including only protein sequences in which amino acids considered important for the fatty acid decarboxylation activity are conserved. The present work presents a new decarboxylase (OleTDRN) with low similarity with OleTJE (32%), its biochemical characterization, and the structure elucidation. Besides that, structure-guided mutations were performed and, according to the functional characterizations, it was observed that some mutations presented different specificity and chemoselectivity by varying the chain-length of FA substrates from 12 to 20 carbons. These results are extremely interesting from a biotechnological perspective as those characteristics could diversify the applications and contribute to better design new enzymes to produce alkenes. Since the knowledge on intriguing molecular mechanisms involved in the decarboxylation preferential from OleTJE is elusive, the elucidation of the OleTDRN structure and its functional characterization provide new information on the CYP152 family. This work also demonstrates the potential applicability of this versatile decarboxylation system for biological routes aiming at the biosynthesis of alkenes.

# Poster

New concepts for valorizing biogenic waste streams

C1 compounds as substrates for industrial biotechnology

Integrating hydrogen into biotechnology

Biotech with hybrid technologies

Bio-based materials with tailored functions

Regional and global effects of the bioeconomy

P001

# An approach on identifying Bavaria's food waste as valuable resource for circular bioeconomic business models

Kathrin Auer[1]; Rogers, Helen[2]

[1] TH Deggendorf, TH Nürnberg & Universität Stuttgart, Germany; [2] TH Nürnberg, Germany

According to the German Federal Environmental Agency , in Germany alone, about one third of all food produced is thrown away without consumption. With this amount of over-production, by-products and waste, resources like human labor, land, water are wasted, and CO2 is being produced.

It is the author's goal to identify the sources, kinds, quantities, and frequencies of food waste occurrence along the food supply chain in Bavaria, especially when it comes to the B2B part of the supply chain. She gathers data about food waste occurrence in the areas and industries of agriculture, food logistics, production, wholesale, retail, hotels, and gastronomy in order to create a market overview and to derive the biggest potentials of food waste as future resource for further upcycling and re-use.

The author uses primary quantitative and qualitative research methods to identify this local potential. She is in possession of an extensive database of companies in the food and related industries to create the broadest overview over food waste occurrence in the B2B sector in Bavaria.

In a second step, the author will identify potential areas of application and customers for these resources to align supply and demand.

It is her goal to share knowledge with potential customers of these newly identified resources to accelerate circular businesses in the area of bioeconomy and to increase the production, consumption and acceptance of their products.

In a following step, factors for success and failure of circular business models in the food sector will be discussed.

P002

# Investigating biocompatible Deep Eutectic Solvents for one pot integrated Bioethanol production from sugarcane bagasse

Vallari Chourasia[1]; Bisht, Meena[2]; Henry, Robert[3]; Pant, Kamal[4]

[1] *The University of Queensland, India;* [2] *Institute of Plant Genetics of the Polish Academy of Sciences, Poland;*
[3] *The University of Queensland, Australia;* [4] *Indian institute of technology Delhi, India*

Lignocellulosic biomass (LBM) represents a sustainable and renewable energy source for addressing the emerging global energy demands and mitigating the environmental damage caused by excessive fuel consumption. Moreover, bioethanol generated from LBM is a potential solution to combat these energy demands of the ever-growing population. However, the process confronts several obstacles, including high operating costs and energy requirements, environmentally hazardous pre-treatment techniques with low process efficiency. Deep eutectic solvent (DES) based LBM pre-treatment has recently evolved as an environmentally friendly technology that offers several benefits over conventional biomass conversion techniques. In addition, CBP, or consolidated bioprocessing, has a number of benefits over conventional multi-reactor biomass processing, and being a relatively new field of study, it can be further explored. In our study, novel DESs were synthesised using choline chloride: ethylene glycol: Lewis acids (1:2:0.016) and utilised for the pre-treatment of sugarcane bagasse (SB) in a microwave assisted process at optimized conditions which resulted up to 82% lignin removal. Furthermore, cellulase enzymes were employed to achieve a saccharification yield of up to 98%, and fermentation with saccharomyces cerevisiae led to the production of 43.56 g/L of ethanol with a volumetric productivity of 0.91 g/L h in a one pot CBP. In this case, the maximum theoretical yield of 50.62 % was attained. Without DESs, ethanol yield was 17.89 g/L after 48 hours of fermentation. Additionally, the cost of producing ethanol was evaluated using NREL databases, and the estimated net selling price for ethanol was $9.75 per gallon, while the environmental assessment using Open LCA software revealed a significant reduction in toxicity compared to typical pre-treatment methodologies.

P003

# Yeast synthetic biology: a powerful tool in bioeconomy

**Fellipe de Mello**[1]; Carazzolle, Marcello[1]; Pereira, Gonçalo[1]

[1] *Unicamp, Brazil*

Microorganisms are praised for the sustainable production of molecules of interest in modern society, and synthetic biology tools comes in handy when better productivity or de novo pathways are preeminent for the establishment of a biotechnological route. When exposed to the industrial environment, the yeast Saccharomyces cerevisiae – the species of choice for most industry – is subjected to stresses that pose challenging conditions for the efficient fermentation of a given substrate. Identification of mutations underlying outstanding resistance traits that endow robustness to this microorganism is paramount for the assembly of top-performing chassis through reverse engineering strategies, representing the starting point for the construction of such biofactories. Regarding bioethanol industry, we have mapped the genetic architecture of S. cerevisiae with extreme tolerance towards low pH, high temperature and elevated concentrations of ethanol and aldehydes – drawbacks for yeast metabolism in the sugarcane and corn ethanol industry. This have uncovered amino acids changes in alleles that eventually enhances yeast phenotype concerning one of these stresses found in large scale production. In parallel, we have developed a CRISPR-Cas9 toolbox that efficiently performs genomic editing of diploid yeast isolated from bioethanol mills. Such system has been applied not only for reverse engineering, but also for the rational editing of yeast metabolism to either improve cell performance or to express heterologous pathways. In the prospect to produce ethanol from neglected biomass or other conventional feedstock, bioinformatic tools were used to uncover new genes encoding xylose isomerases, inulinases and amylases that finally allowed fermentation of xylose, inulin, and starch, respectively. Besides ethanol, we also describe the production of other molecules, such as xylitol, to exemplify the diversity of applications of engineered S. cerevisiae. Next, cell-free strategies will be employed for testing a paradigm shift in the biorefineries, contributing to the expansion of the bioeconomy landscape.

P004

# Prebiotic potential of starch and fiber from banana pseudo-stem

Marcia Maria de Souza Moretti[1]; Kawata Salgaço, Mateus[2]; Sivieri, Katia[2]; Barretto Penna, Ana Lúcia[1]

[1] *São Paulo State University (UNESP), Institute of Biosciences, Humanities and Exact Sciences, Brazil;*
[2] *São Paulo State University (UNESP), School of Pharmaceutical Science, Brazil*

The prebiotic effects of cooked (C) and uncooked (U) starches and fibers extracted from banana pseudo-stem (BPS), maçã (Musa acuminata × M. balbisiana, AAB Group, ‚Silk' [M]) and nanica (Musa acuminata Cavendish Subgroup [N]) varieties, were evaluated. The starch content was quantified in the BPS starches and fibers samples, and approximately 50% were resistant starches. MBPS and NBPS fibers samples presented cellulose (27.0 and 52.4 % w/w) and hemicellulose (25.4 and 33.8 % w/w), respectively. The bioactive compounds contents of MBPS fiber and NBPS fiber included phenolic content (153.5 mg GAE/100g and 193.6 mg GAE/100g) and antioxidant activity estimated by ABTS (145.0 and 50.7 µmol Trolox/g), DPPH (72.5 and 102.8 µmol Trolox/g) and FRAP (14.9 and 12.1 µmol sulfato ferroso/g) assays. Formulated medium supplemented with 1% of BPS starch and BPS fiber was used as substrate for the growth of nine lactobacilli strains (GG, SJRP30, SJRP43, SJRP49, SJRP57, SJRP145, SJRP146, SJRP149 and SJRP169) and one Bifidobacterium strain (Bifidobacterium animalis subsp. lactis BB-12). An initial screening showed that the formulated medium supplemented with 1% the sample UNBPS modulated the proliferation of Limosilactobacillus fermentum SJRP43. The protection of BPS fiber on the viability of probiotic strains was also observed when an in vitro model test was employed under human gastrointestinal conditions, using SHIME®. The ascending colon was inoculated with fecal microbiota from pre-diabetic volunteers. Ammonium ion and microbiota composition were determined by selective ion and microbial counts of different groups of bacteria, respectively. After seven days of prebiotic and probiotic treatment, there was an increase of approximately 4.0 and 1.0 log CFU/mL cycle of total lactobacilli and Bifidobacterium spp., respectively, as well as, an increase of the prebiotic index (PI) of 0.559. Thus, fiber from BPS and L. fermentum SJRP43 are able to stimulate the growth of probiotics bacterial, in SHIME®.

P005

# Enzymatic cocktails targeting fermentable and rare sugars: applications from biomass biorefinery and the dairy industry

**Elisângela Soares Gomes-Pepe[1];** Pereira, Pâmela Aparecida Maldaner[2]; Castellane, Tereza Cristina Luque[1]; Lima, Natália Sarmanho Monteiro[1]; Lemos, Eliana Gertrudes de Macedo[1]

[1] *São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences, Brazil;*
[2] *Institute for Research in Bioenergy (IPBEN) - UNESP Jaboticabal, Brazil*

Agroindustrial residues such as plant biomass and whey constitute an abundant and cheap raw material for the enzymatic production of sugars of biotechnological interest, like fermentable (second generation ethanol) and mono/oligosaccharides for food and pharmaceutical use, such as rare sugars and galacto/fructo-oligosaccharides (GOS/FOS). The combination of enzymes in cocktails, especially when they act in a reaction cascade, is capable of synergistically increasing efficiency, shifting the balance to favor the derivation and yield of products of interest. In addition, enzyme cocktails can be used to in situ modify the constituent sugars of a given food, resulting in new flavors and nutritional enrichment. Thus, we evaluated the creation and optimization of enzymatic cocktails for two applications: biomass saccharification and production of rare sugars. For this purpose, three enzymes previously characterized by our research group were used: two glycoside hydrolases and one oxidase. Additionally, a glycosyltransferase was selected through a study of heterologous expression of eight enzymes prospected from different collections of metagenomes, consortia and genomes, and the construct with the best expression yield was chosen. The best results for biomass saccharification were for bagasse and corn straw under alkaline pretreatment, reaching maximum conversion when the two glycosyl hydrolases and the oxidase were combined. In relation to the production of rare sugars, the results obtained were used to prepare an enzymatic cocktail that aimed to modify the sugars in milk, being adequate to reduce the lactose content by half, making the milk enriched with the rare, hypoglycemic and prebiotic sugar tagatose (244 mM). As an additional effect of the enzymatic synergy, there was a noticeable increase of a peak apparently corresponding to GOS, which will be confirmed by further analysis. These results exemplify the power of green chemistry exerted by enzyme cocktails for agricultural and livestock waste biorefinery and food biotechnology.

P006

# Low-biomass concept for industrial biotechnology with engineered Vibrio natriegens

Maurice Hädrich[1]; Schulze, Clarissa[1]; Blombach, Bastian[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

In recent years, Vibrio natriegens gained attention in biotechnology due to its very fast growth and associated high specific substrate uptake rate (qS). To reach a high volumetric productivity (QP) most industrial production processes must operate at a high final biomass concentration, wasting significant amounts of carbon for biocatalyst generation. Therefore, the choice of future production processes will be low-biomass processes with non-growing but metabolically highly active catalysts. To achieve this, we deleted the aceE gene encoding the E1 subunit of the pyruvate dehydrogenase complex (PDHC). This PDHC-deficient strain is acetate auxotroph with a decoupled catabolite repression, allowing the simultaneous consumption of glucose and acetate. Moreover, the biomass formation can be adjusted by the initial amount of acetate, splitting the whole process into a growth and a production phase in which pyruvate is secreted into the medium. Already during growth on 7.5 g/L glucose and 1 g/L acetate in shaking flasks, up to 4.0 ± 0.3 g/L pyruvate were excreted. Therefore, batch fermentations were performed and up to 22.2 ± 0.8 g/L pyruvate were produced after 10 hours with a yield of 0.59 ± 0.04 g pyruvate / g glucose, which is already in the range of other reported processes between 0.4 and 0.72 g/g. To further enhance the process, an acetate feed to supply the cell's energy demand in the non-growth production phase was added. An acetate feed of 8 mM/h starting when the growth phase ends, increased the titer to 41 ± 2 g/L pyruvate with a cx,max of 6.6 ± 0.4 g/L. Moreover, the qs of the non-growing cells increased to 3.5 g glucose / g CDW * h, close to the qs of exponentially growing cells and therefore over twice as high as the qs of growing E. coli.

P007

# Metatranscriptomics of the gut microbiome of black soldier fly larvae bred on lignocellulose-rich fiber diets

**Eric Gathirwa Kariuki[1];** Tanga, Chrysantus[2]; Kibet, Caleb[2]; Escobar, Juan Paredes[2]; Mboowa, Gerald[3]; Bugg, Timothy D. H.[4]; Masiga, Daniel[2]

*[1] Makerere University, Kenya; [2] International Centre of Insect Physiology and Ecology (icipe), Kenya; [3] Makerere University, Uganda; [4] The University of Warwick, United Kingdom*

Metatranscriptomics of the larval gut of the black soldier fly revealed lignocellulosic biomass-degrading bacteria and CAZyme families that could be applied in, but not limited to, hydrolytic processes in second-generation biofuel production. The Black soldier fly larvae degrade and valorize organic biomass through the gut microbiome. Their rapid growth and broad degradation capabilities present them as a potential source of lignocellulolytic microorganisms and enzymes. The BSF larvae were bred under different diets and selected based on their increasing lignin contents—processed chicken feed (CF), chicken manure (CM), brewers' spent grain (BSG), and water hyacinth (WH)—to identify microorganisms associated with lignocellulolytic activities in the BSF larval microbiome. mRNA libraries from 15 samples (n=15) were prepared, and RNA-Sequencing was conducted using the PCR-cDNA approach with the MinION sequencing platform. We then compared the gut microbiome and functional profiles among the samples. Pooled metatranscriptome samples of larvae bred on highly lignocellulosic diets BSG and WH possessed in high abundance Bacteroides and Dysgonomonas genera known to be involved in lignocellulolytic functions. CAZyme screening revealed arabinofuranosidase families GH43_16 and GH51 in the BSG and WH metatranscriptome samples, respectively. These families are known for degrading arabinoxylan and arabinogalactan hemicellulose fractions. Polysaccharide Utilization Loci (PUL) screening further revealed hits of gene clusters that encode hemicellulolytic arabinofuranosidases in the CAZy family GH51. We conclude that dietary intervention with highly lignocellulosic substrates induced notable changes in gut microbiome profiles of BSF larvae and ultimately CAZyme profiles for lignocellulolytic degrading microorganisms and enzymes.

**Keywords:**

Hermetia illucens, RNA-Sequencing, Bioprospecting, long-read sequencing, polysaccharide utilization loci

P008

# Valorization of Dairy Sidestreams through Selective Magnetic Separation of Valuable Proteins

Eva Krolitzki[1]; Schwaminger, Sebastian[2]; Berensmeier, Sonja[1]

[1] *Technical University of Munich, Germany;* [2] *Medical University of Graz, Austria*

With the increasing demand for high-protein dairy products such as Greek yogurt, world-wide dairy side streams (i.e., sweet and acid whey) had already surpassed 200Mio tons per year in 2018. The processing of acid whey is challenging due to a high salt load and acidity (pH4.3). Since the taste of acid whey powder is slightly bitter, it is less suitable for human consumption and is mainly used as animal feed or disposed of at a high cost. However, acid whey contains the valuable protein lactoferrin (LF) in low concentrations (~0.05mg/mL). LF is a multifunctional protein in most bodily fluids of mammals, with particularly high concentrations in milk (~1-10mg/mL). Since bovine LF is very similar to human LF, it is an essential ingredient in formula. In our innovative, automated magnetic bioseparation process, we use low-cost, superparamagnetic bare iron oxide nanoparticles (BIONs) to isolate the remaining LF from acid whey. Synthetic iron oxides, also known as food colorant E172, are generally recognized as safe (GRAS) for human food use by the FDA. Since BIONs can act as cation exchangers, the cationic LF binds to BIONs in the acid environment in the batch adsorption step. Subsequently, we separate the protein-loaded BIONs from the liquid phase with a commercially available, cGMP-ready rotor-stator high-gradient magnetic separator (RS-HGMS). After several in-line monitored washing cycles, we separate the LF from the BIONs through a salt elution and receive a high-purity LF (>92%) elution with a concentration of 1.1g/L at a recovery rate of 91% and a yield of 60%. Currently, we are working on further automation and digitalization of the process to have a digital twin ready for future applications. Since crude, heterogeneous streams with high loads of solids can be processed effortlessly in the RS-HGMS, this promising technology is also feasible for valorizing other biological streams.

P009

# Extremophile microbial communities: sources for prospecting new biomolecules for application in biorefinery

Pâmela A. Maldaner Pereira[1]; Inácio Gonçalves Funnicelli, Michelli[2]; Bonfá Buzzo, Bárbara[1]; Soares Gomes-Pepe, Elisângela[2]; de Macedo Lemos, Eliana G.[1]

[1] *Institute for Research in Bioenergy (IPBEN) - UNESP Jaboticabal, Brazil;*
[2] *São Paulo State University (UNESP), School of Agricultural and Veterinary Sciences - FCAV, Brazil*

The advent of metagenomics has made it possible to broadly explore the microbial diversity and function of diverse environments, such as the marine environment. This technique is useful to prospect new biomolecules, such as lipolytic enzymes of interest to several industrial sectors, including biorefineries, food, pharmaceutical, and detergent industries, due to the diversity of reactions they catalyze. In this sense, lipases were prospected in marine metagenomes sequenced and deposited in public databases through searches based on HMM profiles, where 2,634 sequences were recovered and screened to select genes not yet characterized and with high biotechnological potential through in silico approaches. The prospection resulted in 73 sequences belonging only to the I.5 subfamily of true lipases and, from the in silico analyses, sequences of pathogenic organisms were excluded, with incomplete domains or with the presence of transmembrane helices, and genes already characterized or with active patents, leaving 19 sequences with desirable characteristics for in vitro studies. From these, the Oleispira antarctica lipase had its three-dimensional model generated through homology modeling and submitted to structural analyses, making it possible to understand a little more about the characterization profile of marine enzymes. Therefore, it was possible to identify the conserved pentapeptide forming a structure known as a nucleophilic elbow and visualize the typical structural folding of α/β hydrolases and the presence of a "lid", consisting of an amphipathic and flexible α-helix on the surface of this enzyme covering its catalytic site. These results show that the marine environment is an important source of lipases with a potential biotechnological application for harboring genes not yet characterized and for its differentiated salinity and temperature characteristics that make its enzymes useful to integrate new processes. Furthermore, the workflow used in this study can be expanded to other enzyme classes and direct in vitro studies.

P010

# Concept for the enzymatic recovery of proteins from agricultural side-streams

Marcel Mayer[1]; Hupfeld, Enrico[1]; Sieber, Volker[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

In the European Union each year large amounts of protein-rich side-streams originate from agricultural processing. Typically, these proteins are treated as waste or are used as low-value animal feed. In the context of a bioeconomy, these proteins would better be valorised to high-value products with lower amounts of waste created, such as dietary supplements and stabilizers. Here, we evaluated the potential of enzymatic crosslinking of proteins for their removal from liquid side-streams in the model system potato fruit juice, which is an abundant, protein-rich side-stream from the production of potato starch. Two oxidases from the enzyme families tyrosinases and laccases showed crosslinking activity on potato proteins. With regard to crosslinking efficiency, the side-stream potato fruit juice was analysed and hurdles and milestones, which need to be overcome for successful industrial application were specified. We also estimate that side-streams from the production of dairy products are an excellent target for enzymatic protein removal.

P011

# Upscaling the production of a monomer for bio-based plastics from lignin

**Jan Notheisen[1];** Gómez-Álvarez, Helena[2]; Nogales, Juan[2]; Díaz, Eduardo[2]; Rashid, Goran[3]; Bugg, Timothy D. H.[3]; Takors, Ralf[1]

*[1] University of Stuttgart, Germany; [2] Spanish National Research Council (CSIC), Spain; [3] University of Warwick, United Kingdom*

Lignin is the second most abundant biopolymer on earth and a waste product of the lignocellulose processing industry. Valorization of lignin appears to be difficult due to its recalcitrant structure. At this point biotechnology can help upcycling this widely underused sustainable resource. Starting from lignin, the production of an aromatic monomer for bioplastic production is possible using two engineered microorganisms.

Rhodococcus jostii can break down the lignin polymer and metabolize released monoaromatics. Thereby, protocatechuic acid (PCA) can accumulate. Engineered Pseudomonas putida enables conversion of PCA to 2,4-pyridinedicarboxylic acid (PDCA). PDCA is an analogue of terephthalic acid and can replace it in common plastics like PBAT or PET.

Previous work[1,2] proofed the feasibility of this approach in small scale. However, it is important to produce PDCA in large quantities at low cost to replace fossil-based plastics. One hurdle on this way is the cultivation of R. jostii, since this bacterium is largely unexplored in the field of bioreactor cultivation. Additionally, a process is required to combine the catalytic potential of both strains. In this work, growth of R. jostii is characterized and optimized to produce up to 35 g L-1 biomass. Furthermore, lignin degrading R. jostii and PDCA producing P. putida are combined in resting cell biotransformation for conversion of lignin to produce a monomer for bio-based plastics.

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P012

# Towards valorization of pectin-rich agro-industrial residues: Engineering of Saccharomyces cerevisiae for co-fermentation of d-galacturonic acid and glycerol

**Andreea Perpelea[1];** Wijaya, Andy Wiranata[1]; Martins, Luís C.[2]; Rippert, Dorthe[1]; Klein, Mathias[1]; Angelov, Angel[3]; Peltonen, Kaisa[4]; Teleki, Attila[5]; Liebl, Wolfgang[3]; Richard, Peter[4]; Thevelein, Johan M.[6]; Takors, Ralf[5]; Sá-Correia, Isabel[2]; Nevoigt, Elke[1]

*[1] Jacobs University Bremen, Germany; [2] Instituto Superior Técnico, Universidade de Lisboa, Portugal; [3] Technical University of Munich, Germany; [4] VTT Technical Research Centre of Finland Ltd, Finland; [5] University of Stuttgart, Germany; [6] NovelYeast, Belgium*

Pectin-rich plant biomass residues represent underutilized feedstocks for industrial biotechnology. The conversion of the oxidized monomer d-galacturonic acid (d-GalUA) to highly reduced fermentation products such as alcohols is impossible due to the lack of electrons. The reduced compound glycerol has therefore been considered an optimal co-substrate, and a cell factory able to efficiently co-ferment these two carbon sources is in demand. Here, we inserted the fungal d-GalUA pathway in a strain of the yeast S. cerevisiae previously equipped with an NAD-dependent glycerol catabolic pathway. The constructed strain was able to consume d-GalUA with the highest reported maximum specific rate of 0.23 g/gCDW/h in synthetic minimal medium when glycerol was added. By means of a 13C isotope-labelling analysis, carbon from both substrates was shown to end up in pyruvate. The study delivers the proof of concept for a co-fermentation of the two 'respiratory' carbon sources to ethanol and demonstrates a fast and complete consumption of d-GalUA in crude sugar beet pulp hydrolysate under aerobic conditions. The future challenge will be to achieve co-fermentation under industrial, quasi-anaerobic conditions.

P013

# Application of novel oxidoreductases for an electrochemical NADPH regeneration system

**Sakoleva, Thaleia[1];** Austin, Harry[1]; Doerr, Mark[1]; Bornscheuer, Uwe[1]

[1] *University of Greifswald, Germany*

In electroenzymatic processes the combination of high enzyme selectivity and electrochemical regeneration of co-factors required for the enzymes has gained the interest in respect of several applications, such as the development of new biotechnological processes for fine and commodity chemicals.[1] The direct utilization of electrons in enzymatic redox transformation is a visionary green and sustainable alternative to chemical electron donor systems, if a sufficient electron donor efficiency is achieved. Thus, special electrode materials need to be developed in conjunction with tailored enzymes specific for new electron transfer pathways and the conditions in an electrochemistry cell. In parallel, for the synthesis of more complex chemicals, enzymatic cascades are needed, while NAD(P)H recycling is essential for many enzyme catalyzed redox reactions.[2-6] To reach this challenging goal, we are optimizing new oxidoreductases, that can be used in enzymatic cascades to selectively build more complex and higher value products. The application of the oxidoreductases in an electrochemical NADPH recycling system towards higher product yields, will also become interesting for industrial applications. Thus, the possibility of tuning enzyme properties to the electroenzymatic reactor's conditions is going to be investigated in this project. Therefore, a biocatalyst toolbox will be used to suggest proper model enzymatic cascade and to adjust enzymes to conditions in the electroenzymatic reactor through electrochemistry expertise with project partners. With this work, we hope to contribute to a less wasteful production of high-value products with a much cleaner and more efficient enzymatic process.

P014

# Domestication of Vibrio natriegens for industrial biotechnology

Clarissa Schulze[1]; Hädrich, Maurice[1]; Blombach, Bastian[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

Exopolysaccharides (EPS) are high molecular weight carbohydrate polymers that are essential for biofilm formation, protect the cell from environmental stress, and play an important role in pathogenicity. EPS are of great interest for industrial use due to their chemical diversity. However, production is challenged by a change in the rheology of the culture broth. This negatively affects the mixing of the broth and leads to a heterogeneous system with reduced mass transfer.

Vibrios are model organisms for studying EPS production and biofilm formation. One of them is Vibrio natriegens, a promising host for industrial biotechnology due to its highest growth rate among non-pathogenic microorganisms and its outstanding high biomass specific substrate uptake rates. The potential for V. natriegens as a production strain for amino and organic acids has already been shown (e.g. for succinate). In such processes, EPS is an unwanted byproduct and complicates the production of the desired product and subsequent downstream processing. A fed batch process was established for the wild type (wt). During the process, EPS was produced which led to an increased viscosity and a poor oxygen transfer rate. To address these issues, several mutant strains carrying gene deletions associated with EPS formation were tested in fed batch fermentations, and viscosity, as an indirect measure of EPS formation, was determined and compared with the wt. The strains showed clear differences during fermentation. Up to 12 hours of process time, viscosity was similar for all strains and no increase was observed. Afterwards, viscosity increased for the wt and ΔwbfF strains. Subsequently, the viscosity decreased again, whereas it continued to increase for the wt. In contrast, viscosity was constantly low for the mutant strains ΔcspR and ΔvpsII. Thus, it was successful to overcome challenges in the operation of bioreactors caused by EPS formation and the associated high viscosities.

P015

# Genome reduced Corynebacterium glutamicum strains as screening platform to identify a hidden pathway relevant for 1,2-propanediol production

**Daniel Siebert**[1]; Wirth, Marie-Theres[1]; Vannahme, Mieke[1]; Saydam, Ezgi[1]; Möggenried, Dominik[1]; Wendisch, Volker F.[2]; Blombach, Bastian[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany;* [2] *University of Bielefeld, Germany*

In the endeavor to build a predictable and easier understandable microbial cell factory, synthetic biology offers the concept of chassis organism. Via a top-down approach such a chassis was constructed with the industrial workhorse Corynebacterium glutamicum leading to the strain C1* with 412 deleted genes (13.4% genome reduction). Strain C1* shows wild-type growth behavior with glucose as sole carbon and energy source in minimal medium. As intermediate steps the pre-chassis strains PC1 (8.8% genome reduction) and PC2 (12.6% genome reduction) were constructed and the latter one was applied as screening platform to improve 1,2-propanediol (1,2-PDO) production in C. glutamicum. After implementing a 1,2- PDO pathway, PC2 showed a strongly decreased 1,2-PDO titer (~25% compared to parental strain GRS), while 50 mM lactate was secreted combined with a growth retardation. This phenotype could be deduced to the missing D-lactate dehydrogenase gene dld in PC2. This indicated a hidden pathway in wild type metabolism probably leading to a loss of carbon for 1,2-PDO production. This sink occurs most likely due to a detoxification of the pathway intermediate methylglyoxal via a glyoxalase-like system known from Escherichia coli, but not described for C. glutamicum yet. While E. coli harbors glutathione as primary thiol for detoxification reactions, Actinobacteria like C. glutamicum use mycothiol (MSH) instead. By deleting mshA, the initial gene of MSH synthesis, the 1,2-PDO yield was improved by 56% (0.53 vs 0.32 mol1,2-PDO per molglucose). This approach showed the successful application of a genome reduced chassis organisms to uncover a yet unknown pathway.

P016

# Generating superior enzymes for biofuel production

Mariko Teshima

*Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

Reducing the dependency on fossil resources and shifting fossil-based chemical industry to be more environmental friendly are major aspects to tackle the challenges of sustainable bioeconomy. In earlier works, we proposed an efficient utilization of glucose obtained from lignocellulosic biomass to produce branched-chain higher alcohol isobutanol as a valuable platform chemical and biofuel in vitro.

In the initial cascade runs with yields of merely above 50 %, we identified some rate-limiting steps, in particular aldehyde dehydrogenase (ALDH), which is the key enzyme for the redirection of the byproduct glyceraldehyde into the cycle to get the second pyruvate molecule.

To tackle the limitations due to remarkably high substrate inhibition, preference of NADP+ over NAD+ and protein folding problems, we first started with a successive genome mining of ALDH. In the first round, we successfully obtained two promissing candidates, one with a 16-fold increase of activity and another one with a higher thermostability. In the second round, we succeeded to combine these characteristics in single enzymes and gained an additional improvement of selectivity for D-glyceraldehyde.

To further increase the abilities of promissing homologs, we developed a microfluidic-based high-throughput screening which enabled a fast screening of 63,000 variants. To exploit all the information supplied from this screening, we performed a novel technology to recombine the mutation candidates. With simultaneous increase of activity, we have currently generated a variant with 252-fold extended half-life at 50 °C.

Furthermore, by combining the information from the screening with semi-rational engineering, we achived 24.1-fold higher substrate specificity and an increase of aldehyde tolerance measured as shift of melting temperature by 10-16 °C.

For validation of the engineered variants, we performed the conversion of D-glucose to ethanol. In these experiments, we could demonstrate > 95% conversion and the highest product titer of 1.3 M.

P017

# Utilizing wheat straw hydrolysate for the production of fatty alcohols with Corynebacterium glutamicum

**Felix Werner[1];** Schwardmann, Lynn S.[2]; Siebert, Daniel[1]; Wendisch, Volker F.[2]; Blombach, Bastian[1]

*[1] Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany; [2] University of Bielefeld, Germany*

Fatty acid (FA)-derived products such fatty alcohols (FAL) find growing application in cosmetic products, lubricants or biofuels. As the biosynthetic pathways of FA and FAL synthesis are similar until they diverge at the acyl-CoA node, a Corynebacterium glutamicum ATCC $^{13032}$-derived strain with deregulated FA synthesis was constructed in this study for the microbial production of FAL. Initial strain and process development was conducted using glucose as carbon source, while the final process was aiming at utilizing wheat straw hydrolysate (Clariant, Muttenz, Switzerland) containing primarily glucose and xylose as carbohydrates. Since nitrogen-limiting conditions were previously shown to be essential for the secretion and accumulation of FA, similar cultivation conditions were initially applied for the FAL production.

Two acyl-CoA reductases of Marinobacter hydrocarbonoclasticus VT8 were screened for sufficient activity in the host strain. While pEKEx2-based expression of both reductases led to the production of FAL in C. glutamicum ΔfasR, only the best-performing reductase Maqu_2220 was used in subsequent experiments.

Further strain engineering aiming at the reduction of the thioesterase-catalyzed competing side reaction showed no positive impact on FAL production. However, when adjusting the cultivation conditions from a nitrogen-limited to a solely carbon-limited minimal medium FAL production was strongly increased. Titers of up to 544 ± 16 mg FAL L-1 were obtained by the best-performing strain C. glutamicum ΔfasR cg2692TTG pEKEx2_maqu2220 when cultivated under those new conditions.

Cultivations in CgXII containing 2 % hydrolysate as carbon source instead of pure glucose resulted in even higher titers of 680 ± 22 mg FAL L-1. To enable an efficient hydrolysate consumption, the evolved xylose-utilization module ΔactA::xylABevol (gX) was additionally implemented, resulting in 705 ± 62 mg FAL L-1.

To our knowledge this is the first report of FAL production in C. glutamicum and will serve as a basis for further metabolic engineering in that context.

P018

# New genetic tools for the acetogen Eubacterium limosum to produce biocommodities from methanol using fluorescent FAST-tagged proteins

Maximilian Flaiz[1]; Ludwig, Gideon; Bengelsdorf, Frank R.[1]; Dürre, Peter[1]

[1] *University of Ulm, Germany*

Acetogens are promising biocatalysts to produce various biocommodities from C1-carbon sources. Especially their ability to utilize carbon dioxide ($CO_2$)- and carbon monoxide (CO)-containing industrial waste gases is outstanding and contributes to fighting global warming. Certainly, gas fermentation also faces some challenges since the mass transfer into the liquid state of media is quite poor. Therefore, synthesis gas-based methanol can serve as an alternative as it is easy to transport, store, completely soluble in water, and can be utilized by several acetogens such as Eubacterium limosum. Since this acetogen is already genetically accessible we aimed to expand its genetic toolbox by establishing recombinant pathways to produce butanol and acetone from the C1-carbon source methanol. Moreover, we established the fluorescence-activating and absorption shifting tag (FAST) as a reporter protein to track protein production during growth.

Butanol production from methanol was achieved by heterologous expression of the bifunctional aldehyde/alcohol dehydrogenase from C. acetobutylicum. Fluorescent C- or N-terminally FAST-tagged fusion proteins were constructed to monitor their production during growth. Production of the respective fusion protein resulted in butanol production and brightly fluorescent cells, also proving that the functionality of the enzyme was not negatively affected. In addition, genes of the natural acetone producer C. acetobutylicum were assembled into an acetone production operon (APO), containing genes encoding thiolase, acetoacetyl-CoA:acetate/butyrate-CoA transferase, and acetoacetate decarboxylase. The last gene of the APO was C- or N-terminally FAST-tagged and expressed in E. limosum together with the remaining genes of the APO. Metabolically engineered strains produced acetone and showed distinct fluorescence during cultivation.

FAST expands the genetic toolbox of E. limosum as an oxygen-independent fluorescent reporter protein. FAST-tagged fusion proteins were successfully used to produce butanol and acetone and we showed that the stability, functionality, and productivity of the resulting enzymes was not negatively affected.

P019

# Systems metabolic engineering of Hydrogenophaga pseudoflava for aerobic gas-based production of fatty acids

Simon Grieshaber[1]; Siebert, Daniel[1]; Blombach, Bastian[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

More efficient and sustainable production of chemicals is becoming increasingly important in times of climate change and global pollution. An intresting approach is the use of CO2- or CO-utilizing microorganisms for the biotechnological production of valuable compounds. Current biotechnological processes are based on acetogenic bacteria, which metabolize gas mixtures anaerobically. An energetically more favorable way to generate costly (ATP-intensive) products may be gas fermentation with aerobic carboxydotrophic bacteria. One promising candidate is the Gram-negative

β-proteobacterium Hydrogenophaga pseudoflava DSM 1084. Gene clusters essentially required for the autotrophic lifestyle, encoding CO dehydrogenase (CODH, cox/cut clusters), enzymes for the Calvin cycle, like ribulose bisphosphate carboxylase (RuBisCo, cbb clusters) and hydrogenases (hox/hyp clusters) are entirely located on the chromosome. Advantages of the bacterium are the (I) high growth rate (μ) of 0.06 h-1 on CO-containing gas mixtures compare to other carboxydotrophs, (II) the CO insensitive aerobic electron transport chain, and (III) the high CO uptake rate by CODH. However, a deep understanding of the autotrophic metabolism, especially the electron transfer, is essential to utilize its potential completely. Engineering H. pseudoflava for fatty acid overproduction is an elegant way to challenge the autotrophic metabolism since the biosynthesis of these acetyl-CoA derived molecules represents a strong electron sink and is ATP-demanding. Therefore, we engineer H. pseudoflava for autotrophic fatty acid production by deregulating fatty acid biosynthesis, increasing precursor availability by avoiding by-product formation and degradation of fatty acids and optimizing the process conditions.

P020

# Electrochemical CO2 reduction to formate as intermediate for biochemical transformations

Dhananjai Pangotra[1]; Bohlen, Barbara[1]; Sieber, Volker[2]; Roth, Arne[1]; Vieira, Luciana[1]

[1] *Fraunhofer IGB, Germany;* [2] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

The substitution of fossil-based production routes with renewable resources is a steadily growing research field. Combining intermittent renewable electricity in such pathways is also appealing, especially considering energy storage in chemical bonds. In this context, biogenic raw materials can be electrocatalytically converted into platform chemicals and further utilized for generating value-added products through power-to-X-to-Y processes. One example is the electrochemical conversion of carbon dioxide (CO2) into valuable feedstocks, such as formate, which can be further utilized in (bio)chemical processes. Formate is a valued product with a wide range of applications, including in the chemical, textile, agricultural, and pharmaceutical industries.

Nevertheless, using CO2-based formate as feedstock for biochemical transformations is a fascinating field because it can enable the production of much more valued products. The focus of our research is to allow a compatible interface for the integration of efficient electrochemical processes with biotechnology. Although the catalyst choice is essential for the reaction efficiency in the electrochemical formate production from CO2,[1] other aspects such as electrolyte, electrochemical setup, and formate production rate must be compatible. Hence, the process interface must be tailored to feed formate into coupled bioreactors containing enzymes or microorganisms efficiently. Herein we present some steps of the compatible electrochemical process development. First is the optimization of bio-compatible electrolytes suitable for efficient electrochemical production of formate. In a second step, we focus on high current densities, electrode composition, structure, and stability for the continuous formate production with maximum productivity rate. We achieve up to 3 molar formate in a bio-compatible buffer electrolyte in an optimized electrochemical process at high current densities. The biochemical utilization of electrochemically produced CO2-based formate is successfully demonstrated in coupled electro-biochemical processes.

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P021

# Sequential C1 fermentation to value added products

**Christina Stark[1];** Gaile, Felix[1]; Seitz, Tom[1]; Schwentner, Andreas[1]; Eikmanns, Bernhard[1]

[1] *Universität Ulm, Germany*

With a growing population, declining resources and global warming, alternative and sustainable processes to produce industrially relevant chemicals are urgently needed. Fermentation of climate-damaging C1 compounds like CO2 by anaerobic acetogenic bacteria represents one such process. However, autotrophically growing acetogens like Acetobacterium woodii are known to be energetically limited, rendering the synthesis of higher-value products difficult. This problem could possibly be overcome by applying a two-step fermentation process with anaerobic acetate production from C1 compounds like CO2 and derivatives thereof such as methanol and formate by an acetogen in a first step and subsequent conversion of acetate to higher-value products by aerobic fermentation in a second step. The few existing examples for such sequential C1 fermentations have recently been reviewed[1].

Optimization of A. woodii medium resulted in formation of up to 24 g/L acetate from CO2 and H2, of up to 6 g/L acetate from 6 g/L methanol and of 3.5 g/L acetate from 9 g/L formate. In the second fermentation step, Corynebacterium glutamicum wildtype was used to produce glutamate from CO2-derived acetate in spent acetogenic medium by induction with PenicillinG with a molar yield of 15%. Metabolically engineered C. glutamicum strains produced 3-hydroxypropionic acid or mevalonate from biological CO2-derived acetate, respectively.

We here show proof of principle of a sequential fermentation process enabling the synthesis of higher-value products from CO2 (and H2), methanol or formate using A. woodii and C. glutamicum strains. This approach constitutes a promising climate-friendly alternative to established sugar-based fermentations.

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P022

# Methanol to low-molecular-weight biochemicals by the yeast Ogataea polymorpha

**Katrin Wefelmeier**[1]; Schmitz, Simone[1]; Blank, Lars Matthias[1]

[1] *RWTH Aachen University, iAMB - Institute of Applied Microbiology, Germany*

The methylotrophic yeast Ogataea polymorpha is an established organism for the production of recombinant proteins due to its many beneficial traits, including its ability for high protein overproduction, high thermotolerance and efficient metabolism without overflow metabolites. In the project METAFOR [1], our goal is to use the yeast's native ability for methanol assimilation to extend its product spectrum by metabolic engineering. As a first step, several promoters and terminators were characterized for their expression patterns on methanol to expand the genetic toolbox of O. polymorpha [2]. For this characterization, a short half-life GFP variant was chosen, which allows a precise temporal resolution of gene expression. By varying the terminators alone, a 6-fold difference in gene expression was achieved with the homologous MOX terminator boosting gene expression on all carbon sources by around 50% compared to the second strongest AOX1 terminator from Pichia pastoris.

We then applied the characterized promoters and terminators as genetic parts to produce lactate from methanol in metabolically engineered O. polymorpha strains. Through strain engineering, adaptive laboratory evolution and optimization of the cultivation conditions, we could achieve titers of up to 3 g/L lactate produced from methanol using an O. polymorpha strain expressing a lactate dehydrogenase from Lactobacillus helveticus.

Apart from lactate, we have also demonstrated the production of acetone and isoprene as heterologous products in O. polymorpha, which highlights how O. polymorpha can be applied as a versatile cell factory for the production of low-molecular-weight biochemicals from methanol.

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P023

# Metabolic engineering of microorganisms for the valorization of C1-compounds out of CO2

Vanessa Wegat[1]; Fabarius, Jonathan Thomas[1]; Sieber, Volker[2]

[1] *Fraunhofer Institute for Interfacial Engineering and Biotechnology, Germany;*
[2] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

Climate change is the greatest environmental threat humanity has ever faced. Combustion of fossil fuels account for over 85% of all CO2 emissions worldwide and are considered as the main cause of the anthropogenic greenhouse effect. Besides diminishing the use of fossil fuels and causing lower emissions, the reduction of CO2 in the atmosphere is another way to mitigate these processes. In a sustainable circular economy, atmospheric and industrial CO2 needs to be captured and converted into chemical products and fuels. Primary products from CO2 reduction are C1 compounds, such as methanol or formate. Such C1 species can be utilized by native or synthetic methylotrophic microbes as feedstock for the production of commercially relevant value-added products.

The presented poster describes the metabolism and the properties of methylotrophic yeasts. In particular, an essential difference between the specific branching points of the methylotrophic metabolism of yeasts and bacteria is evaluated here. A deep understanding of their metabolism serves as the basis for synthetic methylotrophic yeasts and its application in the biotechnological industry. By means of literature research and metabolic flux analysis, the bacterial ribulose monophosphate pathway (RuMP) was identified as advantageous. In addition to the reconstruction of native methylotrophic metabolic pathways, the establishment of new, artificial enzyme cascades represents an attractive alternative. Yeast species like S. cerevisiae or non-conventional yeasts like Y. lipolytica have great potential as hosts for engineering synthetic methylotrophy as they provide distinct advantages (e.g. solvent and pH tolerance or compartmentalization) over organisms such as E. coli for use in industrial fermentation.

Despite the challenges, the future for both, native and synthetic methylotrophy, seems promising, as the tools and technologies are now emerging to push the frontier towards efficient C1 utilization in a modern bioeconomy.

P024

# Cell free enzymatic L-alanine synthesis from carbon dioxide based methanol

Vivian Pascal Willers[1]; Döring, Manuel[1]; Beer, Barbara[2]; Sieber, Volker[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany;* [2] *CASCAT GmbH, Germany*

Today, amino acids are mainly produced to feed livestock for protein extraction or as a direct food additive. In addition, they are used as flavor enhancers, dietary supplements, or precursors for chemicals. However, the production of amino acids for food and feed is mostly based on microbial fermentation, which relies on sugars such as glucose or sucrose as a carbon source 1. To make amino acid production more sustainable and ecologically, alternative resources need to be considered. One advance would be production based on C1 compounds such as CO2, format, methane, or methanol 2, 3. As a contribution to a sustainable vision for the future, we show here a synthetic methanol-alanine pathway (MAP) as a cell-free enzymatic cascade as a case study for a sustainable amino acid supply based on renewable energy. The pathway consists of nine enzymes with an intrinsic ATP and NAD+ recycling system.

P025

# Oxidative Biocatalysis without Oxygen

Ammar Al-Shameri

*Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

P026

# Residents' acceptance of a local production of green hydrogen

Johannes Buchner

*Hochschule Weihenstephan-Triesdorf, TUM Campus Straubing for Biotechnology and Sustainability, Germany*

Green hydrogen is seen as a beacon of hope for the German energy transition. With the use of green hydrogen, fossil fuels can be substituted in significant areas of German industry. However, the production of green hydrogen is currently still very expensive and thus its use on a larger scale is currently economically not viable. In addition to the economic aspects, the acceptance of the local population related to green hydrogen facilities also plays an important role in the successful establishment of this technology.

There are already numerous studies analyzing residents' acceptance of different renewable energy systems. For wind energy, the perceived infrasound, the impact on the landscape or the impact on birds have been identified as factors influencing the acceptance of the local population. For biogas plants, odor emissions or the plate-tank problem have been founded. In scientific research, however, hardly any findings exist so far which factors influence the acceptance of the local population with regard to a green hydrogen plant. This research gap will be investigated in this planned doctoral thesis. For this purpose, an online survey on the acceptance of green hydrogen by the local population will be conducted. In this survey residents living next to green hydrogen production plants (planned or existing) will be asked about their acceptance towards this technology. Thus, the local residents will evaluate the strength of factors that can influence the acceptance regarding green hydrogen. Factors include, for example, influences on the landscape, origin of electricity for green hydrogen production, risk of explosion, participation possibilities, or monetary benefits. Identifying the role of these factors in influencing residents' acceptance is intended to support political decision-makers and project manager in the planning of future projects for the production of green hydrogen and thus further advance the energy transition in Germany.

P027

# Reversible Catalysis for H2 Oxidation and Evolution by a [FeFe] Hydrogenase in a Viologen Modified Film

**Steffen Hardt**[1]; Stapf, Stefanie[2]; Filmon, Dawit Tedros[2]; Birrell, James A.[3]; Rüdiger, Olaf[3]; Fourmond, Vincent[4]; Léger, Christophe[4]; Plumeré, Nicolas[2]

*[1] Ruhr-University Bochum, Germany; [2] Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany; [4] Max Planck Institute for Chemical Energy Conversion, Germany; [5] CNRS, Aix-Marseille Université, France*

Reversible electrocatalysts combine bidirectionality with maximum energy efficiency offering significant currents at a minimum overpotential. Hydrogenases are one example of these highly energy efficient, reversible catalysts; however, they deactivate rapidly in the presence of trace amounts of dioxygen. Viologen-based polymers effectively protect this enzyme, however, mediated electron transfer in the film compromises the enzyme´s intrinsic reversibility resulting in unidirectional catalysis. Here, we describe polymer films with covalently-bound, low-potential 2,2´-methylviologen units allowing for reversible hydrogen conversion when [FeFe]-hydrogenase is embedded. Connecting these films as a hydrogen oxidizing anode to a bilirubin oxidase functionalised cathode delivered an H2/O2 biofuel cell with an open circuit voltage of 1.16 V. This value is close to the thermodynamic limit of 1.23 V setting a benchmark value for H2/O2 biofuel cells relying on redox-polymer films. Operated as an electrolyser cathode, the films exhibit a Faradaic efficiency of almost 100%. Together with the negligible overpotential requirements, this results in significant H2 production at energy efficiencies higher than 90%. These catalytic properties were explained by a kinetic model, which shows that reversible catalysis can be achieved under conditions of both fast and slow electron transfer. Hence, a mediated electron transfer process was demonstrated to operate at energy efficiencies that were exclusively accessible under conditions of direct electron transfer up to now. Considering the added advantage of a protecting polymer environment, this system may serve as a blue print for the future application of (oxygen)sensitive biocatalysts in energy conversion and electrocatalysis highlighting the potential of biotechnology in these seminal sectors.

P028

# Design of chimeric hydrogenases for future light-driven H2 production

**Elisabeth Lettau[1];** Lorent, Christian[2]; Teutloff, Christian[3]; Codero, Paul[1]; Bühler, Bruno[4]; Lauterbach, Lars[1]

*[1] RWTH Aachen University, Germany; [2] Technical University Berlin, Germany; [3] Freie Universität Berlin, Germany; [4] Helmholtz Centre for Environmental Research - UFZ, Germany*

The replacement of fossil fuels with renewable energy sources is indispensable in ensuring sustainable energy supply and counteracting global warming. Since water can be used as an infinite proton resource, engineering hydrogenases to produce photohydrogen by accepting electrons from the photosynthetic electron transport chain represents one of the most elegant solutions to this problem.

Fusions of cyanobacterial and algal hydrogenases with photosystem I [1] as well as integration of an O2-tolerant hydrogenase from Cupriavidus necator (CnSH) into the cyanobacterium Synechocystis sp. PCC6803 [2] were recently successfully demonstrated as a proof of principle. However, sufficient production of photohydrogen is currently still a challenge as efficient electron transfer remains to be resolved. The NAD(H)-converting hydrogenase from Synechocystis (SysSH) accepts electrons from low potential ferredoxins suitable for H+ reduction but is oxygen-sensitive in contrast to CnSH [3]. A fundamental understanding of cofactor composition, electron transfer, and biocatalytic properties of the SysSH reductase module is a prerequisite for our ultimate goal to create O2-tolerant chimeras of CnSH and SysSH for photosynthesis-coupled H2 production.

In this study, we characterized the biocatalytic properties of the SysSH reductase module by photometric activity assays and demonstrated the precise cofactor composition by EPR spectroscopy in comparison to other reductase components [4,5]. Using a rational design, various chimeric hydrogenases composed of different combinations of SysSH reductase and CnSH hydrogenase modules were created, showed promising electronic coupling between two modules.

Our study provides critical insights into the structure and function of metallocofactors in cyanobacterial hydrogenases and has enabled the design of O2-tolerant hydrogenases suitable for future photohydrogen production.

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P029

# Making [FeFe]-hydrogenases fit for applications

Martin Winkler

*Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

[FeFe]-hydrogenases catalyze the reversible interconversion between protons and hydrogen ($H_2$) at moderate conditions and very low overpotentials, reaching the catalytic performance of platinum metals (1, 2). Even though they exhibit high turnover frequencies, in applied systems the turnover numbers and half-lives of [FeFe]-hydrogenases are limited due to their sensitivity to certain system parameters. Most [FeFe]-hydrogenases e.g. share a pronounced sensitivity towards $O_2$ that rapidly leads to the irreversible degradation of their catalytic cofactor (H-cluster), thus limiting their significant application potential (3-5). In contrast to other [FeFe]-hydrogenases, the H-cluster in CbA5H of Clostridium beijerinckii has been shown to reversibly shift between the $O_2$-resistant state ‚Hinact' and the catalytically active Hox-state, irrespective of external protectants, rendering CbA5H the only intrinsically $O_2$-resistant [FeFe]-hydrogenase known so far (6-8). By comparing the crystal structures of air-exposed CbA5H (CbA5Hair, trapped in Hinact) and CpI (Hox) we were able to uncover the mechanism underlying the unique $O_2$-resistance of CbA5H. In the catalytic H-domain of CbA5Hair the peptide loop covering positions T365, S366 and C367 (TSC-loop) is shifted towards the H-cluster, bringing the thiol group of position C367 (known for its decisive function in proton transfer) close enough to the substrate binding site (Fed) to block it against incoming $O_2$ (9). Rational protein-design, protein-film-electrochemistry and Fourier-transform-infrared (FTIR) spectroscopy further allowed us to identify the structural determinants for this potential-dependent mechanism (8). These findings will permit to introduce this protective feature into the structure-function relationships of other [FeFe]-hydrogenases.

P030

# Using adaptive evolution with Escherichia coli DH5α and E2348/69 to grow on sucrose as sole carbon source

**Joneclei Alves Barreto[1];** Andrade, Fernanda Batista de[1]; Jacobus, Ana Paula[1]; Contiero, Jonas[1]; Gross, Jeferson[1]

[1] *Instituto de Pesquisa em Bioenergia - IPBEN/UNESP, Brazil*

Strategies for microbial production of high-value compounds from sucrose are attractive giving the low-cost of this carbon source, specially in Brazil where sugarcane is an abundant feedstock. Metabolic engineering and adaptive laboratory evolution are excellent tools to establish and improve the assimilation of sucrose in microbial workhorses such as Escherichia coli. Here we conducted and evolution study with E. coli DH5α having an episomal expression of the cscBKA operon for sucrose consumption. For comparison, we used the strain E2348/69 which naturally expresses the cscBKA operon. For DH5α the experiment was carried out with three populations, whereas for E2348/69 four replicates were used. In both cases, populations were propagated for 600-800 generations through serial transfers in minimal medium with 2% sucrose as the sole carbon source. In the end of the experiment, maximum growth rates of all final populations showed a marked increase in comparison to the parents. For DH5alpha populations, competition assays supported expressive fitness gains during the evolution. Whole-genome sequencing of the DH5α populations showed that for two populations there was an integration of the cscBKA operon into the genome, and in one population occurred a dramatic decrease in plasmid copy numbers. These results indicated that the major driving force for adaptation was minimizing the costs of episomal expression of the cscBKA operon. Other mutations found were related to the transcriptional apparatus (RNA polymerase subunits), to energy-saving mechanisms (e.g., flagella deletion), and to rescuing the de novo purine biosynthesis in DH5α. The whole-genome sequencing of E2348/69 populations is under analysis. All mutations found will be tested by means of reverse engineering to develop of a strain optimized for growing using sucrose as the sole carbon source. This strain will be used as chassis for implementing the biosynthesis of polyhydroxyalcanoates from sucrose.

P031

# CO2-fixating Bioelectrocatalytic Cascades in Redox-Active Hydrogel for Stereoselective C-C Bond Formation via Reductive Carboxylation

**Leonardo Castaneda-Losada[1];** Adam, David; Sieber, Volker[2]; Erb, Tobias J.; Richter, Michael; Plumeré, Nicolas[2]

*[1] Fraunhofer IGB, Germany; [2] Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

The selectivity of bioelectrocatalysis offers important advantages over classical electrocatalysis for the direct conversion of CO2 into high value-added C2+ products[1]. However, wiring of CO2 reducing enzymes to electrodes is demanding because many of these enzymes require NADPH as natural cofactor for electron transfer. Here, we report the electrically driven regio- and stereoselective incorporation of CO2 into crotonyl CoA by one of the fastest CO2 fixing enzymes, the NADPH-dependent crotonyl-CoA carboxylase/reductase[2]. Specifically, the immobilization of ferredoxin NADP+ reductase within a 2,2´ viologen modified hydrogel[3] allowed the continuous reduction of NADP+ with a faraday efficiency of 98 ± 3 % and a rate of 1.9 ± 0.2 µmol cm-2 h-1. Co-immobilization of crotonyl-CoA carboxylase/reductase within the hydrogel led to the stereoselective formation of (2S)-ethylmalonyl-CoA with 92 ± 6 % faradaic efficiency and a product rate formation of 1.6 ± 0.4 µmol cm-2 h-1. Together, the co-immobilized enzymes constitute an electrically driven cofactor regeneration and coupled CO2 fixation system for the stereoselective (2S)-ethylmalonyl-CoA formation at high rates. Our system provides the proof-of-principle for the electro biocatalyzed CO2-fixation into structurally complex substrates with high regio- and stereocontrol during C-C bond formation. Altogether, the biohybrid system promotes the role of bioelectrochmical CO2 fixation and represents an important step towards the synthetic applications of NADPH-dependent enzymes.

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P032

# Development of a sustainable platform for the production of glutamate derivates

**Milca Rachel da Costa Ribeiro Lins[1];** Mack, Matthias[2]; da Silva, Adilson José[3]; Medeiros de Lima, Alice[3]; Veloso de Paula, Ariela[4]; de Baptista Neto, Álvaro[4]; Bertolazzi Zocca, Vitória Fernanda[4]; Araújo da Silva Amorim, Laura[4]; Pereira Picheli, Flávio[4]; Biscaro Pedrolli, Danielle[4]

*[1] Federal University of ABC (UFABC), Brazil; [2] Mannheim University of Applied Sciences, Germany; [3] The Federal University of São Carlos UFSCar, Brazil; [4] São Paulo State University (UNESP), School of Pharmaceutical Science, Brazil*

Bioeconomy arises from the necessity of industries to overcome CO2 emissions and decrease fossil fuel consumption by developing a sustainable production process for value-added products. Working with biomass from plants and natural microorganisms is an ordinary way for industries to target Bioeconomy goals. However, many bioproducts, such as amino acids, non-ribosomal peptides, and polyketides cannot be sufficiently produced and extracted from natural sources. Therein, Synthetic biology meets Metabolic engineering to strategically manipulate a workhorse‘s genetic code and predict the changes in the metabolic pathway‘s flux, aiming for improvements in upstream processing. Therefore, our research group is employing bio tools for designing and constructing microorganisms with a higher capacity to synthesize glutamate derivates, including indigoidine, a natural blue pigment, and surfactin, a lipopeptide-type biosurfactant. Bacillus subtilis has features to consider it an attractive prokaryotic host for industries: it can secrete proteins to the extracellular environment, and there are plenty of synbio tools available, making it possible to play with it. Besides, the metabolic pathway model of B. subtilis is well established. Flux balance analyses were implemented for understanding the bottlenecks of glutamate biosynthesis. Concurrently, strains were constructed by diversifying genetic modules with different promoters, which were integrated into the genome to evaluate the yield of surfactin production. CRISPR-Cas9 was applied to delete the premature stop codon of the sfp gene, reestablishing the surfactin biosynthesis. Furthermore, auto-inducible promoters, derived from the quorum-sensing system LuxI-LuxR, will be evaluated in the regulation of the srfA operon. These strategies are being applied to obtain a high-yield indigoidine-producing strain as well, which will be heterologously produced by B. subtilis. Some enzymatic reactions in the metabolic pathways for indigoidine will be regulated by small synthetic RNAs. Strategies to manipulate the organisms’ genomes, and taking into consideration metabolism pathways changes, are essential to target Bioeconomy goals.

P033

# Reiterated Mass Selection and Backcrossing diploid is a new protocol for quantitative trait loci mapping in Saccharomyces cerevisiae

**Lucas Souza de Bem[1];** Menegon, Yasmine Alves[2]; Barreto, Joneclei[2]; Gross, Jeferson[2]; Jacobus, Ana Paula[2]

*[1] University of Campinas, Brazil; [2] São Paulo State University UNESP, Brazil*

Quantitative Trait Loci (QTL) mapping is a way to discover regions in the genome that express complex phenotypes. Usually, it is done by crossing strains with opposite phenotypes, followed by selection and analysis of the best recombinants individually. However, to obtain a suitable map, it is necessary to perform many cycles of crossings and selections. To simplify and refine QTL mapping in S. cerevisiae, we have developed a protocol based on Reiterated Mass Selection and backcrosSing (ReMaSSing). From an initial crossing between superior (PE-2_H4, from Brazilian bioethanol) and inferior (S288C) yeast strains, each ReMaSSing cycle generates over 30.000 diploid recombinants that are selected through 12 generations under a stressful condition (lignocellulosic hydrolysate, LCH) to enrich the adaptive alleles of interest. These recombinants are once again crossed, sporulated and subjected to a new propagative selection. The diploid state is maintained and directional crossings are conducted by the use of different allelic antibiotics resistance marks. Many rounds are reiterated leading to the refinement of QTL peaks. After 5 cycles of ReMaSSing with diploid populations, we conducted a whole-genome sequencing of bulk LCH-enriched and control populations (without LCH). As a result, we mapped QTL regions in chromosomes II (IRA1), IV (CRF1), X (VPS70), XII (HAP1), XIII (PHO84) and XIV (MKT1). Using CRISPR/Cas9 we swapped the S288C alleles for the ones from PE-2_H4 and tested their tolerance to the LCH. Through competition analyses and microplate growth assays, we confirmed the importance of amino-acids changes in Vps70 (P199L), Mkt1 (D30G), Pho84 (L259P) and the deletion of a Ty element inserted in the gene HAP1. Most of those QTL have been already found, showing that our protocol has a gene level resolution. Our findings showed that the ReMaSSing Diploid protocol is consistent and can be used to discover QTL regions in S. cerevisiae.

P034

# A microbial supply chain for oleochemicals

Birgitta Ebert

*The University of Queensland, Australia*

In recent decades, there has been a tremendous shift worldwide toward oleochemicals, that is, vegetable and animal oils and fats, as substitutes for petrochemicals. These oleochemicals are important renewable platform molecules for the industrial production of detergents, lubricants, and biodiesel, among other products. However, since the production relies on edible feedstocks, predominately palm oil, their production is not sustainable. Accordingly, there is a demand for alternative sources of oleochemicals that are less reliant on sensitive tropical land use and provide a broader diversity of fatty acids with different physicochemical properties. Metabolic engineering of microbes can provide a more sustainable route to oleochemicals and be an attractive alternative, especially for producing tailored molecules with favourable physicochemical characteristics.

We previously demonstrated the potential of the bacterium Pseudomonas taiwanensis VLB120 as a microbial chassis for fatty acid-derived compounds on the example of recombinant production of methyl ketones, which are promising diesel blendstocks and can be converted into aviation fuel and lubricants. The transformation of the Pseudomonas strain into a superior oleochemical production chassis was supported by model-guided metabolic engineering, which identified crucial competing pathways. Implementing the computed knockout strategies along with overexpression of fatty acid metabolism resulted in a 4-fold improvement of the product titer to 70 g/L in the in situ extractant phase, corresponding to 9.8 g/L in the aqueous medium.

Our current work further leverages the biocatalytic potential of Pseudomonas for fatty aid-derived molecules by diversifying the product range and improving process performance and will be discussed concerning carbon efficiency and sustainability.

P035

# Adaptive evolution of yeast for cellulosic ethanol production

Yasmine Alves Menegon[1]; de Bem, Lucas Souza[2]; Gross, Jeferson[1]; Jacobus, Ana Paula[1]

[1] *São Paulo State University UNESP, Brazil;* [2] *University of Campinas, Brazil*

Second-generation (2G) ethanol results from the S. cerevisiae fermentation of biomass-derived sugars contained in lignocellulosic hydrolysates (LCHs). For 2G bioprocesses, yeast robustness must be improved to withstand fermentation inhibitors (e.g., weak organic acids, phenols, and furan aldehydes) also present in LCHs. This study performed three adaptive laboratory evolution experiments with (1) haploid and (2) diploid CEN.PK113-7D, and with (3) diploid PE-2_H4 (Brazilian bioethanol) strains. Eight populations were subjected to serial transfers (550-750 generations) under increasing amounts of sugarcane bagasse LCHs. Tolerance improvement was the highest for the PE-2_H4 (up to 54% LCH) and CEN.PK113-7D (up to 50% LCHs) populations, while CEN.PK113-7D reached a tolerance plateau at 36% LCH. Competition assays indicated that all populations had marked fitness gains compared to the parental strains. Whole-genome sequencing identified more than 70 genes mutated, as well as duplications related to chromosomes 1 and 9. In addition, parallelism was found in replicate populations of the same background (e.g., CHD1, RET1, MUK1 in CEN.PK113-7D haploid, and SSB2 in PE_2_H4 diploid) between different ploidy states (e.g., HYP2 mutated in both haploid and diploid CEN.PK113-7D) and across strains of different provenances (e.g., SIZ1 disrupted in both CEN.PK113-7D and PE-2_H4). Common adaptation pathways to LCHs involve protein SUMOylation/ubiquitination (SIZ1), chromatin modification (CHD1), rRNA synthesis (RET1), protein stability (SSB2), and regulation of the plasma membrane H+-ATPase (PMA1 and HRK1). Candidate alleles have been validated by reverse engineering into the parental strain using CRISPR/Cas9. Mutations disrupting the functions of SIZ1, MUK1, and CHD1 conferred significant fitness gains of 25%, 24%, and 18% per doubling, respectively. Reverse engineering will guide the construction of a robust LCH tolerant strain to boost 2G ethanol production.

P036

# Immobilization and co-immobilization of enzyme cascades for their use in sustainable chemistry processes

Sara Arana Peña[1]; Sutiono, Samuel[1]; Sieber, Volker[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

The potential of biocatalysis in cascade reactions is reaching new highs, which were unthinkable a few years ago. These multienzyme reactions, are undoubtedly advantageous over classical step-by-step syntheses by eliminating the need of isolation and purification of the reaction intermediates, enabling to work in “one pot”.

One critical drawback of this approach is that each enzyme adds to the cost of the process. Only by achieving high process stability of the enzymes, as well as their reusability, the application of these enzyme cascades for sustainable chemistry becomes feasible.

One solution for this is enzyme immobilization, an extremely useful methodology to improve almost all enzymatic properties if designed correctly and solve the problems associated with the recovery and reuse of enzymes to achieve an economically viable process that facilitates downstream processing and continuous operation.

In this context, enzyme co-immobilization is an impressive opportunity to obtain new synthetic pathways that can be used in extremely complex processes involving cascade reactions. However, immobilization of entire cascades on the same particle is very challenging and can greatly reduce the feasibility of the industrial implementation of the cascade process. With all the complexity, only a few cases have been studied despite the high importance of the topic.

In order to solve the problems inherent to this challenge, different strategies could be developed, involving the use of pre-existing porous supports with different reactive groups in the immobilization of all enzymes involved in the cascade to verifying the suitability of different methods and protocols.

The application of these strategies for the preparation of optimal co-immobilized cascades could allow using, recovering and reusing the biocatalysts and benefiting from all the advantages of immobilization and co-immobilization. This would reduce the cost of chemistry processes and improve reaction yields, providing a promising green alternative to the traditional chemical industry.

P037

# Biocatalytic Nanomachines as Valuable Tools for Biorefinery Approaches

**Zinnia Dsouza[1];** Melse, Okke[1]; Schmermund, Luca[1]; Schenk, Gerhard[2]; Scott, Colin[3]; Sieber, Volker[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany;*
[2] *University of Queensland, Australia;* [3] *CSIRO Land & Water, Australia*

The application of Biocatalysts to optimize various chemical syntheses has flourished into a promising technology due to their high catalytic rates, specificities, and feasibilities. Substantial progress has been made in recent years by substituting isolated enzymes for fermentative-based processes to convert biogenic raw materials into fuels and platform chemicals. The biochemical technique of modular continuous flow also enables flexible assembly of different intensive multi-step reactions into so-called multi-enzyme cascades. However, their effective use has been limited by several challenges; a key obstacle is co-factor-dependent enzymes. Efficient cofactor regeneration is thus crucial for the economic viability of industrial-scale biotransformation.

In the present work, enzymes shall be modified into nanomachines“ to retain and recycle their cofactors. These so-called „Biocatalytic nanomachines“ consist of a cofactor-dependent and a cofactor-regenerating enzyme. The cofactor shall be covalently bound between these two, allowing its direct transfer between the active sites of the enzyme complex. Further, we intend to expand the scope of such“nanomachines“ by exploring their versatility through the lens of biomimetics. Such „nanomachines“ already find applications in the pharmaceutical industry. Thus, a sustainable collaborative effort is anticipated to spur further advances for their application in biorefineries. Here, using an example, namely the already established cascade for the conversion of glucose to isobutanol.

P038

# Enzymatically modified carrageenans from raw algae biomass with improved rheological properties

Alexander Fuchs[1]; Hupfeld, Enrico[1]; Sieber, Volker[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

Carrageenans are a group of sulfated biopolymers that represent the commercially most relevant products obtained from red algae. They are highly appreciated for their gelling behaviour or as thickeners and stabilizers in the food and cosmetic industry. The degree and position of sulfation on the polymer chain has a decisive influence on their rheological properties and thus defines their commercial use. By the targeted cleavage of sulfate groups by sulfatases, carrageenan variants with new physico-chemical properties can be generated. We discovered a novel marine sulfatase that performs desulfation on both ι- and κ-carrageenan to generate α- & β-moieties, respectively, resulting in novel hybrid structures. Importantly, this enzyme could be produced recombinantly in an active form in E. coli. In this work, we successfully used this enzyme to alter the sulfation pattern of carrageenans from different commercially highly relevant species of red algae containing varying amounts of ι- and κ-moieties directly during the aqueous extraction process from algal raw material. The rheological analysis of the purified biotransformation products revealed a significant increase in their viscoelastic moduli as well as an increase in their gelling and melting temperatures, ultimately leading to the production of polymers with superior properties as result of this combined extraction-biotransformation process. A comparison of our directly modified carrageenans to the modified biotransformation products obtained by the conventional extraction method with high pH and temperature revealed a lower gel strength for the one-step extraction-modification process. Presumably, this is caused by the lack of the alkaline conversion of the non-gelling precursor moieties of μ- and ν- carrageenan to the gelling κ- and ι-carrageenan units, respectively. These discoveries make an important contribution for the direct enzymatic modification of carrageenans from algal raw biomass as part of a sustainable extraction process and the utilization of these products in new application fields.

P039

# Functional analysis of a cytochrome P450 gene cluster in the model plant Arabidopsis thaliana

**Erich Glawischnig**[1]; Nicol, Lauren[1]; Müller, Teresa; Böttcher, Christoph

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

Characteristic for plants is the presence of clusters of multiply duplicated genes, which prevent functional assignment via single gene knock-outs. Here, we present a strategy for analysing the biological functions of highly diversified enzyme families in the model plant A. thaliana. We focus on the cytochrome P450 (CYP) subfamily 71B, which contains 33 expressed genes, only a few of which are functionally characterised. Using a CRISPR/Cas9-based approach we have deleted a cluster of 16 transcribed CYP71B genes, which spans 83 kb. Then metabolite compositions were systematically analysed by UHPLC/ESI-QTOFMS in different tissues and in response to abiotic stresses in comparison with the wild type. The metabolic phenotype of the cluster deletion mutant indicates an involvement of the respective enzymes in the biosynthesis and modification of metabolites involved in defence and/or communication. By the analysis of partial deletion and complementation lines, and by heterologous expression in yeast and Nicotiana benthamiana we aim to assign biological functions to specific genes.

P040

# A circular economy-driven business model based on tailored bio-composites

Ruben Casillas[1]; Helen Rogers[2]; Veloso, Luis[1]

[1] *Fungarium, Germany;* [2] *Technische Hochschule Nürnberg Georg Simon Ohm, Germany*

According to the United Nations, desertification of arable land as a result of climate change will require urgent action from all key stakeholders in order to regenerate. Fungarium is a Bavarian-based start-up aiming to make mycelium-based biocomposites for the B2B market and thus help tackle climate change. Mycelium is the root system of fungi and can grow on several organic substrates. Our value proposition is tailoring material properties to meet specific customer needs by controlling the growth parameters using AI technology. Central to our business model is that the production of the material is part of a novel circular economy framework, which first considers the production volume capacity of the local region. For example, if the area grows corn, we will use the corn husks and other associated organic waste to customize the product mix and growth conditions according to the required material properties. Once the material has served its purpose e.g. for packaging, it can then be re-purposed as a soil fertilizer. Fungarium designs a scalable and globally replicable production method of these bio-composites with sufficient volume to supply the industrial demand, repurposing millions of tons of farming waste, avoiding the equivalent mass of plastic being used or produced and repurposing a vast amount of such materials as fertilizer for local soil-regeneration.

## About Fungarium

Fungarium's mission is to achieve high-volume and cost-effective biocomposite production quantities to directly compete in the Styrofoam market within the B2B market. By involving local farmers and soil-monitoring companies, Fungarium aims to add another revenue stream: using soil-regeneration to certify tradeable Carbon bonds. This scheme represents value for local ecosystems, which will solidify the partnerships between farmers, industry and Fungarium, helping this emerging compostable material market gain traction.

P041

# EnzOnomy I – an innovative bio-based technology for the sustainable manufacture of platform chemicals (including biofuels)

**Gary Schenk**[1]; Foley, Gabriel[1]; Pregelj, Lisette[1]; Guddat, Luke[1]; Boden, Mikael[1]; Hine, Damian[1]; Sieber, Volker[2]

[1] *The University of Queensland, Australia;*
[2] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

EnzOnomy is a biomanufacturing platform to produce valuable chemicals and biofuels from a wide range of renewable feedstocks. With increasing demand for sustainable and environmentally friendly industrial processes, EnzOnomy provides an elegant solution to maximise yields and revenue. A process of increasing commercial importance is the conversion of sugar to isobutanol, a valuable platform chemical for the automotive, pharmaceutical, aerospace, bioplastics and solvent industries. Isobutanol can be converted to jet fuel and can be used as a drop-in fuel. Its market was valued at USD1.4 billion in 2021 and is expected to grow to over USD2.5 billion by 2030. Furthermore, it can be refined into chemicals such as isobutene, which has a current market value of USD26 billion.

Within the EnzOnomy process commercialisation analysis is integrated explicitly, using the Tourbillon framework to address the feasibility and avenues to progress a product to market [1]. This combines techno-economic, competitor and market analyses, and defines the viability and current state of intellectual property for a given approach. The integration of the Tourbillon into the EnzOnomy approach allows for evidence-based decisions to dictate paths to market for a specific product (e.g. isobutanol). EnzOnomy relies on the use of enzymes outside their natural, cellular environment, therefore bypassing limitations associated with microbial manufacturing pathways (including metabolic competitions and toxicity of products) [2]. Enzymes can be tailored to be more efficient and stable, and their production cost is steadily decreasing, which removes the major impediment of their widespread application in industrial processes. It is envisioned that cell-free manufacturing processes such as EnzOnomy will become a major component of a carbon-neutral bioeconomy.

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P042

# EnzOnomy II – Enzyme Cascades for the Sustainable Production of Chemicals

**Gerhard (‚Gary') Schenk[1];** Foley, Gabriel[1]; Pedroso, Marcelo[1]; Pregelj, Lisette[1]; Bayaraa, Tenuun[1]; Krco, Stefan[1]; Davis, Samuel[1]; Tule, Sanjana[1]; Martinez, Karen[1]; Marcellin, Esteban[1]; Guddat, Luke[1]; Boden, Mikael[1]; Hine, Damian[1]; Sieber, Volker[2]

[1] *The University of Queensland, Australia;*
[2] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

In the poster "EnzOnomy I – an innovative bio-based technology for the sustainable manufacture of platform chemicals (including biofuels)" we outlined the overall process and application of this emerging biomanufacturing process. Here, we highlight steps integrated into the "EnzOnomy" approach. Our focus is on the conversion of glucose to the platform chemical isobutanol, which has been gaining increased attention as a platform chemical for the manufacture of aviation fuels. The process consists of four steps, i.e. design, engineering, pilot and large-scale, with their respective technology readiness (TRL) levels ranging from 4 to 5. The design step relies on a suite of methods from bioinformatics and genomics to identify suitable biocatalysts for the reaction cascade required to produce isobutanol. In the engineering phase the properties of the biocatalysts and the performance of the cascade are optimised to enhance the product yield obtained by the pilot reactor (Step 3).

The EnzOnomy is, in its conception, a cell-free biomanufacturing process, and as such it presents an alternative to the many microbial systems that have gained significant attention for the sustainable production of bulk and high-value chemicals. Cell-free processes provide significant advantages over microbial systems in terms of higher controllability, higher titres, fewer or no byproducts, and greater flexibility. For instance, while the currently most efficient microbial system reaches a titre of ~20 g/L, the cell-free cascade is already exceeding 275 g/L. Furthermore, with continuously improving bulk productions of the required enzymes the operational costs for cell-free systems is anticipated to become commercially attractive as the processes are scaled up (Step 4).

P043

# Structural and functional insights into the mechanism of the Fe-S cluster-dependent dehydratase from Paralcaligenes ureilyticus

**Gerhard (‚Gary') Schenk[1];** Bayaraa, Tenuun[1]; Lonhienne, Thierry[1]; Sutiono, Samuel[2]; Melse, Okke[2]; Boden, Mikael[1]; Sieber, Volker[2]; Guddat, Luke[1]

[1] *The University of Queensland, Australia;*
[2] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

Enzyme-catalyzed reaction cascades play an increasingly important role in the sustainable manufacture of diverse chemicals from renewable feedstocks. For instance, enzymes from the branched-chain amino acid (BCAA) synthesis pathway have been embedded into a cascade to convert glucose via pyruvate to isobutanol, a platform chemical for the production of aviation fuels and other valuable materials. The third enzyme of the BCAA synthesis pathway, an Fe-S dependent dehydratase from the ilvD/EDD superfamily, plays a central role in the cascade as it catalyzes steps in the conversion of glucose to pyruvate, and another step in the formation of isobutanol from pyruvate. However, this enzyme also represents the rate-limiting step in the cascade as no variant with high and stable activity towards all its substrates in the cascade is currently available.

Recently, we compared members of this dehydratase superfamily and identified several residues that contribute to the activity and substrate selectivity of these enzymes [1,2]. The dehydratase from Paralcaligenes ureilyticus (PuDHT) is very efficient toward larger sugar acid substrates but has minimal activity in presence of other reactants. In an attempt to gain a better understanding of the structure and function of PuDHT we solved its crystal structure to 2.5 A. In combination with in silico docking analyses Thr478 has been identified as crucial for the substrate selectivity of this enzyme. However, as mutagenesis studies demonstrated, single site mutations are unlikely to facilitate a broader substrate acceptance for this enzyme.

**References:**

[1] Sutiono et al., ACS Catal. 2020, 10, 3110

[2] Melse et al., ChemBiochem 2022, 23, e202200088

P044

# Factors explaining the purchase of bio-based products

Sebastian Goerg[1]; Hempel, Corinna[2]; Pondorfer, Andreas[1]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany;*
[2] *Technical University of Munich, Germany*

The transformation from our fossil-based economy to a more sustainable, bio-based economy demands a broad societal acceptance. Therefore, it is essential to learn more about people's opinions, attitudes and purchase intentions towards bio-based products. Using a large-scale online survey representative of the German population at the subnational level (N = 15,000), this study investigates to what extent personal as well as regional macro-economic factors explain consumers' mean stated purchase for a variety of bio-based products. The results reveal that individual factors such as economic preferences (i.e., patience, risk-taking, positive reciprocity, and altruism), universal moral values, and individual factors related to climate change perception, climate policy support and civic engagement to fight climate change are statistically significantly associated with intentions to purchase bio-based products. The influence of regional characteristics (e.g., GDP, and employment sectors) is weaker and less consistent. The aim of this study is to draw a more holistic picture on the factors that determine consumers' preferences, when it comes to the purchase of bio-based products.

P045

# A substitution factor approach for comparing Life Cycle Assessment Studies – the case of timber as a building material

Josef Huber

*Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

Reviewing and comparing results from Life Cycle Assessments (LCA) is crucial and tricky at the same time. On the one hand, comparing different studies is necessary to get a clearer picture of the result reliability. This is extremely important since conducting LCAs provides many degrees of freedom in order to tailor the study to answer the underlying questions. On the other hand, those degrees of freedom often prevent meaningful comparisons between studies. This fact is acknowledged by the standard ISO 14044, which does not see a scientific foundation for direct comparisons between different studies because of several possible biases with regard to assumptions made. In this paper, we develop an approach to overcome the challenges of limited comparability between LCA studies through the development of an approach based on so-called substitution factors and apply this to the comparison of sustainability of timber as building material, which is compared to suitable alternatives (e.g. steel or reinforced concrete). In our approach, we calculate the share to which the observed alternative performs better/worse than the reference within one publication. The obtained values are not directly influenced by methodological choices and further assumptions and results are thus (more) comparable. The approach is employed on the outcome of a structured literature review in the chosen application field. Using a defined search string, we identified 36 studies, which fulfill the requirements as they compare timber with other building materials at a building level. For LCAs from “cradle to construction site” the analysis finds a potential reduction of the GWP of 44 % on average. For a “cradle to grave” perspective, the GWP is reduced by 63 % when timber is used instead of concrete. With the application of our approach to the selected literature, we seek to contribute to more sophisticated comparisons between different LCA studies.

P046

# Identification and analysis of novel bacterial enzymes for pectin degradation

**Yajing Liu[1];** Rühmann, Broder[1]; Sieber, Volker[1]; Liebl, Wolfgang[2]

[1] *Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany;*
[2] *Technical University of Munich, Germany*

Pectic substances are significant components of sugar beet pulp. To find interesting enzymes for pectin degradation, we applied functional screening of metagenomic library. Seventy-one depolymerase-encoding genes were identified. An around 56 kb assembled DNA fragment putatively originating from Xylanivirga thermophila strain or a close relative was analyzed in detail. Seven putative arabinosyl hydrolases from this DNA fragment belonging to GH51 and GH43 were biochemically characterized, the enzyme cocktails composed in this study fully degraded the arabinan substrates and thus could serve for arabinose production in food and biofuel industries.

P047

# High Performance Bio-based Functional Coatings for Wood and Decorative Applications (PerfeCoat)

**Amélie Skopp**[1]; Liu, Yajing[1]; Rühmann, Broder[1]; Sieber, Volker[1]

*Technical University of Munich, Campus Straubing for Biotechnology and Sustainability, Germany*

The pursuit of a climate-neutral Europe and a truly circular economy requires attention to virtually allfields of production if fossil-based materials are to be eliminated. This relates not only to manufactured goods but also the paints and coatings used to protect them; of the almost 1 million tonnes of paints and coatings produced in Europe each year, more than 80% are derived from fossil resources. Reducing the impact of these coatings would represent a major advance in Europe's climate ambitions. To address this challenge, the PERFECOAT project will develop and validate a new generation of industrial wood and decorative coatings with significantly more than 25% bio-based components. The project will address three important markets for coatings: high-volume, UV-curable clear coatings, waterborne trim paints for DIY, and waterborne wall paints. These coatings will reach, and even surpass, the currentquality and sustainability standards.

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