# 6<sup>th</sup> International BioSC Symposium 2022

Towards an Integrated Bioeconomy



August 23, 2022





## **Table of Content**

## Inhalt

Agenda	3
Presentation Abstracts	
SESSION I: Regionalisation as implementation path for the bioeoconomy	
SESSION II: Smart value chains in sustainable food and feed production	
SESSION III: High-value compounds from integrated processes	12
Session IV: Plastics and textiles in a circular bioeconomy	15
Poster Abstracts	18
List of Participants	45











### Agenda

## 23<sup>rd</sup> August 2022 | 09:00 – 17:00 h

## Bonner Universitätsforum, Heussallee 18 - 24, 53113 Bonn, Germany

08:30 h	Registration			
09:00 h	Welcome			
	Ulrich Schurr, Forschungszentrum Jülich/BioSC, DE			
Session I: Regionalisation as implementation path for the bioeconomy				
Moderator: Jan Börner, University of Bonn/BioSC, DE				
	The role of biotechnology in the Green Deal and its implementation via regional			
	and interregional initiatives			
	Ludo Diels, formerly Institute for Technological Research (VITO), BE			
	Regional transformation as the key to a sustainable bioeconomy: the Rheinische			
	Revier as a model case			
09:15 h	Sandra Venghaus, RWTH Aachen/BioSC, DE			
	Challenges for a sustainable and inclusive bioeconomy transition in South			
	America			
	Jorge Sellare, Center for Development Research, University of Bonn, DE			
	Questions & Answers			
10:30 h	Coffee Break			
<b>Session II:</b>	Smart value chains in sustainable food and feed production			
Moderator: Ulrich Schurr, Forschungszentrum Jülich/BioSC, DE				
	Drive stakeholder value through supply chain transparency			
	Drive stakeholder value through supply chain transparency  Alexander Ellebrecht, ChainPoint GmbH, Bonn, DE			
	Alexander Ellebrecht, ChainPoint GmbH, Bonn, DE			
	Alexander Ellebrecht, ChainPoint GmbH, Bonn, DE  Cascade use of lupin plants: plant protein for food and feed and as source for			
10:50 h	Alexander Ellebrecht, ChainPoint GmbH, Bonn, DE  Cascade use of lupin plants: plant protein for food and feed and as source for high-value chemicals			
10:50 h	Alexander Ellebrecht, ChainPoint GmbH, Bonn, DE  Cascade use of lupin plants: plant protein for food and feed and as source for high-value chemicals  Marco Löhrer and Ulrich Schaffrath, RWTH Aachen/BioSC, DE			
10:50 h	Alexander Ellebrecht, ChainPoint GmbH, Bonn, DE  Cascade use of lupin plants: plant protein for food and feed and as source for high-value chemicals  Marco Löhrer and Ulrich Schaffrath, RWTH Aachen/BioSC, DE  Challenges and gaps for building resilient value chains: the case of coastal			
10:50 h	Alexander Ellebrecht, ChainPoint GmbH, Bonn, DE  Cascade use of lupin plants: plant protein for food and feed and as source for high-value chemicals  Marco Löhrer and Ulrich Schaffrath, RWTH Aachen/BioSC, DE  Challenges and gaps for building resilient value chains: the case of coastal lowland quinoa			
10:50 h	Alexander Ellebrecht, ChainPoint GmbH, Bonn, DE  Cascade use of lupin plants: plant protein for food and feed and as source for high-value chemicals  Marco Löhrer and Ulrich Schaffrath, RWTH Aachen/BioSC, DE  Challenges and gaps for building resilient value chains: the case of coastal lowland quinoa  Andrés Zurita-Silva, Chilean Economic Development Agency (CORFO), Dirección			









Session III: High-value compounds from integrated processes				
Moderator: Holger Gohlke, HHU Düsseldorf/BioSC, DE				
	Unlocking full potential of the biomass, producing higher value products by use			
	of new technologies			
	Lene Lange, LL-BioEconomy, Research & Advisory, DK			
	Having the whole process in view: Production of metaraminol from renewables			
14:00 h	using a hybrid process			
14.00 11	Dörte Rother, Forschungszentrum Jülich/BioSC, DE			
	Bioeconomy and climate change mitigation: new enzymes wanted			
	Manuel Ferrer Martinez, Institute of Catalysis and Petrochemistry, Madrid, ES			
	Questions & Answers			
15:15 h	Coffee Break			
Session IV:	Plastics and textiles in a circular bioeconomy			
Moderator: Sc	onja Herres-Pawlis, RWTH Aachen/BioSC, DE			
	Developing a production process for second generation lactic acid, to make PLA			
	and improve sustainability of packaging			
	Albrecht Läufer, BluCon Biotech, Cologne, DE			
	Appetite for trash - fighting the flood of plastics with enzymes and microbes			
15:30 h	Nick Wierckx, Forschungszentrum Jülich/BioSC, DE			
	Challenges in developing a new polymer for textile applications			
	Jeroen van der Vlist, Senbis Group, Emmen, NL			
	Questions & Answers			
16:45 h	Poster Award			
47.00 !	Closing Remarks			
17:00 h	Andreas Jupke, RWTH Aachen/BioSC, DE			
	Get-together Get-together			











#### **Presentation Abstracts**

#### **SESSION I: Regionalisation as implementation path for the bioeoconomy**

#### *Keynote*

# The role of biotechnology in the Green Deal and its implementation via regional and interregional initiatives



**Prof. Dr. Ludo Diels**Formerly Institute for Technological Research (VITO)

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Humans learned during the Holocene to use biomass for food and materials for their survival by using biomass for all kinds of applications (paper, construction, sails, ropes, paints, lamp oils, ...). Especially in the second half of last century humans started to understand and use the biological processes (fermentation and enzymatic processes) in what we call now the biotechnology. Genetic engineering and synthetic biology moved the system to the potential of making all kinds of chemicals/materials with high efficiency, high purity, strong performance and increasing productivity.

So, the availability of new processes and biomass helped agriculture, rural development, new developments to high performing chemicals etc. expanding agriculture to new applications and a new economy.

But it took a while before the advantage was recognized at the level of sustainability, circularity and resilience. Indeed biomass is carbon neutral, bioprocesses are running at lower temperatures compared to fossil processes and everything is feasible via recycling, be it via composting/anaerobic digestion or via mechanical/chemical recycling. On top we see that if biomaterials (e.g. wood) are used over a very long time, it can be seen as a carbon sink. This idea can also be extended to biocarbon-based plastics that can be recycled again and again. It means that the carbon that once is taken from the atmosphere and converted into biomass is brought into a recycling mode. It means that carbon is sucked from the atmosphere and put in recyclable materials.

But biomass grows somewhere and needs to be processed (even in different steps and over different value chains). This brings us to regional development and collaboration. Regions, either specialized in cultivating, fractionation, processing, materials/chemicals production, polymerization work together via regional and interregional initiatives in a systemic approach.

In Flanders, a region with a very strong chemical industry, we see that chemistry in its move toward sustainability is paying more attention to the use of biomass as feedstock and bioprocesses as











conversion technology. We saw in Flanders that, on its move to sustainability, more than 50% of the new R&D projects of catalisti were building on biomass and bioprocesses or circular bioprocesses.

However to make this full change, we need a lot of new integrated research and risk investment. That is why it is so important to start this at a regional strategic level with full support of regional funding with additional European funding. In order to take the risk it is also necessary to collaborate over the regional borders. In that way we started the Biorizon initiative which is a shared research center between the Netherlands and Flanders to make biobased aromatic molecules. To realize a breakthrough in the process from fossil-based to biobased aromatics we had to convince authorities, industry, research organizations etc. and to combine them all in one big initiative which now results in large demo facilities and the first preparations of full commercial bio-aromatics production. The third important chemical industry region in the neighborhood is NRW and via the BIG-Cluster the Biorizon initiative expanded to Germany. In the same way we see now a collaboration on C1chemistry. Finally also via the Vanguard Initiative we have a strong collaboration in Southern Europe with a focus on biowaste and with Northern Europe with a focus on lignin conversion not only to replace niche chemical, but also to move to bulk chemicals in order to really create impact in the chemistry world toward more sustainability.

Biotechnology offers the possibility to collaborate in a sustainable way over several sectors and regions and to make safe, healthy and recyclable but also natural products without needs of any additional fossil carbon molecule. Indeed, we must embrace the WHO-principle of 'one health', which means that we should have an holistic approach towards the equilibrium and health of human, animal, plant, microorganisms as well as on our global system. Finally bio-economy will move from a niche application to become the norm by solving resilience and sustainability problems among other important measures. It started regional and will create a global impact.









#### Regional transformation as the key to a sustainable bioeconomy: the Rheinische Revier as a model case



**Prof. Dr. Sandra Venghaus** Systemforschung und Technologische Entwicklung, Forschungszentrum Jülich and Decision Analysis and Socio-economic Assessment, RWTH Aachen

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Against the backdrop of climate change and the increasing global demand for resources, the German government's 2016 Climate Protection Plan proposed the establishment of a "Commission for Growth, Structural Change and Employment". It was to prepare the phase-out of coal-fired power generation in Germany and at the same time develop policy instruments to manage the associated structural change. In January 2019, the Commission's final report was published, recommending the creation of new value chains to generate new jobs in the areas of energy and industry, innovation and education, landscape and infrastructure, resources and agribusiness, and digitalization, with the aim of making the region a European model region for a sustainable and circular bioeconomy.

To guide the success of this transformation, 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 regionspecific 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.









#### Challenges for a sustainable and inclusive bioeconomy transition in South America



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Bioeconomy is increasingly gaining attention in South America as a potential strategy to foster sustainability transitions. However, bioeconomy development is intertwined with often contested questions of natural resource governance, so it is crucial that its promotion takes place in an inclusive manner. This presentation gives an overview of the preliminary findings from the junior research group Transformation and Sustainability Governance in South American Bioeconomies (SABio). Using case studies from Argentina, Brazil, and Uruguay, we first examine how "Bioeconomy" is conceptualized in these countries. We then analyse why policies sometimes do not have the desired effects and discuss how national legal frameworks, market dynamics, and incentives for policy compliance may affect policy outcomes. We conclude by presenting a conceptual framework that allows us to analyse how welfare distribution in the transition to a sustainable bioeconomy will to a large extent depend on the organisation of bio-based value chains.







#### SESSION II: Smart value chains in sustainable food and feed production

#### <u>Keynote</u>

#### Drive stakeholder value through supply chain transparency



**Dr. Alexander Ellebrecht** *ChainPoint GmbH* 

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The agriculture & food industry is facing severe challenges to keep feeding the world. Global agriculture & food production needs to grow by 60 percent before 2050 to meet the anticipated demand from an increasing world population. Next to that, consumers are demanding ever more insight into the origin of the products they buy. They want to be sure they purchase a product that is produced responsibly, with respect for profit as well as people and planet. Therefore, food companies need to be in control of their supply chain. They must be working closely together with their suppliers - and the suppliers of their suppliers - to improve yield and quality and to reduce environmental impact. This is the only way to ensure a steady supply of sustainably sourced raw materials. Technology can help to tackle these challenges, to facilitate collaboration and providing deep insight by monitoring and analysing key metrics across all stakeholders in agriculture & food supply chains.









#### Cascade use of lupin plants: plant protein for food and feed and as source for high-value chemicals



Dr. Marco Löhrer Plant Physiology, RWTH Aachen University

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Lupin is a valuable alternative protein source suitable for human food and animal feed and presents an attractive alternative to (GMO-)soybean from international markets. Lupin was already well established in Europe and popular in Germany up to the early 1990s, when the fungal disease "anthracnose" led to severe crop losses and in turn to a steep decrease in lupin production. Due to intensive breeding efforts, nowadays more varieties with increased anthracnose resistance are available (mainly L. angustifolius) but many of them still do not reach former yield potential. Species of the genus Lupinus are known for their alkaloid content, which is reduced in the "sweet" varieties, but high in the naturally more disease resistant "bitter" varieties. Alkaloids must be removed before the protein can be further processed for consumption and are up to now treated as waste. From a chemist point of view lupins are of interest, because they contain highly valuable and rare (pre)chiral compounds such as lupanin (2-oxosparteine). In this project we want to explore the use of biostimulants and plant strengtheners in lupin production, for improving yield, increasing pathogen resistance and better adaptation to abiotic stresses. In an exemplary cascade use approach, we want to demonstrate additional benefits of lupin production, by using the hitherto wasted alkaloids as a source for high value chemicals. At the same time, we will investigate the producer's acceptance of this novel concept in order to get it closer to practice. The BioSC BOOST FUND 2.0 Project P<sup>3</sup>roLucas (Optimization of plant performance and products for lupin cascade use) integrates the three different focus topic areas "smart management for plant performance", "modular biotransformations for high-value compounds" and "technological innovations as driver for bio-based social transformation" and is based a successful previous Bioeconomy International project and joint research projects of the core groups within the BioSC.











#### Challenges and Gaps for building resilient value chains: the case of Coastal Lowland Quinoa



Dr. Andrés Zurita-Silva (PhD) Chilean Economic Development Agency (CORFO), Dirección Regional Coquimbo

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Quinoa (Chenopodium quinoa Willd.) is a genetically diverse crop that has gained attention for food security due to its high nutritional content and ability to cope with increasing salinization and aridity. Cultivars from the coastal lowland ecotype are of particular interest due to their insensitivity to photoperiod and their potential to be cultivated at higher latitudes. The aim of this study was to analyze challenges and gaps to fully exploit the potential of this Andean grain, from a bioeconomy perspective, including efforts from fundamental to applied sciences, with multiples stakeholders and engagements from academia to industry.

The results of combined efforts and multidisciplinary approaches to assess genetic characterization, productivity constraints, breeding efforts, determination of valuable compounds, and metabolite profiling are presented to determine drivers and bottlenecks to the sustainable intensification and expansion of this crop. Recent collaborative efforts such as Bioökonomie International 2015: Quinoa Diversity, Breeding program INIA Chile (PMG-Quinoa) and Ancestral Grain Pole FIA-Orafti (functional additives for food industry) are exemplified as virtuous chain links.

The significance of the work is that identifying these critical elements set the ground for further designing an integrative approach with participants from both basic and applied academia, industry, government and international cooperation agencies, cooperatives and producers, who might define the priorities for boosting this value chain. In conclusion, common goals for breeding targets, productivity bottlenecks, strategic values, industry requirements will ultimately drive the whole potential as protein crop for scarce environments, and may boost social, environmental, and economic benefits as adaptive tools for climate crisis.











#### **SESSION III: High-value compounds from integrated processes**

#### **Keynote**

## Unlocking full potential of the biomass, producing higher value products by use of new technologies



**Prof. Dr. Lene Lange** *LL-Bioeconomy, Denmark* 

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Europe is at the forefront of developing a circular biobased economy, having moved swiftly ahead 2014-2021 by the industry-led, public/private collaborative research efforts (BBI-JU). Next in focus: 1. to broaden upscaling beyond the BBI-JU flagship biorefineries, hereby strengthening the biobased-sectors in EU at large; and 2. to implement fully the use of a cascading approach, unlocking the full potential of the biomass (being residues, processing side-streams or wastes). For both objectives, production of higher value products is key, for optimized resource efficiency and commercial viability. Examples: higher value products from the green, the blue, and the red biorefineries as well as from valorization of processing side-streams. Thus, a need for improved bioprocessing agents is created -enzymes as well as bacteria and fungi. To enable and speed up such discoveries, new technologies are needed. Three new discovery technologies will be described: "CUPP", a peptide-based, functional genome (and microbiome) annotation method. "EPR", enzyme profile relatedness, for comparing microbial enzyme profiles, enabled by annotating to integrated "EC-Function; Protein-Family" observations. And "Hotspot" analysis, based on combined use of CUPP and EPR, summing up all Function; Family observations, finding the species richest in digestive capacity or function specificity diversity. Developing the Circular Bio-based Economy, is the single most promising approach to improve global food security, nutrition and health. Thus, it is imperative and urgent to strengthen international collaboration and to make economic calculations, incl estimates of emission reduction and of societal benefits, improved food security, achieved through climate friendly and nutritious, health-promoting products, made from upgrading biomass.











# Having the whole process in view: Production of metaraminol from renewables using a hybrid process



**Prof. Dr. Dörte Rother** *IBG-1: Biotechnology, Forschungszentrum Jülich* 

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Incorporating biological catalysts with classic chemistry has the potential to develop processes that are truly superior to current production methods in terms of efficiency and sustainability. In such hybrid processes microorganisms can, for example, use agricultural and food industry waste streams as substrates to provide simple chemical building blocks. Subsequently, enzymes can be used to diversify these compounds *in vitro*, enabling the construction of product platforms for valuable fine chemicals, complex (chiral) building blocks, and active pharmaceutical ingredients. Furthermore, this product portfolio can be complemented and expanded by chemical transformations.

Such an approach was taken in **the HylmPAct project**, which developed a hybrid process for the biobased production of chiral amino alcohols and active pharmaceutical ingredients, starting from simple sugars<sup>1</sup> like xylose and glucose (Figure 1). The focus of this study included the sympathomimetic metaraminol, an adrenergic receptor agonist used as a vasoconstrictor, and further derived tetrahydroisoquinolines<sup>2</sup>, which are used as scaffolds in numerous bioactive natural products. Challenges and solutions to make these hybrid processes truly advantageous over other strategies, such as the identification of appropriate reaction conditions for each catalyst and the need to integrate downstream processes<sup>3</sup>, will be discussed.

A critical aspect for the economically and environmentally efficient production of metaraminol and its derivates is the operational stability of all (bio)catalysts used. Since enzyme production is one of the main cost drivers of the process chain, reuse and/or continuous application of the enzymes can intensify the process immensely. In HylmPAct, we identified the used carboligase as a critical enzyme in terms of stability. In **the MetaProcess project**, we will address the stability problem of the biocatalysts in particular using a combination of state-of-the-art and novel tools in the field of computational (rational) design and directed evolution techniques<sup>4</sup>. This will not only improve the overall performance of the synthesis of (chiral) amino alcohols such as metaraminol and the derived tetrahydroisoquinolines, but is expected to also lead to the improvement of other value-chains.

[1] Labib M, Grabowski L, Brüsseler C, Kallscheuer N, Wachtendonk L, Fuchs T, Jupke A, Wiechert W, Marienhagen J, Rother D, Noack S. **2022**. *ACS Sus. Chem. Eng.* **10** (16): 5117–28

[2] Erdmann V, Lichman B R, Zhao J, Simon R C, Kroutil W, Ward J M, Hailes H C, Rother D. **2017**. *Angew. Chem. Int. Ed.* 56 (41): 12503-07

[3] Doeker M, Grabowski L, Rother D, Jupke A. 2022. Green Chem. 24 (1): 295-304

[4] Contreras F, Nutschel C, Beust L, Davari M D, Gohlke H, Schwaneberg U. **2021**. *Comput. Struct. Biotechnol. J.* 19: 743–51











#### Bioeconomy and climate change mitigation: new enzymes wanted



**Dr. Manuel Ferrer Martinez** Institute of Catalysis and Petrochemistry, Spanish Council for Scientific Research (CSIC)

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Our understanding of enzymes has demonstrated that they help circular economy and keeping climate change issues from rising. In the context of the circular bioeconomy, the solution is simple: to obtain an enzyme that can be added directly to, or at one of the stages of the production process of, products to make them more sustainable and environmentally friendly. However, the challenges of replacing chemical counterparts with enzymes are manifold, and constant innovation is demanded. In this presentation, M. Ferrer will briefly describe innovations to access enzymatic diversity with which to generate products demanded by industry and consumers. In addition, M. Ferrer will provide an overview of to what extend new enzymes contribute to the circular bioeconomy while also contributing fighting climate change and global warming.











#### Session IV: Plastics and textiles in a circular bioeconomy

#### *Keynote*

# Developing a Production Process for Second Generation Lactic Acid, to make PLA and improve sustainability of Packaging



**Dr. Albrecht Läufer** *BlueCon Biotech GmbH* 

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A novel production process for the PLA (polylactic acid) precursor L-lactic acid will be described. L-lactic acid is formed from pretreated wheat straw or other lignocellulosic biomass, coming e.g. from agricultural or forestry residues, by direct fermentation of the feedstock using a proprietary and patented bacteria. This will supply the plastics industry with a degradable plastic, made from non-food biomass, which in many countries is still incinerated on the fields, at competitive cost. With the BluCon technology, those residues will be incorporated into a new value added cycle.

This so-called consolidated bioprocess for the direct fermentation of lignocellulosic feedstocks is developed by BluCon Biotech GmbH, which was founded in 2017 and is based on the BioCampus in Cologne.

In 2022 BluCon will start a next series of scale-up work and will be the first company in the world demonstrating such a process in the 10 cbm fermentation scale and additionally will generate several 100kg of polymer grade L lactic acid. The scale-up work is performed with four different cooperation partners with which hurdles like availability of suitable industrial-scale equipment can be temporarily overcome but will not be solved for future demonstrational work when it comes to the testing of new technologies and processes in the biotech sector. The scale-up project is supported by a grant from Germany's Ministry of Commerce (BMWK).

BluCon Biotech follows the concept of circular and bioeconomy and by that supports to achieve the Green Deal climate goals for 2050.











#### Appetite for trash - fighting the flood of plastics with enzymes and microbes



**Prof. Dr. Nick Wierckx** *IBG-1: Biotechnology, Forschungszentrum Jülich* 

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Over 300 million tons of plastic waste were produced worldwide in 2015. The environmental impact of these primarily fossil-based plastics has been broadly discussed, although their strength and light weight also often provide comparative advantages. We see great potential in the bio-upcycling of plastic waste, especially when combined with the development of new polymers and composites tailored for end-of-life functionalities such as biodegradability. Such polymers often contain biosimilar C-O and C-N heteroatom linkages, including polyesters, polyamides, and polyurethanes (1). Upon enzymatic or chemical hydrolysis of such polymers, a mixture of monomers is released containing alcohol, acid, or amine groups in the  $\alpha$ , $\omega$ -positions. Depending on the starting substrate the separation of these mixtures into their individual constituents may not be economical. In this case, we propose to use these plastic hydrolysates as carbon source for biotechnology (2). To enable this, we have developed *Pseudomonas* into a plastic monomer-metabolizing biotechnological workhorse, capable of metabolizing a wide range of aliphatic diols (3), dicarboxylates (4), and amines. Currently our main focus is to develop these monomer-metabolizing strains to not just grow on plastic monomers, but to convert plastic hydrolysates into value-added chemicals and biopolymers.









<sup>(1)</sup> Ellis et al. (2021) Chemical and biological catalysis for plastics recycling and upcycling. Nature Catalysis 4:539-556

<sup>(2)</sup> Tiso et al. (2022) The metabolic potential of plastics as biotechnological carbon sources – Review and targets for the future. Metabolic Engineering 71:77-98

<sup>(3)</sup> Li et al. (2020) Unraveling 1,4-Butanediol Metabolism in Pseudomonas putida KT2440. Frontiers in Microbiology 11:382

<sup>(4)</sup> Ackermann et al. (2021) Engineering adipic acid metabolism in Pseudomonas putida. Metabolic Engineering 67:29-40.



#### Challenges in developing a new polymer for textile applications



Dr. Jeroen van der Vlist Senbis Polymer Innovations B.V.

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The textile market is big, well established, and optimized with respect to costs. However, there is a strong need to replace PET, the main polymer in this area, with an alternative. Preferably the new material will be marine degradable in view of the environmental pollution. But the technical requirements are contrary to this requirement. The lecture will elucidate this challenge and show the direction for a solution.









#### **Poster Abstracts**

P1	Nr	<b>Presenting Author</b>	Abstract Title
Tino Polen plant-parasitic nematodes in agri- and horticulture  Cascade use of lupin plants: plant protein for food and feed and source for high-value chemicals  Pathonia Stadler Survey - Evaluation of the potential of utilizing potato-side streams for extraction of alkaloids  PARTHO SAKHA DE PARTHO SAKHA DE PREDIG: Modelling software to predict the enzymatic digestion of biomass  Pathyn Lee Processing of Lignin for Textile Application  Production of a sustainable and tailor-made microbial palm oil substitute from agricultural residues  SSWEEP - Solvent swelling to enhance enzymatic and microbial plastics upcycling  Pof. Nick Wierckx plastics upcycling  Pathonia BioPlastiCycle  Pof. Mirko Dallendörfer Structural Change from Fossil Economy to Bioeconomy  Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy  Processing of Lignin for Textile Application  Production of a sustainable and tailor-made microbial plasm oil substitute from agricultural residues  SSWEEP - Solvent swelling to enhance enzymatic and microbial plastics upcycling  Prof. Nick Wierckx plastics upcycling  Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy  Prof. The role of bio-based value chains in achieving a sustainable and successful regional transition  Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids  Prof. Stephan Thies Surfin - Surface Active Biomolecules for the Chemical Industry  MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing  MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  MetaBolic burden coupled phytase screening for sustainable phosphate recycling  Mire R. E. Dielentheis-  Marie	P1	Alexandra Brautlacht	New Biochars for the Improvement of Agricultural Soils
Pack   Cascade use of lupin plants: plant protein for food and feed and source for high-value chemicals	P2		, ,
P3         Marco Löhrer         source for high-value chemicals           P4         Marília Bueno da Silva, Nina Stadler         ToxPot - Evaluation of the potential of utilizing potato-side streams for extraction of alkaloids           P5         PARTHO SAKHA DE         PREDIG: Modelling software to predict the enzymatic digestion of biomass           P6         Sea-Hyun Lee         Production of a sustainable and tailor-made microbial palm oil substitute from agricultural residues           P7         Marcel Mann         SSWEEP - Solvent swelling to enhance enzymatic and microbial plastics upcycling           P8         Prof. Nick Wierckx         plastics upcycling           P9         Benedikt Wynands         BioPlastiCycle           P10         Mirko Dallendörfer         Structural Change from Fossil Economy to Bioeconomy           P11         Janine Macht         bioeconomy           P12         Ali Abdelshafy         Successful regional transition           P13         Robin Weihmann         Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids           P14         Stephan Thies         Surfin - Surface Active Biomolecules for the Chemical Industry           P15         Stephan Schott-Verdugo         MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing           P16         Berit Rothkranz </td <td>Tino Polen</td> <td></td>		Tino Polen	
Marco Löhrer         Source for high-value chemicals           P4         Marilia Bueno da Silva, Nina Stadler         ToxPot - Evaluation of the potential of utilizing potato-side streams for extraction of alkaloids           P5         PARTHO SAKHA DE         PREDIG: Modelling software to predict the enzymatic digestion of biomass           P6         Sea-Hyun Lee         Processing of Lignin for Textile Application           P7         Marcel Mann         Production of a sustainable and tailor-made microbial palm oil substitute from agricultural residues           P8         Prof. Nick Wierckx         SSWEEP - Solvent swelling to enhance enzymatic and microbial plastics upcycling           P9         Benedikt Wynands         BioPlastiCycle           P10         Mirko Dallendörfer         Structural Change from Fossil Economy to Bioeconomy           P11         Janine Macht         Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy           P12         Ali Abdelshafy         The role of bio-based value chains in achieving a sustainable and successful regional transition           P13         Robin Weihmann         Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids           P14         Stephan Thies         Surfin - Surface Active Biomolecules for the Chemical Industry           P15         Stephan Schott-Verdugo         MetaProcess: Engineeri	Р3		
PARTHO SAKHA DE PREDIG: Modelling software to predict the enzymatic digestion of biomass  PARTHO SAKHA DE LignoTex - Integrated Biorefinery for Sustainable Production and Processing of Lignin for Textile Application  Production of a sustainable and tailor-made microbial palm oil substitute from agricultural residues  Prof. Nick Wierckx plastics upcycling  Paedikt Wynands BioPlasticycle  Pio Mirko Dallendörfer Structural Change from Fossil Economy to Bioeconomy  Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy  The role of bio-based value chains in achieving a sustainable and successful regional transition  Pia Robin Weihmann Viola: Towards violaceines — a mutasynthesis platform for tryptophan-derived alkaloids  Pia Stephan Schott-Verdugo downstream processing odwnstream processing odwnstream processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processes: Engineering enzymes for sustainable and economically feasible metaraminol production  Metabolic burden coupled phytase screening for sustainable phosphate recycling  MK-Scaloop — Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry		Marco Löhrer	source for high-value chemicals
Nina Stadler   Streams for extraction of alkaloids	P4	Marília Bueno da Silva,	ToxPot - Evaluation of the potential of utilizing potato-side
PARTHO SAKHA DE  PARTHO SAKHA DE  PARTHO SAKHA DE  Processing of Lignin for Textile Application  Production of a sustainable and tailor-made microbial palm oil substitute from agricultural residues  Prof. Nick Wierckx  Prof. Nick Wierckx  Pasenedikt Wynands  Prof. Nick Wierckx  Prof. Nick Wierck  Prof. Nick Wierckx  Prof. Nick Wierck  Prof. Nick Wi		Nina Stadler	
PARTHO SAKHA DE    Description	P5		
Processing of Lignin for Textile Application		PARTHO SAKHA DE	
Processing of Lignin for Textile Application	P6		
Marcel Mann   Substitute from agricultural residues		Sea-Hyun Lee	
Prof. Nick Wierckx plastics upcycling  Pendikt Wynands BioPlastiCycle  Prof. Mirko Dallendörfer Structural Change from Fossil Economy to Bioeconomy  Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy  Prof. Mirko Dallendörfer Structural Change from Fossil Economy to Bioeconomy  Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy  The role of bio-based value chains in achieving a sustainable and successful regional transition  Prof. Mirko Dallendörfer Structural Change from Fossil Economy to Bioeconomy  The role of bio-based value chains in achieving a sustainable and successful regional transition  Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids  Prof. Metaphan Thies Surface Active Biomolecules for the Chemical Industry  Metaprocess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing  Prof. Metaprocess: Engineering enzymes for sustainable and economically feasible metaraminol production  Prof. Metaphan Schott-Verdugo Metaphane recycling  Metaphane recycling  Mik-Scaloop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  Liamocin and exophilin – novel surface-active biomolecules and green building blocks for a sustainable chemical industry	P7		·
Prof. Nick Wierckx Plastics upcycling Prof. Nick Wierckx Plastic upcycling Prof. Nick Wierckx Prof. Nick Wie		Marcel Mann	
P9 Benedikt Wynands BioPlastiCycle P10 Mirko Dallendörfer Structural Change from Fossil Economy to Bioeconomy Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy  P12 Ali Abdelshafy The role of bio-based value chains in achieving a sustainable and successful regional transition  P13 Robin Weihmann Tryptophan-derived alkaloids P14 Stephan Thies Surfin - Surface Active Biomolecules for the Chemical Industry MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing  P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  P17 Anna Joelle Ruff MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P18 Moritz Becker Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P8		,
P10 Mirko Dallendörfer Structural Change from Fossil Economy to Bioeconomy  Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy  P12 The role of bio-based value chains in achieving a sustainable and successful regional transition  P13 Robin Weihmann Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids  P14 Stephan Thies Surflin - Surface Active Biomolecules for the Chemical Industry  MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing  P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  P17 Anna Joelle Ruff MetaProcess in moustrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P18 Moritz Becker Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry			†· · · · · · · · · · · · · · · · · · ·
Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy  The role of bio-based value chains in achieving a sustainable and successful regional transition  P13 Robin Weihmann Viola: Towards violaceines — a mutasynthesis platform for tryptophan-derived alkaloids  P14 Stephan Thies Surfin - Surface Active Biomolecules for the Chemical Industry MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing  P16 Berit Rothkranz Economically feasible metaraminol production  P17 Anna Joelle Ruff MK-ScaLoop — Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P19 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P9	, , , , , , , , , , , , , , , , , , ,	,
P11 Janine Macht bioeconomy  P12 Ali Abdelshafy The role of bio-based value chains in achieving a sustainable and successful regional transition  P13 Robin Weihmann Viola: Towards violaceines — a mutasynthesis platform for tryptophan-derived alkaloids  P14 Stephan Thies Surfin — Surface Active Biomolecules for the Chemical Industry MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing  P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  P17 Anna Joelle Ruff MetaProcessing  P18 Moritz Becker Moritz Becker  P19 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P10	Mirko Dallendörfer	·
P12   Ali Abdelshafy   The role of bio-based value chains in achieving a sustainable and successful regional transition     P13   Robin Weihmann   Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids     P14   Stephan Thies   Surfin - Surface Active Biomolecules for the Chemical Industry     P15   MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing     P16   Berit Rothkranz   MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production     P17   Anna Joelle Ruff   Metabolic burden coupled phytase screening for sustainable phosphate recycling     P18   Moritz Becker   Moritz Becker   Moritz Becker   Liamocin and exophilin – novel surface-active biomolecules and green building blocks for a sustainable chemical industry			
P12 Ali Abdelshafy Successful regional transition P13 Robin Weihmann Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids P14 Stephan Thies Surfin - Surface Active Biomolecules for the Chemical Industry P15 MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production P17 Anna Joelle Ruff MetaPolic burden coupled phytase screening for sustainable phosphate recycling MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor P19 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P11		
P12   Ali Abdelshafy   Successful regional transition		Janine Macht	·
P13   Robin Weihmann   Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids	P12		
P13   Robin Weihmann   tryptophan-derived alkaloids		Ali Abdelshafy	
P14 Stephan Thies Surfin - Surface Active Biomolecules for the Chemical Industry  MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing  P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  P17 Anna Joelle Ruff MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  Metabolic burden coupled phytase screening for sustainable phosphate recycling  MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P19 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P13	Bullius Marthuman	, , , , , , , , , , , , , , , , , , , ,
P15 Stephan Schott-Verdugo Stephan Schott-Verdugo downstream processing  P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  P17 Anna Joelle Ruff MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  Metabolic burden coupled phytase screening for sustainable phosphate recycling  MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P19 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	544		1
P15 Stephan Schott-Verdugo enzymatic production of chiral amino alcohols integrating downstream processing  P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  P17 Anna Joelle Ruff MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P18 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P14	Stephan Thies	·
Stephan Schott-Verdugo downstream processing  P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  P17 Anna Joelle Ruff Metabolic burden coupled phytase screening for sustainable phosphate recycling  MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P18 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	545		·
P16 Berit Rothkranz MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production  P17 Anna Joelle Ruff Metabolic burden coupled phytase screening for sustainable phosphate recycling  MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P18 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P15	Storbor Sabatt Vardura	
P17 Berit Rothkranz economically feasible metaraminol production  P17 Metabolic burden coupled phytase screening for sustainable phosphate recycling  MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P18 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry		Stephan Schott-verdugo	·
P17 Anna Joelle Ruff Metabolic burden coupled phytase screening for sustainable phosphate recycling  MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P19 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P16	Porit Pothkranz	
P17 Anna Joelle Ruff phosphate recycling  MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P19 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry		Derit Notikianz	
MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor  P19 Marie R. E. Dielentheis-Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P17	Anna Ioelle Ruff	
P18 biotechnological production of methyl ketones in a novel multiphase loop reactor  P19 Marie R. E. Dielentheis-Frenken, Frederick Haala biotechnological production of methyl ketones in a novel multiphase loop reactor  Liamocin and exophilin – novel surface-active biomolecules and green building blocks for a sustainable chemical industry		Aima sociic Ran	· · · ·
Moritz Becker multiphase loop reactor  Marie R. E. Dielentheis- Liamocin and exophilin – novel surface-active biomolecules and green building blocks for a sustainable chemical industry	P18		· · · · · · · · · · · · · · · · · · ·
P19 Marie R. E. Dielentheis- Frenken, Frederick Haala Liamocin and exophilin – novel surface-active biomolecules and green building blocks for a sustainable chemical industry	10	Moritz Becker	
Frenken, Frederick Haala green building blocks for a sustainable chemical industry	P19		·
raracoccus pantotrophus and its potential as a pioeconomical	P20	, 11 1 1000	Paracoccus pantotrophus and its potential as a bioeconomical
Denise Bachmann chassis organism		Denise Bachmann	· · · ·









Nr	<b>Presenting Author</b>	Abstract Title
		A new bacterial cell factory for the production of microbial
P21		bioplastics – Elucidating metabolic constraints by 13C-based
	Upasana Pal	metabolic flux mapping in Paracoccus pantotrophus
P22		Electro-driven production of fine chemicals using biocatalysts in
	Lars Lauterbach	combination with electrolysis
P23		The geography of agricultural bioeconomic innovations – A case
	Uyen Tran	study of Vietnam's agricultural innovation system
P24		Mycorrhization, wheat growth, and phosphorus transfer
		efficiency in response to fertilization with algal biofilms
	Silvia Schrey	produced on the effluent of municipal wastewater
P25		The project Circular PhytoREVIER – Herbal and medicinal plants
	Mark Mueller-Linow	in the Rheinische Revier











#### P1 - New Biochars for the Improvement of Agricultural Soils

Dr. T. Kraska<sup>1</sup>, Prof. Dr. R. Pude<sup>1</sup>, Prof. Dr. E. Lüdeling<sup>1</sup>, <u>A. Brautlacht<sup>2</sup></u>, M. Lang<sup>2</sup>, Prof. Dr. P. Quicker<sup>2</sup>, Dr. V. Nischwitz<sup>3</sup>, Dr. S. Küppers<sup>3</sup>, Dr. N. Siebers<sup>4</sup>, Prof. Dr. N. Brüggemann<sup>4</sup>, Prof. Dr. H. Vereecken<sup>4</sup>, Dr. S. Schrey<sup>5</sup>, Dr. A. Kuhn<sup>5</sup>, Prof. Dr. U. Schurr<sup>5</sup>, Prof. Dr. G. Walther<sup>6</sup>

Biochars – produced by the pyrolysis of biomass – have recently been promoted as a useful soil amendment with a multifunctional use, e.g., sequester carbon (C), recover nutrient elements from waste-streams, fix excess nutrients in soils to reduce environmental risks, and ameliorate soil in terms of nutrient status and aggregate structure. Our project aims at a multifunctional use of biochars for nutrient recovery from manure and soil amelioration with a cascade-use of biochar feedstocks from horticulture. Thus, a recycling and value-added utilization of agricultural residues through combining technologies such as pyrolysis could increase the recoverable energy, close the nutrient recycle loop, and ensure cleaner agricultural production. The NewBIAS consortium will ensure dissemination and exploitation of technology and products and thus will stimulate the bioeconomy in NRW by developed technology and regional resources with a clear global and worldwide focus on innovation through the delivery of a novel value-added chain.

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20









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#### P2 - ProRNA - Exploring dsRNAs as innovative bio-agents against plant-parasitic nematodes in agriand horticulture

Prof. Dr. Florian Grundler<sup>1</sup>, Dr. Sylvia Schleker<sup>1</sup>, Prof. Dr. Michael Bott<sup>2</sup>, Dr. Philipp Fricke<sup>2</sup>, <u>Dr. Tino</u> Polen<sup>2</sup>

Plant-parasitic nematodes (PPNs) are a top major problem in crop production and thus are scientifically, societally and economically highly relevant for the bioeconomy. Sedentary endoparasitic nematodes, especially Meloidogyne as well as Heterodera and Globodera species are the most important PPNs from an economic point of view. Second-stage juveniles penetrate plant roots and induce the formation of giant-cells as permanent food source leading to root galls, deformed growth and reduced plant health. PPNs also open the door for other plant pathogens such as fungi and bacteria, resulting in the formation of further disease complexes. Since PPNs can target more than 550 hosts the estimated resulting monetary damage is more than 100 billion US dollars anually. Chemical crop protection methods against PPNs are not permitted anymore in most countries due to high environmental toxicity and resistant or tolerant cultivars are scarce and have their limitations. Therefore, there is an urgent need to develop and test new effective methods to control PPNs for sustainable and environmentally friendly production of plants for food and feed.

The ProRNA project is a proof-of-concept-study about exploring applications of dsRNA formulations as a new non-chemical strategy for the practical control of PPNs in soil via RNA interference to protect crops and improve plant health. Knowledge and effective dsRNAs already reported for Heterodera will be used and transferred to Meloidogyne to test and expand dsRNA-based PPN control in vitro and in vivo. First corresponding dsRNA candidates were found in Meloidogyne hapla by BLAST analysis of RNA sequences which will be tested. With effective control of PPNs by dsRNAs in soil, improved plant health and also reduced secondary plant infections by other pathogens are expected. The proposed strategy is also expected to reduce the use of pesticides, fungicides, herbicides, or fertilizers in agricultural and horticultural systems.

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#### P3 - Cascade use of lupin plants: plant protein for food and feed and source for high-value chemicals

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Lupin is a valuable alternative protein source suitable for human food and animal feed and presents an attractive alternative to (GMO-)soybean from international markets. Lupin was already well established in Europe and popular in Germany up to the early 1990s, when the fungal disease "anthracnose" led to severe crop losses and in turn to a steep decrease in lupin production. Due to intensive breeding efforts, nowadays more varieties with increased anthracnose resistance are available (mainly L. angustifolius) but many of them still do not reach former yield potential. Species of the genus Lupinus are known for their alkaloid content, which is reduced in the "sweet" varieties, but high in the naturally more disease resistant "bitter" varieties. Alkaloids must be removed before the protein can be further processed for consumption and are up to now treated as waste. From a chemist point of view lupins are of interest, because they contain highly valuable and rare (pre)chiral compounds such as lupanin (2-oxosparteine). In this project we want to explore the use of biostimulants and plant strengtheners in lupin production, for improving yield, increasing pathogen resistance and better adaptation to abiotic stresses. In an exemplary cascade use approach, we want to demonstrate additional benefits of lupin production, by using the hitherto wasted alkaloids as a source for high value chemicals. At the same time, we will investigate the producer's acceptance of this novel concept in order to get it closer to practice. The BioSC BOOST FUND 2.0 Project P<sup>3</sup>roLucas (Optimization of plant performance and products for lupin cascade use) integrates the three different focus topic areas "smart management for plant performance", "modular bio-transformations for highvalue compounds" and "technological innovations as driver for bio-based social transformation" and is based a successful previous Bioeconomy International project and joint research projects of the core groups within the BioSC.

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#### P4 - ToxPot - Evaluation of the potential of utilizing potato-side streams for extraction of alkaloids

Marília Bueno da Silva<sup>1,3</sup>, Nina Stadler<sup>2</sup>, Sylvia Schleker<sup>3</sup>, Florian M. W. Grundler<sup>3</sup>, Jörg Pietruszka<sup>2,4</sup>, Björn Usadel<sup>1,5</sup>, Anika Wiese-Klinkenberg<sup>1</sup>, Thomas Classen<sup>4</sup>, Franziska Genzel<sup>1</sup>

ToxPot aims at the potential use of secondary ingredients from by-products of potato production, mainly potato berries or flowers, for chemical or enzymatic steroid synthesis for further pharmaceutical use and for use as plant-based pesticides. Leaf and stem material as well as potato flowers or berries remain unused on the field in commercial cultivation or are composted after the tubers have been harvested. These by-products contain high levels of steroidal alkaloids, in particular the glycoalkaloids (GAs)  $\alpha$ -solanine and  $\alpha$ -chaconine. In addition, a large number of other alkaloids have been detected in potato species. Steroidal alkaloids have anti-carcinogenic, anti-inflammatory and anti-fungal properties. With this, alkaloid or alkaloid derivatives as well might be useful for plant protection, and this potential will be evaluated In ToxPot. For this, a new HPLC-MS method was developed to quantify glycoalkaloids in potato tissues. Preliminary results were obtained for quantification of  $\alpha$ -solanine and  $\alpha$ -chaconine from flowers and berries. Several published extraction methods for GAs from potato berries have been evaluated based on their properties regarding purity and complexity. An adapted purification protocol has been established and the optimization process is underway. Next steps combine the application of these methods for further quantification of glycoalkaloids, metabolic profiling of three different genotypes, as well as characterization and semisynthetic modification of  $\alpha$ -solanine, whose effect will be tested on target and non-target organisms.

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#### P5 - PREDIG: Modelling software to predict the enzymatic digestion of biomass

Adélaïde Raguin<sup>1</sup>, Partho Sakha De<sup>1</sup>, Holger Klose<sup>2</sup>, Philipp M. Grande<sup>2</sup>

Lignocellulosic biomass (dry plant material) is a cheap, abundant, and renewable natural resource for the production of biofuels and other useful chemicals. Enzymatic conversions of lignocellulosic biomass are important operations in bio-refinery approaches. However, they are difficult to optimise due to the variability and heterogeneity of the substrate. Predicting lignocellulose saccharification would be crucial for lignocellulosic bio-refinery concepts to achieve economic viability. Several factors are known to contribute to material recalcitrance, such as lignin or product induced enzyme inhibition and crystallinity of the material. A detailed understanding of the underlying molecular mechanisms is crucial and necessitates a multi-disciplinary approach, including advanced computational tools. Our stochastic computational model simulates the enzymatic digestion of the plant cell wall resolved in three dimensions. The substrate is being digested by a cocktail of cellulases and xylanases, depending on its composition, crystallinity properties, and exposure to various pre-treatment processes. Varying severity of phosphoric acid pre-treatment allows us to create different levels of crystallinity in the material for which composition data is already available. The characterisation of crystallinity of the samples is carried out by FT-NIR spectroscopy and cross-validated by X-ray diffraction method. These quantitative experiments allow us to calibrate the model and further enhance its predictive power. Thus, building on the previous work of both teams, PREDIG aims to deliver a free, open-source, and user-friendly software in a Graphical User Interface (GUI). The GUI will include drop-down menus containing the samples studied during the project. This interface will allow users to set substrate properties in terms of composition and crystallinity and simulate the saccharification time courses for various sets of enzymes. It will also allow the user to determine the best adapted enzyme cocktail for the optimal saccharification of a specific biomass.

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#### P6 - LignoTex - Integrated Biorefinery for Sustainable Production and Processing of Lignin for Textile **Application**

Daniel Wolters<sup>1</sup>, Sascha Schriever<sup>1</sup>, Holger Klose<sup>2</sup>, Philipp Grande<sup>2</sup> Sea-Hyun Lee<sup>1</sup>,

In the project LignoTex, the ITA, AVT, NGP2, IAC of the RWTH, IGB-2 of the Forschungszentrum Jülich and ILR of the University Bonn aim to cover the whole process chain from lignin extraction to spinning of lignin-based fibers as well as the application in textiles including societal studies on the perception of bio-based innovations and communication strategies. With a variety of different plants such as woods, grasses, perennial plants and agricultural residues, which will be extracted using the OrganoCat technology adapted and optimized to the different plant materials, the consortium creates a solid foundation of data on which future processes can build on. Once lignin has been extracted, isolated and fractionated by anti-solvent precipitation, the base material can be tested and benchmarked against state of the art lignin types in textile processes. To ensure sufficient quantities of lignin, a scale up of the OrganoCat process to technical scale has been planned. The isolated lignin will be, modified or blended with other (bio-based) polymers such as polylactic acid and polyacrylnitrile. These precursors create the base for state of the art and adapted wet spinning, melt spinning and composite manufacturing processes to determine the influence of molecular properties on their performance in future textile applications. The market potential for different future products will be assessed based on those processes with a focus point in consumer acceptance and communication strategies.

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## P7 – Production of a sustainable and tailor-made microbial palm oil substitute from agricultural residues

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Vegetable oils such as palm oil are used in large quantities in the food industry. The origin of palm oil is often critically perceived since high CO2 emissions are caused by the slash-and-burn clearing of tropical rainforests as well as the oil transport to Europe. Therefore, a microbial palm oil substitute made from the agricultural waste stream corn stover will be produced via fermentation of Ustilago maydis. This fungus is well known for its growth on complex substrates (e.g. plant biomass). The currently available, non-optimized strain already produces 79% oil/cell dry weight under nitrogen limitation. The composition of the microbial oil from preliminary experiments was encouragingly found to be already in the range of palm oil.

By genetic modification of U. maydis and by adjusting the cultivation parameters, the fatty acid profile of the microbial oil can be varied to produce a customized oil. Thus, depending on the requirements of the application, a palm oil substitute with an accordingly tailored fatty acid profile can be produced, enlarging the potential field of application. As an application example, this project will convert an agricultural waste product (corn stover) into a customized palm oil substitute for the production of vegan cheese as the vegan food market is strongly expanding. By adapting the fatty acid profile, milk fat will be substituted even better than it is currently the case with conventional palm oil. The tailored microbial oil is being tested by the start-up Formo for production of vegan cheese specialties. The potential of this concept is particularly high. On the one hand, the national bioeconomy is promoted by a new product with a huge market volume. On the other hand, a new value-adding concept for the waste stream corn stover is created.

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#### P8 - SSWEEP - Solvent swelling to enhance enzymatic and microbial plastics upcycling

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Bio-upcycling of plastic waste is a rapidly developing scientific field with prominent applications in the circular bioeconomy. Especially the development of PETases that hydrolyse polyethylene terephthalate (PET) has rapidly progressed, to the point where these enzymes are now being commercially developed for recycling purposes. However, research is strongly focused on the in vitro application of these enzymes at elevated temperatures and there is a knowledge gap with tremendous untapped potential regarding the underlying microbiology. SSWEEP aims to address this gap with innovative multidisciplinary approaches, aiming at increasing the enzymatic and microbial degradation of PET, and the recycling of PET/PE laminate foils. If successful, the SSWEEP approach offers a plethora of exciting chances for innovation in a circular PET economy that can help to solve the global challenge of plastics pollution, while also providing economic opportunities for hybrid-resource biorefineries by in-cycling cheap carbon feedstock from the excellent German PET collection logistics.

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#### P9 - BioPlastiCycle

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Plastics fulfill many vital functions in modern society, but not enough consideration is given to the endof-life fate of these extremely stable materials. Almost all plastics are currently produced from fossil resources. Thus, plastic pollution is rampant and plastic production significantly contributes to climate change. Because of this, there is a strong societal and regulatory push for the development of more sustainable, biodegradable bioplastics. However, viable recycling options are still lacking for many of these emerging materials, and there is thus an urgent need for technology development towards a circular bioplastics economy. BioPlastiCycle aims to tackle this challenge by developing and evaluating a complete value cycle for bioplastics, focusing on both well-established polymers like polylactide as well as newly developed materials based on alpha-ketoglutarate. We address all major aspects of the value cycle, including bio-based monomer production and purification, model-guided eco-friendly polymerization, tuning of the polymer properties towards biodegradation, bio- and chemical recycling of the resulting bioplastics, as well as assessment of economic and environmental impact of these materials. This highly interdisciplinary project aims to further the transition of plastics into the circular bioeconomy by developing new materials and processes, thereby reducing plastics-associated pollution while also fostering regional economic development.

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#### P10 – Structural Change from Fossil Economy to Bioeconomy

Mirko Dallendörfer<sup>1</sup>, Sophia Dieken<sup>1</sup>, Florian Siekmann<sup>1</sup>, Sandra Venghaus<sup>1,2</sup>

Regions such as the Rheinische Revier are faced with structural change and a political push for a sustainable bioeconomy. This transformation is complex and requires extensive techno-economic and societal changes. In our research, we investigate societal perspectives by pursuing different lines of questioning under the common Transform2Bio project umbrella of identifying transformation pathways. We analyze socio-political factors driving the bioeconomy and the public debate about it, as well as the perception of relevant stakeholders. Methods include discourse analysis and discourse network analysis of statements in textual form, as well as representative quantitative surveys. The results of a media analysis show that the bio-economy has received increased media attention while an in-depth debate is still missing. The overwhelming majority of media articles portrays a technology-based vision of the bioeconomy, while few articles refer to the alternative ecology-based vision. Survey results for the German population show that the bioeconomy concept is largely unknown, while a broadly defined bioeconomy, based on the replacement of fossil resources, the use of new technologies and the sustainability principle, is supported by 68% of the population. These works show that there is a need for information and engagement of the broader society in the transformation process toward a sustainable bioeconomy. The high support for the bioeconomy demonstrates the opportunities to advance a sustainability transformation supported by society, while there is also a risk of disappointments if corresponding high expectations are not met.

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#### P11 - Don't forget the local people: Understanding differences in citizens' acceptance of technologies supporting a sustainable bioeconomy

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Technologies such as biorefineries and aquaponics are seen as key factors in the transition to a sustainable bioeconomy. A core challenge for the long-term market success of new technologies is to identify and address public needs and concerns at an early stage. A lack of citizen acceptance can lead to the rejection of technological innovations and thus increase the risk of such innovations failing, as has been shown in the case of GMO technology or carbon capture. This study investigates citizens' acceptance of the two technologies biorefinery and aquaponics, taking into account regional differences (transition region (Rheinische Revier) vs. non-transition region (remaining NRW)) and differentiating between the general acceptance of this technology and its acceptance when implemented at the local level. Furthermore, the relevance of key determinants, in particular social trust, affect, perceived benefit and risk, for predicting acceptance is investigated using partial least squares structural equation modelling (PLS-SEM). Consistent with the Not in My Backyard (NIMBY) phenomenon, our results show lower local acceptance compared to general acceptance of both biorefineries and aquaponics - although acceptance of the latter was higher. Furthermore, local acceptance was lower in the transition region than in the non-transition region, highlighting the need for effective communication strategies to successfully implement a sustainable transition to a bioeconomy. The results also highlight the role of affect (both positive and negative) in explaining general and local acceptance. While our results additionally point to the importance of perceived benefits in predicting general and local acceptance, perceived trust and risk are only relevant determinants for the latter. Those relevant predictors should be considered when designing information campaigns in the Rheinische Revier or other regions where the establishment of new biobased technologies is planned.

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#### P12 - The role of bio-based value chains in achieving a sustainable and successful regional transition

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The coal phase-out process entails structural changes in the regions where the mining activities take place. The Rheinisches Revier, the largest lignite mining in Europe, yields roughly one half of the German lignite production. Considering that the other half comes from two other regions, the Rheinisches Revier would be the most affected zone in the coming two decades.

In order to respond proactively, Transform2Bio project assesses the potentials of introducing biobased value chains as suitable alternatives to the obsolete fossil-based ones. The poster briefly depicts some aspects of the ongoing analyses and highlights the challenges associated with the phase-out process and the opportunities of establishing a bioeconomy in the region. Although there are many potentials in NRW, still some challenges need to be tackled. Some of these challenges are attributed to the existing industrial system and fossil-based products (lock-in effect), while the other are related to the complexity and novelty of bio-based value chains and bio-technologies.

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#### P13 – Viola: Towards violaceines – a mutasynthesis platform for tryptophan-derived alkaloids

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Natural secondary metabolites provide an ample source of bioactive compounds. Violacein-type indole alkaloids are bioactive compounds with promising properties for applications in pharma and agriculture.

Within "Viola", we will

- i) construct a Pseudomonas putida KT2440 chassis for the conversion of violacein-precursor analogs,
- ii) obtain precursor analogs by chemical synthesis, and
- iii) establish suitable bioprocess protocols for their effective conversion by P. putida to violacein derivatives.

This project thus aims at contributing to the development of sustainable production processes of compounds with high-value properties such as eco-friendly crop protection.

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#### P14 - Surfin - Surface Active Biomolecules for the Chemical Industry

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Within the project "Surfin", we will investigate the production of biosurfactants, which are not yet used by the chemical industry. With years of proven productive cooperation, we aligned a consortium with the necessary know-how, building on the expertise developed in previous BioSC projects to research a biosurfactant production process towards industrial adaptation. Surfln integrates strain engineering of biosurfactant producing microbes with bioprocess engineering for enhanced cultivation conditions and upscaling of the fermentation, chemical engineering for the development of sustainable purification strategies, and finally, social and management sciences for the implementation of the developed technology in relevant industries and markets. For possible exploitation of the Surfln results, industrial experts from world leading companies form part of the

The two target compounds of SurfIn will be the biosurfactants liamocin and serrawettin W1. Both have not been intensively researched and offer a high potential for dissemination and commercial exploitation. Apart from the use in classical surfactant applications (e.g., detergents) both substances feature further areas for usage. Serrawettin W1 for example has been shown to be applicable in crop protection and both molecules are suited as building blocks for bio-based polymers. As heavy oils, liamocins show similar characteristics during production and purification as sophorolipids, a first class of biosurfactants that are successfully exploited in industry. Liamocins feature a high potential to be produced at industrial scale with less development effort than for the extensively foaming biosurfactants such as rhamnolipids. The outcome of SurfIn will be a general workflow for integrated strain and process engineering for biosurfactant production, explicitly a fermentation at 50 L, with titers above 20 g/L, including simple but efficient purification and a defined roadmap for industrial implementation of the technology.

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#### P15 – MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing

Stephan Schott Verdugo<sup>1</sup>, Anna Joelle Ruff<sup>2</sup>, Ulrich Schwaneberg<sup>2</sup>, Andreas Jupke<sup>3</sup>, Dörte Rother<sup>4</sup>, Holger Gohlke<sup>1,5</sup>

Synthesis pathways for chiral amino alcohols, like the vasoconstrictor metaraminol, have relied on classic chemistry based on petrochemical resources and metal-based catalysis reactions. The synthesis of metaraminol can be achieved by combining an enzymatic carboligation step and a subsequent reductive amination step, starting from readily available second-generation feedstocks. However, for an economically and ecological efficient process on larger scale, the biocatalyst must be reused, currently limited by the operational stability of the applied biocatalysts. By incorporating rational design, directed evolution and ad hoc biochemical evaluations, stable carboligase and transaminase variants will be generated, able to withstand repetitive batch/continuous production in a longer time scale. The best candidates will be implemented in a scalable and ready-to-use lab-scale demonstrator plant and evaluated techno-economically. We expect to make amino alcohol production more sustainable and efficient, ultimately lowering the access barrier for otherwise challenging to obtain chemical building blocks and active pharmaceutical ingredients.

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#### P16 - MetaProcess: Engineering enzymes for sustainable and economically feasible metaraminol production

Berit Rothkranz<sup>1,2,3</sup>, Laura Grabowski<sup>1,2,3</sup>, Prof. Dr. Dörte Rother<sup>1,2,3</sup>

Metaraminol is an  $\alpha$ 1-adrenergetic receptor agonist and active pharmaceutical ingredient for the treatment of hypotension. In the past years, our working group has successfully established a multistep enzymatic process for the production of metaraminol as a green alternative to the chemical production

Within the scope of the project "Hylmpact", funded by the Bioeconomy Science Center, the enzymatic cascade, comprising the carboligation step of 3-OH-benzaldehyde with pyruvate to the intermediate (R)-3-OH-phenylacetylcarbinol followed by the transamination to the final product metaraminol, was combined with in situ product removal. As a special goal, most of the initial substrates were obtained from renewable raw materials. An in situ liquid-liquid extraction was applied using 1-octanol as organic solvent with oleic acid as reactive extractant. By using the integrated product recovery, a metaraminol yield of 69 % could be achieved. However, inactivation of the amine transaminase by oleic acid concentrations above 0.5 M in the organic extraction phase could be observed (Doeker et al., 2022). At this point, the project "MetaProcess" comes into play: The operational stability of the applied enzymes, carboligase and amine transaminase, is to be increased by using rational design and directed evolution to enhance the industrial applicability and sustainability of the enzymatic metaraminol synthesis. Particularly, the stability of amine transaminase against high oleic acid concentrations should be improved in order to increase metaraminol yields while reducing catalyst amounts. Additionally, immobilization techniques will further support the operational stability, reuse of the catalysts facilitate (semi-) continuous process In cooperation with our project partners, the inactivation mechanisms of the catalysts should be elucidated by using molecular dynamics simulations to find further enzyme variants for metaraminol production.

Doeker, M. et al. (2022) 'In situ reactive extraction with oleic acid for process intensification in amine transaminase catalyzed reactions', Green Chemistry, 24(1), pp. 295–304.

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#### P17 - Metabolic burden coupled phytase screening for sustainable phosphate recycling

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Phosphate is a main component of fertilizers and therefore essential to feed humankind. Recycling concepts for phosphorus are a key request to ensure a self-sustaining food production in Europe and to avoid rapid depletion of concentrated natural deposits. Renewable resources like meal from food oil manufacturing are locally produced in NRW, available in significant quantities and of high potential for circular P-economy. ScreenP will contribute to phosphate stewardship by developing a product specific screening system, which will strengthen phytase engineering. An increased phytase expression redirects carbon flux and energy consumption from biomass formation to protein production. These dynamics can be monitored via Pichia Pastoris respiratory activity and used for the development of a novel high-throughput screening method. This novel screening assay will increase the screening abilities and the rate of identifying improved variants. Additionally, the product specific detection by NMR ensure to identify hits. The screening system developed in ScreenP will foster enzyme engineering and enable tailor made phytases for industrial applications (food, feed, P-management).

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# P18 – MK-ScaLoop – Towards an industrial-scale process for biotechnological production of methyl ketones in a novel multiphase loop reactor

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Methyl ketones (MKs) are platform chemicals currently used as fragrances, flavors, and precursors in pharmacological production. Furthermore, mixtures of medium-chain length MKs in a range of C11 to C13 show cetane numbers of around 64, making them promising fuel candidates. At present, MKs are industrially produced from petroleum-derived hydrocarbons. To achieve a more sustainable production process and to make MKs industrially accessible as biofuels, current research focuses on biotechnological synthesis by re-arranging the fatty acid metabolism of aerobic microorganisms. [1] However, the efficient biotechnological production of MKs in bioreactors is often hindered by the toxicity of the products. Hence, contacting the aqueous reaction phase with a second organic phase allows for in situ product removal avoiding product toxicity. Since the oxygen needed by the aerobic microbes is supplied by gas sparging and stirring, the organic phase is finely dispersed leading to complicated phase separation and consequently а reduced apparatus To facilitate the phase separation and increase the apparatus' efficiency, while still taking advantage of in situ product removal via a second organic phase, we propose a novel multiphase loop reactor concept. Within this reactor concept, we use an inner compartment (riser) for oxygen supply and an outer compartment (downcomer) introducing the organic phase. Due to the clear segregation, a circulating loop of the aqueous reaction phase within the reactor is forced. Thus, both the oxygen supply needed by the microbes and the generation of organic droplets for in situ product removal with their consecutive coalescence can be controlled. We will implement the novel multi-phase loop reactor on a 100 L scale for fluid dynamic characterization. By combining the resulting knowledge of the conditions inside the reactor with genetic engineering, we optimize the microbes toward the identified prevailing reaction conditions.

Followed by integration of the multiphase loop reactor in a downstream process we conduct a life cycle assessment and costing on an industrial scale to evaluate the potential of our process. On the poster, we present the novel concept described above.

[1] S. C. Nies et al., Metabolic Engineering 62 (2020), 84-94

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## P19 - Liamocin and exophilin - novel surface-active biomolecules and green building blocks for a sustainable chemical industry

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In a world with rising population numbers, climate change, and shrinking fossil resources, the need for replacement of crude-oil based compounds by biotechnologically produced alternatives gains more and more importance every day. One of these alternatives could be an amphiphilic polyol lipid called liamocin, which is produced by the yeast-like versatile fungus Aureobasidium pullulans. Liamocin is a promising biosurfactant and bio-based polymer building-block, while its precursor exophilin can be converted into lubricants or biofuels. The polyextremotolerant fungus Aureobasidium tolerates acidic conditions, high salt concentrations, and low temperatures. Additionally, it can grow on a broad substrate spectrum, allowing the use of renewable substances and industrial waste as carbon source. All these aspects can contribute to a sustainable and economical production. Despite the great potential mentioned above, liamocin production with A. pullulans in larger scale has to date not been intensively investigated. This versatile fungus produces further secondary metabolites like pullulan, melanin, and polymalic acid. Deletion of the genes responsible for by-product formation will be the first attempt to increase liamocin production. In order to realize that, we established a genetic tool in A. pullulans based on the CRISPR/Cas9 method. Further, a minimal medium was developed on a small scale to investigate the potential of suitable carbon sources. This was followed by a successful scaleup to a 1 L bioreactor. Combining genetic engineering and process optimization enables quick advancement in the production of a new and versatile molecule and thus an important contribution to the envisioned circular bioeconomy.

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#### P20 - Paracoccus pantotrophus and its potential as a bioeconomical chassis organism

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In the vein of the circular bioeconomy, biotechnological processes are the focus point to achieve the transition from fossil based to renewable substrate-based products. The pure argument of sustainability is usually not enough, if it is not met with other advantages over established products. Therefore, efforts should be concentrated on bio-exclusive compounds, with, e.g. improved application performance, the production of which would not be feasible in the petrochemical industry. The reduction in complexity and cost of the production process in general is therefore of importance. One way to tackle these challenges is to expand the phenotypic landscape of usual microbial platform organisms used as chassis in biotechnology by exploiting the natural diversity of wild type prokaryotes. For this reason, the wild type strain Paracoccus pantotrophus DSM 11073 was chosen to exemplify the vast unused potential of non-engineered bacteria, in particular the production of the bioplastic polyhydroxyalkanoate (PHA) from glucose. With simple process adjustments and media optimization, it was possible to increase the cell dry weight from 5 g/L to 57 g/L in less than 24 hours in lab-scale fedbatch cultivations. With an adjusted carbon/nitrogen ratio of 32, the amount of PHA in the cells could be increased to 40%. In the latest experiments, PHA was synthesized from ethylene glycol in a 1-liter bioreactor, making P. pantotrophus a promising candidate for plastic upcycling into virgin grade and biodegradable bioplastic. The results will be discussed in the context of "plastic waste to plastic value".

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# P21 - A new bacterial cell factory for the production of microbial bioplastics – Elucidating metabolic constraints by 13C-based metabolic flux mapping in Paracoccus pantotrophus

Upasana Pal<sup>1</sup>, Lars Blank<sup>1</sup>, Till Tiso<sup>1</sup>

To support the circular economy's goal of dissociating environmental pressure from economic growth, producing sustainable and biodegradable bioplastics, such as polyhydroxyalkanoates, contributes to the solution.

In this study, we investigate the untapped potential of the microorganism, Paracoccus pantotrophus DSM 2944T, belonging to the family Rhodobacteraceae and the phylum Alphaproteobacteria. P. pantotrophus possesses a broad substrate range, osmotolerance, the ability to carry out heterotrophic nitrification and aerobic denitrification, and good phenotypic robustness. Moreover, it is a fantastic choice in light of the circular bioeconomy, given its ability to use CO2, organic acids, and low-cost sugars as a carbon source to create polyhydroxyalkanoates. To elucidate the metabolic flux in vivo, an in-depth analysis of the flux was performed in P. pantotrophus DSM 2944T using 13C labeled glucose. The cells were grown using two different tracer strategies. The biomass was harvested at a metabolically steady state and subjected to gas chromatography-mass spectrometry (GC-MS) analysis to determine the flux ratios based on labeled amino acids. The GC-MS data were corrected using iMS2Flux and the flux was calculated using MATLAB-based software INCA. The final metabolic data is mapped. The flux map shows that P. pantotrophus uses the pentose phosphate pathway and Entner-Doudoroff over glycolysis.

This makes an exciting find since glycolysis is often believed to be the predominant metabolic pathway for glucose metabolization. By channeling the flux through the alternative pathways, P. pantotrophus manages surplus co-factor regeneration coupled with energy generation. Moreover, the results pave a path in increasing the acetyl-CoA pool and directing it toward polyhydroxyalkanoate production. The results show that the metabolically versatile P. pantotrophus deserves the title of being an upcoming chassis organism for biotechnology and its potential for achieving the goals of the circular bioeconomy.

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#### P22 - Electro-driven production of fine chemicals using biocatalysts in combination with electrolysis

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Nearly 60% of all small-molecule drugs contain N-heterocycles. However, only limited chemical and biological strategies exist to produce N-heterocycles, especially methylated N-heterocycles. Thus, we designed an artificial enzymatic cascade for the production of methylated N- heterocycles composed of the engineered putrescine oxidase PuO(E203G) [1], a designed NADH-dependent imine reductase (IRED) [2], and the NAD+-reducing hydrogenase (SH) from Ralstonia eutropha [3]. The O2-dependent PuOE(203G) oxidized diamines to the corresponding imines, which were subsequently reduced by a NADH-dependent IRED to the saturated N-heterocycles. The O2-tolerant SH catalysed the H2-driven recycling of NADH [3]. Through powering the cascade with electricity in a flow system, substituted pyrrolidines and piperidines were obtained with up to 99% product formation in a one-pot reaction directly from the corresponding diamine substrates [4]. We extended the applicability of the system to perform regioselective isotope labeling, which provided valuable insights into the enzyme mechanism of SH. This platform represents an important advance in the field of biocatalytic synthesis, and it can be expanded for powering various cofactor-dependent oxidoreductases.

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### P23 - The geography of agricultural bioeconomic innovations - A case study of Vietnam's agricultural innovation system

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Bioclusters are promoted as one of the key strategies for bioeconomic transformation. Previous studies have described their transformation trajectories focusing on the biotechnology sector in developed countries. However, few studies assess transformation determinants at the micro level, and even fewer could clearly determine the boundaries of bioclusters. Therefore, this study aims to understand the spatial patterns of bioeconomic innovations as an input to the design of strategies to foster sustainable bioeconomic transformation. Using a maximum entropy (Maxent) approach, we adopted the viewpoint of ecological species distribution modelers, treated bioeconomic innovations in agriculture as a "species" presence records, and matched them with multiple socio-economic variables representing their suitable development conditions. We derived the locations of the existing innovations from patent data and categorized them into agronomy, plant breeding, livestock and aquaculture, and agrochemicals. The outcome of Maxent is the spatial probability of bioeconomic innovations, or index of innovation suitability. Vietnam, an emerging economy with bioeconomic ambitions and a global key producer of many agro-commodities, is used as a case study. We found that agricultural bioeconomic innovations are clustered in and around Vietnam's municipalities with advantages in finance and infrastructure conditions, including: i) accessibility to large cities, ii) electricity coverage, iii) expenditure in education and iv) revenues from agriculture. Indicators of human and knowledge resources were included in the model, but did not show significant importance for the emergence of agricultural bioeconomic innovations. This study is the first to apply predictive spatial modelling to explain biocluster distribution. With high accuracy and prediction capability from the results, Maxent can be applied to analyze spatial capabilities of innovation systems and to inform and monitor policy makers as well as the private sector about potential place-based bioeconomy strategies.

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## P24 - Mycorrhization, wheat growth, and phosphorus transfer efficiency in response to fertilization with algal biofilms produced on the effluent of municipal wastewater

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Algae biomass is a promising fertilizer. Via so-called "luxury uptake", algal cells can accumulate more phosphorus (P) than necessary for their immediate growth, resulting in up to 4% P per algal dry mass. Algal turf scrubbers (ATS) are low-tech devices that recover nutrients delivered by runoff and leaching of nutrient-rich surface waters, particularly from agricultural overuse of mineral fertilizers, in algal biofilms. The resulting biomass can be used to biofertilize and condition soils, especially those poor in nutrients and carbon. Here, algal biomass can help to build soil structure based on microbial activity that is also required to convert organically bound nutrients into plant-available forms. Because of the variation in nutrient content and ratios the effects of such nutrient sources must be analyzed not only with respect to plants but also in relation to microbes and microbial communities. The availability of nutrients from algal biofilms has been proven in different scenarios but the involvement of microorganisms in the efficiency of nutrient transfer and, in particular, the effect of the alternative nutrient source on plant mycorrhization has not yet been investigated. We conducted a study on the efficiency of nutrient transfer from municipal wastewater effluent to algal biofilm, to soil and, subsequently, wheat plants. We investigated whether P transfer to plants can be enhanced by mycorrhizae and if the effect depends on the P content of the algal biofilm in a marginal substrate. For this purpose, mycorrhized and non-mycorrhized wheat plants were grown in a nutrient-free substrate fertilized with different P levels provided either by algal biofilm or by mineral fertilizer. Plant growth parameters, mycorrhization and P-transfer efficiency were analyzed. Based on our experiments under greenhouse conditions, we provide first insights into the effect of algal biofilm grown on municipal wastewater effluent on the mycorrhiza-mediated P transfer efficiency to wheat.

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#### P25 - The project Circular PhytoREVIER - Herbal and medicinal plants in the Rheinische Revier

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Herbs and medicinal plants are an almost unlimited source for pharmaceutical substances in phytopharmaceuticals and they also display a high value added potential in the agronomic, cosmetic and food industry. At the moment, roughly 90% of required raw products are imported to Germany with a large amount coming from wild harvesting. This approach is neither sustainable nor ecologically recommendable (excessive harvestings, hazards for the biodiversity) and it often leads to unacceptable losses in quality of the active compounds. Therefore, the sustainable production of adequate quantities of herbs and medicinal plants with high quality requires the development and continuation of a highly-efficient and economically viable process chain, which starts at the breeding of plants with optimal yield and continues with the development of new and powerful growing and harvesting technologies and ends up with efficient extraction methods for the provision of active compounds from the raw product.

In the Innovation Lab Circular PhytoREVIER, which is a project within the BioRevierPLUS cluster, we look at essential requirements for an ecofriendly and economically profitable use of herbal and medicinal plants with the aim to create a platform for bio-intelligence and circular added value with high-quality crops. We will use the already established cooperation with agricultural holdings and users from the region such that Circular PhytoREVIER will drive innovation and create new jobs in the Rheinische Revier.

Our focus is on several topics:

- Precision breeding and selection of herbal and medicinal plants with optimized for yield and sites
- Large-scale cultivation, outdoors and in closed systems (greenhouse, phyto-chambers)
- Development of innovative approaches which increase the compound content via biological, physical or chemical stress
- New methods for the non-invasive monitoring of performance parameters (for growth and yield)
- Development of ecofriendly and economic protocols for extraction and further processing (drying, refinement)
- Knowledge transfer to the agronomic and processing industry, politics and society

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