



10<sup>th</sup> European Symposium  
on Biopolymers

10<sup>th</sup> ANNIVERSARY

September

25-27 · 2019

STRAUBING/GERMANY

PROGRAM

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## Welcome to ESBP 2019

Our planet in the year 2019 continues to face many problems such as climate change and a permanently growing population in combination with the need for sustainable industrial solutions. Thus, the basic idea of the European Symposium on Biopolymers has never been as relevant as it is today. For nearly 20 years as a biannual conference, which takes place within a different European country, the ESBP comes back to Germany for its 10<sup>th</sup> anniversary.

Based on the initiation of outstanding scientists and pioneers in their field, the first ESBP was born in the year 2000 and took place in the beautiful city of Münster. From that time on it took place in many different countries to connect academic research with innovative future industrial applications. The 10<sup>th</sup> anniversary of the ESBP reflects the recent development of research and innovation in the field of biopolymers.

Today, new technologies in material processing and state-of-the-art techniques for genetic engineering such as synthetic biology brings novel and innovative materials as well as applications and push the research on biopolymers towards a new level. Ergo, the focus of the 10<sup>th</sup> ESBP is still the same as in 2000, biopolymer production by a vast number of microbes and it will connect young and well-established outstanding researchers with industry to bring innovative and sustainable solutions to the market.

The location of **Straubing** is a perfect place for this anniversary meeting, since the city of Straubing is the center of the region of renewable resources, and the **TUM Campus Straubing of Biotechnology and Sustainability**, together with the **Competence Center of Renewable Resources**, represents a very strong nucleus of research in this field. Together with the **C.A.R.M.E.N e.V.** we organize this 10<sup>th</sup> anniversary meeting to link academia and industry and identify new perspectives on biopolymers. In addition, Joseph von Fraunhofer was born in Straubing, and his scientific spirit is now reflected by the ESBP 2019, to make our world a bit greener and better.

We are very happy to welcome you to Straubing to experience a great and inspiring ESBP 2019 with compelling presentations, discussions, and new network contacts. Next to a great scientific program on all aspects of biopolymers with cutting-edge results, we also will enjoy the local attractions and delicacies such as the Bavarian beer.

Based on your participation, this event will be highly successful and the whole scientific committee will thank you for that.



## Sponsors & partners



Organizer

Scientific organizer

# Program - Day I

## Wednesday 25<sup>th</sup> of September

8:20	Registration, welcome coffee & introduction	
10:00	<b>PLENARY LECTURE</b> <b>Mattheos Koffas</b> – "Production of tailored sulfated glycosaminoglycans such as heparin and chondroitin"	<b>D1.T1</b>
10:40	Coffee break	
11:10	<b>KEY NOTE – CHAIR: JOCHEN SCHMID</b> <b>Kevin O'Connor</b> – "Investigating pathways to PHA accumulation and applications of PHA"	<b>D1.T2</b>
11:40	<b>KEY NOTE</b> <b>Ebru Toksoy Öner</b> – "A systems based approach for microbial EPS production"	<b>D1.T3</b>
12:10	<b>KEY NOTE</b> <b>Auxiliadora Prieto</b> – "About how to functionalize mcl-PHA particles with peptides and alive cargos"	<b>D1.T4</b>
12:40	Lunch & poster session	
14:40	<b>KEY NOTE – METABOLIC ENGINEERING/SYNTHETIC BIOLOGY – CHAIR: KEVIN O'CONNOR</b> <b>Dieter Jendrosseck</b> – "Formation of an organic-inorganic biopolymer: polyhydroxybutyrate-polyphosphate"	<b>D1.T5</b>
15:30	<b>Tatiana Thomas</b> – "Characterization and exploitation of the marine bacterium <i>Halomonas</i> sp. SF2003 for optimization of PHA production"	<b>D1.T7</b>
15:50	Coffee break	
16:20	<b>KEY NOTE – APPLICATIONS OF EPS – CHAIR: MARIA REIS</b> <b>Etienne Paul</b> – "Selection of a microbial population in continuous culture: a way to produce biopolymers for industrial applications?"	<b>D1.T8</b>
16:50	<b>Broder Rühmann</b> – "Identification of novel exopolysaccharides for application in cosmetics, packaging and lubricants"	<b>D1.T9</b>
17:10	<b>Songul Yasar Yildiz</b> – "A promising exopolymer, synthesized by the halophilic bacterium <i>Chromohalobacter canadensis</i> 28, for cosmeceutical applications"	<b>D1.T10</b>
17:30	<b>Patrícia Concórdio-Reis</b> – " <i>In vitro</i> wound healing ability of the fucose-rich polysaccharide FucoPol and its silver nanocomposite"	<b>D1.T11</b>

# Program - Day 2

Thursday 26<sup>th</sup> of September

8:30	<b>KEY NOTE – PHA-PRODUCTION – CHAIR: MANFRED ZINN</b> <b>João Sousa</b> – “Pilot plant production of PHB from waste water”	D2.T1
9:00	<b>Véronique Amstutz</b> – “Characterizing the production of poly(hydroxyalkanoate) from CO <sub>2</sub> ”	D2.T2
9:20	<b>Stanislav Obruca</b> – “Biological and biotechnological consequences of polyhydroxyalkanoates production by extremophiles”	D2.T3
9:40	<b>Nils Hanik</b> – “Influence of unusual co-substrates on biosynthesis of medium-chain-length polyhydroxyalkanoates”	D2.T4
10:00	<b>M. Manuela da Fonseca</b> – “Macroalgal cellulosic waste, a novel platform for the bacterial synthesis of polyhydroxyalkanoates”	D2.T5
10:20	Coffee break	
10:50	<b>KEY NOTE – PHA PRODUCTION AND ANALYTICS – CHAIR: ETIENNE PAUL</b> <b>Alan Werker</b> – “Respiration dynamic response of PHA storing mixed microbial culture biomass”	D2.T6
11:20	<b>Moritz Koch</b> – “Glycogen serves as the main carbon source for PHB synthesis during nitrogen starvation in the unicellular cyanobacterium <i>Synechocystis</i> 6803”	D2.T7
11:40	<b>Camila Utsunomia</b> – “Development of an at-line and automatized method for polyhydroxyalkanoate quantification by flow cytometry”	D2.T8
12:00	<b>Anna Burniol-Figols</b> – “Polyhydroxyalkanoate (PHA) purification through dilute aqueous ammonia digestion at elevated temperatures”	D2.T9
12:20	Lunch & poster session	
14:20	<b>KEY NOTE – APPLICATIONS OF BIOPOLYMERS – CHAIR: MAURO MAJONE</b> <b>Manfred Zinn</b> – “Safety by design concept applied to the synthesis of polymeric nanobiomaterials”	D2.T10
14:50	<b>KEY NOTE – APPLICATIONS OF BIOPOLYMERS</b> <b>Valérie Langlois</b> – “How to modify the properties of PHAs by chemical modifications?”	D2.T11

## Parallel sessions

	Session 1	Session 2
	CHAIR: BERIT STRAND	CHAIR: ALAN WERKER
15:20	<b>Petr Sedlacek</b> – “Combination of time-resolved molecular spectroscopy and advanced morphological analysis in revealing the mechanism of PHA plasticization <i>in vivo</i> ” <b>D2.T12-1</b>	<b>Oscar Vernaez</b> – “High performance PLA for injection molding applications” <b>D2.T12-2</b>
15:40	<b>Ralf Taubner</b> – “Sustainable coated PLA nonwovens for upholstery” <b>D2.T13-1</b>	<b>Markus Neureiter</b> – “Utilization of by-products from sugar beet processing for the production of poly(hydroxyalkanoates)” <b>D2.T13-2</b>
16:00	<b>Audrey Roy</b> – “Water-soluble poly(3-hydroxyalkanoate) sulfonate: versatile biomaterials used as coating for highly porous nano-MOF” <b>D2.T14-1</b>	<b>Serge Gugelmann</b> – “From engineering to technical application – Understanding which technical data are required for the successful development of a bio-based technical product” <b>D2.T14-2</b>
16:20	<b>Davy-Louis Versace</b> – “New photo-induced bio-based coatings for antibacterial applications” <b>D2.T15-1</b>	<b>Jörg Dörrstein</b> – “Technical requirements for biopolymers – from an industrial point of view” <b>D2.T15-2</b>
16:40	Coffee break	
	CHAIR: FRANCESCO VALENTINO	CHAIR: EBRU TOKSOY ÖNER
17:10	<b>Ivan Chodak</b> – “Effect of biodegradation on physical properties of PLA-based blends” <b>D2.T16-1</b>	<b>Berit Strand</b> – “Hydrogels with cyclodextrin-grafted alginates for hydrophobic drug release” <b>D2.T16-2</b>
17:30	<b>Paul Stockmann</b> – “(+)-3-carene: A versatile precursor for high performance bio-polyamides” <b>D2.T17-1</b>	<b>Inês C. Ferreira</b> – “Development of chitin-glucan complex – based polymeric structures using biocompatible ionic liquids” <b>D2.T17-2</b>
17:50	<b>Audrey Magnin</b> – “An innovative and sustainable approach of polyurethane bio-recycling” <b>D2.T18-1</b>	<b>Ana María Hernández-Arriaga</b> – “Bacterial cellulose functionalization for producing biomaterials with antimicrobial capacity” <b>D2.T18-2</b>
18:10	<b>Herbert M. Riepl</b> – “Synthesis of ether-diols with low polarity from long-chained fatty alcohols for use in block copolymers” <b>D2.T19-1</b>	<b>Samira Siyamak</b> – “Functional starch-based hydrogels: sustainable material solutions for environmental applications” <b>D2.T19-2</b>
19:15	<b>Conference Dinner</b>	

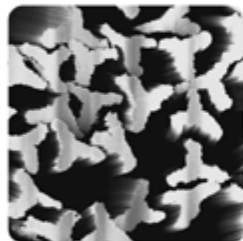
# Program - Day 3

Friday 27<sup>th</sup> of September

8:30	<b>PLENARY LECTURE – CHAIR: VOLKER SIEBER</b> <b>Maria Reis</b> – “Exploring microorganisms and bioresources diversity for biopolymer production: a multibiorefinery concept”	D3.T1
9:10	<b>Francesco Valentino</b> – “An urban bio-refinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas”	D3.T2
9:30	<b>Doris Schieder</b> – “Biorefinery of grass silage for poly-3-hydroxybutyrate (PHB) fermentation”	D3.T3
9:50	<b>Fernando Silva</b> – “Polyhydroxyalkanoates production at pilot scale using fruit waste: enhancing feeding strategies and monitoring”	D3.T4
10:10	Coffee break	
10:40	<b>KEY NOTE – MEDICAL APPLICATIONS OF BIOPOLYMERS – CHAIR: VALÉRIE LANGLOIS</b> <b>Ipsita Roy</b> – “Natural polymers of bacterial origin and their biomedical applications”	D3.T5
11:30	<b>Diana Araújo</b> – “Hydrogels based on chitin-glucan complex: fabrication, characterization and cytotoxicity assay”	D3.T7
11:50	<b>Ana Teresa Rebocho</b> – “From apple pulp waste to high-value biomaterials: development of PHA films for food and biomedical applications”	D3.T8
12:10	<b>Merve Erginer Hasköylü</b> – “ <i>Halomonas</i> levan for cosmeceutical applications”	D3.T9
12:30	Conclusion & poster prizes	
13:30	<b>Friday afternoon event</b>	



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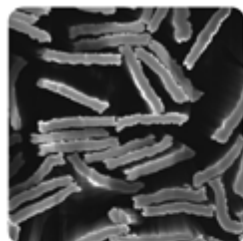


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# Conference Dinner

Thursday 26<sup>th</sup> of September / 19.15 pm

*Enjoy Bavarian haute cuisine in the most traditional tavern of Straubing!*



The theme of the conference dinner is traditional Bavarian food alongside the best-known beverage of Bavaria – beer. For this, we will gather in the oldest tavern in Straubing, which has a history dating back more than 480 years.

The historical restaurant was built in the year 1462, and from then on has nearly continuously served as a brewery and tavern. The modern building is just a part of the original complex, but still evokes the historical atmosphere in a unique manner. The building was completely refurbished in 2001-2002 in order to preserve its valuable facade, as well as the ambience of the late middle age.

Since then, the restaurant “zum Geiss” and its pleasant landlady “Michi” have launched a series of innovative and creative interpretations of traditional Bavarian dishes of the highest quality. The primary ingredients are of regional and seasonal origin in order to meet high sustainability and natural taste requirements. In addition, Michi is one of the rare German female biersommelières. For this reason, the topic of beer is highly appreciated at this tavern and will accompany us for the whole event.

We will enjoy great Bavarian food alongside tasty regional Bavarian beer in a perfect historical setting. Let's meet there and enjoy the Bavarian spirit together.



# Friday Event

For Friday afternoon, you have the choice between visiting different places.

We organized a guided tour (in english) at the **St. Peter Church and the surrounding historical graveyard**. The Church was built in the second half of the 12<sup>th</sup> century, and is distinguished by its two sculpted portals. The surrounding graveyard contains a number of funeral chapels, most notably the Totenkapelle, which is decorated with a cycle of paintings depicting the Totentanz (Dance of the Dead) by the Rococo artist Felix Hölzl.



Alternatively you can get a tour at the **Campus for Biotechnology and Sustainability of the Technical University of Munich**. There you can see the growing campus and the upcoming new buildings, including the NAWAREUM (under construction). As an educational institution, the house becomes the central information and event location for the restructuring of energy and raw material supply in Bavaria.



As third alternative we offer a bus transfer to the **Hafen Straubing-Sand**. There you can visit one of the biggest cargo stopover places in Bavaria as well as the incubator for start-up companies in Straubing.

# Conference Special Issue

## 2019 European Symposium on Biopolymers Special Issue of Bioengineering „Bioengineering – Selected Papers from ESBP 2019 (10<sup>th</sup> European Symposium on Biopolymers)“

Selected, refereed papers from the ESBP 2019 will be published in a special issue of the Bioengineering Journal (IF 3.14). Oral and poster presenters as well as participants of the conference are invited to submit full manuscripts for consideration for inclusion in the special issue. All manuscripts will be subject to the normal process of peer review. All papers submitted and accepted for the ESBP 2019 Special Issue will be published in the next available issue of Bioengineering. Deadline for manuscript submissions: 15 January 2020. The Open Access and Article Processing Charge (APC) is 1000 CHF (Swiss Francs), but we can provide 2 full waiver and five 50 % discounts for attendees of the ESBP 2019. To apply for the waiver or the reduced fees please contact the Special Issue Editor Jochen Schmid.



Manuscripts should be submitted online at [www.mdpi.com](http://www.mdpi.com) by registering and logging in to this website. Once you are registered, click here to go to the submission form. Manuscripts can be submitted until the deadline. All papers will be peer-reviewed. Accepted papers will be published continuously in the journal (as soon as accepted) and will be listed together on the special issue website. Research articles, review articles as well as short communications are invited. For planned papers, a title and short abstract (about 100 words) can be sent to the Editorial Office for announcement on this website.

[www.mdpi.com/journal/bioengineering/special\\_issues/ESBP\\_2019](http://www.mdpi.com/journal/bioengineering/special_issues/ESBP_2019)

## Special Issue Editor

### Guest Editor

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## Presentation awards

### Best poster presentation

We are very happy to announce that we will be able to award a poster prize for the best poster presentation at the ESBP 2019. Members of the Scientific Committee will review all posters and select the winning candidate. The award is sponsored by MDPI (Bioengineering, Polymers).



### Best oral presentation

We are very happy to announce that we will be able to award a prize for the best oral presentation at the ESBP 2019. Members of the Scientific Committee will

select the winning candidate. The award is sponsored by MDPI (Bioengineering, Polymers).

## Telling the story of European biotechnology

**23 – 29 September, 2019 – European Biotech Week: Celebrating Innovation**

The European Biotech Week celebrates biotechnology, an innovative and vibrant sector launched by the discovery of the DNA molecule back in 1953. The first European Biotech Week that took place in 2013 marked the 60th anniversary of this pivotal moment in history.

Biotech associations are key in helping to maximize the potential of biotechnology. In Europe, the National Associations Council, hosted by EuropaBio, represents



15 national biotech associations that together represent over 1,800 small- and medium-sized biotech companies and organisations. In 2019, the week takes place between 23-29 September and the ESBP 2019 is part of that event!

We are happy to participate in this European event, visible beyond Europe. We will place the ESBP on the European Biotechnology web side with the latest news on our program.

## Prof. Dr. Mattheos Koffas

D1.T1

Professor Mattheos Koffas, PhD., received his B.S degree in Chemical Engineering from the National Technical University in Athens, Greece in 1994. He then joined the graduate program of the Department of Chemical Engineering at MIT where he worked on improving amino acid biosynthesis from *Corynebacterium glutamicum*. After completing his PhD in 2000, he joined DuPont's Central Research and Development as a research scientist where he worked on engineering the carotenoid biosynthesis of an obligate methanotroph. In the year 2002 he joined the faculty of the Department of Chemical and Biological Engineering at SUNY Buffalo as a tenure-track Assistant Professor and was promoted to Associate Professor with tenure in the summer of 2008. In January of 2011 Prof. Koffas moved to his current position at RPI. In his research career, Prof. Koffas has worked with a variety of microorganisms and natural products including amino acids, polyphenols, fatty acids and biopolymers.



His main work focuses on the biosynthesis of sulfated glycosaminoglycans, such as heparin and chondroitin sulfate, as well as polyaminoacids, such as polyglutamic acid and polylysine. He greatly combines synthetic biology with biopolymer engineering to realize highly efficient industrial process routes. He has published more than 100 peer-reviewed publications, more than 10 book chapters and holds several patents. He is a member of the editorials boards of various Journals in the area of bioengineering, such as the *Biotechnology Journal*, *Biochemical Engineering Journal*, *Genes*, *Metabolic Engineering*, and he is the Editor of *Biotechnology Advances* and *Metabolic Engineering Communications*. He is a fellow of the American Institute of Medical and Biological Engineers.



D3.T1

## Prof. Maria Reis



Prof. Maria Reis is full Professor in Environmental/ industrial Biotechnology at FCT-UNL. Its main research interests are on the development of sustainable bioprocesses for the removal of pollutants from water and wastewater streams and for the exploitation of industrial wastes for the production of biopolymers and bulk chemicals. Within this research area, she published more than 200 papers in scientific journals with peer review. She is co-author of 4 National patents and 5 International Patents. She coordinated 20 national and international projects (team leader), out of which 9 European Projects and 6 were co-funded by industrial companies, and participated as team member in 22 research projects. She co-supervised 20 PhD students and is currently co-supervisor of 11 PhD students.

Her scientific work has been distinguished with several awards and she was the winner of the Solvay & Hovione Ideas Challenge SHIC'08 Prize-Solvay prize in 2008. Nominated and Elected to the Portuguese Academy of Engineering in 2009 and Elected as IWA Fellow, September 2010. She is Editor of the Water Research (Elsevier).

## Prof. Kevin O'Connor

D1.T2

Professor O'Connor is director of BEACON SFI bioeconomy research centre, Professor of Applied Microbiology and Biotechnology, a Principal investigator in the UCD Earth Institute and a member of the school of Biomolecular and Biomedical Science at University College Dublin, Ireland. He is chairperson of the scientific committee for the €4 billion public private partnership Biobased Industries Joint Undertaking (BBI JU), a former member of the European Commission biobased products expert group and a previous member of European Commission committee entitled the "lead market initiative for Bio-based products (2008 – 2011).



His research interests are integrated biorefining, Biobased chemicals, biodegradable polymers, biocatalysis and protein engineering. Professor O'Connor has published over 100 international peer review articles and made over 200 contributions at national and International conferences. He has filed 8 patents and successfully licensed 3 technologies resulting from his research work. Professor Kevin O'Connor is the founder of Bioplastech a university spin out company with technologies for the conversion of residues and biobased resources to biodegradable polymers and Nova Mentis a second campus company established to commercialise a novel antioxidant with health promoting properties. Professor O'Connor was a key driver behind the development of the €6M rural Bioeconomy campus at Lisheen, Co Tipperary and the Irish EU Model demonstrator region for the sustainable production of chemicals.



D1.T3

## Prof. Dr. Ebru Toksoy Öner



Professor Ebru Toksoy Öner received her PhD degree in Chemical Engineering in the year 2000 and currently she is working as a Professor at the Bioengineering Department of Marmara University (Istanbul, Turkey). She is the Principal Investigator of the Industrial Biotechnology and Systems Biology (IBSB). Prof. Toksoy Öner's current research in IBSB focuses on biomaterials, functional foods and systems microbiology. Ongoing research topics are functional biosurfaces, laser-based technologies, extremophiles, fructans, levan polysaccharide, strain improvement for exopolysaccharide production, production of biopolymers from extremophiles, and optimization of fermentation processes to design high-yield production lines.

Prof. Toksoy Öner has been the coordinator of several national and international projects and partner in COST Actions. She is the Member of European Federation of Biotechnology (EFB), American Nano Society (ANS), American Chemical Society (ACS), International Society for Extremophiles (ISE), Turkish Biotechnology Society and author of various book chapters, reviews and more than 80 peer-reviewed research articles.

## Dr. M. Auxiliadora Prieto

D1.T4

Dr. Auxiliadora Prieto received her PhD in Pharmacy in the year 1996 from the Complutense University of Madrid, followed by two EMBO fellowships. First, at the Federal Institute of Biotechnology, Germany, in the group of Prof. Kenneth Timmis. Then, as Postdoc fellow at the Institute of Biotechnology, ETH Zürich, Switzerland, in the group of Prof. Bernard Witholt. Currently she is Scientific Researcher at the Spanish National Research Council (CSIC). Dr. Prieto is the coordinator of the Interdisciplinary Platform for Sustainable Plastics towards a Circular Economy-SusPlast, which is integrated by eight Institutes of CSIC that count with multidisciplinary biotechnological researchers, dedicated to bioprospecting, biocatalysis, biotransformation, environmental microbiology, synthetic and systems biology, plastics and bioplastics synthesis and biodegradation. In addition, this platform includes specialists in materials engineering, packaging, design of functional materials, modification of surfaces and biomaterials which intensively cooperate with SusPlast groups specialized in waste management for chemical and biotechnological valorisation of bio-based polymers and other value-added products.



At the Biological Research Center (CIB-CSIC) she is the Head of the Polymer Biotechnology group as part of the Plant and Microbial Biotechnology Department. The group aims to explore and exploit the bacterial abilities for producing and degrading bio-based polymers (polyesters and bacterial cellulose) in order to contribute to global sustainability, using tools of molecular biology and metabolic engineering, combined with new omics technologies and synthetic biology. Dr. Prieto has participated in, led and/or coordinated national and international projects and is main inventor of several patents, three of them licensed to bio-tech SMEs dedicated to the production of bio-plastics. She is co-founder of the SME Darwin Bioprospecting Excellence SL. Dr. Prieto has published more than 80 international scientific papers and is Editor in chief of Microbial Biotechnology (Wiley).

D1.T5

## Prof. Dr. Dieter Jendrossek



Professor Dieter Jendrossek studied biology at the University of Göttingen, Germany, where he also completed his PhD (1988) under supervision of Prof. Hans G. Schlegel and Dr. A. Steinbüchel. In 1990 he started to build up his own research group with a focus on the biodegradation of PHB and other PHAs by PHA depolymerases (PhaZs). After completion of his Habilitation in 1995 and a short stay in Prof. H. Schrempf's lab (molecular biology of *Streptomyces*) he broadened his research interests to other topics such as biodegradation of rubbers by haem-dependent rubber dioxygenases, monoterpene metabolism in *Pseudomonas*, biochemistry of squalene hopene cyclases, since 2005 molecular structure and cell biology of PHB granules (carbonosomes) and – most recently – formation, molecular structure and function of polyphosphate (voluntin granules) and acidocalcisomes in alpha- and beta-Proteobacteria. In 1999, he had moved to the University of Stuttgart where he is since then a Professor for Microbiology and Biochemistry. In 2004, Dieter Jendrossek was also a visiting professor at the German University at Cairo (GUC), Egypt, where he established lectures and beginner courses in microbiology.

Prof. Jendrossek has published about 150 original papers and several reviews in international journals. He is on the editorial board of *Applied and Environmental Microbiology* since 2013 and has multiple responsibilities in the German Association for General and Applied Microbiology (VAAM).

## Prof. Etienne Paul

D1.T8

Prof. Etienne Paul studied Biotechnology & bio-engineering at the University of Marseille, France and subsequently conducted his PhD under supervision of Gérard Goma at the University of Toulouse where he is currently Professor. He has twenty seven years of experience in the field of bioprocesses and bioreactors applied for biological treatment of wastewaters and wastes and more recently for their valorisation. His current main research focuses on the production by mixed cultures and characterization of biopolymers (PHAs, bioflocculants, gels, EPS ...) and converting these polymers into useful material.



He has published over 60 SCI-tracked journal papers (H-index 25) and three book chapters in English, participated in 67 international conferences and is the main inventor in 5 patents. He has supervised 30 PhD students and has coordinated approximately 15 national or international research programs.

D2.T1

## Dr. João Sousa



Dr. João Sousa got his PhD degree in Microbiology from Wageningen University in 2017. His PhD work was performed at Wetsus – European Center of Excellence for Sustainable Water Technology where he worked on microbial sulfidogenesis processes to apply in the gas treatment industry. Since 2015 he works at Paques, a global company leading in biological wastewater and gas treatment. Paques has a strong innovative character and a dedicated R&D group located in the Netherlands, being João the Head of Emerging Technologies in this group. His team focus is on developing new technologies to produce higher value products (e.g. short-chain organic acids, medium chain fatty acids and PHBV) from organic waste streams, mainly wastewater and source separated organics. João is involved in several joint projects with different universities to develop further these technologies and explore new ideas.

The main goal of Paques is to bring the most promising technologies to the market. Several technologies have been or are being tested at pilot scale and the team is currently up-scaling the PHBV production from wastewater to demonstration scale.

## Dr. Alan Werker

D2.T6

Dr. Werker is a hybrid environmental engineer and materials scientist with 30 years of experience in both academic and industrial research and development efforts. For 14 years, he led the developments within a trans-disciplinary team from fundamental know-how to bioprocess development in piloting proof of concept of producing polyhydroxyalkanoates from within wastewater treatment facilities. His activities are and have been engaged with national and international academic, corporate, and public/private collaborations in basic research, thesis supervision, EU Consortium Projects, and, as well, practical business development challenges and networking for establishing bio-based value-added chains from renewable resources. Currently, he is a co-owner and consultant with Promiko AB ([www.promiko.se](http://www.promiko.se)) in Sweden, a research theme leader with Wetsus ([www.wetsus.nl](http://www.wetsus.nl)) in the Netherlands, and an Adjunct Professor, School of Chemical Engineering, at the University of Queensland in Australia. His efforts are in continuation to support communities and industries with fundamental as well as practical organic residuals management challenges and opportunities with bioprocesses, that, ideally, lead to renewable resource generation.



## D2.T10

## Prof. Dr. Manfred Zinn



Professor Manfred Zinn studied Biotechnology at ETH Zurich, where he also received his PhD in the research group of Prof. Bernard Witholt studying polyhydroxyalkanoates (PHA) biosynthesis in chemostat cultures. Between 1997 and 2001 he was research associate at the University of Tennessee and at Harvard University at Cambridge, U.S.A. studying the biofilm formation on solid surfaces. Back in Switzerland, he did a postdoc at Eawag Dübendorf in the group of Prof. Thomas Egli focusing on multiple nutrient limited growth conditions. From 2002 until 2011 he was group leader at Empa St. Gallen, developing PHAs for medical applications. Since 2011 he is Professor in Biotechnology at the University of Applied Sciences and Arts Western Switzerland (HES-SO Valais Wallis) and teaching Bioprocess Engineering at EPF Lausanne. At HES-SO Valais-Wallis he is leading the research group Biotechnology and Sustainable Chemistry and is research is focused on designing bioprocesses using sustainable and waste substrates (e.g., syngas and CO<sub>2</sub>), scale up of bioprocesses from micro- to pilot scale (300 L) using process analytical technology and processing of PHAs for medical (e.g. drug delivery by nano- and micro-capsules) and industrial applications (e.g. surface functionalization and 3D printing). Prof. Zinn has published more than 72 papers, 9 book chapters and proceedings, and is Specialty Chief Editor of the journal *Frontiers in Bioengineering and Biotechnology* and on the editorial boards of *Applied Environmental Biotechnology* as well as *International Biodeterioration & Biodegradation*.

## Prof. Valérie Langlois

D2.T11

Valérie Langlois has completed his PhD at the age of 28 years from University Paris VI. She is now deputy director of the East Paris Institute of Chemistry and Materials Science in Thiais, France (Université Paris Est, CNRS). Her main scientific interests are related to biodegradable polyesters, their chemical modifications and synthesis of copolymers. Her research activities are devoted to fundamental aspects of biodegradable polyesters in relation with their biomedical applications such as drug delivery systems, tissue engineering or antibacterial materials. She has published more than eighty publications in this research field.





D3.T5

## Prof. Ipsita Roy



Ipsita Roy is an expert in microbial biotechnology, natural biomaterials and their biomedical applications. She has recently joined the Department of Material Science and Engineering, Faculty of Engineering, at the University of Sheffield as Professor of Biomaterials. Previously she was Professor at the School of Life Sciences, University of Westminster, London. She is also Visiting Professor at NHLI, Faculty of Medicine, Imperial College London. Ipsita was awarded the prestigious Inlaks Scholarship and the Overseas Research Students Award to study for her Ph.D. at the University of Cambridge, where she was awarded the Churchill College Scholarship, the Lundgren Scholarship, Leche Trust Scholarship and the Cambridge University Philosophical Society Fellowship Award.

Ipsita has been at the University of Westminster since 2000, is now the Research Director of the School of Life Sciences and leads the Applied Biotechnology Research Group. She has published over 100 papers in high 'Impact Factor' journals such as Biomaterials, Biomacromolecules, Journal of Royal Society Interface, Acta Biomaterialia and ACS Applied Materials Interfaces. She has presented her work at numerous international conferences. Her group is currently focused on the production of novel polyhydroxyalkanoates (PHAs) and their characterization. She has pioneered the production of PHAs from Gram-positive bacteria which lack immunogenic properties and hence are excellent materials for medical applications. She is an editor of the Journal of Chemical Technology and Biotechnology (JCTB). Many of her projects involve the use of PHAs for medical applications, drug eluting biodegradable stents, nerve guidance conduits, antibacterial polymers and wound healing. Also, she has recently received EPSRC funding for the development of smart wound healing patches (CYCLOPS) and drug eluting biodegradable stents.

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## About how to functionalize mcl-PHA particles with peptides and alive cargos

D1.T4

**M. Auxiliadora Prieto**

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Over the past few decades considerable interest has been shown in developing nano- and micro- carriers with biocompatible and biodegradable materials for medical and biotechnological applications. Nanostructured materials produced by bacteria are becoming increasingly recognized as functionalized beads with great biotechnological and biomedical potential<sup>[1]</sup>. Functionally complex architecture of PHA inclusions, based on interacting proteins embedded/attached to PHA core, has been exploited as a toolbox to display molecules carrying out specific function. Under a wide scope of applications the performance of such engineered PHA beads has been demonstrated in high-affinity bioseparation, enzyme immobilization, protein delivery to natural environments, diagnostics, as an antigen delivery system and many others. BioF, a PHA affinity tag based on the PhaF phasin of *Pseudomonas putida*, has been applied for the functionalization of mcl-PHA beads with fusion proteins both *in vivo* and *in vitro*. The structural model of this tag supports an amphipathic  $\alpha$ -helical conformation with the hydrophobic residues facing the PHA granule. In this work, we rationally design shorter versions of the BioF tag that maintain the affinity for the granule. We studied the *in vivo* localization of the fusion proteins on the PHA, their stability on the surface of the PHA granule against pH, temperature and ionic strength, as well as their possible influence on the PHA synthesis. Based on the results obtained, The MinP tag for PHA functionalization has been proposed which retains similar binding properties but possesses a reduced size.

We also present a new PHA microencapsulation technology capable of bio-plasticize alive cargos such as bacteria by employing a modified double emulsion-solvent evaporation technique. The resulting MPs display a spherical morphology and an average particle size of 10  $\mu$ m. The stability of the MPs was monitored under different conditions of storage and stress.

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# Characterization and exploitation of the marine bacterium *Halomonas* sp. SF2003 for optimization of PHA production

D1.T7

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*Halomonas* sp. SF2003 is a halophilic Gram-negative eubacterium isolated from the Iroise sea which demonstrated its capacity to produce and accumulate polyhydroxyalkanoates (PHA) up to 78 % of the cell dry weight<sup>[1,2]</sup>. Because of their properties, these biopolymers present environmentally and economically interests in order to replace conventional plastics<sup>[3,4]</sup>. Consequently numerous studies are conducting to identify and exploit new bacteria capable of accumulating these biopolymers<sup>[5,6]</sup>. *Halomonas* sp. can naturally produce P3-HB and the co-polymer P3HB-3HV, by use of different carbon sources even those originate from industrial wastes. It also exposes a strong versatility to various temperature and salinity conditions making it a good candidate for biotechnological applications. The aim of our work is to study and manage *Halomonas* sp. metabolism, using genetic, microbiology and bioprocess tools, in order to enhance and to manage its production of PHA. First part of our work consisted to identify and start characterization of the key enzymes involved in the PHA metabolism of *Halomonas* sp. using bioinformatics tools. Sequencing and annotation of *Halomonas* sp. genome allowed identification of different genes including two potential PHA synthases named *phaC1* and *phaC2*. Because of the atypical organization and size of genes presumably involved in PHA biosynthesis, experiments were conducted to isolate and study them. Thus, *phaC1* and *phaC2* genes were individually cloned in a plasmid pBBRMCS2. Recombinant plasmids were express in *Cupriavidus necator* H16 PHB-4, a non-producing PHA strain to study the influence of each gene on PHA production by *Halomonas* sp. Second part of our work was focused on screening of carbon substrates to optimize bacterial growth and PHA production at same time. A selection of several carbohydrates and mix of carbohydrates/acids was tested for PHA production. Screening was conducted using Nile Red agar plates method<sup>[1]</sup>. Assimilation of the carbon sources, bacterial growth and batch PHA production were recorded to compare the performances of wild type and recombinant strains using confocal Laser Scanning Microscopy (CLSM) and gas chromatography GC/FID allowed to study and determine characteristics of each condition/production.

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## Selection of a microbial population in continuous culture: a way to produce biopolymers for industrial applications?

D1.T8

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In the context of the circular economy, materials could be recovered from wastewaters (WW) to produce valuable molecules that could be ideally used in the same WW treatment chain. Interestingly, cellulose fibers coming from toilet paper can be recovered from urban wastewater as they represent the major fraction of the removed COD by an appropriate sieving pre-treatment. Moreover, bacterial exopolymers (EPS) secreted by microbial cells and which remain associated with the surface of the cell (for example in capsule form) may have excellent flocculating properties for some and may thus be a satisfactory alternative to synthetic polymers such as the polyacrylamides (PAM).

In this work, cellulosic material was recovered from WW using sieve and transformed, after hydrolysis, in sugars which then, were used to produce a selected microbial consortia that showed excellent flocculent capability. After concentration up to 18 % (DW) the cellulosic material was hydrolyzed at 50°C using a commercial enzymatic cocktail. The hydrolysate was then used after a coarse filtration to feed a continuous reactor run at ambient temperature and a pH regulated at 7 to produced biomass EPS. In order to evaluate the effect of the hydrolysate in the selection of the microbial population, another reactor run in parallel was fed with a mineral medium and a mixture of glucose and fructose (50/50 in Cmol/Cmol). The two reactors were inoculated with an activated sludge from the treatment plant of Toulouse (France).

The main challenge in our work was to find the appropriate selection pressure to perform a natural stable enrichment of microorganisms with the capacity to overproduce the desired bioflocculent. Therefore, specific operating conditions in terms of substrate nature, dilution rate, feeding strategy and selective settling were investigated.

A reliable and efficient production of EPS with flocculant properties was achieved for a C/P ratio lower than 100 (Cmol/Pmol) and a C/N ratio lower than 9, a dilution ratio of 0.12 h<sup>-1</sup> and selective purging after a sequential decantation to remove filamentous organisms (*Sphaerotilus natans* and fungi). Based on jar tests but also on tests performed on a settler pilot, our results showed that the new bioflocculants have always better performances of pollution removal compared to the use of FeCl<sub>3</sub> alone. Performance as high as +189 % with respect to FeCl<sub>3</sub> + PAM was observed. Cost calculation showed the conditions for which this process could be profitable.

## Identification of novel exopolysaccharides for application in cosmetics, packaging and lubricants

D1.T9

**Broder Rühmann<sup>1</sup>, Christoph Schilling<sup>1</sup>, Tristan Rath<sup>1</sup>, Edilberto Vicente Medina Cabrera<sup>1</sup>, Moritz Gansbiller<sup>1</sup>, Seyedmahdi Ranaei Siadat<sup>1</sup>, Jochen Schmid<sup>1,2</sup>, Volker Sieber<sup>1</sup>**

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In recent years the demand for sustainable polymers as replacement of petro-based polymers or with increased biodegradability has grown continuously. Additionally, there is an ever-increasing need on new polymer properties for diverse specific applications. Microbial exopolysaccharides (EPS) with their bio-based origin and their highly diverse chemical structures – which result in versatile properties – are up to this challenge. Until now, only a few polysaccharides are commercially available. Unfortunately, these polymers are not satisfying all technical requirements. Therefore, the discovery of novel and innovative polysaccharides is essential. A high throughput screening technique in combination with a carbohydrate fingerprint can help to identify new polymer variants and is the major key to trap the full potential of the natural diversity of microbial EPS. Moreover, the presented screening platform is also capable to identify the specific inducing parameters for EPS production (e.g. C/N-ratio, salt or phosphate concentrations) for each individual strain. Thereby, the optimization of the fermentation process is much more focused. The presentation shows that the physiochemical characterization of the EPS is important to find the best application in e.g. cosmetics, lubricants or anti-corrosion protection in order to replace petro-based compounds. Furthermore, different polymer modifications lead to novel properties and enhance the application fields, where commercially available EPS are not reaching the functional properties yet.

## A promising exopolymer, synthesized by the halophilic bacterium *Chromohalobacter canadensis* 28, for cosmeceutical applications

D1.T10

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Cosmetics are substances that are in contact with external part of body and used for cleaning, beautifying and improving appearance (moisturizing, anti-aging etc.). An increased consumer demand for natural polysaccharides worldwide, makes natural polymers popular and especially microbial exopolysaccharides (EPS) are highly preferred with their production and purification easiness, unique structure and properties. Adaptation of microorganisms to extreme conditions like high salinity, acidity, temperature resulted in synthesis of unusual metabolites with novel characteristics including EPSs. Halophilic microorganisms live in high saline environments and can synthesize microbial EPS in non-sterile continuous cultivation processes.

*Chromohalobacter canadensis* 28 is a moderate halophilic bacterium isolated from Pomorie salterns (Bulgaria) and it produces an extracellular polymer (EP) substance containing two fractions, 72 % w/w poly-gamma-glutamic acid (Ð-PGA) and 14 % w/w EP. The hydrogel of this EP is known to show good swelling, high water holding capacity, emulsifying and stabilizing behaviors. The unique properties of this EP clearly pointed to its high potential for applications in the cosmetic industry. In this collaborative systematic study, development of a cost-effective microbial production system for EP synthesized by *C. canadensis* 28 as well as studies on its moisturizing, antiaging, anti-irritant and lifespan enhancer activities, and biocompatibility are the main focuses and the most recent results will be reported.

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# ***In vitro* wound healing ability of the fucose-rich polysaccharide FucoPol and its silver nanocomposite**

D1.T11

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Wound healing efficiency is especially dependent on controlling wound moisture, preventing inflammation and infections and, promoting re-epithelization. The use of sustainable and green processes to obtain formulations for wound healing, especially in chronic situations might be a promising field to explore. Silver has been widely used due to its antimicrobial potential for wound care where recent approaches are focused of a controlled release of silver allowing to retained efficacy. Polysaccharides are biopolymers rich in functional groups that are essential for the stabilization of metal particles, providing great control of shape, size and particle dispersion. Natural polysaccharides are biodegradable, renewable, sustainable and non-toxic, which supports their use as biomaterials for the synthesis of metallic nanoparticles for health applications. Furthermore, many of these macromolecules have interesting biological properties, and physical and structural properties mainly due to their high molecular weight, water retention capacity and molecular structure, which might be interesting for the development of wound dressing products.

In this study, a green, simple, light-assisted method was used for the synthesis of silver nanoparticles (AgNP) using FucoPol, a fucose-containing polysaccharide, as stabilizing agent. The synthesized nanoparticles were spherical in shape and the main population had a particle size ranging between 7 and 60 nm, corresponding to percentiles of 50 and 90, respectively. FucoPol, as well as the functionalized material, besides having no cytotoxicity towards human skin keratinocytes and mouse fibroblasts, also promoted *in vitro* keratinocytes migration. These observations not only show the safety of FucoPol and FucoPol/AgNP biocomposite, but also their wound healing ability. Moreover, the biocomposite proved to have a strong antimicrobial activity against *Staphylococcus aureus* ATCC 6538 (WDCM 00193) and *Klebsiella pneumoniae* CECT 8453 (WDCM 00192), two representative strains of known skin commensal pathogens. These findings demonstrate for the first time the potential of FucoPol to be used for the development of wound healing formulations. Additionally, the use of the FucoPol/AgNP biocomposite would lead to the development of antimicrobial biomaterials, including antibacterial wound healing formulations, which further strengthens the establishment of FucoPol as a bioactive biopolymer.



## Characterizing the production of poly(hydroxyalkanoate) from CO<sub>2</sub>

D2.T2

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Biological capture of CO<sub>2</sub> for the production of bioplastics is an elegant approach to reduce CO<sub>2</sub> emissions, while harnessing the dual challenge of decreasing plastics dependence on fossil fuel and their environmental impact. It is well known that litho-autotrophic bacteria synthesize poly(3-hydroxyalkanoate) (PHA) using CO<sub>2</sub> as sole carbon source and H<sub>2</sub> as energy source, in presence of oxygen<sup>[1]</sup>. However, only a few studies reported a detailed characterization and directed optimization of the fermentation process.

We aim here at presenting our results on the effects of several parameters on the process productivities and carbon capture yields of batch bioprocess in order to provide a benchmark for further improvements. We will focus on the production of poly(3-hydroxybutyrate) (PHB). The experiments were conducted on a 3.6 L bioreactor and yielded more than 15 g L<sup>-1</sup> PHB and specific productivities in the order of 25 mg (PHB) g<sup>-1</sup> (DCW) h<sup>-1</sup>. The litho-autotrophic *Cupriavidus necator* wild-type strain litho-autotrophic was investigated in two-phase bioprocesses. The influence of the biomass concentration on the PHB yield, as well as the effect of nitrogen limitation were optimized by medium composition in order to establish the optimal dual (C, N) limited growth conditions. The obtained yields are currently compared to the theoretical yields deduced from the expected metabolic pathways occurring in litho-autotrophic conditions and to available literature results<sup>[2,3]</sup>.

Furthermore, a discussion regarding the scale-up of the process considering its associated technical challenges will be provided.

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## Biological and biotechnological consequences of polyhydroxyalkanoates production by extremophiles

D2.T3

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Polyhydroxyalkanoates (PHA) are accumulated by numerous prokaryotes primarily as storage materials; nevertheless, we have recently reported that presence of PHA in cells also enhances stress robustness and resistance of the bacteria against various common environmental stressors such as high or low temperature, repeated freezing and thawing, UV irradiation or osmotic pressure. The secondary “protective function” is probably the reason why PHA are biosynthesized by many prokaryotes naturally inhabiting extreme conditions. For instance, we have observed that presence of PHA protects halophiles from adverse effect of osmotic down shock which might be a reason why PHA biosynthesis is so common among halophiles including *Halomonas halophila* which can be, according to our results, considered being prominent candidate strain for PHA production. Furthermore, we have also discovered that presence of PHA protects non-halophiles against dramatic fluctuations in osmotic pressure. Therefore, we utilized exposition to osmotic challenge (hypertonic and subsequent hypotonic treatment) combined with rapid and reliable ATR-FTIR based identification of PHA positive colonies as a novel strategy enabling isolation of PHA producing strains from various microbial consortia. In particular, bearing in mind all the positive aspects of utilization of extremophiles such as reduced sterility or energy demands, we have focused on isolation, identification and biotechnological characterization of PHA producing thermophiles from various suitable microbial consortia such as thermophilic industrial compost or thermophilic activated sludge.

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## Influence of unusual co-substrates on biosynthesis of medium-chain-length polyhydroxyalkanoates

D2.T4

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Biosynthesis of polyhydroxyalkanoates (PHA) bearing unconventional functional groups in the side chains is of high interest since the presence of reactive groups enables tailoring the mechanical and physical properties of a polymer<sup>[1]</sup>. Aromatic substrates are examples of unusual sources of PHA precursors.

The bacterial production of medium-chain-length polyhydroxyalkanoate (mclPHA) in continuous two-stage cultivation was investigated. Chemostats were linked in sequence to separate biomass production in the first stage from PHA accumulation in the second stage. While in the first stage, a single chemostat reactor provided biomass accumulation of *Pseudomonas putida* KTQQ20 with citrate as the only carbon substrate, different binary mixtures of fatty acids were provided to four parallel chemostat bioreactors fed with biomass from the first stage to study the resulting polymer composition during PHA accumulation in the second stage.

With this experimental set-up we were able to produce mcl-PHAs, which are structurally related to the fatty acid substrates. A non-linear correlation between feed composition and polymer composition for specific pairs of fatty acids was observed and a mechanistic model is proposed from the experimental results to explain the distinct molecular weight average dependency on the fatty acid substrate.

[1] Zinn M, Hany R. 2005. Tailored material properties of polyhydroxyalkanoates through biosynthesis and chemical modification. *Adv Eng Mater* 7:408-411.

# Macroalgal cellulosic waste, a novel platform for the bacterial synthesis of polyhydroxyalkanoates

D2.T5

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Our group has been focusing on the production of PHAs from cellulosic biomass wastes, both from terrestrial and marine origin; namely from straw, municipal/garden waste and seaweed. Particularly very high productivities were attained for homo- and copolymers (1.6 g P<sub>3HB</sub> L<sup>-1</sup>h<sup>-1</sup> and 0.5 g P<sub>(3HB-4HB)</sub> L<sup>-1</sup>h<sup>-1</sup>) with *Burkholderia sacchari* in high-cell density fed-batch cultivations on wheat straw hydrolysates.

Recently we have been considering macroalgae as promising feedstock for the production of polyhydroxyalkanoates because of their high carbohydrate content (25–60 % DW) and absence of lignin<sup>[1]</sup>. The later allows a mild chemical pretreatment of the biomass, without the production of toxic compounds which may result from the degradation of the monosaccharides. Besides a glucan fraction (cellulose, starch or laminarin), seaweed possess complex polysaccharides like alginate, agar and carrageenan. These hydrocolloids are extensively used in food technology, microbiology and medicine.

After hydrocolloid extraction, seaweed residues still contain a high carbohydrate content that can be upgraded to produce biodegradable plastics such as PHAs. This not only reduces the amount of waste produced, but also adds value to the residues, contributing to a circular economy.

In this context, we have assayed the residues of the red seaweed *Gelidium sesquipedale*, after industrial agar extraction, as carbon platform. A carbohydrate content of 44 % (w/w) was found in this feedstock, composed mainly of cellulose and agar. Acid catalyzed hydrothermal pretreatment combined with enzymatic cocktails achieved a 82 % total sugar recovery. The released monosaccharides were glucose (93 % total sugars) and galactose (7 %). Algal hydrolysates with ca. 20 g/L total sugars were obtained and tested as C-source. A halotolerant bacterial strain (*Halomonas* sp.) capable of consuming the main monosaccharides in the hydrolysate and accumulate PHA was selected. In shake flasks a P3HB accumulation of 38 % (w/w) on the *Gelidium* hydrolysates was achieved, compared to 40 % on its pure sugar-mix counterpart. Fed-batch cultivations in 2L STR are currently being performed to enhance cell growth and P3HB production.

[1] Cesário MT, da Fonseca MMR, Marques MM, de Almeida MCMD. *Biotechnol Adv*, 36 (2018), 798-817. doi.org/10.1016/j.biotechadv.2018.02.006

## Respiration dynamic response of PHA storing mixed microbial culture biomass

D2.T6

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The present work concerns bioprocesses for mixed microbial culture (MMC) polyhydroxyalkanoate (PHA) production. Our objectives are to characterize and exploit the dynamic respiration response of PHA storing MMCs. Characterization requires distinguishing between stimulation of PHA storage respiration, and trends of respiration with substrate consumption. A series of replicate respiration response experiments were performed with laboratory and pilot scale MMCs. The dynamic response of the biomass was assessed with respect to the substrate concentrations to stimulate maximal respiration rates in PHA storage, versus the substrate concentrations to maintain attained kinetics of substrate consumption. A hysteresis between respiration stimulation, and the trends of substrate consumption rates was observed, and these data were modelled within the frame of Michaelis-Menten kinetics.

Commercial quality PHAs can be produced by using surplus activated sludge from municipal or industrial wastewater treatment. Enrichment for the PHA storing phenotype in MMCs is possible on wastewaters that are not suitable feedstocks for PHA production. Thus, exploitation of the surplus biomass for PHA production needs a supply of regionally available feedstocks in the material flows for industrial scale polymer production activities. Concentration of VFAs in such typical feedstocks can range widely from well under 1 gVFA/L and upwards. Notwithstanding, the literature for MMC PHA production methods at laboratory and pilot scale is dominated by examples of high concentration feedstocks, in excess of 20 gVFA/L. Practical challenges exist when the volume of required feedstock exceeds the working volume of the bioprocess. Up-concentration of fermented streams can be a solution, but robust bioprocesses designed to accommodate dilute VFA rich streams can avoid need for up-concentration so long as such streams do not require transport. Robust methods to drive accumulation with more dilute feedstocks can be made by exploiting the aforementioned respiration stimulation hysteresis. The model from the stimulation-response experiments was applied within Monte-Carlo process simulations from which bioprocess for PHA production on low strength VFA feedstocks was explored. The goal of this presentation will be to relate the findings and significance of respiration hysteresis and considerations in exploitation of this behavior in bioprocesses for MMC PHA production.

# Glycogen serves as the main carbon source for PHB synthesis during nitrogen starvation in the unicellular cyanobacterium *Synechocystis* 6803

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Under nutrient starvation, certain cyanobacteria accumulate several storage polymers like polyhydroxybutyrate (PHB) and glycogen (Klotz *et al.*, 2016). PHB is of high biotechnological relevance since it can be used as a source for bio-degradable plastics. Although the principle reactions of PHB synthesis in cyanobacteria have been analyzed, fundamental questions on PHB metabolism are still unanswered. As recently shown (Dutt & Srivastava, 2018), about 74 % of the carbon from PHB is coming from intracellular metabolites. The precise nature of intracellular carbon, however, remained unknown. In the present study, we analyze the relation between glycogen and PHB metabolism in the model cyanobacterium *Synechocystis* PCC 6803. We demonstrate that the intracellular glycogen pool plays a pivotal role in providing the carbon metabolites for PHB synthesis. In the absence of glycogen degradation, as it is the case in a mutant of glycogen-phosphorylase *glgP1* or in the *glgP1/2* double mutant, PHB synthesis is abrogated. A mutant of the glycogen synthase *glgA1* showed a strong decrease in the overall PHB production, while the amount of glycogen, produced by GlgP2, remained constantly high. These results indicate a functional difference of GlgA1 and GlgA2-produced glycogen, similar to the observation from glycogen utilization during recovery from nitrogen starvation (Doello *et al.*, 2018). Of the various carbon catabolic pathways present in *Synechocystis* (the EMP, ED and OPP pathway), the EMP pathway, which seems dispensable for recovery from nitrogen starvation, plays the most important role in PHB production.

Together, this study clearly indicates that PHB in *Synechocystis* is produced from glycogen catabolism during nitrogen starvation periods (Koch *et al.*, 2019).

Doello S, Klotz A, Makowka A, Gutekunst K & Forchhammer K (2018) A Specific Glycogen Mobilization Strategy Enables Rapid Awakening of Dormant Cyanobacteria from Chlorosis. *Plant physiology* 177: 594-603.

Dutt V & Srivastava S (2018) Novel quantitative insights into carbon sources for synthesis of poly hydroxybutyrate in *Synechocystis* PCC 6803. *Photosynthesis research* 136: 303-314.

Klotz A, Georg J, Bucinska L, Watanabe S, Reimann V, Januszewski W, Sobotka R, Jendrosseck D, Hess WR & Forchhammer K (2016) Awakening of a Dormant Cyanobacterium from Nitrogen Chlorosis Reveals a Genetically Determined Program. *Current biology : CB* 26: 2862-2872.

Koch M, Doello S, Gutekunst K & Forchhammer K (2019) PHB is Produced from Glycogen Turn-over during Nitrogen Starvation in *Synechocystis* sp. PCC 6803. *International journal of molecular sciences* 20.

## Development of an at-line and automatized method for polyhydroxyalkanoate quantification by flow cytometry

D2.T8

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Microbial polyesters, known as polyhydroxyalkanoates (PHA), are intracellularly accumulated as a carbon and energy storage compound under nutrient limited conditions. PHAs are green materials that can have similar properties to petroleum-based plastics with a huge potential for biomedical applications. To optimize the bioprocess at its maximum, the establishment of routine and rapid analytical methods for assessing PHA production and degradation in laboratory and industrial scales are needed. Flow cytometry (FCM) is a powerful analytical tool, which gives detailed insights into cell physiology at single cell level. The staining of cells with BODIPY493/503 and SYTO-62 generate quantitative FCM results on the PHA production in just few minutes<sup>[1]</sup>. Thus, it allows taking rapid responses during the bioprocess, such as the adjustment of PHA precursor feeding and harvest of the cells at the optimal time point.

Here, we established a robust and easy method for quantitative analysis of PHA in native (*Comamonas acidovorans*) and recombinant (*Escherichia coli*<sup>[2]</sup>) PHA producers by FCM. In addition, we have advanced on the implementation of an automatic and real-time analysis of PHA production. As a result of at line FCM analysis, the correlation between the mean fluorescence intensity of the PHA dye and the PHA content (wt %) obtained by gas chromatography of *C. acidovorans* and *E. coli* cultivations were both described by a  $R^2$  of 0.905. The remote control of the FCM and an automatic sample dilution system were created in house as well as the connection between these two parts with a benchtop bioreactor. The challenges, reproducibility of results, and robustness of the real-time analysis of bioprocesses for PHA production will be presented.

[1] Karmann S, Follonier S, Bassas-Galia M, Panke S, Zinn M. 2016. Robust at-line quantification of poly(3-hydroxyalkanoate) biosynthesis by flow cytometry using a BODIPY 493/503-SYTO 62 double-staining. *Journal of Microbiological Methods* 131:166-171.

[2] Le Meur S, Zinn M, Egli T, Thony-Meyer L, Ren Q. 2013. Poly(4-hydroxybutyrate) (P4HB) production in recombinant *Escherichia coli*: P4HB synthesis is uncoupled with cell growth. *Microbial Cell Factories* 12(1):123.

## Polyhydroxyalkanoate (PHA) purification through dilute aqueous ammonia digestion at elevated temperatures

D2.T9

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PHA (polyhydroxyalkanoates) are a family of microbial polyesters with the potential to replace polyethylene or polypropylene in many of their applications. These bioplastics are already in the market, but its presence is still limited, due to – among others – their high production costs or their final properties.<sup>[1]</sup> PHA recovery can have a big impact on both factors, as well as on the overall sustainability of the process.

Dilute ammonia digestion has been considered a promising method for PHA recovery, given the possibility of recycling  $\text{NH}_3$  as a nitrogen source during the PHA production steps. However, most of the studies up to now had achieved low PHA purities and recoveries.<sup>[2]</sup> In the present study, we investigated how the digestion conditions affected the outcome of the purification, and proved that high PHA purity and recovery can be achieved at elevated temperatures (up to 90 %). Moreover, PHA purified through  $\text{NH}_3$  digestion was thermally stable during melting, with almost no reduction of the molar mass at this stage, revealing  $\text{NH}_3$  digestion as a promising method for PHA recovery.

The experiments showed no purity increase from the initial material (64 % PHA) and a low recovery (down to 68 %) at temperatures below 75°C, regardless of the time of incubation and the  $\text{NH}_3$  concentration. The trends were reversed when the temperature increased above 75°C: more impurities were solubilized – attaining higher PHA purities (up to 90 % at 140°C) – and PHA recovery improved (90 %). The results revealed that less PHA monomers were released at temperatures over 75°C, which led to a higher degree of recovery, possibly due to changes in the polymer conformation at elevated temperatures.

On the other hand, severe molar mass reduction was observed at conditions maximizing the purity (140°C). In this regard, sonication proved to be a valuable pre-treatment to enhance the PHA purity at conditions not resulting in severe molar mass reduction. A PHA purity of 86 % and a PHA recovery of 92 % was achieved at 115°C with a previous sonication pre-treatment (molar mass was 200 kg/mol). Despite the polymer was not absolutely pure, it was very stable during melting. It presented only a 10 % reduction of the molar mass at 170°C, comparable to chloroform extracted PHA. On the contrary, PHA purified with other digestion methods (such as  $\text{H}_2\text{SO}_4$  or  $\text{NaOH}$ ), with PHA purities close to 100 %, presented a severe reduction of the molar mass during melting (80 %).

[1] C. Kourmentza, J. Plácido, N. Venetsaneas, A. Burniol-Figols, C. Varrone, H. N. Gavala and M. A. M. Reis, *Bioengineering*, 2017, 4, 1–43.

[2] Y. Jiang, G. Mikova, R. Kleerebezem, L. A. van der Wielen and M. C. Cuellar, *AMB Express*, 2015, 5, 5.



## Safety by design concept applied to the synthesis of polymeric nanobiomaterials

D2.T10

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Nanobiomaterials applied for medical use (nanomedicine) in diagnosis, drug delivery, functional coatings, or nanodevices have been developed successfully in the past decade and have already led to commercialized products. However, compared to the important number of industrial nano-applications and existing proof of concepts, the number of health applications using biodegradable biopolymers remained small on the market due to the unclear situation of the future regulatory assessment of efficacy and safety.

In an international project called GoNanoBiomat<sup>[1]</sup>, the state of the art for poly(hydroxyalkanoate), poly(lactate), and chitosan in research were reviewed in order to elaborate a guideline how to develop polymeric nanobiomaterials for drug delivery applications using the Safer-by-Design (SbD) approach.

In this presentation, decision trees helping in designing, preparing, as well in characterizing polymeric nanobiomaterials will be explained.

[1] [www.gonanobiomat.eu](http://www.gonanobiomat.eu)

## How to modify the properties of PHAs by chemical modifications?

D2.T11

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The question of how to improve the properties of PHAs to enlarge their domain of applications will be addressed by specific needs for the different applications.

These needs are based on properties such as biological, thermal and mechanical ones. The different possibilities which can be addressed by chemical modifications are highlighted and displayed by selected examples. This will include the modification of saturated and unsaturated PHAs by generating:

1. Block copolymers based on PHAs
2. Graft copolymers based on mcl PHAs
3. Graft copolymers based on scl PHAs
4. Networks based on PHAs

The talk will cover the block copolymer synthesis strategies for medical applications such as drug eluting stents based on saturated PHAs. For the unsaturated PHAs, examples for graft copolymers based on mcl PHAs will be given in the field of medical applications and beyond. In that context, efficiency of click-chemistry for various applications will be highlighted and demonstrated. For graft copolymers based on scl PHAs electrospun functionalized scaffolds for tissue engineering will be presented and compared to other methodologies. Finally, the various possibilities for networks based on PHAs will be highlighted to present their possibilities for specific improvements on antimicrobial or (thermo)mechanical properties. This Key-note lecture will summarize the latest insights in the potential of chemical modification of PHAs on a comprehensive level.

## Combination of time-resolved molecular spectroscopy and advanced morphological analysis in revealing the mechanism of PHA plasticization *in vivo*

D2.T12-1

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Although the mechanism that keeps polyhydroxyalkanoates (PHA) amorphous in the cells of their microbial producers has been extensively debated in the last decades, there is still lack of a conclusive experimental justification of proposed molecular and physico-chemical explanations of this phenomenon. In our recent work, we have contributed to this experimental effort with a systematic study of crystallization of polyhydroxybutyrate in model PHA-producing bacterium (*Cupriavidus necator*) induced by an exposure of the cells to various stress factors (elevated temperature, cyclic freezing and thawing, high osmolality, low pH). In this study, we have implemented a powerful combination of spectroscopic (time-resolved ATR-FTIR, Raman), morphological (TEM, cryoSEM) and biological (viability assays) methods in order to consider relationships between changes in the PHA state and other impacts of the stress-exposure, such as an alteration of cell ultrastructure (e.g. coagulation of PHA granules), denaturation of PHA-associated proteins and a general loss of cell viability and reproducibility. Results of this study indicate that the amorphous state of the polymer is stabilized kinetically by the low rate of crystallization in limited volume in small PHA granules and, moreover, water present in PHA granules seems to function as plasticizer protecting the polymer from crystallization. Apart from these particular findings, the study also clearly illustrates that the proposed methodology can serve as an invaluable experimental tool in any studies focused on bacterial stress response.

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## High performance PLA for injection molding applications

D2.T12-2

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A new development of a flame-retardant injection molding material made of Poly(lactic acid) (PLA) with high heat and impact resistance for technical products is presented. The influence of organic and inorganic nucleating agents on the crystallization behavior was tested under different isothermal and variothermal cooling conditions. The influence of organic and mineral flame-retardants on the flammability of PLA compounds was evaluated through UL-94 tests. A core-shell copolymer was used to increase the impact resistance of the PLA. The impact modified PLA was tested in combination with the flame-retardants and the nucleating agents to determine the influence of the different combinations of additives on the material properties. Dynamic scanning calorimetry (DSC) was performed to evaluate the crystallization behavior of nucleated PLA. These compounds were injected under isothermal and under controlled cooling conditions using a switching unit for variothermal control. Tensile tests and heat deflection temperature (HDT) tests were performed to evaluate the influence of the cooling conditions on the mechanical properties. By adding nucleating agents and isothermal cooling with mold temperatures close to the crystallization temperature, the crystallization degree is improved, leading to a significant enhancement of the HDT to values over 140°C. The cycle time could be reduced by 34 % using variothermal control with the same resulting HDT as with the isothermal cooling at mold temperatures close to the crystallization temperature. The crystallization behavior of the organic nucleating agent is regarded to be instantaneous, while for the inorganic nucleating agent it is sporadic, according to the Avrami exponents calculated (2.61 and 3.47 respectively), . According to DSC, the addition of the impact modifier or the mineral flame retardant does not affect the crystallization behavior of the samples, while the organic flame retardant supports the crystallization. The addition of the impact modifier leads to Charpy impact strengths of over 100 kJ/m<sup>2</sup> for unnotched and 10 kJ/m<sup>2</sup> for notched specimen.

From these results, it can be concluded that PLA for demanding technical applications could can be obtained making use of the synergistic effect of the material formulation, with appropriate impact modifier, flame retardant and nucleating agent addition and performing the injection molding under well-defined cooling conditions.

## Sustainable coated PLA nonwovens for upholstery

D2.T13-1

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In this presentation the two research partners will show the creation of a visually appealing, extremely flame-resistant surface material consisting of PLA fine fiber nonwovens with silicone coated surface. For this purpose, high-temperature PLA nonwovens are coated with several, thin layers of silicone including a foamed silicone base coat and finished with high-end silicone top coat. This new material combination will satisfy highest fire safety requirements avoiding flame retardants according to DIN EN 4102-1 B 2 as well as high resistance against disinfectants based on EN 423.

Furthermore, the material compound shows very low levels of dirt and high aging resistance and offers high utility value in terms of comfort, haptics and insulation effect and reveals sustainable and environmentally friendly characteristics. Therefore, this new product will make a significant contribution to advancing the use of resource-efficient and sustainable products in compliance with legal safety standards according to mission-specific fire protection regulations.

## Utilization of by-products from sugar beet processing for the production of poly(hydroxyalkanoates)

D2.T13-2

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About 17 million t of beet sugar are produced annually in the European Union. After the termination of the sugar quota system in 2017, the industry is showing increased interest in the development of high value products derived from sugar beet processing by-products. The goal of the nationally funded ValorPlast project was to evaluate the potential of desugarized sugar beet molasses and acidified sugar beet pulp for the production of poly(hydroxyalkanoates).

*Bacillus megaterium* uyuni S29 is a halophilic and fast growing strain that is able to grow on desugarized sugar beet molasses without additional nutrient supply. Concentrations of up to 18 g/L cell dry mass containing 60 % P(3HB) were obtained in lab-scale batch experiments. A fed-batch approach resulted in increased biomass formation with 46 g/L cell dry mass (50 % P(3HB)). Scale-up to 500 L batch production yielded a total amount of 6.8 kg cell dry mass containing 4.0 kg P(3HB). Desugarized sugar beet molasses appears to be a suitable substrate to establish a simple and robust process for the cultivation of *B. megaterium* and the production of P(3HB).

Sugar beet pulp was converted into volatile fatty acids (VFA) in a mixed culture fermentation, using a 300 L CSTR at mesophilic conditions, pH 6.0 and a hydraulic retention time of 10 d. The acidification process resulted in a mixture of acetic, butyric, propionic and valeric acid with a total VFA concentration of 24.4 g/L. The VFA-mixture served as a carbon source for the production of mcl-PHA with strain *Pseudomonas citronellolis* DSMZ 50332. In order to overcome inhibition problems and enable a constant carbon supply, the VFA-feed was coupled with pH control. In lab-scale, this method yielded maximum concentrations of 14.8 g/L for cell dry mass with a mcl-PHA content of 21 %. The mcl-PHA produced was composed of 1.5 % poly(hydroxyhexanoate), 37.5 % poly(hydroxyoctanoate), and 61 % poly(hydroxydecanoate). We performed two 600 L fermentations based on a defined medium with acetic acid as a carbon source in order to assess the scalability of the process and to obtain larger amounts of polymer for characterization. In total, 18.1 kg cell dry mass containing 4.1 kg mcl-PHA were harvested. The results demonstrate that by-products from the sugar industry are suitable for the production of PHA. In the short term, desugarized beet molasses appears to be more promising, while the utilization of acidified sugar beet pulp still requires optimization.

## Water-soluble poly(3-hydroxyalkanoate) sulfonate: versatile biomaterials used as coating for highly porous nano-MOF

D2.T14-1

Audrey Roy<sup>1</sup>, Caroline Jain-Beuguel<sup>1</sup>, Christelle Simon-Colin<sup>2</sup>, Ruxandra Gref<sup>3</sup>, Estelle Renard<sup>1</sup>, Valérie Langlois<sup>1</sup>

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The objective here was to propose a versatile biodegradable and biocompatible polymeric coating for highly porous nanosized metal organic frameworks (nanoMOFs). NanoMOFs, formed by strong coordination between metal ions and organic ligands, recently emerged in the world of therapeutic nanovectors. These materials offer a particular hydrophilic/hydrophobic microenvironment inside their open porosity, allowing the incorporation of a large variety of either water-soluble or hydrophobic active molecules, by simple one-step impregnation, generally without organic solvent.

In this context, water soluble poly(3-hydroxyalkanoate) containing ionic groups was designed by two successive photo-activated thiol-ene reactions. Sodium-3-mercaptopropyl-ethanesulfonate and poly(ethylene glycol) methyl ether thiol were grafted onto poly(3-hydroxyoctanoate-co-3-hydroxyundecenoate) to both introduce ionic groups and hydrophilic moieties. The grafted copolymers  $\text{PHO}_{(67)}\text{SO}_3^{2-}_{(20)}\text{PEG}_{(13)}$  were used as biocompatible coating of metal-organic frameworks nano-MOF surface. We here developed a new hybrid system, composed of nanoporous MOF coated by a water-soluble PEGylated PHA containing sulfonate groups that are able to strongly interact with nanoMOF. Then, MOF-PHA hybrid nanoparticles were synthesized by a rapid one-step non-covalent method and their structure was investigated by complementary physicochemical methods. The coated nano-MOF are stable in aqueous and physiological fluids and cell proliferation and cytotoxicity tests performed on murine macrophages J774.A1 revealed no toxic side effect. Thus, biocompatibility and stability of these novel hybrid porous MOF structures encourage their use in the development of effective therapeutic nanoparticles.

## From engineering to technical application – Understanding which technical data are required for the successful development of a bio-based technical product

D2.T14-2

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In order to limit their dependence on fossil resources and to lower their carbon footprint, more and more companies are considering switching to bioplastics. With this presentation, we would like to explain which technical steps an engineering company faces in order to achieve the successful development of a bio-based technical plastic part. One of the key points is the characterization of the bioplastic material and this presentation will explain why. Our methodology consists of the following steps:

- 1) Process simulation
- 2) Definition of the load case
- 3) Mapping and
- 4) Nonlinear FEM-simulation.

For each of these stages, data specifically related to the bioplastic used are essential. We have classified these data into three distinct groups:

- A) Process data: rheological, thermal, pVT and shrinkage data necessary for an optimal rheological simulation.
- B) Load case data: constraints, boundary conditions and connectors necessary to define the load case.
- C) Non-linear structural mechanical simulation data: tension and compression stress-strain data allowing a realistic non-linear mechanical simulation.

Using examples from various projects, we would like to highlight the crucial data relating to a bioplastic in order to obtain reliable results. We will also discuss about how to obtain this information and specify the problems related to the lack of key material data. This presentation is intended for bioplastics producers, companies wishing to switch to bio-based plastics, research institutes in the field of bio-based materials and plastic engineers involved in the development of bioplastic parts.



## New photoinduced bio-based coatings for antibacterial applications

D2.T15-1

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Infections by pathogenic microorganisms are of great concern in many fields, particularly in medical devices, hospital surfaces/furniture, and surgery equipment's. Approximately 64 % of hospital-acquired infections worldwide are due to the attachment and the proliferation of bacteria to medical implants, and they are associated with an annual mortality of 100,000 persons in the US as well as an increase in health-care costs. To solve the problem of increasing resistance of bacteria toward antibiotics, much attention has been focused on developing new antimicrobial systems in biomedical industry. Despite the potentially daunting complexity of bacteria populations, new chemical strategies should be developed to limit and prevent the bacterial colonization of materials surfaces. This presentation highlights the synthesis of new antibacterial coatings by innovative photochemical process for preventing the bacterial colonization (particularly against *Escherichia coli* and *Staphylococcus aureus*) on materials surfaces.

The novelty of our researches rests on a new chemical approach which sets out to reduce the amount of energy required for chemical synthesis of the coatings, eliminate the dispersion of harmful chemicals in the environment as the coatings are synthesized without any solvents, maximise the use of renewable resources for developing low cost coatings presenting long-time antibacterial properties.

Therefore, we demonstrate the development of new methodologies for the production of new hybrid coatings which can be conceived and tested at short time according to the "green chemistry" process with:

The use of photochemistry method (cationic or free-radical photopolymerization) at room temperature;

The use of industrially produced epoxidized oils (EVOs) or newly epoxy/vinyl bio-based monomers derived from low cost raw materials such as glycerol, chitosan or polyphenolic compounds.

The use of natural dyes (curcumin, paprika or quercetin) used both as photosensitizers for the initiation of the polymerization and as reactive oxygen species promoter for the synthesis of permanent antibacterial coatings.

Such new coatings could successfully be used to avoid bacteria proliferation; they have demonstrated a tremendous inhibition by more than 99 % of the adherence of *Staphylococcus aureus* or *Escherichia coli* and could be deposited onto disposable paramedical devices like clamp or scalpel that made for few hours single use.

## Technical requirements for biopolymers – from an industrial point of view

D2.T15-2

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The use of biogenic fibers for the production of semi-finished thermoplastic products such as granules, profiles, films etc. has become a versatile and sustainable way to tune several key properties of the final products in terms of mechanical properties, optical properties, biodegradability, design aspects and biobased origin. In this contribution we want to give general informations on the requirements for the industrial use of thermoplastic biopolymers and on their use in fiber-reinforced composites in particular.

The use of biopolyesters in thermoplastic formulations for various customer goods and packaging materials is often limited due to their propensities towards hydrolysis during processing operations (hydrodegradation). This can be overcome by means of end-capping strategies during a reactive compounding step, which render the material stable against hydrolysis. However, this step limits the performance of the biomaterials in terms of enzymatic attack (enzymatic hydrolysis). We want to shed light onto the challenges of polyester stabilization and alternative biopolymers with much lower propensity for hydrodegradation which possess benign (microbial) biodegradability performance. However, these materials possess technological limitations in terms of cycle times and mechanical stability.

Among the most decisive requirements for the production of bioplastics are related to their thermal stabilities. In case of several biopolyesters such as some members from the PHA-family the thermal degradation of the polymer main chain is very close to the melting/plastification point. We report on this issue from the perspective of a compounder of fiber-reinforced plastics where high shear and energy forced are encountered during the compounding and subsequent plastic processing steps such as injection molding to semi-finished and finished products.

With a newly developed product launched in 2018 (BPM® Eco-Spacer®) we present a product novelty in that the granules (typically used for manufacturing processes in the plastics industry, like injection molding) are used as biobased spacers for the transport of concrete goods.

Materials for the horticultural sector are currently dominated by polyolefines for the production of plant pot and seedling trays. These materials are not only petroleum derived but they also don't biodegrade in a considerable time span. We report on a development for substituting conventional materials for the horticultural sector.

## Effect of biodegradation on physical properties of PLA-based blends

D2.T16-1

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We investigated degradation of three polymeric materials in compost, namely virgin polylactic acid (PLA), PLA with a plasticizer glycerol triacetate (TAC), and a mixture of PLA and polyhydroxy butyrate (PHB) containing also TAC, at temperature 58°C for various times up to 16 days and physical properties were measured. The biodegradation degree was determined by measuring the content of evolved carbon dioxide within time period up to 133 days. The degradation rate varied a little for the three samples, presumably due to faster biodegradation of TAC and PHB compared to PLA. The degradation test was performed for virgin PLA also at 25 and 37°C. The biodegradation of PLAs was substantially slower compared to 58°C. Obviously, the key factor for the rate of degradation is the glass transition temperature of PLA, being around 55°C. Therefore, the temperature 58°C was chosen for all samples tested for changes in physical properties.

Physical properties after degradation could be measured only for the first 8 days (the shape of testing specimens intact) or up to 16 days when it was possible to separate the fragments of the material from the compost before the materials have been disintegrated to too small fragments.

Rather complex design of testing methods was made. Determination of changes in molecular weight and molecular weight distribution was made by GPC, supported by measuring the viscosity by rheology. Changes in structure of the materials were determined from changes in T<sub>g</sub> and crystallinity determined by DSC. Mechanical properties of samples after certain time of composting were measured and the data were compared with information obtained from dynamic mechanical analysis (DMTA). The latter method provided also exact data on changes in glass transition temperatures during composting. For estimation of chemical changes, FTIR was applied.

From the courses of changes the conclusions have been made regarding the TAC and PHB presence on the biodegradation of PLA, and changes concerning the structure/mechanical relations.

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## Hydrogels with cyclodextrin-grafted alginates for hydrophobic drug release

D2.T16-2

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Alginates are linear polysaccharides found in brown seaweed and in certain bacterial species. Alginate forms gels in the presence of divalent ions under physiological conditions, rendering alginate an attractive material for cell encapsulation and tissue engineering applications. However, the use of alginate for the encapsulation of drugs has been limited by the rapid diffusion of small molecules out of the gel as well as limitations with solubility in the aqueous phase. We have grafted cyclodextrin (CyD) to alginate to allow the incorporation and slow release of hydrophobic drugs.  $\beta$ -CyD was grafted to alginate in a three-step synthesis using periodate oxidation, reductive amination and copper (I)-catalyzed azide-alkyne cycloaddition. A grafting degree of 4.7 % mol  $\beta$ -CyD/mol sugar residues was obtained and a proof-of-concept study showed that Ca-alginate gel beads with  $\beta$ -CyD-grafted alginate gave a longer and increased release of the model molecule methyl orange. Further, the grafted materials showed reduced gel stiffness (Young's modulus), depending on the degree of oxidation as well as the in mixed gels with non-grafted alginate. The grafted alginate leaked to some extent out of the gels, however, no toxic effects on cells exposed to the leaked material or gels were observed. Paclitaxel was shown to be solubilized in the gels with grafted materials and *in vitro* studies showed toxic effects of the released drug. Hence, the CyD grafted alginate shows potential within drug delivery, as the alginate provides a good delivery vehicle, while the grafted cyclodextrins give increased solubility and slow release of hydrophobic drugs.

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## (+)-3-carene: A versatile precursor for high performance bio-polyamides

D2.T17-1

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A new and facile synthesis for a  $\epsilon$ -lactam derived from the monoterpene (+)-3-carene and the subsequent anionic ring opening polymerization thereof to bio-polyamides is reported. The lactam can selectively be prepared in two diastereomeric configurations which remarkably differ in their polymeric form. Various polymerization conditions regarding activator concentration, reaction temperature and time were tested. The polymers were investigated in terms of structure, molecular weight and thermal properties. Depending on the configuration, the corresponding polyamide either possesses a glass transition at 115°C and a melting point at about 280°C, which is typical for high-performance semi-crystalline polyamides, or only glass transition (120°C), indicating a high level of amorphicity and transparency. The new bio-based lactam is also suitable for copolymerization with commercial caprolactam (PA6) and laurolactam (PA12) and the properties of these co-polyamides exhibit an increased glass transition accompanied by an adjustable degree of amorphicity which possibly broadens the scope of applications for these polyamides. Preparation of the monomer is optimized, resulting in a sustainable four-step one-vessel process with an overall yield of 25 % under application of chemo-enzymatic catalysis for the initial oxidation step. These findings support the establishment of biogenic resources as alternative to fossil oil in the field of polyamides with new promising properties. (*PCT patent filling, paper in preparation*).

## Development of chitin-glucan complex – based polymeric structures using biocompatible ionic liquids

D2.T17-2

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Chitin – Glucan Complex (CGC) is a polysaccharide composed of chitin covalently linked to  $\beta$ -glucans, which has been described as a biocompatible and biodegradable biopolymer, combining antioxidant, antimicrobial and anti-inflammatory properties. These properties makes CGC suitable for a variety of applications that include cosmetics, food and biomedicine<sup>[1]</sup>. However, due to the high number of inter and intra-molecular hydrogen bonds of the macromolecule, CGC is only soluble in corrosive and/or toxic solvents. Ionic liquids (ILs) have been used as alternative solvents for the dissolution of natural biopolymers similar to CGC, such as cellulose and chitin<sup>[2,3]</sup>.

This work aimed at the preparation and characterization of biopolymeric structures based on CGC using biocompatible ILs as solvents. CGC was extrated from *Komagataella pastoris* cell-wall using a hot alkaline treatment. For the dissolution of CGC, three biocompatible cholinium-based ILs namely, choline acetate, choline propionate and choline hexanoate, were used. All ionic liquids successfully dissolved CGC up to a concentration of 5wt. %. Upon applying the phase inversion method, films and hydrogels, depending on the glucan:chitin ratio, were obtained. The CGC films thus obtained possessed good mechanical properties, with decreasing hydrophilicity as a function of increasing IL alkyl chain length. Regarding CGC hydrogels, independently of the IL used for the dissolution, showed a behavior characteristic of strong gels.

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## An innovative and sustainable approach of polyurethane bio-recycling

D2.T18-1

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This year, the International Union of Pure and Applied Chemistry (IUPAC) published a list of ten emerging technologies with the potential to make our planet more sustainable. Among them, technologies allowing for the transformation of plastic material into monomers were highlighted. These recycling technologies allow for limiting plastic wastes with environmental impacts and saving of fossil resources.

Polyurethanes (PUs) rank as the 6th most produced plastic material. The biological depolymerization of PUs is a challenge to be addressed by the development of green recycling processes. For this purpose, both microbial and enzymatic degradation paths were recently studied in our laboratory. On one hand, three efficient strains of filamentous fungi able to degrade two polyester PUs were isolated from PU waste. The identification of degrading enzymes produced by the strain of *Aspergillus* revealed four enzymes as candidates for the hydrolysis of the highly resistant urethane linkage. Alternatively, a collection of hydrolases was screened on two model substrates, leading to the selection of an amidase able to cleave the urethane bond and an esterase able to hydrolyze polyester PU dispersion. Enzymatic activity was then evaluated on four thermoplastic PUs (TPUs). The highest activity was measured for the esterase on a polyester PU with 33 % weight loss after 51 days of incubation at 37°C. Deep cracks on the polymer surface and the presence of oligomers in the remaining TPU pieces revealed eminent polymer degradation. The main degradation product recovered and identified was the 6-hydroxycaproic acid. Mixing esterase and amidase also led to an efficient cleavage of polyester-based PU. New degradation products were detected revealing an efficient urethane bond hydrolysis. For the first time, the synergetic effect of two different enzymes for PUs depolymerization is shown. Monomers recovered after the enzymatic depolymerization can be used for the synthesis of a second-generation polymers in a circular and sustainable Biotech-Chem approach.

# Bacterial cellulose functionalization for producing biomaterials with anti-microbial capacity

D2.T18-2

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In a Circular Economy context, bacterial biopolymers are very attractive materials due to their biodegradability and non-toxicity. They can be produced using wastes as feedstocks in sustainable bioprocesses. We are interested in the production and functionalization of bacterial cellulose (BC) and poly([R]-3-hydroxyalkanoates) (PHAs). BC is being used as wound dressing device due to its characteristics, such as biocompatibility, non-toxicity, mechanical stability and high moisture content. However, it lacks antimicrobial activity which is a crucial function of the skin-barrier that is compromised during wound healing. Another candidate with potential applications in the medical field is the group of bacterial polyesters or PHAs, that constitute a large class of biodegradable biopolymers of minimal tissue toxicity. The physical and mechanical properties of PHAs are influenced by their monomer composition and chemical structure, which can be tailored by metabolic engineering<sup>[1]</sup>. We have developed two approaches to produce BC hydrogels with antimicrobial activity: i) functionalization of BC by the Cellulose Binding Domain from *Clostridium cellulovorans* (CBD<sub>clos</sub> tag) for the immobilization of enzybiotics<sup>[2]</sup> on BC-based devices with antimicrobial activity against *Staphylococcus aureus* and ii) chemical blending of BC with a naturally functionalized bacterial polyhydroxyalkanoate (PHACOS) with demonstrated antimicrobial properties against Methicillin-resistant *S. aureus* (MRSA)<sup>[1]</sup>. Crystalline BC produced from *Komagataeibacter medellinensis*<sup>[3]</sup> from acidic residues was applied to developed functionalized BC hydrogels by CBD<sub>clos</sub>-Enzybiotic fusion tags. The antimicrobial activity of EZB1-CBD-BC against *S. aureus* was demonstrated by an antimicrobial assay specifically designed for these materials. In the second approach, PHACOS was produced in the model bacterium *P. putida* KT2440 as previously described<sup>[1]</sup>. The antimicrobial activities of the BC, and the blended BC/PHA and BC/PHACOS hydrogels were tested showing a reduction in *S. aureus* viability in hydrogels containing PHACOS.

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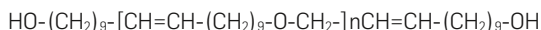
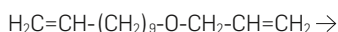
# Synthesis of ether-diols with low polarity from long-chained fatty alcohols for use in block copolymers

D2.T19-1

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Diol-terminated polyethers are important intermediates for the manufacturing of block copolymers, but only a few polyethers other than polyethylene glycols are available for block polyester synthesis on technical scale. Most of them are highly polar. The efficient synthesis of other kind of such ethers without using hazardous chemicals like alkyl bromine in Williamson's ether synthesis is a challenging field. In this work, we describe a sustainable method of synthesis for long chained diol-ethers by using fatty alcohols, from renewable resources. Natural fatty alcohols – converted to similar polyethers – should have a grossly reduced polarity because of a large separation of the oxygen atoms in the C18 chain. A synthesis sequence of polyethers from commercially available fatty alcohols was designed for a future examination of the hydrophobic properties. Palladium catalyzed cleavage of olefinic dialkyl carbonates results in carbon dioxide elimination and subsequent formation of ethers from an allyl-palladium cation. It could be shown that this process with fatty alcohols like undec-10-en-1-ol or oleyl alcohol can be run with appreciable yield.



Ruthenium catalyzed ADMET (acyclic diene metathesis) at both chain terminations then was applied to polymerize the ethers. Dependent on the alkenyl-chain, short oligomers with a DP (degree of polymerization) of about 5-8 seem to be formed according to GPC.

## Functional starch-based hydrogels: sustainable material solutions for environmental applications

D2.T19-2

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Hydrogels are a novel class of functional biopolymers with a unique set of properties such as high swelling capacity, flexibility, and biocompatibility. Their dynamic response to external stimuli differentiates them from other polymeric materials, and their tailorability makes them excellent candidates for the design of smart devices, applicable in a variety of technological fields. Natural polymers such as starch have gained considerable attention as an economic alternative to fossil-based feedstocks, and a sustainable source of materials for the production of hydrogels.

Starch-based hydrogels have recently been identified as having the potential to be successfully employed in a variety of unconventional applications, such as removal of pollutants from wastewater and delivery of agrochemicals. However, the large-scale production of starch hydrogels is currently limited due to the high cost and complexity of their manufacturing methods. Additionally, the undesirable by-products and toxic chemical residues can potentially alter the biodegradability of these hydrogels and negatively influence the life-cycle assessment of the products. In order to address these technical limitations, we have developed a practical and affordable processing method based on reactive extrusion. This method combines the benefits of continuous manufacturing with the use of green chemistry principles to produce functional starch hydrogels.

This study explores the mechanism of grafting starch with acrylic monomers using twin-screw extrusion technology to form starch-based hydrogels. The molecular structures of the products were characterized using NMR and FTIR spectroscopic techniques, as well as thermo-gravimetric and elemental analysis methods. The performance of these starch hydrogels for recovery of reactive nitrogen from water was also evaluated. We found that starch hydrogels prepared by the proposed synthesis method behave similarly to those prepared through conventional synthesis procedures. Our results also indicate that these starch hydrogels are suitable candidates for application as nutrient recovery systems.

# An urban bio-refinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas

D3.T2

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This study focuses on the application of the circular economy concept, where added-value marketable products and energy are created from organic waste and environmental impacts are minimized. Within this purpose, an urban bio-refinery has been developed at pilot scale in the territorial context of the Treviso municipality (northeast Italy) for the production of biopolymers (polyhydroxyalkanoates, PHA) and biogas from urban organic waste. The piloting system (100-400 L) comprised the following units: 1) acidogenic fermentation for volatile fatty acids (VFA) production; 2) two solid/liquid separation step by means of a centrifuge and a tubular ultrafiltration membrane; 3) Sequencing Batch Reactor (SBR) for PHA-storing biomass production; 4) fed-batch PHA accumulation reactor; 5) AD unit for biogas production from the residual bio-waste and excess waste sludge (WAS). The feedstock was a mixture of the organic fraction of municipal solid waste (OFMSW) and WAS (30-35 % and 65-70 % v/v, respectively). The acidogenic fermentation was conducted after a preliminary thermal pre-treatment (72°C, 48 h), in batch mode (5 days) under mesophilic conditions (37°C). The thermal pre-treatment favored a high substrate solubilization, enhancing VFA production, with an overall yield in the range of 0.41-0.44 g COD<sub>VFA</sub>/g VS<sub>red</sub>. A VFA-rich stream around 30 g COD<sub>VFA</sub>/L with high COD<sub>VFA</sub>/COD<sub>SOL</sub> ratio (0.84 ± 0.02) was obtained. This pivotal feature enhanced the PHA-storing biomass selection in SBR by reducing the growth of non-storing microbial population. Under fully aerobic feast-famine regime, the selection reactor was continuously operated for more than 8 months at OLR range 3.2-4.0 g COD/L d, and short HRT (1 day, equal to SRT). The selected biomass was able to accumulate up to 60 % g PHA/g VSS. An overall production yield of 107 g PHA/kg VS was estimated. The mesophilic AD process (HRT 13-14 days, OLR 2-2.5 kg VS/m<sup>3</sup> d) allowed recovering biogas from the solid-rich overflows with a specific gas production (SGP) of 0.4 m<sup>3</sup>/kg VS. Potentially, the scaled-up version (70,000 PE) of this bio-refinery may produce 114 tons PHA/year, 2314 m<sup>3</sup> biogas/d and 5.9 MWh/d of electrical energy. Compared to the actual AD scenario, this scheme can be equally profitable if the produced PHA is marketed at the minimum threshold of 0.82 €/kg. Hence, a higher and still reasonable market price can easily made this multi-step approach more attractive compared to the traditional anaerobic digestion process.

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## Biorefinery of grass silage for poly-3-hydroxybutyrate (PHB) fermentation

D3.T3

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Grass silage offers carbon sources as well as complex nutrients for synthesis of biopolymers by fermentation. Yet, it requires an efficient pre-treatment of the plant matter to make the structural polysaccharides available for fermentation.

A green biorefinery process for treatment of grass silage was established on the lab-scale. It consisted of pulping by screw-pressing and separation of the press juice in the first stage – a procedure which is well acknowledged for green biorefineries – and a high solid load mechano-enzymatic saccharification of the fibers in the second. The goal was to design a technically simple refining procedure suitable for small, decentralized units, to avoid excessive feedstock transport. The remaining solid fraction could be processed further, e.g. for lignin recovery or energy supply.

The nutrient-rich press juice exhibiting a narrow C:N-ratio of 11 proved to be suitable for growth of *Cupriavidus necator* DSM 531 without further supplements. In contrast, the hydrolysate from the enzymatic saccharification of the fibers showed a C:N-ratio of around 37, which was quite well in the range required for poly-3-hydroxybutyrate (PHB) accumulation. A fermentation set-up was established consisting of lactic acid fermentation of the hydrolysate in the first stage to convert saccharides – mainly glucose and xylose – to lactic and acetic acid. The subsequent PHB fed-batch fermentation with *C. necator* was performed using press juice for N-rich medium in the growth phase, while the product of the lactic acid fermentation was supplied as carbon source to the PHB accumulation phase applying pH – stat mode. Converting saccharides to organic acids prior to PHB fermentation enabled the conversion of hexoses and pentoses as well as of organic acids to PHB by *C. necator*. With cell recycling by centrifugation, up to 22 g/L cell dry matter containing up to 42 % PHB were achieved after 1.3 d of fermentation. Improvement of cell recycling and feed control techniques are expected to further enhance the yields.

## Polyhydroxyalkanoates production at pilot scale using fruit waste: enhancing feeding strategies and monitoring

D3.T4

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Polyhydroxyalkanoates (PHA) are polyesters which can be synthesized by bacteria and are completely biodegradable into water and CO<sub>2</sub>. They have a wide variety of properties which make them an interesting alternative to conventional plastics. However, a wider implementation of the production technology has been hindered due its costs and limited productivities. On the other hand, the industrial exploitation has still been restricted to processes involving pure cultures. Mixed microbial cultures (MMC) represent a low-cost alternative to the typical pure culture technology owing to the absence of sterilization and lower costs in aeration and feedstock. Typically, the MMC PHA production process consists in 3 stages: acidogenic fermentation of a feedstock into fermentative products (FP), selection of PHA-accumulating culture and PHA production in fed batch mode.

In this study, a pilot plant consisting in 3 reactors was assembled, inoculated and operated continuously using fruit waste as feedstock for the acidogenic step with the purpose of producing PHA. Specifically, a 100L upflow anaerobic blanket reactor was inoculated with granules from a full scale anaerobic digester to produce FP, which were used in the subsequent stages. In the second stage, a 100L sequential batch reactor (SBR) was inoculated with MMC from a local wastewater treatment plant aiming at the enrichment of the culture in PHA-accumulating microorganisms. This culture was selected based on a feast and famine regime and it was used to inoculate a 60L fed-batch reactor. In the latter stage, the culture selected in the continuous SBR was inoculated and fed with the FP produced in the first stage.

This work aimed at testing the impact of different feeding strategies, namely DO-stat, DO-based pulsewise and pH-stat, in PHA productivity of the fed-batch reactor. Moreover, accumulation of PHA was monitored by near infrared spectroscopy (NIR) and data was used to develop a model for online prediction of microbial PHA content.

## Hydrogels based on chitin-glucan complex: fabrication, characterization and cytotoxicity assay

D3.T7

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Chitin-glucan complex (CGC), a co-polymer extracted from the cell-wall of the yeast *Komagataella pastoris*, was used for the first time for the preparation of hydrogels. Four alkali solvent systems, NaOH and KOH solutions, either at 1 mol/L or 5 mol/L, were used for CGC dissolution using a freeze-thaw procedure (freezing at -20°C and thawing at room temperature; four cycles). The CGC solutions thus obtained were subjected to dialysis that induced the spontaneous gelation of the biopolymer, yielding translucent hydrogels with a yellowish coloration. Although all CGC hydrogels exhibited porous microstructures, high water content (above 97 %) and good mechanical properties, their morphology, viscoelastic properties and texture were influenced by the type of solvent system used for CGC dissolution, as well as by their ionic strength. The best results in terms of mechanical properties, hardness and cohesiveness were obtained for the Na-based CGC hydrogels prepared using NaOH 5 mol/L. Furthermore, all hydrogels were shown to be non-cytotoxic towards L929 fibroblasts and HaCaT keratinocytes. This study demonstrated the feasibility of preparing CGC hydrogels, using alkali solvent systems, yielding biocompatible polymeric structures whose properties render them promising biomaterials.

# From apple pulp waste to high-value biomaterials: development of PHA films for food and biomedical applications

D3.T8

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PHAs display interesting properties as an alternative to petrol-based thermoplastics, elastomers and latexes in several market niches. Amongst PHAs, short-chain-length PHA, specifically PHB, and medium-chain-length PHA (mcl-PHA) are well-known due to crystallinity and thermal as well as mechanical properties. Based on their ability to form films, their gas barrier properties and biodegradability, opens applications in the field of food, hygiene or pharmacy. To obtain PHA films with the desired properties, several approaches are developed, such as the modification of PHA via blending to obtain new polymeric films with more attractive thermal/mechanical behaviour<sup>[2]</sup>. In this work, PHB, mcl-PHA and blends thereof were produced using apple pulp waste from the fruit processing industry as feedstock in batch cultivation of *Cupriavidus necator* and *Pseudomonas citronellolis*. A co-cultivation of both bacteria was performed to yield natural PHA blends. *C. necator* produced PHB with a molecular weight of  $5.0 \times 10^5$  Da and a crystallinity degree of 43 %, with a melting and thermal degradation temperature of 179 and 293°C, respectively. *P. citronellolis* synthesized a mcl-PHA mainly composed of 3-hydroxydecanoate (68 %) and 3-hydroxyoctanoate (22 %), with a molecular weight of  $3.7 \times 10^5$  Da, and melting and thermal degradation temperatures of 53 and 296°C, respectively, and a crystallinity degree of 15 %<sup>[4]</sup>. Films of PHB, mcl-PHA and their blends, obtained by solution casting and solvent evaporation, were characterized regarding morphology, contact angle, mechanical properties and permeability to O<sub>2</sub> and CO<sub>2</sub>. The mcl-PHA films presented best results for O<sub>2</sub> and CO<sub>2</sub> permeability ( $1.1 \times 10^{-9}$  and  $5.3 \times 10^{-9}$  cm<sup>3</sup>.cm/cm<sup>2</sup>.s.cmHg, respectively)<sup>[4]</sup>. Mechanical tests revealed that these blends had higher deformation until break, while PHB had superior tensile strength. This study demonstrates the novel materials to be suitable for PHA based films of interesting features. Their flexible properties renders them for superior food and/or biomedical applications. They might be applied to commodity packaging products, high-value biomaterials in biomedical fields, and as well as dressing, scaffolds for regeneration of skin tissues.

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## *Halomonas* levan for cosmeceutical applications

D3.T9

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Age related skin changes are major concerns in worldwide for both cosmetic and pharmaceutical industries. There is an increased consumer demand on natural bioactive cosmetic ingredients derived from plant, marine and bacterial polysaccharides to maintain longevity and anti-ageing via mimicking natural growth factors and cytokines for biological metabolisms. Levan, hyaluronic acid, and bacterial cellulose are widely used as active ingredient in cosmetics. Levan is a biocompatible, film former, nontoxic, strongly adhesive, water soluble  $\beta$  (2 $\rightarrow$ 6)- linked most common fructan exopolysaccharide in nature and has many application areas from food, medicine, chemical industries, pharmacy to medicine. Levan used as skin moisturizer and whitener in cosmetic industry with its cell proliferating, skin moisturizing and irritation alleviating activities. *Halomonas* levan is an extremophilic organism originated polymer with various features like antioxidant, anticoagulant, anticancer, biocompatible and immunostimulatory effects and could be strong candidate for cosmetic applications. This study investigates the potential uses of *Halomonas* levan based formulations in cosmetics and hence effects of *Halomonas* levan on human dermal fibroblast and keratinocyte cells are investigated in detail *in vitro*. In the light of this study possible application areas of this polymer like wound healing, skin repair and antiaging will be understood to develop new biomaterials and cosmetic formulations.

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Biopolymers from non-natural monomers

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# Assessing PHA synthesis pathway by investigating the role of (*R*)-specific enoyl-coenzyme A Hydratases in *Pseudomonas putida* KT2440 based on CRISPR gene editing technology

P1.1

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Polyhydroxyalkanoates (PHA) are a family of biodegradable and biocompatible biopolymers produced by microorganisms from a range of carbon and energy sources. *Pseudomonas putida* KT 2440 is a well-known model organism for the medium chain length PHA accumulation. (*R*)-Specific enoyl-coenzyme A hydratase (PhaJ) plays an important role in providing monomer units for PHA synthesis by converting the  $\beta$ -oxidation intermediates, trans-2-enoyl-CoA to (*R*)-3-hydroxyacyl-CoA.<sup>[1]</sup> Three PhaJ homologues, named PhaJ1, PhaJ3 and PhaJ4 were annotated in *P. putida* KT2440.<sup>[2]</sup> To assess the role of each PhaJ in PHA biosynthesis in *P. putida* KT2440, a series of *P. putida* KT2440 knockouts with single-, double- and triple- *phaJ* deletions was obtained using CRISPR gene editing tool. Cell growth, PHA content and composition of monomer in WT and mutants under different growth conditions were analysed.

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# Adaptation of PHA producing bacteria *Cupriavidus necator* H16 and *Halomonas halophila* to biotechnologically relevant stressors

P1.2

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Polyhydroxyalkanoates (PHAs) are microbial polymers which could replace traditional petrochemical plastics due to their favorable properties such as biodegradability and biocompatibility. Moreover, properties of PHAs depend on monomer composition therefore materials for selected purpose could be produced. Application of approaches of evolutionary engineering could be used to obtain microorganisms producing PHAs with required properties. Method is useful, quite simple and effective for gaining microbial strains with suitable characteristics on phenotype level (e.g. more effective growth, better productivity, ability of utilization different sources of carbon etc.) with no requirement for knowledge of genetic characteristics of original wild-type strain. In different point of view evolutionary experiments are relatively time-consuming and screening of huge amount of evolved phenotypes is necessary. Selected microorganisms *Cupriavidus necator* H16 (CCM 3726) and *Halomonas halophila* (CCM 3662) have been exposed to several biotechnologically relevant stressors. *C. necator* H16 was exposed to presence of NaCl causing osmotic stress which represented environmental stress factor and to copper representing anthropogenic pollutant. Strain *H. halophila* was exposed to acetic and levulinic acid; components of hydrolysate of lignocellulosic biomass. Evolutionary experiments were provided using multiple serial transfers of cell cultures in Erlenmeyer flasks, always after 48 hours of cultivation. Basic screening accompanying every step included determination of optical density of cell cultures, gravimetric determination of dry cell weight, GC-FID analysis of PHAs in biomass and also determination of organic acids in supernatants of *H. halophila*. After more than 20 and 40 transfers, cultures were preserved for further characterization. Potential of PHAs accumulation, effectivity of utilization of organic acids, testing of robustness, changes in cell morphology, physico-chemical properties of cells and also changes in genome and transcriptome were determined within comparison of evolved strains with wild-type ones.

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# Production of Poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) by recombinant *Burkholderia sacchari* from xylose and fatty acids

P1.3

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The production of polyhydroxyalkanoates from hemicellulosic hydrolysates has been explored as a strategy to reduce production costs and effectively set up the application PHA as biomaterials. *Burkholderia sacchari*, a strain isolated from sugarcane crops in Brazil, is proposed as a biological platform for biotechnological production of PHA, as a producer of diverse hydroxyacyl-CoA monomers from a wide range of carbon sources. Aiming to enhance xylose consumption rates and to produce a polymer composed of 3HB and 3HHx, in the present work we applied the overexpression of a pentose-phosphate-pathway related gene, namely *tktA<sub>BS</sub>*. *tktA<sub>BS</sub>* gene was expressed along with two *Aeromonas* spp. PHA biosynthesis genes, namely *phaPCJ<sub>Ah</sub>* or *phaPCJTSM81*. Sequences were cloned into pBBR1MCS-2 vector and then expressed in PHA-negative *B. sacchari* mutant LFM344. Maximum specific growth rate ( $\mu_{\max}$ ) on xylose was evaluated in plate-reader experiments. Recombinants expressing *tktA<sub>BS</sub>* achieved a  $\mu_{\max}$  of 0.22 h<sup>-1</sup>, the highest induction-independent growth rate on xylose reported for *B. sacchari*. The ability of recombinants harbouring pBBR1MCS-2::*phaPCJ<sub>Ah</sub>*+*tktA<sub>BS</sub>* or pBBR1MCS-2::*phaPCJTSM81*+*tktA<sub>BS</sub>* to produced PHA was evaluated in shaken flasks experiments. Xylose was supplied for growth, followed by the co-feeding of hexanoate (analytical grade) or a mixture of medium chain fatty acids (MCFA), obtained in a mixed microbial fermentation. After 72 hours of cultivation, interesting amounts of P(3HB-co-3HHx) were produced, with considerable 3HHx molar fraction (around 10 mol %). This is the first report of a recombinant overexpressing *tktA<sub>BS</sub>* gene along with *phaPCJ<sub>Aspp</sub>* genes in a PHA production context. This work may contribute to the production of value-added-polyhydroxyalkanoates from waste biomass, and to future advances in biotechnological cost-reduced production of this promising biomaterial.

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## Thermophilic bacteria isolated from compost as producers of polyhydroxyalkanoates

P1.4

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Polyhydroxyalkanoates (PHA) are microbial polyesters produced by many bacteria. PHAs are entirely biodegradable, biocompatible and renewable good alternatives to petrochemical polymers. However, the production costs of PHA are higher than the petrochemical plastics. Two synergistic strategies to reduce the economic costs are the utilization of wastes as feedstock and the use of extremophilic producers.

Extremophilic microorganisms are organisms which live and reproduce under an extreme condition which can be physical (high pressure, low or high temperature) or chemical (low or high pH, salinity). Such conditions are crucial for reducing the cost of the bioprocess because the biotechnological process can be operated in semi- or non-sterile conditions. Sources of extremophilic bacteria can be hot springs, salt lakes, ocean deep or mining water but they are also in compost or activated sludge.

An original isolation protocol was designed for isolating the PHA accumulating bacteria from compost. It is based on the application of osmotic challenge for the selection of PHA producing bacteria since PHA granules protect bacteria against the adverse effect of osmotic stress. We also employed thermophilic conditions to isolate thermophilic PHA producers which were further tested and evaluated in terms of their PHA production ability. Growing and producing conditions were optimized (e.g. different carbon and nitrogen sources, cultivation temperature and precursors). The most promising thermophilic isolated was capable to produce up to 60 % of poly(3-hydroxybutyrate). Furthermore, it was also capable of incorporating 3-hydroxyvalerate and 4-hydroxybutyrate units into the polymer structure when the culture was supplied with the proper precursors. In addition, the most promising strain was characterized by molecular techniques and the expression of genes encoding enzymes involved in PHA metabolism was monitored under PHA production conditions.

## Designer proteins – protein based binders

P2.1

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The aim of the DESIGNER project is to design proteins that can be used as binders in several processes. This could be a promising bio-based alternative since the majority of binders still contain formaldehyde. That applies especially for manufacturing of particle boards and sand constructions. Formaldehyde-based binders are very efficient but since the re-classification of formaldehyde as carcinogenic compound an important goal is to prevent its use and exposure. As bio-based substitutes, proteins are under investigation for this approach for a long time already.

The benefits of proteins are their degradable and non-toxic nature and their functional diversity as well as their strong specific adhesion. However, drawbacks of natural proteins are i.e. their swelling and smelling properties. Avoiding these drawbacks and based on the general principle of a "good glue" the approach addressed here is the combination of adhesive and cohesive protein sequences to meet best binding characteristics. The protein building blocks for the design are inspired by natural proteins. The adhesive sequences are derived from marine organisms such as mussels, barnacles & sandcastle worms. The cohesive blocks contain collagen-like, elastin-like and resilin-like sequences as well as other cohesive domains from structural proteins. The combination of these different building blocks enables the formation of a large design space with various protein properties.

Regarding these unique protein properties as well as the establishment of a cost-effective production process another part of the project is the development of a platform of different production hosts such as bacterial and yeast strains and the optimization of their cultivation from shake flask to bioreactor scale. Subsequently, the downstream process for the respective designer protein is developed and optimized. The functionality and adhesive properties will be investigated with a quartz crystal microbalance (QCM) as well as measurements with a tensile strength testing machine (Zwick) under various conditions.

Currently, we are working on different combinations of six adhesive and five cohesive blocks connected with two different linker sequences and four tags for purification, detection or functionalization. We are convinced that the idea of function-related combination of protein sequences to create new designer protein based binders is a promising approach for future technologies.

# Oxidized low density polyethylene waste; a sustainable substrate for the production of polyhydroxyalkanoates

P3.1

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With the rapid increase in the production of petroleum-based plastics and the resulting accumulation of non-biodegradable plastic waste, there has been an alarming damage caused to the natural environment particularly litter leakage into the oceans that remains a major global concern. As at 2015, 6.3 billion metric tons of plastic waste had been generated by humans, of which only 9 % was recycled<sup>[1]</sup>. The quest to curb these problematic plastics and maintain a clean environment is on a high demand<sup>[1]</sup>. To this end, biodegradable bioplastics are viewed as potential replacement to petroleum based plastics. Among these problematic plastic wastes is low density polyethylene (LDPE) commonly used for bin and laundry bags<sup>[2]</sup>. LDPE waste is proposed in this study as a suitable additional carbon source for the production of bacterial polyhydroxyalkanoates (PHA); a biodegradable, eco-friendly and biocompatible bioplastic. *Cupriavidus necator*; a genetically stable and well known strain for the intracellular accumulation of PHA was employed<sup>[3,4]</sup>. In this study, *C. necator* was grown at 30°C for 48 h in trypton soy broth (TSB) supplemented with oxidized low density PE (LDPE) as additional carbon source. The addition of oxidized LDPE yielded 29 % of PHA (Cell dry weight) as opposed to 0.6 % in cultures without oxidized LDPE. To determine the molecular and chemical structure of the PHA obtained with oxidized LDPE, the polymer was further characterized with gel permeation chromatography (GPC), nuclear magnetic resonance (NMR) and electrospray ionisation tandem mass spectrometry (ESI-MS/MS). GPC analysis revealed the average molar mass of the PHA obtained to be 624 000 g/mol. NMR and ESI-MS/MS showed the presence of 3-hydroxybutyrate (3-HB), 3-hydroxyvalerates (3-HV) and 3-hydroxyhexanoate co-monomer units randomly distributed along the PHA oligomer chain. These co-monomer units of PHA synthesized by *C. necator* can be further used for various industrial applications and the production of PHA oligomers with valuable functional groups.

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## Microbial consortia inside polyhydroxyalkanoates process: selection of PHA-storing in bioreactor and PHA-degrading microorganisms in heavy metal contaminated soil

P3.2

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Polyhydroxyalkanoates (PHA) are biopolymers that arise as eco-friendly and biodegradable plastics with replacement potential of the petrochemical-derived. PHA production costs have been decreased by using microbial mixed cultures (MMC). Also, biodegradation of PHA is performed by microorganisms, the type of soil is an essential factor affecting PHA degradation. The aims were (1) to study the microbial community contained in a batch reactor (SBR) using microbial mixed cultures for polyhydroxybutyrate (PHB) production and (2) to analyze the degradation pattern of PHB in heavy metal (HM) contaminated soil. The SBR was operated in order to select the PHB storing population and for quantifying PHB. The active and PHB-storing populations were analyzed by flow cytometry with Redox Sensor Green and Nile red stains, respectively. Besides, that population was selected by cell sorting and DNA analysis were done. The reactor evolution showed physiology changes along 120 operation cycles (OC) with a final PHB content of 26 % (dw). There were detected an active population with capability to store PHB at 76 OC and it represented a 9 % from the total population (11 % PHB dw). This population increased along the time reaching 34 % of total biomass at 100 OC and 40 % at 120 OC. The active and PHB-storing population was selected by cell sorting and 16s RNA studies were done with the purpose to identify the involved species (in process). It was confirmed that the selective pressure created by the Feast/Famine mode played a dominant role during the OC, which drove the PHB-storing microorganism to become the population with the highest metabolic activity and the dominant population inside the reactor.

In another hand, PHB films of 20x10x2 mm were buried into heavy metal contaminated soil (2-3 mg/kg for Hg). The polymer films were incubated in the soil for 42 days, and degradation analysis was done every 14d. A decrease in average molecular weight and glass temperature were detected after 28 d, but there were not detected changes in crystallinity temperature, even after 6 weeks. The isolation of PHA degraders was done encapsulating soil with PHB films (in process). PHA biodegradation is performed by microorganisms, the soil is the natural environment with the greatest capacity for PHA degradation, but high concentrations of heavy metals in soil have an adverse effect on microorganisms and microbial processes, even so, degradation was detected.

## PHA production from volatile fatty acids with *Bacillus megaterium*

P3.3

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Biobased plastics are an emerging market that depends on finding cheap raw materials for sustainable production. Volatile fatty acids (VFA) have potential as a cheap platform chemical for biopolymer synthesis given that they can be produced from a variety of waste streams.

We have investigated ability of *Bacillus megaterium* uyuni S29 (CECT 7922) to produce PHB from volatile fatty acids. The strain's capabilities were tested on both, fully synthetic media with acetic acid as the sole carbon source, as well as media with various VFA obtained from acidified waste materials. Experiments were conducted with a pH-stat fed-batch feeding regime with 5M acid as feed/pH control. In the case of media from acidification process, we supplied additional VFA from commercial suppliers to reach the concentration in the feed needed for a viable fed-batch process. Nitrogen limitation was used to induce polymer production. Optical density and pH were used as monitoring parameters for the process during growth. After cultivation, we determined cell dry weight, PHA content by GC-FID, as well as the concentration of nitrogen (TKN) and VFA (HPLC) in the substrate.

*B. megaterium* managed to grow and produce PHB on acetic acid, reaching a biomass concentration of up to 10.2 g/l cell dry weight (CDW) and a PHB content of 0.56 g/g CDW. We could also show that this strain is able to utilize a fatty acids mix from an acidification process; in that case it produced 4.9 g/l of CDW with 0.14 g/g CDW of PHBHV.

The diluted nature of the renewable feedstock represents a challenge for the bioprocess development. For a solution we suggest a pH-stat process with a membrane retention system that retains the biomass in the reactor while allowing diluted substrate to pass through the system.

## High rate selection of PHA accumulating mixed cultures in Sequencing Batch Reactors with uncoupled carbon and nitrogen feeding

P3.4

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Polyhydroxyalkanoates (PHA) are completely biodegradable polyesters and it is well known the ability of mixed microbial cultures (MMC) to produce them by using renewable resources (e.g. waste organic streams) as feedstock. MMC-PHA production typically involves a multi-stage process including the selection of PHA-storing micro-organisms from the mixed culture. This is typically carried out in sequencing batch reactors (SBR) operated under feast-famine regime. With a nitrogen-deficient carbon source to be used as feedstock (such as paper mill and olive oil mill wastewaters) for PHA synthesis, a nutrient supply in the SBR is required for an efficient microbial growth. Along this line, research has been currently focusing on the investigation of the optimal strategies for nitrogen supply. In a previous study <sup>[1]</sup>, an uncoupled carbon (C) and nitrogen (N) feeding strategy was adopted by dosing the C-source (a synthetic mixture of acetic and propionic acids) at the beginning of the feast and the N-source (ammonium sulphate) at the beginning of the famine in a lab-scale SBR, operated with a cycle length of 6h and at an overall organic load rate (OLR) of 8.5 gCOD/L d, corresponding to 2.125 gCOD/L cycle. As a main result, it was found that PHA production was more than doubled (up to about  $1300 \pm 64$  mgCOD/L) in comparison with the coupled feeding strategy.

According to these results, in the present study <sup>[2]</sup> the effect of both the SBR cycle length and the applied OLR has been investigated with the uncoupled feeding strategy. More in detail, three SBR runs have been conducted with a 12 h of cycle length and the applied OLR increased from 4.25 to 8.5 and finally to 12.75 g COD/L d, at fixed C/N ratio of 33.4 g COD/g N. A more efficient selective pressure was maintained at lower and intermediate OLR, where the feast phase length was shorter (around 20 % of the whole cycle length). However, at the higher OLR investigated, the PHA content in the biomass reached the value of 0.53 g PHA/g VSS at the end of the feast phase, as consequence of the increased C-source loaded per cycle. Moreover, the 2nd stage PHA productivity was 2.4 g PHA/L d, 1.5 and 3.0 fold higher than those obtained at lower OLR. In this sense, the work highlights the possibility to simplify the process by withdrawing the biomass at the end of the feast phase directly to downstream processing, with no need for the intermediate accumulation step.

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## Moderate thermophilic production of P(HB-co-HV) copolymers by a mixed microbial culture

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Polyhydroxyalkanoates (PHAs) are bio-based polyesters completely biodegradable and biocompatible that could be substitutes of conventional thermoplastics due to their properties. PHA production by mixed microbial cultures (MMCs) using wastes as substrate comprises generally three sequential steps: (i) volatile fatty acid (VFA) production, (ii) MMC enrichment, and (iii) accumulation of PHA. Another common factor for PHA production by MMCs is the use of temperatures within the mesophilic range and even the effect of temperature was studied in the interval of 15-35°C. However, there are no reports about PHA production at higher temperatures, for instance, close to the thermophilic range. The present study aims to address, for the first time to the knowledge of the authors, the enrichment of a MMC and the production of PHAs in the moderate thermophilic range (48°C) and how the kinetic and stoichiometric parameters, or even on the type of P(HB-co-HV) copolymers obtained during the accumulation, are affected.

For this purpose, a sequencing batch reactor with a working volume of 2 L is used to enrich the microbial community under carbon limiting conditions. The carbon source is fed at a loading rate of 90 Cmmol/(L · d) and the composition is a mixture of HAc:HP:HBu with a distribution of 65:20:15 Cmmol %. A volume of 50 mL is added per cycle containing the following concentrations: 13.2 g/L of NH<sub>4</sub>Cl; 6.78 g/L of KH<sub>2</sub>PO<sub>4</sub>; 2.74 g/L of MgSO<sub>4</sub>·7H<sub>2</sub>O; 1.06 g/L of KCl; 3 mL/L of allylthiourea; 30 mL/L of trace compounds. A fed-batch reactor is used for the PHA accumulation experiments, using as inoculum the previously enriched biomass.

Chemical oxygen demand, ammonium, and Volatile and total suspended solids are analyzed according to the Standard Methods. VFAs and PHAs are characterized and quantified by gas chromatography (GC). Specific substrate uptake rates ( $\text{Cmol}_{\text{VFA}}/(\text{Cmol}_x \cdot \text{h})$ ) and specific PHA production rates ( $\text{Cmol}_{\text{PHA}}/(\text{Cmol}_x \cdot \text{h})$ ) are determined by linear regression of the concentrations obtained from each experiment and divided by the average biomass in the experiment. Yields of PHB and PHV ( $\text{Cmol}_{\text{PHB}}/\text{Cmol}_{\text{VFA}}$ ) are obtained from the experimental data by dividing the homopolymer production rate ( $\text{Cmol}_{\text{PHB}}/\text{h}$ ) by the VFA consumption rate ( $\text{Cmol}_{\text{VFA}}/\text{h}$ ).

In this study, the performance of a moderate thermophilic reactor is studied, and it is discussed in detail its comparison with a mesophilic process under the same operational conditions.

# Production of the exopolysaccharide AM-1 by *Gluconacetobacter diazotrophicus* PAL5

P3.6

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The replacement of synthetic materials by sustainable ecofriendly alternatives is of growing interest in the context of bioeconomy. Microbial exopolysaccharides (EPS) are renewable biopolymers, which can be produced on various waste streams. In addition, they are biodegradable and nontoxic. Industrially feasible production of microbial EPS for low cost technical applications must have a high productivity of the microbes to compensate costs for fermentation and downstream processing, where commonly large amounts of solvents are needed. As the production of exopolysaccharides by microbes is differently regulated, for most strains a customized fermentation strategy must be developed to realize optimal EPS production. *Gluconacetobacter diazotrophicus* PAL5 is an endophytic Gram-negative bacterium, which produces an exopolysaccharide build up from the repeating unit AM-1<sup>[1]</sup>. It is discussed if the EPS functions as protection shield of the nitrogenase, helps to solubilize inorganic phosphate from soil or acts as protecting agent against dehydration <sup>[2,3,4]</sup>. However, it turned out that the EPS has a good performance as coating material for different materials in preliminary tests. The relatively low viscosity of the solutions even at high concentrations remains in an area, which is perfect for the coating applications. Though, fermentative production of this EPS is somehow difficult as a part of *Gluconacetobacter diazotrophicus* PAL5 respiratory chain is to oxidize sugars and alcohols at the outer membrane <sup>[5]</sup> resulting in inefficient conversion to EPS. To overcome this limitations and enhance EPS production in this organism, it is necessary to monitor carbon flow and to determine a suitable fermentation strategy where less byproducts are formed and as many carbon as possible is provided for EPS production. Therefore, the productivity on different carbon sources and various process modes are tested and evaluated. Potential usage of waste streams as feedstock for its production, make this EPS a promising and sustainable resource.

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## Farm wastes as feedstock for polyhydroxyalkanoates production with phototrophic purple bacteria operated in anaerobic photo bioreactors

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Polyhydroxyalkanoates (PHA) are biodegradable biopolymers with physical-chemical properties similar to polypropylene and are a sustainable alternative to conventional plastics. In an effort to decrease PHA production costs, mixed microbial cultures fed with cheap agro-industrial wastes have been studied in the last years, and recently, a new PHA producing system has been proposed. It consists in operating phototrophic mixed cultures (PMC) and enriching them in PHA producing phototrophic purple bacteria (PPB). These phototrophic bacteria can obtain energy from light and therefore, do not require aeration, preferring to grow in anaerobic conditions. This is a clear advantage in relation to current PHA producing systems where aeration is continuously or partially present during the system operation, strongly contributing to high operational costs.

Thus far, studies with PHA producing PPB occurred with PMC operated in anaerobic conditions using a permanent carbon feast selection strategy specifically designed for PPB, being attained PHA accumulation levels up to 60 % with acetate feeding. Currently, studies are occurring under the Horizon 2020 European project NoAW. These studies aim to determine, at laboratory scale, what are the optimal operational conditions for valorising fermented farm wastes (mixture of manure and waste maize silage) through PMC's PHA production.

Starting with conventional activated sludge and synthetic feed simulating the organic acids present in the fermented waste mixture, an illuminated photo bioreactor (12h dark/12h light) operated under a permanent carbon feast strategy quickly became enriched with a PMC dominated by phototrophic purple bacteria. The PMC was capable of maintaining a stable PHA content around 20 % PHA/VSS in the selector reactor, a value that could increase up to 35 % PHA/VSS in the accumulator reactor. Microscopic observations clearly indicated that most of the phototrophic population was capable of accumulating PHA. Seeing that these results were obtained in non-aerated photo bioreactors in completely anaerobic conditions, we prospect that phototrophic mixed cultures can lead to more cost-effective and environmentally sustainable PHA production processes. Currently, operating conditions are being adjusted to introduce the real fermented farm waste as influent and further improve the culture PHA production capacity.

## Investigation of parameters influencing biomass and poly(3-hydroxybutyrate) formation of *Bacillus megaterium* uyuni

P3.8

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The halophilic and fast-growing *Bacillus megaterium* uyuni S29 (CECT 7922) displays a high capability to produce poly(3-hydroxybutyrate) (P(3HB)) on a variety of carbon sources. The goal of the study was to explore parameters that influence the growth and P(3HB) formation behaviour of *B. megaterium*. For this purpose, we tested different limitation strategies, as well as the replacement of NaCl with KCl in the fermentation medium.

P(3HB) formation of *B. megaterium* can be triggered by nitrogen, phosphorous and oxygen limitation. Nitrogen and phosphorous limitation were the most effective strategies to induce P(3HB) formation, resulting in P(3HB) concentrations of 0.42 g/g cell dry mass (CDM) and 0.39 g/g CDM, respectively. Oxygen limitation resulted in a lower P(3HB) concentration of 0.21 g/g CDM but triggered the formation of 1 g L<sup>-1</sup> lactic acid. Nitrogen limitation appeared to be less favourable when sucrose was provided as carbon source, as a lack of nitrogen supposedly inhibits the inversion of sucrose. Phosphorous limitation has no such impact on sucrose consumption and is therefore preferable, when this carbon source is used.

Since the strain was isolated from the salt rich environment of the salt lake Uyuni in Bolivia, we also evaluated the influence of different KCl and NaCl concentrations (0.08 M to 1.71 M) on the formation of biomass and P(3HB) at phosphorous limited conditions. As expected, higher salt concentrations resulted in a gradual reduction of the biomass concentration. At all tested concentrations, the experiments with KCl showed higher sugar consumption and P(3HB) formation compared to those with NaCl.

It can be concluded that the combination of KCl in the medium and abundant supply with nitrogen results in fast conversion of sucrose and accumulation of up to 0.50 g/g CDM of P(3HB) in the biomass, when *B. megaterium* is used as production strain at phosphorous limitation.

## Development of immobilized systems for enzymatic production of *Halomonas* levan

P3.9

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Levan is a fructose homopolymer, a fructan, that has been known for more than a century but has attracted renewed interest within the last decade. Levan has been associated with diverse applications in several industrial fields like mainly food, medicine, pharmacy and agriculture however its limited availability is the main bottleneck for its commercialization. Besides several groups working on levan production, levan by *Halomonas smyrnensis*, *Halomonas* levan, has been the focus of interest of our group. As part of ongoing research about the development of cost effective levan production processes, this study aimed at the enzymatic production of *Halomonas* levan via immobilized *Halomonas levansucrase* enzyme. For this, first, halophilic levansucrase enzyme was purified from recombinant *Escherichia coli* cells and then immobilized via different matrices of interest. Optimized conditions for levan production at high salt as well as the reusability levansucrase enzyme stability were investigated.



## Ferulic acid synthesis in engineered *E. coli* is limited by methyl-group supply

P3.10

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The phenylpropanoic acids coumaric acid, ferulic acid and sinapic acid are three precursors of lignin as well as a set of secondary metabolites of which some are predicted to have beneficial biological activity on human health. To identify potential biotechnological routes for their synthesis, we investigated the production of ferulic acid in *E. coli*. We designed an inducible polycistronic expression construct comprising four enzymes catalysing the immediate three step conversions from tyrosine to ferulic acid by desamination (TAL), aromatic hydroxylation (HpaBC) and methylation (OMT). Recombinant cells cultivated in minimal medium were pulse fed with tyrosine and the concentration of intermediates and product were followed by quantitative HPLC measurements. In initial shake flask cultivations a set of homologous enzymes were assessed individually and the best candidates implemented in the synthetic cascade. Cells expressing all enzymes of the cascade transformed 2 mM tyrosine (Y) in 0.5 mM ferulic acid in a shake flask in 24 h, by concomitant accumulation of 0.3 mM caffeic acid. Addition of methionine (M) and serine, precursors of S-adenosyl-methionine, improved the final ferulic acid concentration to 1 mM by improved methylation of caffeic acid. Theoretical flux analysis based on kinetic data of the first two enzymes of the cascade revealed a substantial downshift in the flux by a strong product inhibition of the first enzyme ( $K_i = 16 \mu\text{M}$ ) together with a high  $K_M$  of the second enzyme ( $K_M = 3 \text{ mM}$ ). Product inhibition is partially relieved in a  $\Delta\text{tyrR}$ -background. In a controlled parallel reactor with constant feeding of 1 mM/h (40 h) Y and Y + M was compared. The supply of methyl-precursor M increased ferulic acid production significantly by decreasing accumulation of intermediates and also maintaining the cell vitality. This finally resulted in  $9.6 \pm 1.4 \text{ mM}$  ferulic acid after 48 h.

## Use of carbon dioxide and valeric acid as co-substrates for the production of poly(3-hydroxyalkanoate)

P3.12

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The capture of carbon dioxide from the environment for the production of useful products is naturally performed by autotrophic microorganisms. In this metabolic constitution, CO<sub>2</sub> acts as sole carbon source and is complemented by an energy source that can be supplied in an inorganic chemical form (e.g. hydrogen) for lithoautotrophic microorganisms or in the form of light for photoautotrophic microorganisms. In this study, we focused on the lithoautotrophic bacteria able to grow on CO<sub>2</sub> and hydrogen, and specifically on those capable of producing polyhydroxyalkanoate (PHA) compounds [1-3].

The interesting point is that these bacteria are able to switch between heterotrophic and autotrophic growth and PHA production modes. In this work, we would like to discuss the effects of adding an organic co-substrate to an initially purely lithoautotrophic culture. Especially, these effects are analyzed during the PHA production phase under nitrogen limitation. The composition (relative presence of both 3-hydroxybutyrate and 3-hydroxyvalerate monomers) of the produced PHA, the yields and productivities, the rate of production and of consumption of these PHAs by bacteria will be proposed for discussion.

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## Microbiological analysis of a PHA producing consortium

P3.13

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Polyhydroxyalkanoates (PHAs) are a highly promising class of bioplastics for the transition from fossil fuel-based plastics to biorenewable and biodegradable plastics <sup>[1]</sup>. A laboratory-scale 5L-bioreactor was started in our laboratory with a PHA producing consortium from a pilot-scale reactor. This pilot-scale reactor contains an enriched mixed culture fed with a mixture of fatty acids. On laboratory-scale the culture was kept in a feast/famine regime with acetic acid as the fatty acid. A 16S rDNA microbiological population analysis revealed that majority of the population consists of a Gram-negative beta-proteobacterium belonging to the order of rhodocyclales. The culture produces, in line with other PHA producing species/consortia, polyhydroxybutyrate (PHB) when feeding on acetate <sup>[2]</sup>. The enriched culture will be used for the optimization and validation of environmentally friendly (bio)extraction methods to maintain the green label of PHA.

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## Scleroglucan production by *Sclerotium rolfsii* ATCC 201126 from amylaceous and sugarcane molasses-based media

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Biotechnology tries solving the growing demand on biodegradable polymers of low costs, high quality and viscosifying properties. The large-scale production of microbial exopolysaccharides (EPSs) by controlled fermentation is a promising example of biobased polymers, with world markets representing several hundred million dollars annually <sup>[1,2]</sup>. Scleroglucan (SC) is one of these microbial EPS, produced mainly by fungi of the genus *Sclerotium*, under optimized and standardized cultivation conditions <sup>[2-3]</sup>. Its excellent properties make it especially attractive for various industrial applications ranging from assisted oil recovery to the cosmetic industry <sup>[2-3]</sup>.

To further optimize its production and economy, in this work, nine alternative low-cost C- and twelve N-sources were comparatively assessed. When comparing conventional sucrose-based Production Medium PM (8.41 g C/L + NaNO<sub>3</sub> as N-source) at shake-flask-scale vs. alternative C-source versions, soluble starch and sugarcane molasses led to efficient SC production. On the other hand, oxalic acid byproduct varied with C- and N-sources, with no clear detrimental relationship with SC production.

Afterwards, at 3 L bioreactor scale, a starch-based medium led to the highest SC production (7.95 g/L), best recovery efficiency (~ 52 %) and high values of volumetric and specific productivities (0.11 g SC/L h and 0.018 g SC/g h respectively). Sugarcane molasses, though leading to lower SC production (5.11 g/L), could be envisaged as a promising agroindustrial sub-product as alternative substrates for efficient, low-cost, and scalable SC production, thus opening new perspectives for medium reformulation and sustainable and economic SC production.

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## Protective effect of PHA granules in bacterial cells against UV-irradiation investigated by various spectral techniques

P5.1

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**Abstract:** Bacteria developed various protective strategies to cope with stress conditions including UV irradiation. Polyhydroxyalkanoates (PHA) are polyesters accumulated in form of intracellular granules by numerous bacteria primarily as storage compounds, nevertheless, we investigated that PHA granules are not used only as carbon and energy storage but bacteria can use this compound also as UV-protectant. This statement is based on our observation that PHA accumulating cells of *Cupriavidus necator* H16 survived exposure to UV irradiation challenge much better than its PHA non-accumulating mutant. Mechanisms of protective effect of PHA granules were determined by different spectroscopic approaches such as turbidity measurement, absorption spectrophotometry with integration sphere, nephelometry and continuous fluorescence microphotolysis. Generally, protective mechanisms of PHA granules is predominantly based on very effective scattering of UV radiation with wavelength of about 250 – 290 nm which protect photo-sensitive molecules, especially DNA, from their damage. Furthermore, PHA metabolism also provides reduction power to eliminate radicals induced by irradiation. Therefore, PHA accumulation capability represents very potent strategy to face UV-irradiation.

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## On a usability of various available methods in structural analysis of hydrogel system

P5.2

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Hydrogel is defined as a crosslinked three-dimensional polymeric network structure. Recently, there has been an increasing interest in hydrogels, since they can be used in a broad field of applications (cosmetics, drug delivery systems, etc.). The essence of their unique qualities and technical functionality is rooted in their structure (network architecture, pore size, pore distribution, etc.). For this reason, it is crucial to understand the structure of the hydrogel correctly, and thus to choose the suitable structural analysis technique.

The main aim of our work was testing the applicability of available structural analysis techniques on an appropriate model hydrogel system and assessing the influence of the composition change of the hydrogel system on its internal structure. Hydrogels based on agarose of different concentration were chosen as a model system.

Gels of four agarose concentrations were prepared (0,5 %; 1,0 %; 2,0 % and 4,0 %) and a number of structural assays were executed: for dry (shock-frozen in liquid nitrogen and freeze-dried) samples were selected mercury porosimetry as indirect method and scanning electron microscopy (SEM) as direct method; for native (hydrated) samples were performed turbidimetry as indirect method and cryo SEM as direct method. Native (hydrated) forms are usually used for applications, so it is convenient to examine samples in this state, however, there are not many common and available methods for examining the structure of hydrated samples.

Each method showed some benefits and problems. SEM and cryo SEM provided detailed and realistic visualization of the internal structure, however, during sample preparation, the structure is disrupted, and the results may be distorted. SEM micrographs showed pores slightly larger than cryo SEM, probably due to greater effect of freezing water crystals than cryo SEM. Cryo SEM and turbidimetry provided comparable results of pore diameters (hundreds of nanometers). Mercury porosimetry has proven to be a suitable method only for the determination of larger pores, in the case of small pores, the structure has already been destroyed by high applied pressures.

## Understanding the thermal properties of mcl-PHA: influence of the structure and the crystallization conditions

P5.3

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While thermal characteristics of scl-PHA are becoming more and more understood, for mcl-PHA it is not yet the case. This is mainly caused by the fact that mcl-PHA are copolymers with more variety in their monomeric compositions than scl-PHA. It is thus difficult to compare results from different studies using mcl-PHA with widely different structures. In this study, several mcl-PHA were synthesized by *Ps. Putida* KT2440 on fatty acids (hexanoic, heptanoic and octanoic acids) with glucose. Mcl-PHA with controlled structures were obtained: PHO<sub>93</sub>HHx<sub>7</sub>, PHO<sub>67</sub>HHp<sub>29</sub>HHx<sub>4</sub>, and the almost-homopolymer PHH<sub>98</sub>pHV<sub>2</sub>. Moreover, with the addition of acrylic acid to the substrates, a PHO with less than 1 % of HHx was obtained. Thermal characterizations were done by DSC. First, all samples were melted at 60°C before being aged either at cold (i.e. 1-2°C) or at room temperature (i.e. 19-23°C) for 3 days or 3 weeks. Melting temperatures and enthalpy were then measured from the first heating cycle and glass transition temperatures from the second heating cycle. Results first showed, as expected, that T<sub>g</sub> was independent of the aging conditions and only dependent on the monomeric composition. A linear correlation was found between the measured T<sub>g</sub> and the mean length of the side chains of the mcl-PHA, being lower when the length of side chains increased. The crystallization state and melting behavior, however, showed significant differences between samples with different aging conditions. At low temperature, it was found that there were no differences from samples kept for 3 days or 3 weeks. At low temperature, crystallization equilibrium is already reached over 3 days. On the other hand, at ambient temperature, melting enthalpy of around 20 J/g (similar to the one measured after low temperature aging) were reached at 3 weeks and not at 3 days. Moreover, the melting temperatures were around ten degrees higher on samples aged at room temperature compared to the samples aged at low temperature. The most remarkable result, however, was the fact that for the PHHp97HV2, no crystallization was observed. Whatever the aging conditions, it was completely amorphous. These results showed that even a slight difference in the side chain, e.g. one CH<sub>2</sub> between PHHp97HV2 and PHO, can significantly alter thermal properties. Understanding how much the structure can impact the thermal properties of mcl-PHA will allow to more easily predict which mcl-PHA to use for a given application.

# Rheological characterization & modeling of Sphingan/surfactant mixtures

P5.4

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Due to their outstanding rheological properties, microbial exopolysaccharides (EPS) are used as rheological additives in various industrial applications. In contrast to plant-derived polysaccharides, which still dominate the market, they can be produced under controlled conditions throughout the entire year without local or seasonal restrictions<sup>[1]</sup>. Their broad field of application overlaps with the application of surfactants in many cases, especially in personal care and cosmetics products<sup>[2-4]</sup>. The combined application of these two compounds requires an understanding of potential interactions between them and resulting influences on the rheological properties of the final formulation which ultimately determine the overall product quality<sup>[3]</sup>. Several studies on the structure-function relationship of microbial polysaccharides as well as on the interaction between EPS and surfactants exist<sup>[3,5]</sup>. However, there are little comprehensive studies on the influence of structural features of closely related EPS on interactions with surfactants and the influence of these interactions on the rheological properties. The aim of our work is to establish a structure-function relationship of four different sphingans (Gellan, Welan, Diutan & S-88), which share a common backbone structure and only differ in terms of glycosidic sidechains<sup>[6]</sup> and functional groups as well as the rheological properties of these sphingans in mixtures with model substances from the group of anionic, cationic, zwitterionic and uncharged surfactants. The comparison is facilitated by multi-parameter mechanical models (e.g. Maxwell- and Burgers-Model), which describe the rheological behavior of the systems in a mechanical way. Understanding the interaction of EPS with different structural properties in complex systems will help understanding the physicochemical properties as well as incompatibility effects of resulting mixtures and will facilitate designing and formulating novel products.

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- <sup>[4]</sup> Augustine, Robin, et al., *Biopolymers for Health, Food, and Cosmetic Applications*, in *Handbook of Biopolymer-Based Materials*, 2013, Wiley-VCH Verlag GmbH & Co. KGaA, p. 801-849.
- <sup>[5]</sup> Gansbiller, Moritz, Jochen Schmid, and Volker Sieber, *In-depth rheological characterization of genetically modified xanthan-variants*, *Carbohydrate Polymers*, 2019, 213: p. 236-246.
- <sup>[6]</sup> Pollock, Thomas J., *Sphingan Group of Exopolysaccharides (EPS)*, in *Biopolymers Online*, 2005, Wiley-VCH Verlag GmbH & Co. KGaA.



## Evaluation of the impact of cell lysis on polymeric substances recovered from biological aggregates

P5.5

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Bioaggregation is ubiquitous in natural environment and is of great importance in biological wastewater treatment processes. The analysis of EPS that entrap microbial cells is a prerequisite to understand bioaggregation and how to valorize the produced compounds in valuable materials. Extraction of EPS based on the solubilization of the matrix is often challenging because of the highly diverse physico-chemical properties of these polymers and the diversity of the matrices. Different physical or chemical methods can be employed leading to variable amounts and compositions of the extracted EPS. This variability was generally related to the extractive method that can be more or less adapted for the solubilization of specific biopolymers. The damage of microbial cells during the extraction step may also contribute to this variability through intracellular polymers release. However, very few studies have evaluated the effects of cell lysis on EPS physico-chemical properties. One first objective of our study was to compare the accuracy of various methods for cell lysis evaluation during extraction treatments. The methods were based on the quantification of intracellular components: Glucose-6-phosphate dehydrogenase (G6PD), DNA, ATP and proteins. One important criteria was the robustness of the method towards the extraction treatments. G6PD detection was not adapted for chemical extractions (acidic or alkaline treatments) because of enzyme denaturation. Although robust to chemical treatments, DNA was denaturated by physical extractions such as sonication or thermal ones. ATP measurement was found very challenging because of high lability of this molecule and bad reproducibility of the assays. Finally, protein measurement was found as the most robust method that was compatible with a wide range of chemical and physical treatments. However, the presence of extracellular proteins in many biofilm matrices can be problematic for the evaluation of a precise cell lysis ratio. A second objective was to evaluate the quantitative and qualitative impacts of cell lysis on the recovered EPS fractions. Increasing cell lysis ratio were generated thanks to physical treatments on different types of biological aggregates. Analysis of the extracts showed that whatever the aggregates or extraction methods, the profiles of the biopolymers obtained by size exclusion chromatography (SEC) were significantly impacted by the intracellular components. In particular, SEC monitoring with both UV and Refractive Index showed that additional molecules with very high extinction molar coefficient at 280 nm and high molecular weights were detected after cell lysis. In the perspective of valorization of the biopolymers produced by many bacterial systems, intracellular components could be also considered as positive contaminants leading to new colloids or biomaterials of biotechnological interest.

# Municipal solid waste as a feedstock for P3HB production

P5.6

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Different solutions are being currently sought for the treatment and upgrade of municipal solid waste (MSW). According to EU data, MSW amounts to over 250 million tons per year in Europe<sup>[1]</sup>. The price of the carbon source has a large contribution on the final cost of PHAs<sup>[2]</sup>. The key to obtain an economic carbon source is the use of residual substrates like agricultural and industrial wastes or the organic fraction of municipal solid waste (OFMSW)<sup>[3,4]</sup>. Indeed, the OFMSW is one of the most abundant feedstocks in cities, accounting for approximately 30–40 % of MSW in Europe<sup>[1,5]</sup>. Considering this scenario, it is worthwhile to assess the potential of OFMSW as feedstock to produce added value products through fermentation processes. Recently, Izaguirre *et al.* (2019) reported the production of P(3HB) by *Burkholderia sacchari* DSM 17165 using OFMSW as feedstock<sup>[6]</sup>. Moreover, other authors used OFMSW to produce lipids, lactic acid, biofuels, volatile fatty acids, etc.<sup>[7,8]</sup>. In this work P3HB production from OFMSW was attempted in a three-stage process, ie, a diluted acid pre-treatment followed by an enzymatic hydrolysis and a bacterial cultivation. Sugar recovery was 56 % using a pre-treated waste and an enzyme cocktail of Pentopan 500 BG, Celluclast BG, and Glucoamylase NS 22035. The obtained hydrolysate turned out to be rather adequate for the production of P(3HB) by *B. sacchari*. In shake flask cultivations, it was necessary to supplement the hydrolysate with extra glucose to increase the C/N ratio and with a mineral solution to meet some of the nutritional requirements. P(3HB) accumulation was 58 % (g polymer/ g biomass). In bioreactors (5 L) operated in the fed-batch mode, using the hydrolysate as growth medium and a sugar solution that simulates the hydrolysate as feed, a polymer production and a volumetric productivity of 43 % (g polymer/ g biomass) and 0.56 g·L<sup>-1</sup>·h<sup>-1</sup>, respectively, were achieved.

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## Organosilicon compounds from fatty acids for surface layer polymerization

P7.1

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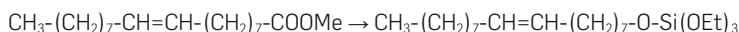
Sealings, adhesives or adhesion promoters, paints, coatings and more are important polymer containing soft matters used in different industry branches. Many of these products are organosilicon compounds, which are a versatile and modifiable substance class due to the combination of an inorganic structure with organic groups. Some of these compounds could also be obtained starting from renewable resources. Fatty acid vinyl esters can polymerize on surfaces when equipped with  $\text{SiOR}_3$  groups and can be synthesized based on materials that are available from plant oils in large scale. This polymer forms a new layer, which has its inorganic part pointing towards and its organic part away from the surface, making it nonpolar and water repellent on the outside and can work as an adhesion promoter. In the same way surface layers for many different purposes could be developed by creating compounds with a functional group that can be polymerized to form a durable, strongly attached layer combined with a group that make their properties suitable for their application. Only one product of this kind is on the market at present.

In order to obtain bifunctional substances for polymerization or surface modification from renewable resources, unsaturated fatty acids are used as starting materials. In a tandem reaction, starting with the isomerization of the internal double bond of the fatty acid to the terminal position followed by the hydrosilylation of the double bond, a product with an alkylsilane and an ester group is formed. An iridium catalyst was designed to catalyze the 8-fold isomerization in combination with triethylsilane as reactant.



Because Alkoxysilanes would be more interesting products for industrial applications the same reaction should be applied with triethoxysilane instead of triethylsilane. However, the iridium catalyst used in the previously mentioned reaction shows no conversion using triethoxysilane. Therefore other catalysts for this purpose are developed.

Triethoxysilylethers can be synthesized from methyl esters of fatty acids and other esters in a platinum-catalyzed reductive hydrosilylation using triethoxysilane as reducing agent and reactant. The starting material is fully converted and the reaction gives high product yields.



## Develop novel bio-epoxy precursors to replace bisphenol A and improve hydroxyl homogeneity through alkoxylation

P7.2

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As Earth's carbon dioxide level in atmosphere hits record high this year, it is a wake-up call for us to further decrease carbon dioxide emission. Bio-based polymers, carbon-neutral polymers, have a low CO<sub>2</sub> footprint and can convert biomass waste into high value products. Several new bio-based polymers have been commercialized such as bio-based polybutylene, bio-based vanillin diglycidyl ether, etc. However, their building blocks generally exist in small portions (< 40 %) of the biomass. For example, many bio-polymers start from tannins which accounts for up to only 20 % of wood. The low conversion rate out of biomass is still a remaining challenge of the bio-based polymer production. Thus, the aim of this study is to liquefy both major components (polysaccharides and lignin) and extractives to increase the total yield. Previous studies show different hydroxyl groups present in the biomass have various pKa values leading to different reaction rates which left unreacted functional groups. To address this issue, the precursors are homogenized using a chain extension technique with propylene oxide and then reacted with epichlorohydrin catalyzed by strong base to produce epoxy monomers. Two types of bark – Japanese Cedar (*Cryptomeria japonica*) and Taiwan Acacia (*Acacia confusa*) – are studied and characterized using Fourier-transform infrared spectroscopy, <sup>1</sup>H, <sup>13</sup>C, <sup>31</sup>P – nuclear magnetic resonance spectroscopy, and gel permeation chromatography. All barks are pretreated by ethanol to remove part of extractives which are used to develop anti-oxidants for another study. Three liquefaction process are conducted – alkaline extraction, phenolation, and alkoxylation. The results show the Taiwan Acacia has very high percentage of condensed tannins which turns the alkaline extraction into a blue solution due to proanthocyanidins and the total conversion rate of Taiwan Acacia bark is higher than that of Japanese Cedar bark. The yield among three conversion methods is phenolation > alkoxylation > alkaline extraction.

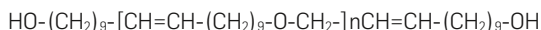
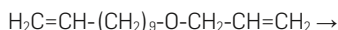
## Synthesis of ether-diols with low polarity from long-chained fatty alcohols for use in block copolymers

P7.3

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Diol-terminated polyethers are important intermediates for the manufacturing of block copolymers, but only a few polyethers other than polyethylene glycols are available for block polyester synthesis on technical scale. Most of them are highly polar. The efficient synthesis of other kind of such ethers without using hazardous chemicals like alkyl bromine in Williamson's ether synthesis is a challenging field. In this work, we describe a sustainable method of synthesis for long chained diol-ethers by using fatty alcohols, from renewable resources. Natural fatty alcohols – converted to similar polyethers – should have a grossly reduced polarity because of a large separation of the oxygen atoms in the C18 chain. A synthesis sequence of polyethers from commercially available fatty alcohols was designed for a future examination of the hydrophobic properties. Palladium catalyzed cleavage of olefinic dialkyl carbonates results in carbon dioxide elimination and subsequent formation of ethers from an allyl-palladium cation. It could be shown that this process with fatty alcohols like undec-10-en-1-ol or oleyl alcohol can be run with appreciable yield.



Ruthenium catalyzed ADMET (acyclic diene metathesis) at both chain terminations then was applied to polymerize the ethers. Dependent on the alkenyl-chain, short oligomers with a DP (degree of polymerization) of about 5-8 seem to be formed according to GPC.

## 3D printed levan scaffolds for cardiac regeneration

P8.1

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There is a clear need for novel scaffolds in cardiac tissue engineering due to the current problems such as deficit of donor tissues, immune rejection, anticoagulation therapy, and limited durability of vascular implants. Recent advances in this field have applied 3D bioprinting as an advanced fabrication technique to produce biocompatible materials, cells and supporting components with great promise for artificial organ printing and regenerative medicine applications. Glycans like alginate and cellulose are commonly used for cardiac tissue engineering applications. As such, levan polysaccharide has been gaining escalating attention due to its distinguishing properties like high biocompatibility, strong adhesively and amphiphilic nature. Levan is a fructose based homopolysaccharide, a fructan, and the sulfated derivative of levan produced by *Halomonas smyrnensis* cultures, *Halomonas* levan, has been shown to be a very promising biomaterial for cardiac tissue engineering applications. This study aims to elucidate the potential of *Halomonas* levan sulfate as a biomaterial in 3D bioprinting technique for cardiac tissue engineering purposes. For this, glycan-based scaffolds were produced with levan and other glycans such as alginate and the scaffolds were characterized comprehensively for their morphological, mechanical, surface and biological properties.

## Synthesis and characterization of octenyl succinic anhydride-modified levan

P8.2

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There is considerable interest in chemical modification of polysaccharides to improve their natural properties and bioactivities and to develop industrial products with specific characteristics. The incorporation of hydrophobic groups into the molecule gives the polysaccharide an amphiphilic nature and thereby surface-active properties. Starches from different botanical origins modified with octenyl succinic anhydride (OSA) are widely used, as emulsifiers and stabilizers, in food industry, pharmacy and cosmetics. Also, OSA derivatives of other polysaccharides such as pullulan, gum Arabic, hyaluronic acid or inulin have been intensively studied.<sup>[1,2]</sup>

The aim of the present study was to optimize the synthesis of OSA-levan derivative. Levan ( $\beta$ -2,6-fructan,  $10^5$  Da) produced by *Bacillus licheniformis* NS032 growing in medium with 400 g sucrose/L was used for synthesis.<sup>[3]</sup> Reaction was carried out in aqueous medium in mild alkaline conditions (pH 8.5). The influence of the following factors on reaction efficiency was investigated: temperature (20-70 °C), levan concentration (25-35 %), OSA concentration (5, 7.5, 10 % in relation to levan weight) and reaction time (2-8 h). FTIR spectroscopy and NMR spectrometry were used for structural characterization.

The synthesis of OSA-levan derivative was confirmed by FTIR spectrum showing two new peaks at 1730 and 1565  $\text{cm}^{-1}$  due to the formation of ester linkage. Degree of substitution (DS) was calculated using  $^1\text{H}$  NMR from the ratio of area of the peak at 0.85 ppm (OSA) to the area of the peaks from 3.45 to 4.18 ppm from levan and found to be from 0.8 to 2.8 %. In comparison to other reaction parameters, the temperature of 40 °C and reaction time of 5 hours were set as the optimum showing the highest reaction efficiency. Increasing the reaction temperature above 50 °C caused a significant reduction in the DS. The efficiency was higher for lower concentration of OSA reagent. Surface tension values for OSA-levan derivatives (0.25 %) were in the range of 55.9-61.2 mN/m.

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## Investigating the effects of *Halomonas* levan on fat deposition, life and healthspan using *Caenorhabditis elegans* as model

P8.3

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Obesity, insulin resistance and type 2 diabetes (T2DM) are closely related diseases that persistently have risen in prevalence consistently worldwide starting from 1980s. Although research is non-conclusive about the root of these diseases; there is scientific consensus over one fact: increased body fat percentage increases the risk developing of insulin resistance, T2DM which are the hallmarks of obesity. *Caenorhabditis elegans* is a free-living nematode which has a relatively short life span and has orthologues of 40 % of genes related to the human diseases. Levan is fructose homopolymer connected with 2,6 beta glycosidic linkages which can be produced by various types of bacteria, archaea, fungi and some plants. In our study, we have shown the fat reducing effect of *Halomonas* levan (levan obtained from *Halomonas smyrnensis* halophilic bacteria, HL) and its hydrolized derivative (hHL) on *C. elegans*. Moreover, levan also significantly reduced intestinal fat deposition when nematodes were treated with in combination with 2 % glucose which is used for model for high glucose diet. Furthermore, preliminary observations of levans positive effect on nematodes' healthspan implicated that *Halomonas* levan's fat reducing effect on *C. elegans* may also positively affect humans and can be used as food additive for weight loss.



## Effect of different compatibilizers types on mechanical and morphological properties of PLA/PP and PLA/bioblends prepared by twin-screw extruder

P8.4

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The Poly (lactic acid) is a biodegradable polymer from renewable sources with potential to replace non-biodegradable polymers derived from petroleum. However, its use is restricted in certain applications due to its high brittleness and rigidity. Thus, blending PLA with soft and tough polymers like PP and PE can enhance mechanical properties, but due to the phase separation of PLA and polyolefins, the addition of a compatibilizer is necessary. In this study, polymer blends of poly (lactic acid) with polypropylene and BioPE were prepared by twin-screw extruder. The addition of polypropylene-graft-maleic anhydride (PP-g-MAH), polyethylene-graft-maleic anhydride (PE-g-MAH) or ethylene/methyl acrylate/glycidyl methacrylate (EMA-GMA) terpolymer as compatibilizer was studied.

**Blends Preparation:** The PLA/PP and PLA/BioPE blends were prepared in a Coperion corrotational twin screw extruder, 35 mm, L/D 40, at 200°C, screw speed of 300 rpm and feeding rate of 30 Kg/h. PLA was processed under the same conditions. Prior to extrusion, PLA was dried under vacuum for 4h. The extruded material was dried under vacuum for 24 h and samples for tensile and impact strength tests were injection molded at 200°C in Haitian injection molding machine.

**Mechanical Properties:** Tensile tests were carried out in a EMIC 100 kN Universal Testing Machine, according to ASTM-D 638, operating at crosshead speed of 50 mm/min. Izod impact strength tests were performed according to ASTM-D 256 in notched samples using a CEAST Resil 5.5 apparatus equipped with a hammer of 2.75J. The results reported are the average of 5 samples.

**Scanning Electron Microscopy (SEM):** SEM micrographs of the fracture surface of cryofractured samples were coated with gold and the morphology was analyzed using a TESCAN VEJA 3 SBH S Scanning Electron Microscope, operating at a voltage of 5kV. The compatibilized PLA/polyolefin blends showed substantial reduction in the droplet sizes of the minor phase, resulting in an increase in the elongation at break, tensile strength at break and Impact Strength.

To continue this work, thermal analyses (TGA/DSC) and X-ray diffraction (XRD) will be done to study that addition of the compatibilizer has effect on crystalline melting temperature of the polymer components as well as on the mechanical properties.

## Operations strategies in brazilian bioplastic supply chain

P9.1

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In the current context, there is a permanent discussion in governments and society about sustainability. In the face of socio-environmental problems, several knowledge areas discuss how to reconcile the economic needs of companies with sustainable development models: socially just, environmentally adequate and economically viable. The appeal of sustainability has also become important at every link in the plastic supply chain. In recent years, initiatives have been implemented to seek and still try to improve the image of plastics in society. Brazil stands out in the development of polymers made from renewable sources from sugarcane, the so-called biopolymers. This paper aims to show that there are new forms of relationship in the supply chain of plastic, that is, in view of the need to obtain sustainable products, there is a new configuration of relationships established between the companies of second transformation of the plastic and the source of raw material, now also coming from the sugarcane sector, in addition to the traditional route of the petrochemical sector. This new configuration generates changes in the Operations Strategies of these different plastic supply chains. A multiple case study was conducted with semi-structured interviews, direct observation and analysis of secondary data sources. In this way, the production strategies adopted in supply chains were identified and compared: that of plastic from a non-renewable source (petrochemical sector) and from plastic from a renewable source (sugar and alcohol industry). The aim was to verify the practices adopted by the companies, identifying the relevant topics, both from theoretical and practical areas, for the continuity of knowledge production in a line of research on operations strategy and sustainable supply chain management.

## RICONE GmbH: Engineering (bio-)plastics – our profession, our passion

P9.2

**Harald Ruhland, Serge Gugelmann**

*RICONE GmbH, Trausnitzstraße 8, D-81671 München*

This poster presentation shows the unique method how RICONE makes plastics and bioplastics engineering.

Why is it important to us to have a clear method and a robust concept when it comes to developing plastic parts?

When developing plastic parts, we have a choice of tens of thousands of different plastics (petro-based) and already today several thousand bioplastics, each one with different properties. Therefore, it is essential to make the right material selection before the actual engineering starts, based on a perfectly fitting requirement profile. This controls to a high degree the further design process of the actual product. The necessary engineering data plays a key role here. These must be known for the selected plastic. If this is the case, we can use our work to develop optimal products from bioplastics. In this way, we are making a decisive contribution to greater confidence in this comparatively young group of materials and, at the same time, to greater sustainability. This is what drives us.

And how do we make plastics engineering in practice?

According to the above-mentioned pre-disciplines of engineering, we consider the optimal plastic construction, the best possible mold concept, stable production and sensible further use at the end of the product life. We do this with our many years of technical expertise, with in-depth coupled simulation techniques and with our recognized methods from the agile and innovative sector, which clearly sets us apart from our competitors in the overall package.

What can we do for you?

We offer our engineering services across all product sectors such as electronics, automotive, mechanical engineering, packaging technology, medical technology, consumer goods, white goods, aerospace, agriculture and forestry, etc.

We would be very pleased if our poster presentation has caught your interest. Take a look at our poster and let us talk about the engineering of bioplastics - we also have a little surprise for you.

## Biodegradable mulch film monomer metabolism: optimization via adaptive laboratory evolution

P11.1

Kristina S. Bitter, R. Nugraha C. Utomo, Lars M. Blank

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The contamination of the environment with plastics is an issue of growing concern. Not only aquatic environments, but also terrestrial habitats are polluted with different plastics. On agricultural and garden soils, plastic mulch films are often applied to enhance plant growth conditions. Traditionally, polyethylene-based mulch films are applied during growing season and removed afterwards. This method gives rise to a risk of hardly biodegradable plastic particles remaining on the field. Further, due to soil clods attached to the mulch film, the material is mostly not suitable for recycling and therefore not sustainably used. Alternatives include so-called biodegradable mulch films which consist of biodegradable polymers. Due to their biodegradability, after growing season, those mulch films are recommended to be left on the field where they are supposed be mineralized by soil microbes.

In this work, the mineralization performances of ubiquitously occurring strains on the monomers of two different biodegradable mulch films are measured and improved via adaptive laboratory evolution (ALE). With evolved strains capable to efficiently mineralize mulch films, the remainings could be completely and quickly removed after growing season and imaginably negative effects of plastics on soil and soil organisms might be avoided.

The polymers used in this study are an oil-based mulch film consisting of polybutylene adipate terephthalate (PBAT) and polylactide (PLA) and a bio-based mulch film consisting of polyhydroxybutyrate (PHB). The degradation rates of the mulch film monomers are tested with strains of ubiquitously occurring *Pseudomonas sp.* and *Bacillus sp.* To examine the degrading capabilities of single strains, first they are sequentially cultured on the monomers 1,4-butanediol, adipic acid, terephthalic acid, lactate and 3-hydroxybutyrate. Via ALE, the growth performances of the strains on the monomers are optimized. After confirmation of successful monomer metabolism, in the next step the evolved strains are cultivated with the mixed monomer compositions of the mulch films. The formation of metabolites and mineralization will be determined using high performance liquid chromatography (HPLC).

The results are discussed in the context of plastic waste to plastic value – up-cycling of low-value plastic fractions.

This research is part of a joint project funded by the European fund for regional development (EFRE).

## Biodegradability and mechanical properties of algal composites

P11.2

**Felix Eckel, Cordt Zollfrank**

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The secondary utilization of algal biomass as additive for injection moulding was studied. Composites of *Chlorella sorokiniana*, *Cylindrospermum alatosporum* and *Chlamydomonas reinhardtii* were manufactured with polypropylene, high density polyethylene (HDPE), polylactic acid (PLA) and polyhydroxybutyrate (PHB). Tensile testing bars with algae contents up to 40 wt % could be obtained. Their respective mechanical properties were determined. While the ultimate strengths and the tensile strengths are lower for all composites with algae compared to the pure matrix polymers, the Youngs moduli are lower for all except for the HDPE composites. The effect is varying with content and different algae strains.

The biodegradation of *Chlorella sorokiniana* and PLA as well as PHB was observed within a time period of three months. The CO<sub>2</sub>-evolution of those composites were analysed in the laboratory and the change of molar masses of the matrix polymers were determined after the samples were subjected to degradation in soil after three months in a field experiment. While PLA composites with algae contents lower than 40 wt % show no significant biodegradation, the composite of PLA with 40 wt % algae shows 33 % of the biodegradation of the same amount of algae. The mineralisation of PHB is also significantly increased by the addition of algae. PHB with 40 wt % algae content shows a biodegradation after three months, which is five times greater than PHB alone and is even better than alpha-cellulose which was used as positive control.

## Technical and environmental aspects of biocomposites (wood-plastics composites) in the injection moulding parts for furniture industrial applications

P13.1

**Carlos do Amaral Razzino<sup>1</sup>, Vivian Karina Bianchini<sup>2</sup>, Glauco F. Bianchini<sup>2</sup>, Eloá Cristina Figueirinha Pelegrino<sup>3</sup>**

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This study wait to contributed to increasing the use of WPC that can reduced environmental impact that can be achieved by offsetting the amount of non-renewable materials used in a product with renewably sourced wood. Future research and innovation must overcome challenges related to polymeric matrix compatibility, rising costs related to increased demand for forest resources as a fuel source, product shortcomings such as exterior durability, impact strength, as well as knowledge gaps and negative perceptions amongst manufacturers in some sectors and consumers.

Two different grades of PP were employed; a homopolymer and a copolymer were used as polymeric matrices for the wood plastic composites. The cellulosic filler used was pinus wood waste milled as wood-flour (WWF). The composite formulations were processed at 200°C, in a co-rotating twin screw extruder. The influence of the cellulosic filler and the efficiency of the coupling agents on thermo-mechanical properties of the composite system were evaluated through tensile, impact and heat deflection temperature measurements and the fractured surfaces were observed by scanning electron microscopy. The influence of coupling agents on the melt rheological properties of composites has been investigated in this work by using capillary rheometer.

The results show that yield stress response to wood-flour content and the coupling agent on both PP matrices are very distinctive. While in the homopolymer composite the addition of the wood-flour reduces the yield stress drastically, in the copolymer such effect is not so intense. This effect has also been reported by many other workers in the current literature as the lack of interfacial adhesion and consequently inefficient load transfer from the matrix to the filler. In such cases, the filler, rather than playing its reinforcement role, works more likely as a stress concentrator reducing the effective yield stress of the matrix. The melt flow properties of the coupling agents influence the composite flow behavior, which also depends on the filler loading. It can be seen that the relative shear viscosity decreases with the shear rate for all of the wood contents studied. It has been shown that the additive plays its role as an internal lubricant in shear flow. Evidences of improved matrix–filler interactions, wettability, and filler dispersion in the presence of the PP-MAH are observed by scanning electron microscopy.

# Evaluation, compassion and induction of EPS from two microalgae producers

P13.2

**Edilberto Vicente Medina Cabrera<sup>1</sup>, Broder Rühmann<sup>1</sup>, Jochen Schmid<sup>1,2</sup> and Volker Sieber<sup>1,3,4,5</sup>**

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Microalgae are a promising source of novel biogenic compounds such as proteins, carotenoids, lipids and carbohydrate polymers. Microbial carbohydrate polymers also called polysaccharides can be distinguished into two types: intracellular polysaccharides and exopolysaccharides (EPS) <sup>[1]</sup>.

The exopolysaccharides show various valuable properties, which leads to their application in different industries like textile, cosmetics, food, agriculture and medicine <sup>[2]</sup>. Currently, EPS are mainly produced by bacteria and fungi. The microalgae EPS are not used in the same extent as other EPS because of two reasons: First, there is limited information on their chemical compositions and rheological properties. Second, the low amounts of EPS that are produced by most microalgae, does not allow industrial scale production. Despite of these problems, there are some highly promising properties of microalgae EPS such as anticoagulant, immunomodulating activity and bioremediation, which could enhance and extend the applications of EPS in different industries <sup>[3]</sup>.

For this reason, a comprehensive evaluation of two microalgae strains was performed in this study. Initially, the growth behavior and nutrient uptake were compared. Followed by the determination of the EPS's chemical compositions and rheological properties via application of diverse techniques <sup>[4]</sup>. Finally, several conditions were tested; such as media composition and abiotic factors to enhance productivity and EPS yields of these two highly promising microalgae strains.

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## LiBerATE – electrochemical lignin degradation for material applications

P13.3

Satya Gultom, Anton Popp, Harald Strittmatter, Volker Sieber

Goal of the European funded project LiBerATE is to deliver a pilot scale electrochemical plant to demonstrate the commercial opportunities of converting low cost lignin feedstock in high value biosustainable chemicals. The LiBerATE project will model and physically integrate renewable energy sources to deliver a process that is capable of synthesizing chemicals with zero carbon dioxide. The renewable energy integration will open up new business models for biorefinery operators to utilize peak renewable energy at discounted rates.

BioCat works in the project on the development of a continuous process for the electrochemical lignin degradation at increased temperature. The challenge in the project is the conversion of a batch process developed by BioCat to a continuous process as well as the adaption of the procedure to the need of pilot scale. Target product of the electrochemical lignin degradation is a mixture of aromatic compounds for the replacement of fossil phenol. Possible applications are the renewable synthesis of phenolic resins, bisphenols or polycarbonates.

In the project the parameters of the electrochemical process will be optimized and a workup procedure suitable for pilot and industrial scale developed. The process will be adapted to the use of renewable energy. A pilot plant will be constructed together with project partners and the new process transferred to their place.



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Tel: +49 (0) 9421 78868-0  
info@hotelasam.de



**2 Hotel Theresientor**  
Theresienplatz 51  
Tel: +49 (0)9421 8490  
straubing@hotel-theresientor.de



**3 Hotel Seethaler**  
Theresienplatz 9  
Tel: +49 (0) 9421 9395-0  
info@hotel-seethaler.de



**4 G1AMAS in Straubing**  
Fraunhofer Straße 26  
Tel: +49 (0) 9421-12992  
hotelg1amas@gmail.com



**5 Hotel Villa**  
Bahnhofplatz 5b  
Tel: +49 (0) 9421 96367-0  
info@villa-straubing.com



**6 GenussHotel by Wenisch**  
Innere Passauer Str. 59  
Tel: +49 (0) 9421 9931-0  
info@genusshotel-wenisch.de

## Main sights



**1 Stadtplatz (Market Place) + Stadtturm**  
(Gothic City Tower) + Carillon (11 a.m./3 p.m.  
– 26 bells are playing various melodies)



**2 Rathaus (Town hall)**



**3 Dreifaltigkeitssäule (Holy Trinity Column)**



**4 Basilika St. Jakob (Basilica of St. Jacob)**



**5 Gäuboden Museum**



**6 Karmelitenkirche Hl. Geist (Carmelite Church of the Holy Spirit)**



**7 Ursulinenkirche (Church of St. Ursula)**



**8 Spitaltor (Northgate)**



**9 Synagoge (Synagogue)**

## Friday Event



**Kirche St. Peter**  
(St. Peter Church)  
Petersgasse 50A



**Technical University of Munich, Campus Straubing for Biotechnology and Sustainability**  
Schulgasse 22



**Hafen Straubing-Sand**  
Europaring 4

## Organizer



**C.A.R.M.E.N. e.V.**  
Schulgasse 18



**Straubing central station**

# Straubing City Map



Donau

Schloss-  
brücke

Uferstraße

Donaugasse



Unterm Rain

Theresienplatz

Ludwigsplatz

Fraunhofer Str.

Burggasse

Stadtgraben

Krankenhausgasse

Schulgasse

Heerstraße

Innere Passauer Straße

Mühlsteingasse

Stadtgraben

Kolbstraße

Bahnstraße

Mittlere Bachstraße

Jahnplatz

Bahnhofplatz

Gabelsbergerstraße

Am Platzl

Steinergasse

Rosengasse

Allachbach

Asamstraße

Dr.-Otto-Hochstet-Strasse

Landshuter Straße

Mittelsbacherhöhe

1

2



3

2

1

8

5

8

7



5

6

## Key facts

### Coat of arms



### State

Bavaria

### Admin. region

Niederbayern

### District

Urban district

### Area

Total: 67,58 km²

### Elevation

322 m

### Population (2018)

Total: 47,794



## Acknowledgements

On behalf of the organizers and the scientific committee, we want to thank all participants, sponsors, partners as well as helping hands of the ESBP 2019. Contributing actively to the ongoing bioeconomy, based on a long time history in the field of biopolymer research, renders the ESBP family a highly innovative and applied community. We hope you enjoy the ESBP 2019 and you will get new input and ideas.

We are looking forward to meet you again at the international version ISBP 2020 ([www.isbp2020.com](http://www.isbp2020.com)) or the ESBP 2021.

In-between, let's pursue the idea of a biobased economy, by replacing as much as possible fossil based polymers.

