7th International BioSC Symposium

Bio-based solutions for a sustainable economy

BioSC
Bioeconomy Science Center

November 6-7, 2023

LVR Landesmuseum Bonn, Colmantstraße 14-16, 53115 Bonn, DE

Table of Content

Agenda

November 6th, 2023	
12:00 h	**Registration** with Light Lunch
13:00 h	**Welcome**
Keynote Lecture Moderator: Ulrich Schurr, Forschungszentrum Jülich/BioSC, DE	
13:15 h	**Genetic resources as the basis for a sustainable crop production** Andreas Stahl, Julius Kühn Institute (JKI), Quedlinburg, DE
Session I: Challenges for sustainable primary production Moderator: Jan Börner, University of Bonn/BioSC, DE	
14:00 h	**Innovations to foster sustainability in global agri-food systems** Jorge Sellare, Center for Development Research, University of Bonn, DE
	Ecological challenges for sustainable primary production Thomas Kastner, Senckenberg Biodiversity and Climate Research Centre, Frankfurt/Main, DE
	New biochars for the improvement of agricultural soils (NewBIAS) Nina Siebers, Forschungszentrum Jülich/BioSC, DE
	Questions & Answers
15:15 h	**Poster Session & Coffee**
Session II: Sufficient, healthy, eco-friendly – Meeting future food demands Moderator: Annaliese Mason, University of Bonn/BioSC, DE	
16:15 h	**Alternative protein perspectives – between promise and reality** Hans-Jürgen Danneel, Institute for Life Science Technologies ILT.NRW, TH Ostwestfalen-Lippe, DE
	Sustainable Plant Oil Alternatives from Corn Residue: Substrate Utilization, Scaling and Research Landscape (NextVegOil) Katharina Miebach, RWTH Aachen University/BioSC, DE
	Innovative technical approaches for regional value chains in the fruit and vegetable sector Kerstin Pasch, DIL German Institute of Food Technologies, DIL Office Brussels, BE
	Questions & Answers
17:30 h	**Poster Session (The exhibition is open all evening)**
18:00 h	**Networking Dinner** and exhibition tours of the LVR museum

November 7th, 2023	
08:30 h	**Welcome Coffee**
09:00 h	**Greetings from Ministries** Sonja Brandt, Ministry of Culture and Science of the State of North Rhine-Westphalia, DE Christian Feiler, Ministry of Economic Affairs, Industry, Climate Action and Energy of the State of North Rhine-Westphalia, DE
Keynote Lecture Moderator: Ulrich Schurr, Forschungszentrum Jülich/BioSC, DE	
09:15 h	**Synthetic Biotechnology as a tool for development of circular bioprocesses** Thomas Brück, Technical University of Munich / German Bioeconomy Council
Session III: Constructing, clothing, packaging – New bio-based materials Moderator: Holger Klose, Forschungszentrum Jülich/BioSC, DE	
10:00 h	**COMP0LIVE - Towards automotive application of new fibres and biocomposites** Inga Wehmeyer, Thomas Baranowski, Ford Research & Advanced Engineering Europe, Aachen, DE
	Technical validation of lignins for textile applications (LignoTex) Sascha Schriever, RWTH Aachen University/BioSC, DE
	Functional & circular paper materials – Towards improved and new applications Markus Biesalski, Technical University of Darmstadt, DE
	Questions & Answers
11:15 h	**Poster Session & Coffee**
Session IV: Teaming up with biology – Next-generation chemistry Moderator: Lars Lauterbach, RWTH Aachen University/BioSC, DE	
12:15 h	**Large-scale production of wood-based chemicals: Experience and perspectives** Gerd Unkelbach, UPM Biochemicals, Leuna, DE
	Surface active molecules for the chemical industry (SurfIn) Till Tiso, RWTH Aachen University/BioSC, DE
	CO_2 and Light: The future resources in chemical manufacturing? Sandy Schmidt, Groningen Research Institute of Pharmacy, NL
	Questions & Answers
13:30 h	**Poster Award** Poster Jury: Anika Wiese-Klinkenberg, Ali Abdelshafy, Stephan Noack
13:45 h	**Closing Remarks** Markus Pauly, Heinrich Heine University Düsseldorf/BioSC, DE
	Farewell Lunch

Presentation Abstracts

Keynote I

Genetic resources as the basis for a sustainable crop production

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Primary plant production is not only the base for food of an increasing world population, but also provides plant-based commodities that are important for numerous utilization paths in the non-food sector. However, crop production is characterized by numerous challenges globally and locally. On the one hand, yields of important crops are substantially affected by drought and heat at critical developmental stages during the crop life cycle. Furthermore, the permanent evolution of pathogens is leading to changes in the spectrum of diseases and pests which are overcoming established resistances. In addition, chemically synthesized pesticides are losing socio-political acceptance, so that alternative and more sustainable concepts of plant production are urgently required. A parallel challenge is the supply of nutrients according to demand. Nitrogen, as the most important nutrient to be fertilized, is essential for achieving high yields and qualities. Unused nitrogen, however, can escape the production system and can either pollute waterways with nitrate or contribute to climate change through gaseous losses to the atmosphere. All those challenges can be addressed by innovations driven improvement of crop production. The use of plant genetic resources in plant breeding is gaining importance as a key strategy for sustainable crop production. This presentation gives an overview of different approaches using innovative digital methods to better describe and understand biodiversity of relevant crops as a particularly valuable treasure. Examples range from non-destructive image-based quantification of pathogens to high-frequency transpiration measurement. The talk underlines the need for collaborations among scientists across disciplines to enhance the phenotype-genotype relationships.

SESSION I: Challenges for sustainable primary production

Innovations to foster sustainability in global agri-food systems

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Global agri-food systems (AFS) are falling short in their ability to provide affordable and nutritious diets while staying within planetary boundaries. AFS are accountable for roughly one-third of all greenhouse gas (GHG) emissions and are among the primary causes of biodiversity loss, primarily driven by land use change, excessive reliance on and overuse of agrochemicals, and the depletion of water resources. Moreover, despite a global reduction in the number of undernourished people, in recent years we have seen that the number of people that do not meet their minimum dietary requirements has bounced back.

A transition towards a bio-based economy, in which we rely on biological resources and technological innovations to drive the transformation of our production structures, could help mitigate some of the negative impacts associated with AFS. Despite the potential of these bio-economic innovations, their adoption is still relatively low. Here, I will first present three innovations that could help address GHG emissions, overuse of agrochemicals, and malnutrition, namely biofuels, bio-inputs, and bio-fortified crops. Then, I will discuss some policies and initiatives that have sought to foster the adoption of these technologies and the challenges associated with increasing their uptake.

Ecological challenges for sustainable primary production

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In the presentation, I will take a global perspective on the production and consumption of biomass products - including uses for food, feed, materials, and energy. Using patterns of ecological net primary production as starting point, I will highlight current patterns of the global biomass metabolism and explore its impact on the carbon cycle, groundwater use and biodiversity patterns. Bringing insights from this line of research together, I will draw conclusions for the potential contribution of a sustainable bioeconomy to ongoing sustainability challenges.

New biochars for the improvement of agricultural soils (NewBIAS)

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Biochar, derived from the pyrolysis of biomass, has emerged as a versatile soil amendment with numerous applications. It can sequester carbon, recover valuable nutrients from waste streams, mitigate environmental risks by retaining excess nutrients in the soil, and improve soil quality, including nutrient status and aggregate structure. Our project is focused on harnessing the versatile potential of biochar. Our goal is to use biochar for nutrient recovery from manure and soil improvement by applying a cascade use approach that incorporates biochar feedstocks from horticulture based on Miscanthus substrates. This innovative approach promotes recycling and value-added use of agricultural residues by integrating various technologies, including pyrolysis. In this way, we aim to increase recoverable energy, close the nutrient recovery loop, and contribute to cleaner agricultural production. The newly designed biochar will be rigorously tested in lab-scale, pot, and greenhouse experiments, evaluating its stability, water-holding capacity, nutrient adsorption and desorption properties, impact on soil aggregate formation, and finally plant-growth. The first results have proven that horticultural Miscanthus substrates are suitable for biochar production, with a high stability after soil application and positive effects on soil structure and plant growth.

SESSION II: Sufficient, healthy, eco-friendly – Meeting future food demands

Alternative protein perspectives – between promise and reality


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The current political and scientific-technological efforts to make alternative proteins marketable as food and feed have, on the one hand, the objective of ensuring protein self-sufficiency in the sense of a circular economy and, on the other hand, limiting livestock utilization in favour of ethical and sustainable protein sources.

Fundamentally, it is not the amount of protein available across the EU that makes achieving both objectives such a challenge, but the way we treat our existing agricultural raw materials. Conventional processes are uncompromisingly focused on traditional primary uses (oil, sugar, alcohol, biogas), and many millions of tons of valuable protein are rendered unusable for further use and processing in the primary processing of crops. The future lies in fundamentally new extraction sequences and value-added cascades.

The "protein revolution" in the food sector is (fortunately) a free consumer decision in our society. It can therefore only succeed if new protein products become by far the better alternative to animal-based products for the vast majority of consumers in terms of prestige, price and taste. An essential prerequisite for good vegan food products is a perfect protein base. The pressure of a waiting market has tempted many manufacturers to carry out product developments with immature protein raw materials. The challenge for Food protein R&D is now to deliver better solutions.

Figures and examples are given in the presentation in order to illustrate and support the contents of this summary.

Sustainable Plant Oil Alternatives from Corn Residue: Substrate Utilization, Scaling and Research Landscape (NextVegOil)

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The global demand for palm oil, a versatile and widely used commodity, has led to rampant deforestation and environmental damage, made worse by its long-distance transportation. This unsustainable practice not only contributes to carbon emissions but also establishes a reliance on foreign resources. To mitigate these issues, we explore alternative sources for palm oil-like compounds. One promising avenue is the microbial production of palm oil substitutes from agricultural waste streams.

We focus on utilizing corn residues as a substrate for microbial fermentation to produce palm oil alternatives. The initial step emphasizes understanding the substrate composition and identifying corn varieties exhibiting more fermentation-suitable properties. Pretreatment strategies for corn residue are investigated to further enhance its fermentability. Subsequently, the desired fatty acid profiles are tailored through both cultivation conditions and genetic engineering techniques. As a significant milestone, we achieved the production of oil-based compounds resembling palm oil using corn residue as a feedstock. The transition from laboratory to industrial scale is initiated through the implementation of stirred-tank reactors and preliminary process modeling. Furthermore, genetic targets for optimizing microbial production are expanded, offering future enhancements. We also delve into the research and startup landscape surrounding sustainable palm oil alternatives, revealing key players and the current level of interconnectedness within the field. In conclusion, we highlight the potential of corn residue for microbial oil production as an alternative to conventional palm oil production.

Partners who participated in the content of the presentation:
Paul Richter, Marcel Mann, Jochen Büchs, Jørgen Magnus – Bioverfahrenstechnik, RWTH Aachen University
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Stefanie Bröring – Entrepreneurship und innovative Geschäftsmodelle, RUB
Janis Goeke, Heiko Hayen – Anorganische und Analytische Chemie, WWU Münster

Innovative technical approaches for regional value chains in the fruit and vegetable sector

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Fostering a more sustainable and resilient food system is the stated policy goal in Europe. The way we process and supply food is a thematic focus in current and future European research programmes. This presentation will highlight innovative processing technologies that are of interest for regional value chains, especially in the fruit and vegetable sector and beyond. Activities, results and conclusions from two EU projects will be presented (FOX, ZeroW) as well as ideas that Startups, supported by the DIL InnovationHub, have taken up on this.
Objective of the FOX project is development and evaluation of mobile or modular processing units for small scale food processors. Different technologies for mild extraction, drying, preservation and packaging are in focus. The three prototypes designed and build are validated for appropriate business models, food products are assessed regarding their sustainability including health aspects. As part of the ZeroW project, the business model 'mobile food valorisation as a service' is further explored for one of the above mentioned prototypes, the mild juice processing-unit. This happens in an systemic innovation living lab in Flanders.
The DIL InnovationHub was founded in 2019 and supports startups and founders by technical and scientific questions around their business ideas for attractive and sustainable products in the sector. Creating value from regionality and up-cycling of side streams is core of many new business.

https://www.fox-foodprocessinginabox.eu
https://www.zerow-project.eu
https://www.dil-innovationhub.de

Keynote II

Innovative technical approaches for regional value chains in the fruit and vegetable sector

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Climate change and the associated volatile markets of fossil resources drive the innovation in industry and society towards climate friendly, sustainable processes for energy carriers, food and chemical commodities.

Biotechnological processes enable fixation, valorisation and finite elimination of atmospheric/industrial CO2. Therefore, they can significantly contribute to mitigate adverse global climate changes scenarios. Synthetic biotechnology is a relatively new scientific discipline utilizing the synergies of computational, synthetic- and systems biology with bioprocess engineering. The resulting technology platforms allow for design of mass- and energy efficient process routes that can generate sustainable chemical entities. The talk will report on advances in the field focusing on development of oleaginous photoautotrophic and heterotrophic microbial platforms that provide for conversion of CO2 to liquid biofuels, polymers and CO2 based carbon fibers. A special focus will be on the use of microalgae based triglycerides carbon fibers and resin formulation that together provide CO2 negative carbon fiber composites. Further processes will report on a circular bioprocess that is based on the use of agricultural residues to generate a sustainable, biodegradable insect repellent, which is non-toxic to useful insects or the aquatic ecosystem but can be applied to agricultural crops for management of crop pests.

SESSION III: Constructing, clothing, packaging – New bio-based materials

COMP0LIVE - Towards automotive application of new fibres and biocomposites

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Comp0live is an EU funded project about the revalorization of pruning waste from olive oil industry into new bio-based composites.

The EU is the leading producer, consumer, and exporter of olive oil. It is estimated that more than 7 million tons of pruning residue are generated every year in Europe but only a marginal amount is used for energy generation. Most of the pruning is shred or burned on the fields.

For the Comp0live project the pruning of the olive trees is collected, milled, sieved, and chemically treated. Afterwards the fibers are compounded with recycled Polypropylene. Different compounds have been developed for furniture and automotive applications.

This project demonstrates that it is possible to substitute 40 % of the Polypropylene with olive tree fibers. Even with this high amount of fibers a good filling behavior of the compound has been achieved.

The new developed Comp0live materials were tested against various Ford specifications and show very promising mechanical properties. Physical test haven been done with Ford prototype and serial tools. They indicate a good processability for the Comp0live material even at the desired low melt and mold temperatures. In addition, validated Moldflow simulations showed appropriate filling and warpage prediction.

Therefore, the usage of the olive pruning waste for Comp0live compounds offers a great opportunity to reduce the amount of fuel based raw materials and to improve the sustainability of future products.

Technical validation of lignins for textile applications (LignoTex)

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Lignin is a promising and abundant coupling product in biorefineries (e.g. paper and textile industry), which in most biorefinery concepts is burned for its energy value. To make biorefineries economically more viable and to reduce the CO2 footprint, a valorization strategy exploiting the high potential of lignin is crucial.

The project LignoTex, covers the whole process chain from lignin extraction to spinning of lignin-based fibers as well as the application in textiles and composites. A variety of different plants such as wood, grasses and perennial plants will be extracted using the OrganoCat technology adapted and optimized to the different plant materials. The extracted lignins will then be isolated and fractionated to produce suitable precursors for the subsequent processing steps. The precursors will then be tested in state of the art and newly developed wet spinning, melt spinning and composite manufacturing processes to determine the influence of molecular properties, e.g. molecular weight, molecular design (linear/branched) and β-O-4 linkages, on their performance in the 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.

Functional & circular paper materials – Towards improved and new applications

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Paper has been known for thousands of years for its unique profile of properties. Despite its classical applications, it has been in focus for several years in very challenging areas, e.g. in microfluidic sensors, light-weight construction materials (e.g. as a honeycomb core in door leaves or shelves), as well as most recently in functional paper-based soft packaging. In this talk I will introduce our recent efforts in understanding and tailoring paper properties by controlled functionalization of the fiber and paper-sheet interfaces with biobased macromolecules. Examples progress from the use of functional biobased wax-polysaccharride coatings for tailoring barrier properties to the cross-linking of polymers with paper fibers to introduce sustainable wet-strength properties in desired soft packaging applications. I will also show how lifetime of packaging materials may be extended by using self-regenerating biogenic hydrophobic top-coatings that can be applied to a number of different base-paper substrates and where water-repellent superhydrophobic coatings can be regenerated by simple heat-cool cycles.

Session IV: Teaming up with biology – Next-generation chemistry

Large-scale production of wood-based chemicals: Experience and perspectives


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Lignocellulosic biomass offer great potential as a sustainable source to replace fossil-based chemicals and improve environmental compatibility in various applications. UPM is building the world's first industrial scale biorefinery in Leuna, Germany based on sustainably sourced hardwood. Due to the structural design a very rigid composite consisting mainly of cellulose, hemicellulose and lignin is built up during plants growth. To allow the maximum valorisation of all wood ingredients, the lignified plant material must be pretreated. The polysaccharides can afterwards be enzymatically monomerized, separated from the lignin and chemically converted into glycols, namely monoethylenglycole (MEG) and monopropylenglycole (MPG). End-use segments for renewable glycols include textiles, PET bottles, packaging, refrigerants, composites, pharmaceuticals, cosmetics, and detergents. The lignin fraction is further converted into a renewable functional filler (RFF), which offer a sustainable alternative to carbon black and precipitated silica in a wide range of rubber and plastics applications.

After a short introduction to wood chemistry, the presentation will provide insights into the development of individual process steps and the challenges of a high degree of integration. Finally, an outlook on further optimisation possibilities will be presented.

Surface active molecules for the chemical industry (SurfIn)


©iAMB

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In the backdrop of growing environmental concerns and the need for sustainable alternatives, our project over the last six months has achieved significant progress in the investigation of surface-active biomolecules for potential applications in the chemical industry. The focus has been on the development of a practical and efficient industrial production process for these biomolecules, thereby contributing to a more sustainable and environmentally friendly chemical sector.
Enhancements have been made in serrawettin W1 production, facilitated by the integration of modular expression cassettes for genome integration. Although initial yields were lower, ongoing optimization of genome-integrated strains is being pursued. Simultaneously, *in situ* extraction and purification methods for serrawettin W1 have been refined, showing promise against pathogenic microbes. Liamocin synthesis witnessed a 25% increase in titer through gene deletions and the optimization of acetate utilization. The successful production of liamocin at a larger scale, including a 10-liter fermenter, was achieved. Current research encompasses online monitoring for growth and liamocin production in *Aureobasidium pullulans*, and sustainable purification strategies are under investigation. Additionally, the analysis of patenting strategies, technological trends, and application testing is being pursued.
In summary, significant strides have been made in biosurfactant production and applications within the project, contributing to more sustainable solutions in the chemical industry. Efforts have been disseminated through presentations and publications, raising awareness within the scientific community and among industrial partners.

Philipp Kohl[1], Sonja Kubicki[2], Max Dicke[3], Rafael Reifsteck[4], Ana Vanacker[5], Lars M. Blank[1], Karl-Erich Jaeger[2], Jørgen Magnus[3], Andreas Jupke[4], Stefanie Bröring[5], Michael Wustmans[5], Marcel Mann[3], Stephan Thies[2], Till Tiso[1]
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CO2 and Light: The future resources in chemical manufacturing?

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The production of chemicals and materials in a sustainable bio-economy is characterized by new process chains in which biocatalysis with microorganisms and enzymes plays an important role. The development of novel bio-based solutions through the development of novel catalysts and catalyst systems goes hand in hand with the integration of alternative resources or even waste streams that would otherwise remain unused. Such alternative resources can be light, which is an ideal "reagent" in chemical synthesis because it is safe to use and readily available, or carbon dioxide (CO2), which is a waste stream, e.g. from industrial waste gases or municipal solid waste gasification, and thus abundantly available. Light as a resource can be integrated into (bio)chemical manufacturing by combining photocatalysis with biocatalysis to expand the chemical repertoire beyond what is currently possible with enzymes alone.[1,2] For example, we have recently shown that combining photocatalytic hydroacylation with enzymatic asymmetric reduction enables the direct conversion of simple aldehydes and unsaturated carboxylic acids, precursors that can potentially be derived from bio-based resources, to chiral �-lactones.[2]

In contrast, the ability of microbes to grow naturally on CO2 as a carbon source can be industrially exploited for the production of complex products.[3] The development of such microbial production strains as a platform technology offers several advantages, including their robustness to contaminants in the gas stream,[4] allowing the direct use of industrial waste gases, as well as broad product flexibility[5–7] to access, for example, bulk chemicals such as isopropanol, fine chemicals such as amino acids, and even pharmaceuticals such as N-heterocycles. In the ConCO2rde project, we are currently combining efforts in chassis strain development and process engineering to increase the competitiveness of these autotrophic biotechnological processes. To achieve this, the development of genetic tools for strain manipulation, the integration of H2-driven biotransformations, new reactor concepts for H2/O2/CO2-based fermentation to achieve high cell densities, and a better understanding of cell physiology during gas fermentation are being explored to increase competitiveness and pave the way for efficient future CO2-based biotechnological processes.

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Poster Abstracts

Nr	Presenting Author	Abstract Title
P1	Mario Andres Murcia Lopez	Bioeconomy as a driver of sustainability transitions in a megadiverse country
P2	Till Kuhn	Using bio-economic farm modelling to assess novel technologies and policies for a sustainable bioeconomy
P3	Johanna Stotz	ScreenP: Metabolic burden coupled phytase screening for sustainable phosphate recycling
P4	Nina Siebers	New Biochars for the Improvement of Agricultural Soils - NewBIAS
P5	Johanna Rütt	Using miscanthus and biochar as sustainable substrates in horticulture: An economic and environmental assessment of their primary and cascading value chains
P6	Otávio dos Anjos Leal	NewBIAS: Miscanthus-biochar reduces N2O emissions in a recently recultivated alkaline but increases in an acidic agricultural silty loam soil in Germany
P7	Ashwaq Najjar	Amendment of poor soil substrate by biochar saturated with biofertilizers (algae, manure) for sustainable production of relevant Palestinian and German crop plants Solanum lycopersicum L. and Hordeum vulgare L.
P8	Mona Sader	Exploring the role of plant-exuded polysaccharides and biochar in the aggregation of different soil types
P9	Marco Loehrer	P^3roLucas: Exploring the benefits of biostimulants in lupin cultivation and introducing a novel cascade use approach
P10	Philipp Sowa	Valuable compounds from lupin plant resource side stream
P11	Mansi Singh	Bioinformatics analysis of selected Lupinus mutabilis accession with the aim of marker development for breeding
P12	Patrick Schwinges	From ash to cash - Utilizing waste streams from locally produced biomaterials for controlling relevant diseases of sugar beet as key crop for the national bioeconomy
P13	Tino Polen	Microbial production and application of dsRNAs for testing control of plant-parasitic nematodes as a new non-chemical crop protection strategy in agri- and horticulture
P14	Marília Bueno da Silva	ToxPot – Potato side-streams and their potential in a circular bioeconomy
P15	Marília Bueno da Silva	ToxPot - Analysing the effect of potato glycoalkaloids (GAs) on different plant pathogens and beneficial organisms
P16	Mohammad Bagshomali	Flycycle: Waste reduction with fly larvae - sustainable protein, fertilizer and biofuel production
P17	Samira Reuscher	Microalgae for Biofuels and Simultaneous Treatment of Paper Mill Wastewater
P18	Andreas Schieder	Chicken Feather Keratin – a Valuable Source for Polythiol Building Blocks
P19	Stephan Schott-Verdugo	MetaProcess: Continuous, stable processes for the sustainable enzymatic production of chiral amino alcohols integrating downstream processing

Nr	Presenting Author	Abstract Title
P20	Diana Wall	MK-ScaLoop – Towards an industrial-scale process for a biotechnological production of methyl ketones in a multiphase loop reactor
P21	Pushpendra	Prospective Life Cycle Assessment and Scale-Up of Methyl Ketones Production from Wheat Straw
P22	Sascha Schriever	Integrated Biorefinery for Sustainable Production and Processing of Lignin for Textile Application
P23	Tim Langletz	Synthesis and Characterization of Lignin-Polylactide Copolymers and their Application in Melt-Spinning for Fiber Production
P24	Philipp Grande	LignoTex - Integrated Biorefinery for Sustainable Production and Processing of Lignin for Textile Application
P25	Philipp Grande	PREDIG: Modelling software to predict the enzymatic digestion of biomass
P26	Vivek Kumar Srivastav	Precision-enabled knowledge extraction: InterText - a scalable and interoperable text-mining framework
P27	Till Tiso	Surface Active Biomolecules for the Chemical Industry
P28	Sonja Kubicki	Engineered Pseudomonas putida KT2440 for the production of tailored biosurfactants showing bioactivities of agricultural interest
P29	Katharina Miebach	Production of a sustainable and tailor-made microbial palm oil and milk fat substitute from agricultural residues (NextVegOil)
P30	Stefan Robertz	Online monitoring of corn stover processing by the fungus Ustilago maydis
P31	Benedikt Wynands	BioPlastiCycle – Transitioning bioplastics to the circular economy
P32	Sara Adeleh	Synthesis of C14-labeled bioplastics for environmental fate assessments
P33	Emine Nil Güreli	Life Cycle Assessment of End-of-Life Scenarios for Polylactic Acid
P34	Tabea Becker	Utilization of Zinc Guanidine Complexe in the Circular Economy of biobased Polyesters
P35	Yannic Ackermann	SSWEEP - Solvent swelling to enhance enzymatic and microbial plastics upcycling
P36	Donato Calabrese	Advancing Green Chemistry: Enzymatic Halogenation of Aliphatic sp3 Carbons
P37	Lars Lauterbach	Towards a sustainable photohydrogen production through oxygen-tolerant hydrogenase chimeras
P38	Guiyeoul Lim	Electr-biocatalysis: production of fine chemicals using renewable energy
P39	Benoit David	CasCAR: Sustainable production of valuable aldehydes using a locally immobilized synthetic enzyme cascade

P1 – Bioeconomy as a driver of sustainability transitions in a megadiverse country

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The concept of bioeconomy has taken center stage in Colombia's public agenda, primarily due to the country's status as a megadiverse nation, which can serve as a comparative advantage in achieving sustainable development. This research aims to analyze the transformative potential of bioeconomy within productive-industrial systems, using a case study approach. It focuses on various value chains related to Brosimum alicastrum (Guaimaro), a native species of the Tropical Dry Forest (TDF). Our findings reveal that a bioeconomy based on biodiversity can offer a means to diversify the region's economic landscape, providing economic opportunities for local communities while fostering positive and desired changes in socio-ecological systems. This has the potential to benefit both the environment and the people living in it.

The utilization of Guáimaro by communities in the TDF has led to shifts in practices, values, and beliefs concerning the forest, creating a conducive environment for its conservation and ecological restoration, along with contributing to food security through the species' food use. Furthermore, our results suggest that one advantage of biodiversity-based businesses is the ability to generate products with varying degrees of value addition. This approach can lead to the establishment of simpler value chains, potentially requiring less infrastructure and initial capital investment, making it a suitable starting point for regions with limited technological resources.

Consequently, this allows for progress in research, technology, innovation, organization, logistics, regulation, and other factors associated with the use of the species. This progress can attract investment and interest from key stakeholders, paving the way for more advanced industries with higher socioeconomic returns, as well as enhanced conservation and well-being within the region.

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P2 – Using bio-economic farm modelling to assess novel technologies and policies for a sustainable bioeconomy

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The transformation towards a sustainable bioeconomy requires technological innovations as well as policy intervention on the production side. The application of models is helpful to assess changes ex-ante before their actual implementation and to take more informed decisions. Bio-economic farm modelling captures bio-physical as well as economic dimensions and focuses on the single farm as the central decision unit of agricultural production. I present two applications of the FarmDyn model (Britz et al. 2021), being part of the terminated BioSC FocusLab Transform2Bio and the Horizon Europe project Lamasus. The first application is on the greenRelease technology that increases the inherence of pesticides to the plant surface and potentially lowers the amount of active ingredients needed (Kuhn et al. 2022). Insights from modelling allow to judge the economic potential of the technology for different crops and products. Results for a typical farm of the German region Rheinische Revier show that the economic potential is largest for systemic fungicides in all assessed crops as well as for herbicides for potato cultivation. Relative to total costs in arable farming, the cost change caused by the greenRelease technology is however small, making the environmental benefits crucial for promoting its use. The second application deals with the German implementation of the new EU Common Agricultural Policy which comprises amongst others a decrease in direct payments, stricter conditional environmental requirements and new optional eco-schemes. First modelling results show that typical German arable farms face a profit loss of around 6% to 9% due to the cutting of direct payments. Under the current environmental obligations and output prices, an exit of the direct payment scheme is however only profitable if direct payments are drastically cut in the future. The applications illustrate the strength of the bio-economic farm modelling for interdisciplinary research on technologies and policies guidance towards a sustainable bioeconomy.

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P3 – ScreenP: Metabolic burden coupled phytase screening for sustainable phosphate recycling

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Phosphorus, which is obtained exclusively from ores, is an essential nutrient in animal and plant breeding.

At RWTH Aachen University, the Institute of Biotechnology and the Institute of Applied Microbiology published a novel production process to mobilize phosphate from plant biomass side streams and produce food-grade polyP. The two-step process of biotechnological bio-polyphosphate production consists of enzymatic phosphate extraction from deoiled seeds and bran, coupled to biotransformation with microorganisms. Enzyme variants hydrolyze phytate, the plant phosphate storage to phosphate, which is converted in yeast to the high-quality food additive polyphosphate (polyP).

In Screen P, the Institute of Biotechnology and the Institute of Biochemical Engineering at RWTH Aachen University successfully developed a screening assay by coupling respiratory activity to enzyme activity to strengthen phytase engineering. An increased phytase expression redirects carbon flux and energy consumption from biomass formation to protein production. The resulting growth-coupled production in Pichia pastoris can be seen as a metabolic burden in the monitored oxygen transfer rate (OTR). An inverse correlation was found between the growth rates of the clones (derived from the OTR) and produced protein amount. Using this correlation, clones with low production rates can be distinguished from clones with higher production rates. Model clones were cultivated in a µTOM device with only phytic acid as phosphorus source. A correlation was found between growth rates of the clones and offline-measured phytase activity. In conclusion, the screening system developed in ScreenP increases the screening abilities, the rate of identifying improved variants and enables tailor made phytases for industrial applications.

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P4 - New Biochars for the Improvement of Agricultural Soils - NewBIAS

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Biochar, derived from the pyrolysis of biomass, has emerged as a versatile soil amendment with numerous applications. It can sequester carbon, recover valuable nutrients from waste streams, mitigate environmental risks by retaining excess nutrients in the soil, and improve soil quality, including nutrient status and aggregate structure. Our project is focused on harnessing the versatile potential of biochar. Our goal is to use biochar for nutrient recovery from manure and soil improvement by applying a cascade use approach that incorporates biochar feedstocks from horticulture based on Miscanthus substrates. This innovative approach promotes recycling and value-added use of agricultural residues by integrating various technologies, including pyrolysis. In this way, we aim to increase recoverable energy, close the nutrient recovery loop, and contribute to cleaner agricultural production. The newly designed biochar will be rigorously tested in lab-scale, pot, and greenhouse experiments, evaluating its stability, water-holding capacity, nutrient adsorption and desorption properties, impact on soil aggregate formation, and finally plant-growth. The first results have proven that horticultural Miscanthus substrates are suitable for biochar production, with a high stability after soil application and positive effects on soil structure and plant growth.

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P5 – Using miscanthus and biochar as sustainable substrates in horticulture: An economic and environmental assessment of their primary and cascading value chains

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The horticultural industry represents a substantial economic sector in Europe with an annual turnover of approximately 1.3 billion euros. Annually, 34.6 million cubic meters of horticultural substrate are used in Europe. Three-quarters of these substrates contain peat, which is an established, cost-effective substrate. However, peat extraction releases fossil carbon, which contributes to climate change significantly. The EU aims to reach climate neutrality in the agriculture and forestry sector by 2035. Thus, the German government prohibited peat utilization within the hobbyist market by 2026. They also decided to significantly reduce peat application within the commercial sector by 2030. Therefore, there is an increasing demand for cost-efficient substrate alternatives with minimal greenhouse gas emissions. Herein, Miscanthus can serve as a climate-friendly substrate alternative. Biochar, produced through pyrolysis, offers potential as a carbon-sequestrating component within horticultural substrates. However, comparing the costs and climate impacts of different substrates is challenging as they have profoundly different production processes, transport distances, and waste treatments.

Hence, this contribution aims at identifying optimal horticultural substrate value chains. The study conducts a combined life cycle assessment and costing of conventional and alternative substrates such as peat, coconut coir, stone wool, miscanthus and blends of miscanthus plus miscanthus biochar. The analysis encompasses the evaluation of techno-economic performance and greenhouse gas emissions, utilizing tomato cultivation in North Rhine-Westphalia as an illustrative case study. In addition to primary substrate use, we examine multi-year cascading uses to analyze the potential of circular economy. The preliminary results reveal that applying miscanthus or miscanthus-biochar mixtures instead of peat and stone wool reduces greenhouse gas emissions significantly. Miscanthus and miscanthus biochar mixtures also offer substantial cost savings compared to stone wool. Therefore, alternative substrates and cascading use can contribute to achieving climate neutrality in the agriculture and forestry sector.

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P6 – NewBIAS: Miscanthus-biochar reduces N2O emissions in a recently recultivated alkaline but increases in an acidic agricultural silty loam soil in Germany

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Following the cascade use of Miscanthus as alternative horticultural substrate, we investigated effects of Miscanthus biochar (MiB) on soil available phosphorus (Pav) and nitrogen (Nav) and their relations to soil CO_2 and N_2O emissions. Two agricultural Luvisols (Campus Klein Altendorf-CKA, and post-mining recultivation Inden-REC) with silty loam texture, but different pH (6.0 and 7.7), organic (1.0 and 0.3%) and inorganic carbon (0.0 and 1.1%) content, were selected. The five experimental treatments of each soil were: control (soil); control+F (soil with Pav and Nav topped up to 60 and 100 mg kg−1 with triple superphosphate and ammonium sulphate, respectively); control+F+5, 10 or 15 Mg ha−1 of MiB. Soils (320 g) were incubated for 93 days at 15°C and 60% water holding capacity (WHC). MiB increased the maximum WHC of CKA from 40.1% up to 56.4% and of REC from 43.9% up to 53.3%, possibly reducing part of aerobic microbial activity and explaining the similarly reduced CO_2 emissions observed in all MiB-amended soils. Overall, MiB did not affect Pav. On average, Pav was 137% higher in CKA (57.5–64.4 mg kg−1) compared to REC (21.2–29.8 mg kg−1) in the fertilized treatments, possibly due to P fixation by calcium in REC. MiB increased CKA pH from 6.0 to 6.4, whereas REC pH remained unaffected (7.7). Together with the higher organic carbon (energy source for microorganisms) and initial nitrate content in CKA (71 mg kg−1) compared to REC (22 mg kg−1), this may explain why MiB increased cumulative N_2O emissions by up to 329% (control+F+5 Mg ha-1 MiB) compared to control+F (1.45 kg N_2O-N ha–1), while MiB reduced REC cumulative N_2O emissions by up to 84% (control+F+10 Mg ha-1 MiB) compared to control+F (6.46 kg N_2O-N ha–1). Soil Nav is being determined. Overall, MiB amendment seems particularly advantageous for REC.

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P7 – Amendment of poor soil substrate by biochar saturated with biofertilizers (algae, manure) for sustainable production of relevant Palestinian and German crop plants Solanum lycopersicum L. and Hordeum vulgare L.

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Soil degradation is a global problem that affects many regions and communities, resulting in poor and stress-prone marginal soils. The potentially positive effects of carbon and nutrient content increase through the addition of nutrient-saturated biochar were investigated on poor and saline substrate in experiments with tomato and barley. Biochar treatments were applied to nutrient- and carbon-poor sandy substrates in two greenhouse pot experiments. Biochar mixed with biofertilizers (algae & pig manure) at three total carbon content levels (0.1, 0.3, 0.5 % C-concentration) was applied to test its effects on tomato growth and soil properties during early growth stages. As a follow-up, carbon addition was increased to 2% via a biochar-sheep manure mixture to test its effect on growth parameters and quality of two barley cultivars (Palestinian & German). Results showed that increasing mineral-fertilizer saturated biochar concentration up to 0.5 % total C in saline soil (4 EC) increased tomato total fruit numbers but delayed the ripening process (24% red fruits in untreated pots, 15% in treated pots). In saline environment, biochar-pig manure mixture led to the highest tomato shoot dry weight (41g) compared to 0% biochar treatments (31g). Increased biochar amount (up to 2%) led to increased shoot fresh weight of up to 1.7g in the German barley cultivar after a 1-month growth period, compared to non-treated (0% biochar) pots at 0.9g, respectively. Increasing biochar up to 2% increased the soil water-holding capacity by up to 17% compared to 0% biochar control. In conclusion, nutrient-saturated biochar constitutes a sustainable solution to condition substrates to improve the quality and fertility of the soil by helping to close the nutrient cycle and increasing the water hold capacity, especially in carbon-poor soil substrate. Further up-scaled greenhouse and field experiments are needed to evaluate longer-term effects on yield and soil parameters.

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P8 – Exploring the role of plant-exuded polysaccharides and biochar in the aggregation of different soil types

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Maintaining a healthy soil structure is the fundamental basis of sustainable agriculture. One key aspect is the formation and stability of soil aggregates through mucilage, the high molecular weight part of root exudates. Up to 80% of root mucilage consist of polysaccharides which are well known as components of plant cell walls, but understudied as part of the root exudate profile. In the presented work, which is a part of the BioSC-funded project "New Biochars for the Improvement of Agricultural Soils (NewBIAS)," we aim to analyze the spectrum of polysaccharides released by plant roots and determine their role in soil aggregate formation as well as identify the effect of biochar on aggregate formation.
We tested the effects of 12 polysaccharides in in vitro soil adhesion assays using four different arable soils, with and without the addition of biochar. We further studied the effects of a 93-day incubated soil-biochar blend on soil adhesion, compared to freshly mixed soil-biochar samples.
Our findings underscore a consistent impact of soil as well as polysaccharide type on soil adhesion. Polysaccharides exhibiting effective binding properties demonstrated this potential across all tested soils. Interestingly, while some polysaccharides displayed no soil specificity (binding all soils uniformly), others exhibited a high degree of specificity as they were less effective in sandy soils compared to those rich in silt and clay content. The 93-day incubation period led to a pH drop in the sandy soils, a factor that is also reflected in soil adhesion patterns.
These nuanced interactions between polysaccharides, soil types, and incubation provide valuable insights into the complex dynamics governing soil adhesion processes. Enhancing marginal soils by identifying polysaccharides that effectively promote soil aggregate formation may provide a nature-based solution for sustainable agriculture.

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P9 – P³roLucas: Exploring the benefits of biostimulants in lupin cultivation and introducing a novel cascade use approach

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The BioSC BOOST FUND 2.0 Project P³roLucas (Optimization of plant performance and products for lupin cascade use) aims at promoting and improving lupin cultivation. Lupin is a valuable alternative protein source suitable for food and feed and presents an attractive alternative to (GMO-) soybean from international markets. Due to breeding efforts, nowadays more varieties with improved agronomic traits, including tolerance against anthracnose disease are available, but yield potential is varying between species. Species of the genus Lupinus are known to have a pronounced alkaloid content, which is positively correlated with resistance against certain diseases, but in turn must be monitored because of its negative impact on human and animal health. In the past, selection and breeding efforts led to the so called "sweet" varieties with a low level of alkaloids. Alternatively, there is a process of debittering established in which alkaloids are technically removed from bitter varieties. Up to now alkaloids, obtained by debittering, are treated as waste. However, they present a valuable source for chiral compounds such as lupanin and sparteine, which are rare at world markets. In an exemplary cascade use approach, we want to make use of this hitherto underestimated resource. Additionally, we will explore the use of biostimulants and plant strengtheners for modulating alkaloid production, improving yield and increasing pathogen- and abiotic stress-tolerance in narrow-leafed lupin. An overview of results from lab-scale experiments is presented here and will later be complemented by initial field trials. Plant responses will be investigated at the metabolic and transcriptomic level, using consolidated and, in frame of this project, newly generated genomic resources (e.g., Andean Lupin genome) as a basis. Transcriptomic analyses will add to our knowledge on alkaloid synthesis pathways and the mechanism(s) for alkaloid reduction in sweet varieties. In a complementary approach we will investigate producer's acceptance of the entire P³roLucas concept to promote lupin production.

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P10 – Valuable compounds from lupin plant resource side stream

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Quinolizidine alkaloids (QAs) are nitrogenous heterocycles derived from L-lysine widely present in the lupin plant family. These secondary metabolites are of particular interest due to their chemical and pharmacological applications. They possess various effects such as anti-inflammatory, antitumor anticonvulsant, antiviral, analgesic, and antimicrobial properties.[1] Generally they are considered to be toxic to humans and thus far hinder the application of resistant bitter lupins as crop plants. The composition of QA alters not only amongst species but also upon stress and aging processes[2].
Synthesizing these compounds for e.g. ligands in enantioselective syntheses is rather difficult and becomes impossible in bulk, although industrial applicators are still highly interested and supplement products are currently under investigation[3].
Using this knowledge, this project aims to exploit the natural compound side stream of lupins in a bio-economic mindset. First, a library of identified alkaloids was set up. Upon identification, the alkaloid profiles of a selected group of lupin species are examined. Afterwards a comparison of these alkaloid profiles shows the effect of stresses, additives, and environments on the production of alkaloids in different parts of the plant. The end result will be a more complete picture of the plant's response to the environment and the selection of an optimal candidate for isolation of individual alkaloids and application to synthesis.

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P11 – Bioinformatics analysis of selected Lupinus mutabilis accession with the aim of marker development for breeding

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The P³roLucas project (Optimization of plant performance and products for lupin cascade), as a part of BioSC, aims at promoting and improving lupin cultivation. Lupins are a valuable source for high grade and healthy protein for human consumption and therefore are a sustainable alternative to soybeans. Lupins' unique ability to fix atmospheric nitrogen into ammonia through rhizobium-root nodule symbiosis makes them resilient to infertile soils and pivotal in reclaiming barren and poor-quality lands. Notably, different lupin species, including Lupinus albus (white lupin), Lupinus angustifolius (narrow leafed lupin), Lupinus luteus (yellow lupin), and Lupinus mutabilis are utilized in agriculture. As an Andean-origin species Lupinus mutabilis is considered an interesting crop for agricultural use in Europe.

While the genomic resources for Lupinus angustifolius and Lupinus albus have reached pan-genome level assembly Lupinus mutabilis, yet no genome assembly is available. We are constructing the first genome assembly of Lupinus mutabilis, utilizing Oxford Nanopore sequencing combined with chromosome conformation capture sequencing to order and orient the sequence contigs.

The initial draft assembly, consisting of exclusively ONT reads, shows good quality and we have applied the Helixer gene prediction tool to identify genes based on the DNA sequence. Our preliminary assembly of Lupinus mutabilis demonstrates a completeness of 98.8%, which is comparable to the pan-genome level assemblies from the other lupins. We performed a functional classification of proteins using Mercator4 and included part of the alkaloid pathway into the MapMan software.

These new resources are intended to further advance lupin breeding and cultivation and ultimately contribute to sustainable regional agriculture of the future.

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P12 – From ash to cash - Utilizing waste streams from locally produced biomaterials for controlling relevant diseases of sugar beet as key crop for the national bioeconomy

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Securing profitable and sustainable production of plant-based biomaterials is key for a functional bioeconomy. To secure crop yields and quality and support establishment of a fully circular bioeconomy, we aim to utilize and valorize existing waste streams as sources for natural plant protectants. Crops, algae, bacteria, and fungi provide a wealth of potentially antimicrobial, repellent or plant health-stimulating molecules such as secondary metabolites, proteins, elicitors, or priming-active compounds. Using established screening tools for assessment of molecular plant performance indicators and assays to robustly evaluate crop performance at stress conditions, we aim to identify such activities encrypted in waste streams of the local bioeconomy to protect sugar beet from economically relevant pathogens and pests that threaten profitable production of this traditional crop which is of exceptional relevance for the local and national bioeconomy. In a tandem approach with an experienced biopesticide regulatory consultant, optimal sources and methods for extraction and purification of crop protectants will be identified and not only evaluated towards their protective capacity but also for their potential to be registered for national and international application as well as for their commercial potential. By this we intend to enforce a streamlined, efficient, and application-centered research strategy for the provision of locally produced bio-actives for effective crop protection.

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P13 – Microbial production and application of dsRNAs for testing control of plant-parasitic nematodes as a new non-chemical crop protection strategy in agri- and horticulture

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Plant-parasitic nematodes (PPNs) such as Meloidogyne penetrating plant roots and leading to root galls, deformed growth and reduced plant health, are a major problem in crop production. Chemical crop protection methods to control PPNs in agri- and horticulture are problematic or no longer allowed due to high environmental toxicity. Hence, there is a need to develop new methods to control PPNs for sustainable and environmentally friendly production of plants for food and feed. The use of double-stranded RNAs (dsRNAs) for non-chemical crop protection strategies represents a promising opportunity to control plant parasites via RNA interference (RNAi). In our study, the objectives are the microbial production of dsRNAs and exploring their applications for the control of PPNs via RNAi to protect crops and improve plant health. Our PPN literature search revealed RNAi experiments almost exclusively for M. incognita. We found more than 20 proven RNAi targets of different functional subgroups. After M. incognita was cultivated and eggs were isolated from roots of infected tomato plants, with total RNA obtained from hatched M. incognita J2s we obtained almost all described DNA fragments based on oligo dT primer used to generate cDNA. Sequencing of generated DNA fragments revealed some RNAi target sequence variations in the M. incognita laboratory strain. Selected DNA fragments were used for construction of microbial dsRNA expression plasmids. The deletion of the RNase III gene rnc in the T7 RNA polymerase strain MB001(DE3) of the model microorganism and industrial workhorse Corynebacterium glutamicum was successful and allows microbial dsRNA production providing sufficient material for in vitro and in vivo RNAi studies with plant/nematode pathosystems.

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P14 – ToxPot – Potato side-streams and their potential in a circular bioeconomy

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The potato (Solanum tuberosum L.) is one of the major agricultural crops cultivated worldwide. Besides its nutritionally valuable tubers, the potato plant is also a source of interesting compounds like the well-known steroidal glycoalkaloids (SGAs)
α-solanine and α-chaconine. These SGAs are highly concentrated in aboveground plant parts (flowers, leaves, berries) which remain in the field as unused biomass after tuber harvest. Due to their anti-inflammatory, anti-fungal, and anti-carcinogenic activities, SGAs are highly interesting for industrial purposes. As a way to improve the bioeconomy in potato fields, this BioSC project aims to evaluate SGAs from potato residuals and their potential as increment for crop protection or pharmaceutical sectors. The analysis of the aboveground plant material revealed strong differences in the content of the main SGAs between tissues with flowers showing significantly higher concentrations than berries. Still, the extractable amount of SGAs was 22 to 27 times higher for berries compared to flowers, because the berry yield strongly exceeded the flower yield under conventional growing conditions. A two-step purification of SGAs from potato berries yielded in a purified mixture of α-chaconine, α-solanine and an additional unknown third mass, which was used for the generation of semi-synthetic derivatives via acidic hydrolysis. Another important part of the project is the study of the impact of SGAs on beneficial and plant pathogenic organisms. The first screening of phytotoxicity showed that SGAs impair germination and development of Arabidopsis thaliana seeds at higher concentrations. Among the pure compounds, α-chaconine had the most negative impact, significantly reducing the development of seeds already at 25 µg/mL, while α-solanine significantly reduced seed development at of 50 µg/mL. Currently, SGAs and their derivatives are being tested for their effect on beneficial and plant pathogenic organisms. So far, the results strengthen the high potential of potato side-streams for use in industrial applications.

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P15 – ToxPot - Analysing the effect of potato glycoalkaloids (GAs) on different plant pathogens and beneficial organisms

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The two glycoalkaloids (GAs) α-solanine and α-chaconine, which are present in different tissues of potato, show interesting antimicrobial activities (Fewell & Roddick, 1993; Li et al., 2023) suggesting their use in plant pathogen control. This study aims at the analysis of the effect of these GAs on growth and survival of different beneficial and plant pathogenic organisms. For this, different concentrations (250 to 0.98 µg/mL) of these GAs were investigated. Preliminary results show a general distinct effect of α-solanine and α-chaconine on different targets. In terms of beneficial organisms, the growth of the beneficial bacterium Bacillus subtilis was affected by the presence of α-solanine in higher concentrations (from 62.5 µg/mL), however, concentrations higher than 1.96 µg/mL of α-chaconine resulted in growth impairments within 74 h. On the other hand, α-chaconine reduced the growth of the beneficial fungus Purpureocillium lilacium only at a concentration of 250 µg/mL, while α-solanine had a strong negative effect in all concentrations tested. For plant pathogenic organisms, no significant differences were observed for both GAs against the bacterium Pseudomonas syringae pv. aptata. For fungi, concentrations higher than 125 µg/mL of α-chaconine inhibited the growth of Leptosphaeria maculans by 62% compared to the mock control. However, same concentrations did not affect the growth of the fungal pathogen Cercospora beticola. Infection assays with the plant-parasitic nematode Heterodera schachtii revealed, that 10 µg/mL of α-chaconine strongly reduced the infection rate to about 1/4th of that of the control. No significant effect was observed for α-solanine. The preliminary results suggest that potato GAs act differently depending on the target but α-chaconine might be an option for the control of some plant pathogens. Nonetheless, more research must be conducted on a larger number of beneficial organisms.

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P16 - Flycycle: Waste reduction with fly larvae - sustainable protein, fertilizer and biofuel production

Prof. Dr. Jürgen Pettrak

Organisms used in gray biotechnology are typically bacteria and funghi, insects play a lesser role, albeit they have a huge potential.
Flycycle is utilizing the larvae of black soldier flies to reduce the amount of organic waste, for instance sewage sludge and manure. Some of the reduced waste is transformed into biomass, mostly into protein and oils, that can be utilized as food, fertilizer or biofuel.
The poster presentation will show data of research at the FH Aachen University of Applied Science in the lab of the subject area Environmental biotechnology. Rearing procedure, waste reduction potential and pupae mass and composition will be shown.

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P17 - Microalgae for Biofuels and Simultaneous Treatment of Paper Mill Wastewater

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In 2021, the Federal Office for Agriculture and Food (Germany) calculated greenhouse gas reductions of 84.4 % using biofuels instead of fossil fuels. Although promising, biofuels are controversial due to their dependence on food crops. Microalgae biomass is discussed as auspicious alternative. Microalgae are fast-growing, CO2-fixing microorganisms containing up to 70 % of lipids suitable for biofuel production. Commercialisation is hindered by high production costs, making demand on reasonable cultivation strategies. An interesting approach is combining microalgae cultivation with additional wastewater treatment, as microalgae have the ability to biodegrade persistent pollutants.
In our research, we investigated the potential of microalgae for removing residues of endocrine disrupting chemicals in paper mill wastewater and subsequent production of biofuels. Three algae strains with different cell shapes were screened concerning their growth in wastewater. The sickle-shaped microalgae Scenedesmus acuminatus achieved the highest biomass yield (7.2 g/L). Biodegradation ability was then validated by cultivating this species in wastewater spiked with 1 mg/L Bisphenol A, reaching 82 % substance reduction. Cultivations were scaled up from laboratory to 6 L Flat Panel Airlift Reactors and the bioprocess was made-up of two phases. In phase 1, Scenedesmus acuminatus was grown in wastewater to maximize biomass. Residues of the endocrine disrupting substances Bisphenol A and Bis(2-ethylhexyl) phthalate dropped below the detection limit (0.05 µg/L and 0.1 µg/L, respectively), demonstrating the success of algae treatment. In phase 2, microalgae were starved under nutrient depletion for stimulating lipid production. Final lipid content accounted for 28 %, leaving room for further enhancement.
Our data is available for the installation of a pilot plant: Establishing good operability could pave the way for the commercialisation of microalgae biofuels, while simultaneously removing pollutants from insufficiently treated wastewater. Overall, the combinatorial process can be stated as highly interesting considering sustainability, climate protection and pollution control.

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P18 – Chicken Feather Keratin – a Valuable Source for Polythiol Building Blocks

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Polythiol-containing compounds are relevant for several industrial sectors due to their potential to enter into various cross-linking reactions. So far, these compounds are mainly fossil-based and must be replaced by bio-based materials.
Chicken feathers, a valuable biomass stream from poultry meat production, have remained mainly underutilised or, at best, are used as a low-grade feed additive despite their high content of keratin. From a biochemist perspective, feather keratin offers the potential to produce specific polythiol-containing peptides due to its natural high L-cysteine content of about 7%.
A controlled enzymatic fragmentation process to produce such specific polythiol-containing peptides from feather keratin was developed by the Fraunhofer IGB within KERAbond, a joint project in cooperation with Henkel AG & Co. KGaA.
High yields of water-soluble keratin-hydrolysis-peptides of up to 88% were achieved, and our controlled hydrolysis approach allowed us to produce hydrolysates containing a high proportion of polythiol-containing peptides.
Furthermore, we could demonstrate the scalability of this selective enzymatic hydrolysis process in batch reactions up to the kilogram level. We also could show further processing of the resulting peptides via chemical or electrochemical reduction without denaturing agents or toxic substances, thereby following the principles of green chemistry. Film formation experiments were performed to explore the application potential of our processed keratin-hydrolysates. The results show that the processed peptides have promising properties which might be interesting for various applications, including adhesives, sealants, coatings, and cosmetics. Additionally, these polythiol-containing peptides serve as a sustainable and cost-effective basis for bioconjugates through selective conversions.
Moreover, our approach was transferred to other protein-containing residual material streams, such as plant-based raw materials. Further extending the substrate scope of the enzymatic hydrolysis will lead to polythiol-containing peptides with different physicochemical properties and thus might pave the way for specific polythiol building blocks from renewable feedstocks for various applications.

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P19 – MetaProcess: Towards a sustainable enzymatic production of metaraminol and related chiral amino alcohols

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In MetaProcess, the biosynthesis of metaraminol using biocatalysts and second-generation feedstocks is being established as a blueprint for synthetic pathways of chiral amino alcohols. While it has been shown that the biosynthesis is technically possible, for being economically competitive and sustainable, the process requires higher stability on the side of the biocatalysts, the pyruvate decarboxylase of Acetobacter pasteurians, ApPDC, and the amine transaminase of Chromobacterium violaceum, Cv2025, proper downstream processing with an in situ product removal (ISPR) step, and a comprehensive life cycle assessment. Currently, we have computationally identified new potential weak spots on the ApPDC structure by rigidity analysis, generated KnowVolution libraries with new variants of ApPDC that preliminarily show a higher activity and concentration resistance, identified reaction conditions that influence/protect ApPDC (from) deactivation, established production strategies depending on the biocatalyst resistance, and generated a model of the overall extraction equilibrium. The preliminary results lead towards a testable process for the production of metaraminol, and related amino alcohols.

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P20 – MK-ScaLoop – Towards an industrial-scale process for a biotechnological production of methyl ketones in a multiphase loop reactor

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Methyl ketones (MKs) are promising platform chemicals due to their wide range of applications from fragrances and flavors to precursors in pharmacological production. Moreover, the mixture of medium-chain length MKs in a range of C11 to C17 offers great potential as a fuel blend candidate. Currently, MKs are produced industrially from petroleum-derived hydrocarbons. By rearranging the fatty acid metabolism of aerobic microorganisms such as Pseudomonas taiwanesis VLB120, MKs can be produced biotechnologically, creating a sustainable production process.

To ensure an efficient production of the biotechnological MKs, product inhibition has to be circumvented. One promising option is in situ extraction: by continuously extracting the hydrophobic methyl ketones into an additional organic phase, longer fermentation times can be realized and thus, the space-time yield and the substrate yield can be increased. However, if the organic phase is added directly to a stirred tank bioreactor, there is a high degree of dispersion induced by high stirrer speeds, resulting in the formation of a stable emulsion. The separation of the organic and the aqueous phase is still a challenging aspect.

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P21 - Prospective Life Cycle Assessment and Scale-Up of Methyl Ketones Production from Wheat Straw

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Life Cycle Assessment (LCA) is a well-known tool for assessing the environmental sustainability of products and services. However, conventional LCA is not well suited to assess the future environmental impact of emerging technologies, which are often only available at a lab scale or pilot scale and have not yet been scaled up to commercial production. To address this challenge, a prospective LCA (PLCA), including a scale-up is used to assess the environmental sustainability of emerging technologies.

Scale-up and PLCA are applied to the production of methyl ketones (MKs) from wheat straw through a fermentation process in a novel multiphase loop reactor (MPLR). The process chain consists of five major steps: wheat straw supply, pretreatment of wheat straw to release fermentable sugars, inoculum preparation, fermenting fermentable sugars to produce MKs, and finally storage of MKs. The data used are taken from lab-scale (5 L fermenter volume) studies of the MK-ScaLoop project, literature, and expert opinion on the technology.

The PLCA results showed that the fermentation stage, followed by the pretreatment stage, are the main hotspot of the process because of the potassium hydroxide (KOH) and electricity demand. Sensitivity analyses of the influential parameters of the process are performed to address the inherent nature of uncertainty in the PLCA, and a best scenario is made from the sensitivity analyses for the industrial scale production of MKs. Moreover, a lab scale comparison revealed a significantly lower environmental impact at the industrial scale, mainly because of the economies of scale achieved.

Overall, this poster demonstrates that scale-up and PLCA can be valuable tools for assessing the environmental impact of emerging biotechnological processes. The findings of this poster can help researchers, process developers, and policymakers identify ways to improve the sustainability of technology and make informed decisions about their deployment.

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P22 - Integrated Biorefinery for Sustainable Production and Processing of Lignin for Textile Application

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Lignin is a promising and abundant coupling product in biorefineries (e.g. paper and textile indus-try), which in most biorefinery concepts is burned for its energy value. To make biorefineries economically more viable and to reduce the CO2 footprint, however, a valorization strategy ex-ploiting the high potential of lignin is crucial.
In the proposed project LignoTex, we want to cover the whole process chain from lignin extraction to spinning of lignin-based fibers as well as the application in textiles. A variety of different plants such as woods, grasses, perennial plants and agricultural residues will be extracted using the OrganoCat technology adapted and optimized to the different plant materials. The extracted lignins will then be isolated and fractionated by anti-solvent precipitation to reduce impurities and narrow the lignin fraction heterogeneity. To produce sufficient quantities of suitable precursors for the subsequent processing steps, the lignin extraction and fractionation will be scaled up into a 50 L scale. The isolated lignins will be chemically modified with polylactic acid (PLA) to adjust key properties for textile production, e.g. tacticity, strength and elongation. The precursors will then be tested in state of the art and newly developed wet spinning, melt spinning and composite manufacturing processes to determine the influence of molecular properties, e.g. molecular weight, molecular design (linear/branched) and β-O-4 linkages, on the their performance in the 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.
With this project, we hope to implement and demonstrate a viable and value-adding process to apply lignin in textiles, which can be integrated in a more holistic valorization of lignocellulose in biorefinery concepts.

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P23 - Synthesis and Characterization of Lignin-Polylactide Copolymers and their Application in Melt-Spinning for Fiber Production

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Plastics and man-made Fibers have become indispensable in the modern world. The vast majority of these materials are made from finite fossil feedstocks which need to be replaced to meet consumer demands in the future. Therefore, intensive research is being conducted into greener catalysts and alternative bio-based feedstocks. One promising pathway is the reintroduction of bio-based raw materials into a natural material cycle at the end of their useful life. Therefore, the production of lignin based thermoplastic man-made fibers is the focus point in the LignoTex project. State-of-the-Art processes in the textile industry for melt spinning are blending lignin with a thermoplastic polymer in order to generate thermoplastic compounds. However, in this working package, star-shaped copolymers consisting of polylactide and different lignins as the center are synthesized using a "grafting-from" approach directly from the lactide melt using a non-toxic zinc-based guanidine catalyst. This method proves to be efficient and copolymers can be produced after 30 minutes to three hours with high lactide conversions. Copolymers of commercially available kraft lignin and OrganoCat lignin isolated in this project were successfully synthesized with varying lignin loadings. First experiments with the use of OrganoSolv lignin were successful as well. Kinetic studies performed to investigate the influence of different lignin loadings on the polymerization rate have also been conducted. Thermal analysis via DSC and TGA reveal an increase of Tg and higher thermal decomposition temperature with increasing lignin content.

Initial melt spinning experiments with the copolymer of kraft lignin were successful and demonstrate improved melt spinning properties. In contrast to the corresponding blend, higher lignin loadings of 30 wt% could be used for melt spinning instead of only 10 wt% for the blend. In addition, the processing temperature during melt spinning could be decreased.

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P24 - LignoTex: Integrated Biorefinery for Sustainable Production and Processing of Lignin for Textile Application

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The project LignoTex incorporates an innovative approach known as OrganoCat, which enables the efficient fractionation of lignin, cellulose and hemicellulose components. This fractionation technique offers several advantages, such as reduced energy consumption and low environmental impact, making it a promising method for lignocellulose processing in future biorefineries. Furthermore, the LignoTex project focuses on upscaling the OrganoCat process, facilitating the testing of valuable lignin-derived products in spinning processes. To maximize the utility of lignin, the project also explores downstream processes, such as anti-solvent fractionation of lignin, which enhances the purity and applicability of lignin-based materials.

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P25 - PREDIG: Modelling software to predict the enzymatic digestion of biomass

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Enzymatic saccharification of lignocellulosic biomass plays a pivotal role in bio-refinery strategies aimed at producing biofuels and other valuable chemicals. Nonetheless, optimising this process faces challenges due to the recalcitrance and variability of the biomass, which hinders economic viability and profitability. Therefore, predicting the saccharification of lignocellulose is essential for achieving economic success in lignocellulosic biorefinery endeavours.
This necessitates a multidisciplinary approach, incorporating advanced computational tools, quantitative experiments, and a profound understanding of the underlying microscopic processes.

To complement both academic and industrial experimental research in this field, the project introduces an advanced web application that encapsulates our in-house developed complex stochastic biophysical model of enzymatic plant cell wall degradation. Named PREDIG (accessible at https://predig.cs.hhu.de/), this free, user-friendly, and fully open-source web application not only enables users to conduct in silico experiments but also facilitates the fitting of model parameters to reproduce uploaded experimental time-course data. This empowers users to quantitatively elucidate the factors contributing to the recalcitrance of their specific biomass material.

This project goes beyond providing a practical tool to address specific applied questions, such as optimising enzyme cocktails for a particular biomass. It also contributes to the accumulation of new fundamental insights into the microscopic mechanisms and kinetics of lignocellulose saccharification.

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P26 - Precision-enabled knowledge extraction: InterText - a scalable and interoperable text-mining framework

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The process of extracting biological data from scientific articles presents a significant challenge, particularly due to the growing number of periodicals and the frequency of publications. Nevertheless, the progress in natural language processing and machine learning has consistently enhanced the functionalities of text mining and data extraction within the realm of scientific research. The advancement of increasingly complex algorithms and the accessibility of extensive datasets have empowered researchers to efficiently extract valuable insights from complete-text articles, notwithstanding the presence of paywalls on certain articles. In order to tackle this particular challenge, we have design InterText framework, a comprehensive suite of interactive software tools that empowers users to perform searches, implement filters, and extract relevant information pertaining to phenomics from scientific articles. The incorporation of MIAPPE (Minimum Information About a Plant Phenotyping Experiment) and ISA-Tab (Investigation Study Assay Tabulation) standards enhances the endeavours aimed at achieving uniformity in data documentation within the field of plant sciences. Regarding the potential future expansion of InterText's functionalities beyond phenomics through the integration of supplementary minimal standards like MIAME (Minimum Information About a Microarray Experiment) and MIAPE (Minimum Information About a Proteomics Experiment), there is significant potential for enhancing the tool's efficacy in various domains of biological investigation. The magnitude of these advancements, however, is contingent upon the level of progress achieved in standardisation initiatives and the accessibility of pertinent datasets for the purpose of training machine learning models.

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P27 - Surface Active Biomolecules for the Chemical Industry

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Within the project “SurfIn”, are investigating 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. SurfIn 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 SurfIn results, industrial experts from world leading companies form part of the consortium.
The two target compounds are the biosurfactants liamocin and serrawettin W1. Both have not been intensively researched and offer a high potential for dissemination and commercial exploitation. Over the past six months, we have ve enhanced serrawettin W1 production and achieved promising effects against pathogenic microbes. Liamocin synthesis has improved by 25%, and cultivation in a 10 L fermenter was successful. Online monitoring techniques are being used to enhance liamocin production in A. pullulans, and sustainable purification strategies are in development. The project is also analyzing patenting strategies and technological trends in biosurfactants. In application-focused efforts, liamocin surfactant properties were successfully characterized, and work with an industrial partner is ongoing. The SurfIn project aims to contribute to greener and more sustainable solutions in the chemical sector through its research and development efforts.

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P28 - Engineered Pseudomonas putida KT2440 for the production of tailored biosurfactants showing bioactivities of agricultural interest

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Biosurfactants are versatile amphipathic compounds with significant applications in industrial, agricultural and medical fields. Conventional production methods often encounter limitations due to complex regulatory mechanisms and challenging cultivation conditions. To overcome these obstacles, we employed Pseudomonas putida KT2440 as a recombinant host, offering improved manageability in the laboratory and a optimized production environment.
Within several BioSC funded projects, we have developed P. putida KT2440 into a robust platform for production of a wide array of rhamnolipids. Using chromosomally stable integrated expression modules, we produced rhamnolipids in diverse compositions, including pure 3-(3-hydroxyalkanoyloxy) alkanoic acid (100%), mono-rhamnolipids (100%), and varying mixtures of mono- and di-rhamnolipids (14-94% mRL), each featuring unique potential properties.
As part of the collaborative project Surfin, we transferred the knowledge gained from rhamnolipid production to the robust production of the lipopeptide biosurfactant serrawettin W1, which is known, for example, for its activity against oomycetes, using recombinant P. putida. This allowed the isolation of suitable quantities for further characterisation of this interesting but little studied biosurfactant, e.g. regarding its antimicrobial capabilities. We tested the bioactivity of serrawettin W1 using a twofold serial dilution on six bacterial strains, including the plant and human pathogens—S marcescens, P. aeruginosa, S. epidermidis, E. faecium, S. aureus, and P. aeruginosa and the mycobacterium C. glutamicum to determine the Minimum Inhibitory Concentration (MIC) and Half Maximal Inhibitory Concentration (IC50) values.
Our study emphasises the capabilities of P. putida KT2440 as a highly effective system for the production of a broad spectrum of biosurfactants. This paves the way for innovative applications in biotechnology, expanding the range of biosurfactants available for various application.

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P29 - Production of a sustainable and tailor-made microbial palm oil and milk fat substitute from agricultural residues (NextVegOil)

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The surging global demand for palm oil, a remarkably versatile and extensively utilized commodity, has led to rampant environmental damage, worsened by its long-distance transportation. This unsustainable practice contributes to carbon emissions and establishes a reliance on foreign resources. To address these issues, we explore alternative sources for palm oil-like compounds in our BioSC research project NextVegOil. Our solution targets the microbial production of palm oil substitutes from agricultural waste streams with the fungus Ustilago maydis. This goal is pursued in partnership with researchers from plant biotechnology, microbiology, bioprocess engineering, economics, and analytical chemistry.

We focus on utilizing non-edible corn residues, like leaves and stems, as a substrate for microbial fermentation to produce our palm oil alternatives. The initial step was to analyze the substrate composition and identify corn varieties exhibiting more fermentation-suitable properties. Pretreatment strategies for corn residue are investigated to further enhance its fermentability. Currently, the desired fatty acid profiles are tailored both through cultivation conditions and genetic engineering techniques. As a significant milestone, we already achieved the production of a fatty acid profile closely resembling palm oil. The transition to a larger production scale was initiated through the implementation of stirred-tank reactors and a preliminary process modeling was created to prepare for an overall cost analysis. Furthermore, genetic targets for optimizing microbial production are continuously expanded, offering future enhancements. We also delve into the research and startup landscape surrounding sustainable palm oil alternatives, identifying key players and the interconnectedness level within the field. Our ecosystem analysis revealed that the microbial oil ecosystem is in the late birth stage, with an ecosystem leader yet to emerge.

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P30 - Online monitoring of corn stover processing by the fungus Ustilago maydis

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A key aspect of a sustainable bioeconomy is the recirculation of renewable, agricultural waste streams as substrates for microbial production of high-value compounds. A promising approach in this direction is the production of sustainable palm oil substitutes with the fungus Ustilago maydis growing on corn stover as the sole nutrient source. However, the utilization of this abundant agricultural waste by U. maydis is often hindered by its complex and heterogeneous nature and the need of an efficient breakdown into a suitable feedstock. The specific corn stover components utilized by U. maydis and the bottlenecks in the fermentation process remain largely unexplored.
To address this challenge within the BioSC project “NextVegOil”, we have developed a platform that enables monitoring U. maydis performance when growing on corn stover. This platform encompasses online measurements of back scattered light, fluorescence intensity, and oxygen transfer rate as a proxy for fungal growth and metabolic activity. By integrating such online data with the determination of the chemical composition of the post-metabolized corn stover residue, we can enhance the understanding of maize biomass degradation by U. maydis.
Our established platform allows the development of more efficient strategies to valorize agricultural waste streams. As an example, the effect of various corn stover pretreatments, the use of genetically engineered fungal strains with enhanced expression of specific lignocellulose-degrading enzymes, and the use of corn stover from genetically diverse corn varieties with varying lignocellulosic properties will be presented. This platform serves as the foundation for the process scale-up towards production of palm oil substitutes from corn stover.

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P31 - BioPlastiCycle – Transitioning bioplastics to the circular economy

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Plastics fulfill many vital functions in modern society, but in the past not enough consideration has been given to the end-of-life fate of these extremely stable materials. There is now a strong societal and technological 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 new technology development towards a more circular bioplastics economy. BioPlastiCycle aims to tackle this challenge by developing and evaluating a complete value cycle for well-established bioplastics and newly developed materials based on α-ketoglutarate.

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P32 - Synthesis of C14-labeled bioplastics for environmental fate assessments

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Due to ever increasing problems in waste management associated with conventional fuel-based plastics, the use of degradable alternative materials appears to be one of the most promising approaches to help prevent the excessive environmental pollution caused by fuel-based plastics disposal and accumulation.

Most of the information about polymer degradation is primarily based on traditional plastics. While various reaction mechanisms and pathways are established for some plastics, the effects of environmental factors such as microorganisms, weathering, pH, water, and structural characteristics on the rate and final level of degradation for many bioplastics are not yet properly known. In environmental fate studies, it also remains uncertain whether polymers introduced into more complex matrices undergo physical breakdown into micro- or nano particles or simply transform into carbon dioxide, water, or microbial biomass, especially when degradation is slow.

The selective labeling of carbon atoms within the polymer backbone, available in both 14C and its stable isotope variant, 13C, has simplified the interpretation of degradation studies. The need to study the fate of bioplastics prompted us to design a synthesis route, starting from C14-labeled lactic acid monomers, transitioning through lactide as an intermediate, and finally culminating in the production of poly 14C-lactic acid chains via a catalytic ring-opening polymerization reaction. Subsequently, after bioplastics conversion into microplastics, these selectively radiolabeled micro-bioplastics are employed for a degradation study in soils under defined conditions. Enhanced understanding of the fate of bioplastics can be achieved by tracking introduced 14C-labeled microplastics in soil up to their ultimate transformation into 14CO2, as a key main degradation product.

In this poster, I will present a synthesis procedure and a setup that we want to use for 14C-labelled poly lactic acid production which subsequent will be used for degradation studies in soil environment.

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P33 - Life Cycle Assessment of End-of-Life Scenarios for Polylactic Acid

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Plastics, with their linear life cycle and poor waste management, pose significant environmental challenges. To address these issues, the European Union pushes towards sustainable plastics through regulations such as directives on single-use plastics, plastic bags, packaging and packaging waste plastic. This effort is particularly relevant as the market share of bioplastics is growing, contributing to an increasing waste stream. This research focuses on the environmental impacts of Polylactic Acid (PLA), a prominent bioplastic. Our goals include quantifying the environmental effects of various PLA end-of-life scenarios with a focus on an emerging chemical recycling technology, depolymerization. An environmental life cycle assessment (E-LCA) approach is used to quantify environmental burdens and compare different end-of-life options. Our study assesses PLA's environmental impacts, considering municipal waste incineration, mechanical recycling, chemical recycling, and composting. By comparing these scenarios to the status quo, we will provide insight into the potential benefits and challenges associated with the growing use of bioplastics. Additionally, we will evaluate the potential of depolymerization as it has the capacity to transform PLA waste into high-quality recycled PLA, in contrast to traditional mechanical recycling which often yields lower-quality outputs. The environmental benefits of each scenario will be calculated through the avoided burden approach, where the valuable output replaces production through traditional routes. Initial findings highlight a lack of PLA end-of-life considerations, especially for depolymerization, in LCA literature. A comprehensive E-LCA of these technologies will reveal their environmental sustainability and their capacity to fit into the existing waste management system. This poster presents an overview of our research's goals, methods, and expected outcomes. We aim to contribute to the understanding of PLA's environmental implications and provide insights into emerging recycling technologies.

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P34 - Utilization of Zinc Guanidine Complexe in the Circular Economy of biobased Polyesters

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Non-degradable plastics, mostly produced from fossil raw materials, are mainly disposed of in landfills or in nature after single usage. The accumulation of plastic waste in the environment is a major problem that needs to be solved. This littering by plastic waste can be remedied by biodegradable bioplastics. Ideally, bioplastics are also recyclable so that they can be returned to feedstock as a new raw material. The combination of polymerization and depolymerization provides the path to a circular economy. Polylactide (PLA) is one of the most promising bio-based, biodegradable bioplastics already being used as a packaging material. In the industrial production of PLA, a toxic catalyst (tin(II) bis(2-ethylhexanoate) is currently used and remains in the polymer. Replacing the catalyst with an environmentally friendly one and incorporate it in the depolymerization of PLA is the challenge. Another problem in the production of polylactide is the cost-intensive method of obtaining lactide via the fermentation of biomass with subsequent dimerization, thus recycling is becoming a focus of research. Using chemical recycling, PLA can be degraded either to recover the polymer into monomer units or into reusable resources. Especially zinc-guanidine complexes are well known as such suitable catalysts in lactide polymerization due to their environmental compatibility. In 2020, the currently fastest, robust, and biocompatible zinc catalyst was reported by Herres-Pawlis et al. It was shown a significantly higher polymerization activity compared to the industrially used Sn(Oct)2. Due to this industrial relevance of the zinc bisguanidine catalyst, its activity was tested in chemical recycling. A combination of production and recycling with a universally applicable catalyst is highly desirable. A different selection of alcohols allows obtaining a wide range of products that can be used as raw materials or green solvents. Furthermore, the recyclability and scalability of the catalyst is significant for a potential industrial application.

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P35 - 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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P36 - Advancing Green Chemistry: Enzymatic Halogenation of Aliphatic sp3 Carbons

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Enzymatic functionalization of aliphatic hydrocarbons has been proven to be an incredibly potent tool for a sustainable organic chemical synthesis. We aim to discover and characterize novel halogenases capable of sp3-carbon functionalization. To do this, we employed cutting-edge computational tools, including the construction of Hidden Markov Models complemented by structural studies using AlphaFold2. Analytical techniques such as GC, HPLC, MS, and NMR were also used to characterize newly discovered enzymes capable of regio- and stereo- specific halogenation(s). Using our computational approach, we identified ten promising novel non-heme Fe(II) alpha-ketoglutarate dependent halogenases, of which two have been optimized for expression and purification. This laid the groundwork for a comprehensive elucidation of these enzymes physiology and fundamental characteristics, such as their O2 tolerance and interaction. The optimized enzymes exhibited remarkable catalytic capabilities across a spectrum of reactions, involving both hydroxylations and halogenations, on selected N-heterocyclic substrates. This and the ongoing assessment of the remaining halogenases will further our understanding of their catalytic potential and expand the available toolbox for a greener and more sustainable chemical synthesis.

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P37 - Towards a sustainable photohydrogen production through oxygen-tolerant hydrogenase chimeras

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The transition from fossil fuels to renewable energy sources is essential for sustainable energy supply and to combat global warming. An elegant solution is emerging in the engineering of hydrogenases for 100% atom-efficient photohydrogen production, using electrons and protons generated through water splitting via the photosystem.[1] Recent work has successfully integrated an oxygen-tolerant hydrogenase from Cupriavidus necator (CnSH) into the cyanobacterium Synechocystis sp. PCC6803.[2] However, achieving sufficient photohydrogen production remains a challenge due to inefficient electron transfer. Although the Synechocystis NAD(P/H)-converting hydrogenase (SynSH) can accept electrons from low-potential ferredoxins suitable for H+ reduction, it is oxygen-sensitive.[3] Our overall goal is to create oxygen-tolerant chimeras containing CnSH and SynSH components for photohydrogen production. This hinges on understanding the cofactor composition, electron transfer mechanisms, and biocatalytic properties of the reductase module of SynSH (SynHoxEFU). Our study systematically characterized SynHoxEFU using photometric activity assays, UV/Vis and electron paramagnetic resonance spectroscopy. We discovered three distinct [2Fe2S] clusters within SynHoxEFU, shedding light on their biocatalytic roles and interactions with other reductase components.[4,5] Using this knowledge, we designed several chimeric hydrogenases combining SynSH reductase and CnSH hydrogenase modules. Our most promising constructs show both electronic coupling of CnHoxYH-SynHoxEFU and ability of accepting electrons from ferredoxin. Our research provides important insights into the structures and functions of metallocofactors in cyanobacterial hydrogenases, paving the way for the design of oxygen-tolerant hydrogenases suitable for future photohydrogen production.

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P38 - Electr-biocatalysis: production of fine chemicals using renewable energy

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Biocatalysis has the advantage of not using environmentally harmful chemicals and operating in milder conditions, producing less by-products. However, many biocatalysts require reduced cofactors for functionality and stoichiometric addition of cofactors is too expensive. The central enzyme in our system is a hydrogenase that uses H2 as a reducing agent and can reduce NAD(P)+, and flavin cofactors. We present an interdisciplinary approach for a sustainable chemical production where the goal is to synthesize fine chemicals in a scalable continuous flow system that uses biocatalysts and electricity. In this system, the biocatalysts were immobilized in a dedicated biotransformation unit for efficient separation and recovery of the substrate/product. Old yellow enzyme from Thermus scotoductus (TsOYE) was used as oxidoreductase for ene-reduction, coupled with the soluble hydrogenase (SH) from Cuprividus necator for H2-driven FMNH2 regeneration instead of stoichiometric addition of NADH. The H2 needed for the reaction was produced in situ via electrolysis using a PEM electrolyzer replacing pressurized gas bottles. The generated H2 was introduced to the flow system through the gas-addition module with H2-permeable Teflon tubing for controlled addition of H2. We demonstrated 99,95 % (ee = 39 % R) conversion of ketoisophorone to levodione with high total turnover number (TTN) of 1,91 x 105 for TsOYE and 1,96 x 105 for SH. A near-complete 99 % conversion was also achieved for the asymmetric reduction of carvone to dihydrocarvone, showing substrate versatility. Additionally, the conversion of ketoisophorone to levodione was demonstrated in an anaerobic glove box with 3% H2, highlighting the adaptability of the setup. Our electro-driven platform separates biocatalysts from the product, allowing for biocatalysts reusability at high TTN. The stability of the system, combined with electro-driven biocatalysis and cofactor regeneration offers promise for sustainable and efficient production of bio-derived and value-added compounds and materials.

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P39 - CasCAR: Sustainable production of valuable aldehydes using a locally immobilized synthetic enzyme cascade

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Carboxylic acid reductases (CARs) are attractive catalysts for the green synthesis of valuable aldehyde building blocks. However, the application of CARs still remains a challenge because CARs depend on essential NAD(P)H and ATP cofactors that need to be regenerated. The CAR enzyme from Nocardia otitidiscavium (NoCAR) has been characterized as one of the most potent enzyme to efficiently convert benzoic acid into benzaldehyde, an important building block in pharmaceutical ingredients. Co-expression or addition of auxiliary enzymes and their integration in a multi-enzyme cascade for cofactor regeneration has been recently validated in E. coli but the long-term use of CARs in whole-cell biotransformations is impaired by the inherent toxicity of accumulating aldehydes. Often preferable, the use of a cell-free approach using purified enzymes in vitro shows reduced catalytic performance as substrate tolerance is decreased compared to in vivo conditions. To overcome this limitation, the CasCAR project aims at designing locally immobilized multi-enzyme constructs with superior catalytic activity and stability in vitro compared to unfused enzymes. To this end, catalytic partners will be fused with optimized peptidic linkers using iterative cycles of structural modeling, simulations, and experimental validation. Coarse-grained models of single and fused enzymes structures, based on prior MD simulations data, will be developed to perform Brownian dynamics simulations. This multi-scale methodology will allow us to effectively explore large conformational spaces and simulation timescales to evaluate the impact of fusion and specifically of linker properties on NoCAR conformational dynamics and substrates diffusion rates in comparison with single (unfused) enzymes. The best fusions candidates will be produced and tested in the lab. We expect this project to provide transferable rational knowledge to guide the integration of CARs into self-sufficient multi-enzyme fusion assemblies for developing green aldehyde synthetic routes.

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