

BioREFINE-2G Project Grant Agreement n°613771

D7.7
Second Project Workshop on
Biopolymers

August 2017



About BioREFINE-2G project

The BioREFINE-2G project - Development of 2nd Generation Biorefineries – Production of Dicarboxylic Acids and Bio-based Polymers Derived Thereof - aims at developing commercially attractive processes for efficient conversion of pentose-rich side-streams from biorefineries into dicarboxylic acids, which can be used as precursors for bio-based polymers including biodegradable polymers. Further information about the project and the partners involved are available under www.biorefine2g.eu.

Project coordinator



Project partners



About this document

This report corresponds to Deliverable D7.7 of the BioREFINE-2G project – Second Project Workshop on Biopolymers. It has been prepared by:

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Introduction - Second Project Workshop on Biopolymers

The **Second BioREFINE-2G Workshop “Utilisation of Waste Streams for Bioproducts and Bioenergy”** was organized on the occasion of the 2017 European Biomass Conference and Exhibition (EUBCE 2017) **on 12 June 2017 in Stockholm, Sweden.**



The main aim of this workshop was to present and discuss results from the BioREFINE-2G project with focus on innovative approaches and value chains towards the efficient utilisation of biorefinery waste streams for bioproducts and bioenergy.

Thereby, EUBCE 2017 offered a suitable environment for dissemination activities of the BioREFINE-2G project with its more than 1500 international participants from university, industry, governmental and non-governmental organizations. As one of the world’s leading R&D conference combined with an international exhibition, EUBCE represents the leading platform for the collection, exchange and dissemination of scientific know-how in the field of biomass.

All presentations of the second BioREFINE-2G workshop are available via the project website under: <http://www.biorefine2g.eu/news-and-events-biorefine2g/events>

The programme of the second BioREFINE-2G workshop in Stockholm is presented in **Annex I** of this report.

The Second BioREFINE-2G Workshop “Utilisation of Waste Streams for Bioproducts and Bioenergy” mobilized more than 50 international participants. The list of workshop participants is presented in **Annex II** of this report.



Workshop Summary

The Second BioREFINE-2G Workshop “Utilisation of Waste Streams for Bioproducts and Bioenergy” on 12 June 2017 in Stockholm, Sweden was opened by **Rainer Janssen**, WIP Renewable Energies.

Irina Borodina, coordinator of the BioREFINE-2G project, DTU - Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark presented an overview of activities and results of the project.

The project **BioREFINE-2G** aims at developing commercially attractive processes for efficient conversion of pentose-rich side-streams from biorefineries into dicarboxylic acids, which can be used as precursors for bio-based polymers including biodegradable polymers. The project covers the whole value chain, from characterization of side streams from forest and other non-food feedstock, development of novel robust industrial yeast cell factories, fermentation and downstream process development, to polymerization methods development for the production of biodegradable polymers applicable as plastics, coatings or adhesives, scale-up and demonstration and to life cycle and economic viability analyses. A schematic outline of the project concept is shown in figure 1.

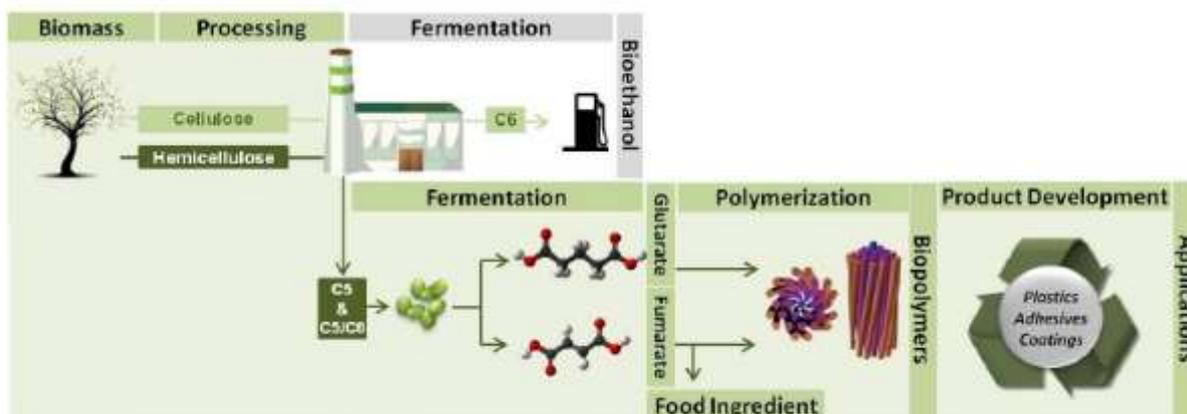


Figure 1: Schematic outline of the BioREFINE-2G concept. The main focus areas included with green background.

In the following, project results in the fields of strain engineering, process development, polymerization methods, scale-up, life cycle analysis and exploitation of results are briefly summarized.

Strain engineering

Microorganisms are among the most powerful resources on earth, but their potential has not yet been fully exploited. BioREFINE-2G aims at engineering the well-known baker’s yeast to produce industrially relevant dicarboxylic acids. In order to develop a sustainable process, the goal is to use industrial wood waste as substrate. Wild-type yeast can neither produce dicarboxylic acids, nor utilize C5 sugars, which are the major fermentable components of the waste streams. Furthermore, the inhibitors usually present in the complex biomass hydrolysates can significantly affect cell growth and performance. With the aid of advanced genetic engineering, modelling tools and

adaptive evolution, these challenges can be overcome and strains with the desired traits can be obtained. The following project results have been achieved:

- Genetic engineering toolbox for rapid and efficient manipulation of industrial yeast strains
- Industrial strains with xylose (C5 sugar) utilization capability
- Industrial yeast tolerating and performing in a selected industrial waste stream
- Industrial strains producing a mixture of dicarboxylic acids. The production titers will be further optimized and the strains will be used for process scale-up.

Process development

The challenges in the process development include firstly designing the fermentation of a complex raw material, and secondly purifying the desired carboxylic acids from the fermented broth to a required degree of purity for polymer applications.

To enable efficient fermentation of raw materials which holds several sugars - including a large fraction of pentose sugars – as well as several other compounds, the yeast *Saccharomyces cerevisiae* is a well-suited host. Process conditions are tuned to reach a suitable compromise between needed fermentation time, product yields and titers. This is done in close collaboration with the strain development efforts by the molecular biologists.

The development of a downstream processing method for the recovery of bio-based dicarboxylic acids faced many challenges to reach the purity standards suitable for polymerization. A multi-stage approach was developed to overcome the issues posed by the high amount of lignocellulosic impurities in the fermentation broth and by the fermentation by-products. Recovered solids from complete process experiments showed that purity specifications were achieved in high product yield. The optimization efforts in recycling and re-use of streams reduced the economic and environmental impact of the process.

Polymerisation methods

The objective of BioREFINE-2G is to prepare bio-polyesters from dicarboxylic acids obtained from genetic engineered yeast. The bio-polyesters are then converted into commercially interesting products such as polyurethane dispersions (PUDs) and thermoplastic polyurethanes (TPUs) used as adhesives and coatings, and polylactide(PLA)-copolymers, which can be used as biodegradable packaging plastics. The following project results have been achieved:

- Bio-polyesters production of different molecular weight by adjusting parameters such as temperature, pressure, reaction time and type of catalyst.
- Bio-polyesters production using lipases as catalyst to perform reactions at lower temperatures.
- Process scale-up by reactive extrusion.
- Bio-polyesters conversion into commercially relevant products, such as PUDs, TPUs and PLA-copolymers as potential bio-adhesives and bio-plastics for packaging.

Scale-up and product development

Within the BioREFINE-2G project scale-up trials are underway to transfer the fermentation technology from lab-scale to industrial application. The process developed at lab scale by ULUND will be implemented at pilot scale at BIOTREND. The resulting scaled-up process will be transferred to Borregard's BALI demo plant. Synergies between partners will be instrumental for a successful scale-up of the fermentations using real raw materials and in conditions suitable for downstream processing.

Life cycle analysis

Life cycle analysis of the BioREFINE-2G process allowed to identify main benefits and drawbacks as displayed in the table below:

Benefits	Drawbacks
<ul style="list-style-type: none"> • Good social performance on wood treatment and fermentation plant • Requires fewer fossil resources than conventional synthesis • Use of waste streams prevents conflicts with food and feed production 	<ul style="list-style-type: none"> • Cost competitiveness requires high yields • Conditioning of bio waste streams requires additional efforts • Agriculture related impacts (land use, eutrophication...) higher than conventional synthesis

Exploitation of R&I results

The following main results of the BioREFINE-2G project offer opportunities for further exploitation beyond the duration of the project. Efforts will be taken to effectively promote the exploitation of these project results. BioREFINE-2G Exploitation Flyers are available at: <http://www.biorefine2g.eu/publications-reports>.

- 1) DTU: Robust xylose-utilizing industrial yeast
- 2) DTU: Genetic engineering toolbox for manipulation of industrial yeast strains
- 3) BIOTREND: Fumaric acid purification process from fermented lignocellulosic wastes
- 4) AIMPLAS: Novel polymerization methods by reactive extrusion to obtain new PLA-Copolymers with enhanced properties
- 5) ECOPOL: Polyether synthesis in batch and reactive extrusion
- 6) IFU: Integrated Life-Cycle-Sustainability-Assessment

Freddy Tjosas, Borregaard AS, presented an industry perspective of more than 70 years of experience in running a leading integrated biorefinery. With its headquarters in Norway, 1050 employees, annual sales of 450 million EUR and production facilities in six countries, Borregaard is a global leading supplier of lignin based performance chemicals as well as the largest producer of ethanol from wood with a production of 20 million liters per year.

Mr. Tjosas emphasised the importance of turning all parts of the wood into products to maximise value creation through full material exploitation, as indicated in figure 2.



Figure 2: High value added through high raw material utilisation

Furthermore, Mr. Tjosas underlined that operating a successful biorefinery over several decades requires an almost continuous adaptation and modification of the biorefinery concept leading to a change of products, through the termination of production lines, the development of new product lines and the utilization of former waste streams. Thus, research and development is of high importance as 14% of company revenues come from new products.

The time evolution of Borregaard’s production lines is presented in figure 3. The production of lignin performance chemicals started around 1940 and now represents the main growth area.

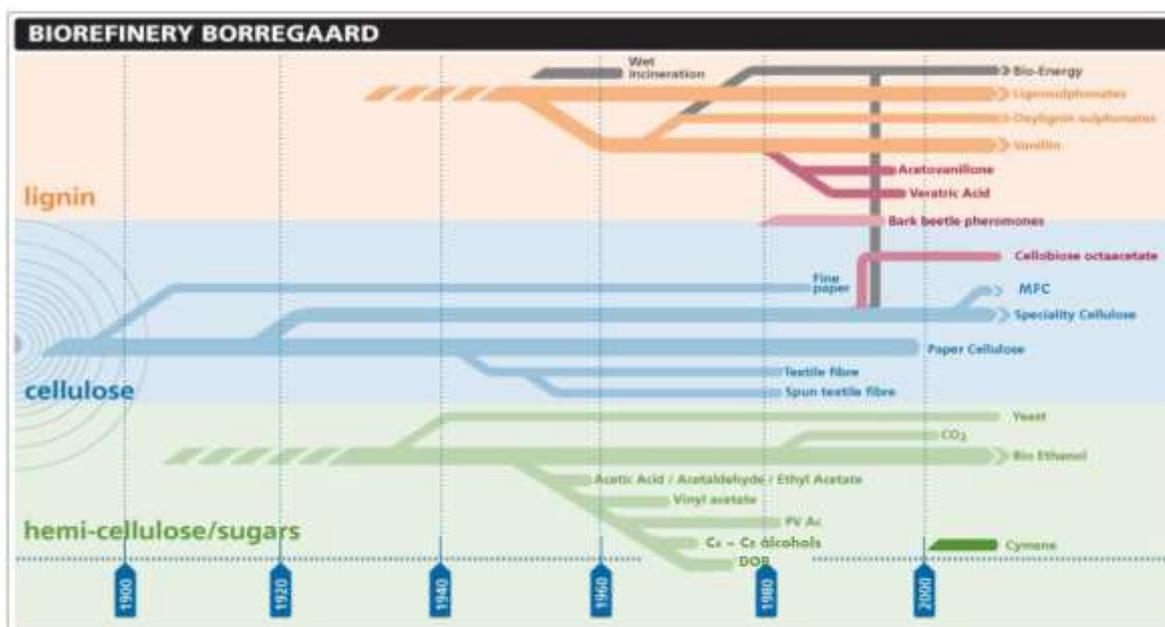


Figure 3: Time evolution of Borregaard’s production lines

In January 2013, Borregaard launched a demo plant utilizing the BALI™ process with the main aim to enable expansion of the lignin business. The BALI™ process involves pre-treatment of biomass, enzymatic hydrolysis and fermentation (see figure 4) and also integrates R&I initiatives on the production of bio-chemicals from process side or waste streams such as the research undertaken in the framework of the BioREFINE-2G project.

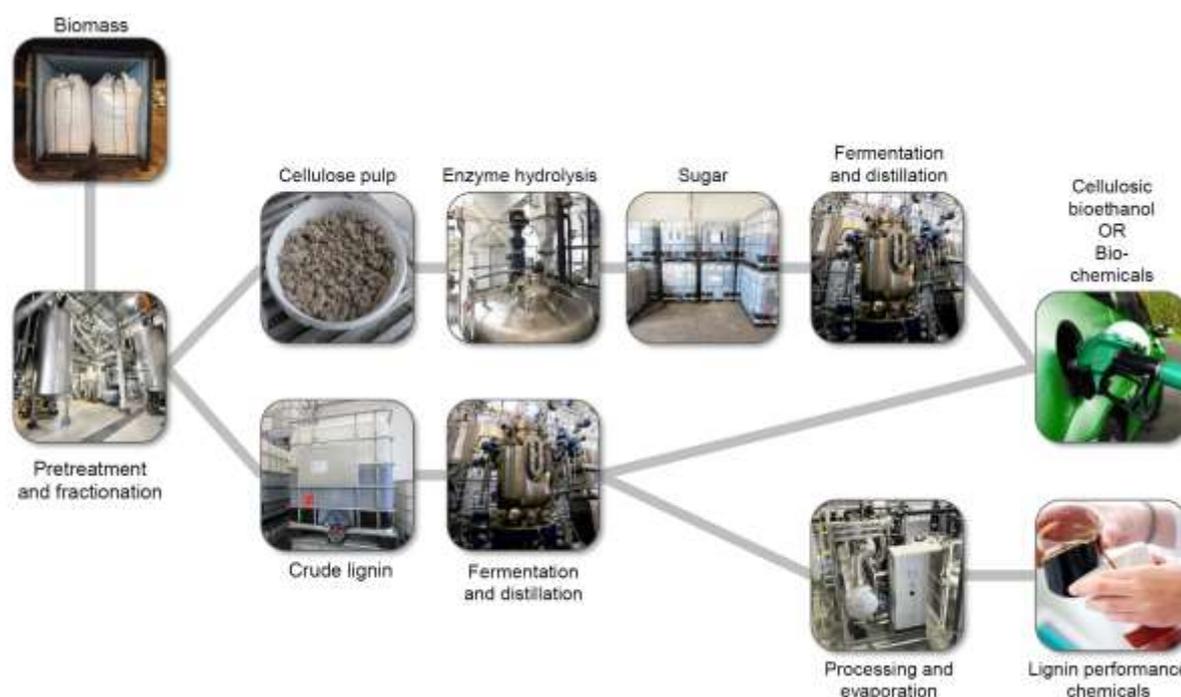


Figure 4: Schematic overview of BALI™ process

Vratislav Stovicek, DTU - Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, presented results on strain development for diacid production achieved in the framework of the Bio-REFINE-2G project.

These activities aim at the development of genetically modified *S. cerevisiae* industrial strains suitable for the production of the selected dicarboxylic acids from side and waste streams rich in C5 sugars. This will require the identification of relevant metabolic routes, the development of a toolbox for engineering industrial strains of *S. cerevisiae* and rational and evolutionary strain engineering. The toolbox allows genome sequencing of novel industrial yeast strains, as well as gives access to tools for characterization of engineered strains using RNAseq, protein and metabolite profiling as well as flux analysis.

A genetic engineering toolbox for engineering polyploid, prototrophic industrial yeast has been established. The toolbox enables to carry out rapid gene deletions and insertions. It consists of advanced molecular tools based on the CRISPR-Cas9 approach (see figure 5). Industrial yeast strains can now be modified almost as fast as laboratory strains.

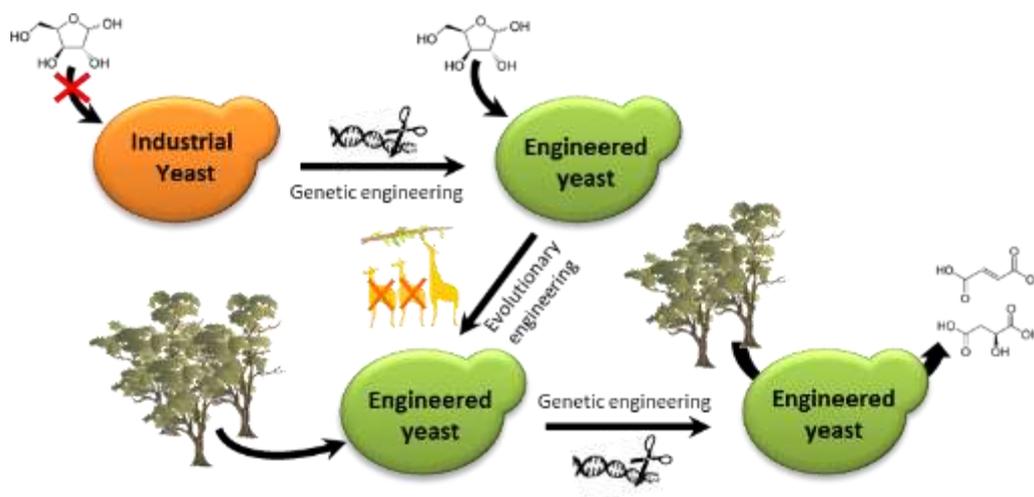


Figure 6: Schematic illustration of the engineering efforts leading to the generation of strains producing a mixture of dicarboxylic acids from a wood waste hydrolysate.

In conclusion, Mr. Stovicek highlighted the following main achievements on strain development realized within the BioREFINE-2G project:

- Development of a molecular toolbox based on CRISPR-Cas9 for fast engineering of industrial yeast strains
- Construction of xylose utilizing industrial strains
- Improved xylose utilization properties via adaptive evolution – platform strain
- Tolerance of the industrial xylose consumers to a C5-rich SSL (Spent Sulfite Liquor) (both YE, MM supplemented) at low pH – platform strain
- Industrial strain tolerant to the SSL engineered for production of dicarboxylic acids converts up to 50% of the xylose content to a mixture of diacids with low proportion of fumaric acid

Amador Garcia, AIMPLAS, Spain presented an overview on the development of polymerization processes implemented in the framework of the BioREFINE-2G project.

The main objective of this activity is to develop new biodegradable bio-based polymers based on natural raw material as potential substitutes of petroleum based polymers. The synthesis of polyesters with similar properties to polyolefin will be optimised by liquid synthesis and by reactive extrusion processes at laboratory scale as well as at pilot plant scale. Thereby, the focus in BioREFINE-2G is to prepare bio-polyesters from dicarboxylic acids which have been obtained from genetic engineered yeast (see figure 7). The bio-polyesters are then converted into commercially interesting products, such as polyurethane dispersions (PUDs) and thermoplastic polyurethanes (TPUs) used as adhesives and coatings, and polylactide(PLA)-copolymers, which can be used as biodegradable packaging plastics. Different synthetic routes are studied and the feasibility to transfer the synthesis to a continuous reaction extrusion process is tested and mathematically simulated.

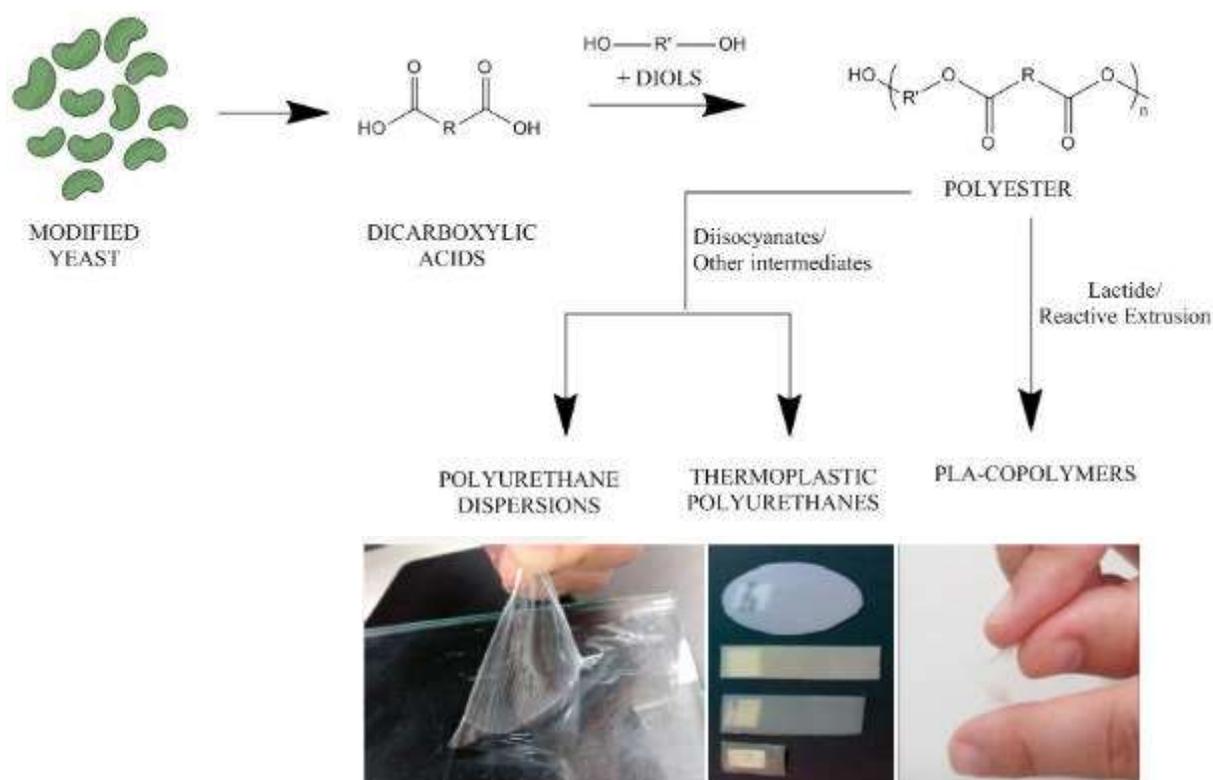


Figure 7: BioREFINE-2G polymerization schemes and samples produced (ECOPOL TECH)

In conclusion, Mr. Garcia highlighted the following main achievements on polymerization processes realized within the BioREFINE-2G project:

- Project partner BIOTREND has optimized biofumaric purification at “polymer grade”
- Biopolyester derived from biofumaric acid have been developed for biomedical applications
- Polymerization of PLA (polylactic acid) glutaric derivatives is available
- Reactive extrusion process is ready for industrial production of PLA and PLA copolymers
- Fast method for MW (molecular weight) characterization is available

Panel Discussion on “Utilisation of Waste Streams for Bioproducts and Bioenergy”

Moderation

- Irina Borodina, Technical University of Denmark

Panellists

- Freddy Tjosas, Borregaard AS, Norway
- Amador Garcia, AIMPLAS, Spain
- Bruno Sommer Ferreira, BIOTREND, Portugal
- Michael Bruns, IFU - Hamburg Institute for Environmental IT
- Maurizio Bettiga, Chalmers University of Technology, Sweden



The panel discussion was launched with brief statements by all panelists and included the opportunity of questions and contributions by the workshop audience. Important topics raised and discussed at the panel are summarized below.

The following **success factors for biorefinery concepts** were highlighted:

- Biorefineries shall focus on the production of (purified and high value) **sugars as building blocks** (from raw materials as well as from side and waste streams) for the bio-chemical market rather than the production of fuel ethanol.
- However, it was stated that high value speciality chemicals represent **rather small markets** and will thus have limited global impact on societal challenges such as climate change mitigation.
- High value chemicals could **improve the overall competitiveness of biorefineries** and thus support market penetration of high volume bulk products such as fuels.

- Furthermore, it was underlined that biorefineries are in competition with well established (fossil based) industries. ***Development of bio-based industries and biorefineries as well as the full exploitation of all side streams will take time*** and in general achievements up to date are encouraging.
- Societal support for bio-based products shall be sought through ***price premiums*** (to cover the current price gap towards fossil based products) paid by environmentally concerned customers.
- Sectors suitable for the introduction of bio-based products need to be identified and focus needs to be placed on branding and communication with society. Thereby, ***cooperation with large companies*** promoting environmentally friendly products (e.g. Coca Cola, Carlsberg, Lego) are important.

The audience raised the topic of ***sustainability of bio-based products*** such as polymers produced from biorefinery side streams within BioREFINE-2G. It was highlighted that a clear focus on sustainability is important to ensure societal support and avoid problems faced by the biofuels industry in recent years.

- BioREFINE-2G includes a dedicated work package on performing a detailed Life Cycle Analysis (LCA) which will be finalized in the coming weeks.
- The use of side streams of existing processes are generally beneficial with respect to sustainability impacts.
- Focus of this LCA is the comparison of bio-based and fossil based polymers (e.g. PLA).
- In addition to the ***quantitative and qualitative evaluation*** of environmental, economic and social impacts, the LCA aims to provide ***feedback for the optimization of BioREFINE-2G process steps***. It was found that the evaporation step dominates the energy consumption of the purification process and thus has strong impact on the overall performance.
- Finally, it was highlighted that LCA methodologies for bio-based products are still under development and further ***harmonization of these methodologies*** needs to be achieved.

Further discussions among the panelists and the audience focused on ***(societal) responsibilities*** for tackling societal challenges such as climate change, resource efficiency, and energy security.

- The ***important role of elementary school teachers*** for the education of society towards a more sustainable and resource aware economy was underlined.
- Societal choices need to be geared towards more environmentally friendly choices by educating and involving young generations. This could create a future market pull for bio-based products.

- For this, the elaboration of **educational material** (for all levels of education, but focusing on early education) is needed.
- It was furthermore stated that societies may need to feel stronger the effects of climate change before more targeted actions are taken.
- However, a certain degree of optimism was voiced due to recent **initiatives towards clean technologies within leading economies** worldwide (e.g. USA, China).

Finally, discussion was raised about which **main technologies and technological approaches** will contribute to the development of a bio-based economy.

- The **role of GMO** within the future bio-based economy and different perceptions towards GMO in Europe and other countries were highlighted.
- Customers may be more supportive of GMO-based plastics (than GMO-based food and feed products), but concerns may arise with respect to GMO packaging material.
- Large companies promoting bio-based plastics (e.g. PLA (polylactic acid) polymers produced by the company NatureWorks from USA) take a **low profile on the GMO origin** of the products.
- The need for **on-going societal discussions and education in Europe** on opportunities and potential negative impacts of GMO bio-based products within the bio-based economy was emphasized.
- It was highlighted that (new) collaboration between different industrial sectors need to be established for the development of a bio-based economy. Thereby sectors need to cooperate which until today have limited “interactions” (e.g. forestry and plastics sectors).
- Focus shall be placed on technologies aiming at the production of **sugars as building blocks** for the bio-chemical market.
- Biorefinery concepts need to ensure **full raw material exploitation** (including all side and waste streams) for the production of a multitude of products to maximise value creation.
- Successful biorefineries require **continuous adaptation and modification of technologies and concepts** aiming at full use of all side streams and the development of new product lines.

Annex I – Workshop Programme



Development of 2nd Generation Biorefineries - Production of Dicarboxylic Acids and Bio-based Polymers Derived Thereof

WORKSHOP

Utilisation of Waste Streams for Bioproducts and Bioenergy

on the occasion of the
2017 European Biomass Conference and Exhibition
(EUBCE 2017)
Stockholm, 12 - 15 June 2017

Date: Monday, 12th June 2017

Timing: 13.00 - 17.00

Location: Room K22, first floor

Contact

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This workshop is organised in the framework of the project BioREFINE-2G (www.biorefine2g.eu) supported by the European Commission in the 7th Framework Programme.

Current second generation biorefineries often utilize less than 20% of the biomass feedstock for ethanol production, and major side-streams are produced such as pentose and lignin waste streams, that are respectively used for biogas and energy production. Converting the carbon from these waste streams into added-value products would increase the otherwise low profitability and improve the environmental benefits of the biorefineries.

The project BioREFINE-2G aims at developing commercially attractive processes for efficient conversion of pentose-rich side-streams from biorefineries into dicarboxylic acids, which can be used as precursors for bio-based polymers including biodegradable polymers.

This workshop will present and discuss results from the BioREFINE-2G project with focus on innovative approaches and value chains towards the efficient utilisation of biorefinery waste streams for bioproducts and bioenergy.



BioREFINE-2G is co-funded by the European Commission in the 7th Framework Programme (Project No. FP7-613771)



WORKSHOP AGENDA

Utilisation of Waste Streams for Bioproducts and Bioenergy

13:00 *Registration and Light Lunch*

13:30 **Welcome to the Workshop**

RAINER JANSSEN, WIP RENEWABLE ENERGIES, GERMANY

13:45 **The BioREFINE-2G Project - Activities and Results**

IRINA BORODINA, TECHNICAL UNIVERSITY OF DENMARK

14:15 **Utilisation of Waste Streams - An Industry Perspective**

FREDDY TJSÓS, BORREGAARD AS, NORWAY

14:45 *Coffee Break*

15:15 **Strain Development for Diacid Production**

VRATISLAV STOVICEK, TECHNICAL UNIVERSITY OF DENMARK

15:45 **Development of Polymerisation Methods**

AMADOR GARCÍA, AIMPLAS - INSTITUTO TECNOLÓGICO DEL PLÁSTICO, SPAIN

16:15 **Panel Discussion on Utilisation of Waste Streams for Bioproducts and Bioenergy**

MODERATION: IRINA BORODINA, TECHNICAL UNIVERSITY OF DENMARK

PANELLISTS:

FREDDY TJSÓS, BORREGAARD AS, NORWAY

AMADOR GARCÍA, AIMPLAS - INSTITUTO TECNOLÓGICO DEL PLÁSTICO, SPAIN

BRUNO SOMMER FERREIRA, BIOTREND, PORTUGAL

JOSEP ROCAS, ECOPOL TECH S.L., SPAIN

MICHAEL BRUNS, IFU - HAMBURG INSTITUTE FOR ENVIRONMENTAL IT, GERMANY

MAURIZIO BETTIGA, CHALMERS UNIVERSITY OF TECHNOLOGY, SWEDEN

16:50 **Summary and Conclusions**

RAINER JANSSEN, WIP RENEWABLE ENERGIES, GERMANY

17:00 *End of the workshop*



Annex II – List of Workshop Participants

First Name	Last Name	Organisation	Country
Rainer	Janssen	WIP Renewable Energies	Germany
Irina	Borodina	Technical University of Denmark	Denmark
Freddy	Tjosas	Borregaard SA	Norway
Vratislav	Stovicek	Technical University of Denmark	Denmark
Amador	Garcia	AIMPLAS	Spain
Bruno	Sommer	Biotrend SA	Portugal
Michael	Bruns	ifu Hamburg GmbH	Germany
Mauricio	Bettiga	Chalmers University of Technology	Sweden
Luc	Pelkmans	VITO	Belgium
Mies	Van Dad	VITO	Belgium
Ozgul	Caliaglu	Penn State University	USA
Uwe	Fritsche	IINAS	Germany
Lisbeth	Olsson	Chalmers University of Technology	Sweden
Dominik	Rutz	WIP Renewable Energies	Germany
Rita	Mergner	WIP Renewable Energies	Germany
Ingo	Ball	WIP Renewable Energies	Germany
Ruben	Guisson	VITO	Belgium
Kees	Kwant	RVO	The Netherlands
Regis	Leal	CTBE/CNPEM	Brazil
Mina	Bajaj	SYNCOM	Germany
Rocio	Diaz-Chavez	Imperial College	UK
Viktoria	Leitner	Kompetenzzentrum GmbH	Austria
Florian	Gattermayr	Kompetenzzentrum GmbH	Austria
Rory	Monaghan	Nui Galway	Ireland
Netta	Sharma	ENEA	Italy
Marlous	Van Dyk	Chalmers University of Technology	Sweden
Emma	Karlsson	Chalmers University of Technology	Sweden
Andres	Moreno	UCLM	Spain
Herbert	Baset	DST	South Africa
Jonas	Erikson	City of Stockholm	Sweden
Swarnima	Aqnihotri	HB	Sweden
Hannes	Gruber	TU Wien	Austria
Frank	Hedlund	COWI	Denmark

First Name	Last Name	Organisation	Country
Jonathan	Wong	Hong Kong Baptist University	Hong Kong
Arturo	Infante	FedeBiocombustibles	Colombia
Silvia	Maltagliati	ARPAT – DG RTD.F2	Italy/Belgium
Carl Johan	Franzen	Chalmers University of Technology	Sweden
Dodiek	Candra	Hochschule Aschaffenburg	Germany
Ilona	Sarvari Horvath	University of Boras	Sweden
Annarita	Salladini	Processi Innovativi SRL	Italy
Mirjam	Roeder	University of Manchester	UK
Cristina	Negri	Argonne National Laboratory	USA
Bernd	Krautkremer	Fraunhofer IWES	Germany
Francesco	Ometto	Scandinavian Biogas Fuels	Sweden
Sepehr	Shakeri Yekta	Linköping University	Sweden
Huang	Chiang-Fang	Institute of Nuclear Energy Research	Taiwan
Matteo	Romano	Politecnico de Milano	Italy
Juergen	Loipersboeck	Bioenergy 2020+	Austria
Eric	Peterson	ICESI University	Colombia
Nathalie	Devriendt	VITO	Belgium
Johanna	Olofsson	Lund University	Sweden
Suneerat	Fukuda	King Mongkut's University	Thailand
Wim	Van Swaaij	Twente University	The Netherlands
Sata	Errasti	Natural Resources Institute Finland	Finland
Michael	Daramola	University of Witwatersrand	South Africa
Patrick	Mukumba	University of Fort Hare	South Africa
Augustine	Ayemi	University of Witwatersrand	South Africa