Session 3   14:00 - 18:00   Netherlands III

Innovative Bioproduction for Sustainable Society

14:00 Opening: Prof. Akihiko Kondo, Kobe University

14:05 Dr. Hideki Fukuda, President of Kobe University
"Innovative Bio-production in Kobe: iBioK"

14:15 Prof. Akihiko Kondo, Kobe University
"Production of Bio-based Chemicals and Fuels from Biomass"

14:40 Prof. Ken-ichi Yoshida, Kobe University
*"Bacillus subtilis Cell Factory for scyllo-Inositol Production:
Discovery of an Inducible NADPH Regeneration"*

15:05 Assoc. Prof. Tsutomu Tanaka, Kobe University
*"Bio-based Chemicals Producution from Oligosaccharides
by Engineered Bacterial Cells"*

15:30 - Coffee Break -

15:50 Prof. Dr. François Reniers, Vice-president of GREENWIN
"Overview on Biomass Research in French Speaking Part of Belgium"

16:00 Prof.Em. dr.ir. Erick Vandamme, Ghent University
"Biomass Research in Ghent University"

16:10 Prof. Dr. Jozef Anné, KU Leuven
*"Greener Industry with Improved Production Strains for Heterologous Proteins
Using Streptomyces Lividans as a Model"*

16:35 Prof. dr. ir. Wim Soetaert, Ghent University
"Bio Base Europe: Open Innovation and Education for the Biobased Economy"

17:00 Dr. ir. Inge Van Bogaert, Ghent University
"Engineering of Candida Bombicola for the Production of Tailor-made Biosurfactants"

17:25 Prof. Dr. Philippe Dubois, Vice-rector of University of Mons UMONS
*"Bio-sourced Lactic Acid-based Polymers:
From Reactive Extrusion to High Performance Materials"*

17:50 Summary

The theme of session 3 was Innovative Bioproduction for Sustainable Society. Participants were researchers from Kobe University, Ghent University, KU Leuven, Université Libre de Bruxelles, and University of Mons. President Fukuda began the session by introducing Kobe University's collaborative research project "Innovative BioProduction Kobe." Then distinguished researchers made presentations on various bio-production projects and educational programmes undertaken in the EU nations led by Belgium. The session became an opportunity for the participating researchers to find many common interests with other researchers and seek ways to collaborate with each other.

Hideki Fukuda, Ph.D.
President of Kobe University

"Innovative Bio-production in Kobe: iBioK"

To build a sustainable, low carbon society, shifting Green Innovation from the oil-refinery to bio-refineries must a key area of research. "Bio-refinery" is a excellent technology to produce biofuels, bio-plastics, bio-fibres and bio-chemicals from biomass using carbon dioxide as a recyclable resource. iBioK has pioneered "BioProduction", which can produce fuels and chemicals from cellulosic biomass. A big paradigm shift made possible by the iBioK project is the changing from oil-dependent target products produced from oils to bio-based products.

Introducing bio-based products into markets from iBioK achieves green innovation by shifting from oil-based products to bio-based products. iBioK is concerned with the following three areas; bulk chemicals such as "Biofuels and Biochemicals", "Bioplastics and Biofibers", and "BioFineChemicals" with high additional values. Alcohols, diols, organic acids, diamines, amino acids, aromatic compounds have been produced as biofuels, biochemicals, bioplastics and biofibres with significant impact. Value-added sugars, functional inositols, peptides, phospholipids have also been produced as BioFineChemicals. As a fundamental technology, biomass resouces, cellulases, bioreactors and separation processes have been developed to widely diffuse iBioK BioProduction.

Establishment of Bio-production can generate huge, novel markets through a number of target products. IBioK can potentially have a large impact on reducing oil dependency, biomass conversion, reduced CO_2 emissions, agricultural production, forestry and fishery industries.

Akihiko Kondo, Ph.D.
Professor, Department of Chemical Science and Engineering
Director of Biorefinery center, Kobe University

"Production of Bio-based Chemicals and Fuels from Biomass"

To build an energy and material secure future, we must pioneer the next generation of renewable fuels and chemicals using environmentally-friendly production process. To meet this goals, we have promoted many kinds of research projects related the field of bioproduction. One of the key technologies is cell surface display, which is a powerful tool to engineer and functionalize many microorganisms. Using the technology, various kinds of functional proteins such as enzymes can be expressed on the cell surface without loss of their activities. The other key technology is synthetic bioengineering, which is also powerful tool to engineer the metabolic pathway for efficient and robust Bioproduction from biomass.

Metabolic profiling and flux analysis allow to pathway optimization and pathway design for biofuels and biochemicals production. We established the whole metabolites analysis system based on systematic bioengineering, which can accelerate the development of product yield, production speed, and the tolerance against some inhibitor. We should proceed with bioproduction research and practical realization more and more, to build sustainable society.

Ken-ichi YOSHIDA, Ph.D.
Professor of Applied Microbiology, Department of Agrobioscience, Graduate School of Agricultural Science, Kobe University

"Bacillus subtilis Cell Factory for scyllo-Inositol Production: Discovery of an Inducible NADPH Regeneration"

Inositol stands for a class of compounds forming nine stereoisomers through epimerization of the six hydroxyl-groups. *myo*-Inositol (MI) is one of the isomers, most abundant in nature, and supplied cheap from phytin in rice-bran. Some of the other isomers are rare and thus very expensive but reported to possess interesting biological functions for the treatment of diseases difficult to treat. For instance, *scyllo*-Inositol (SI) is undergoing clinical investigation for the treatment of Alzheimer's disease, as it has received fast track designation from the U.S. FDA. We demonstrated that manipulating the inositol metabolism in *Bacillus subtilis* enabled an efficient cell factory to covert cheap MI to valuable SI, by which almost 50% of MI initially contained in the medium was converted to SI after 48-h cultivation. Transcriptomic analyses were performed to understand the efficient bioconversion to reveal that a global change in metabolic pathways might occur to fulfill the demand of required coenzyme regeneration.

Tsutomu TANAKA, Ph.D.
Associate Professor, Department of Chemical Science and Engineering, Graduate School of Engineering, Kobe University

"Bio-based Chemicals Producution from Oligosaccharides by Engineered Bacterial Cells"

We demonstrated direct assimilation of cellooligosaccharides and xylooligosaccharides using beta-glucosidase (BGL) and beta-xylosidase co-displaying *E. coli*. After screening active BGLs, Tfu0937, which is a BGL from *Thermobifida fusca* YX, was successfully displayed on the *E. coli* cell surface and directly assimilated cellobiose as a carbon source. Then, we screened for a suitable XYL for xylooligosaccharides assimilation. Active XYL was successfully displayed on the *E. coli* cell surface and directly assimilated xylooligosaccharides as a carbon source. Finally, we created BGL/XYL co-displaying *E. coli* and successfully demonstrated co-assimilation of cellobiose/xylooligosaccharides mixture as carbon sources without carbon catabolite repression. The engineered *E. coli* is a candidate for a platform of bio-chemicals production from biomass, and bio-chemical production is demonstrated. In addition, using similar approaches, we created BGL-displaying *Corynebacterium glutamicum* and demonstrated Lysine production from cellobiose directly.

Prof. Dr. François Reniers

Dean of the Faculty of Sciences, Université Libre de Bruxelles
Vice-president of GREENWIN

"Overview on Biomass Research in French Speaking Part of Belgium"

Biomass is seen nowadays as a new renewable source of chemicals, materials, and energy. However, it is only one component of a new approach of chemistry and industrial processes, that focus nowadays on concepts such as life cycle analysis, recycling, cradle to cradle, energy efficiency. A brief overview of the status of research involving biomass, and more generally sustainable chemistry, in the French speaking part of Belgium will be presented. The different funding agencies and their basic rules will be explained. The overview identifies the actors (university laboratories, research centres, private companies). Some recent research projects will be highlighted.

Prof.Em. dr.ir. Erick Vandamme

Centre of Expertise -Industrial Biotechnology and Biocatalysis, Dept.Biochemical and Microbial Technology, Fac.Bioscience Engineering,
Ghent University, Ghent, Belgium

"Biomass Research at Ghent University"

At the Centre for Industrial Biotechnology and Biocatalysis (InBio), improved processes are being developed for the conversion of renewable resources into various biobased products, such as prebiotic oligosaccharides, pharmaceutical glycosides and glycolipid surfactants. These goals can be achieved thanks to the collaboration of three research professors, i.e. Tom Desmet (biocatalysis and enzyme engineering), Marjan De Mey (fermentation and metabolic engineering) and Wim Soetaert (process integration and scale-up). In addition, state-of-the-art tools are available for biotechnological research, including a high-throughput screening platform, software for *in silico* modelling and a range of fermentors with automated process control.
Recently, a Multidisciplinary Research Platform (MRP 'Ghent Bio-Economy') has been established at Ghent University that wants to bridge the gap between plant ('green') and industrial ('white') biotechnology. To that end, 12 different laboratories have joined forces to integrate the different steps of the biobased economy, i.e. from the primary production of plants, over the (bio) chemical conversion of biomass, to the management of waste streams and the recycling of nutrients. Besides the sustainable production of biofuels, biochemicals and biomaterials, the project also aims to alleviate the problem of CO_2 emissions by storing the unconverted biomass in the soil in the form of biochar.

Prof. Dr. Jozef Anné

Laboratory of Molecular Bacteriology, KU Leuven, Belgium

"Greener Industry with Improved Production Strains for Heterologous Proteins Using Streptomyces Lividans as a Model"

Both in molecular and medical research and in the bio-industry, recombinant protein production is an important tool, for which several prokaryotic and eukaryotic expression systems are available or being tested. Among the bacterial systems applied as platform for the production of biopharmaceuticals and for industrial enzymes, *Streptomyces* is considered an attractive host, because several strains have inherently a high secretion capacity and low endogenous protease activity. While several proteins are secreted to commercially acceptable levels using this host, some others are poorly secreted, indicating the need for further optimization. Bottlenecks can be located at different levels, ranging from transcription and translation of the heterologous gene to the secretion process and the secretome-metabolome interactions.
To get a better insight in possible bottlenecks, a systems

biology approach could be helpful to identify genes/proteins with key roles in protein secretion and their interrelationship with cell growth, secretion stress control and energy production/consumption. Such information permits targeted manipulation of specific genes or metabolic pathways for a better energy generation, and hence to make the system more sustainable.

Prof. dr. ir. Wim Soetaert
Centre of Expertise for Industrial Biotechnology and Biocatalysis (InBio.be)
Ghent University, Faculty of BioScience Engineering

"Bio Base Europe: Open Innovation and Education for the Biobased Economy"

The bio-based economy is strongly developing today as a consequence of the strong price increase for fossil resources such as petroleum, the drive towards sustainable production processes and to reduce the emission of greenhouse gases such as CO_2. The combination of these factors causes a strong penetration of biobased products and processes in a multitude of industrial sectors, particularly in the chemical industry, the energy sector, and the agro-industry. As a consequence, the transition from a fossil-based economy to a biobased economy has clearly begun.

The development of the biobased economy is seriously handicapped by a number of problems. First of all there is a serious gap in the innovation chain, caused by the lack of pilot and demonstration facilities. These facilities are required to scale up a process from a laboratory setting to an industrial production plant. The lack of pilot facilities for biobased processes seriously limits most industrial and academic players to realize their plans and to valorise their knowledge. As a second problem, there is a general shortage of well-trained process operators with experience in biobased processes. Apart from a generally decreasing interest for technical professions by youth, the problem is reinforced through the lack of specific training facilities for biobased activities.

Bio Base Europe is a joint initiative by Europe, Belgium and the Netherlands to alleviate these problems. They have united their forces in order to speed up the development of a sustainable biobased economy in Europe. Bio Base Europe has built research and training facilities for the biobased economy with an overall budget of 21 M€. The Bio Base Europe Pilot Plant is a flexible and diversified pilot plant, capable of scaling up and optimising a broad variety of biobased processes up to the multi-ton scale. The pilot plant contains fermenters and chemical reactors up to 15 m^3 scale, as well as a large variety of equipment for biorefining and up- and down-stream processing. The pilot plant is a one-stop-shop, capable of performing the entire value chain in a single plant, from the green resources up to the final product. The Bio Base Europe Pilot Plant is a completely independent facility that is operating according to the open innovation model. As such, the Bio Base Europe Pilot Plant is open to all players of the bio-based economy that can use the equipment to develop biobased products and processes. The Bio Base Europe Training Center houses a number of training facilities for biobased activities, and is operating according to an open education model. Companies as well as schools can rent these facilities for tailor-made training programs of their personnel or students.

Bio Based Europe is an important building block for the development of the biobased economy in Europe. This research and training infrastructure is expected to improve economic growth, innovation and sustainable development. This will lead to a strong innovation dynamic, a flood of new projects, diversified contacts, networking and collaboration and in general a reinforcement of the open innovation and education model for biobased activities.

More information on Bio Base Europe can be found on www.bbeu.org

Dr. ir. Inge Van Bogaert
Centre of Expertise for Industrial Biotechnology and Biocatalysis, Ghent University

"Engineering of Candida Bombicola for the Production of Tailor-made Biosurfactants"

The yeast Candida bombicola is able to synthesize and secrete one of the most promising bio-surfactants or biological surface active agents: sophorolipids, and this at economical relevant yields of over 400 g/L.

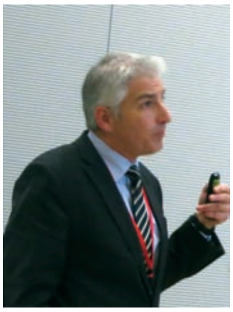
Just like their chemical counterparts, biosurfactants such as sophorolipids find applications in the food, pharmaceutical, cosmetic, and cleaning industries. Furthermore, sophorolipids display specific biological properties. Biosurfactants produced by fermentation offer a worthy alternative to traditional surfactants, which are typically derived from non-renewable petrochemical resources and may cause environmental problems due to their ecotoxicity and poor biodegradability.

The large majority of the research on sophorolipids is conducted on optimization of the feeding strategy and fermentation parameters, while the clarification of the biosynthetic pathway remains mainly neglected. Yet, insight in the biochemical process is a fundamental prerequisite for profound understanding, controlling and engineering of the production process. Therefore, we de novo sequenced the full genome of the yeast and set up extensive transcriptomics and proteomics experiments. This among others resulted in the identification of the six sophorolipid core enzymes and research is ongoing on their regulation and expression profiles.

Finally, now the sophorolipid biochemical pathway is identified and characterized, genetic engineering strategies can be applied in order to produce new-to-nature biosurfactants with novel properties, in this way broadening the application potential of biosurfactants. Several examples will be discussed.

Prof. Dr. Philippe Dubois
Laboratory of Polymeric and Composite Materials, Center of Innovation and Research in Materials & Polymers, Materials Research Institute, University of Mons UMONS

"Bio-sourced Lactic Acid-based Polymers: From Reactive Extrusion to High Performance Materials"



Bio-sourced polymers, so-called bioplastics, are currently receiving considerable attention for applications such as packaging films as well as textile fibers, and more recently, as nanocomposites for durable technical applications in automotive and electronic industries.

Polylactic acid or polylactide (PLA) certainly represents one of the most investigated and industrially developed bioplastics. However, the use of PLAs is still restricted by their relatively high production cost and limited mechanical properties compared to commodity (petro) plastics. In this contribution, we will show how reactive extrusion (REx) technology can serve on the sustainability and future growth of high performance bioplastics like PLAs. First continuous PLA production through catalyzed ring-opening polymerization will be presented. Subsequently, in order to better suit their properties to specific applications, chemical modification of PLAs by reactive extrusion (e.g., internal plasticization via *in situ* reactive grafting) will be discussed. Furthermore, PLA-based nanocomposites have been prepared starting from various (functionalized) nanoparticles such as organo-modified nanoclays and zinc oxide nanoparticles. In order to reach high thermo-mechanical, antibacterial, UV absorption and gas barrier properties, it is required to properly control the interfacial compatibilization between the finely dispersed nanofillers and the polyester matrix. This is precisely where REx can be useful paving the way to applications as high performance films and fibers.