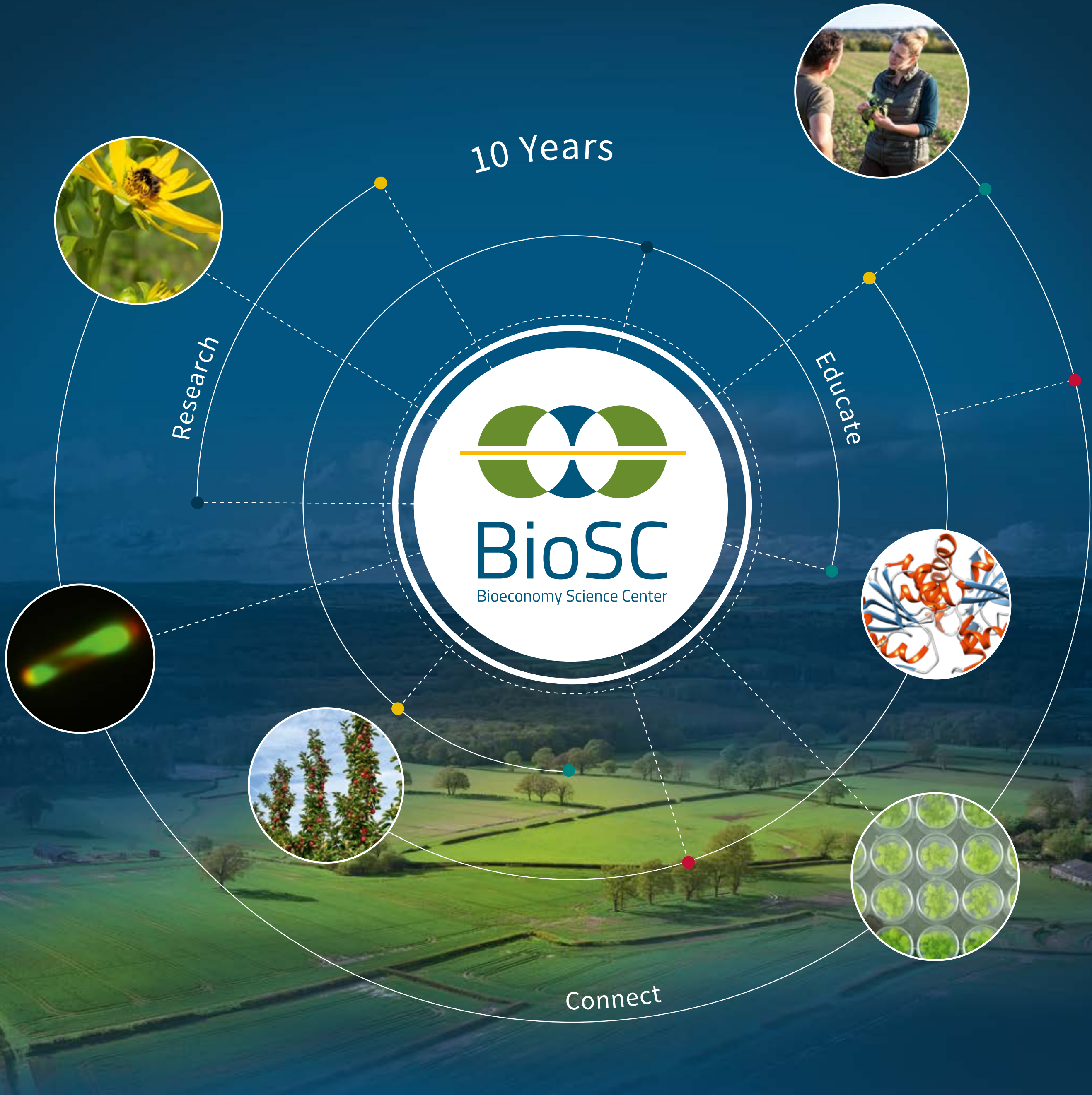
For a Sustainable Bioeconomy



RWTH AACHEN UNIVERSITY

UNIVERSITÄT BONN

hhu Heinrich Heine Universität Düsseldorf

JÜLICH Forschungszentrum

A Successful Experiment

Prof. Dr. Ulrich Schurr
Forschungszentrum Jülich
Spokesperson of the BioSC Executive Board

The idea for a major scientific network for bioeconomy research in the region was developed by scientists in the region. Initial talks with the representatives of the RWTH Aachen University and the HHU Düsseldorf began as early as 2008. “Together we are stronger” was convincing – the scientific profiles complemented the concept perfectly. It quickly became clear that the University of Bonn with its competence in agricultural sciences must be a founding member. Promising approaches to a bioeconomy could function only in collaboration with a broad competence in crop and agricultural research, the procedural conversion of biomass and biotechnology. However, technical options alone do not result in sustainable implementation – the experiences from other fields of technology made clear that socio-economic research needed to be involved directly.

The persons responsible at the universities and at Forschungszentrum Jülich were convinced about the opportunities including the financing of the strategy project with the support of the Ministry of Research of the State of North Rhine-Westphalia (at that time under the leadership of Professor Andreas Pinkwart). The route was open for the first competence center for a sustainable bioeconomy.

It was a major undertaking to bring together the wide range of required expertise. However, the value-added content and the structural opportunity for the Rhineland was obvious and became explicit also at the event of the presentation of the grant agreement by the Minister of Science, Svenja Schulze. Nevertheless, there was much work ahead: “Can this experiment succeed?” However, the tireless dedication of scientists, employees of the managing body and the advisory board, always critical but inspired by the idea, resulted in quick successes: at the end of the first phase, almost 80% of the projects founded in the course of the strategic project included partners of all four research areas. Innovative ideas were the rule and not the exception. Enthusiasm rose with increasing attention from other regions of Germany, Europe and the world. The BioSC became a brand that others also followed.

After the broad collecting approach of phase 1, consolidation to focus topics was needed. A special quality of the BioSC became apparent, which remains a key to success today and for the future: the BioSC represents a learning and agile system. And: funding bodies and those responsible in the institutions provided the BioSC with the opportunity to draw conclusions from what it had learned and to implement them. This trust is the basis for the extraordinary development of the BioSC as a benchmark for sustainable bioeconomy and regional science cooperation.

The BioSC continues to represent a unique opportunity in a very dynamic field of science: bringing together areas of science that otherwise have few points of contact but which are essential for developing a sustainable bioeconomy for the future. The BioSC is a successful experiment for which much remains to be done! Let us shape this future together!

Prof. Dr. Thomas-Müller-Kirschbaum
tmk-expertise
Member of the BioSC Advisory Board

If the Bioeconomy Center did not already exist, it would have to be invented! How fortunate that the scientists in Aachen, Bonn, Düsseldorf and Jülich, together with the Government of North Rhine-Westphalia, had the vision and courage to commit to this project back in 2010. All involved can be proud of this and of what has been achieved. I would like to expressly thank all who have actively contributed to this.

I am especially impressed that the BioSc has thought and worked ahead at a time when the terms “circular economy” and “climate neutrality” or the consideration of the UN Sustainable Development Goals were not as self-evident as they are today.

The unique, interdisciplinary and systemic overall concept of the BioSC is very convincing. The quickly organized networking and continuously developed cooperation of the scientific/technical and the economic or social science disciplines created the basis for this excellent competence center. It has been shown that research becomes more holistic and better with this multidisciplinary approach.

The systemic concept also improves the competence of the researchers. The training and promotion of young scientists at the BioSC benefits from the interdisciplinary cooperation in two ways. On the one side, knowledge expands beyond one’s own discipline. On the other side, the scientists develop an understanding for the different ways of thinking and proceeding in other disciplines. Successful work in multidisciplinary teams is an excellent preparation especially for those who want to continue their career in an industrial environment.

Those who want a climate-neutral economy and closed raw material cycles will not be able to get along without a sustainable bioeconomy. The BioSC has made a unique contribution to this over the last ten years. I wish all researchers that this success will continue in the future for the BioSC and for them personally.

Congratulations on the anniversary and all the best for the future!

Sen.-Prof. Dr. Peter Westhoff
HHU Düsseldorf
BioSC Founding Member

The Bioeconomy Science Center is celebrating its tenth anniversary. This is an occasion to look back but also – much more important – to look ahead: where should the journey lead? It has long been clear to the rationally thinking part of humanity that we cannot continue to manage our energy, material and food economics as before. We need to move away from production methods based on fossil energies and materials and turn to a sustainable economic form that preferably uses renewable energies and avoids waste wherever possible or sees it as raw material in a circular bioeconomy.

This question was taken up by the Bioeconomy Science Center ten years ago when it began to develop methods and techniques for such a circular bioeconomy. The researchers did not need to look too far ahead, as nature has been showing us how to do it for millions of years – it is a master of recycling. The entry into a sustainable and circular bioeconomy requires scientists and engineers who learn these methods and techniques from nature and develop them to application maturity.

What is also needed is the cold view of economy: can the newly developed process exist on the market at all and if so, when could it be competitive? And finally, people need to get enthusiastic about this enormous task. Researchers must leave their ivory towers and regard it as their noblest task to contribute to solving the major issues of mankind. Such researchers, who are enthusiastic about their work, will then easily succeed in inspiring the population and take them along on this journey. Since its foundation, the Bioeconomy Science Center has included all these aspects in its strategy. This is why it deserves enormous praise!

In my many years as Vice Rector for Research and Transfer of the Heinrich Heine University Düsseldorf, the Bioeconomy Science Center has always been a matter close to my heart – and will remain so! I wish the Bioeconomy Science Center all the best for the future and the courage to carry on working with spirit on the great transformation of the economy in our region.

Prof. Dr. Sandra Venghaus
Forschungszentrum Jülich
Spokesperson of the FocusLab Heads

Ten years ago, the Bioeconomy Science Center was established - a research network to initiate a scientific rethinking towards a sustainable bioeconomy. This was to be achieved through interdisciplinary collaboration between natural, agricultural, engineering, economic and social sciences across the entire spectrum from basic research to demonstration and application.

Within the framework of the NRW Strategy Project BioSC, a funding concept for large multidisciplinary collaborative projects was implemented - the FocusLabs, with young scientists entrusted with the leadership. The willingness and ability to look beyond “one´s own nose” and get involved with completely different research disciplines were essential prerequisites for mastering this task. In many cases, interdisciplinary cooperation first meant learning to understand each other before one could speak a “common language”.

It is the variety of perspectives that is crucial for the complex task of a transformation towards a sustainable bioeconomy. Within the framework of the BioSC FocusLabs, the interdisciplinary idea is already being introduced into the various sub-areas of the bioeconomy, shaping collaborations with industry partners and the research in the disciplines involved.

We will continue this collaborative research aimed at a systemic approach in the future in order to further shape the path towards a knowledge-based bioeconomy. We are therefore already implementing our ideas and concepts on site in field trials and pilot projects and are also demonstrating new perspectives beyond the BioSC.

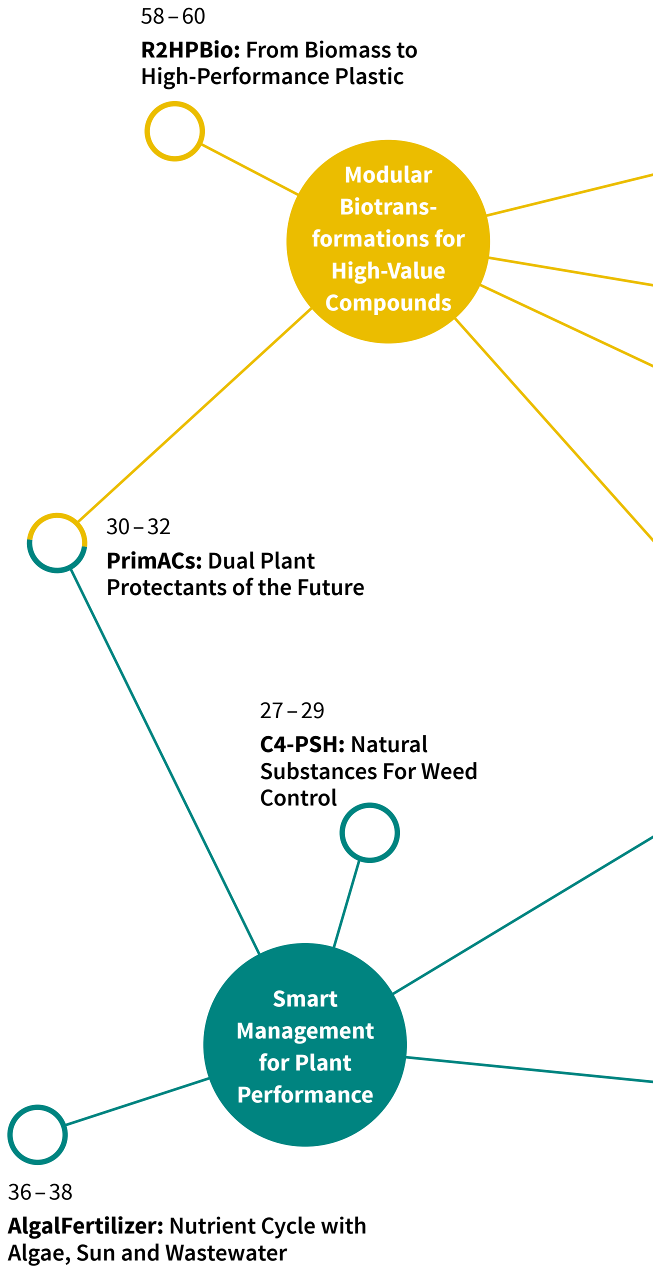
The management of such comprehensive projects across different sites has allowed us to assume a high degree of responsibility for the implementation of a major project already at an early stage of our scientific careers. For this reason, on behalf of all the FocusLab leaders, I would like to take this opportunity to thank all those who have made this possible for an excellent and successful collaboration.

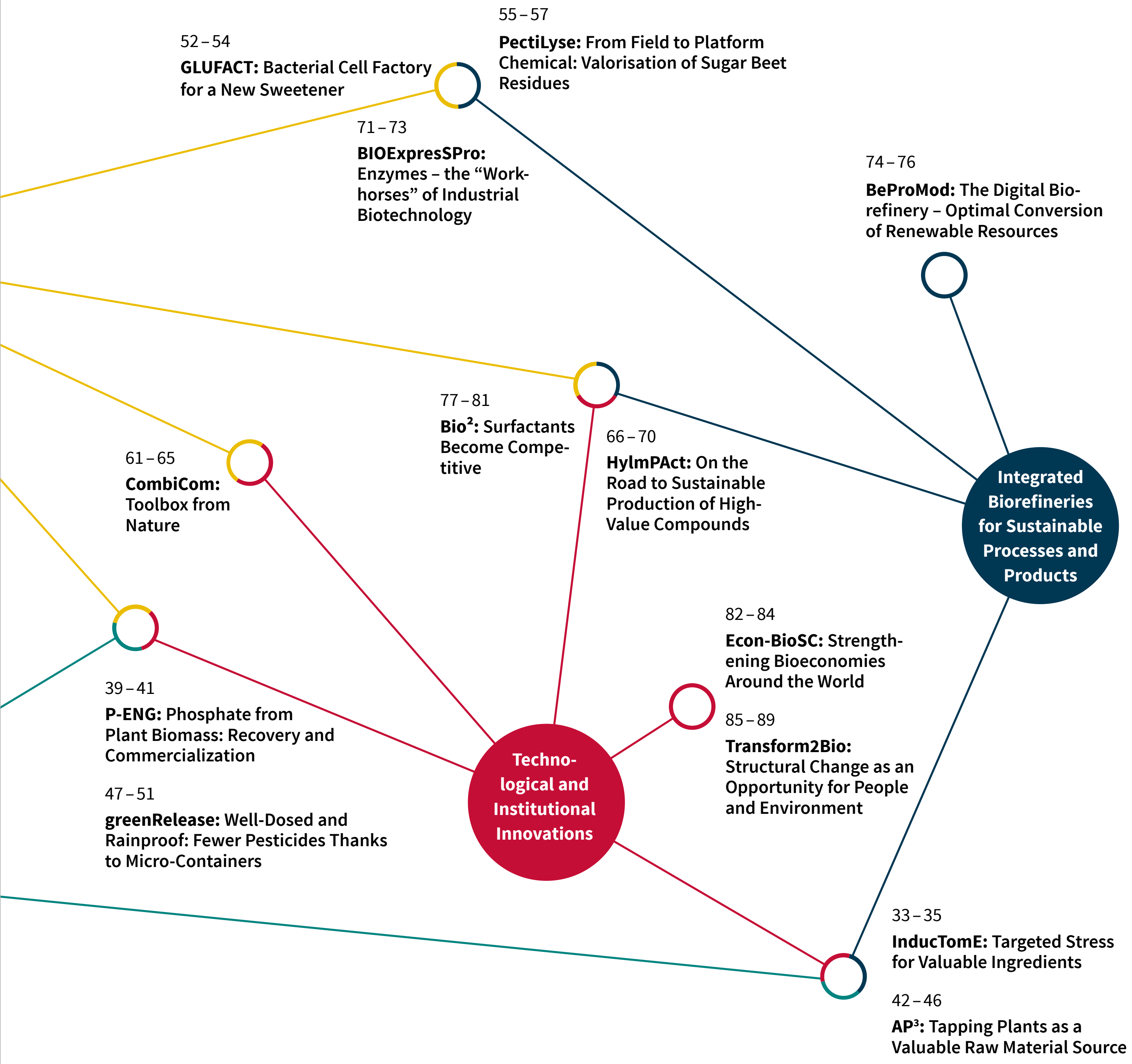
With this in mind, we are looking forward to presenting key findings and perspectives from the FocusLabs in this brochure and wish you a pleasant read.

Research • Educate • Connect

For a Sustainable Bioeconomy

CONTENTS


58 – 60
R2HPBio: From Biomass to High-Performance Plastic
Modular Biotrans-formations for High-Value Compounds
30 – 32
PrimACs: Dual Plant Protectants of the Future
27 – 29
C4-PSH: Natural Substances For Weed Control
Smart Management for Plant Performance
36 – 38
AlgalFertilizer: Nutrient Cycle with Algae, Sun and Wastewater


52 – 54
GLUFACT: Bacterial Cell Factory for a New Sweetener
55 – 57
PectiLyse: From Field to Platform Chemical: Valorisation of Sugar Beet Residues
71 – 73
BIOExpresSPro: Enzymes – the “Work-horses” of Industrial Biotechnology
74 – 76
BeProMod: The Digital Bio-refinery – Optimal Conversion of Renewable Resources
77 – 81
Bio²: Surfactants Become Compe-titive
66 – 70
HylmPAct: On the Road to Sustainable Production of High-Value Compounds
61 – 65
CombiCom: Toolbox from Nature
Integrated Biorefineries for Sustainable Processes and Products
82 – 84
Econ-BioSC: Strength-ening Bioeconomies Around the World
85 – 89
Transform2Bio: Structural Change as an Opportunity for People and Environment
39 – 41
P-ENG: Phosphate from Plant Biomass: Recovery and Commercialization
47 – 51
greenRelease: Well-Dosed and Rainproof: Fewer Pesticides Thanks to Micro-Containers
Techno-logical and Institutional Innovations
33 – 35
InducTomE: Targeted Stress for Valuable Ingredients
42 – 46
AP³: Tapping Plants as a Valuable Raw Material Source

The Bioeconomy Science Center

Mankind is facing major challenges: climate change, resources becoming scarcer and a growing world population require rethinking and new, more sustainable action and economies than before. These major societal challenges can be met only through joint action from the regional to the global level. The United Nations (UN) has also recognized this and in 2015 adopted the Agenda 2030, which formulates seventeen objectives and measures for sustainable development, the so-called Sustainable Development Goals (SDGs, figure below). These objectives are to be achieved within a period of fifteen years.

Achieving these goals requires a major joint effort by all nations and actors involved, the generation of cross-sectoral and multidisciplinary linking of new knowledge and modern, environmentally-friendly technologies, the protection and conservation of natural resources, a change of the raw material base for industrial production and various measures for societal transformations. These are all basic principles that also underlie the bioeconomy. The bioeconomy is closely linked to thirteen of the seventeen Sustainability Development Goals.

The challenges for the technological, economic and social transformation to a sustainable, bio-based economic activity are also diverse and great. All actors – from science, the economy, society and politics – are facing new questions that can result in the necessary transformation only through joint strategies and cooperations. Only a sustainable production of healthy food and renewable resources, modern, bio-based production processes for high-quality products and climate-neutral energy sources can lead the necessary transformation process to success.

Already in 2008, scientists of the Bioeconomy Science Center (BioSC) recognized the necessity and potentials of a sustainable, knowledge-based bioeconomy (KBBE), the concept of which originated at the European level in 2005. The idea to establish a joint competence center for bioeconomy research developed on the basis of a broad scientific excellence, high synergy potentials and cooperations in bioeconomy research fields that already existed between Forschungszentrum Jülich, Rheinisch-Westfälische Technische Hochschule Aachen, Heinrich-Heine-Universität Düsseldorf and Rheinische Friedrich-Wilhelms-Universität Bonn. Following the development of a cooperation concept designed for the long term, the founding treaty for the Bioeconomy Science Center was signed in the fall of 2010 – the same year in

Plants have a key position in the establishment of a sustainable bioeconomy.

which the government also adopted the National Research Strategy Bioeconomy 2030.

With the BioSC, the four partner institutions established a cross-institutional and multidisciplinary cooperation in research and training for the transformation towards a sustainable bioeconomy that is still unique today. Research at the BioSC is based on a systemic, integrated approach. The integrated overall concept

In the future, chemicals and materials will increasingly be produced on the basis of biogenic resources.

consisting of basic research and application-oriented research in inter- and transdisciplinary research teams (e.g. biotechnologists, plant scientists, engineers, economists), as well as the development and use of modern technology platforms offers unique conditions in the regional cooperation for developing bio-based production procedures and bioeconomy concepts. The BioSC conducts research and training on sustainable plant production for food and feed, renewable resources and their molecular and microbial conversion into high-quality, bio-based products (e.g. fine chemicals, biopolymers, pharmaceuticals, enzymes, proteins, biofuels). In addition, scientists at the BioSC develop technical processes and integrated biorefinery concepts, for example for multiple, closed-loop and recycling material use, and apply them at various scales. In another professional focus, the relation between technical innovations and market, politics and society is established and investigated. Different scales are used to examine how the economic and social changes in the transition from a fossil-based to a bio-based economy can be managed and designed so that they are ecologically compatible, technically feasible and socially accepted. Currently, 67 member institutes and chairs (Core Groups) with approximately 1,900 employees from the four institutions form the core of the BioSC.

The NRW Strategy Project BioSC, which has been funded by the State of North Rhine-Westphalia in three funding phases since 2013, is especially focused on the integration of research disciplines and topics within the framework of the systemic approach. Specific scientific questions are addressed in multidisciplinary, cross-institutional research projects.

The first funding phase was focused on establishing the BioSC as regional research infrastructure in NRW and on identifying and bringing together the scientific potential of the BioSC Core Groups across disciplines. A thematic focus of multidisciplinary research topics was compiled at the end of the first funding phase. This resulted in the definition of four thematic priorities, which represent the content framework of the strategy project.

The four focus topic areas are:
- Smart management for plant performance
- Modular biotransformations for high-value compounds
- Integrated biorefineries for sustainable processes and products
- Technological and institutional innovations as drivers of bio-based social transformations.

The project formats in the NRW BioSC Strategy Project range from Seed Fund projects lasting one or two years, in which completely new explorative project topics are tested, to Boost Fund projects lasting three years, in which bioeconomy-relevant research approaches from the BioSC are further developed, up to large, multidisciplinary collaborative projects, the so-called FocusLabs, which were established in the second funding phase and are managed by young scientists.

All project formats have one thing in common: following the multidisciplinary and cross-sectional integrative approach of the BioSC, projects must always involve scientists from at least two research areas and two BioSC partner institutions. In the thematic chapters of the brochure, 17 projects from a total of 58 projects are presented as examples.

In addition to an intensive cooperation of the 67 Core Groups, cooperations with partners from science and industry in the regional, national and international area play an important role. The BioSC`s combined scientific strength and innovation potential as well as its established bioeconomy community in the region has significantly promoted the development of the structural change initiative BioökonomieREVIER. Since its establishment in 2019, this initiative aims to develop a model region for resource-efficient, sustainable economic activity in the Rhenish mining area (see Chapter V). The BioSC supports the initiative through systemic research and, through its long-term funding, creates a knowledge base and innovation potential for the development of a sustainable bioeconomy in the region and beyond.

The transformation to resource-saving and bio-based actions and economies requires new training concepts for young scientists and further training in business and science (see Chapter IV) and an intensive dialogue with society, which is practiced, for example, through BioSC exhibitions, participation in science nights and citizens' forums.

Ten years after its founding, the BioSC has developed into a renowned competence center for bioeconomy research in Germany and Europe, where numerous solution-oriented innovation approaches have been developed. Since 2013, around 180 publications in scientific journals as well as numerous patents have resulted from the NRW Strategy Project BioSC. The training of young scientists with excellent expertise and profound bioeconomy knowledge is also a success story that, in addition to educational contributions to lifelong learning, will be an important element in the BioSC mission in the future.

The BioSC is now on the threshold of the third funding phase of the NRW Strategy Project BioSC. Topics with high relevance for an integrated sustainable bioeconomy will be continued and further developed to implement the systemic perspective of the BioSC for bioeconomy research. The BioSC is well positioned for this task in order to make the vision of a sustainable, bio-based economy a reality together with regional, national and international partners in science, industry and society.

Exhibitions, science nights and citizens' forums offer an opportunity for intensive exchange with civil society.

Smart Management for Plant Performance

Plants have a key position in the establishment of a sustainable bioeconomy. They must provide the basis for healthy nutrition for a growing world population and will also be needed as a renewable raw material basis to replace fossil resources such as crude oil in the future. However, the areas globally available for agriculture can no longer be expanded considerably. The increase in plant biomass production must be realized on the same area and with the least possible environmental impact. For these reasons, intelligent solutions are needed to increase crop yields while protecting the environment. This includes the efficient and economical use of water, nutrients and plant protection products, protection of soils, closing of nutrient cycles and the development of new concepts for plant protection. At the same time, post-harvest losses such as the rotting of foods must be reduced significantly and plants must be adapted to changing climate and environmental conditions through breeding.

Over the past 15 years, plants that, for example, can use water or nutrients more efficiently or are resistant to pathogens have frequently been bred using the methods of modern plant breeding. Precision farming uses sensors to distribute quantities of fertilizer, pesticides and water that are precisely adapted to the current need within the acreage. Soil scientists are investigating how the composition of microorganisms in the soil influences plant growth and are developing new soil protecting cultivation concepts together with farmers. Land requirements and usage competitions can be minimized by the development and combination of different cultivation systems and technologies for the simultaneous production of energy and plants on one area (agrophotovoltaics). The combination of different approaches and expertise is the key to efficient and environmentally friendly plant production.

Research at the BioSC

In BioSC projects, the following main focuses of the focus topic area have been investigated to date:

- Development of new crop protection strategies through application of new selective active substances or activators to improve crop yield and plant health.
- New technology platforms for controlled release and application of bioactive substances in agricultural systems to significantly reduce the use of pesticides, fungicides and herbicides, for example, and to minimize yield losses in cultivation and after harvests.
- Identification and cultivation of plants with improved resource utilization, higher yields and suitable reproducible characteristics for material and energy use.
- Recovery of phosphate from plant residues and wastewater to close nutrient cycles.

Developments of the focus topic area are supported by two major research infrastructures in the BioSC: a) the Jülich Plant Phenotyping Center (JPPC), which provides technology platforms for non-invasive phenotyping of plants at shoot, root and stock level in high-throughput, and b) the Kleinaltendorf Campus, which, as a modernly equipped agricultural experimental station of the University of Bonn, offers a wide variety of cultivation systems in the field and in greenhouses.

BioSC Research Projects in the Focus Topic Area

C4-PSH 01/14 – 12/15	C4 plant selective herbicides: a new approach to combat C4 weeds in arable crops	Biochemical Plant Physiology, Pharmaceutical and Medical Chemistry (HHU) Systemic Microbiology, Plant Sciences (FZJ)
OrCaCel 07/14 – 09/16	OrganoCat plant & pulping combinations for the full valorization of lignocellulose from perennial plants from marginal land	Botany and Molecular Genetics, Technical Chemistry and Petrochemistry (RWTH) Plant Sciences (FZJ)
EtMeD 08/14 – 07/15	Development of an ethylene measuring device for ethylene receptor characterization of plants	Biochemical Engineering (RWTH) Biochemical Plant Physiology (HHU)
MisQual 11/14 – 12/15	Genotype and environment as driving factors for quality of lignocelluloses from Miscanthus as raw material for industry	Renewable Resources (U Bonn) Microbial Genetics, Botany and Molecular Genetics (RWTH)
GreenGel 11/14 – 12/15	Bifunctional nanogel-based fertilizers for controlled nutrition of plants	Biotechnology, Functional and Interactive Polymers (RWTH) Plant Nutrition (U Bonn)
LIPANO 01/15 – 12/15	Glucosinolate break-down products as modifiers of soil fungal lipids, protein profiles and allelopathy: impacts on nodulation, mycorrhiza and growth of Fabaceae	Biotechnology of Plants (U Bonn) Molecular Enzyme Technology (HHU)
P-ENG 01/15 – 12/16	Efficient phosphate recovery from agro waste streams by enzyme, strain and process engineering	Microbiology, Biotechnology (RWTH) Systems Biotechnology (FZJ) Technology and Innovation Management (U Bonn)
NovoSurf 04/15 – 03/16	Identification of novel natural microbial surfactants	Molecular Phytomedicine (U Bonn) Microbiology (RWTH)
BiFuProts 04/15 – 06/17	Bifunctional proteins for plant protection	Biotechnology, Plant Physiology (RWTH) Biochemistry I (HHU) Horticultural Science (U Bonn)
PhytaPhoS 04/15 – 03/18	Optimizing the phosphorus cycle in the sugar beet production process by phytase supplement	Biotechnology (RWTH) Plant Sciences (FZJ)
RIPE 10/15 – 12/17	Ripening delay of climacteric fruits by peptides	Biochemical Plant Physiology, Pharmaceutical and Medical Chemistry (HHU) Biotechnology, Functional and Interactive Polymers (RWTH) Horticultural Science (U Bonn)
AlgalFertilizer 11/15 – 10/17	Algae delivering waste phosphorus to soil and crops	Quantitative and Theoretical Biology , Biochemistry of Plants (HHU) Plant Sciences, Agrosphere (FZJ) Soil Sciences (U Bonn)
PrimACs 11/15 – 10/17	Priming-active compounds for plant protection	Plant Physiology, Biochemical Engineering (RWTH) Bioorganic Chemistry (HHU)

BioSC Research Projects in the Focus Topic Area

InducTomE 11/15 – 12/17	Induction of secondary metabolites in tomato by-products for extraction and economic evaluation of the model process	Botany and Molecular Genetics, Fluid Process Engineering (RWTH) Plant Sciences (FZJ) Horticultural Sciences, Technology and Innovation Management (U Bonn)
SPREAD 11/15 – 10/18	Silphium perfoliatum – resource evaluation and development	Developmental and Molecular Biology of Plants (HHU) Renewable Resources (U Bonn) Enzyme Process Technology (RWTH)
PlaMint 11/15 – 10/18	Investigate plant-microbe interactions to improve plant health and productivity	Chemical Signalling (U Bonn) Microbiology (HHU) Plant Physiology, Botany and Molecular Genetics (RWTH) Plant Sciences (FZJ)
AP³ 04/17 – 12/20	Advanced pulping for perennial plants: a holistic and sustainable integrated lignocellulose biorefinery concept	Plant Sciences (FZJ) Botany and Molecular Genetics, Technical Chemistry and Petrochemistry, Fluid Process Engineering, Process Systems Engineering (RWTH) Developmental and Molecular Biology of Plants, Plant Cell Biology and Biotechnology (HHU) Technology and Innovation Management (U Bonn)
greenRelease 01/18 – 06/21	GreenRelease for plant health	Biotechnology, Plant Physiology, Functional and Interactive Polymers (RWTH) Technology and Innovation Management, Horticultural Sciences, Molecular Biology of the Rhizosphere (U Bonn) Biochemical Plant Physiology, Pharmaceutical and Medical Chemistry (HHU) Plant Sciences (FZJ)
QuantiP 11/18 – 12/19	P-quantification in vivo and in vitro by Raman spectroscopy and NMR	Biotechnology (RWTH) Plant Sciences, Analytics (FZJ)
iBiomass 11/18 – 12/19	Improve maize biomass for processing applying OrganoCat technology	Microbiology, Plant Cell Biology and Biotechnology (HHU) Technical Chemistry and Petrochemistry (RWTH)
PepUse 09/19 – 02/21	Peptide adhesion promoters for user centered plant health applications	Biotechnology (RWTH) Physical Biology (HHU) Technology and Innovation Management (U Bonn)
TaiLead 08/20 – 10/21	Lead verification of tailored prodiginine derivatives	Molecular Enzyme Technology (HHU) Bioorganic Chemistry (HHU) Molecular Phytomedicine (U Bonn)

FOCUS TOPIC AREA

Modular Biotransformations for High-Value Compounds

The production of chemicals and materials in a sustainable bioeconomy is marked by new process chains in which biocatalysis with microorganisms and enzymes plays an important role. Biogenic raw materials include a wide range, from perennial biomass plants to agricultural waste streams. Increasingly, these are joined by non-biogenic carbon sources such as CO_2 or municipal waste streams such as plastic waste. In order to cover this broad range of source materials, the conventional "one substrate – one product" concept must be further developed into flexible and modular "multi substrate – multi product" process chains in which chemocatalysis and biocatalysis must be combined.

Synthetic biology as the driving force for molecular biotechnology provides a variety of new concepts to meet these changing demands. One of the greatest challenges is to make the resulting production processes and products usable and competitive compared with existing oil-based processes. The greatest prospects for success are in the area of high-value compounds, some of which have new functionalities such as fine chemicals, natural substances or proteins. Since these classes of substances have complex synthesis pathways, modular multi-step processes are the most promising. In addition, considerable added value can be achieved in the use of side-streams of bioeconomic process chains if further valuable fine chemicals or pharmaceutically usable substances are obtained in addition to the main products.

Research at the BioSC

In BioSC projects, the following main focuses of the focus topic area have been investigated to date:

- Hybrid processes that modularly combine whole-cell transformations, enzymatic and chemical transformations using customized bio-and chemocatalysts.
- Identification of naturally occurring bioactive substances and their synthesis pathways and modelling of new bioactive substances and synthesis pathways using bioinformatics methods.
- Establishment of synthesis pathways for bioactive substances, especially for secondary metabolites, and of synthetic enzyme cascades for the flexible modular synthesis of fine chemicals.
- Development of synthetic microcompartments and enzyme immobilization for handling low concentration, insoluble, unstable or toxic intermediate compounds.
- Provision of well-characterized and genetically accessible platform organisms (chassis organisms, microbial and plant cell factories).

The developments in the focus topic area are supported by two major research infrastructures: a) the "Microbial Phenotyping Center" (JMPC) established at Forschungszentrum Jülich, which allows detailed quantitative characterization of bioprocesses at all levels of the omics methods, single-cell analysis and process characterization, and b) the platforms for bioanalytics and process characterization existing in Düsseldorf, Jülich and Aachen which allow the topics to be addressed using state-of-the-art methods.

BioSC Research Projects in the Focus Topic Area		
CoSens 01/14 – 12/14	Genetically encoded biosensors for dynamic and quantitative sensing of metabolic cofactors in subcellular compartments	Chemical Signalling (U Bonn) Molecular Enzyme Technology, Biochemistry of Plants (HHU)
UstiLyse 01/14 – 12/14	Improving plant biomass conversion by Ustilago maydis for sustainable production of platform chemicals	Microbiology (HHU) Microbiology (RWTH)
PNP-EXPRESS 01/14 – 12/15	Discovery and microbial production of natural products from plants	Systemic Microbiology (FZJ) Molecular Enzyme Technology (HHU) Botany and Molecular Genetics (RWTH)
BIOEx-presSPro 01/14 – 12/16	Research and technology platform for the identification, production, secretion and optimization of enzymes	Molecular Enzyme Technology (HHU) Systems Biotechnology, Systemic Microbiology (FZJ) Botany and Molecular Genetics, Biotechnology (RWTH)
GLUFACT 01/14 – 12/16	Gluconobacter-Factory: strains and processes for stereoselective oxidative conversion of renewable carbon sources to industrial products	Systemic Microbiology (FZJ) Botany and Molecular Genetics, Biochemical Engineering (RWTH) Microbiology und Biotechnology (U Bonn)
FlowCom 07/14 – 09/15	Everything in flow – new compartments for cascade reactions	Physical Chemistry, Enzyme Process Technology (RWTH) Bioorganic Chemistry (HHU)
MoRe-Plants 07/14 – 06/16	Towards alkaloids: monooxygenases from plants in reaction cascades	Bioorganic Chemistry, Biochemistry II (HHU) Biochemical Engineering , Biotechnology, Botany and Molecular Genetics (RWTH)
GreenGel 11/14 – 12/15	Bifunctional nanogel-based fertilizers for controlled nutrition of plants	Biotechnology, Functional and Interactive Polymers (RWTH) Plant Nutrition (U Bonn)
BioBreak 11/14 – 12/16	Enzymes for biomass breakdown	Molecular Enzyme Technology, Biochemistry I (HHU) Enzyme Process Technology , Biochemical Engineering (RWTH)
TPOT 01/15 – 12/15	A proof of concept for an E. coli hydroxylation platform with efficient terpene/terpenoid translocation through the outer membrane	Biotechnology, Botany and Molecular Genetics (RWTH) Biochemistry II (HHU)
P-ENG 01/15 – 12/16	Efficient phosphate recovery from agro waste streams by enzyme, strain and process engineering	Microbiology, Biotechnology (RWTH) Systems Biotechnology (FZJ) Technology and Innovation Management (U Bonn)
CatIBs 01/15 – 12/17	Catalytically active inclusion bodies: new carrier-free enzyme immobilizes for biocatalysis	Molecular Enzyme Technology (HHU) Biochemical Engineering (RWTH) Systems Biotechnology (FZJ)
UstiOpt 03/15 – 03/16	Defining optimum growth conditions for Ustilago maydis to increase the production of recombinant proteins	Microbiology (HHU) Biochemical Engineering (RWTH)

BioSC Research Projects in the Focus Topic Area		
DiPro 04/15 – 03/16	Dirigent proteins in bioeconomy	Bioorganic Chemistry (HHU) Botany and Molecular Genetics (RWTH)
ROXENSE 04/15 – 03/16	Efficient redox sensing and engineering for optimization of biocatalysis	Microbiology (RWTH) Chemical Signalling (U Bonn)
MoniCon 04/15 – 03/16	Establishing online methodology to monitor growth of single species in fungal consortia	Biochemical Engineering (RWTH) Microbiology (HHU)
BiFuProts 04/15 – 06/17	Bifunctional proteins for plant protection	Biotechnology, Plant Physiology (RWTH) Biochemistry I (HHU) Horticultural Science (U Bonn)
VariSurf 09/15 – 11/16	Production of glycolipid variants for industrial application by smart genetic engineering	Molecular Enzyme Technology, Microbiology (HHU) Biochemical Engineering (RWTH)
BioSAF 10/15 – 12/16	Carotenoid cleaving enzymes for efficient biosynthesis of Saffron	Biotechnology, Botany and Molecular Genetics (RWTH) Molecular Enzyme Technology (HHU)
RIPE 10/15 – 12/17	Ripening delay of climatic fruits by peptides	Biochemical Plant Physiology, Computational Pharmaceutical Chemistry and Molecular Bioinformatics (HHU) Biotechnology, Functional and Interactive Polymers, (RWTH) Horticultural Science (U Bonn)
PrimACs 11/15 – 10/17	Priming-active compounds for plant protection	Plant Physiology, Biochemical Engineering (RWTH) Bioorganic Chemistry (HHU)
PectiLyse 11/15 – 10/17	Activation of intrinsic enzymes for degradation of plant biomass side-streams	Microbiology (HHU) Microbiology, Biochemical Engineering (RWTH)
BioCaPS 01/16 – 12/16	Structural insights of biohybrid catalysts for polyactide synthesis	Organometallic Chemistry, Biotechnology (RWTH) Biochemical Plant Physiology, Physical Biology (HHU)
HiQFlux 11/16 – 12/17	Quantifying metabolic network operation in compartmentalized organisms – yeast as a eucaryotic model	Microbiology (RWTH) Systems Biotechnology (FZJ)
CombiCom 05/17 – 12/20	Combinatorial creation of structural diversity for novel high-value compounds	Molecular Enzyme Technology, Microbiology, Synthetic Biology, Bioorganic Chemistry (HHU) Biotechnology, Biochemical Engineering (RWTH), Plant Sciences (FZJ) Molecular Phytomedicine, Technology and Innovation Management (U Bonn)
Bio² 05/17 – 12/20	Integration of next generation biosurfactant production into biorefinery processes	Microbiology, Molecular Enzyme Technology (HHU) Biochemical Engineering , Microbiology, Fluid Process Engineering, Chemical Process Engineering (RWTH) Systems Analysis and Technology Evaluation (FZJ)

BioSC Research Projects in the Focus Topic Area

greenRelease 01/18 – 06/21	GreenRelease for plant health	Biotechnology, Plant Physiology, Functional and Interactive Polymers (RWTH) Technology and Innovation Management, Horticultural Sciences, Molecular Biology of the Rhizosphere (U Bonn) Biochemical Plant Physiology, Pharmaceutical and Medical Chemistry (HHU) Plant Sciences (FZJ)
HyImPAct 01/18 – 09/21	Hybrid processes for important precursor and active pharmaceutical ingredient	Systems Biotechnology (FZJ) Bioorganic Chemistry (HHU) Fluid Process Engineering, Operations Management (RWTH)
HySyn 10/18 – 12/19	Fatty acid decarboxylases for hydrocarbon synthesis	Molecular Enzyme Technology (HHU) Botany and Molecular Genetics (RWTH) Plant Sciences (FZJ)
R2HPBio 10/18 – 12/20	Renewables to high-performance bioplastics through sustainable production ways	Bioinorganic Chemistry, Fluid Process Engineering (RWTH) Macromolecular Chemistry (HHU)
XyloSens 10/18 – 03/21	Development of a xylose sensor toolbox for microbial process monitoring and control	Systems Biotechnology (FZJ) Physical Biology (HHU) Biochemical Engineering (RWTH)
QuantiP 11/18 – 12/19	P-quantification in vivo and in vitro by Raman spectroscopy and NMR	Biotechnology (RWTH) Plant Sciences, Analytics (FZJ)
GlycoHype 09/19 – 10/20	Synthesis of glycosides by hyperthermophilic glycosidases	Biotechnology and Biomaterials, Biochemical Engineering (RWTH) Molecular Enzyme Technology, Pharmaceutical and Medical Chemistry (HHU)
PepUse 09/19 – 02/21	Peptide adhesion promoters for user centered plant health applications	Biotechnology (RWTH) Physical Biology (HHU) Technology and Innovation Management (U Bonn)
HaloEnz 09/19 – 08/21	Enzymatic halogenation: enzyme identification, characterization, application	Pharmaceutical and Medical Chemistry, Bioorganic Chemistry (HHU) Botany and Molecular Genetics (RWTH)
TaiLead 08/20 – 10/21	Lead verification of tailored prodiginine derivatives	Molecular Enzyme Technology (HHU) Bioorganic Chemistry (HHU) Molecular Phytomedicine (U Bonn)
DesignR 08/20 – 10/21	Tailoring Biosurfactants – Production of Designer Rhamnolipids	Biochemical Engineering (RWTH) Molecular Enzyme Technology (HHU) Fluid Process Engineering (RWTH)

Integrated Biorefineries for Sustainable Processes and Products

The development of biorefinery concepts and systems in which the provision of biomass and its conversion into products are integrated represent a key element for the transformation from an oil-based economy to a bioeconomy. Different bio-based raw materials must be utilized for the process: plants specifically cultivated for energy or material use, which may have been improved for later use by breeding, aquatic plants, waste materials or side streams from agriculture, the food industry and forestry, as well as residual materials from the paper industry. For energy or material use, perennial plants might be preferred if these can provide appropriate yields on otherwise unused poor soils that are unsuitable for agricultural production of food.

A biorefinery must not only be able to process a broader and more variable range of feedstocks than conventional oil refineries. Rather, completely new processes and procedures must be developed. In established petrochemical processes, the feedstocks are transformed in organic solvents, often at high temperatures, whereas the components of biomass require conversion processes at low temperatures in electrolytic solvents. Biotechnological processes for the production of platform chemicals require large quantities of auxiliaries and water. In order to allow up-scaling to production scale, methods are needed for recycling of solvents, catalysts and process water within a biorefinery. For residual material from the processed biomass, such as minerals or organic residues, opportunities must be created for recycling into material cycles, such as agricultural fertilizer.

Research at the BioSC

In BioSC projects, the following main focuses of the focus topic area have been investigated to date:

- Development and optimization of chemical methods for the digestion of lignocellulosic biomass.
- Identification of suitable enzymes for the digestion of lignocellulosic biomass and development of production platforms for their cost-effective production.
- Microbial exploitation of sugar beet residues.
- Methods for the gentle separation of valuable plant-based ingedients such as lignin or secondary metabolites.
- Integrated processes for the production and purification of high-value compounds using new technologies.
- Computer-aided modelling of biorefinery processes.

The developments in the focus topic area are supported by the research biorefinery NGP² of RWTH Aachen. The flexible and modular concept of the NGP² biorefinery allows the use of diverse biomass sources and residue streams as well as the separation of valuable plant-based ingredients and the recycling of solvents and auxiliaries. This provides the basis for a techno-economic analysis which is the basis for the assessment of possible business models.

BioSC Research Projects in the Focus Topic Area		
UstiLyse 01/14 – 12/14	Improving plant biomass conversion by Ustilago	Microbiology (HHU) Microbiology (RWTH)
EnZIP 01/14 – 12/15	maydis for sustainable production of platform chemicals	Molecular Biotechnology, Botany and Molecular Genetics (RWTH) Biochemistry II (HHU) Plant Sciences (FZJ)
GLUFACT 01/14 – 12/16	Gluconobacter-Factory: strains and processes for stereoselective oxidative conversion of renewable carbon sources to industrial products	Systemic Microbiology (FZJ) Molecular Enzyme Technology (HHU) Botany and Molecular Genetics (RWTH)
BIOEx-presSPro 01/14 – 12/16	Research and technology platform for the identification, production, secretion and optimization of enzymes	Molecular Enzyme Technology (HHU) Systems Biotechnology, Systemic Microbiology (FZJ) Botany and Molecular Genetics, Biotechnology (RWTH)
OrCaCel 07/14 – 09/16	OrganoCat plant & pulping combinations for the full valorization of lignocellulose from perennial plants from marginal land	Botany and Molecular Genetics, Technical Chemistry and Petrochemistry (RWTH) Plant Sciences (FZJ)
BEProMod 07/14 – 06/17	Incremental multi-scale and multi-disciplinary modeling of processes in Bioeconomy	Systems Biotechnology (FZJ) Process Systems Engineering, Software and Tools for Computational Engineering (RWTH)
BioBreak 11/14 – 12/16	Enzymes for biomass breakdown	Molecular Enzyme Technology, Biochemistry I (HHU) Enzyme Process Technology , Biochemical Engineering (RWTH)
MoniCon 04/15 – 03/16	Establishing online methodology to monitor growth of single species in fungal consortia	Biochemical Engineering (RWTH) Microbiology (HHU)
PectiLyse 11/15 – 10/17	Activation of intrinsic enzymes for degradation of plant biomass side-streams	Microbiology (HHU) Microbiology, Biochemical Engineering (RWTH)
InducTomE 11/15 – 12/17	Induction of secondary metabolites in tomato by-products for extraction and economic evaluation of the model process	Botany and Molecular Genetics, Fluid Process Engineering (RWTH) Plant Sciences (FZJ) Horticultural Sciences, Technology and Innovation Management (U Bonn)
SPREAD 11/15 – 10/18	Silphium Perfoliatum – resource evaluation and development	Developmental and Molecular Biology of Plants (HHU) Renewable Resources (U Bonn) Enzyme Process Technology (RWTH)
MetEvo 01/16 – 12/16	Metabolic evolution of microorganisms for the efficient utilization of carbon sources	Biochemical Engineering (RWTH) Systemic Microbiology (FZJ)
AquaPro 04/16 – 03/17	Using invasive alien aquatic plant biomass for bioenergy production	Biochemistry of Plants (HHU) Biochemical Engineering (RWTH)
BioDeg 12/16 – 12/17	Boosting plant biomass degradation by combined use of lignin- and cellulose-degrading enzymes	Biochemistry II (HHU) Botany and Molecular Genetics (RWTH)

BioSC Research Projects in the Focus Topic Area

AP³ 04/17 – 12/20	Advanced pulping for perennial plants: a holistic and sustainable integrated lignocellulose biorefinery concept	Plant Sciences (FZJ) Botany and Molecular Genetics, Technical Chemistry and Petrochemistry, Fluid Process Engineering, Process Systems Engineering, Technology and Innovation Management (RWTH) Developmental and Molecular Biology of Plants, Plant Cell Biology and Biotechnology (HHU)
Bio² 05/17 – 12/20	Integration of next generation biosurfactant production into biorefinery processes	Bioprocess Engineering , Microbiology, Fluid Process Engineering, Chemical Process Engineering (RWTH) Microbiology, Molecular Enzyme Technology (HHU) Systems Analysis and Technology Evaluation (FZJ)
HyImPAct 01/18 – 09/21	Hybrid processes for important precursor and active pharmaceutical ingredient	Systems Biotechnology (FZJ) Bioorganic Chemistry (HHU) Fluid Process Engineering, Operations Management (RWTH)
XyloSens 10/18 – 03/21	Development of a xylose sensor toolbox for microbial process monitoring and control	Systems Biotechnology (FZJ) Physical Biology (HHU) Biochemical Engineering (RWTH)
iBiomass 11/18 – 12/19	Improve maize biomass for processing applying OrganoCat technology	Microbiology, Plant Cell Biology and Biotechnology (HHU) Technical Chemistry and Petrochemistry (RWTH)
Lignin2Value 09/19 – 02/21	Valorization of lignin from agricultural residues for integrated biorefinery	Biochemistry II (HHU) Plant Sciences, Systems Biotechnology (FZJ)
DesignR 08/20 – 10/21	Tailoring Biosurfactants – Production of Designer Rhamnolipids	Biochemical Engineering (RWTH) Molecular Enzyme Technology (HHU) Fluid Process Engineering (RWTH)
LIFT 07/20 – 6/21	Lignin fractionation and separation to produce different technical lignins	Plant Sciences (FZJ) Fluid Process Engineering (RWTH)

Technological and Institutional Innovations as Drivers of Bio-Based Social Transformations

The goal of an economically, ecologically and socially sustainable bioeconomy is to secure the prosperity of present and future generations within the planetary boundaries. This will only be possible with comprehensive social and economic changes. New goods must be produced using new raw materials and processes, but this can succeed only if they are demanded and socially accepted. The transition from a fossil-based to bio-based economy will take place only with substantial changes in, for example, consumption patterns, value networks, business models, infrastructures and regulatory frameworks.

Technological and institutional innovations are key drivers of such transformation processes. However, they must be accompanied by the analysis of potential conflicts of goals such as nutrition versus material use of plants, by the analysis of the competitiveness of new products versus established oil-based alternatives and by investigations of the social acceptance of new technologies. Thus, transformation pathways can systematically be identified that are at the same time a) desirable from the sustainability perspective, b) possible from the techno-economic point of view and c) acceptable from the societal point of view.

Research at the BioSC

In BioSC projects, the following main focuses of the focus topic area have been investigated to date:

- Markets in the bioeconomy: What are the societal needs and challenges today and in the future? How can the developments of new technologiesbe reconciled with needs and markets?
- Innovation and technology transfer: What factors promote or hinder innovations? How can new key technologies be recognized early? What are the potentials and risks of different application areas of biomass?
- Value chains: How can existing industry sectors and value chains be networked so that new technologies can be introduced? Which factors promote or hinder the emergence of new value chains?
- Sustainability: How are benefits and risks of new technologies such as synthetic biology perceived by society? How can conflicting goals such as usage competition be mitigated? How do regional bioeconomy strategies and different regulatory frameworks affect the development of new regional bioeconomies?

The BioSC offers competencies and scientific expertise in agricultural economics, economic sciences, political sciences, economic development, consumer psychology and socio-technical systems analysis. Their bundling and integration with scientific-technical competence allows an integrating, systematic development of system and transformation concepts for the implementation of a bioeconomy that is sustainable in all three dimensions in regional and global approaches.

BioSC Research Projects in the Focus Topic Area		
Econ-BioSC 03/15 – 02/16	Biomass flows and technological innovation win the bioeconomy: a global scenario analysis	Economics of Sustainable Land Use and Bioeconomy, Technology and Innovation Management (U Bonn)
P-ENG 01/15 – 12/16	Efficient phosphate recovery from agro waste streams by enzyme, strain and process engineering	Microbiology, Biotechnology (RWTH) Systems Biotechnology (FZJ) Technology and Innovation Management (U Bonn)
InducTomE 11/15 – 12/17	Induction of secondary metabolites in tomato by-products for extraction and economic evaluation of the model process	Botany and Molecular Genetics, Fluid Process Engineering (RWTH) Plant Sciences (FZJ) Horticultural Sciences, Technology and Innovation Management (U Bonn)
AP³ 04/17 – 12/20	Advanced pulping for perennial plants: a holistic and sustainable integrated lignocellulose biorefinery concept	Plant Sciences (FZJ) Botany and Molecular Genetics, Technical Chemistry and Petrochemistry, Fluid Process Engineering, Process Systems Engineering, Technology and Innovation Management (RWTH) Developmental and Molecular Biology of Plants, Plant Cell Biology and Biotechnology (HHU)
CombiCom 05/17 – 12/20	Combinatorial creation of structural diversity for novel high-value compounds	Molecular Enzyme Technology, Microbiology, Synthetic Biology, Bioorganic Chemistry (HHU) Biotechnology, Biochemical Engineering (RWTH) Molecular Phytomedicine, Technology and Innovation Management (U Bonn) Plant Sciences (FZJ)
Bio² 05/17 – 12/20	Integration of next generation biosurfactant production into biorefinery processes	Bioprocess Engineering, Microbiology, Fluid Process Engineering, Chemical Process Engineering (RWTH) Microbiology, Molecular Enzyme Technology (HHU) Systems Analysis and Technology Evaluation (FZJ)
greenRelease 01/18 – 06/21	GreenRelease for plant health	Biotechnology, Plant Physiology , Functional and Interactive Polymers (RWTH), Technology and Innovation Management, Horticultural Sciences, Molecular Biology of the Rhizosphere (U Bonn) Biochemical Plant Physiology, Pharmaceutical and Medical Chemistry (HHU) Plant Sciences (FZJ)
HyImPAct 01/18 – 09/21	Hybrid processes for important precursor and active pharmaceutical ingredient	Systems Biotechnology (FZJ) Bioorganic Chemistry (HHU) Fluid Process Engineering, Operations Management (RWTH)

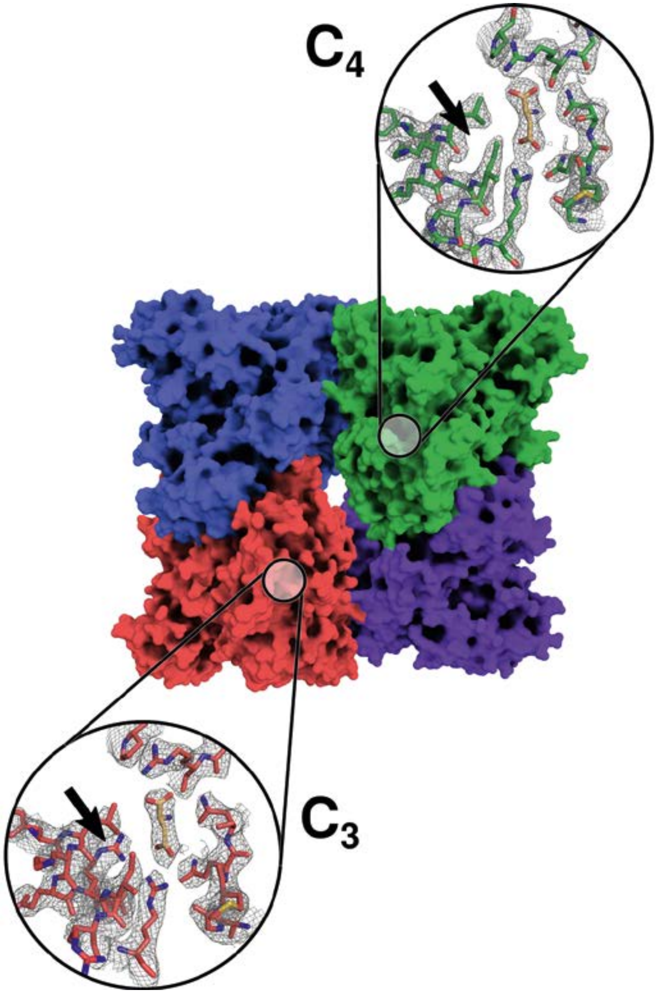
BioSC Research Projects in the Focus Topic Area		
PepUse 09/19 – 02/21	Peptide adhesion promoters for user centered plant health applications	Biotechnology (RWTH) Physical Biology (HHU) Technology and Innovation Management (U Bonn)
GreenToxiCon-omy 09/19 – 08/21	Green toxicology for a green bioeconomy	Environmental Research, Microbiology, Functional and Interactive Polymers (RWTH) Microbiology (HHU)
Transform2Bio 09/19 – 08/22	Integrated transformation processes and their regional implementations: structural change from fossil economy to bioeconomy	Systems Analysis and Technology Evaluation, Plant Sciences (FZJ) Economics of Sustainable Land Use and Bioeconomy, Economic and Technological Change, Economic Modeling of Agricultural Systems, Technology and Innovation Management, Agricultural and Food Market Research , Economic and Agricultural Policy, Production Economics (U Bonn) Operations Management (RWTH)

BOOST FUND **C4-PSH**

Natural Substances For Weed Control

A small but subtle difference in the photosynthesis pathways of many crop plants and associated yield-reducing weeds opens the door for BioSC scientists for selective and sustainable plant protection. Key elements in this concept are an enzyme that is substantially involved in biomass production in many weeds, and a natural substance from wood waste that inhibits it.

Domestic and global agriculture will face numerous challenges over the coming years to ensure the sustainable supply of the population with food and other agricultural products. These include the increasing shortage of the production factors soil and water, the strong dependence of harvest yields on fertilizers and plant protection products, as well as increasing climate change. Current climate models forecast increasingly prolonged droughts and higher average temperatures in summer, the main vegetation period in our agriculture.



3D structure of the plant photosynthetic key enzyme phosphoenolpyruvate carboxylase. The circular insertion on both sides shows the molecular differences that are relevant for selective binding of the natural substance okanin in the binding site of the C_3 and C_4 form of the enzyme.

Small difference – great effect

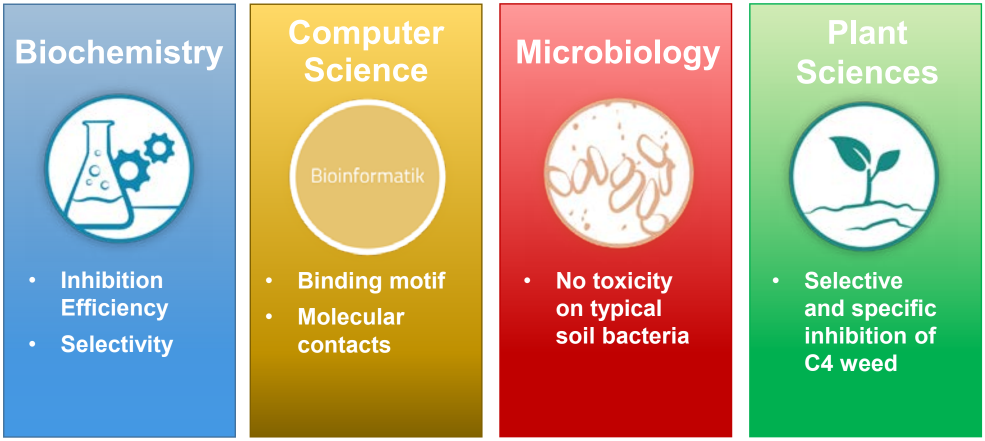
The metabolic pathway by which our crop plants form nutrients from sunlight, water, and carbon dioxide is not designed to tolerate prolonged drought or high temperatures. Domestic crop plants that use this metabolic pathway, known as "C_3 photosynthesis", for their growth and development, include oilseed rape and sugar beet. In contrast to these two typical representatives of the C_3 photosynthesis pathway, common amaranth, which is adapted to drier and warmer habitats, as well as many other ubiquitous, highly aggressive weeds use another metabolic pathway for their growth and development. This pathway, known as "C_4 photosynthesis", makes these plants exceptionally strong competitors of our crop plants, particularly with regard to the coming climate changes.

Investigation of the C_4 pathway and how it differs from the C_3 metabolism applied by most crop plants was the focus of the BioSC Boost Fund Project C4-PSH. The objective of the project was to identify molecular differences in the two photosynthesis pathways which can be used as a target for developing environmental-friendly plant protection products that specifically affect the C_4 pathway used by weeds but not the C_3 pathway used by crop plants.

Biochemical and structural studies at the Heinrich Heine University in Düsseldorf resulted in the identification of phosphoenolpyruvate carboxylase (PEPC), a key enzyme of the C_4 photosynthesis pathway, as an effective target. Although this enzyme is also present in C_3 plants, it is not of crucial importance for their growth and development. In contrast, C_4 plants cannot grow or survive without a functional form of this enzyme. By comparing the molecular structure of the C_4 form with the enzyme isoform found in C_3 plants, the Düsseldorf team was able to identify differences in a specific binding area of this complex molecule that can be used to shut down enzyme function.

Meticulous search pays off

Based on the structural differences of the two enzyme isoforms, the researchers used computer-based methods to identify a number of substances that bind to the C_4 isoform but not or only weakly to the C_3 form. They screened the most promising candi-



Contributions of the individual project groups in the interdisciplinary and cross-research field consortium to the discovery of the natural substance okanin for selective plant protection.

dates from comprehensive substance libraries consisting of more than 20 million compounds. In these analyses okanin, a natural substance, was identified which almost perfectly fits into the binding site of the C_4 enzyme. Although okanin can be produced synthetically, the natural product is also available by direct extraction from the wood of various industrially used tree species. In contrast to the specific binding to C_4 PEPCs, the compound shows no or extremely limited access to the corresponding binding pocket in C_3 PEPC. Subsequent laboratory experiments with both purified PEPC isoforms confirmed the selective inhibition of the C_4 enzyme.

Comparative plant studies at Forschungszentrum Jülich proved that the natural substance okanin not only causes the inhibition of the purified C_4 phosphoenolpyruvate carboxylase in the test tube but also inhibits the growth of typical C_4 weeds without affecting C_3 crop plants such as oilseed rape and sugar beet. Microbial tests carried out in the Jülich laboratories also showed that okanin has no negative effects on typical representatives of the bacterial soil flora. Okanin as well as derived natural and synthetic derivatives are thus attractive new compounds for selective plant protection.

The interdisciplinary research in the fields of structural research, molecular bioinformatics, microbiology, and plant sciences in the framework of the C4-PSH project, therefore, showed how the discovery of an environmentally friendly plant protection product can succeed and initial basic research can result in applied, socially relevant research.

Contact

Prof. Dr. Georg Groth,
Institute of Biochemical Plant Physiology, HHU Düsseldorf,
georg.groth@hhu.de

Core Groups involved

Prof. Dr. Georg Groth, Dr. Giang Nguyen,
Institute of Biochemical Plant Physiology, HHU Düsseldorf
Prof. Dr. Holger Gohlke, Dr. German Erlenkamp,
Computational Pharmaceutical Chemistry and Molecular Bioinformatics, HHU Düsseldorf
Prof. Dr. Michael Bott, Dr. Andreas Küberl,
IBG-1 Biotechnology, Forschungszentrum Jülich
Prof. Dr. Ulrich Schurr, Dr. Fabio Fiorani,
IBG-2 Plant Sciences, Forschungszentrum Jülich

Dual Plant Protectants of the Future

To minimize crop yield loss, there is increasing interest in natural or near-natural substances for effective and environmentally-safe and consumer-friendly plant protection. Of promise are chemical substances that not only fight a potential harmful organism but also strengthen the plant immune system and prepare the plant for possible future infection. Such substances have been identified in the boost fund project "Priming-Active Compounds for Plant Protection" (PrimACs).

The development of active substances has a long-standing tradition and is of high importance to Germany. Over the past decades, compound development has resulted in the marketing of many drugs and pesticides, which have an essential share in the economic success of the large pharmaceutical and agricultural industries. Domestic sales of German plant protectants was approximately 1.2 billion euros in 2019, for example. However, despite the great effectiveness of pesticides, at least 40 percent of potential crop yield is still lost to diseases and environmental stresses such as drought. This yield loss poses a serious threat that needs to be to be addressed. On the one hand, the world

population steadily increases whereas, on the other hand, plants are increasingly used not only for food production, but also for the provision of energy and plant-based products such as fibers or metabolites. Due to the low acceptance of genetically modified plants in Europe but with the simultaneous desire for environment-friendly agriculture, there is a great interest in natural or near-natural substances that enable effective and sustainable plant protection.

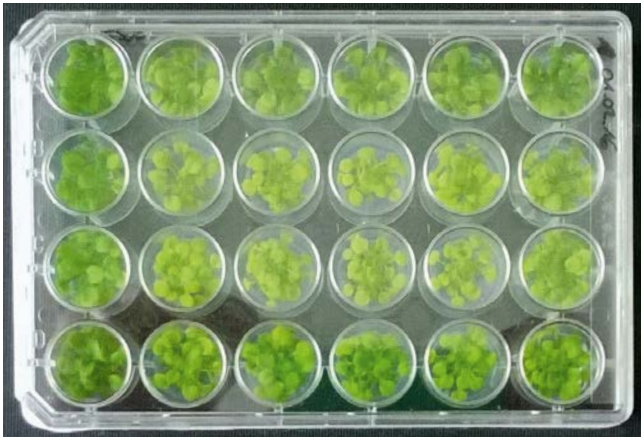
Advantages of dually active substances
Natural or near-natural substances are especially promising for environment-friendly plant protection if they not only kill a pathogen or pest but also prime the plant immune system for enhanced defense. Thus, the plant is prepared for a potential infection by pathogens or insect pests. Such dual acting substances otherwise hardly affect the plant. Unlike substances that directly activate the immune response of plants, PrimACs do not reduce yield. In addition, plant pathogens and pests cannot develop insensitivity to dual-acting substances. Therefore, active substances that combat pathogens or pests and simultaneously strengthen the plant immune system are the top sellers of agroindustry. However, plant protectants were often developed solely based on their toxic effect against pathogens or pests, so that their immune-stimulating effect, the so-called priming activity, was recognized only after their market launch. Targeted optimization as a dual-active substance was, therefore, not carried out during product development. This optimization requires appropriate test systems that are suitable for detecting the immune-stimulating activity of substances.

Finding new substances in a targeted approach
This need is addressed in the PrimACs project. A so far successfully used assay for spotting priming-inducing chemistry at RWTH Aachen uses suspension cells of parsley. If the cells are primed by initial contact with a pathogen or upon treatment with a PrimAC, they will secrete higher amounts of anti-microbial substances to the culture medium than unprimed cells. Since the substances fluoresce in ultraviolet light, they can easily be detected and quantified. The parsley test system has proven useful in the search for PrimACs in many ways for many years, for example regarding reliability and speed. However, the test is limited to undifferentiated culture cells and the parsley species. To identify PrimACs using intact plants of other species, the experts of different disciplines at the University Düsseldorf and RWTH Aachen University teamed up to identify new PrimACs.

First, the researchers synthesized enough quantities of more than 50 new, functionally and structurally diverse substances of good to excellent quality. In addition, they included a commercial reference library in the selection, so that a total of 76 substances were tested for their ability to activate priming in plants. While doing so, the collaborators used a novel miniature test system, the so-called µRAMOS, in which seedlings of thale cress were cultivated in microtiter plates. After treatment of the seedlings with a candidate substance, the respiration of the seed-

Novel miniature test system (µRAMOS), enabling determination of respiratory activity in each well of a microtiter plate thus spotting PrimACs.

lings served as a selection criterion, because respiration increases during activation of priming. Eight new substances were identified using this approach. They included 1-(3,4-dihydroxyphenyl)-2-oxocyclopentane-1-carbonic acid methyl ester, Tyr020 for short, and some other Tyr derivates synthesized by the interdisciplinary team. The priming function of the newly identified PrimACs was subsequently tested in the laboratory using molecular-biological and biochemical analyses. They included the analysis of genes and enzymes with a role in plant disease resistance. The analyses revealed that treatment of the thale cress seedlings with the newly identified PrimACs resulted in stronger

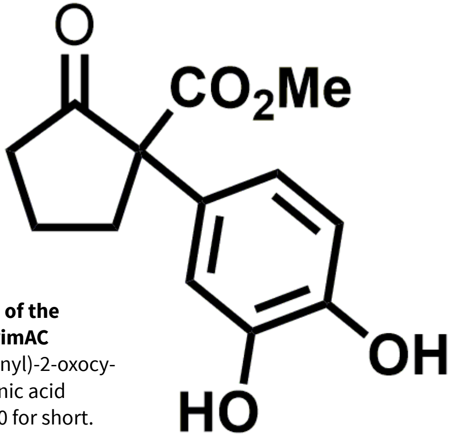


Seedlings of thale cress (*Arabidopsis thaliana*) in a microtiter plate

activation of defense responses upon subsequent contact with pathogens. This finding confirmed the priming effect of the newly identified PrimAC.

Successful greenhouse tests

The consortium then transferred the six most promising PrimACs to an agricultural company that tested the substances for their suitability for applied plant protection in the greenhouse. It turned out that Tyr020 protected cucumber from anthracnose disease. However, Tyr020 did not protect sufficiently against other diseases. The protective potential of the other PrimACs was overall too low to develop a marketable plant protectant. Nevertheless, the methodology developed provides a new and promising approach for identifying new substances for plant protection.



Chemical structure of the newly identified PrimAC
1-(3,4-dihydroxyphenyl)-2-oxocyclopentane-1-carbonic acid methyl ester), Tyr020 for short.

Contact

Prof. Dr. Uwe Conrath,
ABBt – Plant Physiology, RWTH Aachen,
uwe.conrath@bio3.rwth-aachen.de

Core Groups involved

Prof. Dr.-Ing. Jochen Büchs,
AVT – Bioprocess Engineering, RWTH Aachen

Prof. Dr. Jörg Pietruszka,
Institute of Bioorganic Chemistry, HHU Düsseldorf

Prof. Dr. Uwe Conrath,
ABBt – Plant Physiology, RWTH Aachen

Targeted Stress for Valuable Ingredients

Tomatoes are cultivated in greenhouses over a period of many months. The remaining tomato plants are composted at the end of the production phase in autumn. Is there any way to make better use of these leftovers? One idea for adding value to them was established in the project InducTomE. Tomato leaves contain, among other substances, rutin and solanesol, which are already marketed as food supplements or drugs. In order to significantly increase the concentration of these phytochemicals, the tomato plants were deliberately subjected to environmental stresses.

Tomatoes are one of the most widely cultivated fruits in the world. In Germany, tomatoes are mostly grown in greenhouses. There, the plants produce their fruits over months. At the end of tomato production in autumn, the plants are impressively tall. The large plants remain as residual biomass and leaves are also repeatedly removed from the plants between the individual fruit harvests. These leaves and residual plants contain bioactive ingredients that can be of industrial value. For example, they have antioxidant, antimicrobial or also virucidal effects. Such so-called phytochemicals often also have pharmacological effects and can be used as dietary supplements. Thus, the flavonoid

rutin contained in tomato leaves shows antioxidant and anti-inflammatory effects and rutin derivates are used in vein therapy. The isoprenoid solanesol is the precursor for coenzyme Q10 und vitamin K, which are used in the cosmetic industry and as food supplements.

Multiple benefits

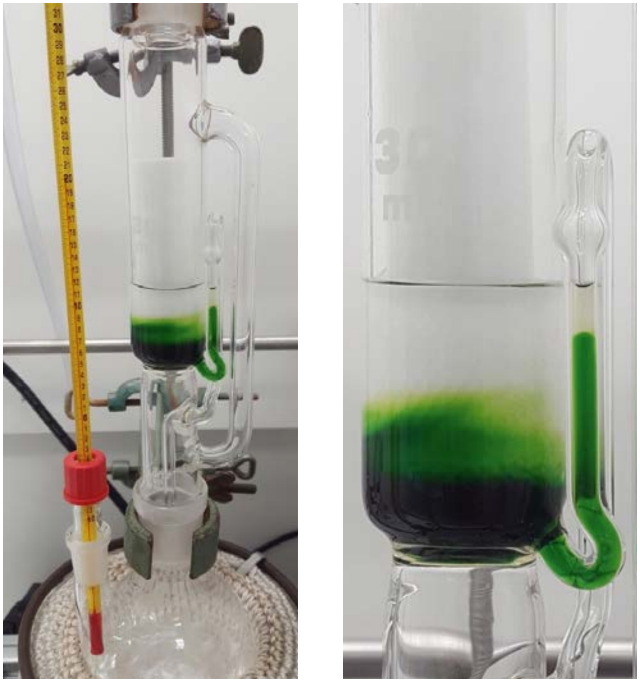
Plants produce such bioactive substances to protect themselves against environmental influences and to interact with the environment. In doing so, some phytochemicals are produced in greater quantities during environmental stress and protect plants against damage. The scientists working on the InducTomE project wanted to make use of this fact: following the last fruit harvest, the tomato plants were exposed to targeted environmental stresses such as heat, cold or nutrient deficiency to accumulate the desired ingredients rutin and solanesol. This would add value to plant residues that otherwise would usually be used only as compost at the end of the tomato production. The dual use of the plants for tomato production and for the extraction of valuable ingredients means an expansion of the value chain. In addition, the biomass can be used to produce energy and so-called bulk chemicals such as sugar or bioethanol. This is already planned in the project. Such value chains are resource-friendly since the cultivation of the corresponding energy or medical plants is saved.

Measurements of fluorescence parameters in the greenhouse

Plant scientists from Forschungszentrum Jülich and RWTH Aachen, together with horticultural scientists of the University of Bonn, tested the environmental conditions that cause the enrichment of rutin and solanesol. Many different stress conditions were initially tested on young plants and the most suitable were transferred to production greenhouses. The combination of nutrient deficiency, cold and increased light intensity resulted in a maximum increase in rutin content. Heat increased the solanesol content. In addition, the option to change the cultivation conditions already during fruit production and the effect on the ingredient content of the leaves as well as on fruit quality and yield was investigated. In order to also identify further usable phytochemicals, gene expression studies and untargeted metabolite analyses were carried out. These new identified ingredients could then also be produced and marketed by extraction or by biotechnological production after elucidation of their biosynthesis pathways.

Proof at the plant

To verify the successful application of stress conditions, optical methods were developed for detecting the increase in the quantity of rutin and solanesol. Different optical methods were tested, which can also be carried out by horticultural producers in greenhouses. Fluorescence measurements as well as simple quantification of the leaf color could be established to check the success of the applied stress conditions. The Chamber of Agriculture North Rhine-Westphalia at the Experimental Center Horticulture in Straelen supported the project by actively providing experimental setups and advising the project partners. The task of the process engineer at RWTH Aachen was to develop processes for the extraction and purification of phytochemicals from tomato leaves and subsequent biorefining of the plant residues. Concepts for an efficient, environmentally friendly process were developed that met the requirements of the potential



Extraction of ingredients from tomato leaves

market sectors for rutin and solanesol. An economic assessment of the process showed that the total costs could be significantly reduced by sequential extraction of the ingredients. In addition, the further usage of the tomato biomass in biorefinery processes turned out to be a suitable and efficient process.

Increasing profitability

Agricultural economists at the University of Bonn investigated possible ways to introduce the dual usage of tomato plants into the market. Two sectors were initially identified in which rutin and solanesol could be marketed and the corresponding regulations were checked. The participants involved in the production chains were then identified and their willingness to innovate was investigated. In addition, various market entry barriers that may be an obstacle for the new value chain were assessed. The results show that the economic efficiency can be significantly increased: first, by extracting and marketing several bioactive substances and, second, by reducing production costs, for example by using central extraction plants.

Meanwhile, the InducTomE consortium has transferred the idea to produce phytochemicals from leaves and residual plants to bell pepper production. The BMBF project TaReCa ran from 2017 to 2020 in collaboration with an advisory board from the aroma and fragrance industry, the biocide industry and horticulture, which brought in the interests and possibilities of potential industry partners.

Contact

Dr. Alexandra Wormit,
ABBt – Botany and Molecular Genetics, RWTH Aachen,
awormit@bio1.rwth-aachen.de

Core Groups involved

Prof. Dr. Björn Usadel[1], Dr. Alexandra Wormit, Dr. Julia J. Reimer,
ABBt – Botany and Molecular Genetics, RWTH Aachen

Prof. Dr. Ulrich Schurr, Dr. Anika Wiese-Klinkenberg[1], Dr. Björn Thiele, Dr. Laura Junker-Frohn,
IBG-2 Plant Sciences, Forschungszentrum Jülich

Prof. Dr. Georg Noga, PD Dr. Mauricio Hunsche, Dr. Simone Röhlen-Schmittgen, Dr. Tanja Groher,
INRES – Horticultural Sciences, University of Bonn

Prof. Dr. Andreas Jupke, Andreas Bednarz, Manuel Lück,
AVT – Fluid Process Engineering, RWTH Aachen

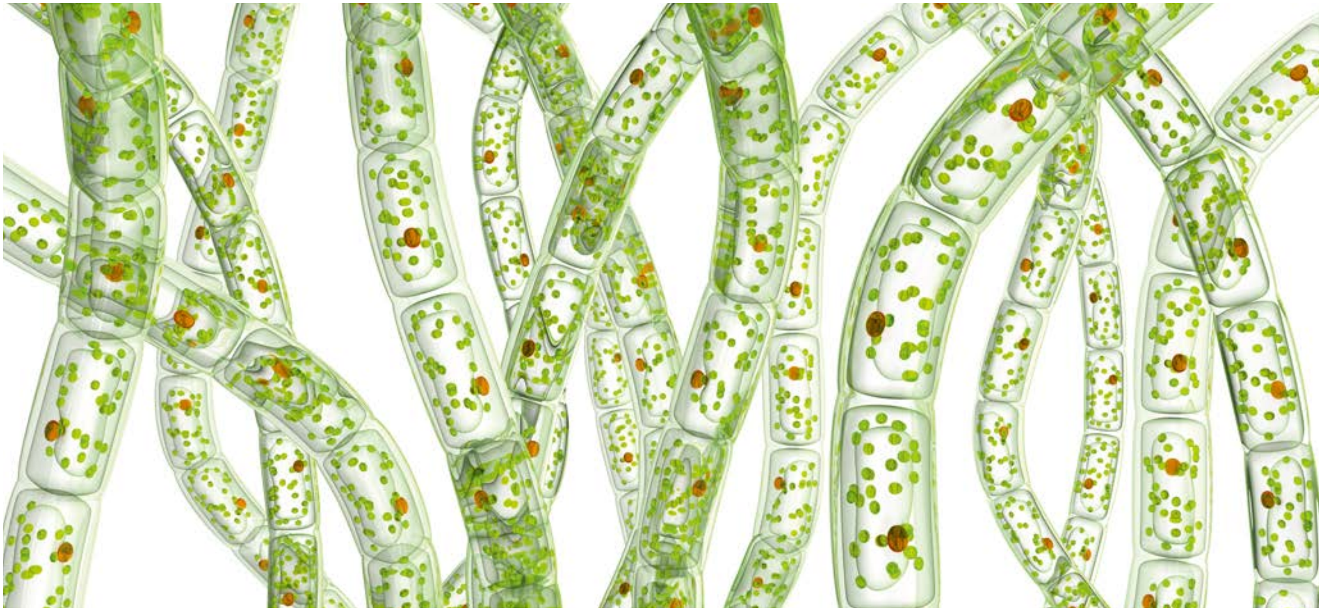
Prof. Dr. Stefanie Bröring, Dr. Laura Carraresi, Joana Wensing,
ILR – Technology, Innovation Management and Entrepreneurship, University of Bonn

[1] New address: IBG-4 Bioinformatics, Forschungszentrum Jülich

AlgalFertilizer – Nutrient Cycle with Algae, Sun and Wastewater

Our nutrient streams are inefficient and in some cases wasteful in terms of substance, energy, and water. Every opportunity to establish a real cycle should be seized to become more independent and future-oriented. Fast-growing and ingredient-rich microalgae are a promising and flexible resource of the future. Effective nutrient transfer back to the plant via microalgae fertilizer was shown in the AlgalFertilizer project.

In addition to light and water, plants also need nutrients, in particular a lot of phosphorus and nitrogen. Until now, large quantities of mineral fertilizer have been used. The phosphorus contained in these fertilizers is extracted from phosphorus-containing rocks that have formed over millions of years. Global deposits are limited and concentrated in a few high-yield locations. In view of independent food production, especially with regard to phosphorus, the most efficient resource recovery possible is an important investment in the future. Algae can play an important role here – they take up phosphorus very effectively from



their environment and grow much faster than land plants under appropriate light conditions and by uptake of climate-damaging CO_2. The AlgalFertilizer project with its five Core Groups in the BioSC has investigated the phosphorus uptake of microalgae from phosphate-containing water and wastewater and the transfer of this phosphorus from the microalgae biomass to wheat plants.

Algae are cultivated in tubes in the greenhouse or in fields and later used as fertilizer.

Good for the environment

Initially, the researchers were interested in how exactly the efficient phosphorus uptake in algae cells works. With this scientific understanding, they could identify especially effective algae species and implement further optimizations. Subsequently, they investigated whether these algae take up phosphorus completely from the water. In doing so, the algae could make an important contribution to water purification in treatment plants since the limit values for phosphorus in purified wastewater will be further lowered in the future – effective separation methods are therefore essential. At the same time, algae also take up nitrogen and both nutrients cause eutrophication, an overfertilization, in rivers and lakes if present in excess.

Another question is whether a plant can be adequately provided with the input of nutrient-rich algae biomass. The algae cells studied in the project contain the nutrients phosphorus and nitrogen in an optimal mixture for plant growth. Do these nutrients also reach the plant? Is an algae-based fertilizer even superior to a mineral fertilizer since the nutrients are released only gradually from the algae cells and thus can act more long-term as a kind of depot nutrient? To answer these questions, nutrients, water, algae, soil, and plants must be brought together experimentally to precisely monitor the nutrient transfer.

Successful tests

The algae were initially produced in a biomimetic bioreactor at Forschungszentrum Jülich. This produced cell densities more than twice as high due to especially effective light distribution. These algae were produced for the characterization of phosphorus uptake. For the significantly larger quantities of algae biomass needed for the field and pot experiments, the researchers produced the same algae in tubes in the greenhouse. One liter of algae solution usually yields only one to two grams of dried algae biomass. Thus, several cubic meters of algae solution must be cultivated for a sufficient quantity of fertilizer. Since this is carried out using sunlight in the greenhouse, this kind of production is less consistent than in the laboratory but reliably provides the required biomass.

Nutrient uptake from water showed that certain previously starved algae of the *Chlorella* species take up much more phosphorus than they need for their metabolism. This so-called luxury uptake accounts for up to eight percent of the dry mass while the phosphorus content is usually only one percent. To understand the underlying processes, the scientists analyzed the actual amount of phosphorus in the cells and the forms in which it is present. For example, the phosphorus can occur as free phosphate, as polyphosphate chains bound together or organically bound.

These forms affect plant availability of phosphorus after degradation of the algae biomass in the soil. It could be shown that the phosphorus accumulates in the cell in polyphosphate chains, a quickly available storage form. A complementary computer model from the Heinrich Heine University of the phosphorus metabolism simulates the growth of the algae with the data thus obtained. It describes the dynamic and the conversion between the different phosphorus forms in the cell. Conclusion:

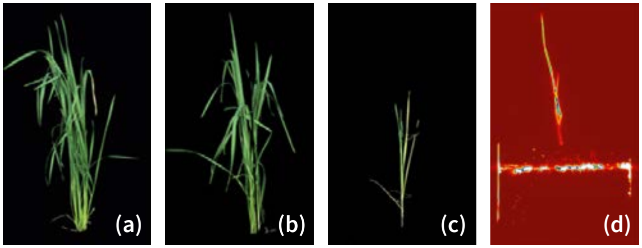
algae offer very good conditions to remove phosphorus from water or wastewater. Based on the results obtained, a promising algae fertilizer strategy can be developed.

Confirmed in nature

In an initial field experiment on previously normally fertilized soil, researchers of the University of Bonn compared the effect of algae, chicken dung, and mineral fertilizer on the germination and growth of wheat plants. The result: the plants grew unhindered in all three experimental designs. This was followed by automated pot experiments jointly designed by Forschungszentrum Jülich and the University of Bonn to compare the form of application: dry or fresh algal biomass and mineral fertilizer as a control in standard and shortage fertilization. Analyses of the growth and irrigation were carried out with a crane system equipped with a camera and enabled statistical evaluation. The wheat plants fertilized with algal biomass, dry as well as fresh algae fertilizer, were almost equivalent to the mineral fertilizer.

The question remained whether the phosphorus taken up by the plants actually comes from the algae or from microorganisms composting the algae or whether phosphorus already bound in the soil is dissolved again by these processes. Thus, the algae were supplied with radioactive ^{33}phosphorus in a controlled laboratory experiment and used as fertilizer in so-called rhizotron experiments. The wheat plants fertilized in this way were characterized at different development stages using a bio-imager for the visualization of radioactivity.

It could be shown in roots and leaves that indeed the algal phosphorus was taken up by the plants – initially delayed compared to the mineralized phosphorus but to the same extent after approximately three weeks. The delayed availability suggests an upstream release mechanism and thus a longer bioavailable residence in the topsoil. Algal-borne phosphorus thus delays fast phosphorus binding to minerals such as transfer to the subsoil and minimizes the loss of the nutrient with regard to the use by plants.



The growth of wheat plants with mineral fertilizer (a), with algae fertilizer (b), and without fertilizer (c). A special camera shows that the phosphorus taken up by the plant (d, bright red) indeed originates from the algae.

With these results, AlgalFertilizer confirms the potential of algae in the nutrient cycle. Future projects should then allow bioeconomic evaluation and usage.

Contact

Prof. Dr. Oliver Ebenhöh,
Institute of Quantitative and Theoretical Biology, HHU Düsseldorf
oliver.ebenhoeh@hhu.de

Core Groups involved

Prof. Dr. Ulrich Schurr, Dr. Christina Kuchendorf (author), Dr. Ladislav Nedbal (author),
IBG-2 Plant Sciences, Forschungszentrum Jülich

Prof. Dr. Wulf Amelung, Dr. Henning Schiedung,
INRES – Soil Science and Soil Ecology, University of Bonn

Prof. Dr. Harry Vereecken, Dr. Diana Hofmann, Dr. Nina Siebers,
IBG-3 Agrosphere, Forschungszentrum Jülich

Prof. Dr. Andreas Weber, Dr. Tabea Mettler-Altmann,
Institute for Biochemistry of Plants, HHU Düsseldorf

Prof. Dr. Oliver Ebenhöh,
Institute of Quantitative and Theoretical Biology, HHU Düsseldorf

Phosphate from Plant Biomass: Recovery and Commercialization

The need to recover essential resources, such as phosphate from side- or waste streams, confronts existing industries with numerous challenges. Bio-based technologies can be the solution here. However, existing obstacles include high conversion costs, lack of quality and industry standards and uncertain legal frameworks. The P-ENG project establishes a method for the recovery of phosphate from rapeseed press cakes and investigates how value chains that allow commercialization of this phosphate might look.

Phosphorous as a fossil resource is finite and is mined only in a few regions in the world, including Morocco. However, the raw material is an irreplaceable resource for living beings and many industries, such as fertilizer in agriculture or as an acidity regulator in food production. The world's phosphate deposits will last only approximately 300 years. Thus, strategies for sustainable phosphate usage and processes for the recovery of phosphate from unused side streams must be found. One example is the production of cooking oil in oil mills. In addition to the cooking

oil, which amounts to about 40% of the production, the by-product oil press cake is also produced. The remaining 60 percent of the total production contains a considerable quantity of plant-bound phosphorous in the form of the storage molecule phytate. In the current value chain, oil press cakes are further processed mainly in feed production.

Yeasts as helpers

This was the starting point for the BioSC project P-ENG. Initially, the recovery of phosphate from oil press cake was established using a phytase. Phytases are enzymes that can release phosphate by separating it from phytate. Using biotechnological methods, the phytase was optimized so that it is heat stable. The phytase was produced using a yeast that can use methanol as a carbon source.

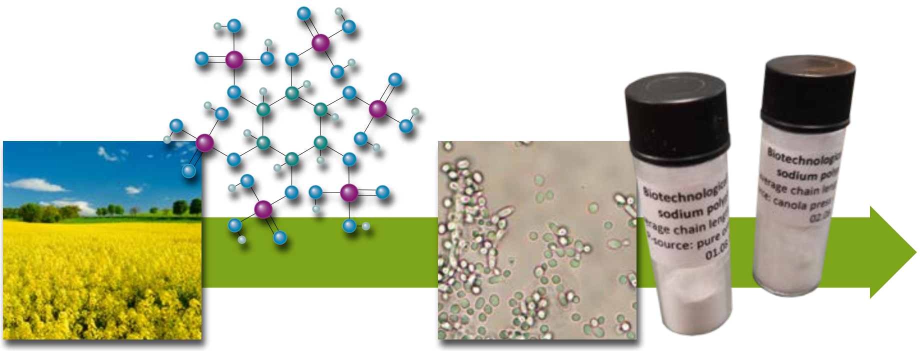
The concentration of phosphate was investigated using yeasts, which can store phosphate as polyphosphate. Polyphosphate-rich yeast extract or pure bio-based polyphosphate can be used as a food additive and thus presents a biological alternative to chemically produced polyphosphate.

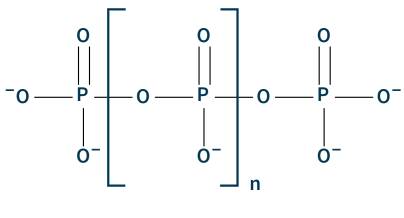
Furthermore, this technology offers the chance to produce feed with a lower phosphate content. This is because livestock such as pigs or poultry cannot adequately digest plant-bound phosphate in feed, so that unused phosphate reaches wastewater through the animals' excretions or onto fields through manure fertilization. The phosphates then pollute rivers and lakes. The recovery of phosphate before further processing to feed would counteract this problem.

New networking of markets

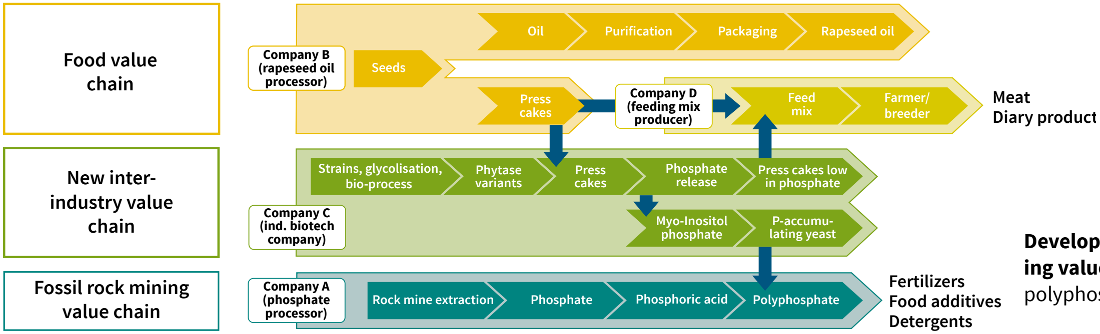
However, market implementation of this technical process is accompanied by challenges and changes in existing value chains. Within the framework of the P-ENG project, experts from classic polyphosphate producers, oil mills, enzyme producers and feed producers were interviewed to investigate these challenges for the existing market.

At the moment, there are no connections between the value chain of the oil mill and the polyphosphate producers. In the case of phosphate recovery from oil press cakes and the subsequent processing into polyphosphate, new value-adding networks must be established. The research teams derived different scenarios, depending on which actor assumes the process of enzymatic phosphorus separation. An important prerequisite for the implementation of the process is the investment in a bioreactor, which none of the actors already owns. Assuming that the classic polyphosphate producer undertakes the phosphate extraction, it would have to face the challenges of the lack of the biotechnological know-how and the geographic fragmentation of the oil sector. Furthermore, specific legal and customer-specific requirements on the polyphosphate must be met depending on the field of application. The new bio-based polyphosphate is associated with uncertainties since its exact characteristics remain to be investigated. The lack of standards for the new bio-based





Production of bio-based polyphosphate using plant material treated with phytase and phosphate-accumulating yeast.



Development of a new networking value chain for new bio-based polyphosphates.

polyphosphate also represents an obstacle that makes market implementation difficult. Depending on which actor assumes phosphorous extraction, a forward (e. g. oil mill) or backward (e.g. phosphate producer) integration is necessary in the value chain. Overall, organizational, regulatory, economic, geographic and product-related challenges were identified for the entire value chain.

Biotechnology as mediator

In contrast, there are the enormous opportunities for companies to contribute to a sustainable phosphate economy. The result of the interviews carried out in the framework of P-ENG with existing market participants was a completely new scenario: the researchers established an additional actor – the so-called collector. Since none of the industry sectors currently active in the market possesses all the necessary competences, the new actor could function as mediator between the previously separate value chains. It should be positioned favorably geographically in order to create a kind of bio-based polyphosphate district, which would help to lower logistics and transport costs and make the entire process more sustainable.

Based on reliable data on efficiency, the technology must reach a higher degree of maturity. This is the only way to change the existing business models of established actors and facilitate market introduction of the new technology. Closer networking between science and industry is essential to realize technology transfer and meet the market requirements.

In follow-up projects at the RWTH Aachen, the DBU project Value-PP and the MWIDE project Business-P, a process has been developed over the last years from the release of phosphate using phytase to the generation of phosphate-accumulating yeast cells up to the production of bio-based polyphosphate. The future will show whether the value chain outlined above is feasible.

Contact

Prof. Dr. Lars M. Blank,
ABBt – Applied Microbiology, RWTH Aachen,
lars.blank@rwth-aachen.de

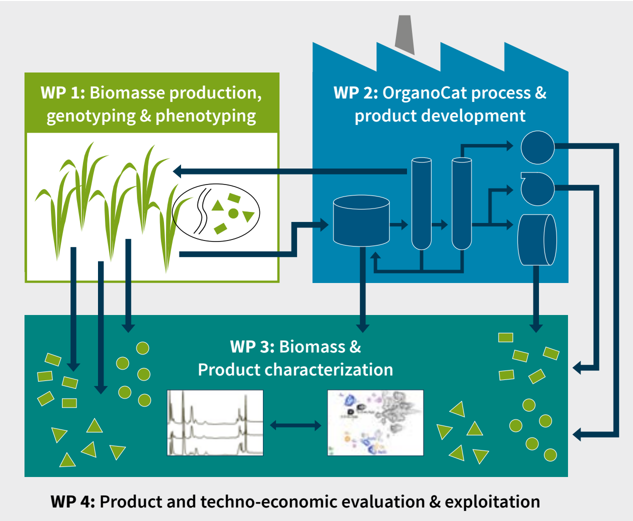
Core Groups involved

Prof. Dr. Lars M. Blank, Jana Fees (author),
ABBT – Applied Microbiology, RWTH Aachen
Prof. Dr. Stefanie Bröring, Dr. Laura Carraresi, Carolin Block (author),
ILR – Technology, Innovation Management and Entrepreneurship, University of Bonn
Prof. Dr. Ulrich Schwaneberg, Dr. Anna Joëlle Ruff,
ABBt – Biotechnology, RWTH Aachen
Prof. Dr. Wolfgang Wiechert, Prof. Dr. Marco Oldiges,
IBG-1 Biotechnology, Jülich Research Center

Tapping Plants as a Valuable Raw Material Source

Second generation biorefinery concepts use the plant raw material lignocellulose and can make a promising contribution to a bio-based economic system if ecological considerations and aspects of sustainability are also considered in their development. The AP³ FocusLab aimed to investigate and establish novel value chains based on holistic utilization of lignocellulose. The project focused on plants that do not or hardly compete with food or feed crops for cultivation area.

Lignocelluloses represent up to 95% of dry mass in land plants and thus are important resources for biogenic products. Their complete utilization can constitute a major economic and ecologic progress. To convert lignocellulose into platform chemicals, it must first be digested and then separated into its chemical components – lignin, cellulose and hemicellulose. These components can be used as raw materials for a broad product range such as chemicals, materials and fuels. In particular, the extraction of high-quality lignin can improve the economic efficiency of such concepts.



Research structure of the AP³ FocusLabs with four scientific work packages and their cooperation with each other.

The OrganoCat-technology represents a promising innovation in this field. A two-phase system of water and biogenic solvent is used. An organic acid, for example oxalic acid, is used as a catalyst to digest lignocellulose and obtain lignin, cellulose and hemicellulose in three separate phases. These are separated in subsequent steps and then further enhanced or used as valuable raw materials.

In the framework of the precursor project OrCaCel, the OrganoCat technology was successfully tested on various herbaceous plants with variable composition. As a result of the project, the use of the catalyst and solvent could be successfully reduced and the process stream could be recycled.

Beautiful and useful

In order to prevent land-use conflicts, it is necessary to investigate the use of plants that do not or hardly compete with food or feed plants for cultivation space. In particular, perennial plants that can be grown on poor soils with little fertilization are promising alternatives to conventional crops. The OrCaCel and SPREAD projects investigated the potential of perennial shrubs such as the mallow *Sida hermaphrodita* and the composite *Silphium perfoliatum*. Both grow up to three meters high and can be a source of food for bees and other insects due to their numerous blossoms and long flowering period. The project investigated different cultivation strategies for these plants and a range of promising scenarios for the production of lignocellulose-containing biomass by these plants. In addition, collections of different genotypes of the individual plant species were created and, with its help, genetic studies were carried out, which formed an important basis for subsequent studies in AP³.

Nitrogen source is decisive

Plant nutrition or the kind of fertilization can have a significant impact on the quantity and composition of the plant biomass

The cup plant *(Silphium perfoliatum)* is one of the undemanding perennial plants that can be considered as a sustainable biogenic raw material source.

and ultimately on the efficiency of any subsequent utilization. Nitrogen is an important plant nutrient and occurs in different chemical forms in mineral and organic fertilizers. The effect of these nitrogen forms was tested on various genetically different varieties of Sida and the model plant *Brachypodium distachyon*, an annual sweet grass. *Sida* plants respond differently on nitrate or ammonium under greenhouse conditions. Under controlled conditions in growth chambers, it was shown that the composition of lignocellulose changes depending on the nitrogen source in *Brachypodium*, especially in the early growth phases. It could also be shown in the straw of these plants that the lignin content in particular changes in response to nitrogen fertilization. These results demonstrate the possibility to adjust the composition of lignocellulose for special utilization by targeted plant nutrition.

Designing profitable biorefineries

Due to its complexity and variability, lignocellulose has a high resistance to subsequent utilization. This represents a major challenge in biorefineries. This resilience is influenced by specific factors. For example, the crystallinity of the cellulose, lignin content, as well as structure and composition are important factors. Resistance not only differs between the plant species and the stages of development of the plants, but also depends on the selected digestion process, since different chemicals and process conditions can cause different product yields and qualities. In the framework of AP³, a selection of very different lignocellulose-containing biomasses was analyzed with the aim of identifying common characteristics that affect resistance to the OrganoCat process in particular. The accessibility of the separated cellulose for enzymatic degradation, the efficiency of hemicellulose break down and the yield and quality of the extracted lignin were assessed.

The results showed that especially the relative ratio between the lignin compounds sinapyl alcohol and coniferyl alcohol has a major effect. Furthermore, specific parameters of the contained polysaccharides correlate with the resistance to the OrganoCat process. For grasses, a small proportion of arabinosyl residues is advantageous for processing. It is possible to predict product yields and qualities in OrganoCat processes based on the chemical profile of the biomass. Energy grasses in particular show good predictability with regard to effective processing and fractioning.

Energy-efficient strategy found

The utilization of lignin can be a decisive step towards economically viable biorefineries. This is why special attention was paid in the project to the development of strategies for the purification of lignin. Initially, a process to separate lignin from the organic solvent was developed that also allows recycling of the solvent. The obvious approach – recovery by evaporation – is unfavorable on a technical scale, since complete evaporation is an energy-intensive process and causes incrustations on the evaporation surface. For this reason, a new concept based on the addition of precipitants has been successfully established and developed.

Fractionation reactors for realizing the OrganoCat-process in the pilot biorefinery NGP² at RWTH Aachen.

In a first step, various precipitants were evaluated that precipitate lignin from the organic solvent. Known as a cooling and foaming agent, n-pentane generated high yields in lignin precipitation and could be recovered in an energy-efficient way. Based on experimental data and process simulations, an energetically optimized concept was thus developed for n-pentane. This consists of solvent pre-evaporation, lignin precipitation and recovery of the precipitant by rectification. This concept was successfully carried out on the laboratory scale and resulted in lignin that could be separated from the organic phase by filtration. Subsequent investigations showed that different fractions obtained at different added concentrations of the precipitant also showed different molecular characteristics. Thus, even defined, homogenous lignin fractions can be produced in this way.

Successfully implemented on a larger scale

One of the main objectives in AP³ was to improve the technology maturity of the OrganoCat technology. The process was therefore carried out on a large scale in the modular biorefinery in the technical center of the RWTH Aachen. Using oxalic acid, 2.5 kilograms of beech wood was successfully fractionated into lignin, cellulose and hemicellulose – as hydrolysate. Realization of the process on a larger scale proved the fundamental feasibility of the concept. In particular, the particle formation of lignin and its composition under different process conditions will be further investigated in the future.

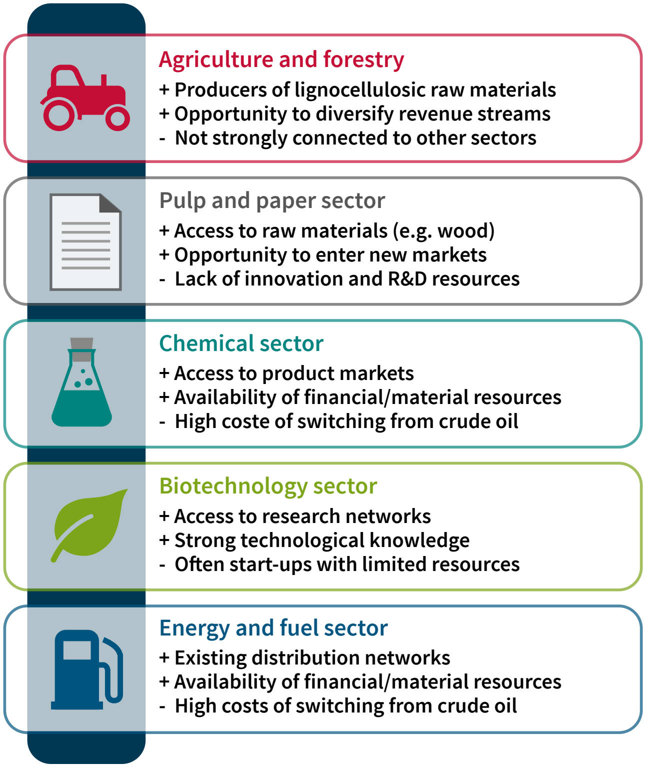
Biorefinery technologies as developed by AP³ demonstrate the complex reality that must be faced by emerging sustainability-oriented technologies. On the one hand, their development can open up the full utilization of lignocellulose into marketable products. On the other hand, they are still in a formative stage and their profitability is often uncertain. Successful development of such technologies requires deep understanding and knowledge from different disciplines. Greater insight in the underlying principles of the entire innovation system of a lignocellulose biorefinery is needed. Answers to the questions of which industry sectors or companies are relevant for the development of technologies or are already active in this field and in what way they play a role in the new value chain are crucial to identify obstacles and pitfalls of these concepts.

Search for partners

AP³ identified the following relevant sectors: agriculture and forestry in the role of raw material suppliers, the pulp and paper industry with the potential to integrate biorefinery technologies into their existing infrastructure, the chemical and energy sector where a market can be found for the products derived from biorefineries and the biotechnology sector where entrepreneurs with scientific background often develop new processing technologies or product applications. This study, focused on Germany, showed that the research environment here, with its well-developed networks consisting of funding bodies, start-up centers and universities, can be supportive. However, what is still missing in the further development of these technologies towards commercial application, are in particular, industrial actors who provide resources and invest in further developed biorefinery facilities on a commercial scale.

Waste from the wood industry represents a major resource for new value chains.

Furthermore, the market for biorefinery products has been shaped by policies towards biofuels over the last decades, but the development of new products - biochemicals, innovative materials – is becoming more and more important. Technologies based on the use of biomass should be holistic, sustainable and efficient. In this context, biorefinery concepts to utilize lignocellulose need actors to develop, test and commercialize processing technologies for different types of biomass. AP³ identified industry



Relevant sectors for the development of biorefinery technologies for lignocellulose-containing biomass.

actors who could already fill these roles. Companies in the chemical, textile, pulp and paper industries have already implemented similar concepts on a commercial scale or have started building such facilities. At the same time, innovative start-ups as well as small and medium-sized companies are creating technology and product variability.

The scientific findings gained in the AP³ FocusLab broaden the basis for the integration of lignocellulose-based biorefinery concepts in industrial applications. These scientific and technological advances are valuable components for implementing such concepts and technologies in the near future and for establishing them in industry.

Contact

Dr. Holger Klose,
IBG-2 Plant Sciences, Forschungszentrum Jülich,
h.klose@fz-juelich.de

Core Groups involved

Prof. Dr. Ulrich Schurr, Dr. Holger Klose, Dr. Silvia Schrey, Dr. Philipp M. Grande,
IBG-2 Plant Sciences, Forschungszentrum Jülich

Prof. Dr. Björn Usadel[1], Sabine Dietrich,
ABBt – Botany and Molecular Genetics, RWTH Aachen

Prof. Dr. Walter Leitner, Dennis Weidener,
ITMC – Technical Chemistry and Petrochemistry, RWTH Aachen

Prof. Dr. Peter Westhoff, Dr. Elena Pestsova,
Institute of Developmental and Molecular Genetics of Plants, HHU Düsseldorf

Prof. Dr. Markus Pauly, Dr. Murali Dama,
Institute of Plant Cell and Biotechnology, HHU Düsseldorf

Prof. Dr.-Ing. Andreas Jupke, Arne Holtz, Martin Leipnitz,
AVT – Fluid Process Engineering, RWTH Aachen

Prof. Dr.-Ing. Alexander Mitsos, Dr.-Ing. Jörn Viell, Alexandra Weber-Bernard,
AVT – Process Systems Engineering, RWTH Aachen

Prof. Dr. Stefanie Bröring, Dr. Laura Carraresi, Lora Tsvetanova,
ILR – Technology, Innovation Management and Entrepreneurship, University of Bonn

[1] New address: IBG-4 Bioinformatics, Forschungszentrum Jülich

FOCUSLAB **greenRelease**

Well-Dosed and Rainproof: Fewer Pesticides Thanks to Micro-Containers

The overarching objective of the greenRelease FocusLab is to significantly reduce the use of plant protection agents and thus contribute to sustainable agriculture and bioeconomy. The crux of the greenRelease technology developed and successfully patented by the scientists are microgels that are loaded with active substances and adhere to the plants over a long period of time by means of customized anchoring molecules.

During the course of the project, the researchers developed the greenRelease technology, which was initiated in the BioSC projects: GreenGel, BiFuProts and RIPE. It was subsequently developed into a platform technology that is robust and validated in the field for plant protection. In 2020, the North Rhine-Westphalia Chamber of Agriculture carried out field tests and validated the technology. The tests show that the same protection against apple scab was achieved with a three-fold reduction in fungicide use compared to commercial products. In parallel, a regulatory and economic assessment of the technology was carried out in order to evaluate the technology transfer potential of greenRelease.

Defying rain

Many plant protection products have an inherent weakness: the active substances do not adhere sufficiently to the plants and are leached into the soil and groundwater by rain. These challenges are addressed by the greenRelease technology. It is based on the combination of biocompatible microgel containers, which measure a mere 100 nanometers to ten micrometers, and special molecules known as anchor peptides, which are also minute and less than ten nanometers in size. Microgels are smooth, porous polymer colloids loaded with active substances and adhere to plant leaves at ambient temperature through customized anchor peptides via simple spray applications.

The main advantage of the greenRelease technology over existing release techniques is the controlled and induced release of active substances over weeks and months. In addition, the new technology minimizes losses due to high rainfastness and superb plant compatibility. Furthermore, the biodegradability is adjustable. In 2018, the greenRelease technology was awarded the Innovation Prize of BioRegions in Germany.

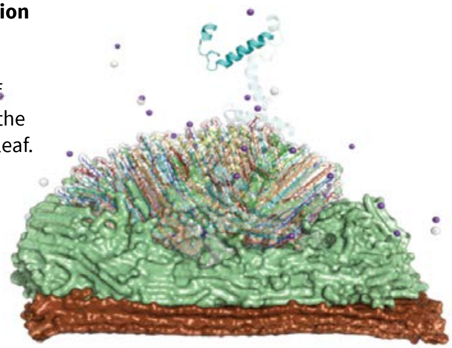
Customized molecules for better adherence

Anchor peptides are tiny molecules consisting of only ten to 100 amino acids. Due to their defined 3D-structure, they bind to surfaces at room temperature even with simple spray application. The binding strength of the anchor peptide to a particular surface can be tailored for the prevailing conditions by protein engineering. In the framework of the greenRelease project, anchor peptides that strongly bind to the leaves and fruits of all selected target crops such as apple, sugar beet, potato and barley, have been identified. The researchers developed a protocol for the directed evolution of anchor peptides to further increase the rainfastness of these molecules. To further optimize the anchor peptides for rainfast binding, it is important to understand the interactions between anchor peptides and the leaf surface. Therefore, directed evolution was complemented by computer simulation and the first knowledge of the binding of anchor peptides to plant leaves was generated.

Targeted release of active substances

Microgels are “intelligent” materials that show a fast reaction to external stimuli. They are promising candidates for many applications, such as transport of proteins, micronutrients and vitamins or targeted release of drugs. The team developed microgel containers with polar or non-polar domains and loaded them with various active substances, such as pesticides and herbicides, so that the active substances are subsequently released over several weeks. Using molecular dynamic stimulations, insights could be gained not only into how the smallest microgel components react to different temperatures but also into their growth process. These models were used to describe the uptake and release processes of fungicides and herbicides. Based on the findings, the researchers subsequently optimized the microgels for slow and long-term release of active substances.

Within just two years, it has been possible to increase the product quantity of the greenRelease formulation from only 150 milliliters for laboratory experiments to 120 liters for initial field applications. In 2020, the first field experiments started at an apple orchard of the North Rhine-Westphalia Chamber of Agriculture.



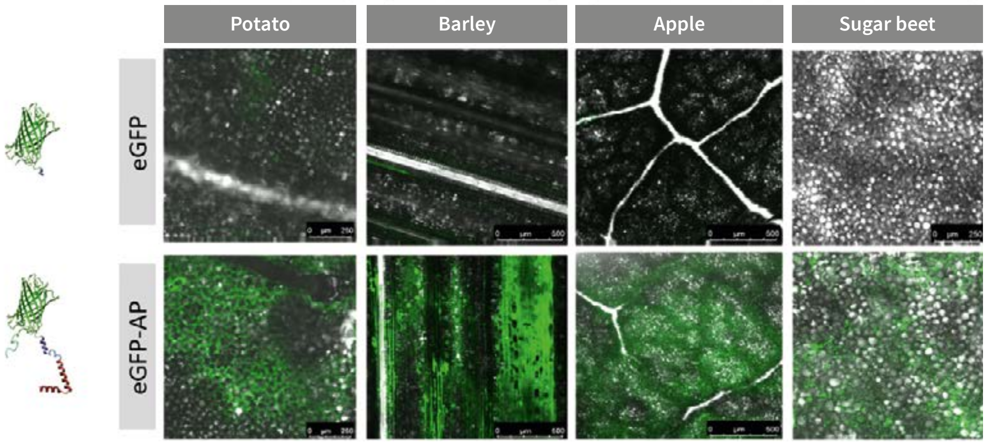
Controlled simulation of molecular dynamics to understand the binding of anchor peptides to the surface of an apple leaf.

Powerful duo

To ensure better adhesion of the micro-containers loaded with active substances to leaf or fruit surfaces, they were decorated with anchor peptides. These biohybrids are referred to below as greenRelease microgels. Anchor peptide-mediated microgel binding to the surface of the selected target plants was confirmed using fluorescence and electron microscopy. Anchor peptides that bind microgels to the leaf surface in a rainfast manner were identified. In addition, electron microscopy showed that the microgels evenly distribute in a homogenous layer on the leaf surface after spraying and and thus ensure optimal protection against pathogens.

The greenRelease microgels were loaded with two fungicides and two herbicides and their release characteristics were determined. Special focus was placed on the contact fungicide copper, since reduction of the application quantity is of great importance for both integrated and organic farming. Spray application of microgels did not negatively alter or affect photosynthetic activity or leaf growth, which indicates no cytotoxic effects on crop plants. Another aspect of the biocompatibility tests and overall sustainability is the assessment of potential effects of the greenRelease technology on soil- and leaf-associated microbiomes (biodiversity). Soil microorganisms can extensively contribute to plant growth and health and are an important factor in the assessment of environmental impacts of various substances.

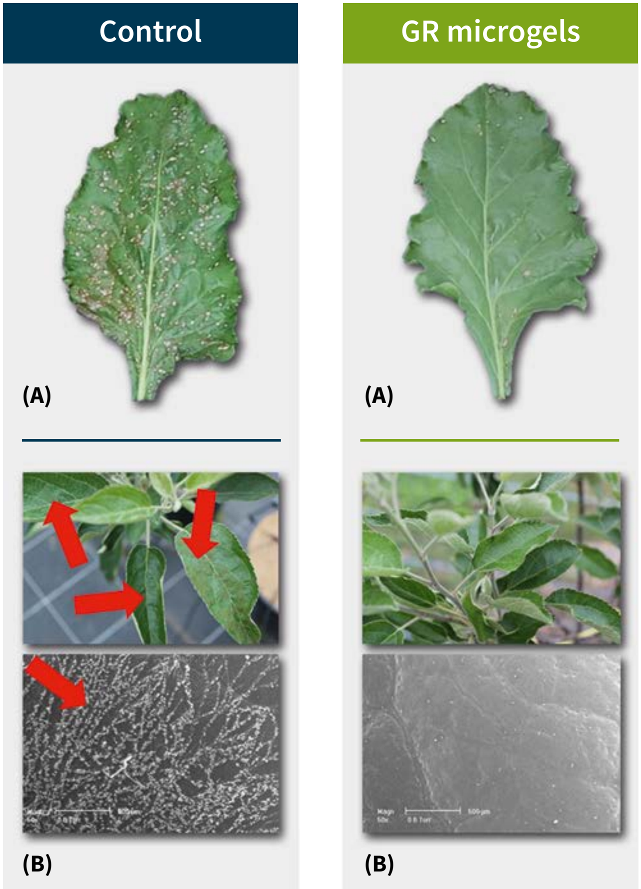
The effects of the microgels were investigated in laboratory and in field experiments. The extent to which activity and composition of the microbial community changes when soil samples are treated with different components of the greenRelease technology was observed in laboratory experiments. This was determined using high-throughput sequencing to analyze microbial communities and microbial respiration measurements to analyze the respiratory activity of the cultures. The apparent increase in the relative abundance of a few bacterial strains over time as a consequence of incubation with greenRelease microgels, coupled with a temporary increase in respiratory activity, suggests a response by soil microorganisms. These results will be further investigated in detail in ongoing experiments until the end of the project. So far, our experiments confirm that the greenRelease technology presents a promising formulation of agrochemicals without negative effects on plant growth, plant health and the surrounding environment, such as the microbiome.



Microgels loaded with anchor peptides under the microscope. A fluorescence marker makes the anchor molecules visible.

Low application – great impact

The protective effect of the greenRelease technology was assessed for sugar beets and apples. The efficiency of microgels loaded with copper was confirmed in field experiments. It could be shown that treating sugar beets with copper-loaded greenRelease microgels significantly reduces the symptoms of the cercospora leaf spot disease caused by the fungus *Cercospora beticola*. In addition, infestation of apple trees with apple scab caused by the fungus *Venturia inaequalis*, which is undesirable in fruit growing, was considerably reduced after treatment with copper-loaded microgels.



greenRelease-microgels (GR-microgels) with incorporated copper protect sugar beets and apples against an infection with fungal pathogens. The protection of the sugar beets against the fungus *Cercospora beticola* can even be seen with the naked eye (A). The protection of apples against the fungus *Venturia inaequalis*, feared in orcharding, becomes visible under the microscope (B).

Under controlled laboratory conditions, copper concentrations approximately 40 times lower than those typical in commercial reference products were already sufficient to prevent *Cercospora beticola* infestation. At these lower concentrations, the loaded greenRelease microgels provide better protection than commercial reference products, especially when rainfall was simulated to wash off the fungicides.

In experiments with apples, the greenRelease microgels loaded with copper in very low concentrations provided better protection against *Venturia inaequalis* infestation than the commercial products used, both under controlled conditions and in field experiments. Here too, the copper concentration was approximately 40 times lower than in commercial reference products.

In particular, the field experiments carried out by the North Rhine-Westphalia Chamber of Agriculture impressively confirmed that the use of the greenRelease technology can help to significantly reduce the total copper usage per season in the future. Compared to standard copper formulations, the greenRelease technology used three times less copper to effectively protect apple plants against apple scab in the field. Further development of the technology is expected to result in an additional significant reduction of the quantity under field conditions, since the reduction potential has already been demonstrated under controlled conditions.

Successful in competition

The technology development is complemented by an economic and regulatory assessment of the greenRelease technology. Among others aspects, the technology landscape of microgels and anchor peptides for plant health was evaluated. The researchers furthermore assessed different market entry options and technology transfer mechanisms in order to get an overall view of the technology transfer potential of the greenRelease

Overview of the experimental field in Cologne at the North Rhine-Westphalia Chamber of Agriculture. The concept of greenRelease technology was validated here in field experiments.

technology. Different market entry options were derived and assessed through the development of a business plan.

The research team carried out several expert interviews with farmers, two business model workshops with project partners and a structured user survey with 150 potential users of the greenRelease technology. Furthermore, the researchers developed customer journey maps for the cultivation of different crops to identify farming-specific problem fields that show at which point during a season a farmer is facing certain challenges related to the cultivation of a specific crop.

Based on the specific problem areas and points of contact, it was shown how greenRelease can address certain challenges and support farmers in their daily work. In order to better anticipate the market potential of the greenRelease technology, the scientists discussed the benefits of the greenRelease technology with industry partners from our technology transfer advisory board as well as with additional actors along the value chain, such as agricultural suppliers. The scientists summarized the business idea, including the target customer group, the results of the user survey and the business model workshops in a business plan.

This extensively researched and sophisticated business plan was submitted for the "Bio-Gründer Wettbewerb" start-up competition. Here, the jurors from various sectors, including politicians, were convinced of the high transfer potential, placing it first ahead of 40 other teams.

The postulated objective to develop a field validated release technology was successfully achieved by the partners and the greenRelease technology is now ready for subsequent projects and innovations in a sustainable agriculture and bioeconomy.

Contact

Dr. Felix Jakob,
ABBt – Biotechnology, RWTH Aachen,
f.jakob@biotec.rwth-aachen.de

Core Groups involved

Prof. Dr. Ulrich Schwaneberg, Dr. Felix Jakob, Christin Brethauer, Liudmyla Goncharenko,
ABBt – Biotechnology, RWTH Aachen

Prof. Dr. Uwe Conrath, Dr. Caspar Langenbach, Patrick Schwinges,
ABBt – Plant Physiology, RWTH Aachen

Prof. Dr. Andrij Pich, Alexander Töpel, Tim Sassmann, Xu Wenjing,
ITMC – Functional and Interactive Polymers, RWTH Aachen

Prof. Dr. Stefanie Bröring, Dr. Michael Wustmans,
ILR – Technology, Innovation Management and Entrepreneurship, University of Bonn

Prof. Dr. Georg Groth, Dr. Alexander Hofmann,
Institute of Biochemical Plant Physiology, HHU Düsseldorf

Prof. Dr. Holger Gohlke, Jonas Dittrich,
Computational Pharmaceutical Chemistry and Molecular Bioinformatics, HHU Düsseldorf

Prof. Dr. Georg Noga, Dr. Shyam Pariyar,
INRES – Horticultural Sciences, University of Bonn

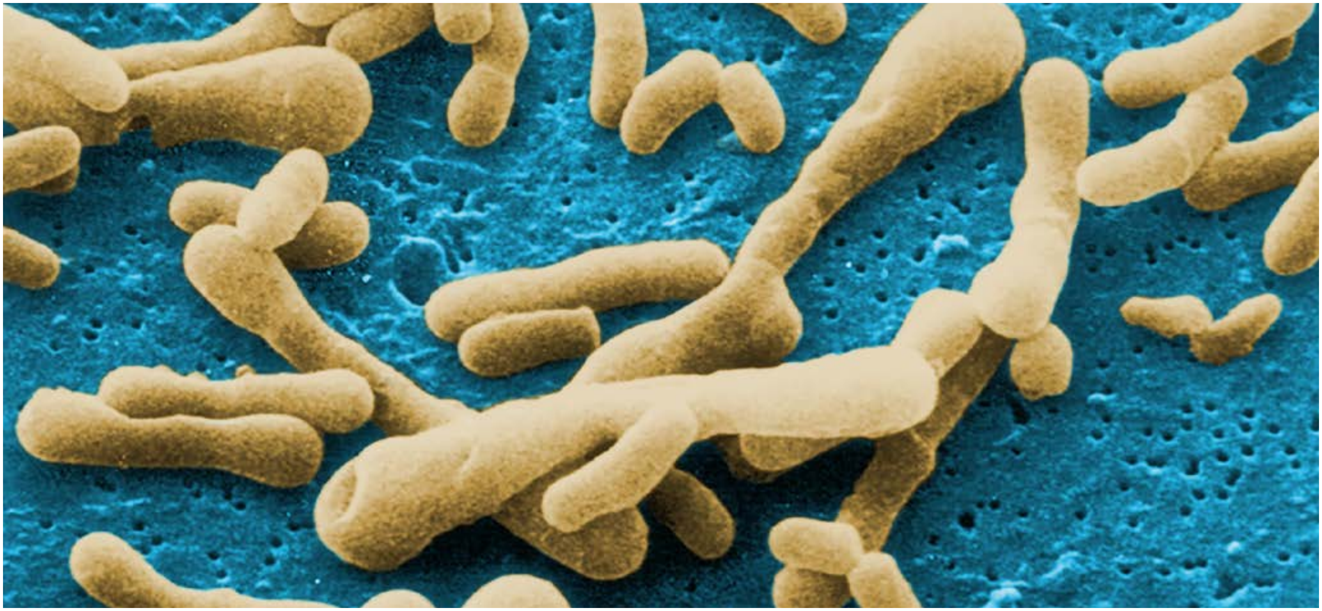
Prof. Dr. Claudia Knief, Janina Zierul, Romy Geer,
INRES – Molecular Biology of the Rhizosphere, University of Bonn

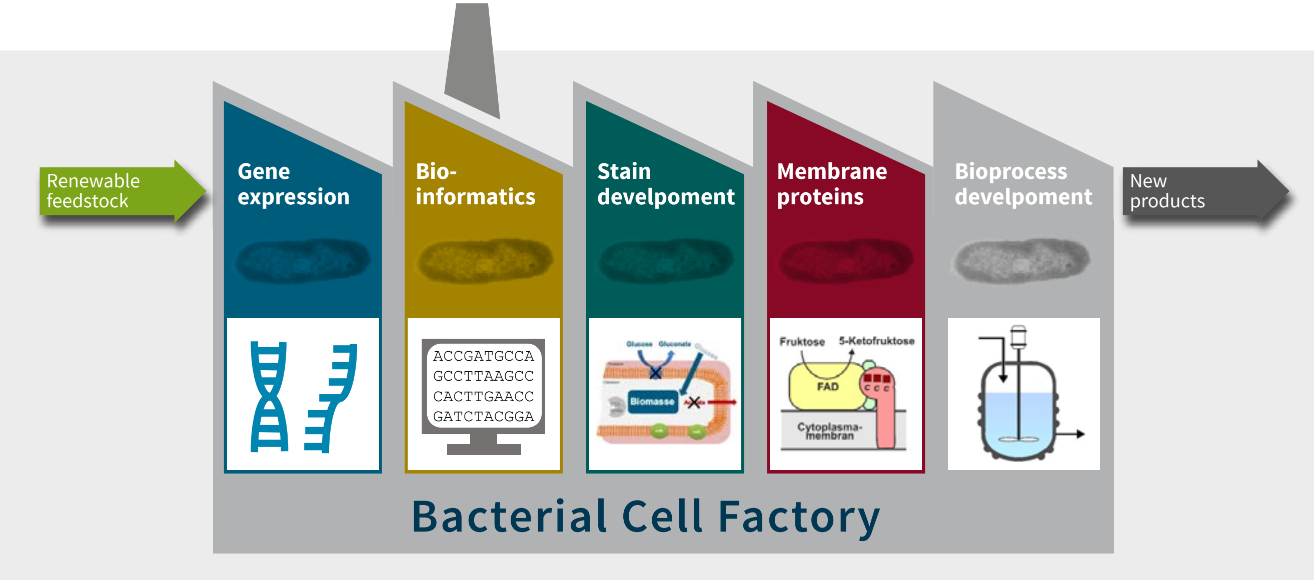
Prof. Dr. Ulrich Schurr, Dr. Fabio Fiorani, Dr. Henning Lenz,
IBG-2 Plant Sciences, Forschungszentrum Jülich

Bacterial Cell Factory for a New Sweetener

Bioprocesses with microorganisms as biocatalysts play a decisive role in establishing a sustainable bioeconomy. In the framework of the BioSC project "Gluconobacter Factory", an interdisciplinary research approach was used to explore the acetic acid bacterium *Gluconobacter oxydans* with regard to fundamental properties and application potentials. Among other findings, this resulted in a highly efficient production process for a potential new sweetener that is natural and low in calories.

Many biotechnological bioprocesses start with sugars such as glucose, fructose or sucrose. Microorganisms usually transport them initially into the interior of the cell, the cytoplasm, and then convert them via a variety of enzyme-catalyzed steps into the desired product, which then has to be exported from the cytoplasm. In *Gluconobacter*, however, numerous production processes take place outside the cytoplasm in the periplasm, which is the space between the cytoplasmic membrane and the outer membrane. Enzymes called dehydrogenases are located there, which oxidize sugars and other carbohydrates highly specifically at a specific carbon atom. The electrons released in the process





are transferred via other enzymes (cytochrome oxidases) to oxygen, which is converted to water. Such specific oxidations are difficult or not at all possible to achieve purely chemically, so the superior capabilities of microorganisms are in demand. For these reasons, the bacterium *Gluconobacter oxydans* has already been used industrially for the production of vitamin C since the 1930s. In order to make even better and broader use of the biocatalytic potential of this bacterium, an interdisciplinary team worked on a detailed analysis of the genetic information and the development of new production strains and products.

Analysis of 2,664 genes

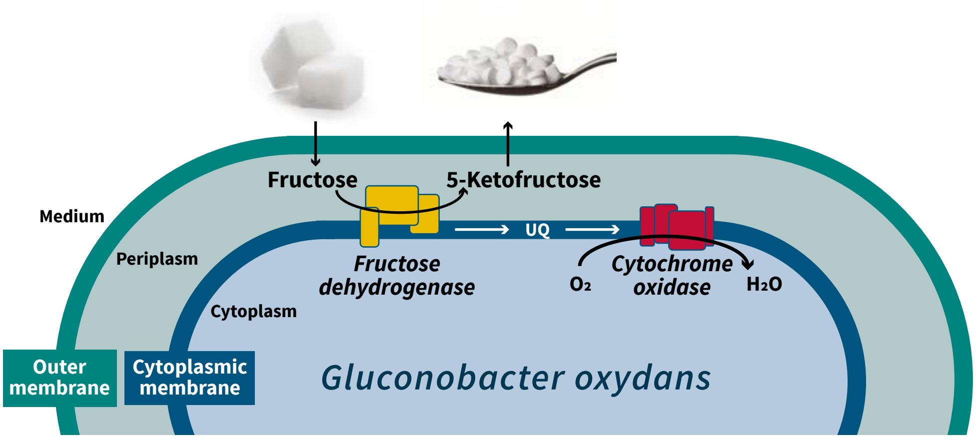
The genetic information of *Gluconobacter oxydans* in the form of deoxyribonucleic acid (DNA) is organized in a circular chromosome of 2.7 million base pairs with 2,432 genes and five plasmids with again a total of 232 genes. The question of how these more than 2,600 genes of *Gluconobacter oxydans* are organized into transcription units and which control sequences direct the transcription of DNA in ribonucleic acid (RNA) was answered by sequencing the RNA and subsequent bioinformatics analyses. This extensive information was made available to the scientific community on a freely accessible, web-based bioinformatics platform at www.gluconobacterfactory.de.

In the BioSC Boost Fund project Gluconobacter Factory, microbiologists, molecular geneticists, bioinformaticians and bioprocess engineers worked together to exploit the special capabilities of the microbial cell factory *Gluconobacter oxydans* for the development of new bioprocesses contributing to a sustainable bioeconomy.

For the industrial use of *Gluconobacter oxydans*, large quantities of bacterial cells must first be cultivated. Here the special characteristics of *Gluconobacter oxydans* are disadvantageous: only about 100 g cells are formed from one kilogram of glucose, whereas for bacteria with a standard type of metabolism about 500 g cells are obtained. This means that the production of cells for biotransformations is considerably more expensive when *Gluconobacter oxydans* is used. By targeted modification of glucose metabolism, an approach called metabolic engineering, a strain gaining 160 g cells per kilogram of glucose could be constructed and thus allows reduction of the costs for production of cells.

Production of a low-calorie sweetener

Due to its specific characteristics, *Gluconobacter oxydans* is highly suited for synthesizing membrane-bound, periplasmatic en-



Biotransformation of fructose to 5-ketofructose with *Gluconobacter oxydans*. Fructose is oxidized to 5-ketofructose by the enzyme fructose dehydrogenase and the released electrons are transferred to oxygen, which is reduced by terminal oxidases to water.

zymes also from other bacteria in order to investigate their characteristics and application potentials. Fructose dehydrogenase from the related bacterium *Gluconobacter japonicus* proved to be especially promising. This enzyme consists of three different subunits and catalyzes the oxidation of fructose to 5-ketofructose. 5-ketofructose is a very interesting product since the compound has a comparable taste and a similar sweetening power as fructose itself, but unlike fructose, it seems to be not or only marginally metabolized by our intestinal flora. These are exactly the characteristics a low-calorie sweetener should have. The potential for the technical production of 5-ketofructose from fructose using *Gluconobacter oxydans* cells, which overproduce fructose dehydrogenase, was evaluated and optimized by the project team using methods of biochemical engineering. A process was developed that could produce 490 g of 5-ketofructose per liter with a product yield of 0.92 g per g fructose consumed and a space-time-yield of 7 g per liter and hour. This indicated that an economical production of the potential sweetener is possible. Further studies must now prove the tolerability of 5-ketofructose.

Patent application

The project shows that the concept of interdisciplinary cooperation in the framework of Boost Fund research projects is very successful and promising also in the future. It enables basic research as a prerequisite for innovations and the establishment of a circular bioeconomy based on renewable resources. The GLUFACT project resulted in a patent application and seven original publications in peer-reviewed journals. It was followed by two BMBF-funded projects (IMPRES und IMPRES-2), in which 5-ketofructose production was further developed and new products for human nutrition were established.

Contact

Prof. Dr. Michael Bott,
IBG-1 Biotechnology, Forschungszentrum Jülich,
m.bott@fz-juelich.de

Core Groups involved

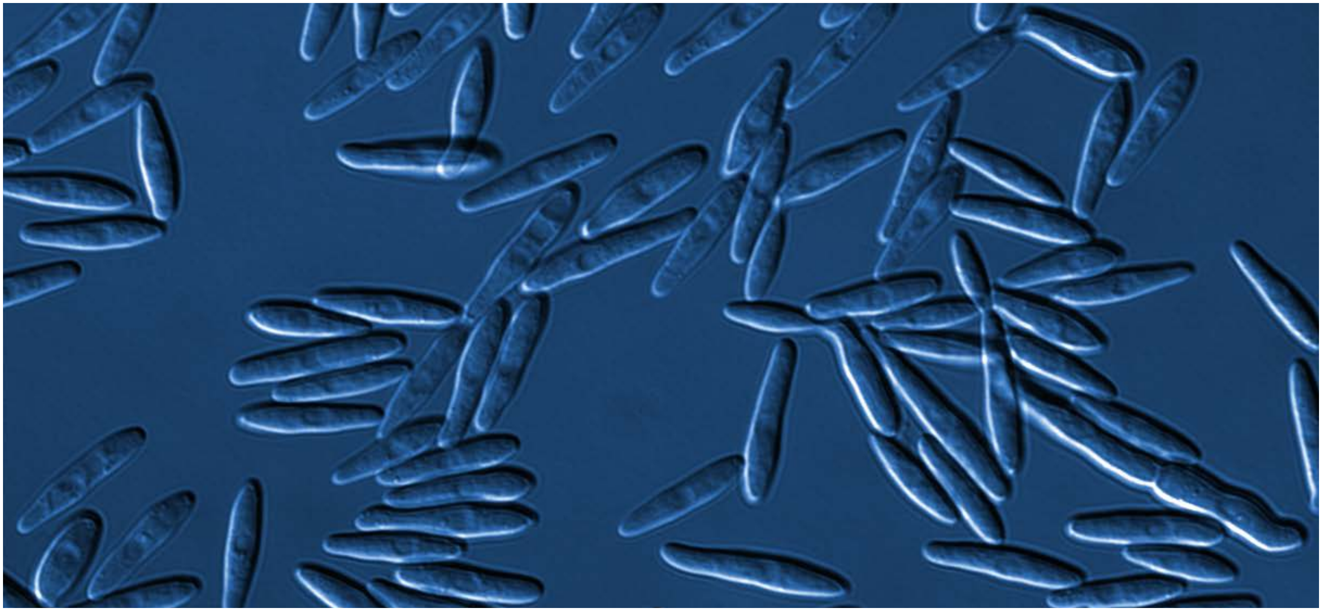
Prof. Dr. Michael Bott, Dr. Stephanie Bringer, Dr. Ines Kiefler,
Dr. Tino Polen, Dr. Angela Kranz,
IBG-1 Biotechnology, Forschungszentrum Jülich
Prof. Dr. Björn Usadel, Dr. Alexander Vogel,
IBG-4 Bioinformatics, Forschungszentrum Jülich
Prof. Dr. Uwe Deppenmeier, Dr. Konrad Kosciow, Dr. Anna Siemen,
Institute for Microbiology and Biotechnology, University of Bonn
Prof. Dr.-Ing. Jochen Büchs, Dr. Elena Herweg,
AVT – Bioprocess Engineering, RWTH Aachen

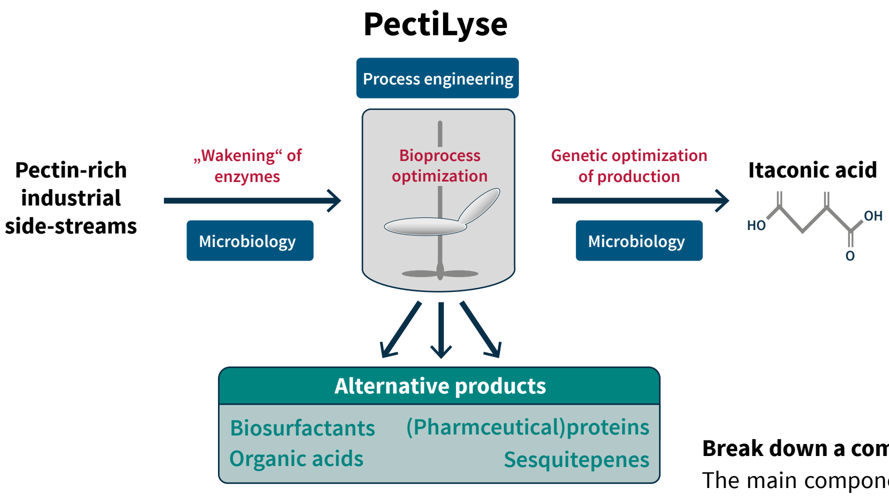
BOOST FUND **PectiLyse**

From Field to Platform Chemical: Valorisation of Sugar Beet Residues

The BioSC Boost Fund project PectiLyse had the objective to use the fungal model organism *Ustilago maydis* for the degradation of pectin-rich biomass. Large quantities of organic side streams arise during sugar production from sugar beet. These largely consist of the complex polysaccharide pectin. Currently, the pectin-rich side streams are sold as feed with low added value. The PectiLyse project should establish basic methods to convert these residues into valuable products in an integrated bioprocess in the future.

A consortium of researchers from different disciplines fostered an interdisciplinary approach to the project: microbiologists with expertise in genetic manipulation of the fungus as well as the production of organic acids teamed up with leading bioprocess engineers. This supported a holistic approach in which optimized strains could be efficiently characterized using online analytics.





Enhancement of pectin-rich industrial side streams in a sustainable microbial bioprocess.

The fungus *Ustilago maydis* has some attributes that especially qualifies it for the development of an integrated bioprocess. In contrast to other known pectin-degrading fungi, *Ustilago maydis* is able to grow filamentously as well as yeast-like. This offers decisive advantages in handling and especially in cultivation. *Ustilago maydis* infects corn plants and causes the so-called corn smut. However, it is not dangerous for humans. Infected corn cobs are even suitable for human consumption and sold as a delicacy in Mexico. Yeast-like growing laboratory strains are not infectious for plants and are ideal for biotechnology.

Its pathogenic lifestyle is reflected in the enzyme repertoire of *Ustilago maydis*. In contrast to other model fungi, such as baker´s yeast, *Ustilago maydis* already encodes enzymes to metabolize individual sugars contained in pectin. In addition, other enzymes useful for the degradation of pectin into these individual sugars are present. Unfortunately, these are synthesized only in the infectious phase during corn colonization. To solve this problem, the corresponding enzymes were kind of awakened and thus activated by molecular biological methods. Applying this “sleeping beauty” principle, the desired enzymes could successfully be produced in the yeast phase and secreted into the extracellular space. This allows an expansion of the substrate spectrum.

Break down a complex molecule

The main component of pectin is polygalacturonic acid, which forms the backbone of the complex molecule. The team thus first developed several *Ustilago maydis* strains that produce and secrete different enzymes for the degradation of polygalacturonic acid. For example, the researchers investigated the fungal enzyme endopolygalacturonase and assessed its effectivity. In addition, they transplanted already characterized, potent enzymes from other bacteria and fungi into their model organism *Ustilago maydis* by gene transfer. The result: the foreign enzymes were also produced successfully by this laboratory fungus and released into the culture medium without any problems. For production of the bacterial enzymes, the fact that *Ustilago maydis* has an unconventional secretion pathway that exports these enzymes without foreign modifications – with full activity potential – into the extracellular space was exploited.

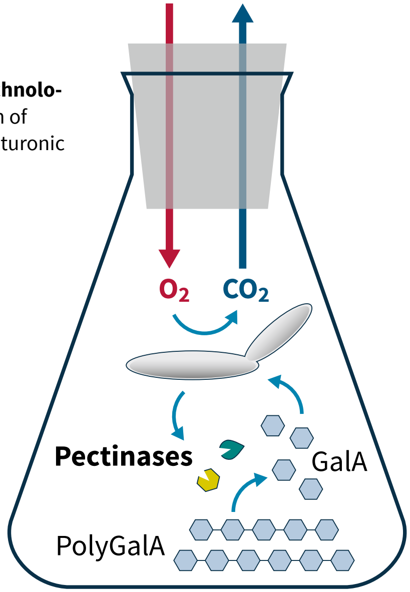
This broad approach allowed the identification of fungal strains that export different active pectinolytic enzymes. Using mixed cultures consisting of strains with complementary enzyme activities, efficient degradation of polygalacturonic acid (PolyGalA) to the component galacturonic acid (GalA), subsequently metabolized by the fungi, could finally be achieved. The project thus lays the foundation for further steps towards the degradation of pectin-containing biomass.

In order to successfully establish polygalacturonic acid degradation, modern online analytics from the biochemical engineers were key to evaluating the produced strains. The fungal strains were cultivated on polygalacturonic acid and, at the same time, their respiration rate was determined during the course of cultivation. Data analysis supported characterization of the individual strains with regard to the respective substrate turnover and enzyme activity. Indirect quantification of the polygalacturonic acid concentration could thus be achieved based on the respiration rate. Mixed cultures could also be analyzed efficiently in this way.

Further products in the focus

As an example of a valuable industrial product, the research team initially focused on the organic acid itaconate, which is naturally produced by *Ustilago maydis* under nitrogen limiting conditions. Itaconate is an important platform chemical in polymer chemistry. Its fields of application range from the production of paints and varnishes to thickeners for fats, pharmaceuticals or herbicides up to biologically degradable polymers. The biosynthesis of itaconate is regulated by a gene cluster and proved to be highly variable when compared in different *Ustilago maydis* strains. Very low itaconate production could be observed especially in the strain background used for secretion of pectin-degrading enzymes. New genetic approaches to remedy these variations could be developed by comparing biosynthetic enzymes of related smut fungi at the genome level. The team succeeded in permanently activating the central regulator of the gene cluster by a genetic trick. This turned out to be an excellent set screw for a high basic level of itaconate production. The method lays the foundation for the targeted bioprocess.

The PectiLyse project could thus establish the first basic steps on the way to an efficient bioprocess both at the level of substrate degradation as well as at the level of itaconate production. This was possible only by a team cooperating in the fields of microbiology, biotechnology and bioengineering. The researchers will further optimize the fungus for degradation of pectin in the future. At the same time, they are working on a consolidated bioprocess in which further valuable molecules are produced from the released fermentable sugars. In addition to itaconate, other products such as biosurfactants, organic acids, sesquiterpenes, which are used as flavorings or fragrances, or pharmaceutical proteins are conceivable. The production of biosurfactants was successfully established in the Bio² BioSC FocusLab. Based on the interdisciplinary work in the framework of the BioSC, *Ustilago maydis* could serve as a flexible platform organism for industrial biotechnology in the near future.



Online measurement technology for the characterization of fungi growth on polygalacturonic acid.

Contact

Dr. Kerstin Schipper,
Institute of Microbiology, HHU Düsseldorf,
schipper@hhu.de

Core Groups involved

Prof. Dr. Michael Feldbrügge, Dr. Kerstin Schipper,
Dr. Benedikt Steuten, Dr. Peter Stoffels,
Institute of Microbiology, HHU Düsseldorf

Prof. Dr. Lars M. Blank, Prof. Dr. Nick Wierckx[1],
Dr. Elena von Helden (née Geiser),
ABBt – Applied Microbiology, RWTH Aachen

Prof. Dr.-Ing. Jochen Büchs, Dr. Markus Müller, Dr. Tino Schlepütz,
AVT – Bioprocess Engineering, RWTH Aachen

[1] New address: IBG-1 Biotechnology, Forschungszentrum Jülich

SEED FUND 2.0 **R2HPBio**

From Biomass to High-Performance Plastic

The R2HPBio project pursued an integrative approach along the entire value chain for the production of bioplastics. A specially developed crystallization process purifies a range of potential platform chemicals from a fermentation broth. Using novel catalysts, these are processed to plastics that can be given customized characteristics through subsequent modifications. The resulting materials are biodegradable, can be produced from renewable resources and have potential for a variety of practical applications.

R2HPBio covers the entire life cycle of novel bioplastics. The project is closely linked to the HyImPAct FocusLab that produces important platform chemicals from renewable resources and offers subsequent purification. In the framework of the R2HPBio project, platform chemicals were purified using modern, electrochemically induced separation methods to convert these to linear, bifunctional polyesters with robust metal complex catalysts. Subsequently, the bioplastics were optimized for specific applications by functionalization and investigated for their degradability.

Reducing costs

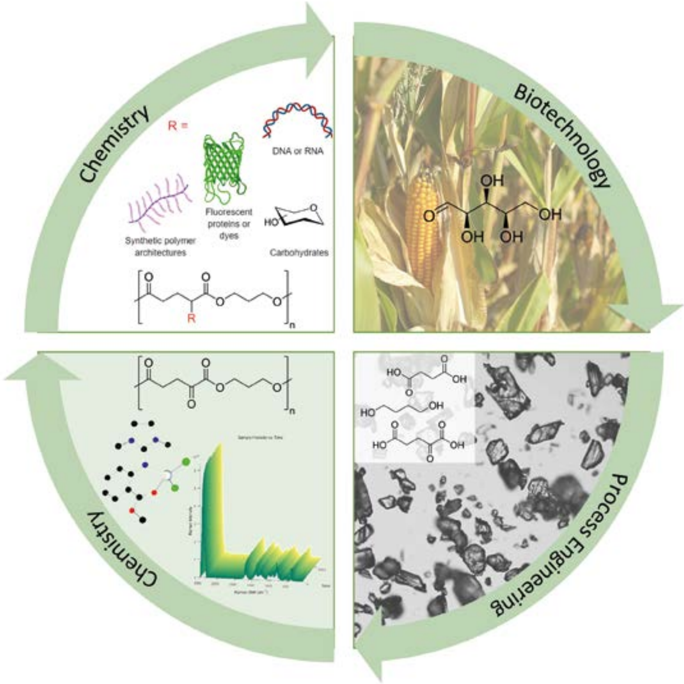
The platform molecules produced in the HyImPAct FocusLab such as succinic acid, 1,4-butanediol and protocatechuic acid are promising monomers for novel bioplastics. However, purification of these molecules from aqueous fermentation media is complex and cost-intensive. The R2HPBio established a new purification method based on the fact that carboxylic acids such as succinic acid and protocatechuic acid have a lower solubility at lower pH values and can be extracted. Electrochemical separation methods are a novel procedure for the separation of these carboxylic acids since the pH value can be adjusted specifically without additional additives such as hydrochloric acid or sodium hydroxide.

Neutral compounds cannot be separated as easily as salts. Therefore, the formation of carboxylates is induced by lowering pH using water splitting and the formation of neutral salts is avoided. For optimal separation, measuring the pH-dependent balance between dissolved and precipitated substance is essential. An automated installation was developed to measure the solubility of carboxylic acids in aqueous media for different temperatures and pH values. These data allowed the interpretation and implementation of electrochemically induced crystallization of succinic acid and protocatechuic acid.

Controlled growing

For the production of bioplastics, the purified monomers are converted into the desired polyesters in the next step via polycondensation. Polycondensation is a stepwise growth reaction of molecules. In this process, bifunctional monomers or already formed oligomers, i.e. more than one molecule already accumulated spontaneously, react to form linear polymers by splitting off low-molecular compounds such as water. This project converted the monomers succinic acid and 1,4-butanediol to the known polyester polybutylene succinate.

A customized, biocompatible zinc catalyst was used as the catalyst. What is special in the use of a specific metal complex is not only its biocompatibility but also its robustness against water formed during polymerization. The complex thus allows the synthesis of long chains. The objective is to initially produce medi-



The R2HPBio project is based on the interdisciplinary cooperation between biotechnology, process engineering as well as inorganic and macromolecular chemistry.

um-sized polyester chains, which then will be extended to biodegradable polyurethane. Cold-foam mattresses, thermal insulting panels or dishwashing sponges consist of polyurethanes, for example. This plastic is very difficult to recycle in its previous oil-based form.

Environmentally friendly production

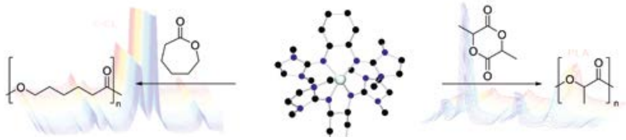
Another sub-project developed a new, robust zinc-bisguanidine catalyst for the polymerization of the renewable monomers lactide and ε-caprolactone. Both cyclic monomers can be transformed into the biodegradable polyesters polylactide and polycaprolactone by catalytic ring-opening polymerization Polylactide is currently one of the most commonly produced bioplastics. The catalyst most widely used in industry in the

Design of the electrochemically induced separation method: two electrode cages each with cathode (left) and anode (right), agitators and intermediate membrane.

polymerization of lactide is a complex containing the metal tin, which is harmful to cells. The catalyst, which only controls the reaction and is not used up, is difficult to remove from the polymer. Thus, the catalyst remains in the plastic for economic reasons. The plastic, which can be degraded to 90 percent within 90 days under industrial composting conditions, leaves the toxic metal in the compost and thus constitutes a threat to the environment.

The zinc catalyst developed in this cooperation represents a good alternative not only due to its non-toxic characteristics. In addition, the zinc catalyst exceeds the polymerization rate of the industrial used complex many times over. This was investigated using Raman spectroscopy, a method that follows the reaction to polymer in a time-resolved manner. Hereby, a colorless, long-chain polymer with high molar masses is formed. In addition, polymers produced with the zinc catalyst show high crystallinities, which are especially advantageous for medical applications such as artificial shoulder bones and bone screws, since the higher crystalline content in polylactide causes greater stability in an aqueous environment. In the medical field, the slow degradation of the bioplastics to lactic acid takes place after months or years, depending on the material characteristics, making a second surgical intervention unnecessary. If the bioplastics are used in packaging, they are expected to degrade or be recycled more quickly. Here it was shown that zinc-guanidine complexes excellently catalyze not only simple degradation to lactic acid but also the reaction to other platform chemicals, such as ethyl acetate, as a recycling step. In this way, the renewable resource can be used several times in the sense of a recycling economy and additional waste is avoided.

The R2HPBio project thus took a central step towards a circular bioeconomy through interdisciplinary cooperation, which will establish the sustainable bioeconomy in the long term.



The production of bio-based and biodegradable polylactide and polycaprolactone mediated by a zinc catalyst is observed using Raman spectroscopy.

Contact

Prof. Dr. Sonja Herres-Pawlis,
Chair of Bioinorganic Chemistry, RWTH Aachen,
sonja.herres-pawlis@ac.rwth-aachen.de

Core Groups involved

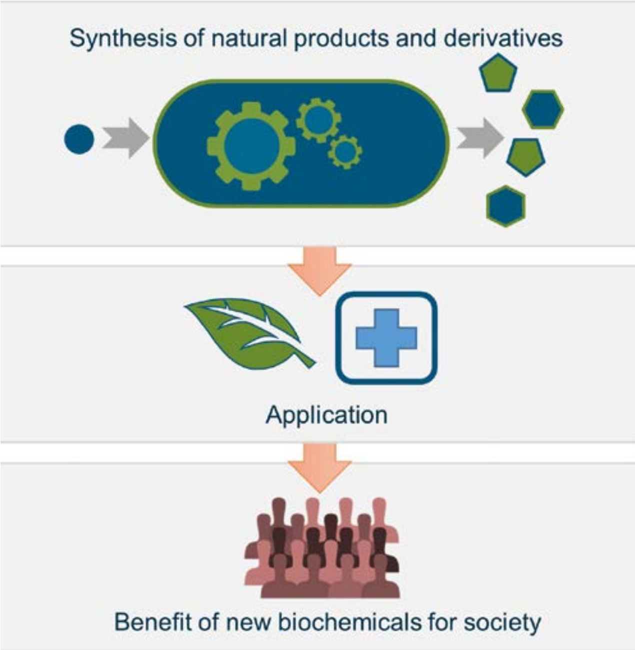
Prof. Dr. Sonja Herres-Pawlis, Alina Herrmann,
Chair of Bioinorganic Chemistry, RWTH Aachen
Prof. Dr. Andreas Jupke, Jonas Görtz,
AVT – Fluid Process Engineering, RWTH Aachen
Prof. Dr. Laura Hartmann, Dr. Stephen Hill,
Chair of Macromolecular Chemistry, HHU Düsseldorf

Toolbox from Nature

Bacteria, fungi and plants produce a colorful bouquet of natural substances that are not essential to survive but which are often bioactive. These so-called secondary metabolites serve the organisms for pest control or chemical communication with each other, for example. Scientists use the basic structure of such substances to develop new chemicals that are much needed to safeguard nutrition and health in the agricultural or pharmaceutical sectors.

The objective of the CombiCom project was to realize secondary metabolite metabolic pathways in microbial bioproduction and thus to obtain sufficient quantities of the respective natural substances. These were also tested for spectrum range of biological activities. New derivatives were developed from their basic structure that can show an extended or even completely new bioactivity. These substances are highly promising as new active and valuable substances for plant protection, medicine or the feed and food industry. With regard to a potential market launch, the social acceptance of products made in synthetic biological processes was also evaluated.

The involved groups developed the scientific basis for this largely in phase 1 of the strategy project, primarily in the BioSC projects BioSAF, VariSurf, AlgalFertilizer, MoRe-Plants, NovoSurf and Econ-BioSC. In phase 2, the researchers established a synthetic biology platform in the framework of CombiCom using the bacteria *Rhodobacter capsulatus* and *Pseudomonas putida,* the fungus *Ustilago maydis* and green algae of the *Chlorellaceae* family in order to exploit non-photosynthetic and photosynthetic organisms for natural substance production. The respective hosts were specifically selected to equip them with genetic modules for the production of various natural substances, such as terpenoids, prodiginines, pyrrole alkaloids and glycolipids and their derivatives.



CombiCom motivation: microbial production of new bioactive natural substances for a bio-based economy. Sustainable approaches for production, diversity of products and corresponding application possibilities and public acceptance are crucial for success.

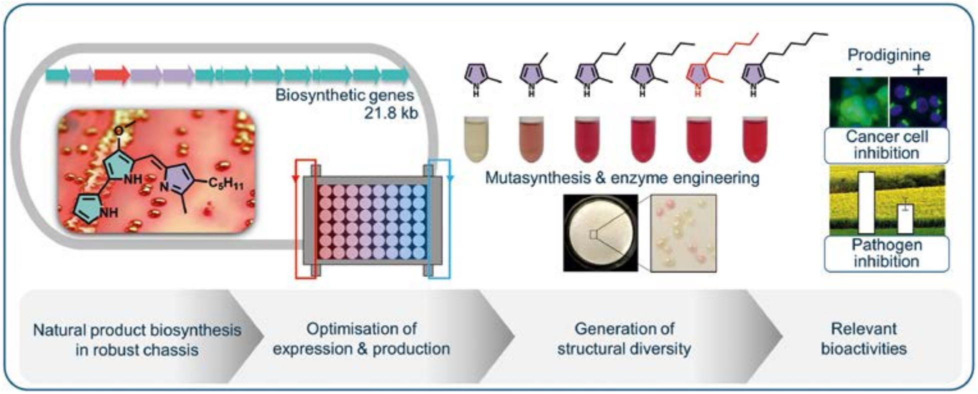
Bioactive substances from bacteria

Certain bacteria produce so-called prodiginines, which show a typical red color and belong to the pyrrole alkaloids. The naturally occurring structural differences of prodiginines are known to result in a broad spectrum of different bioactivities such as cytostatic or antibiotic activity. The prodiginine family thus presented an attractive starting point for the production and investigation of natural and nature-inspired compounds. For this reason, the team implemented prodiginine biosynthesis into the laboratory bacterium *Pseudomonas putida*, which is easy to handle and has a remarkable tolerance to these compounds that are toxic to other species. In doing so, molecular genetic tools that allow the transfer of biosynthetic genes were optimized. Integration of the genetic information into the host organism at a particularly suitable position resulted in production of over 0.2 grams of the desired prodiginines per liter of nutrient solution. In addition, the production could be monitored by newly developed online monitoring methods and optimized, for example, with regard to the cultivation temperature.

On this basis, the research team produced a variety of new prodiginines, exploiting the modularity of the prodiginine biosynthesis pathway. This allowed the introduction of variants of the precursor molecules obtained by chemical synthesis. These could be converted to new prodiginine compounds in the bacterium. The key enzyme that must accept the foreign component was adapted accordingly. In this way, the researchers could eventually produce more than 40 differently substituted prodiginines. The work showed that the combination of the necessary synthetic biological tools and the synthetic chemistry was the key to product diversity.

Fungi and plants are inventive

With more than 80,000 variants, terpenoids are one of the largest and most diverse natural substance classes. Vitamin A, for example, is one of them. Terpenoids are made of C5 isoprene units and converted into a multitude of different structures by specialized enzymes, the terpene synthases. Plants in particular, but also fungi, seem to have developed a large number of different terpene compounds with specific ecological function during evolution. Due to diverse bioactivities, they can be used



Production of bioactive prodiginines and natural substance derivatives in the bacterium *Pseudomonas putida*. The robust bacterium allows the production of diverse structure variants so that the investigation of relevant bioactivities against cancer cells or plant pathogens becomes possible.

as nature-inspired active substances in plant protection, for example. The researchers thus developed the bacterium *Rhodobacter capsulatus* and the fungus *Ustilago maydis* to complement well-established platforms such as *Saccharomyces cerevisiae* and *Escherichia coli.*

The bacterium *Rhodobacter capsulatus* is able to use sunlight as an energy source via photosynthesis. Its special internal cell structure offers a suitable environment for the production of various molecules of the terpenoid biosynthesis pathway. Using specific expression tools, the team adapted the isoprene metabolism of the bacterium specifically for terpene formation. This allowed the production of different terpenoids such as (+)-valencene, an aroma component of citrus fruits, and β-caryophyllene, which has anti-inflammatory properties, with titers of more than 0.1 grams per liter. This work thus underlines that *Rhodobacter* species can be used as microbial cell factories for the production of terpenes. The fungus *Ustilago maydis* is a relative of the fruiting body forming hat fungi, which can be cultured yeast-like and manipulated genetically. The research of the project shows that *Ustilago maydis* has increased resistance to antifungal compounds and thus lends itself to their production. However, only few expression tools of synthetic biology have been available for *Ustilago* until now. The team thus established a molecular toolbox of synthetic biological elements for the fungus. In addition, the scientists developed *Ustilago maydis* species for the production of large quantities of glycolipids, which belong to the natural biosynthesis repertoire of the fungus and are relevant as bioactive and surfactant compounds. Finally, the researchers could produce the terpenes (+)-valencene and α-cuprenene for the first time and determined a specific suitability of the host for the production of the latter compound that originated from a fungus. This demonstrated for the first time that *Ustilago maydis* can be used as promising new organism for terpene production.

Versatile algae

Phototrophic microalgae are enjoying an increasing market potential and particularly high consumer acceptance. They are used as a source for health-promoting food, sustainably produced feed and platform chemicals. The researchers thus developed techniques to improve the permeability of the cell wall, since cell walls are physical barriers that currently hinder the effective use of microalgae. An approach of mild enzymatic treatment was established. Potential applications including milking, for example, which is the continuous non-destructive product extraction from cells, or the introduction of DNA for molecular genetic manipulation. The team thus demonstrated a new approach for the permeabilization of microalgae that has the potential to accelerate the scientific progress for sustainable algae biotechnology, for example for terpene production, as a key technology. The work showed that the collaborative development of new host platforms is worthwhile and significant for the exploration and exploitation of the naturally wide chemical space of terpenes.

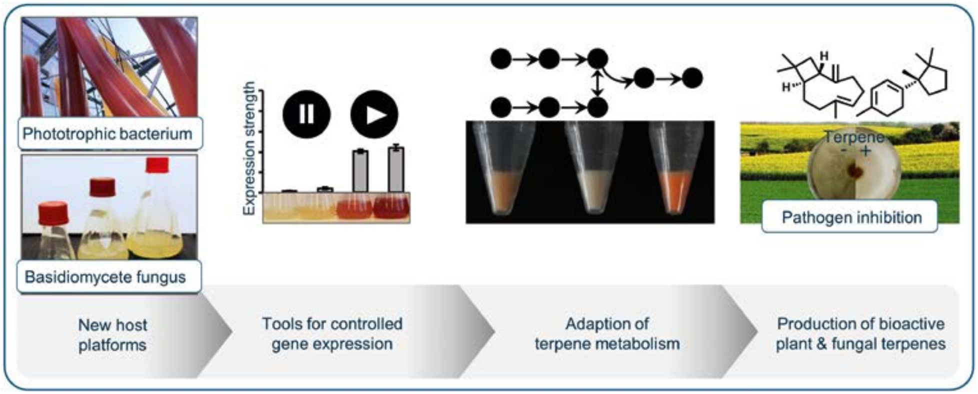
Broad application is possible

New, effective and at the same time gentle bioactive substances are needed in particular in the agricultural and medical sectors. Analysis of the bioactivity of the compounds addressed in CombiCom showed a number of promising effects. Some prodiginines had an antibacterial and combinatorial effect with biosurfactants. One of the derivatives showed antiproliferative activity through autophagy modulation and induction of apoptosis in breast cancer cells. In addition, several prodiginines and terpenoids were active against plant pathogens. They inhibited the fungi *Phoma lingam* and *Sclerotinia sclerotiorum* in particular, which cause blackleg in cruciferous plants such as canola or beet crop plants or white mold in various host plants such as vegetables, canola and even ornamental plants. A number of prodiginines and one terpene reduced the infestation of plants with *Heterodera schachtii*, a parasitic nematode that causes serious problems in the cultivation of canola or sugar beet, for example. Some prodiginines even stimulated plant growth. These interdisciplinary studies demonstrated that, here in particular using the example of prodiginines, how desired properties can be evolved through structural modifications starting from a bioactive lead compound. The project thus contributed to the discovery and provision of bioactive compounds with the potential to develop new plant protection products.

Questioning social acceptance

The research field summarized under the term synthetic biology promises sustainable microorganism-based solutions of some of today's major environmental and societal challenges. This also was the motivation for the CombiCom project. However, the technical questions are not the only relevant aspect in the emergence of such microorganism-based technologies involving synthetic biology. These may also be of a social nature since, for example, questions of public acceptance and potential innovation barriers arise. Scientists therefore analyzed these aspects in more detail.

In general, the fear of public fear is a central concern among researchers and innovators. Such fears can prevent or slow the establishment of promising research approaches or technologies. A central factor for the acceptance of new technologies is information and knowledge transfer. In the case of synthetic biology, acceptance is quite low. In addition, project studies showed that acceptance can be influenced mainly by the potential benefit as well as by statements of important actors such as farmers. Steps to proactively present the potential of new technologies in a positive light, including for society as a whole, are thus crucial.



Establishment of the production of bioactive terpenoids in the bacterium *Rhodobacter capsulatus* and the fungus *Ustilago maydis*: in order to use them, tools for controlled gene expression first had to be developed. After adapting the metabolic pathways, different terpenes could finally be produced that can be used as inhibitors of plant pathogens.

Intensive discussions were held to determine which factors are decisive for the establishment and acceptance of a new technology such as synthetic biology.

On this basis, the researchers developed a concept for an interdisciplinary invention ecosystem that arises in the center of the multifaceted influences of different actors and stakeholder groups. From this point of view, teams of the Heinrich Heine University Düsseldorf evaluated the situation. Significant influence factors for the emergence of synthetic life sciences could thus be determined, as well as differences in perception and weighting of these for representatives of all stakeholder groups, such as researchers, students, administration and also industry partners. In doing so, the relevance of administrative actors became clear, since these can establish a coherent and common leading vision between less compatible stakeholder groups. Among other things, proactive positive communication by experts was defined here as essential. Based on the aspects that were deemed important and modifiable, the scientists could formulate suggestions for initiatives that can support the establishment and acceptance of new technologies. The interdisciplinary work in the CombiCom project thus addressed the promising potential applications of synthetic biology and, in doing so, on the one hand the technological challenges and on the other hand the socioeconomic implications. These findings can be used as a basis for the development and improvement of much needed bioactive products as well as for their future commercialization.

Contact

Dr. Anita Loeschcke,
Institute of Molecular Enzyme Technology, HHU Düsseldorf,
a.loeschcke@fz-juelich.de

Core Groups involved

Prof. Dr. Karl-Erich Jaeger, Dr. Anita Loeschcke, Dr. Thomas Drepper, Robin Weihmann, Fabienne Hilgers,
Institute of Molecular Enzyme Technology, HHU Düsseldorf

Prof. Dr. Michael Feldbrügge, Dr. Kerstin Schipper, Jungho Lee, Peter Stoffels,
Institute of Microbiology, HHU Düsseldorf

Prof. Dr. Matias Zurbriggen, Nicole Heucken, Lisa Hüsemann,
Institute of Synthetic Biology, HHU Düsseldorf

Prof. Dr. Jörg Pietruszka, Dr. Thomas Classen, Jan Gebauer, Hannah Brass,
Institute of Bioorganic Chemistry, HHU Düsseldorf

Prof. Dr. Ulrich Schwaneberg, Dr. Anna Joëlle Ruff, Dr. Johannes Schiffels, Stefanie Brands,
ABBt – Biotechnology, RWTH Aachen

Prof. Dr.-Ing. Jochen Büchs, Dr. Nina Ihling, Carl Brehl,
AVT – Bioprocess Engineering, RWTH Aachen

Prof. Dr. Florian Grundler, Dr. Sylvia Schleker, Dr. Samer Habash,
INRES – Molecular Phytomedicine, University of Bonn

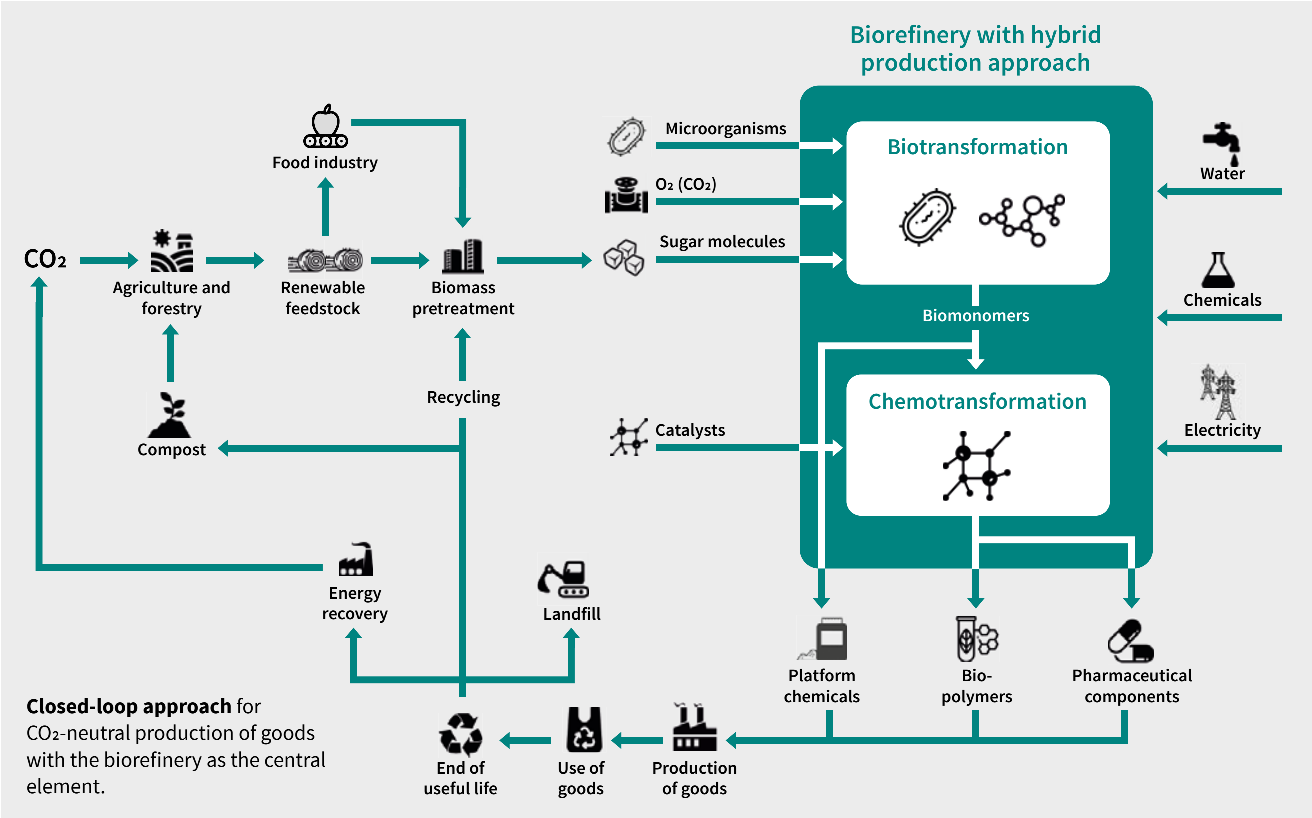
Prof. Dr. Ulrich Schurr, Dr. Holger Klose, Dr. Diana Reinecke-Levi,
IBG-2 Plant Sciences, Forschungszentrum Jülich

Prof. Dr. Stefanie Bröring, Dr. Chad M. Baum,
ILR – Technology, Innovation Management and Entrepreneurship, University of Bonn

On the Road to Sustainable Production of High-Value Compounds

In order to build economically viable biorefinery concepts, the development of closely linked bio- and chemo-transformation approaches and customized down-stream processing is required. On the basis of these hybrid production processes, one-dimensional value chains can be overcome and biorefineries can be developed into ecologically and economically operating "multi substrate to multi product" factories. An initial hybrid demonstrator process to produce platform chemicals and pharmaceutical compounds was developed in the BioSC HyImPAct project.

CO_2-neutral production requires the development of an industrial bioeconomy based on renewable raw materials such as plant biomass. In this context, biorefineries occupy a central place in order to be able to realize closed-loop approaches with a low carbon footprint. Starting with biomass – ideally regional residue streams such as wastes from agriculture and forestry, as well as from the food-processing industry – chemical precursors, biopolymers and pharmaceutical compounds can be produced in biorefineries using bio- or chemocatalytic substance transformations.



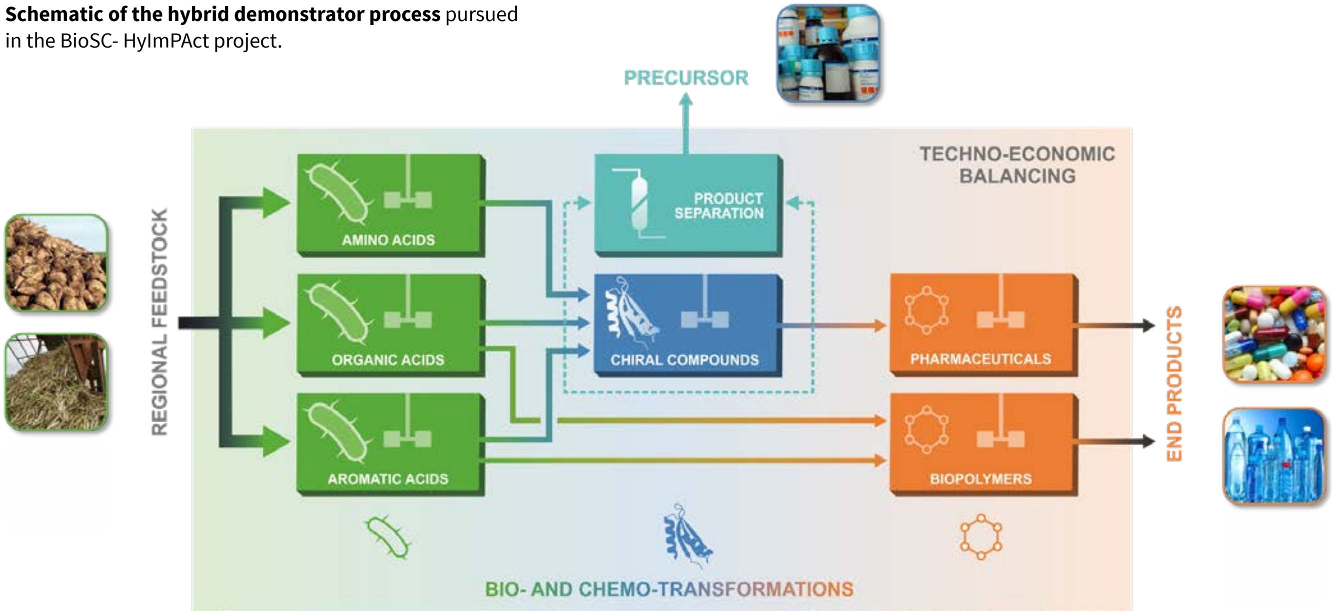
However, such a broad product range will be accessible only if there is a consequent development of existing biorefinery concepts towards "multi substrate – multi product" factories. This approach, hereinafter referred to as a "hybrid production process", is not fundamentally new but has been pursued in many different fields of industrial production for some time.

Combination of precursors and end products

In the framework of the BioSC-funded "HylmPAct" FocusLab, hybrid production processes are being developed based on renewable resources. The focus is on biological and technical approaches based on a close integration of bio- and chemotransformations and down-stream processing. The work is supported by techno-economic analyses that deal specifically with the comparison of the alternative methods developed in "HylmPAct" with already established production processes. Simultaneously, new models and tools to plan, evaluate and optimize hybrid processes were developed. In doing so, experiences and approaches from the field of digital biorefinery modeling already collected and established in the previous "BeProMod" BioSC project can serve as a basis.

The current status of the hybrid demonstrator process developed in "HylmPAct" will be discussed below. Two different products from the field of platform chemicals and active pharmaceutical ingredients are considered.

Schematic of the hybrid demonstrator process pursued in the BioSC- HyImPAct project.



Lowering of the carbon footprint through biotechnology

Succinic acid, for example, is an important platform chemical in the chemical industry. What makes it so interesting is that - due to its chemical properties – it can be converted to other precursors for important substances such as 1,4-butanediol. It thus forms the basis for a wide variety of industrial applications. In addition, succinic acid is a central building block for various polyesters, including the biodegradable polybutylene succinate. According to estimates of the nova Institute, the industrial demand for succinic acid will increase to 94,000 metric tons per year over the next 5 years. If produced based on only fossil resources, the corresponding fourfold quantity of carbon would be withdrawn from the earth and ultimately fed into the CO_2 cycle. In the framework of “HyImPAct”, three alternative transformation approaches for the provision of succinic acid were investigated. The platform organism *Corynebacterium glutamicum*, well known at Forschungszentrum Jülich, was further developed to utilize lignocellulose-containing biomass in order to produce high-value compounds. Lignocelluloses stabilize the cell walls of many plants and thus are available in large quantities in form of plant and wood wastes. Using the developed producer strains, a carbon-efficient transformation of the sugars glucose and xylose – derived from lignocellulose – into succinic acid has already been successful at lab-scale. In addition, other precursors such as the organic acid pyruvate or the amino acid alanine are produced simultaneously. In the hybrid process approach both are important for the production of pharmaceuticals.

An alleged disadvantage of the selected organism *Corynebacterium glutamicum* for biotransformation is that succinic acid production must necessarily be carried out under neutral pH conditions. The subsequent down-stream processing traditionally involves several pH adjustments by addition of acids and bases. This results in a high auxiliary production of neutral salts with negative economic and ecological effects. However, this high salinity can be avoided completely by regulating the pH value electrochemically via water splitting. The underlying electrochemical “power-to-purity” process concept was developed at the RWTH Aachen University and adapted and optimized during “HyImPAct” for the separation of various organic acids. Subsequently, a life cycle assessment with evaluation of the carbon footprint was carried out, based on the experimental data of two realized lab-scale processes for succinic acid and the optimal production processes simulated in the "BeProMod" project. For an economically competitive scenario, maize stover as a potential regional residue stream was comparatively investigated in three established pretreatment processes of this biomass.

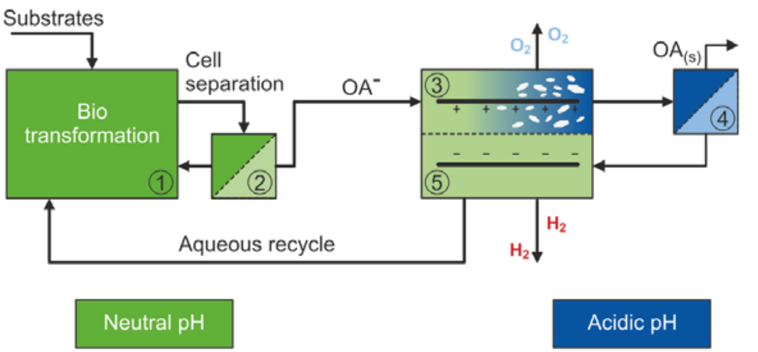
In contrast to the wide application of platform chemicals in the chemical industry, connected with large-scale productions, high-value products such as pharmaceuticals are needed in considerably smaller quantities. As a result, the carbon footprint is not the decisive criterion for life cycle assessment.

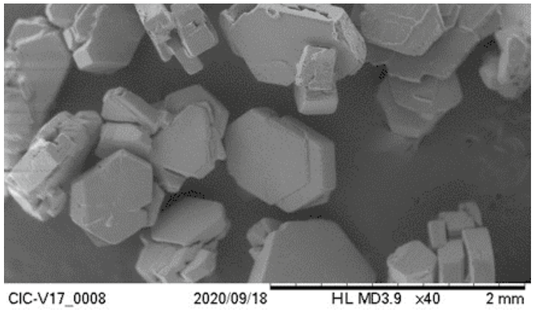
Environmentally friendly production of bio-based pharmaceuticals

Active pharmaceutical ingredients are often chiral substances in which the atoms can take up different spatial arrangements. Substances with identical molecular formula but different spatial structures are called enantiomers. Nevertheless, chirality is of enormous importance for the correct activity of a pharmaceutical since the biological activity is mostly based on a specific enantiomer.

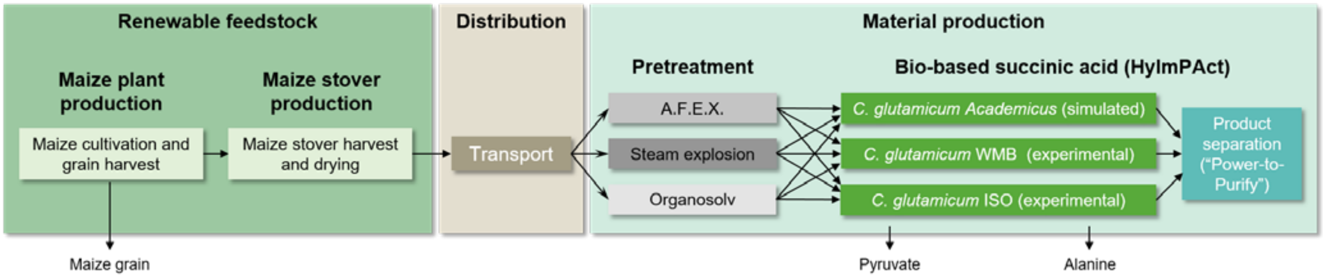
In order to specifically produce individual enantiomers, a multi-phase synthesis using protecting group chemistry and toxic adjuvants is traditionally required. These methods thus contribute to a significant waste streams formation. Bio-transformations based on enzymatic cascades are an ecologically and economically meaningful alternative. Using biodegradable and highly selective biocatalysts, high-value molecules from bio-based materials can be produced under mild reaction conditions. At this point the hybrid demonstration process pursued in the "HyImPAct" project comes in with the specific product metaraminol and the product class of tetrahydroisoquinolines (THIQs). Metaraminol is an active pharmaceutical ingredient used to treat low blood pressure and simultaneously serves as chiral precursor for more complex bioactive compounds such as the THIQs. Initially, the bio-based precursors 3-hydroxybenzoate, pyruvate and alanine are converted into metaraminol using a three-step enzymatic cascade. Suitable biocatalysts with high activity could be obtained for all cascade steps, particular for the conversion of real substrates from cell-free supernatants of the upstream microbial bio-transformation.

However, a greater challenge arises for the third cascade step. Here alanine is used as a bio-based co-substrate. The involved





Electrochemical power-to-purity-process concept for the separation of biotechnologically produced organic acids. Left: process diagram, 1) pH-neutral fermentation, 2) cell separation, 3) low-pH crystallization, 4) product separation and 5) electrochemical pH management for biotransformation (high pH). Right: bio-based succinic acid crystals after down-stream processing.



Life cycle assessment for bio-based succinic acid from maize stover compared to fossil fuel based production. For biomass pretreatment, three established methods (ammonia fiber expansion (AFEX), steam explosion and organosolv) were compared.

reaction takes place close to thermodynamic equilibrium. Without technical support measures, this leads to low metaraminol yields. To increase the reaction yield, an *in-situ* product removal method was developed. The concept allows a higher metaraminol yield and minimizes waste streams, since production and a first down-stream processing step could be realized in a common process.

In a final step, metaraminol can be converted into a broad range of THIQs. Chemical synthesis -based on the Pictet–Spengler reaction- was developed and optimized for the use of bio-based metaraminol as substrate.

The current results show that hybrid production processes allow sustainable production of platform chemicals and active pharmaceutical ingredients based on renewable resources. Hybrid process approaches are thus essential for the achievement of already decided climate targets regarding their CO_2-neutrality and a consequent implementation of existing environmental standards in the production of valuable substances and thus will inevitably gain in economic importance.

Contact

Dr.-Ing. Stephan Noack,
IBG-1 Biotechnology, Forschungszentrum Jülich,
s.noack@fz-juelich.de

Core Groups involved

Prof. Dr.-Ing. Wolfgang Wiechert, Dr.-Ing. Stephan Noack, Prof. Dr. Dörte Rother, Prof. Dr. Jan Marienhagen, Mohamed Labib, Christian Brüsseler, Laura Grabowski, Kevin Mack, Douglas Weber, Jochem Gätgens,
IBG-1 Biotechnology, Forschungszentrum Jülich

Prof. Dr. Jörg Pietruszka, Andreas Sebastian Klein, Anna Christina Albrecht,
Institute of Bioorganic Chemistry, HHU Düsseldorf

Prof. Dr.-Ing. Andreas Jupke, Moritz Doeker, Christian Kocks, Thomas Fuchs,
AVT – Fluid Process Engineering, RWTH Aachen

Prof. Dr. Grit Walther, Dr. Angel Merchán, Michael Wolff,
Chair of Operations Management, RWTH Aachen

Enzymes – the "Workhorses" of Industrial Biotechnology

The technology platform BIOExpresSPro provides tools for the identification and production of industry-relevant enzymes that can degrade plant biomass effectively, for example. Using innovative protein-based fluorescence biosensors, secretion processes can now be monitored directly. An automated mini-pilot plant micro bioreactor system with higher process throughput was validated and is now available for strain screening.

An essential part of the bioeconomy is exploiting and utilizing biological resources to produce sustainable and bio-based products. Plants have a key position here since they produce biomass using water, carbon dioxide, and sunlight. They thus represent an almost inexhaustible source of raw material for bioeconomic utilization and industrial value creation. However, to use these plant raw materials sustainably, they must first be made accessible. One strategy to gain this access under environmentally friendly conditions is the application of enzymes.

Hidden champions

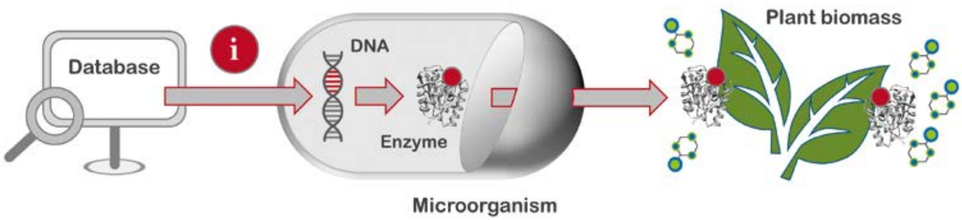
Enzymes are biocatalysts that accelerate chemical reactions. In doing so, enzymes are very efficient. An enzyme catalyzes a chemical or biochemical reaction by converting a specific starting molecule, the substrate, into a product. The enzyme itself emerges unaltered from this reaction. Enzymes are thus “hidden champions”, the “workhorses” of industrial biotechnology. Among other things, they are used as helpers in the food and feed industry but also in the cosmetics, textile, paper and pulp industries, the chemical industry, fuel production, the agricultural industry, and the medical and pharmaceutical industries.

The objective of the BioSC Boost Fund project BIOExpresSPro was to identify in a first step naturally occurring enzymes that degrade plant biomass. Such enzymes can occur in fungi and bacteria, for example, which in nature ensure the decomposition of everything from leaves to entire tree trunks. However, these microorganisms occurring in nature are often not suitable for use in the laboratory. Their demands on the environment, such as nutrient supply and temperature, are usually too complex. Thus, the second objective was to transfer the genes encoding these specialized enzymes into easy-to-handle laboratory organisms, producing and secreting the desired enzymes. In a further step, the enzymes were to be optimized. To achieve these goals, broad expertise from biology, biochemistry, biotechnology, bioprocess engineering, bioanalytics, and bioinformatics was required. Furthermore, BIOExpresSPro has been advised by two associated industry partners throughout the project. These partners were AB Enzymes GmbH and evocatal GmbH, which Advanced Enzyme Technologies Ltd. has since acquired as evoxx technologies GmbH.

Pooling of expertise

As a technology platform, BIOExpresSPro channels this broadly based expertise and industry knowledge into so-called pipelines. Thus, a prediction pipeline was initially established to identify new biomass-degrading enzymes. Using sophisticated algorithms, publicly accessible databases such as BRENDA and UniProt were bioinformatically searched. By doing so, unknown enzymes were found whose features are in great demand also by industry partners. Such enzymes were found in the bacterial genera *Arthrobacter* and *Pseudomonas*, for example. To use such enzymes in bioeconomic processes, they must be produced in sufficient quantities. Hence, in another pipeline, the genes of the bioinformatically identified project-relevant enzymes were introduced into easy-to-handle microbial hosts using state-of-the-art cloning methods. These hosts then synthesized the sought-after enzymes in large quantities. By directed evolution of the host cells, the scientists also ensured that the desired enzymes were released in larger quantities from the host cells and did not remain in the cell interior. This so-called secretion facilitates the subsequent harvest of the enzymes immensely.

An extensive genetic library of the enzyme cutinase from the fungus *Fusarium solani pisi* was generated and used in different host organisms to demonstrate the method’s feasibility. Cutinases can crack the waxy protective armor of plants. They open the gates into the plant and make them accessible for further

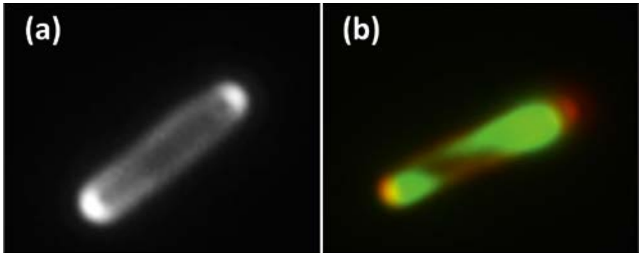


The BIOExpresSPro technology platform aims to provide enzymes that can be used to degrade plant biomass. In the framework of a BioSC Boost Fund project, new enzymes were identified using bioinformatic methods. Their genes were cloned and expressed in bacteria, and thus novel enzymes were produced that can degrade plant biomass.

degradation processes. These enzymes thus play an essential role in the industrial utilization of plant biomass. In a subsequent step, it was possible to optimize cutinase production in *Escherichia coli* and *Corynebacterium glutamicum*, which are widely used bacteria in biotechnology.

Successful production of enzymes

Scientists of the technology platform BIOExpressSPro also developed novel biosensors to monitor the secretion of the synthesized desired enzyme from the cell in a concentration- and location-dependent manner. The process is based on a fluorescent protein that lights up cells that secrete particularly heavily. Using this technique, it was possible to isolate a new hypersecretion mutant of *C. glutamicum* that secreted significantly higher quantities of the desired enzyme into the medium than the wild-type strain. In parallel, another novel fluorescent sensor was established, which is used to analyze secretion and protein localization in *E. coli*. This biosensor allows localizing the selected target protein, for example the desired enzyme, in the cell due to its fluorescence emission. Since this biosensor has a different fluorescence inside the cells than outside, it can also determine the secretion rate of the desired enzyme across the inner cell membrane. In addition, an automated mini-pilot plant micro bioreactor system was developed and validated at the level of process development. This bioreactor system helps to find, isolate and cultivate bacterial strains with the desired features in a short time. The results obtained and the methods newly developed in the framework of BIOExpressSPro were so promising that they had been incorporated into further projects that have been and are being funded by the Federal Government, the state of NRW, and the EU.



Novel biosensors can show where proteins are located in a bacteria cell. (a) Monochrome fluorescence microscope image of an *E. coli* cell with a biosensor in the periplasm, which is the area between the two membranes that surround the cell. The entire periplasm of the cell fluoresces; a strong fluorescence appears at the poles of the cells. (b) Two-color image of an *E. coli* cell with a biosensor in the cytoplasm (green fluorescence) and the periplasm (red fluorescence). There is less biosensor in the cytoplasm so that even the weak fluorescence from the periplasm is visible.

Contact

Dr. Achim Heck,
Institute of Molecular Enzyme Technology, HHU Düsseldorf,
a.heck@fz-juelich.de

Core Groups involved

Prof. Dr. Karl-Erich Jaeger, Dr. Achim Heck, Dr. Thomas Drepper, Dr. Katrin Troost, Andreas Woop,
Institute of Molecular Enzyme Technology, HHU Düsseldorf

Prof. Dr. Michael Bott, Prof. Dr. Roland Freudl, Sarah-Kristin Jurischka,
IBG-1 Biotechnology, Forschungszentrum Jülich

Prof. Dr. Wolfgang Wiechert, Prof. Dr. Marco Oldiges, Dr. Jannick Kappelmann, Dr. Johannes Hemmerich,
IBG-1 Biotechnology, Forschungszentrum Jülich

Prof. Dr. Björn Usadel, Dr. Alexander Vogel,
IBG-4 Bioinformatics, Forschungszentrum Jülich

Prof. Dr. Ulrich Schwaneberg, Dr. Anna Joëlle Ruff, Dr. Wei Long,
ABBt – Biotechnology, RWTH Aachen

Industry partners (associated partners)

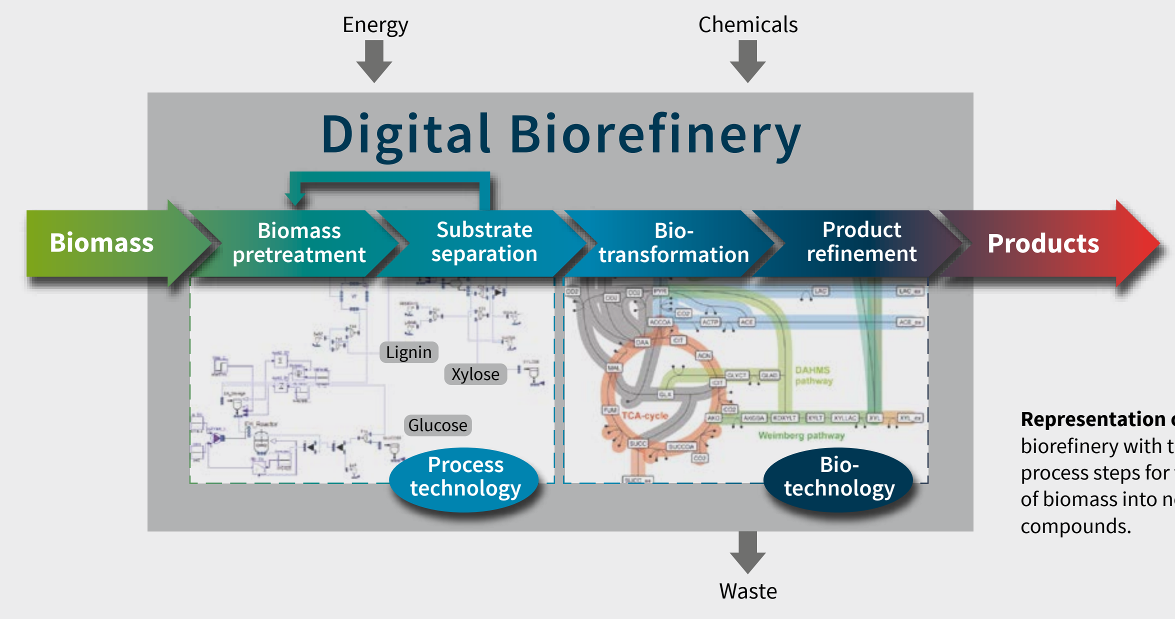
Dr. Christian Degering, Dr. Michael Puls, Dr. Christian Leggewie,
evocatal GmbH, Düsseldorf (currently: evoxx technologies)

Dr. Jari Vehmaanperä, Prof. Dr. Karl-Heinz Maurer,
AB Enzymes GmbH, Darmstadt

The Digital Biorefinery – Optimal Conversion of Renewable Resources

Computer models are playing an increasingly important role in enhancing the competitiveness of bio-based procedures. With their help, it is possible to precisely predict and thus optimize biological and technical processes. This applies in particular to the operation of biorefineries. Their efficiency depends to a large extent on the degree of utilization of the applied renewable resources and their effective conversion into new valuable substances. In the framework of the BeProMod Boost Fund project, a digital biorefinery was developed that can map optimal operation strategies in facilities with high modularity and changing input materials.

Renewable resources such as plant or animal biomass are rich in carbohydrates, fats, oils and proteins. Thus, they represent an alternative to crude oil in the production of chemicals. The conversion of biomass into new valuable materials take place in biorefineries and involves a variety of process steps. In order to be competitive compared to crude oil-based procedures, individual steps of the biological and technical processes as well as the overall process need to be optimized. As with crude oil-based refineries, the operation of biorefineries will be economical only if all chemical components are used to the maximum and with the highest efficiency.



Representation of the digital biorefinery with the essential process steps for the conversion of biomass into new high-value compounds.

Depending on the composition of the biomass and the type of the intermediate and end products desired, there are different biorefinery concepts. On the one hand, research teams are designing biorefineries that can cope with changing input materials, from straw and reeds to wood waste. On the other hand, they are developing optimal operating strategies for facilities that are modular in design and thus can be used flexibly. The highly complex questions involved can no longer be solved purely empirically, that is, by more or less targeted trial and error. It is more effective to digitally model a biorefinery with all its facets and possibilities using a computer model, before it is actually built. The development of such a digital biorefinery was the core task of the “BeProMod” project in the framework of a BioSC Boost Fund. This project was mainly focused on the utilization of lignocellulose-containing biomass. Lignocelluloses provide stability in the cell walls of many plants and even fend off fungi and bacteria with their dense structure. They account for up to 95 percent of the dry biomass in terrestrial plants – thus, they are available in large quantities but are difficult to utilize due to their robustness.

Cracking unruly lignocellulose

At the center of a lignocellulose biorefinery is a biochemical conversion process with microorganisms as the key players. However, these microorganisms initially cannot handle the lignocellulose for its long-chain, highly branched molecular structure and need a technical jump start. The biomass is thus pretreated by mechanical and enzymatic processes. One such process is, for example, the OrganoCat process developed at RWTH Aachen University and the Forschungszentrum Jülich. This process was established on the basis of waste from the wood processing industry and then further developed in the “AP³” FocusLab for the treatment of a wide variety of lignocellulose-containing residue streams (see AP³ article). The substance mixture obtained by the pretreatment is then further separated, resulting in a solids-containing cellulose fraction and an aqueous phase with dissolved sugars and lignin as an additional complex component. While the subsequent enzymatic breakdown of the cellulose into glucose is technically solved, the decomposition of lignin still poses a major challenge and is the subject of current research at the BioSC. Therefore, lignocellulose cannot be entirely utilized - yet. However, two material flows result, containing high yields of sugars with six carbon atoms (glucose) or five carbon atoms (xylose). This small but distinctive difference is vitally important for further microbial bio-transformation.

The sugars released from lignocellulose can be converted by suitable microorganisms and their natural enzymes into desired intermediate and final products. However, there are two basic biological issues to be considered: First, there is no SINGLE industrially applicable microorganism that naturally utilizes all the different sugars from the pretreatment stages. Second, based on different cell types and metabolic pathways, there are always several pathways that allow the utilization of a particular sugar. The task of the researchers was to find the optimal procedure. Depending on the defined starting material (e.g. C5- or C6 sugar) and the desired target product, the conversion can cause a more or less by-product formation of CO_2. This positively or negatively affects the efficiency of the carbon use in the overall process of the biorefinery and thus also the economic efficiency as well as the ecological footprint.
At this point digital comes into play.

Many small models result in the big picture
Computer-aided process modeling and optimization steadily increase the competitiveness of bio-based procedures compared to existing conventional methods. Due to the outlined complexity of a biorefinery, the creation of a digital computer model requires the expertise of scientists from different disciplines – above all bio- and system- process engineering. In the framework of the BeProMod BioSC project, scientists of the RWTH Aachen University and the Forschungszentrum Jülich created a first demonstration model of a digital biorefinery.

The level of detail ranges from the biological description of individual enzyme-kinetics reactions to complex microbial metabolic networks and from the technical implementation of various basic operations and reactor types to complete process chains of a biorefinery. The model approach was chosen in a way that future further extensions based on new experimental data and in-depth knowledge is easily possible. Special attention was paid to user-friendliness and reusability of individual model components to allow modelling of dynamic processes from other scopes of application. In a first concrete case study, a production process was modelled in which microorganisms provide the organic acid succinate as an important precursor for industrial plastics production. The key player in this process was the model organism *Corynebacterium glutamicum*, which was optimized for this task.

With the help of the computer model, ideal strain and process variants could be identified for a scalable production of succinate based on OrganoCat substrates. The jump from the digital world into a real-world biorefinery is being prepared in the "HyImPAct" BioSC FocusLab, among others. The experimental implementation and further techno-economic questions concerning the best possible production process are assessed here.

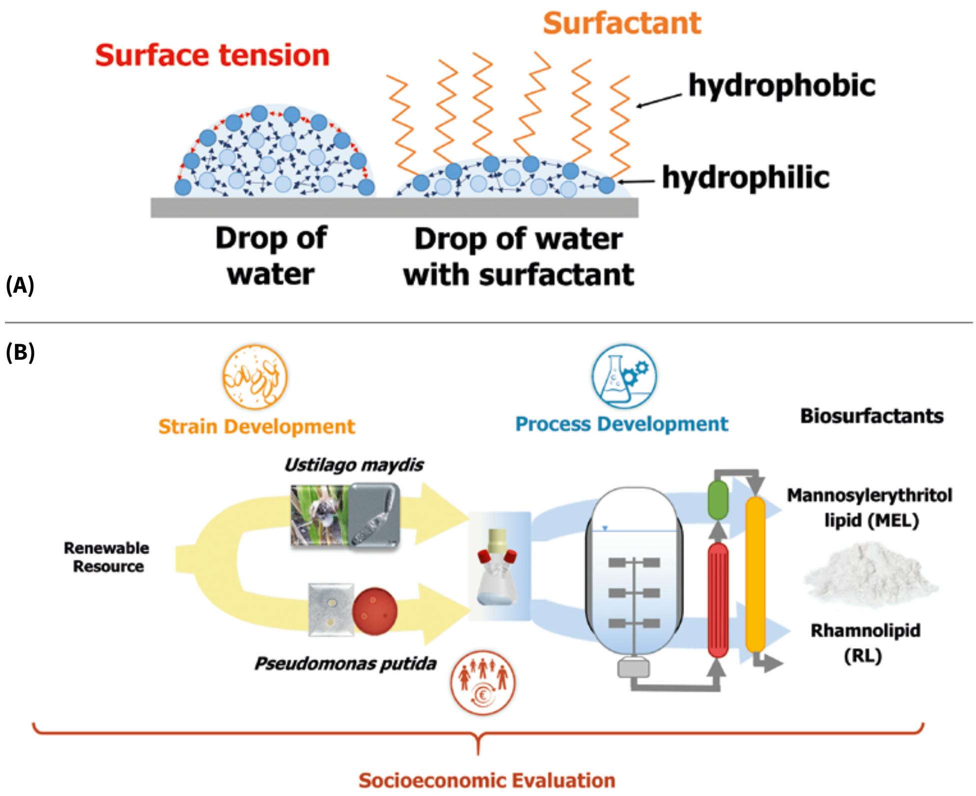
Contact
Dr.-Ing. Stephan Noack,
IBG-1 Biotechnology, Forschungszentrum Jülich,
s.noack@fz-juelich.de

Beteiligte Core Groups
Prof. Dr.-Ing. Wolfgang Wiechert, Dr.-Ing. Stephan Noack,
Dr.-Ing. Eric von Lieres, Dr.-Ing. Xiao Zhao,
IBG-1 Biotechnology, Forschungszentrum Jülich
Prof. Dr.-Ing. Alexander Mitsos, Dr. Ralf Hannemann-Tamás,
Tobias Ploch,
AVT – Process Systems Engineering, RWTH Aachen
Prof. Dr. Uwe Naumann, Jonathan Hüser,
Software and Tools for Computational Engineering, RWTH Aachen

Surfactants Become Competitive

The Bio² FocusLab was focused on the development of a competitive biorefinery process for bio-based surfactants. The production routes of two biosurfactants using bacteria and fungi were selected as example processes. In contrast to surfactants conventionally produced from crude oil, biosurfactants can be obtained from renewable resources. However, competitive production is complicated by various challenges that were addressed along the entire process chain at the same time.

Surfactants reduce the surface tension of liquids and the interfacial tension in substance mixtures. This opens up a wide range of applications. For example, surfactants are used in cleaners, as foaming agents in firefighting and also as emulsifiers in the food industry. Completely bio-based surfactants, called biosurfactants, present a sustainable alternative for surfactants produced from crude oil. Biosurfactants also include natural substances produced by bacteria and fungi.



(A) Mode of action of surfactants: They consist of a hydrophilic and a hydrophobic element. Therefore, they are able to reduce the surface tension of liquids or moderate oil-water emulsions.

(B) Process chain for the production of biosurfactants from renewable resources in the Bio² project.

Holistic approach

Microbial biosurfactants face several challenges. These include high production costs resulting from the use of expensive raw materials such as purified sugars or plant-based oils on the one hand. It is thus more effective to use raw materials that are cheaper and at the same time not in competition with food production. On the other hand, so far the yields are rather low when bacteria are used for production. Biosurfactants are often produced by bacteria that are pathogenic for humans and are thus not suitable for process development. In addition, process control for the production of biosurfactants in classical stirred tank bioreactors is made difficult by massive foam formation. This can be reduced by the addition of antifoam agents but results in complicated purification.

Within the framework of Bio², all the above-mentioned challenges were addressed in a holistic approach along the entire process chain. Strain and process development were considered simultaneously. A continuous socio-economic process analysis assessed the process on the basis of ecological, economic and social aspects and suggested possible improvements. This holistic approach could be implemented only in an interdisciplinary team.

Biosurfactants from natural sources

The bacterium *Pseudomonas putida* is known as a non-pathogenic representative of the Pseudomonas bacteria family and is especially suitable for the construction of genetically modified production strains for biosurfactants of the rhamnolipids class.

Since *Pseudomonas putida* does not produce such biosurfactants in nature, the corresponding genes must be transferred from the closely related but human-pathogenic bacterium *Pseudomonas aeruginosa*.

The metabolism of *Pseudomonas putida* was optimized and numerous systems for the expression of the rhamnolipid biosynthesis genes were developed in various subprojects. Thus, the researchers succeeded in producing a potent rhamnolipid production strain by combining both systems.

Use of plant residues and alternative sugars

To be able to use renewable raw materials as food for the microorganisms, the bacterial strain was adapted in the laboratory to use xylose and ethanol as carbon sources. The sugar xylose is an important component of plant material that can be readily obtained at low cost, and which could serve as an energy source for the bacteria and can thus increase the added value of the process. Three different pathways to utilize xylose were introduced into *Pseudomonas putida* using genetic engineering and now allow growth on this simple sugar. Subsequently, productivity could be further improved using so-called adaptive laboratory evolution. Ethanol is another interesting substrate that can be produced inexpensively from biomass. This alcohol can be used particularly efficiently for the production of rhamnolipids with *Pseudomonas putida*, since the bacteria have the natural ability to metabolize ethanol. This process could also be improved significantly by adaptive laboratory evolution. It could be demonstrated for the new strains that the production of rhamnolipids based on alternative substrates is comparable or even better than when glucose was used. At the same time, metabolism was optimized by removing energy-intensive processes. In addition, synthesis pathways that compete with the rhamnolipid biosynthesis were switched off.

A new tool that allows the construction of genetically stable production strains was developed to express the biosynthetic genes. Controllable expression systems, applied in rhamnolipid production for the first time, showed efficient and controllable production combined with excellent stability of the production strains. Finally, the broader applicability of the strains could be shown by targeted production of different rhamnolipid variants.

Fungi produce biosurfactants

The fungus *Ustilago maydis* produces biosurfactants naturally. In nature, however, this strain produces a mixture of glycolipids. The objective of the work was to obtain only one of these glycolipids, mannosylerythritol lipid-D, in purest form. Mannosylerythritol lipids are produced under conditions of nitrogen starvation in nature. If renewable resources are used, the nitrogen content cannot be controlled arbitrarily. In order to achieve the production of mannosylerythritol lipid-D from renewable resources, a strategy was established for artificial regulation of the biosynthesis steps. In addition, various enzymes of the corresponding biosynthesis pathway were relocalized within the cell to generate biosurfactant variants with new properties.

The substrate spectrum was also expanded for this microorganism to be able to use renewable resources more efficiently; in this case polygalacturonic acid. This organic acid is a major component of pectin, which can be found in sugar beet pulp, for example. Therefore, work of the UstiLyse Seed Fund and the PectiLyse Boost Fund projects was continued. The already extensive enzyme repertoire of the fungus for biomass utilization was extended by bacterial enzymes that can degrade pectin.

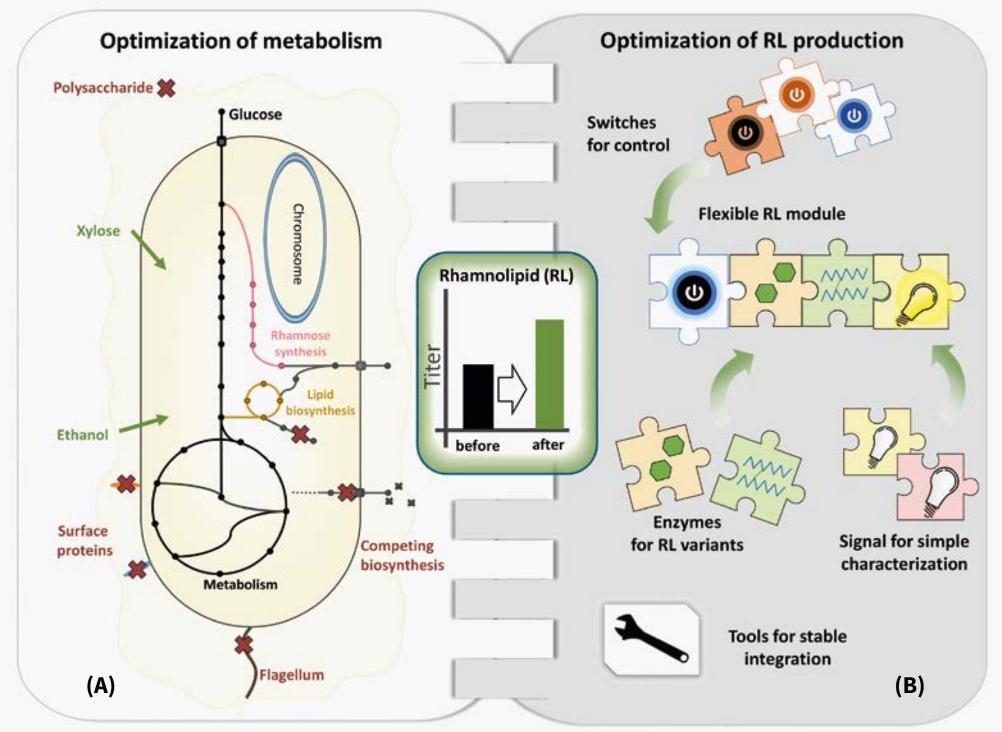
Far-reaching analyses

One prerequisite for the parallel evaluation of the performance of different bacterial and fungal strains are simple methods that allow the rapid and reliable detection and quantification of biosurfactants. In the framework of the project, the so-called Victoria Pure Blue BO test was newly developed for rapid quantification of various biosurfactants from fermentation broth. While these and similar methods can be used to gain a first impression about the obtained titers, the analytics platform installed within the project provides insight into the exact quantity and chemical composition of the biosurfactants.

The production of microbial biosurfactants is linked to the idea of a progressive and sustainable process design. Accordingly, among others, an integrated approach was taken to directly couple fermentation of the optimized microorganisms with the

(A) Optimization of the metabolism of *Pseudomonas putida* (red X symbols label switched off processes)

(B) Optimization of the rhamnolipid production using a flexible toolbox approach. (Center) Qualitative change of rhamnolipid titers before and after optimization.



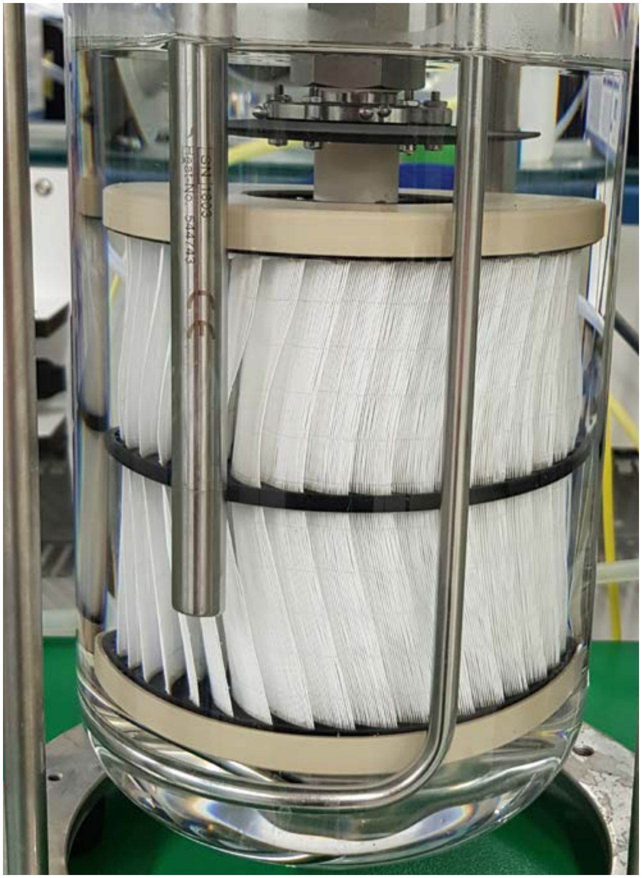
product purification. Based on studies in the laboratory and at pilot scale, a life cycle assessment, a life cycle account analysis and a social life cycle analysis of the processes were carried out simultaneously. This allows starting points for optimizations to be identified as early as possible. It was also investigated to what extent the biomass remaining at the end of the process can be used in subsequent fermentations to further utilize arising waste streams in the sense of a sustainable process design.

A new concept for the cultivation of microorganisms – membrane aeration – was developed in the framework of the project and registered for a patent. This module combines the advantages of bubble-free oxygen supply with unique control functions within the fermentation process and thus makes methods for mechanical and chemical foam destruction obsolete. The possibility to integrate the respective modules in existing bioreactors allows for easy application. In addition, interconnection of the membrane module with a filtration membrane for cell retention is the basis for product separation integrated into the fermentation process.

Improved competitiveness

In the framework of the project, various strategies for the purification of biosurfactants from the fermentation broth were developed and evaluated. Potential separation methods were initially studied in experimental and model-based approaches at the laboratory scale in order to investigate a variety of process parameters in the most resource-efficient way possible. Subsequently, the separation operations were combined into purification processes with the aim of minimizing losses and maximizing the purity of the biosurfactants.

The process chains resulting from the interdisciplinary collaboration of all groups were evaluated from a socio-economic point of view, assessing processes from biomass provision to the final biosurfactant. With regard to the economic evaluation, a classification compared to the current market development could be carried out by analyzing market data, economic key figures and specific product prices. By opening up new raw material sources, increasing the yields, developing new cultivation concepts and establishing new analytical methods, the project contributed to improving the opportunities for biosurfactants on the market.



Module for bubble-free membrane gassing integrated into a bioreactor.

Contact

Dr. Nina Ihling,
AVT – Bioprocess Engineering, RWTH Aachen,
Nina.Ihling@avt.rwth-aachen.de

Core Groups involved

Prof. Dr.-Ing. Jochen Büchs, Dr. Nina Ihling, Maximilian Schelden, Katharina Miebach,
AVT – Bioprocess Engineering, RWTH Aachen

Prof. Dr. Michael Feldbrügge, Dr. Kerstin Schipper, Dr. Silke Jankowski, Dr. Marius Terfrüchte, Magnus Philipp,
Institute of Microbiology, HHU Düsseldorf

Prof. Dr. Lars Blank, Dr. Till Tiso, Dr. Isabel Bator,
ABBt – Applied Microbiology, RWTH Aachen

Prof. Dr. Karl-Erich Jaeger, Dr. Stephan Thies, Dr. Sonja Kubicki,
Institute of Molecular Enzyme Technology, HHU Düsseldorf

Prof. Dr.-Ing. Andreas Jupke, Andreas Biselli,
AVT – Fluid Process Engineering, RWTH Aachen

Prof. Dr. Matthias Wessling, Robert Keller, Patrick Bongartz,
AVT – Chemical Process Engineering, RWTH Aachen

Prof. Dr. Jürgen-Friedrich Hake, Dr. Wilhelm Kuckshinrichs, Dr. Petra Zapp, Dr. Andrea Schreiber, Andreas Schonhoff,
IEK-STE Systems Analysis and Technology Evaluation, Forschungszentrum Jülich

SEED FUND **Econ-BioSC**

Strengthening Bioeconomies Around the World

Bioeconomies are increasingly globally interconnected through biomass trade and international knowledge transfer that promotes technological innovations. Quantitative approaches such as modelling and scenario development can help to assess the sustainability impacts of bioeconomic change and allow prospective analysis of potential innovation paths including the convergence of previously separate technology sectors. The Econ-BioSC project enabled the development of an innovative model system and a roadmapping approach for applied sustainable research and policy advice.

Technological innovation in bio-based value chains is essential to supply the growing world population with food and feed as well as biofuel and biomaterials. For example, improved agricultural technologies enable the production of food while using fewer resources such as land or water. Accordingly, the more efficient conversion of biomass in the processing industry and the recovery of valuable components from waste streams can increase the value added in bio-based supply chains. However, such benefits do not always accrue automatically when new technologies are introduced in complex global value chains. Instead, sustainability often requires appropriate socioeconomic

and political frameworks, which can vary substantially at global scale and across biomass exporting and importing countries.

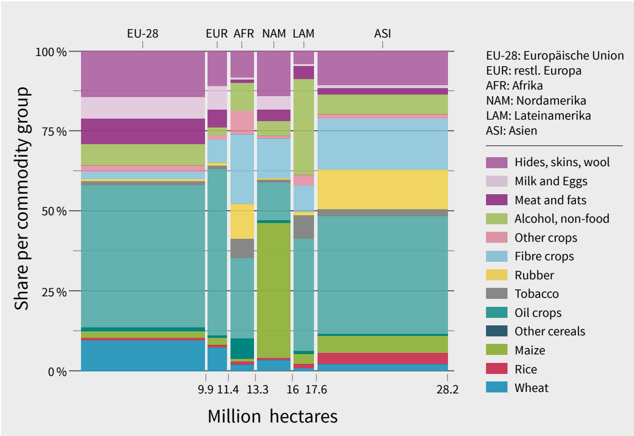
Europe leaves an enormous footprint

The Food and Agriculture Biomass Input-Output Model was developed in the framework of the Econ-BioSC project. It quantifies the land footprint of global trade with bio-based products over time. The researchers investigated the dynamics of the non-food biomass footprint of the European Union in terms of land requirements. The land footprint is the amount of land needed to produce the equivalent biomass used by an average EU citizen in one year. The researchers found that the total EU non-food biomass footprint has increased from 477 to 562 square meters between 1995 and 2010. For 2010, two thirds of this footprint occurred abroad, for example, in Asia or Latin America. Palm oil and soya account for the lion's share of EU non-food biomass imports and are often used as animal feed.

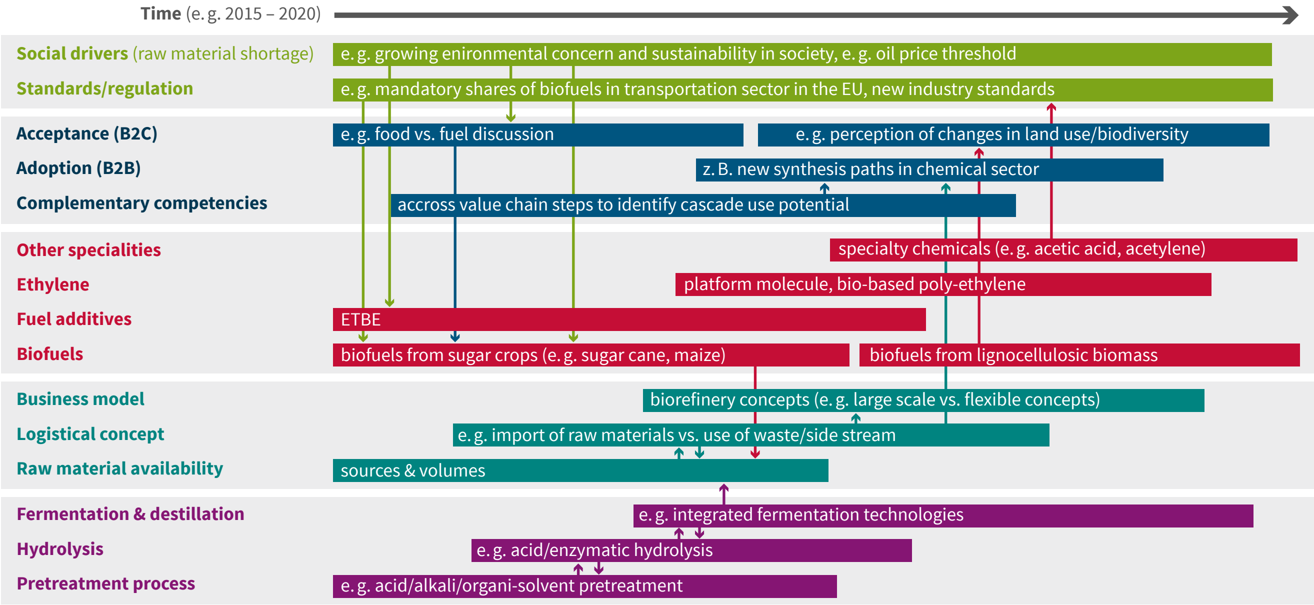
In order to further understand the sustainability effects of the corresponding biomass imports, the team quantified the carbon emissions of selected biomass streams such as soy, which is mainly imported to the European Union from Brazil. The carbon footprint of EU soy imports from Brazil is one of the highest in the world, in part because the EU sources from from regions in Brazil that are characterized by comparatively high levels of both legal and illegal deforestation. The research suggest that the undesirable environmental impact of EU soy imports is mainly caused by illegal deforestation. Tropical forest losses can also be driven by technological innovations in agriculture, which allow the production in fragile ecosystems previously unsuitable for soy cultivation. Good news comes from further studies that show how well-designed environmental policies in Brazil have been temporarily effective in reducing illegal deforestation while simultaneously increasing agricultural productivity. Technological innovation thus often requires accompanying regulatory policy action in order to unfold its potential for sustainable transformation.

Intelligent networking of industrial sectors

Together with the bioeconomy, the connection between the agricultural and energy sectors increases, potentially leading to so called industry convergence. This is due to multiple cross-sector interactions, since both sectors increasingly share the same raw material base. In addition, they share technologies associated with the processing of biomass as well as bio-based materials from side-streams classified as renewable energy. Since technological interfaces between sectors have been described as sources of radical innovation, the cross-sector application of the most important basic technologies has the potential to trigger novel bioeconomic value chains. To elucidate the status quo and the dynamics involved with the development of the bioeconomy, the scientists conducted a patent analysis to map the technology innovation system around second-generation bioethanol production. This revealed that the technological development in lignocellulosic bioethanol is diverse. Lignocelluloses make up about 95 percent of the dry mass of land plants. They are available in large quantities and addresses numerous application fields, including fuel and material uses. However, lignocellulosic ethanol production has not yet reached full commercialization. A subsequent analysis of business news highlighted that pilot production plants are often established in close collaboration between research institutes and industry partners, stressing the need to form novel knowledge ecosystems involving partners from different sectors.



Global cropland footprint of the EU consumption of non-food products in 2010 according to region and raw material



Roadmapping for the emergence of secondary biomass and related value chains (by the fields Society, Market, Product, Value chain, Technology)

Implications for the future

Sustainable bioeconomic transformation requires supportive consumption, policy and management decisions based on knowledge-based impact assessment. The Econ-BioSC project developed and applied innovative assessment and planning tools that assist in estimating past as well as future sustainability effects of bio-based consumption and improve the understanding of the underlying innovation systems. There are limits to value chain transparency and the reliability of scenario assumptions. Nonetheless, synergies between digitalization and the availability of new data, as well as increasing consumer awareness, can assist in making bioeconomic transformations more sustainable.

Contact

Prof. Dr. Jan Börner,
ILR – Economics of Sustainable Land Use and Bioeconomy,
Universität Bonn,
jborner@uni-bonn.de

Core Groups involved

Prof. Dr. Stefanie Bröring, Dr. Nina Preschitschek,
ILR – Technology, Innovation Management and Entrepreneurship,
University of Bonn

Prof. Dr. Ulrich Schurr, Dr. Martin Bruckner,
IBG-2 Plant Sciences, Forschungszentrum Jülich

Prof. Dr. Jan Börner,
ILR – Economics of Sustainable Land Use and Bioeconomy,
University of Bonn

● FOCUSLAB **Transform2Bio**

Structural Change as an Opportunity for People and Environment

The bioeconomy not only contributes to environmental and climate protection directly but can also help to achieve the Sustainable Development Goals (SDGs). The accompanying transformation simultaneously implies far-reaching economic and social changes. The design possibilities are varied. Regional transformation pathways and their implementation are thus not universal. They depend to a large extent on regional conditions.

In order to systemically understand how a regional transformation towards a sustainable bioeconomy can be successful, scientists accompany and analyze the development in the *Living Lab Rheinisches Revier* and prepare comparative studies with other regions undergoing (structural) change. The driving forces of structural change in the Rhenish mining area are in particular climate change and the resolved lignite phase-out. The region will reposition itself in the coming years and aims to become a model region for a sustainable bioeconomy. This social endeavor allows rare insights into how such transformation processes work.

Wordcloud based on the “Wirtschafts- und Strukturprogramm für das Rheinische Zukunftsrevier 1.0” (first iteration of the economic development and structural program for the Rhenish mining area) (Source: Zukunftsagentur Rheinisches Revier/Sandra Venghaus and Florian Siekmann).

In the Transform2Bio FocusLab, the researchers are thus interested in how such a social transformation process proceeds – and what can be learned from it. Even though the Rhenish mining area as a model region is embedded in a very exciting and dynamic context, some framework conditions and processes are by no means limited to the region itself and can therefore provide valuable insights applicable far beyond the Rhenish mining area.

The direction and goal of the regional transformation paths strongly depend on the given point of departure. This is particularly favorable in the Rhenish mining area. The region is already characterized by productive agriculture and surrounded by markets that require sustainable raw materials – the traditionally strong food industry, chemical and pharmaceutical industries, as well as paper and textile industries. The Rhenish mining area therefore has ideal conditions to become a model region for a sustainable bioeconomy.

The *Rheinische Revier* as a *Living Lab*

The Rhenish mining area functions as a so-called *Living Lab*, in which new bioeconomic approaches are tested and the transformation dynamics are analyzed at the same time. The Rhenish mining area in North Rhine-Westphalia is the largest mining area in Germany and the largest connected lignite deposit in Europe. It currently includes three open-pit mining areas: Garzweiler, Hambach and Inden. For this reason, it is particularly affected by the lignite phase-out planned by 2038 and simultaneously offers the opportunity to design and analyze major structural changes. This includes the need to create new employment possibilities for approximately 9,000 directly and close to 93,000 indirectly affected jobs. At the same time, a common vision is being developed for a future regional economic model built on biological resources and knowledge. A regional transformation of this magnitude is thus not only a technological and economic challenge but also a social one.

The concept of a sustainable bioeconomy requires the collaboration of actors, among others, from biomass production, development of (bio)technologies and bio-based products and, last but not least, consumers. The Rhenish mining area is characterized by excellent starting conditions for a bioeconomy. Due to the favorable climate and fertile soils, agricultural production

View over the Blausteinsee in Eschweiler-Dürwiß.

The change from fossil- to bio-based value chains.

plays an important role in large parts of the region, not least as a supplier to the regional food industry. In addition, the region is characterized by the centuries of lignite mining. As a result of the close proximity to the large metropolitan areas at the Rhine and the provision of previously inexpensive energy, many industrial plants have settled here. In particular, the chemical industry is of great importance – 30 percent of Germany´s capacities for cracking and refining are located in North Rhine-Westphalia. The Rhenish mining area is also home to a large number of innovative small- and medium-sized enterprises. The strong research landscape in the region should also be emphasized. In addition to numerous universities and universities of applied sciences, several non-university research institutions as well as the Bioeconomy Science Center research association are located here.

Due to the large-scale protests against the lignite phase-out, in particular around the Hambach Forest at the Hambach surface mine, the region has attracted considerable attention far beyond the region. Following the decision by the Federal Government to phase out lignite power, there has been a dynamic engagement of a large number of social actors and interest groups who want to actively participate in shaping the future design of the Rhenish mining area. The Rhenish region is therefore better suited than most other regions to successfully implement the regional transformation towards a sustainable bioeconomy and for monitoring and analyzing the social dynamics.

Identifying and modeling transformation pathways

The research approach of Transform2Bio combines scenario development and the use of economic models that capture central relations between production, demand and trade in the bioeconomy. It employs two detailed simulation models. The (bio)economic operating model FarmDyn is used for analyses on the level of the individual agricultural holding. It captures operational management and scientific interrelationships with a high level of detail. The general economic equilibrium model CGEBox charts the connection between all economic sectors to analyze the impact of policy guidelines and available technologies across all relevant sectors. It is globally aligned and thus allows other regions to be considered in addition to the Rhenish mining area, both nationally and internationally.

Transformation of the entire region can succeed only through dialogue.

Scenario development is based on the manifold stakeholder visions in the region. Far-reaching changes in the use of natural resources affect different economic sectors and areas of everyday life. Actors concerned, the so-called stakeholders, therefore represent different interests and attitudes towards the development direction and implementation of a bioeconomy. Bringing together these positions is a central challenge for the success of the transformation. Thus, the research also addresses the question of how to succeed in bringing together the divergent interests of different social groups. After all, perspectives of companies, farmers and citizens associations can differ fundamentally. In order to successfully design the transformation processes, social consensus is required.

The Rhenish mining area includes numerous small and medium-sized enterprises.

Question, analyze, involve, and co-design

The analysis of stakeholder visions immediately prompts questions concerning the involvement of various social groups. The mere knowledge of different perspectives does not necessarily lead to a balanced consideration. Previous experiences, amongst others the German Energiewende, have shown that some forms of participation in decision processes are promising – whereas others are not. At worst, the misguided or missing participation of the involved actors can lead to social groups resigning from or even blocking the transformation of their region. The scientists in the project thus search for opportunities and means to support a successful implementation of a sustainable bioeconomy. An insight into the general social perspectives is provided by the reporting on the bioeconomy in the national media in Germany. It is characterized by a strong focus on the development of new technologies and the goals of competitiveness and growth. This reflects the research-oriented approach of the Federal Government and the European Union. Considerations of biomass production and rural development are less frequent and reflect the predominantly technology-based understanding of the bioeconomy. Critical voices are raised regarding the social and ecologic sustainability of such a transformation, but present only a marginal aspect of the public debate.

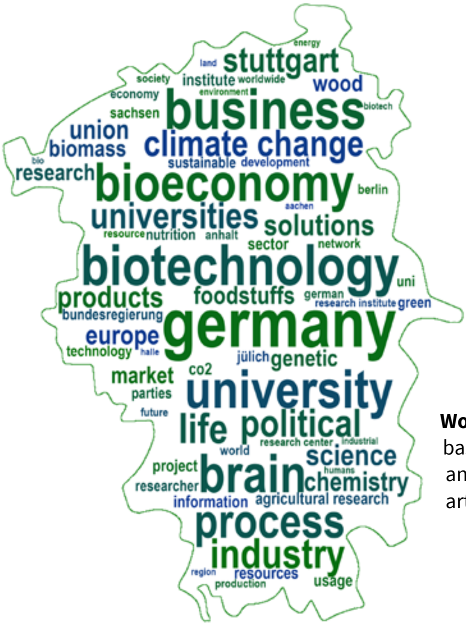
Agriculture provides an interesting example of a specific stakeholder group, with farmers who, as raw material providers in the region, represent a central actor for the success of a sustainable bioeconomy. Farmers are regularly faced with complex decisions and often have to plan well in advance since the cultivation of certain crops can shape the soil for many years to come. In addition, there are changing demands on people and nature resulting from the effects of climate change – but also exciting opportunities offered by new technologies or developments such as digitization. Furthermore, societal demands for sustainable cultivation and resilient supply chains play an increasingly important role, which is reflected in reforms of the complex political framework. For these reasons, it is important to understand what factors are central to farmers and on what considerations their decisions are based. It is equally important to consider how the biomass produced is subsequently used. The individual farmer prefers a certain degree of planning security regarding demand for their produce by consumers and industry. At the same time, however, the industry must also find the corresponding conditions to produce innovative and at the same time sustainable products.

In order to be able to make decisions in the interest of the general public in a comprehensive and transparent way, a multitude of interests and mutual dependencies needs to be considered.

The researchers pool the common knowledge of the stakeholders and use a multi-criteria decision analysis approach to support decision-makers in such complex decisions. In doing so, scientists are able to recognize and consider which decision criteria are of particular importance to different actors and how these preferences can influence finding a common compromise.

The resulting knowledge is central to ensuring that society as a whole continues to achieve common goals in the future. The researchers are certain that the results of Transform2Bio can also contribute to success in future transformation processes in other regions.

View into the surface mine



Wordcloud
based on the results of an analysis of German media articles 2010 – 2019

Contact

Prof. Dr. Sandra Venghaus,
IEK-STE Systems Analysis and Technology Evaluation,
Forschungszentrum Jülich,
s.venghaus@fz-juelich.de

Core Groups involved

Dr. Wilhelm Kuckshinrichs, Prof. Dr. Sandra Venghaus, Mirko Dallendörfer, Sophia Dieken, Florian Siekmann,
IEK–STE Systems Analysis and Technology Evaluation,
Forschungszentrum Jülich

Prof. Dr. Jan Börner, Dr. Sascha Stark,
ILR – Economics of Sustainable Land Use and Bioeconomy,
University of Bonn

Prof. Dr. Joachim von Braun,
ZEF – Economic and Technological Change, University of Bonn

PD Dr. Wolfgang Britz, Dr. Till Kuhn,
ILR – Economic Modelling of Agricultural Systems, University of Bonn

Prof. Dr. Stefanie Bröring, Simon Ohlert, Anna Waßenhoven,
ILR – Technology, Innovation Management and Entrepreneurship,
University of Bonn

Prof. Dr. Monika Hartmann, Janine Macht, Jeanette Klink-Lehmann,
ILR – Agricultural and Food Market Research,
University of Bonn

Prof. Dr. Thomas Heckelei, Dr. Yaghoob Jafari,
ILR – Economic and Agricultural Policy, University of Bonn

Prof. Dr. Silke Hüttel, Dr. Stefan Seifert,
ILR – Production Economics, University of Bonn

Prof. Dr. Ulrich Schurr, Dr. Leonie Göbel,
IBG-2 Plant Sciences, Forschungszentrum Jülich

Prof. Dr. Grit Walther, Ali Abdelshafy,
Lehrstuhl für Operations Management, RWTH Aachen

Can Research for Bioeconomy be Learned?

Training and communication have been cornerstones of the BioSC from the beginning. Why? The bioeconomy can work only if – in the sense of a sustainable value chain - the most diverse disciplines and all stages of necessary processes are involved. This ranges from biomass availability and production to its transport, processing, conversion and utilization, to its sale and social acceptance. In order to achieve this, awareness of the possibilities and an understanding of the concepts and content of the related disciplines must be generated in all persons involved. This includes the ability to combine their own disciplinary excellence together with other experts to exchange ideas and jointly develop solutions for complex questions. This interdisciplinary problem-solving competence is at the heart of the BioSC training concept.

Over the past ten years, the BioSC has tested and evaluated numerous concepts and has addressed a wide range of target groups. Very early on, the focus was on the training and networking of young scientists. An important target group was and still is the group of PhD students and young postdocs, since these communicate the contents and concepts of the bioeconomy to science and society in long-term. In addition to their specialist skills, the aforementioned ability for interdisciplinary work and thus the overarching collaboration are of central importance. In international Season Schools, organized by BioSC scientists and addressing current topics of the bioeconomy, young scientists and PhD students can acquire content-related professional and interdisciplinary competences. The BioSC has organized summer schools on topics such as "From plant to product. Value chains in a sustainable bioeconomy using the example of Costa Rica and Germany" or "From plants to pilot plants. Holistic process development in a sustainable bioeconomy".

Young scientists and PhD students working directly in BioSC projects must fit into interdisciplinary research groups from the beginning and thus face special challenges. Their discipline-specific training is carried out in one of the Core Groups. However, the BioSC specifically requires PhD students, who network intensively with other PhD students and scientists – also and especially from other disciplines. They meet on common retreats to exchange and discuss their work. They are given the opportunity to develop small joint projects and apply for their own funding for these. They can establish contacts with industry through excursions, which – whenever possible – are combined with workshops and substantive exchange with the employees working there. One example is a recent visit to BRAIN AG in Zwingenberg. The workshop there was so successful that the company expressed the willingness to repeat such workshops on a regular basis. And certainly, the PhD students offer significant contributions at the scientific level to the annual international BioSC Symposia and a wide variety of topic specific conferences.

The annual NRW PhD Student Day "Future Bioeconomy" also creates the opportunity to network with other PhD students from throughout North Rhine-Westphalia, beyond the group of direct BioSC PhD students. For this PhD student day, the BioSC, together with its network of graduate schools and associations engaged in bioeconomy-related training, invites personalities from industry, start-ups or interdisciplinary research associations and networks. The aim of this event is to offer PhD students a low-threshold opportunity to discuss in small groups with the invited speakers – after their keynote talks – both the implementation and relevance of the bioeconomy, the personal careers of the speakers as well as the prospects for young scientists in the bioeconomy.

However, promotion of “bioeconomic thinking” in PhD students requires that there is the willingness and awareness on the side of the scientists and direct supervisors who train them. This is exactly where the annually awarded BioSC Supervision Award comes in: a prize endowed with 25,000 euros in research funds for outstanding achievements in PhD student promotion towards networking, scientific autonomy and excellence, interdisciplinary thinking and promotion of “thinking outside the box”. The special feature of this award is the nomination – usually made jointly – by a Core Group leader and the supervised PhD students. The nomination proves the scientific excellence and outstanding supervision work of the nominee. The fact that already two BioSC FocusLab leaders received the award and thus a young female scientist and a young male scientist, who were entrusted with the coordination of a large interdisciplinary bioeconomy project early in their careers, shows that the concept of promoting young scientists in the BioSC is successful on diverse levels. This is proven not least by the fact that the BioSC has already been selected twice as the lead member of the group of coordinators for the Education Workshop of the Global Bioeconomy Summits (2018 and 2020). Overall, this resulted in networking with other European groups addressing the topic of bioeconomy training, among them the Community of Practice for Bioeconomy Education (CoP Bio-Edu) and the Association for European Life Science Universities (ICA), with the common goal of helping to shape the development of training concepts for bioeconomy internationally.

BioökonomieREVIER Rheinland – A Model Region for Sustainable Bioeconomy

A key topic of structural change in the Rhenish mining region is the development of a model region for a sustainable economy. The bioeconomy is a key subject. New regional value chains and business models are to be established based on bio-based innovations. The Rhenish mining region offers excellent scientific, economic and social conditions. With the bioeconomy, the change from a mining region to a model region for sustainability and climate protection can succeed.

In 2019, the structural change initiative BioökonomieREVIER was initiated by the Institute for Plant Sciences at Forschungszentrum Jülich. Fifteen innovation laboratories and the coordination office together with actors from business, municipalities, agriculture, science, education, politics as well as the public in the region developed approaches on how a transformation can be carried out successfully. Promising bio-based value-added concepts are directly translated into practical and regional solutions for companies, farmers, municipalities and society.

The Bioeconomy Science Center was a decisive pioneer in the development of BioökonomieREVIER. The bundling of regional bioeconomy research, the elaboration of thematic research focuses and the development of a bioeconomy community in the Rhineland, which could be quickly activated, were of particular importance. Within the framework of the NRW strategy project BioSC, a regional research network was developed in the field of systemic bioeconomy research. For years, the inter- and transdisciplinary BioSC research projects have provided solution-oriented and economically viable innovation concepts for the region. This extraordinary potential was an important prerequisite for the inclusion of the bioeconomy as an important future field in the regional economic and structural development. The collaboration with several “Revierknoten” and numerous partners from practice opens up additional opportunities for necessary economic structural changes. The development of bio-based value-adding concepts offers the possibility to reconcile utilization and protection of natural resources. Bio-based business models provide the basis for effectiveness in all dimensions (income, jobs, sustainability) of structural change and the social perspectives on sustainable living and working receive innovative impulses. The Rhenish mining region is developing into a unique real laboratory for bio-based technology and service concepts.

Alongside the three thematic clusters “Innovative Agriculture”, “Integrated Biorefineries” and “Biotechnology & Plastics Technology”, the partner institutions of BioökonomieREVIER develop research concepts and technologies with high application potential in innovation laboratories. This is just the beginning. The region is to become a beacon for the transfer of bioinnovations into real economic practice both regionally and internationally. One key to this is the networking of scientific key players with actors who are focused on innovations for accomplishing operational and social challenges. In innovation partnerships, business models are renewed in the sense of sustainability (green deal) and new companies are founded. The BIOBoosteRR accelerator program, specifically tailored for the bioeconomy, was established for start-up support in the BioökonomieREVIER. Founding teams are supported in business development and scaling; a direct transfer is also facilitated from the BioSC projects into commercial application. Bioeconomy profile locations in the Rhenish mining area develop a special incentive for the implementation of sustainable bioeconomic solutions. Profile locations have a thematic focus and special infrastructure facilities (e.g. a hightech field laboratory) and proximity to excellent partners from research, industry and agriculture.

The Rhenish mining area is also an area on which research is carried out: the Transform2Bio BioSC competence platform uses the accesses to elaborate knowledge on transformation research and thus important contributions to the design of transformation in the Rhineland and other regions. Transform2Bio studies the transformation process systemically and shows potential transformation paths. Here in particular it becomes clear how BioökonomieREVIER and BioSC can promote successful regional and structural development by closely interlocking their activities.

The BioökonomieREVIER work will result in regional recommendations for the Rhenish mining region to be presented in the fall of 2021. In parallel, practical implementation routes are already being pursued. Economic innovations as well as social and societal topics are being addressed. Important milestones on this path include networking of regional actors, collecting and evaluating material flow from the local agriculture and food industry, developing regional innovation partnerships between companies, agriculture and science, creating municipal bioeconomy profiles and analyzing education and training opportunities. Interactive exhibition formats are accessible for the interested public. The process is accompanied by citizen participation.

Contact

Dr. Christian Klar
Koordinierungsstelle BioökonomieREVIER,
IBG-2 Plant Sciences, Forschungszentrum Jülich
c.klar@fz-juelich.de, www.biooekonomierevier.de

Cooperation for the Development of a Bioeconomy

The transformation from an economy based on fossil resources to an economy based on sustainable, renewable resources is a challenge that requires targeted and long-term collaborations on regional, national, European and global levels. All actors are equally challenged: business, science and society. The European Commission laid important foundations for a European cooperation for a knowledge-based, circular bioeconomy more than 15 years ago with the publication of the Knowledge-Based-Bio-Economy (KBBE) as an overarching concept and guiding principle for solving the major societal challenges. In the subsequent years, the concept was followed by policy strategies, roadmaps and action plans. These were implemented by the European research framework program Horizon 2020, advisory boards such as the European Bioeconomy Council and legal directives on renewable energies, among others, to accelerate a climate- and resource-saving, competitive economy in Europe. The European Green Deal to transform the EU economy for a sustainable future, published in December 2019, targets numerous bioeconomy guidelines.

The development of bioeconomy has also increased in importance at the global level in recent years. One example of this is the organization of the Global Bioeconomy Summit (GBS), which was first held in Berlin in 2015 and brought together about 3,000 bioeconomy experts from science, business, politics and civil society from more than 50 countries for the third time in November 2020. The GBS and its international advisory board is thus the central platform worldwide for discussing the current developments and challenges of the global bioeconomy and elaborating recommendations for action to advance the transformation. Scientists from the BioSC contributed significantly to the design and execution of the Global Summit in the third, digital GBS. They co-shaped and -led four of a total of twelve interactive workshops that spanned topics such as biorefineries, regionalization of the bioeconomy, bioeconomy training and transformation concepts.

Numerous partnerships and research collaborations exist at European and international level. For example, this includes the strategic project associations and networks for modern research infrastructures in Europe, such as the European Infrastructure for Multi-scale Plant Phenotyping and Simulation for Food Safety and Climate Change (EMPHASIS), the European Plant Phenotyping Network (EPPN) or the European infrastructure project IBISBA – Accelerator for Innovations in Industrial Biotechnology and Synthetic Biology. On the other hand, there are longstanding research cooperations and strategic partnerships in various fields in the Asian region with, among others, the National Science and Technology Development Agency Thailand (NSTDA), the Chinese Academy of Sciences (CAS), with research institutions in India and Malaysia as well as the Russian Federation. Countries such as Brazil, Argentina and Uruguay are examples in Latin America. In addition, there are cooperation projects with the US, Australia and Africa.

The BioSC is closely connected with three different excellence clusters in the region through its partner institutions and the scientists involved: CEPLAS – the excellence cluster for Plant Sciences, PhenoRob – Robotics and Phenotyping for Sustainable Crop Production, and the Fuel Science Center (FSC) for customized biofuels. Due to the multiple participations of scientists, the excellence clusters are interlinked in terms of personnel and content. Such a concentration of scientific excellence in bioeconomy-relevant topics in one region is unique not only in Germany but also internationally and is an example of the competence of the BioSC in serving as a basis for networking scientific

The Thai-German CassavaStore BMBF research project deals with the improved and resource-saving cultivation of cassava, one of the most important food and resource plants in Asia and Africa.

actors beyond itself. In addition to strong scientific cooperations, there are also collaborations with application-oriented associations such as the Cluster Industrial Biotechnology (CLIB), BIO.NRW, the Network for Biotechnology in NRW or the ABC/J Geo Association for earth sciences.

The national cooperations reach far beyond the borders of North Rhine-Westphalia. Since the founding of the BioSC, numerous other cluster initiatives on the bioeconomy in Germany have followed, with which partnerships contacts and complementary cooperations exist. Examples include the ScienceCampus Halle for Plant-based Bioeconomy, the BioEconomy Cluster in Central Germany, the BioPro-Cluster in Baden-Wuerttemberg, the Straubing Campus for Biotechnology and Sustainability of the Technical University of Munich and the DBFZ (Deutsches Biomasseforschungszentrum) in Leipzig.

To realize bio-based processes and bio-based social transformation concepts, cross-disciplinary networking between scientists as well as close collaboration at eye level between science and economy is an essential prerequisite. This must include all fields relevant to the bioeconomy and also develop networking between different industrial sectors, which has been poorly developed in some cases to date. For example, residual materials from the food industry can be used as basic materials for the production of bioplastics. Through such novel cooperations, additional innovation and value networks can develop that can contribute to the competitiveness of bio-based processes and products and can create new markets and jobs. The role of the BioSC is to study scientific issues concerning the technological, economic and social feasibility of such new processes and products. In cooperation with small and medium-sized enterprises, industrial companies or through spin-offs, developed processes,

The workshop for education and training at the Global Bioeconomy Summit 2018 was co-designed by the BioSC.

technologies or transformation concepts are then brought towards application. In the transfer of developments from laboratory to application in the region, the BioökonomieREVIER structural change project opens up excellent access to regional cooperation partners. With its network of companies and actors and innovation measures (e.g. InnoLabs), targeted further development tailored to the possibilities and prerequisites of the region and transfer of research results into application becomes possible.

Close collaboration with regional to international partners from science, industry, civil society and politics will continue to be an important pillar and activity of the BioSC in the coming years.

The PuresBioAshes BMBF project, a Brazilian-German research cooperation, investigated the utilization of agricultural residue streams.


10 Years
Research
Educate
Connect
BioSC
Bioeconomy Science Center

IMPRINT

10 Years Bioeconomy Science Center

Publisher
BioSC Geschäftsstelle
Forschungszentrum Jülich GmbH
IBG-2: Plant Sciences
D-52425 Jülich
biosc@fz-juelich.de
www.biosc.de

Last revised
April 2021

Editors
Dr. Heike Baron (Coordination), Brigitte Stahl-Busse,
Dr. Heike Slusarczyk, Prof. Dr. Ingar Janzik, Prof. Dr. Ulrich Schurr

Authors of the articles on the research projects and the BioökonomieREVIER project are the contact persons named below the articles, unless otherwise noted.

Graphics und Layout
Grafische Medien, Forschungszentrum Jülich GmbH

Printing: Schloemer + Partner GmbH
Copies: 300

This publication is part of the public relations work in the framework of the NRWStrategy Project BioSC, funded by the Ministry of Culture and Science of the State of North Rhine-Westphalia. It is provided free of charge and is not intended for sale.

Picture credits

istock.com:
halbergman, Kuzmalo, fotografixx: Titel; wundervisuals: p. 23; Maksud_kr: p. 27; BONDART: p. 30; wirOman: p. 36; Tomas Miskinis: p. 39; Andreas Vitting, Sinhyu: p. 40; JudiParkinson: p. 42; Andrea Sommer: p. 45; Kuzmalo: p. 47; rob_lan, fireworks_pixels: p. 54; Stas_V: p. 58; Arkadij Schell: p. 61; greenleaf123: p. 66; Yuuji: p. 74; Patrick Daxenbichler: p. 77; richcarey: p. 82; fotografixx: p. 87; davelogan: pS. 88

Forschungszentrum Jülich: p. 65, p. 91
Ralf-Uwe Limbach: p. 3, p. 16, p. 37
Sascha Kreklau: p. 26, p. 85, p. 86 rechts, p. 89, p. 93
IBG-1: p. 20, p. 52, p. 53, p. 54, p. 67 – 70, p. 75
IBG-2: p. 11, p. 13, p. 43, p. 46, p. 95, p. 96 oben, p. 96 below (Dr. Nicolai Jablonowski)

HHU Düsseldorf:
Computational Pharmaceutical Chemistry and Molecular Bioinformatics: p. 28, p. 48 (Jonas Dittrich)
Institute of Bioorganic Chemistry: p. 32
Institute of Biochemical Plant Physiology: p. 29
Institute of Microbiology: p. 56
Institute of Molecular Enzyme Technology: p. 62, p. 63 (using a figure from Brands et al., Chemical Communications 56, 8631-8634 (2020)), p. 64, p. 72 (Dr. Achim Heck), p. 73 (Andreas Woop in collaboration with Dr. Thomas Gensch (IBG-1), Forschungszentrum Jülich)

RWTH Aachen: p. 44
AVT – Biochemical Engineering: p. 12, p. 31, p. 78
AVT – Fluid Process Engineering: p. 35 (Manuel Lück), p. 60 left
AVT – Chemical Process Engineering: p. 81 (Patrick Bongartz)
ABBt – Plant Physiology: p. 32, p. 50 (A) (Patrick Schwinges)
ABBt – Applied Microbiology: p. 40, p. 80
ABBt – Biotechnology: p. 49 (Liudmyla Goncharenko)
Chair of Bioinorganic Chemistry: p. 59, p. 60 right
Chair of Operations Management:
p. 87 left (own presentation/Ali Abdelshafy)

University of Bonn:

INRES – Horticultural Sciences: p. 34 (Dr. Simone Röhlen-Schmittgen), p. 50 (B) (Dr. Shyam Pariyar)

INRES – Molecular Biology of the Rhizosphere: p. 51 (Janina Zierul)

p. 4: Prof. Dr. Thomas Müller-Kirschbaum

p. 5: Prof. Dr. Peter Westhoff

p. 6: Prof. Dr. Sandra Venghaus

p. 10: United Nations

p. 33: stock.adobe.com/photlook

p. 38a – c: from doi.org/10.1007/s10811-018-1390-9 (Schreiber et al. 2018)

p. 38d: Poster for Biogeomon 2017, Hofmann et al., ^{33}P Labeling of C. vulgaris algae for rhizotrone imaging studies

p. 41: Carraresi, L., Berg, S., & Bröring, p. (2018), Journal of Cleaner Production, 183, 87-101

p. 57: modified according to Müller et al., J Biol Eng 12, 34 (2018)

p. 71: *Image of cutinase 1CUS (C. Martinez, P. De Geus, M. Lauwereys, G. Matthyssens, C. Cambillau (1992) Fusarium solani cutinase is a lipolytic enzyme with a catalytic serine accessible to solvent Nature 356: 615-618) created with UCSF Chimera (E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin (2004) UCSF Chimera - a visualization system for exploratory research and analysis J Comput Chem 25: 1605-1612).*

Image of alcohol dehydrogenase 4W6Z (S.B. Raj, S. Ramaswamy, B.V. Plapp (2014) Yeast alcohol dehydrogenase structure and catalysis Biochemistry 53: 5791-5803) created with UCSF Chimera (E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, T.E. Ferrin (2004) UCSF Chimera – a visualization system for exploratory research and Analysis J Comput Chem 25: 1605-1612).

p. 83: modified according to Bruckner et al., Environ. Res. Lett., 14, 045011 (2019)

p. 84: based on Bröring (2016) Roadmapping the circular economy to align technological development with social needs. Working Paper, University of Bonn

p. 86 left: Zukunftsagentur Rheinisches Revier/Sandra Venghaus and Florian Siekmann

p. 89 left: Sophia Dieken and Sandra Venghaus: Sustainability 2020 (12), 7987 www.biosc.de