

**BioTech 2017
and
7th Czech-Swiss Symposium with Exhibition**

Book of Abstracts



Czech Biotechnology Society
and
University of Chemistry and Technology, Prague
and
Institute of Biotechnology, LFSM, ZHAW Wädenswil

BioTech 2017
and
7th Czech-Swiss Symposium with Exhibition

Prague, June 13-17, 2017

Book of Abstracts

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Patronage

The Organizers are proud to announce that the conference is organized under the patronage of Dr. Pavel Bělobrádek, Deputy Prime Minister of the Czech Republic for the Science, Research and Innovation, Chairman of the Research, Development and Innovation Council, prof. Eva Zažímalová, President of the Czech Academy of Sciences and Mgr. Ondřej Kolář, Mayor of the Municipal District of Prague 6.





Welcome Address by Eva Zažímalová

Dear Ladies, dear Gentlemen,
dear Colleagues,

It is an honour and pleasure for me to be invited as one of the patrons of the “BioTech 2017 and 7th Czech-Swiss Symposium”. On behalf of the Czech Academy of Sciences, I would also like to welcome all of you, whether colleagues-scientists or partners from industry or young students or just sympathisers, who have come to discuss topics of advanced biotechnologies. Undoubtedly, the “BioTech 2017 and 7th Czech-Swiss Symposium” has established itself as a very important event, the aim of which is not only to exchange information at the biotechnology-related topics but also to further support necessary collaboration between the academic and applied spheres.

Contemporary scientific and technological development entails an increasing expectation of society towards science. The main challenges in the current world include sustainability of the quality of life and the environment, reasonable usage of natural resources, assurance of economic development, social solidarity, and - last

but not least - control of the influence of rapid technological changes on nature, society and individuals. The possibilities of resolving these challenges at the level of individual countries are limited and also determined by the means that are available at the given country. At the same time, it is increasingly evident that only large interdisciplinary and mostly international teams can find answers to a number of present research problems and challenges. Therefore, we are always looking for new ways to connect and integrate the research activities at national and international levels, or how to make use of limited public resources more effective.

Considering the above-mentioned context, in 2014 the Czech Academy of Sciences adopted the so-called Strategy AV21, through which we flexibly respond to specific social problems and whose implementation reflects awareness of the Czech Academy of Sciences of its obligation to the public. Hence the motto of the Strategy AV21 is “Top research in the public interest”. Importantly, and at the same time, the research programmes of the Strategy are from the very beginning open to partners from universities, the business sphere and institutions of public administration as well as to foreign research groups and organizations. Out of a total of 18 research programs, I could mention some of the topics that have high application potential in the area of biotechnology, such as: Quality of life in health and disease, Food for the future, Molecules and materials for the life, and Preclinical testing of potential drugs.

We also place great hopes in the BIOCEV project, in which two leading re-

search institutions in the Czech Republic – the Czech Academy of Sciences and Charles University have interconnected their research and human potential to jointly build an international centre of excellence in advanced biotechnology and biomedical research. The aim of the project’s scientific programme is a detailed understanding of cellular mechanisms at the molecular level, which will be an inspiration for applied research and the development of new medical treatments, early diagnosis, biologically active agents, including chemotherapeutic agents, protein engineering and other technologies. Similarly to Strategy AV21, the latest technology in centralized laboratories will also be made available to interested parties outside BIOCEV – to teams from other research institutions or to firms. I am convinced that this centre will achieve European-wide importance and make possible highly desirable deve-

lopment of an advanced biotechnological industry. Besides BIOCEV, in recent years the Czech Academy of Sciences achieved a series of important scientific results that also have application potential in the field of biotechnology and namely in medicine, such as e.g. new methods for the preparation of biomaterials for regenerative medicine and tissue engineering or very promising polymeric nanoparticles for drug carriers or as an imaging agent in diagnostic applications (involving both diagnosis and therapy).

At the conclusion of my welcome address, I wish you successful and productive “BioTech 2017 and 7th Czech-Swiss Symposium”, fruitful discussions, many new friends, and a pleasant stay in beautiful Prague.

Eva Zažímalová
President of the Czech Academy of Sciences



Welcome Address by Ondřej Kolář

Dear participants of
the BioTech 2017 Conference,
ladies and gentleman,

It is my great pleasure to welcome you all in Prague 6 – a district of the capital city of the Czech Republic, which has a few interesting superlatives. Apart from being the biggest and greenest of all the city’s districts, it is also a home to the biggest number of technical and science universities and to a world-renowned and respected institute. The Institute of Organic Chemistry and Biochemistry is truly something to be proud of, as well as the Czech Technical University, the University of Chemistry and Technology and the National Library of Technology, which all together make and jointly form what we call “The Dejvice Campus”. I am glad that our municipality can participate with these great institutions in creating a better environment for both the academic public as well as common Prague 6 citizens who only pass through the Campus on their

way home.

To be honest, to me the word “biotechnology” sounds like a phrase from a language of an alien race from a distant galaxy, which has come to visit Earth to enrich our vocabulary with a few words of their own. Yet whenever I hear or see the word in written form, something tells me that it is something worth being respected, but that it is also something mysterious. What is biotechnology? What does it do? Where did it come from and where is it going? Who are the people behind? What are the outcomes of this field of science? How does biotechnology affect our everyday life? I am sure that many people ask such questions and are somewhat eager to get the right answers. I am more than certain that many of us, laymen, don’t see that biotechnology surrounds us and influences our lives in many ways. Be it through new medicinal techniques to research in agriculture and other areas of human activity. Not only biotechnology, but science and research as such are something that pushes the mankind forward. Science gave us all we have and will certainly give us more in the future. I am glad that Prague 6 will once again become the home of an important scientific event, where experts from all around the world will share their ideas and knowledge. This again makes Prague 6 special and gives us another superlative.

I am grateful to co-host the Biotech 2017 and the 7th Czech-Swiss Symposium and wish all of you both a great stay in Prague 6 and an inspiring and creative conference.

Ondřej Kolář
Mayor of Prague 6



Welcome Address by Karel Melzoch

Distinguished Guests,
Ladies and Gentlemen!

I have great pleasure to welcome you to the BioTech 2017 symposium. It is an honour for me and my colleagues from the University of Chemistry and Technology that we can co-organize bilateral Czech-Swiss scientific event in close cooperation with our partners from Switzerland. Over the years, we have welcomed many distinguished guests and speakers who have presented significant and interesting lectures. At the same time, BioTech symposia have brought together many people during informal discussions that have helped to grow a unique network of experts in biotechnology within academia and industry. Also this year's BioTech 2017 will cover all important aspects of advanced biotechnologies. The main scope of the symposium is to pro-

mote the exchange of information among academic and industrial researchers regarding technologies utilizing life-based systems to produce useful products and services. Lectures will cover areas such as Medical and Pharmaceutical Biotechnology, Microalgae Biotechnology, Environmental Biotechnology, Food and Agriculture Biotechnology, Biorefinery and Industrial Biotechnology.

Let me introduce you briefly our institution, the University of Chemistry and Technology, Prague (formerly the Institute of Chemical Technology Prague, ICT Prague), the hosting institution of the BioTech 2017. We are a public university providing education and pursuing scientific research and development, and its implementation. Our University is known for both the depth and breadth of its education and research in almost all branches of chemistry, including chemical engineering, food chemistry and technology, biochemistry, refining, water-treatment, power, biological sciences and technologies, as well as environmental protection, material sciences and other chemistry-based fields of study. The UCT Prague consists of four faculties, Chemical Technology, Environmental Technology, Food and Biochemical Technology, and Chemical Engineering. UCT Prague, that this year celebrates 65 years of independent existence, is the world's largest, independent university specialising in chemical education with a rich network of international ties. Among the famous names, connected with our University, can be listed outstanding chemists such as Professor Otto Wichterle, the inventor of soft contact lenses, and Vladimír Prelog, Professor at the ETH Zürich, who was awarded the

Nobel Prize for Chemistry in 1975.

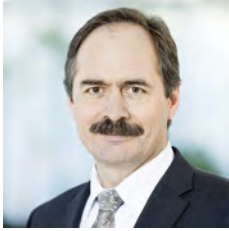
At this place, I would like to express my gratitude to our patrons, Dr. Pavel Bělobrádek, Deputy Prime Minister of the Czech Republic for the Science, Research and Innovation, Chairman of the Research, Development and Innovation Council, prof. Eva Zažímalová, President of the Czech Academy of Sciences and Mgr. Ondřej Kolář, Mayor of the Municipal District of Prague 6 for their support. I would also like to thank to the sponsors and organizational team for all the work that was (and will be!) done.

And, last but not least, I thank you,

our visitors and participants, for giving us credit by coming to participate at Biotech 2017 – I wish you a pleasant stay in the city of Prague and I hope you find here many fruitful and inspiring ideas and partnerships that would further enhance your scientific work and biotechnology in general.

Karel Melzoch

*Rector of the University of Chemistry and
Technology, Prague*



Welcome Address by Urs Hilber

Once again three years have passed since the last Czech-Swiss Symposium, the 6th, back in 2014. We are proud and honoured to once again be able to contribute to this important scientific exchange, and to help strengthen the ties between our countries and universities.

A good deal has happened during this short time. As a scientist or engineer, one may look back at past ground-breaking achievements in science and technology that have shaped the future for a long time to come – the invention of the integrated circuit for example, or the discovery and unravelling of the genetic code. One might even feel a little pang of sadness – how interesting must it have been, back then, to witness and take part in what was a revolution in the making?

But such concerns are actually unfounded as we now find ourselves in the middle of a rapid development step in biotechnology.

A number of individual developments in different fields are presently converging and new, highly versatile tools have been established. Crispr/Cas is just one exam-

ple that has considerably simplified key processes. The still young field of “data science” – powerful hardware with novel algorithms – and artificial intelligence seem to offer the potential to help turn mountains of seemingly loosely-related data into concepts, knowledge and understanding.

These new tools are direly needed. Affordable personalized healthcare, climate-benign sources of energy and raw materials, and sustainable production of healthy food are all examples of key questions that need to be answered in the near future. There is little doubt that biotechnology is going to be of central importance in addressing these issues.

But new tools are only one part of the equation. The other and possibly more important factor is a coming generation of scientists and engineers who are comfortable working across the boundaries of disciplines, cultures and languages. It is here that the partnership of our two universities at this 7th Czech-Swiss Symposium holds promise and creates exciting opportunities.

On behalf of the ZHAW management, professors, co-workers and students, we wish all parties a successful and rewarding exchange at the BioTech 2017 and 7th Czech-Swiss Symposium and are looking forward to ensuing partnerships.

Urs Hilber
*Dean School of Life Sciences and Facility
Management in Wädenswil, Switzerland
Zurich University of Applied Sciences*



**Welcome Address by
Jan Káš and Daniel Hanus**

Dear ladies and gentlemen,
dear colleagues,

It is our very great pleasure to warmly welcome you to the BioTech 2017 conference that is taking place in the heart of Europe in Prague, the Golden City with one hundred spires. The tradition of this series of symposia was born out of the willingness of Czech and Swiss biotechnologists to share experiences and knowledge, and to stimulate progress in biotechnology in favour of both countries. The first event took place in Prague in the building of the University of Chemistry and Technology (located in the front of the present National Technical Library) in 1999 and it was a great success. At this meeting, we decided to get together every three years. Since the year 2005 we have welcomed biotechnologists from other neighbouring and distant countries and have created a platform for a larger international event. We started to organize the programme in several parallel sessions offering more opportunities for oral presentations and widening the scope of biotechnological topics. Each section is introduced by first class opening lecture by well-

known scientists. Attention is also paid to the presentation of posters which topics are unlimited. Importance of poster presentations is supported by awards for best posters. The organizers are trying to arrange pleasant atmosphere for breaks by supplementation with good lunches, drinks and snacks. Of course, the exhibitors are prepared to give symposium participants all necessary information about their products.

Important part of the symposium is traditionally carefully selected social programme. Welcome party will open opportunity to meet old friends and to start new friendships for next days and maybe also for the rest of life. Gala dinner offers chance to see exceptionally nice Kaiserstein palace located in the Lesser Town Square situated in wonderful old Prague district and to know Czech cuisine. For those who still do not know Prague a sightseeing tour may be warmly recommended. Traditional trip at the end of the symposium is this year also devoted to a visit of an important biotechnological company (world famous Pilsner Urquell Brewery) and Abbey of Kladruby, founded in 13.century.

We believe that both scientific and social programmes will contribute to start new friendships among symposium participants and will create new mutual collaboration in favour of biotechnology progress in the world.

Jan Káš and Daniel Hanus

*On behalf of
Czech Biotechnology Society,
Czech Association of Scientific and
Technical Societies (CASTS)
and Swiss partner organizations*

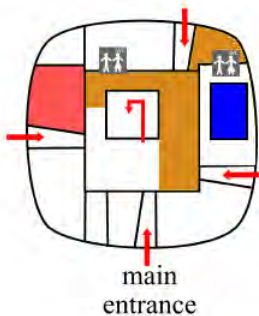
Sponsors



Exhibitors



Venue

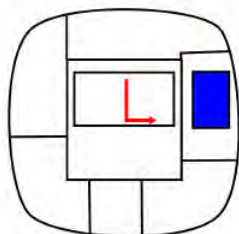


Ground floor

Balling hall

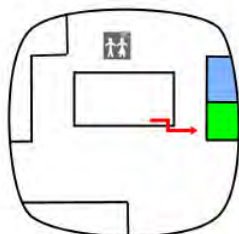
Poster sessions
and Lunches

Welcome party



1st floor

Balling hall



2nd floor

Hall 01

Hall 02

Committees

Organizing Committee (in alphabetical order)

Jan Káš

Czech Biotechnology Society, CZ

Karin Kovar

ZHAW, CH (special responsibility NBO-Workshop)

Jan Lipov

Department of Biochemistry and Microbiology, UCT Prague, CZ

Jan Masák

Department of Biotechnology, UCT Prague, CZ

Olga Mařátková

Department of Biotechnology, UCT Prague, CZ

Leona Paulová

Department of Biotechnology, UCT Prague, CZ

Diyana Petrova

Academic Programmes Unit, ZHAW, CH

Dana Pokorná

Czech Biotechnology Society, CZ

Vojtěch Spiwok

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Jana Zábranská

Department of Water Technology and Environmental Engineering, UCT Prague, CZ

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Urs Baier

Institute of Chemistry and Biotechnology, ZHAW, CH

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Department of Phototrophic Micoorganisms, the Czech Academy of Sciences, CZ

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Ivana Márová

Institute of Food Science and Biotechnology, Brno University of Technology, CZ

Karel Melzoch

Rector of UCT Prague, CZ

Murray Moo-Young

University of Waterloo, Canada

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Michaela Rumlová

Department of Biotechnology, UCT Prague, CZ

Diego Schmidhalter

Lonza AG, Visp, CH

Peter Šebo

Institute of Microbiology, the Czech Academy of Sciences, CZ

Miroslav Šoóš

Department of Chemical Engineering, UCT Prague, CZ

Program

Tuesday, June 13, 2017

Pre-Conference Events

Venue: National Technical Library, Balling Hall

9:00 NBO-Workshop: Reinforcing the links between industry, research and biotechnology education

Chairperson: **Karin Kovar**, *Institute of Biotechnology, ZHAW, Switzerland* (Open for public)

13:00 Publishing in academic journals: tips to help you succeed

Lecturer: **Jennifer Stokes**, *Taylor & Francis Group* (Open for public)

Venue: National Technical Library, Hall 1

10:00 Production of biobutanol and succinic/lactic acid from lignocellulose biomass: bilateral Czech-Chinese Inter-Action project, teamwork meeting

Chairpersons: **Leona Paulová** *Department of Biotechnology, UCT Prague, Czech Republic*, **Jianan Zhang** *Institute of Nuclear and New Energy Technology, Tsinghua University, China* (Private session)

Tuesday, June 13, 2017

Conference Events

Venue: National Technical Library, Balling Hall

15:00 Official Conference Opening

15:10 Welcome by **Pavel Bělobrádek**, Deputy Prime Minister for the Science, Research and Innovation, Chairman of the Research, Development and Innovation Council

15:20 Welcome by **Eva Zažímalová**, President of the Czech Academy of Sciences

15:30 Welcome by **Ondřej Kolář**, Mayor of Prague 6, Czech Republic

15:40 Welcome by **Karel Melzoch**, rector of UCT Prague, Czech Republic

15:50 Welcome by **Urs Hilber**, dean of ZHAW, Switzerland

16:00 Welcome by **Ulf Grawunder**, vice president of the Swiss Biotech Association

16:15 Plenary Lecture:

FROM AVIDIN-BIOTIN TO CELLULOSOME TECHNOLOGIES: A JOURNEY THROUGH TIME...

Ed Bayer *Department of Biomaterial Sciences, The Weizmann Institute of Science, Israel*

18:00 Welcome Party (*Venue: Gallery of National Technical Library*)

Wednesday, June 14, 2017

Venue: National Technical Library, Balling Hall

9:00 Plenary Lecture:

MOLECULAR MECHANISM OF THE GENOME EDITOR NUCLEASE CAS9

Martin Jinek *University of Zurich, Switzerland*

10:00 Coffee break

Biorefinery

Venue: National Technical Library, Balling Hall

Chairpersons: **Petra Patáková** *Department of Biotechnology, UCT Prague, Czech Republic*, **Ivana Márová** *Institute of Food Science and Biotechnology, Brno University of Technology, Czech Republic*, **Murray Moo-Young** *University of Waterloo, Canada*

10:30 Syngas fermentation design for hybrid biorefineries (Invited lecture)

Henk Noorman *Delft University of Technology, Netherland*

10:55 The phototroph biorefinery: emerging microalgae in the bioeconomy (Invited lecture)

Spiros Agathos *Bioengineering Laboratory, Catholic University Louvain, Belgium*

11:20 Microbial production of required metabolites in plant/animal biorefinery (Invited lecture)

Petra Patáková *Department of Biotechnology, UCT Prague, Czech Republic*

11:45 Fermentation of glycerol by *Paracoccus denitrificans* under sub-lethal high pressure levels

Maria João Mota *University of Aveiro, Portugal*

12:10 Production of fuels from Macroalgae (Invited lecture)

Ana Lopez-Contreras *Wageningen University, Netherlands*

12:30-13:30 Lunch

13:30 Leveraging yeast potential for the valorization of biomasses into valuable bioproducts (Invited lecture)

Danilo Porro, Paola Branduardi *Department of Biotechnology and Bioscience, University of Milano, Italy*

13:55 Genome mining for biorefinery use (Invited lecture)

Karel Sedlář *Department of Biomedical Engineering, Brno University of Technology, Czech Republic*

14:20 Gene expression of sporulation factors in *Clostridium beijerinckii* during solventogenesis (Invited lecture)

Jan Kolek *Department of Biotechnology, UCT Prague, Czech Republic*

- 14:45** Solvent production in sporulation deficient *Clostridium* (Invited lecture)
Mamou Diallo *Wageningen University & Research, Food & Biobased Research, Netherlands*

Pharmaceutical Biotechnology

Venue: National Technical Library, Hall 01

Chairpersons: **Flavia Marinelli** *Department of Biotechnology, University of Insubria, Italy*,
Michaela Rumlová *Department of Biotechnology, UCT Prague, Czech Republic*, **Vera Luginbühl** *Institute of Chemistry and Biotechnology, ZHAW, Switzerland*

- 10:30** Marine antimicrobial compounds and their potential as lead compounds for novel antibiotics (Invited lecture)
Klara Stensvåg *University of Tromsø, Norway*
- 10:55** Old and novel glycopeptide antibiotics: from product to gene and back (Invited lecture)
Flavia Marinelli *Department of Biotechnology, University of Insubria, Italy*
- 11:20** 5-Aminolevulinic acid in glioblastoma surgery and intracellular tumour targeting by a shiga toxin subunit B (Invited lecture)
Vera Luginbühl *Institute of Chemistry and Biotechnology, ZHAW, Switzerland*
- 11:45** Assembly and disassembly of HIV-1 as a target for screening and rational design of anti-HIV-1 compounds (Invited lecture)
Michaela Rumlová *Department of Biotechnology, UCT Prague, Czech Republic*
- 12:10** Uncovering citrinin biosynthesis in *Monascus ruber* by reconstructing its biosynthetic pathway in the secondary host *Aspergillus oryzae* (Invited lecture)
Yi He *College of Food Science and Engineering, Wuhan Polytechnic University, China*
- 12:30-13:30** Lunch
- 13:30** Biofilms of opportunistically pathogenic microorganisms and possibilities of their regulation (Invited lecture)
Jan Masák *Department of Biotechnology, UCT Prague, Czech Republic*
- 13:55** The nanoliter-reactor platform for high throughput screening of cell libraries secreting bioactive compounds
Andreas Meyer *FGen GmbH, Basel, Switzerland*
- 14:20** The Sugar Code as a Door Opener at Resistant Uptake Barriers – Biotechnological Concepts for Glycotargeted Drug Delivery in Bladder Cancer (Invited lecture)
Lukas Neutsch *Institute of Chemistry and Biotechnology, ZHAW, Switzerland*
-

- 14:45** Highly Potent and Immune Stimulatory Antibody Drug Conjugates with a novel Anthracycline Toxin
Ulf Grawunder *NBE-Therapeutics Ltd., Switzerland*
- 15:10** Smart nanostructures for bioapplications
Petr Slepíčka *Department of Solid State Engineering, UCT Prague, Czech Republic*
- 15:35** An enzymatic tool-box for modification of hyaluronan
Dzianis Smirnou *Contipro, Czech Republic*

Food and Agriculture Biotechnology

Venue: National Technical Library, Hall 02

Chairpersons: **Pavel Dostálek** *Department of Biotechnology, UCT Prague, Czech Republic*, **Brion Duffy** *Institute of Chemistry and Biotechnology, ZHAW, Switzerland*, **Lars Fieseler** *Institute of Chemistry and Biotechnology, ZHAW, Switzerland*

- 10:30** Enhancing brewing yeast performance (Invited lecture)
Pavel Dostálek *Department of Biotechnology, UCT Prague, Czech Republic*
- 10:55** Microbial innovation for microbreweries (Invited lecture)
Marilena Budroni *Department of Agricultural Sciences, University of Sassari, Sassari, Italy*
- 11:20** Moderate Electric Fields application as a biotechnological tool in food processing (Invited lecture)
Antonio Vicente *University of Minho, Braga, Portugal*
- 11:45** Yeast for production of alcohol free beer (Invited lecture)
Daniela Šmogrovičová *Department of Biochemical Technology, Slovak University of Technology in Bratislava, Slovakia*
- 12:10** Probiotic effect of microorganisms (Invited lecture)
Šárka Horácková *Department of Dairy, Fat and Cosmetics, UCT Prague, Czech Republic*
- 12:30-13:30** Lunch
- 13:30** Chitinases from metagenome as pest biocontrol agents (Invited lecture)
Francesca Berini *University of Insubria, Italy*
- 13:55** Recent researches of *Monascus* spp. and their metabolites in China (Invited lecture)
Fusheng Chen *Huazhong Agricultural University, Wuhan, China*
- 14:20** Effect of various factors on the buffering capacity of wort (Invited lecture)
Gabriella Kun-Farkas *Szent István University, Hungary*
- 14:45** Development of a fed-batch system for the fermentation of grape must with high sugar content
Matthias Kowalczyk *Institute for Viticulture and Oenology, Germany*

16:00 Poster Session with Refreshment and Beer Party

Venue: Orchestra of National Technical Library

Thursday, June 15, 2017

Venue: National Technical Library, Balling Hall

9:00 Plenary Lecture:

THE ENERGY-ENVIRONMENT ENIGMA DILEMMA

Murray Moo-Young *University of Waterloo, Canada*

10:00 Coffee break

Biorefinery

Venue: National Technical Library, Balling Hall

Chairpersons: **Petra Patáková** *Department of Biotechnology, UCT Prague, Czech Republic*, **Ivana Márová** *Institute of Food Science and Biotechnology, Brno University of Technology, Czech Republic*, **Murray Moo-Young** *University of Waterloo, Canada*

10:30 How to convert lignocellulose to biosurfactants within a biorefinery (Invited lecture)

Suzanne Zibek *Fraunhofer Institute for Interfacial Engineering and Biotechnology, Germany*

10:55 Vibrational spectroscopy for monitoring lipogenesis in microbial cells (Invited lecture)

Volha Shapaval, Achim Kohler *Norwegian University of Life Sciences, Norway*

11:20 Integrating production of PHA into bio-refinery concept – shedding light also on specific consequences of non-optimal cultivation conditions (Invited lecture)

Stanislav Obruča *Institute of Food Science and Biotechnology, Brno University of Technology, Czech Republic*

11:45 Bench-scale production of polyhydroxyalkanoates and other valuable biomaterials from xylose-rich lignocellulosic hydrolysates (Invited lecture)

Maria Catarina Marques Dias de Almeida *Technical University of Lisboa, Portugal*

12:10 Consolidated production of Volatile Fatty Acids from plant biomass using defined and natural microbial consortia

Charilaos Xiros *School of Agricultural, Forest and Food Sciences, Bern University of Applied Sciences, Switzerland*

12:30-13:30 Lunch

13:30 Comprehensive Characterization of Lignin and its Degradation Products: Approaches and Challenges (Invited lecture)

Alena Kubátová *University of North Dakota, USA*

13:55 *Rhodothermus marinus* – A New Platform Organism for Industrial Biotechnology (Invited lecture)

Eva Nordberg Karlsson

- 14:20** Simultaneous saccharification and fermentation of steam pretreated beech wood with in situ *Irpex lacteus* treatment (Invited lecture)
Simone Brethauer *School of Agricultural, Forest and Food Sciences, Bern University of Applied Sciences, Switzerland*
- 14:55-15:30** Coffee break
- 15:30** The holistic biorefinery concept: from by-product to high value – case study
Marcin Łukaszewicz *Uniwersytet Wrocławski, Faculty of Biotechnology, Poland*
- 15:55** Application of onsite produced cellulase in lignocellulosic biorefinery for bioethanol and lactic acid production using freshly pretreated materials
Abdul Sattar Qureshi *University of Sindh, Pakistan*

Industrial Biotechnology

Venue: National Technical Library, Hall 01

Chairpersons: **Diego Schmidhalter** *Lonza AG, Visp, Switzerland*, **Miroslav Šooš** *Department of Chemical Engineering, UCT Prague, Czech Republic*

- 10:30** Model-driven process design for improved antibody glycosylation
Cleo Kontoravdi *Department of Chemical Engineering, Imperial College London, Great Britain*
- 10:55** Medium and Process Development for Next-Generation Perfusion Processes (Invited lecture)
Jochen Sieck *Merck KGaA, Darmstadt, Germany*
- 11:20** Optimization of mAb glycosylation produced in mammalian cell cultures (Invited lecture)
Miroslav Šooš *Department of Chemical Engineering, UCT Prague, Czech Republic*
- 11:45** Scale-up in the single use age: Does geometry matter? (Invited lecture)
Colin Jaques *Lonza Biologics, Slough, Great Britain*
- 12:10** Optimization of microbial hyaluronic acid fermentation by metabolomic approach (Invited lecture)
Lukáš Franke *Contipro, Czech Republic*
- 12:30-13:30** Lunch
- 13:30** Systems and synthetic biology approaches to accelerate bio-systems engineering (Invited lecture)
Beat Christen *Institute of Molecular Systems Biology, ETH Zürich, Switzerland*
- 13:55** Cell-free expression as a new alternative for difficult-to express-proteins (Invited lecture)
Sandra Cortes *Synthelis, Grenoble, France*

- 14:20** White Biotechnology for the Production of Flavor and Fragrance ingredients (Invited lecture)
Michel Schalk *Firmenich SA, Switzerland*
- 14:45-15:30** Coffee break
- 15:30** Concurrent optimization strategies for high level protein production: Process, strain and expression engineering (Invited lecture)
Astrid Weninger *TU Graz, Austria*
- 15:55** Fast track expression and production of proteins with Lonza's *XSTM* Pichia Platform (Invited lecture)
Christoph Kiziak *Lonza AG, Visp, Switzerland*
- 16:20** Using a systematic approach to accelerate bioprocess development: methanol-free manufacturing with *Pichia pastoris* (Invited lecture)
Verena Looser *Zürich University of Applied Sciences ZHAW, Wädenswil, Switzerland*
- 16:45** USP and DSP projects of the Biotechnological Pilot Plant - the Institute of Microbiology, the Czech Academy of Sciences (Invited lecture)
Aleš Prell *Institute of Microbiology, the Czech Academy of Sciences, Czech Republic*
- 17:05** Model based high cell density continuous cultivation of *A. latus* for biopolymer (PHB) production
Ashok Kumar Srivastava *Department of Biochemical Engineering and Biotechnology, IIT Delhi, India*

Food and Agriculture Biotechnology

Venue: National Technical Library, Hall 02

Chairpersons: **Pavel Dostálek** *Department of Biotechnology, UCT Prague, Czech Republic*, **Brion Duffy** *Institute of Chemistry and Biotechnology, ZHAW, Switzerland*, **Lars Fieseler** *Institute of Chemistry and Biotechnology, ZHAW, Switzerland*

- 10:30** Bacteriophages for the control of pathogenic bacteria (Invited lecture)
Lars Fieseler *Institute of Food and Beverage Innovation, Zurich University of Applied Sciences, Switzerland*
- 10:55** Phages to combat *Listeria* and *Salmonella* (Invited lecture)
Steven Hagens *Micreos Food Safety, The Netherlands*
- 11:20** Synthetic biology of designer endolysins (Invited lecture)
Yves Briers *Laboratory of Applied Biotechnology, Ghent University, Belgium*
- 11:45** Antimicrobial and defence elicitor peptides in plant disease control (Invited lecture)
Emilio Montesinos *Institute of Food and Agricultural Technology, University of Girona, Spain*

- 12:10** Antifungal lactic acid bacteria in food fermentations (Invited lecture)
Susanne Miescher Schwenninger *Institute of Food and Beverage Innovation, Zurich University of Applied Sciences, Switzerland*
- 12:30-13:30** Lunch
- 13:30** Beneficial cardiovascular effects of (-)epicatechin-containing foods (Invited lecture)
Iveta Bernátová *Institute of Normal and Pathological Physiology, Slovak Academy of Sciences, Slovakia*
- 13:55** Biosensors: Potentials and Applications in Food Industry
Alireza Shirazinejad *Islamic Azad University, Iran*
- 14:20** Enzyme-mediated Hypoallergenic Milk and Dairy products – Present scenario and Future prospects
Klára Pásztoriné Huszár *Szent István University, Hungary*
- 14:45-15:30** Coffee break
- 15:30** Industrial biotechnology for the production of microbial pesticides
Mirata Marco *Lonza Ltd, Switzerland*
- 15:55** Two-dimensional GC: a suitable tool to enhance the understanding of complex volatile profiles released from biological materials such as fermented tobacco leaves
Manuel Mazenauer *Zurich University of Applied Sciences, Switzerland*
- 19:30 Galadinner (not included in conference fee)**
Venue: Kaiserstein Palace, Lesser Town Square

Friday, June 16, 2017

Venue: National Technical Library, Balling Hall

9:00 Plenary Lecture:

PERSPECTIVES FOR DNA SEQUENCING TECHNIQUES, APPLICATIONS IN GENOMICS and MEDICINE

Wilhelm Ansorge *ETH Lausanne, Switzerland*

10:00 Coffee break

Medical Biotechnology

Venue: National Technical Library, Balling Hall

Chairpersons: **Jana Pěkníková** *Institute of Biotechnology, the Czech Academy of Sciences, Czech Republic*, **Peter Šebo** *Institute of Microbiology, the Czech Academy of Sciences, Czech Republic*, **Jan Dohnálek** *Institute of Biotechnology, the Czech Academy of Sciences, Czech Republic*

10:30 Electron transport chain disruption as a new strategy to suppress Her2high breast cancer (Invited lecture)

Kateřina Rohlenová *Institute of Biotechnology, the Czech Academy of Sciences, Czech Republic*

10:55 Immunogenic cell death in lung cancer immunotherapy (Invited lecture)

Irena Adkins Sotio, *Czech Republic*

11:20 Evaluating the quality of male sperm cells with monoclonal antibodies (Invited lecture)

Jana Pěkníková *Institute of Biotechnology, the Czech Academy of Sciences, Czech Republic*

11:45 Monoclonal and polyclonal antibodies as tools for the study of proteins in the male reproductive tract (Invited lecture)

Pavla Postlerova *Institute of Biotechnology, the Czech Academy of Sciences, Czech Republic*

12:10 Zinc-dependent 3' nucleases related to treatment of human diseases (Invited lecture)

Tomáš Koval *Institute of Biotechnology, the Czech Academy of Sciences, Czech Republic*

12:30-13:30 Lunch

13:30 Development of novel biologics for prostate cancer imaging (Invited lecture)

Zora Nováková *Institute of Biotechnology, the Czech Academy of Sciences, Czech Republic*

- 13:55** (Glyco)peptide mimetics derived from albumin-binding domain scaffold as tools for induction of neutralizing antibodies against HIV-1 gp120 glycoprotein (Invited lecture)
Petr Malý *Institute of Biotechnology, the Czech Academy of Sciences, Czech Republic*
- 14:20** Streptavidine-based system for antigen delivery and vaccination
Ondrej Staňek *Institute of Microbiology, the Czech Academy of Sciences, Czech Republic*
- 14:45** Biotechnology aspects of PIXL methodology (photo-induced cross-linking): A tool for structural biochemistry
Miroslav Šulc *Department of Biochemistry, Faculty of Science, Charles University, Czech Republic*
- 15:10** Bordetella pertussis and its adenylate cyclase toxin: How it works and how to use it (Invited lecture)
Peter Šebo *Institute of Microbiology, the Czech Academy of Sciences, Czech Republic*
- 15:35** Computer-assisted engineering of fibroblast growth factors
Gabriela Daňková *Loschmidt Laboratories, Department of Experimental Biology & RECETOX, Masaryk University, Czech Republic*
- 16:00** Targeting the stress signaling in cancer (Invited lecture)
Jaroslav Zelenka *Department of Biochemistry and Microbiology, UCT Prague, Czech Republic*

Microalgae Biotechnology

Venue: National Technical Library, Hall 01

Chairpersons: **Kateřina Bišová** *Institute of Microbiology, the Czech Academy of Sciences, Czech Republic*, **Yusuf Chisti** *Massey University, New Zealand*

- 10:30** Microalgae grown in stable isotopes (Invited lecture)
Kateřina Bišová *Institute of Microbiology, the Czech Academy of Sciences, Czech Republic*
- 10:55** Towards industrial products from microalgae (Invited lecture)
Maria Barbosa *Wageningen University, Netherlands*
- 11:20** Influence of inorganic salt precipitates on autoflocculation of microalgae (Invited lecture)
Tomas Brányik *Department of Biotechnology, UCT Prague, Czech Republic*
- 11:45** Screening cyanobacteria for novel compounds with potential anticancer activity (Invited lecture)

Pavel Hrouzek *Institute of Microbiology, the Czech Academy of Sciences, Czech Republic*

12:10 Study of up- and downstream processes in *Microcystis aeruginosa* cultivation – One approach, two distinct objectives

Pedro Geada *University of Minho, Portugal*

12:30-13:30 Lunch

13:30 Identifying knowledge gaps for an efficient anaerobic digestion of microalgae biomass (Invited lecture)

Cristina Gonzalez *IMDEA Energy Institute, Spain*

13:55 Endopolyploidy, fragmentation and reconstitution of chromosomes by the heavy-ion beam irradiation in *Parachlorella kessleri* (Invited lecture)

Shigeyuki Kawano *University of Tokyo, Japan*

14:20 Isolation and structural characterization of exopolysaccharides from microalgae *Dictyosphaerium chlorelloides* and *Porphyridium purpureum*

Roman Bleha *Department of Carbohydrates and Cereals, UCT Prague, Czech Republic*

14:55 Wood hydrolysates as potential feedstocks for microalgae biomass, fatty acid and pigment production

Krystian Miazek *University of Liege-Gembloux Agro-Bio Tech, Belgium*

Environmental Biotechnology

Venue: National Technical Library, Hall 02

Chairpersons: **Pavel Jeníček** *Department of Water Technology and Environmental Engineering, UCT Prague, Czech Republic*, **Martin Halecký** *Department of Biotechnology, UCT Prague, Czech Republic*, **Urs Baier** *Institute of Chemistry and Biotechnology, ZHAW, Switzerland*

10:30 Microbial Ecology in Anaerobic digesters: status and perspectives (Invited Lecture)

Irini Angelidaki *TU Lyngby, Denmark*

11:20 Physico-chemical characterization of EPS-based biomaterial recovered from anammox granular sludge (Invited Lecture)

Tommaso Lotti *Politecnico di Milano, Italy*

11:45 Olive Mill Solid Waste Biorefinery: Comparative of thermal pre-treatments for phenols recovery and biomethanation (Invited Lecture)

Fernando Feroso *Instituto de la Grasa Sevilla, Spain*

12:10 Critical metals – the challenges of the present, solutions for the future

Alina Butu *National Institute of Research and Development for Biological Sciences, Romania*

12:30-13:30 Lunch

13:30 Separate carbon and nutrients removal from sewage: Making the concept reality (Invited Lecture)

Jan Bartáček *Department of Water Technology and Environmental Engineering, UCT Prague, Czech Republic*

13:55 Biotechnology of nutrient removal from waste water by autotrophic denitrification with sulfides from biogas (Invited Lecture)

Jana Záborská *Department of Water Technology and Environmental Engineering, UCT Prague, Czech Republic*

14:20 Adaptation of anammox to low temperature in response to cold shock (Invited Lecture)

Vojtěch Kouba *Department of Water Technology and Environmental Engineering, UCT Prague, Czech Republic*

14:45 Hybrid treatment methodologies for biotransformation of color industrial effluents – A bioreaction calorimetric study

Bhuvanesh Kumar Shanmugam *Department of Chemical Engineering, CLRI., Adyar, India*

15:10 Bioleaching for heavy metal recovery and detoxification of ashes from thermal power plants

Tingyue Gu *Ohio University, USA*

15:35 Efficacies of Seventeen organically made Northern fertilisers on sustainable crops production in acidic soil of food security under climate change

Durlave Roy *Northern Agro Services Ltd, Bangladesh*

Best Poster Awards and Closing Ceremony

Venue: National Technical Library, Balling Hall

17:00 Best Poster Awards and Closing Ceremony in Balling Hall

18:30 Prague Sightseeing (not included in conference fee)

Saturday, June 17, 2017

8:30 Excursion to Pilsner Urquell Brewery with lunch, Kladruby Abbey Sightseeing (not included in conference fee). Expected arrival to Prague in late afternoon.

Workshop: NBO-workshop at BioTech2017, Prague

Reinforcing the links between industry, research and biotechnology education

Tuesday, June 13, 2017

Venue: National Technical Library, Balling Hall

- 09:00** Welcome and introduction to the NBO-workshop
Karin Kovar *Zurich University of Applied Sciences ZHAW, Wädenswil, CH*, **Mike Cook** *Cook Business Consulting, Wädenswil, CH*
- 09:10** NBO-analysis: course alumni perspectives
Marcel Straumann & Iwo Zamora *Zurich University of Applied Sciences ZHAW, Wädenswil, CH*
- 09:30** Collaborating on the NBO-programme: an industry perspective
Diego Schmidhalter *Lonza, Visp, CH*
- 09:40** Discussion I: What is the future of university education?
- 10:00** Changing perspectives: a biologist as entrepreneur and political consultant
Christine Lang *ORGANOBALANCE GmbH & German Bioeconomy Council, Berlin, DE*
- 10:30** Spinning-off from academia: visions versus reality based on the story of FGen
Andreas Meyer *FGen GmbH, Basel, CH*
- 10:50** Discussion II: What are the needs of society and industry?
- 11:05** SynBiT: a novel biological engineering platform for producing chemicals by biosynthesis
Beat Christen & Matthias Christen *Experimental Systems Biology, ETH Zürich, CH*, **Karin Kovar** *Zurich University of Applied Sciences ZHAW, Wädenswil, CH*
- 11:30** Discussion III: How should we proceed? (NBO-topics 2017/18 and future collaboration)
- 12:00** Individual lunch

Target group: This workshop is open to everybody. In particular, students making decisions about their future careers and SMEs seeking collaboration with universities are encouraged to participate in the discussions.

Scope: The objective of this workshop is to facilitate a discussion on the future of university education in biotechnology and to stimulate the mutually beneficial links between research, education and industry (encompassing both global corporations and SMEs). The implications for current biotechnology students completing Master's or PhD programmes as well as future graduates will be explored. Scientists from universities and industry, students and lecturers, experienced European entrepreneurs, key opinion leaders and policymakers will

discuss their personal experience of scouting out and developing new business opportunities in (microbial) biotechnology.

New Business Opportunity (NBO) Analysis represents an innovative, cross-disciplinary teaching/learning format that uniquely combines the development of entrepreneurial skills with in-depth subject-specific knowledge. Current stakeholders in the NBO-programme at the ZHAW in Wädenswil will share their vision and experience, paving the way for a discussion on future collaboration between academia and industry on biotechnology education.

Further information and registration on the NBO is available at: www.zhaw.ch/icbt/nbo

Workshop: Production of biobutanol and succinic/lactic acid from lignocellulose biomass:

bilateral Czech-Chinese Inter-Action project, project meeting

Tuesday, June 13, 2017

Venue: National Technical Library, Hall 1

Scope: This workshop is proposed as a project meeting between the bilateral partners of Inter-Action project, UCT Prague, Czech Republic and Tsinghua University, China. The research activities of both project teams will be presented, the project outputs will be evaluated and the plan of next activities will be discussed.

Chairpersons: **Leona Paulová** *Institute of Biotechnology, UCT Prague, Czech Republic*, **Jianan Zhang** *Institute of Nuclear and New Energy Technology, Tsinghua University, China*

10:00 Workshop opening

Karel Melzoch *rector of UCT Prague, Czech Republic*

10:15 Overview of project research activities performed at UCT Prague

Leona Paulová *UCT Prague, Czech Republic*

10:30 Production of butanol using lignocellulosic material: selection of high tolerant strains and consolidated process arrangement

Petra Patáková and Maryna Vasilkyvska *UCT Prague, Czech Republic*

10:50 Production of lactic acid using lignocellulose and other waste materials

Marek Drahokoupil and Leona Paulová *UCT Prague, Czech Republic*

11:05 Flow cytometry as a convenient tool for process control

Barbora Branská *UCT Prague, Czech Republic*

11:20 Overview of project research activities performed at Tsinghua University

Jianan Zhang *Tsinghua University, China*

11:40 Production of butanol with a new symbiotic system TSH06 under micro-aerobic condition

Hongjuan Liu *Institute of Nuclear and New Energy Technology, Tsinghua University, China*

12:00 Production of succinic acid by fermentation: mutation and selection of microorganism by ARTP

Xiang Yan *Institute of Nuclear and New Energy Technology, Tsinghua University, China*

12:20 Informal discussion with refreshment

Workshop: Publishing in academic journals: tips to help you succeed

Lecturer: **Jennifer Stokes** *Taylor & Francis Group*

Tuesday, June 13, 2017, 13:00-14:00

Venue: National Technical Library, Balling Hall

Target group: This workshop is open to anyone who would like to learn more about the publishing process in academic journals. The talk is particularly aimed at early career researchers or PhD students who may be new to the publishing process.

Scope: This talk will guide you through the process of getting an article published – from choosing a journal, to what to think about when writing to improve your chances of publication, and then on to how to navigate the peer review process and what you can do once your article is published to increase its impact. The workshop will cover article metrics, use of social media, how to respond to reviewers' comments plus more. By the end of the session you will have a thorough understanding of the steps involved for authors in publishing a journal article, the key information sources you should be aware of, and what you can be doing to help get your research published.

Plenary Lectures

PL1

From avidin-biotin to cellulosome technologies: A journey through time...

E. Bayer¹

¹ *The Weizmann Institute of Science, Department of Biomolecular Sciences, Rehovot, Israel*

Synthetic biology is a very broadly defined approach, in which biology, biotechnology and bioengineering are closely interwoven. In the past several decades, our work has contributed to this modern-day field that continues to provide significant insight into the mechanisms of biological systems with extensive applied import. In early work, we exploited the tenacious affinity between the egg-white glycoprotein, avidin, and the vitamin biotin. We synthesized a large array of chemically reactive biotin-containing reagents for labeling different types of biological molecules, including proteins, complex carbohydrates, lipids, nucleic acids, etc. In parallel, we produced a variety of avidin-conjugated molecules and probes. The interaction between the biotinylated materials and avidin-conjugates provided the basis of the avidin-biotin system, which remains a staple of many areas of basic, clinical, medical and industrial research and technology. Another decade passed, when we discovered the multienzyme cellulosome system, which can efficiently deconstruct recalcitrant cellulosic biomass. In contrast to the free enzyme paradigm, the cellulosome

some comprises a set of Lego-like multimodular components – some structural and some enzymatic, contained in a discrete complex. More recently, our lab has focused on dismantling the cellulosome into its component parts and reassembling them into "designer cellulosomes" of precise enzymatic content and configuration, thereby providing an alternative type of synthetic biology. Rational bioengineering of cellulase and cellulosomal components for production of tailor-made multi-functional enzymes, or "designer cellulosomes", is now being developed for improved cellulose degradation. The designer cellulosome approach shows promise for understanding the rationale behind its catalytic efficiency, and knowledge gained from these studies may provide the basis for creating improved designer cellulosomes for conversion of plant-derived biomass into liquid biofuels. Integration of alternative enzymes and/or noncatalytic macromolecules into designer cellulosome-like complexes provides a general platform for self-assembly of biologically active nanomaterials for a wide variety of applications.

PL2

Molecular mechanism of the genome editor nuclease cas9

M. Jinek¹

¹ *Department of Biochemistry, University of Zurich, Winterthurerstr. 190, Zurich, Switzerland*

CRISPR-Cas systems have emerged as a powerful technology for precision genome editing in cells and organisms. The CRISPR-associated protein Cas9 is an RNA-guided DNA nuclease that associates with an unusual dual-RNA guide structure and cleaves double-stranded DNA sequences complementary to a 20-nucleotide sequence in the guide RNA. The enzyme can be programmed using single-molecule guide RNAs to induce double-strand DNA breaks in genomic DNA, paving the way for RNA-guided genetic genome editing. Our recent work has focused on obtaining structural insights into the molecular function of Cas9 and related genome editor nucleases. To this end, we have determined crystal structures of Cas9 and its complexes with a guide RNA and a DNA target. The structures shed light on the molecular mechanism of Cas9-mediated DNA binding and cleavage and reveal the conformational transitions occurring during the process. Our current studies focus on exploring the mechanism of Cas9 in greater detail and on understanding complementary genome editing tools such as Cpf1/Cas12. These studies provide the structural framework for the ongoing development of CRISPR-Cas for a new generation of genome editing tools and tech-

nologies.

PL3

The energy-environment enigma: biomass dilemmas

M. Moo-Young¹, C.P. Chou²

¹ *Centre for Bioengineering and Biotechnology, Department of Chemical Engineering, University of Waterloo, Canada,*

² *Department of Chemical Engineering, University of Waterloo, Canada*

Renewable energy, biomass-derived fuels, climate change, and other related topics are popular these days. However, relatively few realistic strategies have been proposed or implemented commercially. The favorable economics of petroleum continues to drive the global industrial manufacturing and vehicle transportation operations, which mainly cause world pollution problems. Clearly, various major culture-changes are required; hence, the ongoing enigma and dilemmas of biomass utilization scenarios. Most communities are still involving in a pervasive "throw-away" society and government research organizations promoting political agendas, while scientific researchers are concerned less with practical applications rather with fundamental scientific discoveries. Here, noting our own research contributions to the complex scenario, we are developing biotechnology innovations for the production of biofuels and bioproducts using biomass waste-residues as feedstock. Example inputs are our creation of

genetically-modified microbes and bioprocessing strategies to overcome the current low-yield limitations and the poor utilization of cheap feedstocks, in a collaborative and multidisciplinary approach. In this talk, we will examine the options and controversies which we have encountered at present.

References

1. K. Srirangan, L. Akawi, M. Moo-Young, and C. P. Chou (2012), Towards sustainable production of clean energy carriers from biomass resources, *Applied Energy*, **100**, 172-186.
2. R. Gheshlaghi, J. M. Schärer, M. Moo-Young, and C. P. Chou (2009), Metabolic pathways of Clostridia for producing butanol, *Biotechnology Advances*, **27**, 764-781
3. K. Srirangan, X. Liu, A. Westbrook, L. Akawi, M. E. Pyne, M. Moo-Young, and C. P. Chou (2014), Biochemical, genetic, and metabolic engineering strategies to enhance coproduction of 1-propanol and ethanol in engineered *Escherichia coli*, *Applied Microbiology and Biotechnology*, **98**, 9499-9515.
4. M. Pyne, M. Moo-Young, D. Chung, CP Chou (2013) Development of an electrotransformation protocol for genetic manipulation of *Clostridium pasteurianum*. *Biotechnology for Biofuels*, **6**, 50.
5. K. Srirangan, X. Liu, L. Akawi, M. Bruder, M. Moo-Young, and C. P. Chou (2016), Engineering *Escherichia coli* for microbial production of butanone, *Applied and Environmental Microbiology*, **82**, 2574-2584.

PL4

Perspectives for dna sequencing techniques, applications in genomics and medicine

W. Ansorge¹

¹ *ETH Lausanne, Route Cantonale, Lausanne, Switzerland*

Development and applications of DNA sequencing techniques in the last years have exceeded by far the expectations. Historical development of the field from the start up to the initial Next Generation systems, technical principles of the platforms existing 6 years ago and many applications, have been described in the previous reviews (e.g. 1,2). In this updated review will be discussed the status of the technology since the second half of the year 2015, both of the novel commercially available platforms, and of the systems in development. Mentioned will be techniques with potential for future DNA sequencing techniques. Discussed will be some of the not solved challenges for the platforms, the price to performance ratio, complexity of sample preparation, and detection of structural variations in genome. The aims for the technology will be to lower the cost of the equipment and biochemicals involved, increasing simultaneously the reproducibility, reliability and simplicity of the techniques and protocols in operation, of importance for diagnostics and applications in the clinics. During the last few years have been rapidly developing technologies and methods that permit analysis of the genome and transcriptome of a single cell. The first observations suggest that both ge-

omic and transcriptomic heterogeneities within an organism are more common than expected, during normal development and disease. Recent public support for precision medicine, for novel therapeutic approaches and efforts for improvements in the healthcare, will be a motivation for further innovations and developments in the field.

References

1. Ansorge WJ (2009) Next Generation DNA Sequencing Techniques and Applications. Review in New Journal of Biotechnology *N Biotechnoll.* **25**:195-203.
2. Ansorge WJ (2010) Novel Next-Generation DNA Sequencing Techniques for Ultra High Throughput Applications in Bio-Medicine, Molecular Diagnostics (2ndedn), ScienceDirect

Oral Presentations – Biorefinery

BR1

Syngas fermentation design for hybrid biorefineries

H. Noorman¹

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Today, 1st generation industrial processes annually produce about 140 billion liters of bioethanol from food crops such as corn and sugar cane in approximately 1000 plants worldwide. Co-products and biorefineries with multiple product outlets are gaining importance to maximize economic returns from the value chains.

At the same time, industries are implementing 2nd generation processes to bioethanol, based on non-food, lignocellulosic feedstocks. Biomass pre-treatment and handling, lignocellulose-degrading enzymes and pentose-fermenting yeast are new technologies, facing minor (enzyme-yeast) and sometimes major (biomass-related) scale-up challenges.

A separate route is gasification of domestic/industrial waste (including CO from the steel industry) and forestry/agro-residues to syngas, followed by fermentation to bioethanol. This alternative 2nd generation process is approaching commercialization.

We have investigated the techno-economical design of such processes, with some emphasis on the fermentation step, and explored other products than bioethanol. This presents a bridge to the

4th generation processes (bypassing algae-based processes, which form the 3rd generation) where the scope of syngas fermentation is extended with H₂ from surplus solar/wind energy or direct electricity input in combination with CO₂ as carbon feedstock. This all is an embryonic field but has abundant growth potential.

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BR2

The phototroph biorefinery: emerging microalgae in the bioeconomy

S. Agathos^{1,4}, C. Jeffryes², B.S. Grama³

¹ *Université Catholique de Louvain, Place Croix du Sud 2, Box L7.05.19, Louvain-la-Neuve, Belgium*, ² *Lamar University, Beaumont, TX 77710, USA*, ³ *Université Larbi Ben M'hidi, Oum El Bouaghi, Algeria*, ⁴ *Yachay Tech University, 100119 San Miguel de Urcuquí, Ecuador*

Phototrophs like algae and cyanobacteria are generating about one third of the Earth's primary biomass by consuming solar energy and carbon dioxide. Consequently they can contribute to the bioeconomy for the sustainable production of biofuels and value-added chemicals. The biotechnology of phototrophs is entirely compatible with the biorefinery concept whereby their biomass is converted in an integrated manner to a multiplicity of product

and energy streams. This is illustrated by our current work with novel extremophilic microalgal strains to exploit their metabolic characteristics and devise innovative bioprocessing through the rational design of scalable and energy-efficient photobioreactor systems. Photoheterotrophy conditions with glycerol addition to the new microalgal strain *Dactylococcus dissociatus* MT1 can simultaneously increase biomass production and reduce or eliminate the need for gas sparging. This behaviour is modelled mechanistically by linking the oxygen exchange between the cells and the culture medium as a function of glycerol concentration, cellular chlorophyll content, and photosynthetic efficiency to biomass productivity. The latter is greatest when the photosynthetic and respiratory pathways are nearly balanced. This implies that an intracellular recycling of O₂ and CO₂ increases biomass production efficiency and that a process set-point can be reached which eliminates the need to provide supplementary O₂ or CO₂. In the same strain, environmental stressors can induce the production of the valuable carotenoids canthaxanthin, adonixanthin and astaxanthin. Light intensity has a positive influence on the accumulation of the major carotenoid, canthaxanthin, similarly to salinity stress, while nitrate deprivation has more of an effect on lipid production, an additional product stream. Nitrate depletion and salinity stress in combination increase both lipid and carotenoid accumulation. The growth and carotenogenesis phases can be initiated, reversed and repeated at regular intervals, thus opening the way to a scalable and sustainable bioprocess

BR3

Microbial production of required metabolites in plant/animal biorefinery

P. Pataková¹, L. Paulova¹, B. Branska¹, J. Kolek¹, K. Sedlar², I. Provazník²

¹ *University of Chemistry and Technology Prague, Technická 5, Prague, Czech Republic,* ² *Brno University of Technology*

The basic idea of a biorefinery which is to mimic oil processing refinery in a way where oil is alternated with biomass, preferably with the biomass unusable directly for food production, attracts attention of both laical and expert public. Although the concept seems promising, there are still many issues that prevent its application in pilot and industrial scale. The main microbial biorefinery problems are dependent on the way of biomass exploitation and may include necessity of expensive treatment of originally low cost material prior its use, low concentration of fermentable sugars in final hydrolysate often containing mixture of hexoses and pentoses (not fermentable for every microorganism), generation of inhibitors derived from lignin/cellulose/hemicellulose during biomass pretreatment and high energy/water/chemicals consumption. The presentation addresses some of these bottlenecks using examples of butanol, ethanol and lactic acid production in biorefinery régime. For butanol production by clostridia, there will be demonstrated advantageous properties of clostridia for biorefinery use and a potential offered by different methodical approaches toward the problem

solution i.e. joining of classical microbiology/biotechnology with bioinformatical *in silico* approach. Production of lactic acid or ethanol will be compared using different cultivation arrangements. In all mentioned cases, there will be shown promising combination of plant and animal waste as a complete microbial substrate. Future perspectives will be outlined.

Acknowledgement: This work has been supported by grant project GACR 17-00551S, TACR BIORAF No. TE01020080 and Inter-Action LTACH-17006.

BR4

Ethylene production from cyanobacteria

J. Yu¹

¹ National Renewable Energy Laboratory, 15013 Denver West Parkway Golden, Colorado, United States

Ethylene is the most widely produced petrochemical feedstock globally. It is currently produced from fossil resources, and its production via steam cracking is the largest CO₂-emitting process in the chemical industry. A potentially more sustainable alternative is a biological process that converts CO₂ to ethylene by photosynthesis. The *efe* gene encoding an ethylene-forming enzyme from the bacterium *Pseudomonas syringae* was expressed in the cyanobacterium *Synechocystis* 6803, leading to continuous ethylene production. Ethylene productivity has been increased by enhancing *efe* expression levels, such that up to 20% of photosynthetically fixed carbons are re-directed from biomass growth to ethylene

formation. Detailed characterization in the ethylene-producing strains using metabolic flux analysis identified global adjustments in carbon and energy metabolism, that support ethylene production without necessarily slowing down cell growth. Current challenges and approaches will also be discussed.

BR5

Fermentation of glycerol by *Paracoccus denitrificans* under sub-lethal high pressure levels

M.J. Mota¹, R.P. Lopes¹, M.M.Q. Simoes¹, I. Delgado¹, J.A. Saraiva¹

¹ Research Unit of Organic Chemistry, Natural and Agro-food Products (QOPNA), Chemistry Department, University of Aveiro, Aveiro, Portugal

The microbial conversion of glycerol into value-added products constitutes a promising approach to dispose the excess of glycerol generated during biodiesel production. *Paracoccus denitrificans* is a facultative methylotrophic bacterium, able to synthesize polyhydroxyalcanoates (PHAs) from many carbon sources, including glycerol^[1]. PHAs are a class of naturally occurring bacterial polyesters with unique and interesting features, such as biodegradability, biocompatibility, water resistance, and oxygen impermeability^[2]. It has recently been reported that microbial fermentations performed under sub-lethal high pressure (HP) can result in useful

process modifications, such as the increase of fermentation rates and yields, and/or the formation of products with different features^[3].

Therefore, the present work intended to evaluate the effects of HP on *P. denitrificans* growth and metabolism during glycerol fermentation. To meet that purpose, the fermentation process was tested under different sub-lethal pressures levels (10, 25, 35 and 50 MPa) at 35 °C. In parallel, control samples were incubated at atmospheric pressure (0.1 MPa) and 35 °C. The samples collected over time were analyzed in terms of cell growth, glycerol consumption and products formation.

HP showed an inhibitory effect on both cell growth and glycerol consumption, with the effect being more pronounced at higher pressure levels. In fact, no variation was observed on both parameters when fermentation was performed at 50 MPa. Regarding products formation, the results obtained so far suggest possible modification of the PHA composition, namely changes in the monomers chain length. Other metabolic changes were observed, such as the production of succinic acid in samples fermented under lower air availability. Overall, the findings of this work revealed interesting features of *P. denitrificans* metabolism, indicating that HP, as well as air availability, affect the metabolic pathways used by *P. denitrificans* during fermentation and lead to the formation of different products.

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BR6

Production of fuels and chemicals from Macroalgae

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Seaweeds are worldwide used as food and as source of chemicals (i.e. gelling agents and phycocolloids). Annually, 7-8 million tonnes seaweeds are harvested, with an estimated total value of the products of US\$ 5-6 billion^[1], with an increasing market and production capacity^[2]. Because their special chemical composition and the possibility of large scale cultivation in the ocean, or in combination with aquaculture (Integrated Multitrophic Aquaculture systems, IMTA) with high yields, they are potential feedstocks for production of renewable chemicals and fuels^[3].

The biomass of seaweeds is highly suited as raw material for the co-production of chemicals, biofuels and energy via the biorefinery approach. In recent projects, strategies have been developed for the biorefinery of native Atlantic seaweed species. In these processes, the main components,

sugars, proteins and minerals are converted into bulk chemicals and energy carriers. Several seaweeds (including green, brown and red species) were characterized and biorefinery routes have been assessed. The sugars in the seaweed biomass have been fermented into fuels (butanol) or polymer precursors (lactic acid), while the other components have been characterized for a variety of uses, including animal feed.

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BR7

Leveraging yeast potential for the valorisation of biomasses into valuable bioproducts

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Natural and engineered yeast cell factories are today extensively used for commercial productions. While the scientific and technological platforms leading to the production of a heterologous protein is under consolidation, with the exception of

membrane proteins, the production of heterologous and endogenous metabolites by metabolically engineered yeast cell factories always suffers from extensive regulation of cellular metabolism, which easily evolves in order to ensure the cellular homeostasis. This cellular homeostasis often leads to a low accumulation of the desired product.

While on the market we can find about 600 recombinant pharma-proteins, the amount of recombinant metabolites is undoubtedly much lower. Nevertheless, yeasts are among the workhorses for the commercialization of recombinant metabolites.

The main research efforts in our laboratory are related to the design of robust and stable production strains and bioprocesses to match the stress conditions occurring during industrial fermentation for both metabolite and protein productions. Here we will present how this can be reached both by manipulating specific targets involved in the pathway of interest or by modulating "hub elements" responsible for a general cellular reorganization. Finally, an innovative non-invasive strategy to monitor the accumulation of the "final product" will be presented.

BR8

Genome mining for biorefinery use

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Although a current biorefinery can hardly compete with a far less costly petroleum refinery, sustainable production of bio-based products and bioenergy appears as the future direction. Its wider utilization, however, requires thorough understanding of molecular processes in organisms used for biorefinery. Fortunately, the massive reduction in costs made the genome sequencing widely available and bioinformatic analyses on a genome wide scale became a common procedure in modern biotechnological research. Phylogenomic analyses, using PhyloPhlan, digital DNA-DNA hybridization or other tools, helps infer the proper taxonomic classification. Especially in cases where phenotype based classification can be misleading as we demonstrated during reclassification of promising butanol producer *C. beijerinckii* NRRL B-598, formerly misclassified as *C. pasteurianum*. Correct taxonomic assignment then permits better understanding of the behavior of an organism and is crucial for planning further genetic modification to improve its features like tolerance to toxic product of fermentation.

This can be done by following analysis of particular genes, in that case those coding efflux pumps, using aligning algorithms, e.g. Clustal Omega, Needleman-Wunsch, and their comparison based on hidden Markov models or sequence logos. Besides the slightest differences in sequence composition, such analysis can discover differences in whole signal pathways responsible for the specific behavior of an organism. On the other hand, in some cases even an exhausting analysis of a genome does not provide any satisfactory answer. Fortunately, sequencing itself can provide additional information. Sequencing platforms of the third generation, e.g. PacBio, allows also sequencing of a methylome on a genome wide scale. A bioinformatic analysis of these modifications then complements a standard genomic analysis with epigenetic information. Eventually, mapping reads from RNA sequencing with BWA, Bowtie or other mapping algorithm can detect level of transcription and post-transcriptional modifications.

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BR9

Gene expression of sporulation factors in *Clostridium beijerinckii* during solventogenesis

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Clostridia represent a huge group of Gram-positive, strictly anaerobic microorganisms including e.g. many well-known pathogenic species, natural polymers decomposers and also solvent producers. All *Clostridium* bacteria are able to form endospores which are generally recognized as the one of the most resistant form of life on Earth and are highly resistant to heat, UV as well as gamma radiation, desiccation, chemical substances including strong disinfectants etc. Sporulation is characterized by several, typical consecutive steps while each of them is characterized by specific cell morphology as well as gene expression. Unfortunately, sporulation program and its genetic background was studied thoroughly especially in *Bacillus* bacteria; the best described is model of sporulation in strain *Bacillus subtilis* 168 and many aspects of sporulation in *Clostridium* bacteria is still missing. Interconnection of sporulation and solvents production in solventogenic clostridia is also often discussed question and opinions about this issues differ considerably.

Clostridium beijerinckii represents sol-

ventogenic clostridium with ability of production of butanol which can be used as an important raw chemical for subsequent chemical synthesis or directly as a potential fuel additive. Amount of produced spores is generally highly dependent on chosen culture condition. The expression profile of several main genes of sporulation cascade, each from different stage of sporulation, namely *spo0A*, *spoIIE*, *sigG* and *spoVD*, were monitored in three *C. beijerinckii* strains: NCIMB 8052, NRRL B-593 and NRRL B-598. Similarly, expression of single genes connected to solventogenesis and acid production, namely *ald* and *buk*, were monitored as well in course of ABE fermentation with and without sporulation of culture using optimized TYA and RCM media and with the aim to clarify if sporulation is really necessary for satisfactory solvent production in this bacterium.

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BR10

Solvent production in sporulation deficient *Clostridium*

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Clostridium acetobutylicum and *beijerinckii* are spore forming bacteria, living

in anaerobic conditions. They are able to utilize a wide range of sugars to produce solvents such as acetone, ethanol, butanol (ABE) and isopropanol. These strains were used world wide in the ABE fermentation for the production of butanol and acetone from molasses at an industrial scale during 20th century. But this bioprocess was gradually replaced by a petroleum based process in the 1980s^[1].

However interest in these bacteria re-emerged since the end of the 90s with the craze for bio based chemicals^[2]. While the solvent production pathway has been intensely investigated, the sporulation mechanism in these organisms is still unclear^[3]. Several studies show a link between sporulation and solvent production in solventogenic *Clostridia*^[4,5] but none was able to explain the involved mechanism.

To fill the knowledge gaps in this field, we decided to generate sporulation deficient strains in *C.beijerinckii*, by deleting sporulation genes. SpoIIE is a phosphatase involved in the sporulation cascade. Previous studies show that spoIIE deficient *C.acetobutylicum* strains are asporogenous^[6,7]. We generated an asporogenous *C.beijerinckii* strain by deletion of the spoIIE gene. This deletion was obtained thanks to a novel efficient CRISPR cas9 system developed in our laboratory. This new method uses two plasmids to couple the inducible expression of the cas9 nuclease from *Streptococcus pyogenes* carried on one plasmid and the transcription of a guide RNA (gRNA) carried on a second plasmid. The separation of the cas9 gene and the DNA template enables to design larger DNA template and enhanced the DNA Cargo capa-

city of the system. The method used to generate this mutant and the characterization of the *spoIIE* mutant will be described in this presentation.

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BR11

How to convert lignocellulose to biosurfactants within a biorefinery

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Currently, sustainable surfactant products with decreased carbon footprint and complete biodegradability receive increasing demand. The production of these biosurfactants from second generation substrates is favored due to ecological benefits but also to make the process economically feasible.

Our research is focused on cellobiose lipids (CL) and mannosylerythritol lipids (MEL), which are among the most promising microbial biosurfactants with application potential in personal care, technical uses and pharmaceuticals. MEL and CL are produced by smut fungi of the genera *Ustilago* and *Pseudozyma*. Interestingly, the molecular structure and hence product composition of both biosurfactants can be tailored to meet specific demands.

In various projects we have created a value chain starting from lignocellulosic feedstock like beech wood and finally ending with high purity biosurfactants. The feedstock is first subjected to an organosolv process yielding the cellulosic pulp and a soluble lignin fraction. While the lignin fraction can be used for other product streams within a biorefinery, the cellulosic fibres are further hydrolyzed by enzymatic treatment to provide a substrate for the

microbial fermentation of biosurfactants.

Our optimized fermentation processes currently deliver product concentrations of 30 g/L for cellobiose lipids and over 120 g/L for mannosylerythritol lipids. However, increasing space-time yields, up-scaling and cost-reduction are still topics that need to be addressed here. Downstream processing as another critical step in cost-effective production is a further focus. Using different approaches like extraction with "green" solvents, sedimentation or foam fractionation, sufficient amounts of surfactants in desired purity are generated. Lastly, metabolic engineering creates the possibility to select or generate strains that are capable of producing a certain type of biosurfactant with high yields.

BR12

Vibrational spectroscopy for monitoring lipogenesis in microbial cells

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The use of vibrational spectroscopy (FTIR and Raman spectroscopy) for the characterization of lipogenesis in oleaginous microorganisms is gaining an interest from both fundamental and application point of view. Both, FTIR and Raman

spectroscopy is the biophysical methods that are used for characterizing the biochemical composition of microbial cells. Both techniques are non-invasive, do not require labeling and rapid. The acquired FTIR and Raman spectra are complementary and represent a molecular 'fingerprint' of the total cell chemical composition which constitute of cell lipids, proteins, nucleic acids and carbohydrates. The application of vibrational spectroscopy in microbial lipidomics is broad and it is related to analyzing both lipid associated parameters as the analysis of the total lipid content, length of fatty acid chain and the unsaturation level, and cell change associated with the lipogenesis.

In the present study, the authors show the approach based on vibrational spectroscopy that provides robust prediction of total lipid and fatty acid profile in fungal cells grown on different substrates. The evaluation of FTIR and Raman spectroscopy for the analysis of lipids and prediction of total lipid and fatty acid profile has been performed for filamentous fungi and yeasts^[1,2]. In addition, a high-throughput approach based on FTIR spectroscopy and micro-cultivation in Microtiter Plate System was developed for rapid screening and optimization of oleaginous microorganisms. Further, it has been shown a successful use of FTIR spectroscopy for monitoring lipid extraction processes.

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biochemical fingerprinting of *Saccharomyces cerevisiae* by Fourier transform infrared spectroscopy. *PlosOne*. **10**(2):e0118052

BR13

Integrating production of PHA into bio-refinery concept – shedding light also on specific consequences of non-optimal cultivation conditions

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Polyhydroxyalkanoates (PHA) are microbial polyesters produced and accumulated by numerous bacteria primarily as storage of carbon and energy. Nowadays, PHA are considered being biodegradable alternative to petrochemical plastics such as polypropylene or polyethylene. Moreover, their production can be integrated into various bio-refinery concepts which both enhance ecological nature of these polymers and also substantially improve economic feasibility of the PHA production by fermentation. Therefore, we investigated biotechnological production of PHA using waste frying oils as a cheap animal food

chain non-competing carbon substrate. In addition we also investigated utilization of inexpensive waste-originating complex nitrogen sources such as proteolytic hydrolysate of cheese whey or alkaline hydrolysate of chicken feather which substantially improves process of PHA production by supporting growth of bacterial cultures but also by enhancing PHA accumulation in bacterial cells. Furthermore, we also aimed at valorization of low-cost lignocellulose materials such as spent coffee grounds or waste wood biomass employing bio-refinery concept in which PHA production represent one of the key processes. For instance, spent coffee grounds can be completely utilized in sequential process, in which oil can be extracted from spent coffee grounds and used for PHA production employing *Cupriavidus necator*. Further, the solid residues after oil extraction can be hydrolysed yielding fermentable sugars, which are further used as a substrate for production of PHAs employing *Burkholderia cepacia*. Finally, solids after SCG hydrolysis possess high calorific value and can be used as a fuel to at least partially cover energetic demands of the process.

Furthermore, utilization of waste substrates is usually associated with non-optimal cultivation conditions induced by presence of various microbial inhibitors. Therefore, we also focused on influence of stress conditions on PHA production and, oppositely, also on involvement of PHA in stress resistance of bacteria. It is very interesting that numerous stress conditions usually connected with waste substrates such as lignocellulose hydrolysates (e.g. osmotic pressure, oxidative pressure, presence of weak organic acids etc.) sti-

mulates PHA biosynthesis in bacterial cells when applied at mild level. Oppositely, presence of PHA in bacterial cells considerably enhances their resistance against numerous stress factors such as osmotic pressure, temperature or oxidative stress. Therefore, it seems that microbial production of PHA is very interesting process robust to specific conditions associated with utilization of complex and non-optimal substrates in the concept of bio-refinery.

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BR14

Bench-scale production of polyhydroxyalkanoates and other valuable biomaterials from xylose-rich lignocellulosic hydrolysates

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The set of work developed at Instituto Superior Técnico addressed the optimization of polyhydroxyalkanoates (PHA)

production from wheat straw lignocellulosic hydrolysates (WSH). PHAs are biodegradable and bioproduced polymers, suitable for applications in fields such as agriculture, food packaging, medicine and pharmacy^[1,2]. In most PHA producing bacterial strains, poly-3-hydroxybutyrate (P(3HB)) accumulation as carbon and energy storage granules is favored by an excess carbon source and a low supply of macronutrients (N, P, O₂) or micronutrients (e.g. Mg). A range of different C-sources is metabolized to produce PHA co- and terpolymers. Research efforts have been devoted to trying to decrease production and extraction costs in order to increase the market share of these polymers^[2].

Burkholderia sacchari DSM 17165 was chosen due to its simultaneous ability (i) to metabolize C-5 and C-6 sugars and (ii) to produce PHA. Polymer accumulation was triggered by P-limitation. At *biorefinery.de*, lignocellulosic biomass (chopped wheat straw) was pretreated using the AFEX process followed by enzymatic hydrolysis and a concentration step, originating WSH with different glucose/xylose ratios. C-sources were quantified by HPLC, PHA and gamma-butyrolactone (GBL) by GC^[3,4]. For PHA recovery from lyophilized cells, solvent extraction was followed by precipitation with C₂H₅OH^[5]. Fed-batch experiments were run on 2L bench scale stirred tank bioreactors, under controlled conditions with on-line data acquisition.

Assays ran with real WSH were compared to "simulated" hydrolysates with different glucose/xylose ratios. Feedback from bench-scale assays allowed for WSH improvement (sugars:organic acids: inhi-

bitors ratio) by *biorefinery.de*. Remarkable P(3HB) volumetric productivities (Prod_{v,ol}) of 1.7 g/L³h were obtained for P(3HB) production from WSH as sole C-source. These high productivities resulted from process optimization, involving the choice of the strain and cultivation media, and fed-batch operating conditions. Cell density and P(3HB) Prod_{v,ol} obtained were similar to those reached in control cultivations with mixtures of commercial sugars. Additionally, fed-batch strategies for the production of P(3HB-co-4HB) on glucose and GBL were developed, and led to copolymer accumulation with Prod_{v,ol} reaching 0.5 g/L³h using wheat straw hydrolysates as major carbon source^[6].

Recently, the authors reported for the first time that *B. sacchari* DSM 17165 is also able to produce xylitol and xyloonic acid from low glucose/xylose ratio mixtures^[7]. Further optimization is under way to find the best culture conditions to favor either PHA, xylitol or xyloonic acid production from xylose rich hydrolysates, towards an integrated biorefinery concept.

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BR15

Consolidated production of Volatile Fatty Acids from plant biomass using defined and natural microbial consortia

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A process design approach for consolidated production of Volatile Fatty Acids (VFAs) from plant biomass was developed. The design of the reactor enables simultaneous aerobic and anaerobic conditions in direct physical closeness and thus allow different microorganisms to coexist, grow and co-operate. Both defined synthetic microbial consortia as well as natural undefined ones were used to hydrolyze the plant biomass and convert the released sugars to the desired fatty acids. The production can be directed towards different carboxylic acids by either selecting the microorganisms in the case of defined con-

sortia or by controlling the process conditions (in the case of natural ones).

A synthetic fungal-bacterial consortium for the direct production of lactic acid from cellulosic biomass was developed. The aerobic fungus *Trichoderma reesei* was introduced as producer of cellulolytic enzymes and the facultative anaerobic bacterium *Lactobacillus pentosus* was used as the product forming microorganism. The cellulolytic activity of the system was investigated and the addition of *Aspergillus niger* as a second enzymes producer was evaluated.

The use of natural microbial consortia for the production of VFAs was also studied. We examined the ability of our system to provide suitable growth conditions to different microbial members of the consortium, for the direct production of VFAs from biomass. Furthermore, we studied the effect of the introduction of a cellulolytic enzyme producer, on VFAs yields and productivities.

BR16

Comprehensive Characterization of Lignin and its Degradation Products: Approaches and Challenges

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Lignin, a major constituent of lignocellulose, is an abundant natural heteropolymer with potential to be an excellent feedstock for renewable chemicals as a replacement for petroleum-based analogs. Lignin's ability to crosslink and repolymerize provides structure for membranes and plant walls. However, these same features make its decomposition and analysis challenging.

In our research, we address a comprehensive characterization of both the initial feedstock and lignin breakdown products. Our strategies include 1) a newly developed thermal carbon analysis (TCA) protocol providing carbon mass balance closure, 2) phosphitylation followed by nuclear magnetic resonance (^{31}P NMR) spectroscopy enabling the evaluation of main structural features, and 3) gel permeation chromatography with mass spectrometry (GPC-MS) to determine molecular weight (MW) distribution.

Based on quantification of thermally evolving carbon fractions obtained with TCA, we were able to differentiate thermally desorbing monomeric and dimeric phenolic species from pyrolyzed large-MW compounds, as well as the "coke" fraction evolving only in the presence of oxygen at high temperatures. The TCA quantification provided results similar to thermogravimetric analysis, while being more effective in characterization of thermally evolved fractions not visible by TGA and specific to carbon, i.e., distinguishing the organic matter of lignin from water and other inorganic impurities. The TCA also provided complementary data to detailed thermal desorption/pyrolysis gas chromatographic/mass spectrometric ana-

lysis.

Phosphitylation followed by ^{31}P NMR spectroscopic analysis allows for identification and quantification of the majority of OH-containing compounds formed during lignin hydrotreatment. Particularly, this method readily identifies phenols, alcohols, carboxylic acids as well as hemiacetal groups. Comparison of lignin ^{31}P NMR spectra before and after hydrotreatment has provided useful information on possible repolymerization of degradation products, i.e., structure of nano-size oligomers.

GPC and gel filtration chromatography (GFC) are typically employed for determination of MW distribution of lignin and its major degradation products. However, lignin is a heteropolymer of an uncertain shape, so these methods may provide an incorrect MW value. The resulting MW may also be affected by the superposition of the molecular sieve effect and unwanted analyte-column interactions occurring due to functional groups of lignin. The complexity of lignin characterization is further magnified by the lack of representative standards, which are usually replaced by polystyrene fractions only inadequately mimicking the behavior of lignin.

Thus, representative mono- to oligomeric standards have been synthesized and a protocol for their analysis has been optimized. We have confirmed that GFC and GPC methods are affected by other interactions besides the size exclusion effect. In our study carried out using a high-resolution time of flight MS, we have demonstrated advantages of detection with electrospray-high resolution (MS) providing accurate masses for high

MW species and showed that detailed optimization is essential to prevent excessive fragmentation and loss of molecular ions. Our results have also been evaluated with regard to other common detectors suitable for quantification, i.e., evaporative light scattering detector and traditional UV detection.

BR17

***Rhodothermus marinus* – A New Platform Organism for Industrial Biotechnology**

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For a long time, industrial biotechnology focused on only a few organisms. However, that paradigm will certainly change as our understanding improves and molecular tools for additional organisms become widely available. The thermophilic bacterium *R. marinus* is for this purpose a promising organism owing to: 1) wide metabolic range of sugars (glucose, xylose, arabinose, mannose and galactose and glucose as well as uronic acids), 2) Production of an array of polysaccharides degrading enzymes – allowing use of polymeric substrates, 3) robustness as a thermophile, and 5) interesting products, such as carotenoids.

Thermophilic microorganisms include ecological generalists that have evolved wide substrate degradation systems enabling efficient utilization of complex carbohydrates in biomass. Thermophiles are also robust by nature. *R. marinus* is an aerobic thermophile producing a great diversity of polysaccharide-degrading enzymes using abundant sugars in both terrestrial and marine feedstocks, i.e., glucose, xylose, arabinose, mannose and galactose and glucose. Strains of *R. marinus* are thus capable of utilizing a large fraction of the constituent carbohydrates in plant including wood lignocellulose and algal polysaccharides, which are major components of third generation biomass and effective degradation is accomplished by synergetic action of enzymes of different activities.

The ability to grow at high temperatures in bioreactors also minimizes contamination of spoilage bacteria. Thus far, a few thermophiles have been metabolically engineered for production of biofuels, with encouraging results. However, wider industrial use of thermophiles awaits development of their cultivation technology. Amongst thermophiles, primarily anaerobes have been investigated. *R. marinus* in contrast is an aerobic marine thermophile. Here, we are undertaking increased investigations on *R. marinus* to gain understanding of the link between the utilization of different types of biomass feedstocks for production of cell mass and metabolites of industrial interest.

BR18

Simultaneous saccharification and fermentation of steam pretreated beech wood with *in situ* *Irpex lacteus* treatment

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Hardwood residues are attractive lignocellulosic feedstocks for biorefineries due to their year-round availability and their high energy density. However, they typically show a higher lignin content and are more recalcitrant to enzymatic sugar release than typical herbaceous residues. Consequently, harsh pretreatment conditions are required, leading to an increased consumption of energy and to the formation of enzyme and fermentation inhibitors.

In order to enable efficient simultaneous saccharification and fermentation (SSF) of beech wood to ethanol, we investigated the combination of steam pretreatment and biological treatment with lignin degrading fungal strains. To select a suitable fungal strain, a screening of seven white rot fungi was performed by growing them in submerged and membrane aerated biofilm cultures on mildly steam pretreated beech wood followed by enzymatic hydrolysis of the residual solids. The treatment with *Irpex lacteus* for 14 days in a biofilm reactor almost doubled the glucose yields in enzymatic hydrolysis of washed solids compared to the control. Furthermore, we could show that *Irpex lacteus* detoxified the steam pretreatment hydrolysate thereby ci-

rcumventing cellulase inhibition in whole slurry enzymatic hydrolysis experiments. We then aimed to investigate an advanced process configuration, where SSF is performed with *in situ* fungal delignification and detoxification. To this end, a membrane aerated stirred tank reactor was first inoculated with *Irpex lacteus*. After a visible biofilm was formed, yeast and cellulytic enzymes were added to start ethanol production. The presence of *Irpex lacteus* increased whole slurry SSF ethanol yield from 60 to 80%.

The *in situ* fungal treatment of lignocellulosic biomass is an interesting method to improve whole slurry SSF. Advantages compared to separate fungal pretreatment include the circumvention of an extra reactor for fungal treatment and the reduced sugar loss due to competition of the yeast and the fungi for the easy available sugars.

BR19

The holistic biorefinery concept: from by-product to high value – case study

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The most effective holistic approach of circular economy based on biotransformation of the agricultural biomass, which has been developed in the last few years,

is the concept of biorefineries. In the biorefinery approach assuming zero waste, each process stream is exploited to the full through careful fractionation to produce commercially valuable products or through reuse of by-products and wastes. The biorefinery approach has already been introduced to this area through a consideration of biodiesel and bioethanol, but very interesting seems multitude of other application especially in green chemistry to obtain high value added compounds. The main objective of this case study (demonstration plant) is to focus on possibilities using GRAS microorganisms such as *Bacillus subtilis* in biotransformation of press cake or meal remaining after oil extraction from oilseeds and subsequent fractionation in biorefinery. The key to this is the assertion that a complex mixed component material can be exploited in a variety of ways with some components used to produced new materials while others can be directly fractionated and separated into commercially highly valuable materials. Most of high value added products are synthesised in relatively low quantities e.g., biosurfactants making often the production process unprofitable. The recent advances in technology coupled with system biology and the rise of biotechnology as an industrially viable concept now make it possible to examine in more detail the traditional processes and to seek new ways to satisfy the demands for sustainability and environmentally acceptable processes and new products.

BR20

Application of onsite produced cellulase in lignocellulosic biorefinery for bioethanol and lactic acid production using freshly pretreated materials

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Cellulosic ethanol and lactic acid production is considered a promising strategy to enhance alternative resources for increasing demands as fuel for transportation and biodegradable plastics. Commercialization of bioethanol production has recently started but still several methodologies need proper solution. One of the major difficulty is of pretreatment inhibitors. These toxic inhibitors are released during pretreatment, those negatively affect the saccharification of lignocellulosic materials, microbial growth, ethanol production and yield. As an attempt to overcome this problem, in the present study, evolutionary adaptation was evaluated of *Saccharomyces cerevisiae* Angel for ethanol production and *Bacillus coagulans*, *Lactobacillus sp*, and *Pediococcus sp*. All microbial strains were continuously transferred on the wheat straw hydrolysate with regular interval until stable cell growth, glucose consumption and ethanol and lactic acid titer. This strategy improved fermentative performances of adapted strains compared to parental strains in both hydrolysate and in

simultaneous saccharification and fermentation. Ethanol yield reached more than 90 % when wheat straw hydrolysate or freshly pretreated rice straw and wheat straw were used as carbon source. In addition, lactic acid yield reached to 75 %. Moreover, in the saccharification of freshly pretreated lignocellulosic biomass on-site produced cellulase was applied. Utilization of the on-site produced cellulase in bioethanol and lactic acid production saved the costs of enzyme purification, packing and transportation and consequently reduce ethanol and lactic acid production cost.

Oral Presentations – Pharmaceutical Biotechnology

PB1

Marine antimicrobial compounds and their potential as lead compounds for novel antibiotics

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Marine organisms like animals and bacteria together with other biological material of marine origin, that also include organic matter usable for food, are a source of huge potential for exploring novel bioactive components with activities that can be exploited. In this context, marine proteins and peptides are very interesting.

The wide repertoire of biological functions that such natural peptides have makes them exciting for bioprospecting and drug discovery. Among them, antimicrobial peptides (AMPs) are important since the increased spreading of bacterial resistance in human- and other pathogenic bacteria worldwide against commercial available antibiotics have stimulated the search for novel antimicrobials. AMPs are believed to be important components of the innate defense system in invertebrates in addition to also be an important source for potential new drug leads of antimicrobials. In general, small peptides have advantages in their rather specific activity, small size,

short half-lives, usually non-immunogenic properties and low toxicity.

Many of the drugs approved today are based on knowledge from natural products (over 60%) from terrestrial sources. During the last decades, however, there has been a growing interest of bioprospecting of marine resources for their unique bioactive properties in addition for being usable as food or feed.

We have characterized several new cases of such AMPs and explored their mode of action. The organisms have been collected from the Arctic or/and sub-Arctic region and can be very diverse covering biological resources from microalgae to invertebrates. Novel bioactive peptides can be isolated, characterised and explored by traditional bioassay-guided purification of organic extracts in combination with genetic approaches and virtual screening. In addition, a more extensive screening, performing biosensor tests can reveal the mechanism of action of the peptides towards the membrane or integral targets of the bacteria. SAR studies can give information about important pharmacophores of the molecules and thus be the basis for improved marine natural products mimics that can be candidates of novel drug leads with specific properties.

PB2

Old and novel glycopeptide antibiotics: from product to gene and back

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Glycopeptides are currently considered drugs of last resort for life-threatening infections caused by multi-drug resistant Gram-positive pathogens such as *Staphylococcus aureus*, *Enterococci spp.* and *Clostridium difficile*. They arrest bacterial cell wall biosynthesis by binding to the acyl-D-alanyl-D-alanine terminus of the nascent peptidoglycan, blocking its extracellular polymerization, and subsequently inhibiting cell growth and division. These agents are glycosylated, halogenated, and in some cases lipidated, non-ribosomal heptapeptides produced by a diverse group of filamentous actinomycetes. First generation glycopeptides include vancomycin, made by *Amycolatopsis orientalis*, and teicoplanin, produced by *Actinoplanes teichomyceticus*. The spread of resistance to glycopeptides in enterococci since 1988 and the recent emergence of high level of glycopeptide resistance in clinical isolates of methicillin-resistant *Staphylococcus aureus* have prompted the search for second-generation glycopeptides (dalbavancin, oritavancin, telavancin) that are produced by chemical modification of natural products and recently introduced in clinical practice. In this presentation, a brief history on how teicoplanin and

the teicoplanin-like A40926 (the natural precursor of semi-synthetic dalbavancin) were discovered by natural product screening will introduce the concept "from product to gene". Current knowledge on the genomes of the two producing organisms (respectively *Actinoplanes teichomyceticus* and *Nonomuraea gerenzanensis*) and on regulation of their glycopeptide biosynthetic gene clusters will be overviewed. This up-to-date information is currently used for rational engineering of the two producing actinomycetes by targeted DNA manipulation, with the aim to improve the production process of the two glycopeptide antibiotics ("from product to gene"). Although glycopeptide scaffold could be chemically synthesized, the complexity of these natural products renders fermentation the only viable route for producing them pharmaceutically. Low yield of fermentation is a serious limitation on the way to the development of these drugs and their novel derivatives. In the past, several classical strategies have been applied in attempts to increase the production of glycopeptides including empirical mutagenesis of the producing organisms and optimization of fermentation media and conditions. Innovative approaches based on genomic information can successfully complement classical strategies.

PB3

5-Aminolevulinic acid in glioblastoma surgery and intracellular tumour targeting by a shiga toxin subunit B

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Glioblastoma multiforme (GBM) is the most common but also most malignant primary brain tumour. Treatment of GBM remains very challenging, as no contemporary treatments are curative. GBM cells show an enormous heterogeneity, are invasive and exhibit many drug resistance strategies. Standard treatment of GBM patients consists of maximal surgical resection, radiotherapy, and concomitant chemotherapy. To improve the degree of resection, 5-aminolevulinic acid (5-ALA) is used to differentiate normal cerebral from tumor tissue. Oral administration of 5-ALA leads to an accumulation of fluorescent protoporphyrin IX (PpIX) in the malignant glioma and the fluorescence signal can be visualized during the operation with a fluorescence microscope^[1].

In our work, we have established several *in vitro* glioma cell culture models to study the effects of 5-ALA. We found that glioma cells with a migratory phenotype or when cultured under physiological (hypoxic) oxygen conditions are able to accumulate PpIX but may be less sensitive to camptothecin treatment and photodynamic the-

rapy. Co-medication with the antiepileptic drug phenytoin reduced the efficacy of 5-ALA induced PpIX fluorescence *in vitro*^[2].

Further, we developed a novel targeting approach for glioma cells using a shiga toxin subunit B as a potential carrier protein for antineoplastic drugs. Shiga toxin is taken up into the cytosol through internalization via the binding to glycosphingolipid globotriaosylceramide^[3]. In our studies, we were able to produce the non-toxic shiga toxin subunit B in an *E.coli* strain with high efficiencies. The purified and fluorescent dye-labeled toxin was taken up intracellularly into Vero cells much more efficiently (550:1) than the control dextran molecule of the same size.

Intracellular targeting using shiga toxin seems to be a promising approach to image and to target GBM cells. However, further research is needed to elucidate its role in GBM targeting and to investigate its safety profile *in vitro* and *in vivo*.

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PB4

Assembly and disassembly of HIV-1 as a target for screening and rational design of anti-HIV-1 compounds

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HIV-1, a member of retroviral family, infects the key cells of human immune system, specifically CD4+ T cells and macrophages. HIV-1 infection results in gradual depletion of T cells, which in non-treated individuals leads to the development of acquired immune deficiency syndrome (AIDS). Current therapy is based on a combination of several inhibitors targeting viral enzymes: mostly reverse transcriptase and protease. Inhibition of integrase or virus entry is another possibility. However, due to development of multidrug resistance, there is a continuing demand for identification of anti-HIV compounds that would exhibit novel mechanisms of action allowing thus to expand potential treatment options and overcome the problems with the resistant strains.

Attractive inhibitor targets in the HIV-1 life cycle are the assembly of HIV-1 immature particles as well as the subsequent step i.e. the disassembly of mature core. We have developed a method for *in vitro* screening and testing of anti-HIV compounds interfering with the assembly of both, immature as well as mature HIV-1 particles. We have also established a method for testing of compounds blocking the processes connected with the HIV-1 un-

coating. Both methods were verified by already published inhibitors as well as used for screening of mixtures of natural compounds and rationally designed set of inhibitors.

PB5

Uncovering citrinin biosynthesis in *Monascus ruber* by reconstructing its biosynthetic pathway in the secondary host *Aspergillus oryzae*

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Citrinin, a nephrotoxic mycotoxin, belonging to the azaphilone family^[1], is originally discovered from *Penicillium citrinum* in the 1930s and later from *Aspergillus species* and *Monascus species*. Although citrinin was among the first compounds to be identified as a polyketide and its biosynthesis was investigated from the 1950s^[2], however, remarkably little is known of the individual chemical assembly steps of its biosynthesis.

Citrinin is widely regarded as a model compound, especially in the arena of fungal polyketide biosynthesis, where it is related to many bioactive metabolites such as

the azaphilones, sorbicillinoids and tropolones. We thus sought to definitively clarify its biosynthesis. In our research, the individual steps of citrinin biosynthesis in *Monasascus ruber* M7 were thoroughly and systematically investigated by a combination of targeted gene knockout and heterologous gene expression in the secondary host *Aspergillus oryzae*^[3]. The pathway involves the synthesis of an unreduced trimethylated pentaketide by a non-reducing polyketide synthase (nrPKS) known as CitS. Reductive release yields the keto-aldehyde 2 as the first enzyme-free intermediate. The nrPKS appears to be assisted by an as-yet cryptic hydrolysis step catalysed by CitA which was previously wrongly annotated as an oxidase. CitB is a non-heme iron oxidase which oxidises the 12-methyl of 2 to an alcohol. Subsequent steps are catalysed by CitC which oxidises the 12-alcohol to an aldehyde and CitD which converts the 12-aldehyde to a carboxylic acid. Final reduction of C-3 by CitE yields citrinin^[4]. The pathway rules out alternatives involving intramolecular rearrangements, and fully defines the molecular steps for the first time and corrects previous misleading interpretations in the literature.

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PB6

Biofilms of opportunistically pathogenic microorganisms and possibilities of their regulation

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Pseudomonas aeruginosa is an important source of nosocomial infections, especially in patients with compromised host defense mechanisms. The increasing resistance to current drugs and its ability to form biofilms poses a serious challenge to immune defense systems and antibiotherapy. The objective of our work was to investigate the effect of natural products with different biological activity (baicalein, chitosan, rhamnolipids, stilbenes, antibiotics etc.) on *P. aeruginosa* planktonic and biofilm growth and level of regulation molecules involved in quorum sensing system.

Quorum sensing allows microorganisms to coordinate their behavior based on cell density. *P. aeruginosa* uses for this purpose N-acyl-homoserine lactones. Production of these molecules closely follows the adhesion and biofilm formation processes and therefore its inhibition may be an interesting tool in virulence and infection control.

The interaction of biologically active substances with the initial stages of adhesion and with the pre-formed biofilms

was studied and the viability of cells, the total biofilm biomass and the N-acyl-homoserine lactones production was determined with the aim of finding an effective approach to suppress *P. aeruginosa* biofilm formation. The production of QS molecules in *P. aeruginosa* was determined using *Agrobacterium tumefaciens* NTL4 (pZLR4) biosensor.

PB7

The nanoliter-reactor platform for high throughput screening of cell libraries secreting bioactive compounds

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The discovery of biological activities and the development of efficient cell lines for the production of biologics often relies on the screening of large cell libraries. We will present the nanoliter-reactor (NLR) platform that consists in the encapsulation and cultivation of individual library members in gel-microcarriers and the subsequent analysis of the candidates based on a phenotypic assay using flow cytometry. The NLR technology is highly flexible with respect to cell types, assay

formats, and analyzed products, and enables a throughput of up to 1 million candidate clones per day.

We will highlight the versatility of the NLR-platform by showing case studies with bacterial and mammalian cells and two exemplary assay formats. The first assay principle is based on the co-cultivation of candidate cells with sensor cells that react on compounds secreted by the candidates. Such assays can be used for the screening of growth inhibiting or toxic compounds, such as antibiotics, as well as for growth promoting molecules. As a second assay format the application of functionalized NLRs for the screening of antibody secreting cells will be shown, a valuable protocol for the development of highly productive cell lines or for the discovery of antibodies binding to difficult targets.

The presented NLR-platform offers a powerful tool for the analysis of large cell libraries by various assay formats, thus enabling the discovery of rare biological activities and the optimization of production strains and cell lines.

PB8

The Sugar Code as a Door Opener at Resistant Uptake Barriers – Biotechnological Concepts for Glycotargeted Drug Delivery in Bladder Cancer

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Protein bioconjugates or truncated derivatives that are reduced to the functional domain(s) are important tools in the development of enhanced treatment concepts for a wide range of diseases. In many cases, a binding domain or recognition element is combined with an effector domain with cytotoxic properties or catalytic activity. Targeted adhesion to specific surface receptors, e.g. via antibodies, often presents the decisive trigger to induce endocytic uptake, and can be used to exert control over the subsequent processing steps via intracellular trafficking routes. Up to now, the production of such multifunctional bioconjugates is mainly reliant on conventional coupling biochemistry, which, however, entails problems in scalability, batch-to-batch variability and stability of the final product. The possibility for biotechnological production as one single entity in a recombinant host system offers alluring prospects to implement safe

and economically sustainable supply chains, but requires detailed knowledge on the impact of molecular and configurational variations (e.g. glycosylation) on delivery performance and pharmacological efficacy.

The limited impact of intravesical chemotherapy in bladder cancer (BCa) provides a good example for the need and benefits of enhanced delivery concepts at resistant epithelial barriers. It has been demonstrated that bioadhesion to carbohydrate epitopes via lectins may offer an elegant way to counteract urine washout and escape the endosomal/lysosomal degradation pathway. Bioconjugates of lectins with horseradish peroxidase (HRP) can be utilized to realize targeted enzyme-prodrug therapies in combination with orally absorbable radical precursors (lectin-delivered enzyme/prodrug therapy; LeDEPT). However, an integrated development approach with close feedback from biological performance on upstream and downstream process design is required to harness the full potential of such innovative strategies. Here, case-specific findings and general implications of research efforts directed at advancing LeDEPT and similar bioconjugate-based concepts from partial to fully recombinant production settings are presented and critically discussed.

PB9

Highly Potent and Immune Stimulatory Antibody Drug Conjugates with a novel Anthracycline Toxin

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The development of antibody drug conjugates (ADCs) is associated with the challenge of selective delivery of highly potent toxins to tumors. ADC stability, homogeneity, binding specificity and the engagement of immune-mediated cell death (ICD) mechanisms are critical factors to achieve an optimal therapeutic index of ADCs, especially, if ultra-potent toxic payloads are employed. Data for the anti-tumor activity in various *in vitro* and *in vivo* tumor models with novel, next-generation ADCs comprising a novel ultra-potent anthracycline toxin will be presented.

PB10

Smart nanostructures for bio-applications

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Three major applications of laser induced structures are introduced, cytocompatibility control, application as anti-bacterial substrate and plasmonic-based detection system. Laser induced regular structures on the surface of aromatic polymers and their interaction with different cell types were studied. The augmentation of the surface area enabled more pronounced cell-material interaction and elevated formation of filopodia and focal adhesions. Modification of biopolymer surface by laser with high number of pulses and fluence may lead to creation of surface wrinkle-like structures with high roughness. The laser treatment of biopolymer foil guide the cell growth and exhibit a natural barrier for cell growth.

We have focused on *in vitro* cytotoxicity assessment and antibacterial activity of Pd and Ag nanostructures in its various forms (nano- layers, droplets and wires) supported on a biocompatible polymer. For the construction of a second generation antibacterials we used the synergic effect of (i) patterning of polymeric materials by a laser, and (ii) deposition of noble metals in their nanostructured forms. We prepared highly-ordered periodic structures (ripples) on polyethylene naphthalate (PEN) with fully separated Ag nanowires. The results of antibacterial tests predetermine these novel structures as promising materials able to fight against a broad spectrum of microorganisms.

One of the more promising fields of excimer-created periodical nanostructure application is the plasmonic-based de-

tection system. The further covering of patterned surface by thin metal films allows preparation of substrates, able to efficiently support the surface plasmon-polariton (SPP) excitation and propagation. SPP excitation leads to efficient "focusing" of light energy in the near-metal space and significant enhancement of closed (bio)organic molecules optical response. This phenomenon is commonly used for the design and creation of sensorics platform with unprecedented sensitivity and ability to perform (bio)medical analysis in the fast and unpretentious way.

PB11

An enzymatic tool-box for modification of hyaluronan

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Hyaluronan (HA) is a high-molecular glycosaminoglycan, which fulfils important functions in human organism. Due to unique properties and biocompatibility, HA is abundantly used in medicine and cosmetics. Recent studies indicate that biological activity of HA oligosaccharides differ from that of high-molecular polysaccharide. We elaborated processes for production of enzymes, which cleave HA macromolecules into fragments of various length and structure.

HA-hydrolase from mould fungus *Talaromyces stipitatus* was expressed in *Pichia pastoris* host and produced in amounts

of 895 kU/L medium. The recombinant enzyme cleaved β -(1,4) bonds of HA in non-processive fashion and yielded odd-numbered HA oligosaccharides with N-acetyl-D-glucosamine on the reducing terminus. *T. stipitatus* recombinant hyaluronidase was characterised by high thermostability and specificity to HA.

Another hyaluronan hydrolase, this time with β -(1,3) activity, was obtained from *Hirudinaria manillensis* leech. The enzyme was expressed in *Pichia pastoris* and produced in amounts of 109 kU/L medium. Products of HA digestion with this enzyme were odd-numbered HA oligosaccharides with D-glucuronic acid on the reducing terminus.

For non-hydrolytic cleavage of HA, HA-lyase from bacteria *Streptococcus pneumoniae* (SpHyl) was used. The enzyme was expressed in *E.coli* and produced in amounts of 220 kU/L medium. SpHyl cleaved β -(1,4) bonds of HA in the reaction of β -elimination in a processive exolytic fashion. The product of enzymatic reaction was dimer of HA with unsaturated D-glucuronic acid on the non-reducing terminus.

Larger HA oligosaccharides with the same structure were obtained by the action of bacteriophage-associated HA-lyase HylP1. The enzyme was produced in the similar expression system in amounts of 2.3 kU/L medium. HylP1 cleaved HA in the non-processive endolytic fashion, and the reaction products were different size odd-numbered HA oligomers.

By applying the above described enzymatic tool-box all types of biologically active HA oligosaccharides can be derived.

Oral Presentations – Food and Agriculture Biotechnology

FAB1

Enhancing brewing yeast performance

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Brewing yeast strains can be divided into top-fermenting and bottom-fermenting strains. Among the top-fermenting yeasts belong the many species of the genus *Saccharomyces*, most of them being closely related to the species *Saccharomyces cerevisiae*, creating a heterogeneous group of polyploid and sometimes hybrid yeast strains. Bottom-fermenting strains belong to the species *Saccharomyces pastorianus* and are genetic hybrids of *Saccharomyces cerevisiae* and *Saccharomyces bayanus*. Targeted genetic modification of a yeast strain is one way to increase beer quality and to improve the economics of beer production. Despite difficulties in many laboratories around the world, various transformed brewer's yeast strains have been prepared. Examples include modifications to enable extended stability of flavour and beer foam, limits to ethanol production, and shortened fermentation times, all of which contribute to significantly more ef-

ficient beer production.

The aim of our work was to apply a surface protein display technique for modifying the *Saccharomyces* cell wall and to determine whether this technique would be useful for improving brewing yeast performances. The cell surface display technique is a powerful tool for endowing novel functions on the host cell by displaying functional proteins on its surface. Proteins covalently bound to the yeast surface are more active than free proteins in solution. We applied this methodology for the development of a brewing yeast with a truncated, active α -acetolactate decarboxylase (ALDC) from *Acetobacter aceti* ssp. *xylinum* attached to the cell wall. With these cells, no biacetyl was present in the wort after primary fermentation. Another application was with a proline-rich peptide, QPF, attached to the cell wall. Yeast cells expressing the cell wall-bound QPF peptide were able to bind about 20 % more proanthocyanidins compared with a control strain displaying just the anchoring domain. Since these modifications did not affect the yeast fermentation performance, this technique appears to be a promising approach for enhancing brewing yeasts and improving the efficiency of beer production.

FAB2

Microbial innovation for microbreweries

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Despite overall negative trends in 2016 economy, Italian craft brewing continues increasing the economic and occupational assets, as well as the quality and innovation of products and processes. In particular, the strong demand for local beers is pushing small yet very dynamic producers towards the utilization of high quality and healthy raw materials. In this respect, the Microbee®project, funded by EU (POR Sardegna 2007-2013), laid the foundations for the creation and development of a production chain of local beers by evaluating the technological and healthy value of local raw materials and by emphasizing the role of biodiversity for product differentiation. Mycotoxigenic or pathogenic fungi were not found in the field and in kernels of local barley and wheat sampled for three years. On the contrary, mycotoxins produced by saprophytic fungi were found in malt subjected to prolonged storage. Moreover, six yeast strains, belonging to *Saccharomyces cerevisiae* species, isolated from sourdough and wine were selected as starters for lager and ale beer production in micro-breweries. Thus, baker's and wine strains of *S. cerevisiae* could represent a reservoir of biodiversity for the selection

of starter strains for craft beer production. Moreover, the use of local raw material, besides leading to the production of high quality craft beers, could trigger the development of a sustainable production chain.

FAB3

Moderate Electric Fields application as a biotechnological tool in food processing

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Moderate Electric Fields (MEF) provide a uniform, rapid and energetically very efficient means of processing foods (mostly by heat). This has been known for over a century and has finally found its place among food processors, as MEF industrial equipments are being installed worldwide in growing numbers and in a variety of applications. This happened after technological issues such as electrode corrosion and adequate temperature and power control systems were solved.

Less unanimous are the so-called "electrical effects" of MEF, namely over microorganisms and enzymes. Despite of this, research efforts have consistently shown that it is possible that the presence of an electric current may induce changes leading to microbial or enzyme inactivation. These effects may be further enhanced by temperature, in a synergistic combination that may be advantageous when applied to the processing of foods: decreased degradation of nutrients, colour, aroma or

texture; lower energy consumption; reduced processing time, or a combination of these.

Our proposal is that it is possible to take advantage of the "electrical effects" of MEF to induce changes in biomolecules (e.g. proteins) which will in turn change their functionality. If the origin of such changes and their nature are understood, it will be possible to tailor such molecules towards specific applications (e.g. gelling agents, texturizers, activation/deactivation of functional groups, etc.).

In this work we will summarize the activities of our group regarding the use of MEF in food processing applications, with a particular emphasis on their potential to change the functional properties of proteins and applications thereof.

FAB4

Yeast for production of alcohol free beer

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An alternative way to produce beer with a reduced ethanol concentration involves the use of genetically modified yeasts that form less ethanol during complete wort fermentation. The aim of our work was to investigate the potential to produce beer with a reduced ethanol content using *S. cerevisiae* cells deficient in the synthesis of any of the tricarboxylic acid cycle enzymes. We studied a set of fourteen ye-

ast strains deficient in one of the TCA cycle genes *S. cerevisiae* BY4743 (MAT α his3 δ 1/his3 δ 1 leu2 δ 0/leu2 δ 0 lys2 δ 0/LYS2 MET15/met15 δ 0ura3 δ /ura3 δ 0) was from the *Saccharomyces Genome Deletion Project*, in which the respective open reading frames for *ACO1*, *CIT1*, *CIT3*, *FUM1*, *IDH1*, *IDH2*, *KGD1*, *KGD2*, *LSC1*, *LSC2*, *MDH1*, *SDH1*, *SDH2* or *SDH3* were replaced with the *KanMX4* gene. A strain deficient in the gene encoding alcohol dehydrogenase subunit *ADH1*, and a strain deficient in the *LIP5* gene encoding lipoic acid synthase were also investigated for reduced ethanol production. Non-alcoholic beer can be made by the yeast deficient in TCA cycle genes with disruptions in the *FUM1*, *KGD1* and *KGD2* genes, corresponding to fumarase and α -ketoglutarate dehydrogenase.

FAB5

Probiotic effect of microorganisms

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Currently, lactic acid bacteria and bifidobacteria are the most widely used types of probiotic microorganisms. The presentation summarizes the most important species of probiotics, including information regarding new probiotic agents. It is specifically focused on issues related to the application of probiotics in foods, in particular their stability in different food matrices

and the determination of their number in mixtures with other bacteria.

In further detail several probiotic properties are discussed, such as the prevalence of bile salt hydrolase (BSH) in lactobacilli (*L. acidophilus* and *L. casei*) isolated from different sources, as well as methods of its determination. Two methods were compared – plate method with addition of CaCl_2 and thin layer chromatography, which showed more accurate results. Next, primers for the detection of genes for production of BSH enzymes in both lactobacilli species were tested. Further, the ability to grow in an environment with oxbile and released cholic acid, influencing of the lag phase and the growth rate of BSH positive and BSH negative bacterial strains were investigated. The effect of cultivation in the presence of bile salts on the surface properties of the cells, measured by auto-aggregation and cells hydrophobicity was also examined.

FAB6

Chitinases from metagenome as pest biocontrol agents

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The growing reluctance to use hazardous pesticides in agriculture has encouraged the search of alternative environmen-

tally friendly practices for controlling plant diseases. Among them, the biocontrol, i.e. the employment of natural agents for suppressing plant pathogens with the minimal impact on the environment, holds the greatest promise. Bacteria and fungi are used as biocontrol agents, but their in-field application is debated. Some of the limits of using alive cells are overcome by replacing them with cocktails of microbial hydrolytic enzymes: the combined use of pesticides with microbial enzymes (such as chitinases, proteases, glucosidases) that favour pesticide action allowing a better penetration into the targeted phytopathogens, may significantly reduce the use of chemicals. Chitinases, enzymes able to degrade chitin – a polysaccharide that is absent in plants, but plays essential structural roles in insects, fungi and nematodes – represent optimal candidates for the development of integrated pest management (IPM) strategies^[1]. In the past decades, numerous bacterial and fungal chitinolytic enzymes were identified by traditional screening approaches, but their development as biocontrol agents was limited by different factors, such as scarce activity or lack of robust systems for their production.

With the aim of identifying novel chitinolytic enzymes, in the frame of the EU MetaExplore consortium, *ad hoc* activity- and/or sequence-based screenings were applied to different metagenomic libraries, leading to the discovery of two novel chitinases, Chi18H8^[2,3] and 53D1^[4]. The biochemical and functional characterization revealed interesting features for both proteins, such as activity and stability in a wide range of conditions^[3,4], as well as antifungal properties^[2,3]. We developed efficient

protocols for their production/purification, both in conventional (*Escherichia coli*) and unconventional (*Streptomyces spp.*) hosts, and we are testing their in vitro and in vivo biocontrol activity towards insect pests and phytopathogen fungi.

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FAB7

Bio-production of lipid nutrition chemicals: polyunsaturated fatty acids and vitamin K2

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The Nutrient Chemicals are a number of chemicals with multiple functions which are important for good health, such as anti-oxidant, anti-radiation, improving memory, regulating blood lipid etc^[1]. Our work is concentrated on two kinds of lipid nutrient chemicals, polyunsaturated fatty acids docosahexaenoic acid and vitamin K2.

Docosahexaenoic acid is an important w-3 fatty acid, and contributes greatly to infant mental development and cardiovascular diseases prevention^[2]. It has been widely used in food additives and pharmaceutical industries. The research work in our group mainly focused on how to realize the efficient DHA production by marine microorganism and improve the product quality. The detailed research work include strain improvement, nutrition and oxygen regulation, improvement of lipid quality, pathway modification, omics study and bioreactor design and scale up.

Vitamin K2 (menaquinone 7), a fat-soluble vitamin, is a polyene compound containing 2-methyl-1,4-naphthoquinone. According to the number of repeat isoprene units of a side chain at the 3-position, it can be indicated as MK-4 to MK-13^[3]. As one of the indispensable vitamins in human, vitamin K2 plays an important role in the prevention of osteoporosis, arterial calcification, cardiovascular disease, cancer, and Parkinson's psychosis with other vital physiological functions^[4]. Vitamin K2 is widely used as dietary supplements or drug treatments in the food, pharmaceutical, and healthcare industries. Currently, the production of MK-7 has been gradually shifted from the traditional chemical synthesis to microbial fermentation. Our work reports fermentation characteristics of Ba-

cillus natto by a new isolated strain, and designed some regulation strategy to improve the VK2 production. In addition, we also developed a new nutrient food by mixing VK2 with mushroom powder.

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FAB8

Recent researches of *Monascus* spp. and their metabolites in China

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Monascus spp. are fungi to produce traditional fermentation product Hongqu, also called red yeast rice in China. Nowadays, the industrial Hongqu products in China have been widely used as food pigments, brewing starters, acridine and functional foods. It is estimated that the annual output of Hongqu amounts to several tens of thousands of tons.

Monascus spp. can produce an abundant of secondary metabolites during the growth process, which mainly include *Monascus* pigments (MPs), monacolin K, γ -aminobutyric acid, dimeric acid, acetylcholine, ergosterol, citrinin and so on. MPs have been used as food colorants, and their yield is the fastest growing food colorants in recent years in China. Functional Hongqu has been successfully developed as a cholesterol-lowering drug on account of its main functional component monacolin K in China. Furthermore, a variety of functional foods produced with Hongqu, which richens other functional ingredients, as the main raw materials, has also been successfully developed in China. In the aspect of citrinin control, the citrinin limit standard ($50\mu\text{g} / \text{Kg}$) of Chinese functional Hongqu is far below the European Union standard ($2000\mu\text{g} / \text{Kg}$).

Around the last two decades, a lot of researches about the type and structure of MPs have also been investigated in China. By the end of 2016, more than 50% of MPs were discovered by Chinese researchers in the nearly 100 kinds of MPs. The research in the molecular biology of *Monascus* spp. in China was also developed rapidly. Molecular biology has been successfully applied into strain classification and identification, cloning and functional analysis of secondary metabolite genes (clusters), biosynthesis pathways investigation of MPs and citrinin. Currently, the biosynthetic pathways of MPs and citrinin have been basically clear. By the end of 2016, the complete genomes of 5 *Monascus* strains had been sequenced, 3 of which is completed by Chinese researchers.

FAB9

Effect of various factors on the buffering capacity of wort

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Throughout the brewing process and in the quality of beer pH has great importance. However brewers have limited possibility to influence it, and it is most often achieved by altering pH of the preceding wort. Buffering capacity of wort defines the extent of the change of pH in case acid is added during mashing. Thus, it is an important quality parameter of wort.

Buffering substances originate from mainly from malt but other technological factors influence their amount. Some of these factors were studied, like application of rice adjunct in different proportion, execution of infusion mashing (varying number of temperature rest) or addition of acidifying reagents (phosphoric acid, acetic acid and lactic acid).

Analysis of the regression between buffering capacity of wort and the proportion of rice adjuncts indicated that buffering capacity of wort linearly fell with the increase in the percentage of rice adjuncts and the rice adjuncts contributed just approximately half as much buffering substances as malt. The wort from multi-step infusion mashes had a relatively high buffering capacity. The buffering capacity tended to

drop with the increase in the amount of addition of calcium salt. Phosphoric acid, lactic acid and acetic acid can be used for acidifying mash to target pH. The buffering capacity of the wort using acetic acid as acidifying reagent was the highest and the buffering capacity of the wort using phosphoric acid as acidifying reagent was the smallest. Organic acids, such as lactic acid and acetic acid had stronger buffering capacity than phosphoric acid at the pH of wort.

Wort may be regarded as mixture of buffering agents. Its capacity is affected by many factors that have not been extensively studied. Further exploration of this complicated system is necessary.

FAB10

Development of a fed-batch system for the fermentation of grape must with high sugar content

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Sweet wines, such as "Spätlese", "Troddenbeerauslesen" or ice wine are

produced from high-sugar grape musts, containing 200 g/L of glucose and fructose. Fermentations of these musts often stagnate due to hyperosmotic stress of yeasts^[1]. As a result, secondary products such as acetaldehyde, pyruvate and acetic acid are formed. The aim of this research is to develop a fed-batch process for the fermentation of high-sugar grape must to lower these undesirable metabolites. On-line analyzers for sugar, ethanol, CO₂, and density have been evaluated for an automatic fed-batch system consisting of a reservoir tank (filled with fresh must) and a fermentation tank. The fermentations are controlled by continuous measurement of the aforementioned metabolic parameters. As soon as the fermentation rate descends below a target value, fresh grape must is automatically transferred from the reservoir tank to the fermenter via a PID-controlled pump. Our experiments aim to compare fed-batch with conventional fermentations of high-sugar grape musts. Fed-batch fermentations are conducted with a constant sugar concentration at 50 g/L as proposed by others^[2]. Acetaldehyde content and acetic acid concentration could be decreased by 50 % entailing a considerably lower SO₂ demand for the wines. To monitor the osmotic stress of yeasts during alcoholic fermentation, specific genes related to the cellular stress were monitored by real-time PCR. *GPD1* and *HSP12*, encoding proteins, that are responsible for the biosynthesis of glycerol and maintaining membrane stability, respectively, showed lower expression during fed-batch fermentation [3, 4]. The genetic and metabolic analysis revealed the advantages of a fed-batch system for the fermentation of high-sugar grape must. However,

the headspace volume in the fed-batch fermenter may still cause unwanted oxidation. Any contamination with microorganisms in the reservoir tank and in the pipes has to be avoided. Current work focus on these issues to be overcome.

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FAB11

Bacteriophages for the control of pathogenic bacteria

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The application of bacteriophages is a promising alternative to conventional an-

tibiotics. The strictly virulent and non-transducing bacteriophages are powerful tools to specifically treat pathogenic bacteria in food and agriculture. Taking into account the great diversity of bacteriophages, the isolation of a suitable biocontrol phage can be a challenging and time consuming task. In addition not every phage isolate can be used for biocontrol until it is not sufficiently characterized and approved. To enhance directed phage finding we developed a PCR driven approach to rapidly detect phages for biocontrol of *Salmonella*, *Erwinia amylovora* and other members of the *Enterobacteriaceae*. Isolated bacteriophages were initially characterized in terms of host range and genome size. Genomes of selected isolates were sequenced and subjected to *in silico* analyses. Because phage resistance can be observed after application, a combination of different phages, i.e. phage cocktails, with different host receptor specificities may be utilized. To identify phage receptors in target bacteria we established an easy to handle high throughput assay. Receptors of virulent bacteriophages with overlapping host ranges were identified and the effectiveness of the different bacteriophages, either applied alone, in different combinations, or after genetic modification, was tested *in vitro*, in food and *in planta*, depending on the targeted bacteria. It seems that Enterobacteria can be more efficiently controlled by phage cocktails than by single phage isolates.

FAB12

Phages to combat *Listeria* and *Salmonella*

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Bacteriophages are a novel tool in food-safety. Not all phages are suitable for bio-control and criteria that need to be met will be discussed on the basis of two examples, a single phage product against *Listeria* and a two phage cocktail effective against *Salmonella*. Laboratory data demonstrating the efficacy of phages will be discussed. Food manufacturers need to comply with rules governing the presence of these pathogens in food and the possibilities of integrating phages into these frameworks will be shown. The issue of resistance will be addressed. It will be shown that phages cannot mask bad hygiene and that phage use certainly cannot replace hygiene in any way. Lastly, the transition from laboratory bench and the challenges thereof will be discussed, from a simple application such as in smeared cheeses to treatment of composite foodstuffs. This will show both possibilities as well as limitations of using phages as bio-control agents in food manufacture.

FAB13

Synthetic biology of designer endolysins

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A recent portfolio overview of the antibacterial development pipeline has highlighted bacteriophage-encoded endolysins as an emerging class of antibiotics with the highest potential. These enzymes are produced at the end of the lytic cycle of bacterial viruses (bacteriophages) in the infected host cell. They degrade the peptidoglycan layer of the cell, resulting in osmotic lysis and dispersion of the newly produced phage particles. Addition of purified endolysins to Gram-positive cells results in a rapid killing of multi-drug resistant strain with a low probability to provoke resistance development. Fusion of a peptide with specific physicochemical properties to either the N- or C-terminus expands the applicability of endolysins drastically. Cationic and amphipathic peptides with outer membrane permeabilizing activity have demonstrated to transfer the endolysin moiety across the outer membrane, resulting in a quick lysis of Gram-negative bacteria. Recombinant fusion with similar cationic peptides also increases the enzymatic and bactericidal activity of endolysins acting against Gram-positive species due to an increased affinity for the cell wall. The progress in the field during the last decade has now yielded a multitude of (engineered) endolysins against many major Gram-positive and Gram-

negative pathogens. Many endolysins have a modular structure, comprising a cell wall-binding domain and an enzymatically active domain. Domain swapping efforts have allowed to engineer endolysins with desired and improved properties. We expect that we are only at the beginning of the synthetic biology of modular endolysins, which bears the potential to produce customized antibacterials against any bacterial pathogen.

FAB14

Antimicrobial and defense elicitor peptides in plant disease control

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Chemical control of plant diseases is currently achieved with copper compounds and certain antibiotics, that have undesirable non target effects in human health and negative environmental impact. Regulatory restrictions in many countries tend to promote the use of natural compounds that are effective but with a low risk for consumer health and the environment. Furthermore, emerging and devastating bacterial diseases caused by xylem and phloem limited bacteria are lacking of efficient means of control. As a consequence, there is a need of novel compounds for plant disease management.

Antimicrobial peptides are interesting as alternatives or complement to conventio-

nal products for plant disease control. They are produced by animals and plants to defend themselves from microbial infection, or by microorganisms to compete for resources. Most antimicrobial peptides have primarily a lytic effect on bacterial or fungal pathogens. However, natural peptides are present at low concentrations or may have non-target effects.

Synthetic analogs or *de novo* designed compounds have been developed to optimize control of several fungal and bacterial diseases of plants, with minimized toxicity. Most of the peptides have a lytic effect on pathogens, but certain peptides can penetrate plant cells without a lytic effect on their membranes and can be used as carriers for cargo proteins to interact with key cell functions. Other peptides can trigger plant defence mechanisms by inducing the expression of genes involved in the pathways of plant resistance to pathogens or stress. Theoretically it is also possible to develop peptides that combine several of these properties.

However, the synthesis of peptides is costly when using chemical methods like solid phase synthesis, and it is not feasible for an application in agriculture in plant protection. In the past years several synthetic peptides have been produced using microbial and plant biofactories. As in other fields, methods of gene editing are expected to provide a powerful tool to develop and produce novel functional peptides for plant protection.

FAB15

Antifungal lactic acid bacteria in food fermentations

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The group of lactic acid bacteria (LAB) plays a central role in food fermentation processes and has a long and safe history of application. In fermented foods, LAB initiate rapid acidification of the raw material concomitant with the development of further functional compounds influencing organoleptic, technological, and nutritional or health aspects. Recently, functional microbial food cultures are being developed complementary to classical starter cultures focusing on specific functionalities in the respective fermented food. Antifungal LAB comprise a specific group of functional microorganisms and their application seems a promising solution to control fungal spoilage of fermented foods.

This study shows recent developments in the selection of antifungal LAB from natural sources including two exemplary applications in sourdough as well as in post-harvest processing of cocoa beans. Furthermore, insight into antifungal metabolites as well as interactions between antifungal LAB and their target fungi is given.

FAB16

Beneficial cardiovascular effects of (–)-epicatechin-containing foods

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Various epidemiological reports indicate that consumption of foods and beverages rich in natural polyphenols is associated with lower incidence of cardiovascular diseases. (–)-Epicatechin (Epi) is a flavanol that is present in various foods, including red wine and beer. Red wine polyphenols were shown to reduce cardiovascular risk in general as well as to decrease blood pressure (BP) in various experimental models of hypertension. Various polyphenols (among them mainly Epi and resveratrol) affect various molecular mechanisms participating on regulation of cardiovascular functions. These include improvement of endothelial function, normalisation of reactive oxygen species and peroxynitrite formation, up-regulation of endothelial nitric oxide (NO) production and reduction of endothelin-1. In addition, we showed that red wine polyphenol extract can maintain equilibrium between endothelium-derived vasoconstrictor and vasodilating factors in chronic social stress-exposed rats.

Recently, much attention has been paid to Epi itself. The highest amount of Epi was determined in cocoa-derived products. Epi is absorbed well from the gastrointestinal tract and it can cross the blood-brain barrier, thus it may affect a broad

spectrum of physiological processes. Studies have shown that cocoa flavanol consumption may reduce BP, improve cognitive and motor functions in humans as well as affect vascular function and behavior of rats. We showed that short-term treatment of rats with Epi may reduce BP and improve vascular function in adult hypertensive rats (SHR) and prevent the development of hypertension in young SHR. In this model, the mechanism of Epi action was associated with reduced superoxide production, increased plasma total antioxidant capacity, improved endothelial NO bioavailability, NO-dependent vasorelaxation and increased erythrocyte deformability. In humans, single ingestion of dark chocolate also improved erythrocyte deformability despite unchanged NO production. Thus, Epi-containing foods may be a useful tool in maintenance of cardiovascular health in humans.

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FAB17

Biosensors: Potentials and Applications in Food Industry

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Most of the food-borne diseases caused by food pathogenic bacteria such as *Salmonella* spp., *Escherichia coli*, *Streptococcus* spp., *Staphylococcus* spp., *Vibrio* spp. and *Listeria monocytogenes*. The

detection and identification of the bacteria using conventional methods; such as morphological, cultural and biochemical evaluations have been an essential concern for many years. These classical methods are considered the multistep assay and consequently time consuming process. Current detection techniques for instance for Salmonella such as fluorescent-antibody (FA), enzyme-linked immunosorbent assay (ELISA), and polymerase chain reactions (PCR) are also time-consuming and cumbersome with limited sensitivity. Biosensor technology is showing a great potential for detection of food-borne pathogenic bacteria with high accuracy^[1]. Biosensors for bacterial detection generally involve a biological recognition component such as receptors, nucleic acids, or antibodies attached to an appropriate transducer. Transducers, including electrochemical, optical, piezoelectric, thermal, and magnetic devices, can quantitatively monitor the biochemical reaction. Nowadays, biosensors integrated with new technologies (molecular biology and nanotechnology) have various applications in agricultural production and food industry. They can provide a reliable, fast, cheap, miniaturized and multi-analyte analysis with rapid and real-time detection of different food pathogens, pesticides, antibiotics, and toxins^[2]. This paper presents an overview of the biosensors with more emphasis on electrochemical biosensors, their recent developments and applications for detection of pathogens.

Keywords: Electrochemical biosensors, Detection of pathogens, Agriculture, Food safety
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FAB18

Enzyme-mediated Hypoallergenic Milk and Dairy Products – Present Scenario and Future Prospects

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Milk is considered as a functional food regardless of ages and genders around the world. Due to unique biological properties and activities of milk components, mainly protein and carbohydrate, milk reduces the risk of several diseases and promotes sustainable health. However, the members of milk sensitive community experience with non IgE-mediated protein allergy/ IgE-mediated protein allergy/ combination of non IgE- and IgE-mediated protein allergy/ lactose intolerance. In many cases cereal-based milk/ fermented dairy products are recommended instead of consumption of processed milk. Besides that, great efforts were placed to make hypoallergenic milk and dairy-based products. Microbial synthesized β -galactosidase was prevalently

applied to produce low-lactose milk and fermented dairy products. Individual heat-, microwave-, high pressure- and proteolytic enzyme (α -chymotrypsin, trypsin, pronase, corolase, papain, neutrase, alcalase)-treatment as well as their combined effects were investigated to reduce the allergenic activity of whey-protein (β -globulin) and milk protein (α s1-casein). Enzymatic hydrolyzate of total whey proteins and casein were popularly accepted as energy drink and infant formula. Some research groups reported about plasmin-mediated hypoallergenic yoghurt. However, there are conflicts about the sensory properties of enzyme hydrolyzed milk and hydrolyzed milk-derived products. The counter statement of this debate is proteolytic digestion might itself generate new antigenic substances; those offer benefits for the immune system against other allergens. Interestingly, it was reported that papain hydrolyzate dairy products have high organoleptic properties. Hypoallergenic property of laccase-mediated dairy product is also reported. Often laccase-/ trans-glutaminase-/ tyrosinase-mediated processes were considered to provide the stable structure of protease hydrolyzed milk products. There is a major challenge about the production cost of enzyme-intercede hypoallergenic dairy products in industrial scale. Therefore in this review, process and techno-economical analysis for the production of enzyme-mediated hypoallergenic dairy products are described in judicious way, which may be the first effort in the field of dairy technology.

FAB19

Industrial biotechnology for the production of microbial pesticides

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Over the past decades, the crop protection market has relied heavily on chemical pesticides. Today, the evolution of the resistance of chemical pesticides in pest populations and increasing regulatory hurdles are resulting in a significant trend towards bio pesticides in the agrochemical industry. As a consequence of the fast growing biological crop protection market, industrial biotechnology is taking an increasing role in the agrochemical industry for the development of environmentally benign pesticides. In this context, recent examples of biological processes developed to produce microbial pesticides using biotechnology are presented.

FAB20

Two-dimensional GC: a suitable tool to enhance the understanding of complex volatile profiles released from biological materials such as fermented tobacco leaves

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The analysis of volatile organic compounds (VOC) released from complex biological matrices is a challenging task, as separation efficiency and peak capacity in one-dimensional GC are often not sufficient to fully separate and assign the vast amount of compounds present in the samples. But for the understanding of biological processes such as the fermentation of tobacco leaves, next to the most prominent substances such as nicotine and its derivatives, the modulations of "small peaks" may be valuable as well for creating a chemical map of the compounds present at different fermentation time points.

Therefore, we have developed a heart cutting direct headspace GC-FID-GC-MS method using static headspace to complement our standard GC-MS method, where we use dynamic headspace for improved sensitivity. The first dimension chromatogram was obtained on a primary DB-5 column which was coupled to a FID detector.

Subsequently a 30-seconds time-window of the first dimension chromatogram was cut and fully resolved on the secondary DB-Wax column, hence achieving a systematic screening of unresolved peaks. The second dimension column was coupled to a mass spectrometer for identification of the co-eluting substances. This heart cutting procedure using a Deans Switching System from Agilent was performed along the whole chromatogram in overlapping steps to find the elution order of all peaks relative to the elution of n-alkanes, as comparison.

The chemical information on the elution order of those identified substances was then used to enhance the substance identification, precision of peak integration and subsequently the quantification of compounds obtained by our standard dynamic headspace GC-MS method. Combining the higher sensitivity from dynamic headspace analyses and the optimized resolution from GC-GC analyses, many more compounds could be identified as well as quantified with high reliability.

Oral Presentations – Industrial Biotechnology

IB1

Model-driven process design for improved antibody glycosylation

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Exerting control over the glycan moieties of glycoprotein therapeutics is highly desirable from a product safety and efficacy perspective. Strategies to improve specific protein productivity may compromise quality, while interventions for improving glycoform distribution can adversely affect cell growth and productivity. Process design therefore needs to consider the trade-offs between preserving cellular health and productivity and enhancing protein quality. In this work, we present a modelling platform that can be used to diagnose intracellular bottlenecks for antibody glycosylation and design improved processes. Using this platform we have been able to establish the impact of process temperature changes and glycosylation precursor feeding on cellular metabolism and the expression of Golgi-resident transport proteins and glycosyltransferases. We have used this knowledge to identify the process input space in terms of the culture dynamic feeding regime that satisfies both titre and glycan distribution constraints using a novel algorithm for constrained global sensitivity analysis. Selected feeding strategies

have been implemented experimentally and have successfully demonstrated the validity of model-driven process design. This work supports the implementation of Quality by Design in Bioprocessing and is expected to significantly increase operational flexibility.

IB2

Medium and Process Development for Next-Generation Perfusion Processes

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Interest in perfusion as means for increasing upstream process yields and reduction of manufacturing costs rises throughout the biopharmaceutical industry. As many companies focused on fed-batch process development for decades, medium development knowledge for perfusion is a gap for the wider adoption of perfusion in biologics manufacturing today.

A critical tool for medium development are robust and representative high-throughput screening systems. Screening tools should represent the large scale system in the most critical aspects. In our case study, different Scale-Down Models for perfusion medium development were tested and compared based on the cellular metabolic behavior in these systems.

Compared to fed-batch, perfusion processes have fundamentally different requirements and challenges in terms of medium composition and formulation. The

main challenge in perfusion medium development is to align cellular nutrient demands with their concentrations. The application of cell culture media designed for fed-batch can be a quick route to a baseline perfusion process, but there may be limitations to possible optimization with this approach, in terms of performance as well as cost. Best results were achieved with media specifically designed for perfusion.

IB3

Optimization of mAb glycosylation produced in mammalian cell cultures

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Monoclonal antibodies (mAb) secreted by mammalian cells are becoming major target-oriented biotherapeutics. In the presented work an effect of various environmental conditions (i.e. hydrodynamic stress, pH, ammonia concentration etc.) and media components (i.e. carbon sources and trace elements) on the cell metabolism and mAb glycosylation pattern will be studied through combination of experiments and mathematical modeling. A mechanistic model of a mAb glycosylation will be used to optimize target glycosylation pattern. Model predictions will be compared with available experimental data collected in various bioreactor scales and applying fed-batch as well as perfusion operation mode.

IB4

SCALE-UP IN THE SINGLE USE AGE: DOES GEOMETRY MATTER?

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Single use bioreactors (SUBs) are becoming standard work horses in the biopharmaceutical industry. These SUBs are supplied by vendors as off the shelf designs limiting the cell culture engineer's ability to match the geometry of the SUB to the geometry of their existing stirred tank reactor (STR) capacity. The first generation of SUBs departed from conventional stirred tank bioreactor (STR) geometry in terms of impeller number, and orientation and sparger hole diameter. Moreover, one marked feature of SUB bioreactors was that they could be operated at lower volumes than conventional STRs, bringing considerable operational flexibility. This practice, however, further negated the principle of geometric similarity. This presentation considers the implications of changing reactor geometry on scale up of mammalian cell culture processes using multivariate data analysis to compare different geometries and different fill volumes. This approach uncovered a surprising result when working at half volume, which may not have been spotted using conventional data analysis methods.

Mass transfer studies were performed with two manufacturing scale SUB systems and a miniature SUB system using the gassing-out approach. The results have been compared to results generated using

Lonza's proprietary STR geometry from 10 to 20,000 L. Vessel geometry is shown to have a substantial impact on mass transfer.

Cell culture evaluations were performed with a model cell line in all three systems. The results were compared to historical data obtained in 10 L STR and airlift vessels. Multivariate analysis of the data showed that there were substantial differences in cell culture performance between different STR vessels.

The impact of operating at half volume was investigated for one vessel design at two different vessel volumes. Multivariate data analysis showed that there was considerable difference in behavior of the cultures performed at half volume when compared to cultures performed in the conventional scale-down model. Furthermore, the analysis indicated that there was also a difference in behavior of the half-volume cultures in different size vessels. This indicated a lack of scalability between half-volume cultures performed in different scale vessels, which was not apparent when the same vessels were run at full volume.

It was concluded that SUB geometry does matter when scaling processes up and should be a key consideration in a quality by design approach to minimizing differences in culture behavior during cell culture process scale up. Moreover, multivariate data analysis can provide useful supplemental insight in bioreactor process performance comparisons.

IB5

Optimization of microbial hyaluronic acid fermentation by metabolomic approach

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Hyaluronic acid (HA) is a linear glycosaminoglycan which is applied in cosmetic and pharmaceutical industry. In the last years HA production is predominantly based on *Streptococci* fermentation. Nascent HA chain is elongated by alternating addition of activated subunits UDP-glucuronic acid (UDP-GlcUA) and UDP-N-acetyl glucosamine (UDP-GlcNAc). Availability of these precursors or their ratio can play crucial role in HA biosynthesis optimizing. In this work we focused on changes in intracellular activated precursors concentrations and evaluation of the effect of these processes on HA production and molecular weight (Mw).

HA production and cell wall biosynthesis compete for the UDP precursors. In order to reveal the relationship between these processes mutant *Streptococcus equi* subsp. *zooepidemicus* (SEZ) strain with hyaluronan synthase deletion (SEZ δ hasA) was constructed. The elimination of hyaluronic acid biosynthesis led to 15 % increase of biomass formation and 14 % decrease of lactic acid production. Surprisingly just a negligible portion of UDP precursors pool was redirected towards biomass formation.

In order to increase HA production,

cultivation medium was enriched by UDP precursors. It was found that external supplementation with UDP-GlcNAc increased HA production and its Mw. Surprisingly, external supplementation did not increase the intracellular UDP precursors pools. Therefore it is unlikely that UDP-GlcNAc is used as a direct substrate for HA synthesis. Method of comparative RNA arrays revealed a significant changes in gene expressions depending on UDP-GlcNAc supplementation. These data could be used for screening of candidates genes affecting HA production.

IB6

Systems and synthetic biology approaches to accelerate bio-systems engineering

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Recent advances in high-throughput sequencing and low-cost *de novo* DNA synthesis technologies provides now for the first time the capabilities to program biological systems by writing long DNA molecules. However, fast-paced navigation of the design-build-test (DBT) cycle of bio-engineering still represents a major challenge for efficient manufacturing of gene-circuits, pathways and entire platform organisms with fully defined genetic makeup. Our research focuses on systems and synthetic biology approaches to accelerate

the DBT-cycle and obtaining new fundamental insights into the biology of microbial cells. We will present insights into novel experimental systems biology strategies that combine transposon mutagenesis with next generation sequencing to define genome-wide DNA parts that serve as building blocks for the precision engineering of complex biological systems. Furthermore, we will highlight computational sequence refactoring approaches and assembly optimization algorithms to assist in designing and building large-scale bio-systems form individual DNA parts in a reliable, cost and time efficient manner. In sum, our approaches are broadly applicable to harness the genomic diversity of microbes for application-related engineering of biological systems.

IB7

Cell-free expression as a new alternative for difficult-to-express-proteins

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Cell-free expression systems provide a real alternative for membrane protein expression, enabling the study of structure and function of membrane proteins. In this presentation, we will first introduce Synthelis and the cell-free expression system features and advantages. Then, we will present few case studies to illustrate this know-how and technology. Our core expertise is the implementation of the most

suitable hydrophobic matrix for membrane proteins. This could be detergents or liposomes to solubilize and stabilize membrane proteins straight from the expression and provide a format fitting to the final application. These proteins can be used for immunization (vaccine or antibody development project), reagents for screening and display technology, structural biology, IVD and vectorization (protein therapy). Almost all target families has been successfully expressed in our system from mammalian targets (GPCRs, ion channels, etc...) to bacterial and viral proteins.

As an example of success of our cell-free technology in producing active proteins, we have reported that human Bak protein integrated in proteoliposomes was able, *in vitro*, to activate the mitochondrial apoptotic pathway in glioblastoma cancer cells.

Another interesting example is the CXCR4 protein that we successfully produced in both proteoliposome and detergent format and characterized structurally and functionally.

Hence, the cell-free technology is suitable for structural studies as we are able to produce high yields of membrane proteins using this system.

IB8

White Biotechnology for the Production of Flavor and Fragrance ingredients

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White biotechnology opens up new possibilities of developing cost-effective and sustainable manufacturing processes for molecules not easily available by organic chemistry or by extraction from natural resources. Because of the structural diversity and broad range of properties of terpenes, the terpenoid biosynthetic pathway has been extensively studied in recent years and economically viable solutions for the biochemical production of this family of molecules are emerging.

In the flavor and fragrance industry, terpenoids represent a class of secondary metabolites of great economic importance, with unique olfactory properties, often difficult to replace by synthetic analogs. These molecules are mostly derived from plants and have thus the disadvantage of being subject to fluctuations in price and quality due to climatic or geo-politic factors and are sometimes available only in small concentration in the raw material or only from non-sustainable resources. In addition, given the structural complexity of terpene molecules, cost-effective chemical routes are not available for many terpene compounds important for the industry.

We have therefore investigated the biosynthesis of terpene molecules that are constituents of key perfume ingredi-

ents. The approaches used for the molecular characterization of new terpene biosynthetic pathways will be discussed and examples of successfully elucidated pathways leading to terpene molecules of high value for the perfumery industry will be presented. Finally, recent achievements in cost-effective industrial production of terpene compounds, such as the ClearwoodTM ingredient, will be discussed.

IB9

Concurrent optimization strategies for high level protein production: Process, strain and expression engineering

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The development of economically feasible production processes is largely attributed to the robustness of the production strain. In order to achieve competitive enzyme production by tailored production strains, low expression levels and insufficient enzyme yields must be overcome. Therefore, strain and expression engineering have to go hand in hand with cultivation process optimization.

In the recent years, we have developed a large collection of expression tools and

strain engineering techniques for industrially important yeasts *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Pichia pastoris* (*Komagataella phaffii*).

In this study, we combined expression and strain optimization with first steps for process engineering to obtain high level human cytochrome P450 (CYP) producing *P. pastoris* strains for whole-cell biocatalysis. Engineering of human P450 isoenzymes was performed by random and multi-site-saturation mutagenesis and high through-put screening procedures were developed. Media optimization led to the identification of relevant cultivation parameters. High-level production strains were created by means of synthetic biology: The co-expression of the monooxygenase and its electron transfer partner cytochrome P450 reductase (CPR) was fine-tuned by screening a pool of artificial, bidirectional promoters. *P. pastoris* strains were engineered using CRISPR/Cas9 to improve the cells for whole cell biotransformations. By combining these optimization strategies, the product yields in whole cell biotransformations for the synthesis of human drug metabolites were several fold improved compared to initial experiments.

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IB10

Fast track expression and production of proteins with Lonza's XStm Pichia Platform

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Pichia pastoris has been developed as a valuable alternative to *E. coli* and CHO cells for production of novel protein formats such as multi-specific antibody mimetics. Lonza has developed a new XStm Pichia expression and manufacturing platform designed to provide high product titers for these novel compounds along with a fast, robust and scalable manufacturing process suitable for commercial production.

The newly developed G1-3 platform enables a speed fermentation process that makes use of short and straightforward fermentation regimes for optimized space time yield. Moreover a model based feed optimization enables a customizable process setup based on the requirements of the full production process and the specifications of the production plants. Additional benefits include:

- Simple screening and fermentation setup
- Higher titers in short "E. coli-like" total fermentation times of 2-3 days
- Methanol-free induction
- High cell viability due to reduced cellular stress
- Lower host cell proteins from reduced cell lysis
- Improved product quality due to shortened residence time

These advantages make XS Pichia G1-3 not only a valuable production system for biopharmaceuticals, but open up a wider

field of applications where high amounts of protein products have to be produced within a short time frame at good quality and purity.

IB11

Using a systematic approach to accelerate bioprocess development: methanol-free manufacturing with *Pichia pastoris*

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Pichia pastoris (synonym: textitKoma-gataella sp) is an important system for the manufacture of heterologous proteins. Most commonly, the AOX1 promoter is used for methanol-inducible and strictly regulated production, and the GAP promoter for constitutive production. Due to their disadvantages (methanol is a toxic and explosive substrate, and production is constitutive and non-inducible with the GAP promoter), new promoter systems are being sought in which product formation can be controlled without methanol induction (Kovar *et al.*, 2010; Looser *et al.*, 2015 and 2017). Beside advances in genetic design

(i.e. new promoters and production strain constructs), appropriate bioprocesses also need to be established. In general, the optimum growth conditions to achieve maximum recombinant product formation in such new systems are not predictable *a priori*, and must be empirically researched for each new combination of promoter, host and heterologous gene. To cope with the demand for increasing the efficiency of bioprocess development, a paradigm shift is needed from approaches based on high experimental load (i.e. trial-and-error high-throughput) to rational, systematic ones which are based on models or big data.

A systematic approach to optimum design feed addition profiles in fedbatch cultures with new recombinant *Pichia pastoris* strains by performing a minimum of cultivation experiments is described. The model-based strategy presented is a case study for recombinant *Pichia pastoris* secreting *Candida antarctica* lipase B (CALB) under the control of a novel methanol-free promoter. As CALB has previously been used to characterise numerous different promoters (Looser *et al.*, 2017), a comparison of the results is feasible. The generic model applied collects and combines information from multiple scientific publications, most of which have not yet been systematically analysed. Finally, for different recombinant strains with both different promoters (i.e. AOX1, GAP and several newly published methanol-free promoters) and various recombinant proteins (i.e. enzymes, antibodies, hormones and growth factors), the particular relationships between secreted product formation and biomass growth are described using the same model.

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IB12

USP and DSP projects of the Biotechnological Pilot Plant – the Institute of Microbiology CAS

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The Biotechnological Pilot Plant of the Institute was established and equipped in the mid eighties to transfer research results into practice. This task is fulfilled yet, even that under different conditions. The technical equipment is upgraded and replenished continuously, also technology showed some progress the from the beginning.

Currently there are many biotechnological projects of public and contract research dealt with. Since the equipment is mostly designed for aerobic submerged cultivation technologies and the subsequent down-stream processing, we are correspondingly engaged in cultivation of microorganisms, manufacturing of microbial products, optimization of bioprocesses and, first of all, in scale-up of the microbial productions.

Our department currently operates in the areas of:

- human and veterinary medicinals,
- antibiotics, vitamins, carriers, vaccines, biologically active compounds, proteins
- nutrition and feeds
- proteins, polysaccharides, lipids, enzymes
- enzyme technology

- detergents, pharmacy, food and feed industry
- decontamination
- environmental biotechnology
- bioenergetics
- biogas, agricultural waste treatment
- soil amendments, fertilizers.

IB13

Model based high cell density continuous cultivation of *A. latus* for biopolymer (PHB) production

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Indiscriminate use of plastics has led to serious threat to the environment, primarily due to their non-degradable nature and their production from disappearing fossil stocks. Hence there is a desperate need for sustainable alternatives to plastics. PHAs produced from renewable substrates have properties similar to petroleum derived polymers & are biodegradable in nature. Poly-hydroxy-alkanoates (PHAs) are a class of biopolymers synthesised by bacteria when they are subjected to nutrient stress like nitrogen, phosphate and even oxygen limitation in presence of excess carbon source. The batch kinetics of *A. latus* featured a biomass and PHB accumulation of 9.27 g/l and 7.1 g/l in 36 h. This was used to develop a mathematical model which was, thereafter, extrapolated

to design integrated fed-batch-continuous cultivation strategies with cell retention by spin filter to improve biomass &/or PHB accumulation. Several off line model simulations were done to identify appropriate integrated fed-batch/continuous cultivation strategy(ies).

Out of several computer simulated strategies, best integrated fed-batch-continuous cultivation was identified for experimental implementation. The cultivation was initiated as batch for 21 h followed by fed-batch with inlet sucrose & nitrogen concentrations being 150 g/l & 3.6 g/l respectively at a constant rate of 100 ml/h till 42 hours. The bioreactor was there after converted to continuous mode (volume 4 L) with feeding of sucrose and nitrogen along with other nutrient at a dilution rate of 0.03 h^{-1} . Upon experimental implementation, it was observed that the steady state accumulation of biomass, PHB and residual sucrose were 32.4 g/l, 23.3 g/l and 5.86 g/l (PHB productivity 0.69 g/l-h) respectively for cell retention system.

The integrated fed-batch /continuous cultivation with cell retention was then scaled – up to 15 liter bioreactor (using constant $K_L a$ as scale up criteria) which featured similar biomass & PHB accumulation as that of 7L bioreactor.

Oral Presentations – Medical Biotechnology

MB1

Electron transport chain disruption as a new strategy to suppress Her2^{high} breast cancer

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Expression of the Her2 oncogene in breast cancer is associated with resistance to treatment, and Her2 may regulate bioenergetics. Therefore, we investigated whether disruption of the electron transport chain is a viable strategy to eliminate Her2^{high} disease. We demonstrate that Her2^{high} cells and tumours have increased assembly of respiratory supercomplexes and increased complex I-driven respiration *in vitro* and *in vivo*. They are also highly sensitive to MitoTam, a novel mitochondrial-targeted derivative of tamoxifen. Unlike tamoxifen, MitoTam efficiently suppresses experimental Her2^{high} tumours without systemic toxicity. Mechanistically, MitoTam inhibits complex I-driven respiration and disrupts respiratory supercomplexes in Her2^{-high} background *in vitro* and *in vivo*, leading to elevated reactive oxygen species production and cell

death. Intriguingly, higher sensitivity of Her2^{high} cells to MitoTam is dependent on the mitochondrial fraction of Her2. This shows that oncogenes such as Her2 can restructure electron transport chain, creating a previously unrecognized therapeutic vulnerability exploitable by supercomplex-disrupting agents such as MitoTam.

MB2

Immunogenic cell death in lung cancer immunotherapy

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Several cytotoxic drugs, oncolytic viruses and some physical modalities were shown to induce immunogenic cell death (ICD) of tumor cells, which results in the induction of effective anti-tumor immune responses *in vitro* and *in vivo*. We have recently described that high hydrostatic pressure (HHP) induces ICD of tumor cells

which is suitable for generation of immunogenic tumor cells for active cancer immunotherapy approaches. In our following studies we showed that dendritic cells (DC) pulsed with HHP-killed allogeneic lung cancer cells lines and poly(I:C) enhanced DC maturation, chemotactic migration and production of pro-inflammatory cytokines after 24h. DC-based HHP lung cancer vaccine stimulated IFN- γ -producing tumor antigen-specific CD4⁺ and CD8⁺ T cells from non-small cell lung cancer patients. Similarly, we newly identified severe heat shock treatment (HS, 47°C), but not mild HS treatment (42°C), of tumor cells as another ICD inducer which can be used for generation of immunogenic cell-based anti-tumor vaccines. We described in detail the molecular mechanisms underlying ICD induced by sHS as defined by the induction of ER stress response and ROS generation, cell surface exposure of calreticulin, HSP70 and HSP90, decrease of cell surface CD47, release of ATP and HMGB1 immunogenic molecules. DCs loaded with sHS-treated tumor cells stimulated IFN- γ -producing CD8⁺ T cells without any additional adjuvants *in vitro* and elicited protective anti-tumor immunity *in vivo* in mouse colorectal cancer model. Our results represent important preclinical data for ongoing NSCLC Phase I/II clinical trial using DC-based active cellular immunotherapy (DCVAC/LuCa) in combination with chemotherapy and immune enhancers.

MB3

Evaluating the quality of male sperm cells with monoclonal antibodies

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Recent studies shows that infertility affects estimated 15% of couples. Male infertility is the primary cause in 60% of these cases. For these reason we analyzed sperm proteins with normal and pathological spermograms. We found that intracrosomal sperm proteins VCP (valosin-containing protein), GAPDHS (sperm glyceraldehyde phosphate dehydrogenase), and ATP synthase (cAMP-dependent protein kinase II, PRKAR2A) identified by our monoclonal antibodies are differentially expressed in normal healthy men and asthenozoospermics, with a reduced expression in asthenozoospermics. These proteins are involved in energy metabolism and apoptosis of cells. Our results indicate the possibility of evaluating sperm quality in reproductive medicine by monoclonal antibodies against selected sperm proteins. This work was supported by the Agency for Healthcare Research of the Czech Republic, grant No. AZV 15-30880A and by BIOCEV project CZ.1.05/1.1.00/02.0109 from the ERDF.

MB4

Monoclonal and polyclonal antibodies as tools for the study of proteins in the male reproductive tract

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Study of molecular mechanisms in mammalian reproduction is an essential for the understanding of this outstanding process. We study proteins originated in reproductive organs and spermatozoa using various biochemical methods and use specific antibodies for their detection and localization. We extract sperm proteins using different approaches and kits for proteins from the sperm surface and subcellular compartments. The proteins of reproductive organ fluids are separated by chromatographic methods, such as size exclusion chromatography, high-performance liquid chromatography with reverse phase (RP-HPLC) and affinity chromatography on matrices with various ligands. We subject isolated proteins to SDS- or 2D-electrophoresis for their characterization and comparison throughout sperm functional development in various mammalian species. Electrophoretically separated proteins can transfer onto nitrocellulose membrane (Western blot) for

antibody detection or binding studies with lectin-labelled ligands (lectins, polysaccharides, *zona pellucida* glycoproteins). The immunoprecipitation method with specific antibody is used for protein determination followed by the MALDI identification. We study protein origin and localization by immunofluorescent techniques on/in spermatogenic cells and tissue sections from reproductive organs using monoclonal and polyclonal antibodies. Altogether, the isolation of proteins from reproductive tissues and fluids, and their antibody detection are a crucial for the studying of reproductive proteins origin and localization.

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MB5

Zinc-dependent 3'nucleases related to treatment of human diseases

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Family of zinc-dependent S1-P1 nucleases (Pfam PF02265, EC: 3.1.30.1) is present in plants, fungi, protozoan parasites and some bacteria. Nucleases from this family play versatile native roles in different organisms. In plants, they are utilized in specific apoptotic functions, in tissue differentiation, or in the response to viroid

pathogenesis [1]. In fungi, they are usually used as extracellular enzymes for scavenging of nutrients (phosphate or nucleotides) [2]. Their role in protozoan parasites and in some genera of bacteria are the least understood, however there is evidence for involvement of these nucleases in interactions between host and symbiont (pathogen) [3] or in DNA repair [4]. The S1-P1 nuclease family can be utilized in biochemistry, biotechnology and medicine. S1 nuclease can be used in nuclease protection assay. Any of these nucleases can be potentially utilized in production of mononucleotides. Tomato bifunctional nuclease TBN1 showed inhibition effects in a study using mice bearing human tumors [1]. Protozoan parasites in the amastigote phase produce P4 nuclease and their homologues. Infected mammals produce specific antigens against these nucleases [4]. So while the specific role of S1-P1 nucleases in these parasites is not yet known, they can be potentially utilized in vaccination or specifically inhibited as a part of the treatment.

S1-P1 nucleases are phosphoesterases cleaving the P-O3' bond and producing 5'-mononucleotides or nucleosides and phosphate ions as end products [1]. Generally, they are all zinc dependent glycoproteins (except bacterial homologs) active on both RNA and DNA with acidic pH optima. Our recent studies on TBN1 [5] and single-strand-specific S1 nuclease from *Aspergillus oryzae* [6] revealed structural features responsible for their high thermal stability, explained the role of glycosylation and brought insight into the substrate recognition mechanism of this promiscuous family of nucleases.

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MB6

Development of novel biologics for prostate cancer imaging

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Prostate carcinoma (PCa) is one of the most frequently diagnosed cancers in countries with high standard of living.

Therefore, intensive search for new markers enabling highly specific and sensitive detection of PCa is currently of paramount importance. Prostate-specific membrane antigen (PSMA) has been recognized as a highly specific marker of PCa for a long time, as its increased expression coincides well with the malignant transformation of the prostate tissue. Among PSMA-specific reagents developed for diagnostics and therapeutic purposes antibodies occupy prominent positions due to their strict selectivity for PSMA. Furthermore, unfavorable pharmacokinetic parameters of intact immunoglobulins can be alleviated by the engineering of low-molecular antibody derivatives.

Our group is interested in the development of PSMA-specific reagents including high-affinity antibody molecules and artificial scaffold proteins, such as Anticalins. Recently, we have isolated and characterized two PSMA-specific monoclonal antibodies designated 5B1 and 5D3. Microscopy, ELISA and flow cytometry analyses revealed high affinity and specificity of both molecules for PSMA localized on the cell surface in its native conformation. These results imply these antibodies are promising candidates for the development of new PSMA-specific diagnostic and therapeutic tools. To develop such reagents suitable for *in vivo* applications, we are currently focusing on designing and production of recombinant antibody derivatives including Fab, scFv and diabody fragments and mouse-human chimeras.

MB7

(Glyco)peptide mimetics derived from albumin-binding domain scaffold as tools for induction of neutralizing antibodies against HIV-1 gp120 glycoprotein

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HIV-1 infection belongs to one of the most critical global medical challenges as more than 35 million people are infected by the HIV-1 virus and this number increases every year. Infected individuals are exposed to development of AIDS and about 1.5 million die each year due to this disease. To combat with HIV-1-mediated AIDS, several types of vaccines have been developed but these lack a sufficient efficacy and, in addition, they function only on a limited part of human population. Therefore, generation of more efficient vaccines is highly required. Recent research of broadly-neutralizing antibodies (bn-Ab) identified in human HIV-1 non-progressors suggested that carbohydrates carried by gp120/gp41 protein complex of the HIV-1 Env seem to be a promising target for generation of specific bn-Ab. However, carbohydrates-based immunogens are generally less effective in generation of long-lasting antibody responses, thus proteins mimicking glycan epitopes represent a promising alternative. We used

our established concept of a high-complex combinatorial library derived from scaffold of 46 amino acid albumin-binding domain (ABD) and, in combination with ribosome display, targeted several types of bn-IgG and identified unique binding candidates targeting antigen-binding-domain of the tested bn-IgG. These ABD variants as potential (glyco)peptide mimetics are currently being characterized and will be tested for the stimulation of HIV-1 gp120-specific neutralizing antibody response. Thus, ABD-derived recombinant mimotopes could serve as a useful molecular tool for more efficient HIV-1 vaccine development.

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MB8

Streptavidine-based system for antigen delivery and vaccination

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For various diagnostic and vaccine applications it is important to stimulate antigen-specific immune responses of T lymphocytes. This represents the technical challenge of targeted delivery of antigens

into the cytosol or the endosomes of professional antigen presenting cells (APCs), such as dendritic cells, where the antigens can be processed, loaded onto MHC class I or II molecules and can be next exposed on APC surface to stimulate antigen-specific T cells.

We have developed a novel system for antigen delivery into APCs that offers a high flexibility of targeting of various endocytic receptors. This system is based on genetic fusion of the antigen of choice with streptavidin (SA-Ag), which in its tetrameric form binds a biotinylated targeting antibody specific for an endocytic receptor. Upon endocytic uptake, the antigen is processed for presentation on the surface of APCs in complex with MHC molecules.

Recently, we have improved the expression vectors for production of SA-Ag by insertion of a sequence that encodes a flexible linker separating the SA and Ag protein moieties. This allows production and isolation of higher amounts of more stable and pure SA-Ag tetrameric fusion proteins. The SA-Ag fusions are currently produced into inclusion bodies in *Escherichia coli*, with subsequent purification of the denatured monomeric protein in 8 M Urea by two chromatographic steps. Finally, the monomeric form of SA-Ag folds into tetramers in the course of dialysis against 50 mM (NH₄)₂CO₃.

Here we have used the adenoviral proteins Hexon and Hexon-associated protein to evaluate the ability of the improved SA-Ag:biot-antibody complexes to induce protective immune responses against an adenoviral infection of chicken. When the complexes were directed to APCs through the chicken Ly75 C-type lectin, a signifi-

cant protection against adenoviral infection was induced.

MB9

Biotechnology aspects of PIXL methodology (photo-induced cross-linking): a tool for structural biochemistry

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The photo-induced crosslinking (PIXL) technique employs a photolabile diazirine analogue of amino acid that is introduced into protein sequence during its recombinant expression. We utilized the structural analogues of methionine (L-2-amino-5,5-azi-hexanoic acid) and leucine (L-2-amino-4,4-azi-pentanoic acid) to substitute canonical amino acids in protein sequence. The studied proteins prepared in such a way can serve as photolabile nanoprobes to study their protein-protein interactions and to map these protein-protein interfaces structurally, to authenticate their 3D protein structures, to determine their changes during catalytic/enzymatic function, or to study the electron transfer during redox reactions realized via amino acid residues in protein. The PIXL technique workflow typically includes: recombinant expression of studied protein, the photolabile protein nanoprobe purification and validation of the

incorporation of used structural analog, the photolabile nanoprobe incubation in a designed system with following activation of introduced diazirine groups by UV irradiation forming reactive carbene (this species is able to attack any molecule in its close vicinity bellow 5Å), and finally the identification of resulting covalent crosslinks (intermolecular or intramolecular) formed during PIXL (e.g. crosslinks formed between the interacting protein parts). The high resolution or tandem mass spectrometry analysis are employed after sample processing to identify crosslinks and to determine their constrain-distances that can serve for subsequent 3D model building or model evaluation.

The introduced PIXL technique was successfully applied to several medically significant subjects/questions comprising: (i) the structure-functional relationships in the eukaryotic microsomal Mixed Function Oxidase complex (responsible for biotransformation of many hydrophobic endogenous compounds and chemical carcinogens, drugs or other xenobiotics), (ii) the homodimer interface mapping and binding partners determination of human 14-3-3zeta regulatory protein, (iii) the protein fold validation and electron transfer description of the metal-labeled blue copper protein azurin of *Pseudomonas aeruginosa* capable of electron tunneling through its peptide β-strands together with fast and reversible switching between the Cu^[II] and Cu^[I] oxidation states.

This work has been supported by Charles University (UNCE204025/2012).

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MB10

***Bordetella pertussis* and its adenylate cyclase toxin: How it works and how to use it**

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Bordetella adenylate cyclase toxin (ACT) is secreted from bacteria by a 'push-pull' mechanism through a T1SS 'channel-tunnel' assembly. This is promoted by the C-terminal folding nucleus that emerges with the C-terminal secretion signal from the T1SS conduit on bacterial surface and facilitates Ca²⁺-driven stacking of adjacent RTX repeats blocks. These form β -roll structures, serving as Brownian ratchets that promote vectorial folding of the translocating ACT polypeptide as it emerges from the T1SS duct. The secreted toxin then targets myeloid phagocytes bearing the complement receptor 3 (CR3, $\alpha_M\beta_2$ integrin CD11b/CD18 or Mac-1), such as neutrophil, macrophage or dendritic cells (DC, CD11b^{high}). ACT recognizes a positively charged loop of the CD11b subunit of CR3 near the hinge region outside of the I-domain of CD11b and inserts directly across phagocyte membrane. ACT-mediated Ca²⁺ influx then induces calpain-mediated cleavage of talin, enabling ACT to hijack the receptor and mobilize it into membrane lipid rafts. There, translocation of the AC domain across cell

membrane is completed across a tightly sealed protein-lipid interface. The AC binds cytosolic calmodulin and catalyzes conversion of ATP to cAMP, generating supra-physiologic cAMP levels that subvert phagocyte functions, causing phagocyte impotence due to inactivation of the Syk kinase and block of signaling of leukocyte receptors. Activation of PKA through cAMP next provokes transient inactivation of the small GTP-ase RhoA, causing rapid and unproductive cell ruffling. In parallel, activation of Epac causes inhibition of oxidative burst in neutrophils through inhibition of PLC and of NADPH complex assembly. Transient activation of the tyrosine phosphatase SHP-1 occurs by an as yet unknown PKA-dependent mechanism and blocks of expression of iNOS and of bactericidal NO production in macrophages. Simultaneously, activated SHP-1 causes stabilization of BimEL and activation of Bax, provoking induction of apoptosis. Influx of calcium ions and relocation into membrane rafts also allows ACT to escape rapid endocytic removal from cell surface, thus enabling a subpopulation of ACT molecules to oligomerize into small cation-selective pores that permeabilize cells for potassium efflux. This contributes to induction of maturation of dendritic cells that is, however, hijacked by cAMP signaling and compromises the capacity of DCs to stimulate antigen-specific T cell immune responses. Migration of the incompletely mature DCs into lymph nodes then likely contributes to suppression of adaptive host immune responses to the pathogen and support bacterial colonization of the host in early stages of infection. Later in infection, ACT action provokes NALP3 inflammasome activation

in dendritic cells, which likely contributes to late inflammatory response and eventual development of Th1/Th17 polarized immune responses that support eventual clearance of the bacterial infection.

MB11

Computer-assisted engineering of fibroblast growth factors

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Fibroblast growth factors display a great therapeutic potential, particularly in regenerative medicine, cardiovascular disease therapy, and therapy of metabolism associated conditions^[1]. FGF2 is a promising agent for the treatment of cardiovascular

diseases, cancer and mood disorders. It also shows efficiency in wound healing and tissues engineering. Moreover, FGF2 has an irreplaceable role as an essential component of media for cultivation of pluripotent stem cells^[2] and is attractive for anti-aging cosmetics and as a supplement promoting hair growth^[3,4]. Long-term maintenance of FGF2 in the tissue or media is desirable for both protein therapies and stem cell culturing but is hindered by its low stability. The application potential of FGFs is therefore limited due to their natural instability and a limited half-life at 37 °C^[5,6]. Protein stability can be increased by the introduction of computationally designed stabilizing mutations. We have previously developed a robust computer-assisted engineering strategy combining energy-based and evolution-based computational analysis FireProt^[7]. Here we applied FireProt in combination with focused directed evolution on FGF2^[8]. The nine-point mutant of FGF2 exhibits melting temperature increased by 19 °C and *in vitro* functional half-life at 37 °C improved from 10 hours to more than 20 days. Preserved biological activity of FGF2 variant was confirmed *in vitro* and *in vivo*. This construct can be directly applied in stem cell culturing and broadens the use of FGF2 in medicine and cosmetics. As we demonstrated convenience of FireProt strategy, we plan to utilize it for stabilization of other therapeutically important FGFs. The strategy can be applied to any protein for which a tertiary structure and homologous sequences are available.

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MB12

Targeting the stress signaling in cancer

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Tumor tissue differs from the normal one not only by bearing the oncogenic mutations but also by the presence of hypoxia, lactic acidosis and lack of glucose and glutamine as a result of a rapid growth and an insufficient capillary network. Therefore, tumor cells are exposed to a metabolic and oxidative stress which could sensitize them to some kinds of therapy. On the other hand, the stress activates several prosurvival pathways including HIF1alpha, AMPK, sirtuins, PGC1alpha, heme oxygenase-1, and an antioxidant transcription factor Nrf2. The higher activity of these pathways was associated with the higher aggressiveness and invasiveness of cancer tissue and with the higher chemo and radioresistance. Interestingly, known activators of the stress signaling including statins, metformin, and the physical activity were associated with a lower cancer incidence. The complex understanding of the cancer metabolism and the stress response is necessary for the development of a rational therapy targeting the tumor microenvironment. Here, we discuss the approaches which could switch the tumor tissue stress from the resistance mediator to the damaging factor.

Oral Presentations – Microalgae Biotechnology

MAB1

Microalgae grown in stable isotopes

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Stable isotopes, the non-radioactive variants of elements with the same number of protons but with a varying number of neutrons, have been widely used for metabolic labeling to study metabolic fluxes as well as for quantitative proteomics. Deuterium, the heavy stable isotope of hydrogen, has strong isotopic effects and at high levels causes major defects in cell physiology. The borderline for its safe incorporation into plant and animal cells is 20 %. Yet some green algae are able to incorporate 100 % deuterium into their cells and are still able to grow and divide. It is not clear what mechanisms enable cells to tolerate high levels deuteration. Here, we study the effect of growth in deuterated water, D₂O, on two green algae, *Parachlorella kessleri* and *Chlamydomonas reinhardtii*. Of the two, *P. kessleri* is more resistant

and can grow up to the 99 % incorporated deuterium. In contrast, *C. reinhardtii* can only grow and divide until the ratio of D₂O/H₂O reaches 0.70, but can be acclimated to growth even to higher ratios. We compare *in situ* incorporation of deuterium into different biological molecules in the two microalgae by Raman microscopy. Furthermore, we perform a mutagenesis screen to isolate genes allowing growth of *C. reinhardtii* to higher D₂O/H₂O ratios. This work was supported by the GA CR (grant no. 17-06264S).

MAB2

Towards industrial products from microalgae

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Microalgae show an enormous potential as sustainable feedstock for numerous bioproducts. The current work analyzes the feasibility of business cases for different markets of products from microalgae.

We perform a techno-economic evaluation of the whole process chain including cultivation, biorefinery and market exploitation for a 100 hectares' facility in six locations. Our projections show a current cost per unit of dry biomass of 3.4 e/kg for phototrophic microalgae cultivation in Spain (excluding biorefining products), with an expected reduction to 0.5 e/kg in ten years. A sensitivity analysis reveals the roadmap to achieve this. Production of high-value products (e.g. pigments) would be currently profitable.

Markets aimed at food and chemical commodities require further cost reductions for cost competitiveness, reachable in the next decade.

Techno-economics of phototrophically produced microalgae will be compared with heterotrophic production.

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Ruiz J., Olivieri G., de Vree J., Bosma R., Willems P., Reith J.H., Eppink M.H.M., Kleinegris D.M.M., Wijffels R.H., Barbosa M.J. (2016) Towards industrial products from microalgae. *Energy & Environmental Science* 9: 3036-3043.

MAB3

Influence of inorganic salt precipitates on autoflocculation of microalgae

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Unicellular photosynthetic microorganisms have a significant biotechnological potential, which is often constrained by the high costs of cultivation and separation of biomass. Separation of the biomass is particularly costly because of the low harvesting densities of these microorganisms. The separation is therefore usually a two-stage process, where the final harvesting of biomass by centrifugation is preceded by some type of flocculation. For the most cost effective, however, somewhat unreliable, is considered autoflocculation induced by inorganic salt precipitation at high

pH. This work studied the flocculation of *Chlorella vulgaris* promoted by precipitates (e.g. calcium and magnesium phosphates) both experimentally and theoretically. Experimentally were characterized the surface properties of the cells and precipitates (charge, contact angle), and quantified the flocculation efficiencies under different environmental conditions (pH, ionic strength, presence of cellular organic matter). Simultaneously, colloidal interaction models were created for the interaction of cells with precipitate particles and after their confrontation with experimental data the driving forces and mechanisms of flocculation were identified.

MAB4

Study of up- and downstream processes in *Microcystis aeruginosa* cultivation – One approach, two distinct objectives

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The cyanobacterium *Microcystis aeruginosa* and the accumulation of its cyanotoxin microcystin (MC) have been responsible for several human/animal deaths and intoxication incidents. Therefore, the

World Health Organization established recommendation values for MC in water, giving rise to an increasing demand for MC's analytical standards to be used as laboratory standards both in human and environmental risk assessment studies. These Cyanotoxins are also considered promising anticancer/antitumor drugs as well as antifungal, antialgal and insecticide agents. Despite the interest, commercial MC availability is still limited due to constraints found in production, which inflate the final price to values as high as 28000 €/mg.

Therefore, in order to implement a cost-effective MC production, the objectives of this work were the following: 1) evaluate the effect of environmental factors on *M. aeruginosa* growth and MC accumulation; 2) implement cultivation strategies (e.g. co-cultivation) to optimize cyanobacteria growth and MC productivity; 3) optimize downstream processing steps in order to increase cost-effectiveness.

The combined influence of light intensity, CO₂ concentration, temperature and medium pH on *Microcystis aeruginosa* toxicity, biomass concentration and productivity was evaluated, setting the bases to explore combinations of environmental variables as a means of limiting cell growth and/or toxin production or to boost its industrial production. Higher MC concentrations were obtained at low light intensities and CO₂ concentrations while approximately 1000-fold lower MC concentrations were achieved by simultaneous use of high values of light intensity, CO₂ concentration and temperatures.

In order to ensure cost-effective downstream processing, six different disruption methodologies (microwave, high-speed ho-

mogenizer, sonication, freeze-thaw cycles and bead mill) and several harvesting approaches were tested. Disruption and harvesting efficiencies up to 97 % and 95 %, respectively, were attained.

This study allow us to achieve two distinct objectives: i) increase cost-effectiveness of MC production; and ii) improve controlling and predicting mechanisms regarding *M. aeruginosa* blooms.

MAB5

Identifying knowledge gaps for an efficient anaerobic digestion of microalgae biomass

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Photosynthetic microorganisms, such as microalgae, are nowadays studied as potential feedstock for next generation biofuels as an alternative to fossil fuels. Among biofuels, biogas generation seems to be the least complex. Out of the subsequent stages involved in anaerobic digestion, hydrolysis is the rate limiting stage in the case of microalgae. The main challenge for an efficient anaerobic digestion using microalgal substrates is the optimization of cell wall disruption in order to make accessible organic matter to the anaerobic bacteria. To breakdown the cell wall of *Chlorella vulgaris*, two pretreatments have been assessed herein included biocatalyst (protease) addition and thermal application. These two

pretreatments resulted in an enhanced methane yield in batch mode assays. More specifically, thermally and biological pretreated biomass enhanced methane yield by 1.87 and 1.57-fold, respectively. This enhancement was attributed to the higher organic matter solubilisation as a result of the cell wall disruption that took place during the pretreatments. The solubilized organic matter increased from 2-3% of soluble organic matter before pretreatment up to 18% and 49% after thermal and biological pretreatment, respectively.

The biogas production from pretreated biomass was further assessed by moving forward from batch to semicontinuous digestion mode (CSTR). As in Batch experiments, CSTR fed with pretreated biomasses increased methane yield by 1.5-fold in comparison to raw biomass. However, methane yields in CSTRs were considerably lower than those obtained in batch (48%), revealing that some kind of inhibitory mechanisms occurred. The lower methane yield attained in CSTR fed with protease pretreated biomass can be attributed to ammonium inhibition (1900 mg L^{-1}) that resulted in volatile fatty acids (VFAs) accumulation. Given the fact that methanogens are more sensitive to ammonium/ammonia inhibition, microbial imbalance between acidogens/acetogens and methanogens was taken place. In CSTR fed with thermally pretreated biomass, no VFAs accumulation was measured and ammonium/ammonia concentrations were not in the threshold limits; therefore, other unidentified inhibition mechanisms should be responsible for methane yield reduction. Overall, this study highlights the need to study in depth batch mode promising pretreat-

ments to cope with semicontinuous digestion drawbacks not identified in batch. Likewise, the anaerobic microbiome should be followed to clearly identified microorganisms imbalances during digestion process.

MAB6

Endopolyploidy, fragmentation and reconstitution of chromosomes by the heavy-ion beam irradiation in *Parachlorella kessleri*

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Heavy-ion mutagenesis is a technology used for effective production of genetic mutants. We have demonstrated that algal breeding using a unicellular alga, *Parachlorella kessleri*, by heavy-ion mutagenesis can improve lipid yield in laboratory experiments^[1]. Heavy-ion beams are powerful mutagens. These mutagenic effects are due to dense ionization in a localized region along the ion particle path. We'd like to investigate the direct effect

of Fe heavy-ion beams on chromosomes in *P. kessleri*. However, in *Chlorella*, chromosome analysis has not been developed yet and there is no report on karyotype of *P. kessleri*. In this study, we investigated the karyotype and ploidy of *P. kessleri* and then clarified the chromosome composition of heavy-ion beam irradiated strains. The chromosomes were stained by SYBR Green I and analyzed by pulsed-field gel electrophoresis; the chromosome number of *P. kessleri* was set to $n = 7 + 3B$. The cells with B chromosomes made about 20 %. The nuclear DNA content increased to 9.7 C in the logarithmic growth phase, but chromosome number stayed unaffected. Thus, the chromosome of *P. kessleri* should be polytene-like like a polytene chromosome. When irradiated with the Fe heavy-ion beam (75 Gy), the cells with more than 30 fragmented chromosomes appeared in 14 % of total cells immediately after irradiation. Such vigorously chromosome-fragmented cells disappeared after one subculture. In the cloned strain (Fe75-1-3H), four SNPs: one deletion and three inter-chromosomal translocations, are involved in only one part of its scaffold (No 15) suggesting more local and more complex mutations being caused by the heavy-ion beam irradiation.

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MAB7

Isolation and structural characterization of exopolysaccharides from microalgae *Dictyosphaerium chlorelloides* and *Porphyridium purpureum*

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Microalgae have full attention in these days due to their potential as food supplement, source of biological active compounds [1] and are also used as sensitive tester for purity of water streams [2].

This work is devoted to isolation and structural characterization of exopolysaccharides (EPS) from *Dictyosphaerium chlorelloides* and *Porphyridium purpureum*. EPS were separated from cultivation media and also isolated from algal biomass. Several fractions were obtained and further purified by the treatment with acidic ethanol and enzymes (amylase, pepsin) to remove α -glucan and proteins. EPS fractions from both sources con-

tained carboxylic and sulphate semi-ester groups. FTIR and FT Raman spectroscopy were used for purity control and structural evaluation. Neutral sugars and linkage positions were determined by GC/FID and GC/MS respectively. Correlation NMR was used for more specific structural analysis. These EPS are interesting as potential biologically active compounds.

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MAB8

Wood hydrolysates as potential feedstocks for microalgae biomass, fatty acid and pigment production

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In this study, the effect of various wood hydrolysates on microalgae biomass, fatty acids and pigments production is evaluated. Microalgae are photoautotrophic microorganisms containing valuable compounds such as lipids and pigments that can be harnessed for industrial applications. Wood biomass is a lignocellulosic material containing various organic molecules, such as sugars, organic acids and phenolics. Microalgae strains, such as *Chlorella*, are capable of using organic substrates to support growth and biomass production during heterotrophic and mixotrophic cultivation. Hence, wood hydrolysates containing organic carbon have a potential to improve microalgae growth, in light presence or in case of lack of light^[1].

Beech *Fagus sylvatica* wood hydrolysates were tested in terms of their effect on *Chlorella* growth and fatty acid and pigment production during heterotrophic and mixotrophic cultivation in a multitube photobioreactor.

It was observed that wood hydrolysates supported *Chlorella* growth in light and in dark. Hydrolysates were stimulatory at lower loadings and inhibitory at higher lo-

adings. Organic and mineral compounds from wood hydrolysates affected *Chlorella* growth. Fatty acids and pigments were produced from *Chlorella* cultivated on wood hydrolysates. Light had influence on composition of fatty acids and pigments in *Chlorella* cultures cultivated on wood hydrolysates.

This study shows that beech wood hydrolysates have potential to support *Chlorella* growth and improve fatty acid and pigment productivity during heterotrophic and mixotrophic cultivation. Such an approach can possibly find application in biorefineries and for industrial production of high value-added compounds^[2].

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Oral Presentations – Environmental Biotechnology

EB1

Microbial Ecology in Anaerobic digesters: status and perspectives

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Anaerobic digesters are typical engineered microbial cell factories, in which complex consortia perform a series of subsequent metabolic activities that lead to waste treatment and valorisation, renewable energy production and generation of high value added compounds. The anaerobic digestion (AD) microbiome consists mainly of Eubacterial and Archaeal species, whose structure is still far from being completely elucidated. Interestingly, some of these species belong to the major uncharted branches of the tree of life, the so-called "microbial dark matter". Till now, the composition of AD ecosystem has been generally determined via traditional molecular techniques, mostly targeting marker genes such as the 16S rRNA gene. However, since the majority of the participating microbes still resist cultivation efforts, it is challenging to perform an accurate identification and to understand the mechanisms beyond the interconnected steps of the AD process. Recent advances in the era of -omics enabled a fine-scale definition of population dynamics and provided fundamental information for poten-

tial assignment of functional roles to specific microbes. Thus, the combination of different meta-omics tools (e.g. metagenomic, metatranscriptomic and metaproteomic) seems to be the Ariadne's thread that should be followed in order to decipher specific metabolic properties of microbial populations, unravel their collective behaviour and discover novel aspects of AD ecology (e.g. new phyla, interspecies communication). It is important to highlight that this research field is quite interdisciplinary and demands to employ diverse knowledge, ranging from engineering science to biochemistry, microbiology and bioinformatics. It is envisioned that by gaining deeper insights into the AD ecosystem it would be feasible to overcome a number of currently existing technical challenges of biogas plants (e.g. ammonia, foaming) by designing a portfolio of advance tools (e.g. microbial cocktails) and manipulate the process towards certain outcomes (e.g. biogas upgrade).

EB2

Physico-chemical characterization of EPS-based biomaterial recovered from anammox granular sludge

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Autotrophic biological processes based on the metabolism of anaerobic ammonium oxidizing bacteria (*anammox*) allow removing ammonium from wastewater with significant savings in terms of energy spent for oxygen supply, organic carbon supply and excess sludge production. Anammox bacteria easily form granules which, compared to conventional activated sludge, have better settleability resulting in efficient biomass retention and thus in systems characterized by faster kinetics and smaller required volume. The recovery of biomaterial from excess granular sludge to be applied in other industrial sectors would substantially increase the sustainability and economics of wastewater treatment and would promote the development of a circular economy. In this contribution, structural extracellular polymeric substances (EPS) were extracted under alkaline conditions from anammox granular sludge, recovered in acidic conditions and finally used to form a viscoelastic biomaterial. A physico-chemical characterization of the recovered biomaterial was then performed, investigating its rheological properties as function of the EPS concentration, which is considered a prerequisite for the exploration of its potential applications. The average yield of extraction was 0.223 ± 0.051 gEPS/gVS. The sum of total proteins and carbohydrates fractions accounted on average for the 91% of the extracted EPS, with a mutual ratio of 2.35 ± 1.06 gPN/gPS. Lyophilised extracted EPS were used for the preparation of hydrogels of physical nature with a clear dependency of the storage modulus

G' from the EPS concentration. The complex viscosity of the recovered biomaterial sharply increases above 17.5% wt% indicating the formation of an extended 3-D network which seems to be constituted by the physical interaction of askew fibrils. This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 661429

EB3

Olive mill solid waste biorefinery: comparative of thermal pre-treatments for phenols recovery and biomethanization

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Two-phase Olive Mill Solid Waste (OMSW) is a residual olive oil by-product composed by the olive husk, olive pulp, and olive vegetation water. OMSW must be correctly managed to avoid severe environmental impacts. OMSW is a lignocellulosic biomass with a significant concentration of phenolic compounds, which could be recovered due to its high economic interest. Among the different phenolic compounds present in OMSW, hydroxytyrosol is one of the most important because it has excellent properties as a pharmacological and antioxidant agent. Different thermal pretreatments have been studied in order

to recover economically interesting phenols. Their effects on the subsequent anaerobic digestion process were also evaluated. Three different thermal pretreatments have been studied. 1) Low Temperature (LT) pretreatment, at 65°C, 90 min, and atmospheric pressure. 2) High Temperature (HT) pretreatment, at 170°C, 60 min, and at an operating pressure of 8 kg/cm² 3) Steam Explosion (SE), at 200°C, 5 min at an operating pressure of 26 kg/cm². The three pretreatments facilitated the separation of a solid phase, where most of the organic compounds remained, and a liquid phase, where most of the phenolic compounds were concentrated and separated by a chromatographic column for their revalorization. Although the separation of a liquid and a solid phase have been observed for the three applied pretreatments, there are significant differences on the effect of the pretreatment over the organic matter solubilization and further anaerobic digestion process. The LT pretreatment did not entitle a high solubilization of COD, but a disaggregation of particulate COD, which did not increase solubilize COD. The HT pretreatment entitiled an important increase of soluble COD, but there was the formation of inhibitors of anaerobic digestion such as hydroxymethylfurfural. The SE pretreatment entitiled an increase of soluble COD and lignin removal. The removal of lignin entitiled a formation of polyphenols which highly inhibited the methanogenic process.

EB4

Critical metals – the challenges of the present, solutions for the future

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The European Commission defines the critical raw materials as being both of high economic importance for the EU and vulnerable to supply disruption^[1]. Early in 2010 and later on in 2014, a list of 14 and respectively 20 raw materials was set up, identifying critical raw materials for the European Union industry. The criticality is given by their high supply risk due to uneven geographical distribution and the economic importance due to emerging technologies developed by the high tech, electronics and defense industry^[2]. The extraction of critical raw materials from low-grade ores, wastes and industrial residues by conventional methods has proved to be economically inefficient and causing various environmental issues. Therefore, innovative mining and/or recycling concepts for materials containing critical metals are considered of immediate priority. The lecture focus on critical metals extraction from various sources by using microorganisms both isolated from mine waste sample or from collection cultures. This di-

rection is receiving attention among the scientific research groups studying integrated ways to improve resource use and recovery of raw materials from alternative sources. The study presents experiments performed for the isolation and characterization of critical metal resistant microorganisms from samples collected from mine tailings existent on Romanian territory. Preliminary characterization was done based on cell growth, Gram staining reactions, microscopic observation and other standard biochemical tests. Biotechnologically engineered processes with practical use in biomining will open novel prospects for the recovery of critical metals, thus fostering the improvement of resource use.

Acknowledgements: This work was supported by a grant of the Romanian National Authority for Scientific Research and Innovation, CCCDI – UEFISCDI, project number 18/2016, within PN-CDI III.

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EB6

Biotechnology of nutrient removal from waste water by autotrophic denitrification with sulfides from biogas

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Autotrophic denitrification represents the specific part of biological wastewater treatment, which couples the reduction of oxidized forms of nitrogen and the oxidation of sulfidic sulfur by denitrifying sulfur bacteria.^[1] Sulfides can come from biogas desulfurization by water scrubbing that is relatively simple and costly effective method^[2].

The efficiency of this biotechnology depends on many factors influencing the activity of autotrophic denitrifying bacteria (ADB): in particular sulfide and nitrate loading rates, molar ratio of S/N, pH and temperature. Especially temperature is very important parameter positively affecting the activity. Its effect was studied during continuous operation of anoxic upflow bioreactor with biomass of ADB immobilized on plastic carrier. Immobilization can cumulate a high amount of active bacteria to a small volume and assure the high effectivity of bioreactor.

Sulfide loading rate 170 mg/(L·d) was a limiting value for stable operation of bioreactor at 20 °C. By increasing of tem-

perature to 25 °C and 30 °C, the bioreactor can be stable and effectively operated at the sulfide loading rate higher by 27 % and 106 %, respectively. The molar ratio S/N was set on value 0.55 to obtain sulfates as end products of sulfides oxidation, so that the highest loading rates of sulfides and nitrates were 350.9 mg/(L·d) and 258.6 mg/(L·d), respectively.

The efficiency of bioreactor operation was high and stable even at high values of pH (over 10) owing to presence of mainly *Paracoccus denitrificans* (its growth optimal pH 6.5-10.5 [2]); further *Thiobacillus denitrificans* and *Thiobacillus thioparus* were also identified by FISH analysis.

Results of our research confirmed the suitability of coupling biogas desulphurization and autotrophic denitrification in the external bioreactor with immobilized ADB. This biotechnology can be advantageous especially in the biological wastewater treatment line, saving operation costs of denitrification.

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EB7

Adaptation of anammox to low temperature in response to cold shock

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Adaptation of anammox to the low temperatures in main stream of municipal WWTP is necessary to enable stable high nitrogen removal in the winter months of mild climates. An efficient adaptation strategy would open the possibility to significantly reduce the consumption of energy for aeration and organic carbon for denitrification, reducing an economic and environmental footprint of WWTP.

But, current methods for the adaptation of anammox to low temperatures are based on a slow reduction of operational temperature, which takes months or even years. Inspired by a work of bacteria adaptation to cold, we suggest a new approach: exposing anammox microorganisms to an abrupt temporary reduction of temperature described as "cold shock".

Anammox biomass in a moving bed biofilm reactor was subjected to two subsequent cold shocks of 5 °C with a duration of eight hours. Control biomass was operated at same conditions without the shocks. After each shock, activity of anammox microorganisms was determined ex-situ using batch assays at temperature of (10, 13, 16, and 20 °C).

First cold shock increased the activity of anammox bacteria at low temperatures.

At 10, 13, 16 and 20 °C, the activity of anammox was 0.30, 0.37, 0.762 and 1.85 kg-N.m⁻³.d⁻¹. In comparison, the activities of control biomass were 0.065, 0.116, 0.202 and 0.206 kg-N.m⁻³.d⁻¹. The second shock further increased anammox activity at 10 °C to 0.374 kg-N.m⁻³.d⁻¹ while reducing the activity at 20 °C to 1.2 kg-N.m⁻³.d⁻¹. This demonstrates, that cold shocks have the potential to be a fast and highly effective strategy for the adaptation of anammox to low temperatures.

EB8

Hybrid treatment methodologies for biotransformation of color industrial effluents – A bioreaction calorimetric study

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The impact caused by industrial effluent discharge on environment is well known. Chemical and biological treatment processes although offer partial remediation, intense research activities are going on to resolve problems in handling higher effluent toxic load^[1], selection of robust bacterial consortia, and treatment of color effluents with dye concentrations above 0.8 g/l ^[2]. Further, in real treatment processes, there is a fluctuating toxic load and this may affect the consortia efficiency. In order to have optimal degradation, effluent pretreatment is required. The study

explores a hybrid method i.e. combining biological treatment (by a defined bacterial consortium) with Fenton oxidation for active treatment of an effluent containing toxic azo dye (acid blue 113). Fenton concentration was optimized and kept minimal in order to protect the sustainability of bacteria (f/m ratio). Pre-treatment of dye with Fenton (H₂O₂ & Fe²⁺), considerably reduced the initial toxic load resulting to a decreased HRT (Hydraulic retention time) of 12 hrs and an increased process efficacy (97%) for dye concentrations of 0.9-1.4 g/l. The bioprocess was investigated in a bioreaction calorimetry (Bi-oRc1e) for effective monitoring and control. Heat profile pattern, bioenergetics data along with CER (Carbon di oxide emission rate) and OUR (Oxygen uptake rate) provided vital information for a feasible application in commercial level^[3,4]. FACS (Fluorescence-activated cell sorting) analysis reveals a better bacterial cell viability and proliferation in a pre-treated experiment with higher initial dye concentrations. Use of marginal chemical reagent makes the process cost effective for large-scale operations. The toxicity of the treated effluent was assessed using MTT assay (human cell lines of keratinocytes – Ha-CaT), and the final metabolic product obtained was identified using GC-MS and FT-IR analysis. The final treated effluent was non-toxic in nature. The novel combination of Fenton and the mixed microbial population is a competitive technology for industrial effluent treatment processes.

Keywords: Biocalorimetry, Fenton pretreatment, FACS, Bacterial consortium, Effluent treatment Biography

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EB9

Bioleaching for heavy metal recovery and detoxification of ashes from thermal power plants

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Environmental pollution is a major problem especially in developing countries. This presentation discusses how bioleaching may be used to recover heavy metals from ashes produced by coal-firing and oil-firing thermal power plants to reduce pollution. Valuable metals such as vanadium, nickel and copper can be recovered with very good yields. Detoxified ashes are well below environmental safety thresholds for safe disposal. This presentation will provide an example using autotrophic *Acidithiobacillus thiooxidans* to recover V,

Ni and Cu from fuel-oil ashes from a power plant. Up to 4% (w/v) of fuel-oil ash particles suspended in the culture medium was tolerated by the bacterium through gradual adaptation. Maximum recoveries for V, Ni and Cu were 96.4%, 100% and 99.2%, respectively at an ash concentration of 1% after nine days of incubation in 100 ml shake flask cultures. Bioleaching performances in a 2.4 L bubble column will also be discussed.

EB10

Efficacies of seventeen organically made northern fertilizers on sustainable crops production in acidic soil of food security under climate change

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Different field study was conducted to evaluate the efficacies of Northern organically made fertilizers on yield and quality of crops in a study. BF(Rice) M-54, Yield increased by 7-19%, Urea saved by 50%, BF(Wheat)M-51, Yield increased by 17-19%, BF(Potato)M-48, Yield increased by 23%, BF(Mustard)M-50, Yield increased by 28.5%, BF(Maize)M-53, Yield increased by 28%, BF(Onion)M-47, Yield increased by 5kg per decimal average, Northern

BF(Banana)M-49, Saving in Urea fertilizer per plant 50-100g, BF(Cauliflower)M-66, Saving in urea fertilizer per decimal 250-280g, BF(Sugarcane) M-63, Yield increased by 36%, BF(Garlic)M-201, weight loss during storage is very less, BF(Tomato)M-199, Chemical fertiliser saves by 50%, BF(Cabbage)M-198, saving in urea fertilizer per decimal 250-280g, BF(Chili)M-200, saving in urea fertilizer per decimal 200-250g, BF(Brinjal)M-403, saving in urea fertilizer per decimal 200-230g, BF(Watermelon)M-402, Saving in Urea per decimal by 150-200g, Shakti Fertiliser (Mango, Tea, Vegetables, Cotton), M-52, Yield increased by 45.8% of Mango, 15% of Tea, Chemical fertiliser saves by 50% of Rice and Vegetables, Higher yield growth of 2550kg per hectare of Cotton, Organic Fertilizer (Potato, Sugarcane, Betel Leaf, Mango, Tea, Vegetables)M-65, Yield increased by 45.8% of Mango, 9% of Tea, chemical fertilizer saves by 50% of Rice and Vegetables, Higher yield growth of 68.41 tons per hectare of Sugarcane, 8850 betel Leafs per decimal, 23.68 tons per hectare of Potato.

Keywords: Efficacy, Northern Fertiliser, Sustainable, Production, Crop

Posters

P1

Antibiotic resistance of *Pseudomonas aeruginosa* biofilm

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A biofilm can be defined as a community of microorganisms stored in protective matrix of EPS (exopolymeric substances) adhering to various surfaces. Microbial biofilms cause chronic human diseases (e.g. pneumonia in cystic fibrosis patients, endocarditis, middle ear infections and implant-/catheter-associated infections) and the treatment is very difficult. The cells in biofilm are significantly more resistant than suspension cells to environmental influences and they also show resistance to antibiotics and disinfectants. This study is focused on monitoring the ability of polymyxin B in comparison with first-line antibiotics (gentamicin and ceftazidime) to disrupt biofilm formation of opportunistically pathogenic Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 10145 and ATCC 15442. We observed anti-adhesive properties of biofilm to 96-wells polystyrene microtiter plates using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay for determining the metabolic activity of the cells in biofilm and crystal violet staining method to quantify the amount of biofilm biomass. Optical density was measured at 600 nm to quantify the cells presented in the surroundings

of *P. aeruginosa* biofilm. We determined the minimum biofilm inhibitory concentration (MBIC) of polymyxin B in quite low concentrations (10 mg/l) for both tested strains of *P. aeruginosa*. The other tested antibiotics had lower activity than polymyxin B.

P2

Preparation of tyrosyl beta-rutinoside catalysed by crude plant material

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Tyrosyl β -rutinoside(**1**) was prepared in our laboratory as derivative of natural compound salidroside which has many proven health benefit effects as antioxidant^[1,2], anti-cancer^[3], anti-viral^[4], anti-inflammatory^[5], anti-diabetic^[6], neuroprotective^[7], cardioprotective^[1,8] and hepatoprotective^[9] properties.

Homogenized defatted seed material from *Fagopyrum tataricum* var. *Mada-waska* and flower buds from *Sophora japonica* were tested for rutinoidase and transrutinosidase activities. Rutinosidase from *F. tataricum* was not effective in transrutinosylation of tyrosol, but useful in hydrolysis of rutin and therefore preparation of free rutinose (yield 80 %). This source of catalyst has been also successfully applied in transrutinosylation with 57 % yield of 2,2,2-trifluoroethyl rutinoside (**2**).

Rutin (**3**) and (**2**) were tested as donors for transglycosylation. Compound (**2**) was partially hydrolyzed, anyway no or only negligible transrutinosylation occurred, i.e. this compound is not suitable donor for our purposes. Product (**1**) has been prepared by transrutinosylation from rutin as glycosyl donor. Preparation of (**1**) was achieved using rutinoidase present in flower buds from *Sophora japonica* with 164 % yield according to rutin added to reaction. The excessively high yield of the product was caused by natural occurrence of rutin in flower buds. Structures of isolated products were elucidated by NMR spectroscopy.

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P3

Biological activities of *Monascus purpureus* pigments

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The filamentous fungus *Monascus purpureus* is capable of producing secondary metabolites such as red, yellow and orange pigments, cholesterol lowering agent monacolin K or mycotoxin citrinin. Those biologically active compounds may possess specific properties like antimicrobial, anti-oxidant, anti-cancer, anti-obesity, anti-inflammation or anti-diabetes activities. Potential food or pharmaceutical application of microbial pigments depends also on their biological activity which can be used for the benefit of the quality and safety of the product. Simultaneously, the citrinin production should be kept at minimal level.

In our research, we were focused on the certain cultivation conditions under which especially the yellow pigments monascin and ankaflavin are produced. Further, we tested selected biological activities of extracts obtained from fungal mycelium. The purified pigments were obtained through HPLC fraction collector and used for testing of anti-microbial properties against G⁺ bacterial strains of *Bacillus* and *Clostridium*. In addition, the pigments and extracts were examined for their potential cytotoxicity and mutagenicity.

The highest concentration of monascin and ankaflavin was obtained by cultivation of the strain, isolated from Chinese red yeast rice, using sodium nitrate as a nitrogen source. Moreover, mycotoxin citrinin was not detected in ethanol extract from fungal mycelium. The extracts from rice substrate inhibited germination of *Clostridium pasteurianum* and *Clostridium beijerinckii* spores. Hence, the crude extracts exhibited bacteriostatic effect against *Bacillus subtilis*. The pigments and extracts did not show any cytotoxic or mutagenic activity in Ames test.

We believe that pigments may offer interesting usage in food industry as food colourants or flavouring agents, but could also be used as a preservative.

P4

Microbial production of lactic acid using the keratin hydrolysate and straw hydrolysate

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Lactic acid is a chemical compound with a broad spectrum of applications, for example in food, tanning, cosmetics or pharmaceuticals. In addition, lactic acid can be considered a precursor for the synthesis of different polymers, which may be more environmentally friendly in comparison with traditional plastics.

We tried to design and optimize the process of microbial production of lactic acid by the strain *Lactobacillus casei* using chicken feather as a low-cost source of nitrogen and wheat straw as a low-cost source of carbon. Firstly, feather was hydrolysed with sodium hydroxide at a concentrations of 2, 5, 10, 15 and 20 % by weight and tested as a replacement of all nitrogen sources present in MRS medium. Taking the growth of biomass and the final concentration of lactic acid as the main criteria, 20 % hydrolysate of chicken feather was chosen for the next experiments, where it was used not only as a source of nitrogen but also as a neutralizing agent during bioreactor fermentations. This highly alkalic hydrolysate was efficient neutralizing agent and also increased production of lactic acid in comparison with sodium hydroxide of the same concentration. The final reached concentration of lactic acid in the medium was 58 g/l.

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P5

Mutagenesis and adaptation as tools for the development of *Clostridium beijerinckii* strains with increased butanol tolerance and production

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Acetone-butanol-ethanol fermentation using *Clostridium* species is a promising way of butanol production. However, its use is limited by the achievement of low final solvent concentration, probably caused by high butanol toxicity towards the production cells. To overcome this limitation, we developed *C. beijerinckii* NRRL B-598 strains with enhanced butanol tolerance using mutagenesis and adaptation techniques. Random chemical mutagenesis using ethyl methanesulfonate was used to obtain mutants, that were selected based on their ability to survive different concentrations of ethidium bromide and/or concentrations of butanol lethal for the parental strain. Strains tolerant to butanol were also prepared from the parental strain using repetitive evolutionary domestication^[1] as an adaptation strategy. Single survived mutant colonies as well as adapted cultures were tested for their butanol resistance in microtiter plates with the medium containing bromocresol purple as a growth indicator. Both adapted and mutant strains with the highest butanol tolerance were cultivated under substrate non-limiting conditions (with a high amount of sugar in

medium) and analyzed for substrate consumption and product formation by HPLC. Results of the research show that, in most cases, the increase of butanol tolerance does not lead to higher butanol production. However, strains with the both features will be used for the further research aimed to solvent tolerance and butanol production.

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P6

Cytocompatibility of argon plasma-treated fluorinated ethylene propylene: Comparing keratinocytes and primary dermal fibroblasts

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Surface modifications are often necessary for the use of synthetic bioinert polymers, such as fluorinated ethylene propylene (FEP), as biocompatible materials allowing cell adhesion. We prepared matrices of FEP modified for 0-240 s using argon plasma treatment with the power of 3, resp. 8 W. This simple procedure leads to increased surface roughness and wettability. We employed the human keratinocytes HaCaT and primary human dermal fibroblasts HDF2 as models for cytocompatibility testing of the prepared FEP matrices. The modification enhanced cell adhesion and proliferation of both HaCaT and HDF2 on the tested matrices. Compared to those on pristine FEP, cells of both cell lines on the modified matrices showed also improved spread-out morphology as well as metabolic activity similar to the control (standard polystyrene tissue culture dish). Using immunofluorescent labelling of the focal adhesion protein talin 1, we also observed focal adhesions of cells cultured on FEP matrices. Our findings suggest that Ar plasma treatment constitutes a straightforward method for a significant improvement of cell adhesion on the otherwise bioinert fluorinated ethylene propylene copolymer.

MW1

miwelt: a visible and invisible world of microorganisms

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The experience of a microbial world, which is at a first glance not visible to the naked eye, is facilitated in a non-fiction children's book developed in the scope of the miwelt-project: 'The world is full of things which are not visible to the naked eye.'

miwelt is a science communication project initiated under the Agora scheme of the Swiss National Science Foundation (SNSF). Scientists, artists and journalists have jointly developed illustrated materials, thematic excursions, and laboratory experiments on the subject of microbial biotechnology for children from the age of 7 to 11.

As part of the miwelt project, scientists have been encouraged to explain both the content of and methods used in their everyday work to children and their families as well as to teachers who have only a basic grounding in science. For this purpose, they are aided by illustrations and analogies with everyday life, produced by a professional illustrator and journalists engaged in dialogue with the scientists.

For more information visit:

www.miwelt.net

P7

Estimation of the biogas productivity potential of cow manure and aerobic sludge: Effect of inoculum/substrate ratio and salts addition

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During the recent decades the world has been looking at renewable fuels as result of greenhouse gas pollution and the depletion of traditional fossil fuels. The demand for renewable energies has driven the efforts of scientists around the world to find a sustainable source of energy which eventually can replace traditional fuels. Anaerobic digestion is becoming an efficient system not just for treatment of wastes but for the generation of pipeline-quality biogas. Different types of waste have shown to be suitable for such purpose. Besides gas production, anaerobic digestion generates a digestate rich in nutrients that can be used as fertilizer in agricultural activities, resulting in a sustainable closed loop for biogas production.

In this study, we investigated the influence of inoculum/substrate ratio and salts

addition on the biochemical methane potential of anaerobic digestion of cow manure and aerobic sludge under mesophilic conditions. Current research is underway to improve further the anaerobic digestion by investigating the process conditions. The existing results show that different ratios influence the biogas yield and quality. Therefore, the objective of this study is to test the effect of the inoculum/substrate ratio and minerals additions on the process performance of the anaerobic digestion for these 2 substrates. Also, current problems are summarized and several future development directions are pointed out for enhanced biogas production from other agricultural waste.

P8

A novel method for monitoring the unfolded protein response in *Pichia pastoris*

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Pichia pastoris is a preferred microbial host for the production and secretion of high-quality recombinant proteins. However, in such an efficient species, incorrectly folded proteins may occur. Failure to properly fold proteins can lead

to loss of protein function, protein degradation or a reduction in cell viability, resulting in reduced or impaired productivity. To cope with impaired protein synthesis, the cells trigger a signalling cascade called the Unfolded Protein Response (UPR) pathway. To achieve high productivity in a biotechnological production process, a systematic understanding of the mechanism of UPR induction is required. We developed a novel UPR-reporter expression cassette, consisting of a *KAR2* upstream region controlling the expression of super folder GFP (sfGFP). After integration of the cassette into the *P. pastoris* genome, UPR induction was assessed as sfGFP fluorescence at-line and in a non-invasive manner using flow cytometry. To determine the relationship between UPR and the production of heterologous protein, we studied the induction of UPR in *P. pastoris* expressing a recombinant penicillin G acylase (PGA) gene from a methanol-inducible alcoholoxidase (*AOX1*) promoter and under controlled bioreactor conditions. PGA is an appropriate model system since bottlenecks in protein production and secretion could be observed during controlled bioreactor cultivations, and these triggered the UPR. In a control non-producing strain, no significant increase in fluorescence was observed, excluding environmental stress effects and indicating that the UPR was solely caused by PGA production. This application-inspired basic research suggests a novel approach to systematic bioprocess analysis, in which product formation kinetics and physiological cellular states are correlated.

P9

Searching for novel cold-active hydrolases from different psychrophilic and psychrotrophic microbial sources

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Organisms successfully coping with low temperatures in permanently cold areas are valuable sources of so-called cold-active enzymes. Due to many unique properties of these biocatalysts such as high catalytic efficiency at low temperatures associated with a high thermosensitivity, they can participate in various industry processes. Despite the fact that many of them are well recognized and widely used, searching for cold-active enzymes displaying new useful characteristics is still attractive. In this work we searched for selected cold-active hydrolases (protease, lipase/esterase, amylase, carboxymethylcellulase, α -glucosidase, β -glucosidase, β -galactosidase and α -fucosidase) in different microbial samples containing cold adapted microorganisms. For this purpose the six cold-active bacterial isolates originating from Antarctica, the six isolates of psychrophilic yeasts and the sediment sample from the cold lake located in Romania containing an undefined mixture of cold adapted microorganisms were used. The samples were cultivated on 1/2 LB medium agar plates with substrates for the selective detection of the enzymatic activity.

ties at 4 and 15 °C. Single strains collected from the individual cultivations of the sediment together with the positive Antarctic bacterial and yeast strains were then cultivated in the liquid medium in a large scale. Both grow mediums and cell lysates after lysis were tested to obtain temperature activity profiles of the selected extracellular or intracellular enzymes. For future experiments we will choose the strains producing the hydrolases with the best cold-active properties. Finally the genes coding the sequences of these biocatalysts will be searched in the genomes of their producers in order to synthesize the enzymes in recombinant forms.

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P10

Mesenchymal stem cells as a suitable source for cell therapy of male infertility: what are our findings and how far is the goal?

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In this presentation we are going to explain the results of our five in vitro and in vivo studies on using mesenchymal stem cells (MSCs) for generation of germ cells (GCs). At first, we observed that zinc ions could not induce differentiation into germline cells in bone marrow (BM) MSCs but they have a regulatory effect on the expression of some GC-specific genes. Next we found that the optimum concentration of retinoic acid (RA) for induction of differentiation into GCs was 10 μ M. In our next study we found that among three different morphogenes, TGFb1 (10 ng/ml) induced generation of adult germ-like cells from MSCs, while BMP4 and BMP8b caused expression of primordial GCs characteristics in treated MSCs, although the efficiency of GC generation in all groups was very low. Then, we treated ram BM-MSCs with RA and TGFb1 separately and transplanted the produced germ-like cells into the testes of ram lambs. Evaluation revealed that TGFb1 caused formation of GCs more efficiently than RA. Moreover, the percentage of TGFb1 treated

cells which located on the basement membrane of seminiferous tubules and expressed GC-specific marker-PGP9.5- was greater than that of RA treated cells, although the homed cells did not participate in host spermatogenesis. In another investigation, we found that mouse amniotic membrane MSCs can differentiate into GCs under the effects of BMP4+RA. And finally in our last study, we observed that although BM-MSCs survived in the testis at least for two month and also differentiated into adult GCs, the efficiency was very low and the produced germ-like cells did not rescued spermatogenesis in infertile recipient rats. From these results, and the results reported by other researchers, it could be concluded that although the results are hopeful so many detailed studies are still necessary to achieve to the final goal- treatment of infertility using stem cell therapy

P11

Screening for phosphate-solubilizing bacteria to promote growth of *Aegiceras corniculatum* (L.) Blanco seedlings for rehabilitation of degraded mangrove ecosystems

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This study evaluated the cell density and P-solubilizing characteristics of cultu-

rable PSB strains from the mangrove rhizosphere of Zhangjiang estuary. A modified strategy for screening of PSB strains based on various organic and inorganic phosphate forms (i.e. $\text{Ca}_3(\text{PO}_4)_2$, FePO_4 , AlPO_4 and lecithin) was applied in this study. However, a total of 37 inorganic phosphate solubilizing bacteria (IPSB) and 179 organic phosphate dissolving bacteria (OPSB) were isolated only in agar plates with $\text{Ca}_3(\text{PO}_4)_2$ and lecithin, respectively. In liquid culture all isolated IPSB strains displayed no P-solubilizing ability with AlPO_4 and showed a very low P-solubilizing activity with FePO_4 . And medium acidification and organic acids release were associated with the two principal mechanisms for P-solubilization with $\text{Ca}_3(\text{PO}_4)_2$. A collection of five efficient IPSB bacteria (*Gallaecimonas* sp., *Mangrovibacter* sp. and three strains of *Bacillus* sp.) were investigated in a short-term pot experiment for their biological effects on growth parameters, anatomical structure, nutrient uptake, leaf photosynthetic capacity and soil biochemical parameters of *A. corniculatum* seedlings. Additionally, their performance in rhizosphere microbial activities were also tested. Results indicated that the application of IPSB strains, especially IPSB28 strain, was really effective in increasing plant growth promotion. The positive growth response with IPSB strains might be attributed to the synergic combination of P-solubilizing ability and plant growth regulators production (indole-3-acetic acid (IAA) and siderophores). Therefore, our results suggested that the efficient IPSB28 strain (*Mangrovibacter* sp.) which was first reported as PSB with PGP attributes had great potential for-

mangrove rehabilitation and reforestation.

P12

Thermophilic biofiltration of complex VOC mixture in bubble column reactor

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Although thermophilic biofiltration is thought to have potential technological advantages over the mesophilic process for hot waste air treatment, little has been reported on the thermophilic biofiltration of complex hydrophilic/hydrophobic VOC mixtures using a bubble column reactor. Therefore, we investigated the biodegradation of a complex VOC mixture in a reactor inoculated with a mixed thermophilic culture at 55 °C. The mixture, based on the compostation waste air composition, contained acetone, dimethyl sulfide, ethyl acetate, propionic acid, triethylamine and α -pinene. Initial experiments focused on the effect of elevated temperature and air flow rate on the oxygen mass transfer from polluted air to liquid media. While the oxygen solubility in the media decreased with rising temperature, volumetric mass transfer coefficient increased. Within the range of tested air flow rates the overall oxygen mass transfer was not affected by the temperature change. Subsequently the reactor was inoculated with

mixed thermophilic culture, after successful start-up period the organic load of the pollutant mixture ranged from 61.5 to 292.3 g*m⁻³*h⁻¹. During reactor operation, only propionic acid and ethyl acetate were degraded completely, but the degradation of the other components was limited. We show that this limitation was not related to the dissolved oxygen concentration, and was probably caused by the mutual inhibitory relationships between the individual compounds. Our results suggest that a thermophilic bubble column reactor has some potential for use in the treatment of waste air with this composition.

P13

Domain-based understanding of structural factors for enzyme activity and stability

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An enzyme with improved activity and stability is required for enzymatic processes to be economically feasible. Enhancement of activity is in many cases resulted in the decrease of stability and increase of stability also resulted in the decrease of activity. In this study, structural factors affecting enzyme activity and stability were investigated based on domain analysis. In selecting mutant sites for the improvement of enzymes, strengthening specific interactions or flexibility in the specific domain can be an efficient approach instead of considering all the amino acid residues in the

enzyme. As the results, strategies to improve the activity through flexibility modulation, improvement of stability by molecular modification in specific domains were suggested. Since this approach is based on the understanding of enzyme structure and function, it will become easier to understand the enzyme's function and to design the enzyme for improved activity and stability. The details will be presented and discussed.

P14

Production and Some properties of a new thermostable xylanase from *Bacillus oceanisediminis* SJ3 isolated in Algerian soil

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A new *Bacillus oceanisediminis* SJ3 strain which produces thermostable xylanases was isolated from Algerian soil, it has been selected as a promising strain for xylanase production. The activity was

20.24 U/ml after 2 days in the presence of oat spelt xylan. The xylanase activity was characterized in terms of temperature and pH profiles and thermostability and effect of the metal ions and solvents. The results indicated that the enzyme was optimally active at pH 7.0 and was stable over a broad pH range of 5.0-10.0. The optimum temperature for xylanase activity was 55°C. At this temperature, the half life was of 6 h and the enzyme retained 50 % of its activity after its incubation for 2 h at 95°C. These properties qualify the enzyme to be highly thermostable and potentially important for application in some industrial processes. The enzyme resist to SDS and β -mercaptoethanol, while all the ions tested do not affect the enzyme activity. This latter is, at least, stable in all organic solvents tested except in propanol where a reduction of 46.5 % was observed, the stability of the xylanase was higher in hydrophobic solvents where a maximum stability was observed with cyclohexane

P15

Simultaneous desizing and scouring of cotton fabric using amylase and pectinase from *Aspergillus aculeatus* by single batch production: laboratory to pilot scale testing

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Development of eco-friendly enzymatic textile processing has received increasing attention as a way to reduce uses of chemical, energy, and waste treatment. In this study, a combined one-step desizing and scouring of cotton fabric was developed using a dual activity enzyme from solid state fermentation of *Aspergillus aculeatus* BCC17849. According to optimization by Plackett-Burman method, the optimized fermentation media contained cassava pulp and defatted soybean meal and mineral solution with cultivation time at 30°C for 5 days, which led to maximum amylase and pectinase activities of 23.33 and 76.46 IU/g, respectively. The crude enzyme was further concentrated 10- to 15-fold by

ultrafiltration prior to use in cotton preparing process. Application of the concentrated crude enzyme to one-step desizing and scouring of cotton fabrics under pH 5.5 and 55°C for 1 h led to efficient starch and wax removal with Tegawa scale of 6-7 and water absorbency of \leq 30 s, which were in the same range to those obtained in sequential process using respective commercial enzymes. Pilot-scale trial of the enzyme using cold patch batch and exhaust processes showed similar desizing and scouring efficiencies to the lab-scale testing. The work demonstrates the potential of the combined enzymatic desizing and scouring process for eco-friendly textile industry.

P16

Steam-blanching and drying effect on celery leaves bioactive compounds

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Celery leaves are commonly used in foods as additive- a good source of carbohydrates and contain high amount of compounds with antioxidant and antimicrobial properties. Using various thermal and non-thermal processing methods, chemical composition may change in foods. The aim of this study was to analyse the effect of steam-blanching, convective and microwave-vacuum (MV) drying on colour changes, bioactive compounds (total carotenoids, total phenolics, individual phe-

nolic composition and organic acids) and their antiradical scavenging activity. Integrated evaluation was used to select the most suitable drying method for maintaining nutritional value and biological active compounds of celery leaves. Fresh celeries were steam-blanching, convective drying was performed at $45\pm 1^\circ\text{C}$; MV drying at $35\pm 1^\circ\text{C}$; and steam-blanching (1.5 and 3.0 min) followed by MV drying. Total carotenoids and total phenolics (phenolics, phenolic acids, flavonoids, flavonols) were detected in high amounts in celeries which increased significantly after processing; the highest increases were observed in celeries after steam-blanching followed by MV drying. 4-hydroxybenzoic, sinapic and 2-hydroxycinnamic acids, rutin and quercetin, oxalic, malonic, citric and ascorbic acid were detected in the highest amounts in celery leaves. Previously mentioned compounds changed differently depending on the processing method used. Ascorbic acid decreased with processing, but lowest decreases were observed for MV dried samples. Processing influenced the extractability of the investigated compounds due to cell damaging thus explaining increases in total carotenoids and phenolic compounds; also the formation of cis- trans- isomers in carotenoids that could lead to increased total amount. It was concluded that most suitable drying method for maintenance of nutritional value and highest biological active compounds content could be steam-blanching followed by MV drying. Research was supported by AgroBioRes 2014-2017.

Keywords: celeries, phenolics, organic acids, carotenoids, processing

P17

Effects of low direct electric current on aerobic biological treatment of urban wastewater

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Background and aim: Wastewater is one of the principal source of organic and inorganic contamination of the environment. It is because of its complex composition (proteins, carbohydrates, oils, urea and synthetic organic compounds). Application of electric current in wastewater treatment processes has become, nowadays, a phenomenon of increasing importance. In this work, we proposed to study, in vitro, the effect of low electric current treatment on the performances of the microorganisms present in activated sludge obtained from the aeration basin of a wastewater treatment plant located in Tizi-Ouzou (Algeria).

Methods: The trials were carried out in an experimental setup by varying two parameters: the distance between electrodes ($d = 2\text{cm}$, $d = 6\text{cm}$) and the density of the electric current ($D = 1.4\text{ mA/cm}^2$, $D = 3.8\text{ mA/cm}^2$, $D = 7\text{ mA/cm}^2$). Samples were removed at regular time intervals (2h) and were subjected to the following measurements: electrical conductivity, mixed liquor suspended solids, turbidity, and dominant

microbial flora study.

Results: The results obtained showed a significant improvement in the abatement rate of the two following parameters: turbidity and suspended matters, demonstrating the effectiveness of the approach used in this study. Regarding the results inherent to the bacteriological parameters, it was apparent that electric current affected the composition of the microbial flora of the activated sludge used. Indeed, with the exception of sulfite-reducing clostridia count, it was observed that the numbers of the whole germs under consideration, significantly decreased as a result of direct electric current application.

Conclusion: This treatment method might, in the future, lead the way towards profitable application in wastewater treatment plants as an alternative disinfection technique. Besides, it can be useful to optimize and model the impact of electricity using response surface methodology (RSM).

Keywords: Activated sludge, electric current, pathogens, stainless steel plate, wastewater

P18

Lipid profile of yeast growing on n-alkanes

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Yeast have a rather unique ability to utilize n-alkanes, which are major environmental pollutants. Alkanes can alter the lipid composition of yeast, which could thus

produce nutritionally important fatty acids. We tested the ability of three yeast strains (*Candida krusei* DBM 2136; *Trichosporon cutaneum* CCY 30-5-10; *Yarrowia lipolytica* CCY 30-26-36) to utilize various concentrations of n-alkanes (C15 and C17) as a carbon source. The identification and quantification of fatty acids was performed using GC-MS FAME. The yeast strains were grown in modified YPD or TCM media with or without the addition of natural biosurfactants, rhamnolipids. All three yeast strains were able to utilize n-alkanes as a sole source of carbon and energy. The addition of rhamnolipids increased the amount of biomass except for *Yarrowia lipolytica* since this strain is known to produce its own biosurfactants. However, the addition of rhamnolipids triggered an increased production odd-chain fatty acids in all tested yeast strains.

P19

An Optimized Yield of Streptazolin Produced by the River Sediment Derived Strain *Streptomyces* Sp. SRC3

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Efficiency of currently used antibiotics is worldwide decreasing, due to resistance phenomenon and because people are faced with new and emerged pathogens.

Most active natural products are isolated from Actinobacteria which are a prolific source of diverse classes of secondary metabolites showing a series of promising bioactivities.

The main aims of this study were i) the exploration of Algerian river sediments as a source of actinobacteria producing bioactive metabolites, ii) the isolation and structural identification of the molecules responsible for the bioactivity, iii) the optimization of culture conditions for the production of these bioactive metabolites.

In detail, 39 different actinobacteria strains were isolated and subjected to antagonistic activity test against human pathogenic germs.

The most active antibiotic producer SRC3 (active against *Salmonella Typhi* ATCC14028, *Vibrio cholerae* ATCC14035, MRSA ATCC 43300 and *Candida albicans* ATCC10231) was selected and identified as *Streptomyces sp.*

A bioassay-directed fractionation of its ethyl acetate crude extract provided one major compound, identified as the known antibiotic and antifungal streptazolin by extensive 1D and 2D NMR studies and mass spectrometric analysis. Furthermore, the MIC value of the pure compound was also determined.

In order to increase the low natural concentration of streptazolin in SRC3

strain, a culture media optimization was carried out by RSM statistical strategy and Plackett-Burman design to screen the media components affecting the antimicrobial production. This statistical strategy allowed to select the following optimal conditions to maximize the production of streptazolin: KCl (0.051 %), MgSO₄ · 7H₂O (0.05 %) and 5 day- incubation for *C. albicans* pathogenic germ.

In conclusion, this optimized production of streptazolin by SRC3 strain, coupled to the easy procedure of its purification from the crude extract, is competitive with the reported availability by a chiral synthesis involving more than 10 reaction steps and a relatively low global yield.

Keywords: River sediments, Streptazolin, Response surface methodology

P20

Continuous isolation of mannose using SMB unit

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Guar gum, obtained from endosperm of plant *Cyamopsis tetragonoloba*, is one of the material rich in galactomannans. According to the type of hydrolysis, guar gum can provide a set of monosaccharides and other higher saccharides^[1]. These saccharides can be isolated from hydrolyzed solution using different separations methods. In

rule, the first separation step is membrane filtration aimed to remove higher saccharides. If necessary, the second step is desalination of the solution containing lower saccharides using membrane separation^[2], or electro dialysis. The final step for isolating of monosaccharides is chromatography. Monosaccharides can be chromatographically separated using a variety sorbents and methods^[3]. Especially, mannose has wide area of applications^[4]. The products from batch chromatography are almost diluted, so is better to use one of the continuous chromatographic method, best with hybrid connection with the nanofiltration for concentrating the injecting material before injection or any other flow during the separation.

The objective of this work was to study continuous separation of the guar gum hydrolysates in SMB unit^[5] in consequence of previous work^[6]. Obtained guar gum hydrolysates were rich in galactose and mannose content and there were tried methods for isolation them using chromatography separation on catex and anex sorbents. At the beginning it was necessary to identify selected chromatographic separation model using measured data from pulse experiments. Identified parameters for each experiment were passed for the simulation of continuous mode of separation with initial operational parameters up to steady state. Using script for regulator based on fuzzy logic the parameters were tuned to get optimized operational parameters for maximum mannose purity in extract stream. All calculations were done in Matlab® software environment using newly developed scripts and algorithms, which allows the processing of the chromatograms and si-

mulating with regulation of the separation for the equilibrium dispersive model with linear or extended Langmuir isotherm.

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P21

Selecting an DNA motif that enhances the thermal stability of reverse transcriptase

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Due to its role in techniques such as RT-PCR, reverse transcription is used by scientists in a wide range of disciplines. However, highly structured RNA templates are not efficiently transcribed into cDNA in some cases, which can lead to imprecisions in the readout of data. This limitation is related to the low reaction temperature during reverse transcription (typically 50-60°C), which is not always sufficient to denature stable RNA structures.

Here, we used *in vitro* selection to isolate DNA motifs that, when fused to a regular primer, enabled reverse transcription to occur at temperatures $\geq 70^{\circ}\text{C}$. These results suggest that our system will facilitate reverse transcription of difficult RNA templates with minimal additional cost and effort.

P22

Interactions between non-functionalized nanofiber fabrics and bacteria *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*

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Various skin injuries, including both acute ones caused by traumas (burns, stab wounds) and especially chronic ones (many types of ulcers), represent a common global clinical issue and it is generally known that the regeneration process can be strongly influenced by the wound dressing material.

In recent years, there has been increasing concern about nonwoven nanofiber membranes mainly produced by electrospinning approach. The main advantages of such fabrics are high surface area to volume ratio, very high porosity and

possibility to be synthesised from various biocompatible and biodegradable polymers.

Apart from facilitation of a healing process by appropriate air permeability, moisture control etc., another desirable property of such dressing material could be protection against microbial contamination and possible secondary infection. This can be achieved by incorporation of numerous antimicrobial agents or simply functioning as a physical barrier.

Our study was focused on interaction between selected bacteria (*Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*) and pure unmodified nanofiber membrane electrospun from solution of 16 wt% polycaprolactone (PCL). Chosen bacteria differed in size, cell shape, cell wall composition and ability to move. Polycaprolactone is biodegradable and biocompatible polyester and series of experiments were performed to assess its penetrability for above mentioned bacteria and its possible ability to inhibit their growth.

P23

Influence of chitosan on biofilm formation of *Aspergillus fumigatus*

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The ability of microorganisms to colonize medical devices and human tissues, to change their phenotype into biofilm structures, to cause serious complications in the healthcare and even fatal diseases, and especially their ability to develop resistance against the usual used drugs is in the forefront of interest of many research groups around the whole world. Nowadays there exists much information about the bacterial biofilms, less about the biofilm of yeasts and the least about the biofilm of filamentous fungi. Among the pathogenic filamentous fungi with ability to form biofilm is included *Aspergillus fumigatus* which causes e.g. aspergillom, invasive aspergillosis or allergic bronchopulmonary aspergillosis.

This study was focused on the effectivity of natural polysaccharide chitosan to inhibit biofilm formation of *Aspergillus fumigatus* DBM 4057 and compare it with the effectivity of antibiotic amphotericin B. The compared factors were metabolic activity of biofilm cells (XTT assay) and total biofilm biomass (crystal violet staining) with or without the presence of anti-biofilm substances. The selected samples

were also visualised by automatic inverse microscope Cellavista and spinning disc confocal microscope. We found out that both anti-biofilm substances are able to inhibit the biofilm formation of *A. fumigatus* during 24 and 48 hours of biofilm formation but chitosan is more effective in inhibition after 72 hours. The metabolic activity of biofilm cells and also total biofilm biomass decrease by 80 % in the presence of 25 µg/ml of chitosan after 72 hours of *A. fumigatus* biofilm formation.

P24

Rice straw biorefinery: feasibility and challenges in Iran

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Rice straw is known as one of the major and abundant lignocellulosic wastes with annual production of about 731 million tons in the world. The annual production of rice straw in Iran alone is about 6 million tons per year out of which more than 50% remains unprocessed and is subjected to open burning in rice fields. The biorefinery approach for the production of different valuable bio-based products is gaining more and more attraction in recent

years. The present paper discuss the feasibility and challenges of rice straw based biorefinery in Iran. The review focuses the availability and the potential of this residue for the production of biofuels (bioethanol, biogas, biobutanol and microbial fuels cells), food additives (e.g. xylitol), biocompost and vermicompost, biochar, nanocellulosic fibers and crystals and nanosilica in Iran. For instance, our recent results in development of a robust and economic biorefinery process for continuous co-production of ethanol and xylitol from rice straw in a membrane bioreactor will be discussed. The process included pretreatment, enzymatic hydrolysis, detoxification, yeast strains selection, and continuous co-fermentation of *Saccharomyces cerevisiae* and *Candida tropicalis* in a membrane bioreactor. In addition, our economic process for fast production of enriched biocompost from rice straw using bioprocess engineering and biotechnological approaches will be presented.

P25

The new perspective on ABE fermentation by microbial consortia to extend substrate utilization and fermentation environment

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The production of ABE (acetone-butanol-ethanol) solvents under anaerobic condition is restricted to solventogenic clostridia, such as *Clostridium acetobutylicum*, *Clostridium beijerinckii* and *Clostridium pasteurianum*. By now, mono-cultures of these bacteria continue to dominate the landscape of ABE fermentation. However, all of the known solventogenic clostridia were strict anaerobes, which must be cultured under anaerobic conditions with sugars or starch as substrates, and no single strain has been isolated or genetically engineered to produce butanol effectively from lignocellulose. The potential for synergistic utilization of the metabolic pathways of all involved strains in microbial consortia have come to appreciate in recent researches. This article highlights some examples of natural or engineered microbial consortia with the ability to use lignocellulose as substrate directly or produce solvents under aerobic conditions. By co-cultured solventogenic clostridia and cellulose-decomposing microorganisms (e.g. *Clostridium cele-vecrescens*, *Clostridium thermocellum* and *Clostridium cellulovorans*)^[1], the consortia could degraded cellulose to sugars and then shifted to solvents. The microbial consortia consisted of anaerobic clostridia and aerobic microorganisms (e.g. *Bacillus subtilis*, *Bacillus cereus*) were able to grow and produce solvents under aerobic or micro-aerobic conditions^[2]. In our research, a symbiotic system TSH06 including *Clostridium acetobutylicum* TSH1 and *Bacillus cereus* TSH2 was isolated to be capable of producing butanol under

micro-aerobic conditions, and 11.2 g/L butanol in a 5 L bioreactor even with continuous 0.15 L/min air sparging was obtained, and high ratio of *Clostridium* and low ratio of *Bacillus* in TSH06 was spontaneously formed during the fermentation^[3]. These results showed that different strains in microbial consortia cooperatively provided a new perspective on ABE fermentation to extend substrate utilization and a promising feasibility to produce solvents under aerobic conditions.

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P26

***Caldicoprobacter* species, isolated from an Algerian hot springs, producing new thermostable enzymes (xylanase, protease and keratinase)**

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New phylogenetic thermophilic lineages of unknown species were anaerobically isolated and determined through molecular techniques and the choice of the 16S RNA as scalable scoring from terrestrial hydrothermal vents. These prokaryotes have very different physiological and metabolic characteristics and are very attractive as source of new thermostable enzymes. Thanks to their physico-chemical characteristics that make them particularly interesting tools for many applications, their use has led to the development of high value products, used in various industries. Currently, thermostable proteases, xylanases and keratinases form the enzyme group the most sought, due to their advantages. Our work allowed us to isolate and characterize under anaerobic conditions at 70 °C, new bacterial species belonging to the genus *Caldicoprobacter* (*Clostridiales*) from the Hammam D' Bagh hot spring (Algeria). Xylanase activities of (250U/ml) and keratinase (21000U/ml) in *Caldicoprobacter algeriensis*; and Protease (23,000 U/mL) in *Caldicoprobacter guelmensis* are found between 70 and 90 °C. The results of the characterization of three enzymes are very promising and could lead to new bio-

technological applications.

Keywords: Caldicoprobacter, Anaerobiosis, Hot spring, Enzymes.

P28

Microbial synthesis of silver nanoparticles by microorganisms

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Nowadays intensive search for new approaches to synthesize nanoparticles is stipulated by their unique physical characteristics and wide spectrum of their possible application. Microorganisms as potential sources for receipt of nanoparticles possess a number of advantages: directed growth of their biomass, receipt of nanoparticles with controlled characteristics, environment safe technology and so on. Nanoparticles obtained by physical and chemical methods tend to faster aggregation.

Development of safe methods of synthesis of silver nanoparticles is of special importance. Silver nanoparticles possess antimicrobial activity towards a number of bacteria resistant to antibiotics and possess antifungal activity as well.

In these regards, we studied possibility of synthesis of silver nanoparticles by different microorganisms isolated from soils polluted with heavy metals and from active silt of water treatment facilities. These

microorganisms possess high resistance to the action of pollutants and metals.

Solutions of argentic nitrate containing from 10 to 1000 mg/l of Ag⁺ were used in this study. Mixture of cells and silver ions were incubated on rotwry shaker (180 rpm; 280 °C) from 1 to 72 h.

Five microbial cultures (2 *Bacillus*, 2 *Pseudomonas* and one *Arthrobacter* strains) resting silver ions with production of metallic particles were studied. The selection of these strain was stipulated by their ability to survive conditions of high concentration of silver on account of production of protective polysaccharide capsules. It was established that out of five studied cultures two strains (*Bacillus sp.* 1 and *Pseudomonas sp.* 2) expressed ability to form nanoparticles. Optimum conditions for biosynthesis were determined: concentration of Ag⁺ 100 mg/l, period of cells contact with silver ions 48 h. In these conditions, yield of silver nanoparticles by these cultures was 21 and 29%, respectively.

MW2

miwelt: where information comes from?

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A comic story illustrates the principle of an investigative dialogue and demon-

strates how information can be obtained from different sources, while highlighting the importance of asking questions.

miwelt is a science communication project initiated under the Agora scheme of the Swiss National Science Foundation (SNSF). Scientists, artists and journalists have jointly developed illustrated materials, thematic excursions, and laboratory experiments on the subject of microbial biotechnology for children from the age of 7 to 11.

As part of the miwelt project, scientists have been encouraged to explain both the content of and methods used in their everyday work to children and their families as well as to teachers who have only a basic grounding in science. For this purpose, they are aided by illustrations and analogies with everyday life, produced by a professional illustrator and journalists engaged in dialogue with the scientists.

For more information visit:
www.miwelt.net

P29

Effect of point mutations in the nuclear localization signal of visfatin on the cancer cell proliferation

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Visfatin is an enzyme and a secreted factor affecting metabolism and immunity. Visfatin catalyzes the rate-limiting step in NAD biosynthesis in the cell. Therefore, it seems to be a promising target for cancer therapy. However, inhibitors of the enzymatic activity failed in clinical trials. Our previous work has shown that visfatin is located in the nucleus of cells and its nuclear localization signal was found. In the nucleus, NAD is used for many regulatory processes. Therefore, the inhibition of nuclear import of visfatin could be a new and effective approach in cancer treatment.

We prepared cancer cell lines HepG2 with the production of wild type visfatin, and visfatin with point mutation in nuclear localization signal. Cytosolic localization of visfatin with point mutations was verified by immunofluorescence staining. Generation time of the cells was determined by their counting at various time intervals. We found that increased production of wild type visfatin accelerated the proliferation of cancer cells HepG2. Mutations in the nuclear localization signal of visfatin canceled this effect. It was demonstrated that the slowing of cell growth did not cause changes in the catalytic activity of visfatin. Our results show that point mutations in the nuclear localization signal of visfatin reduced the growth rate of cancer cells. For this reason, point mutations of visfatin nuclear localization signal might be used in cancer treatment.

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P30

Screening and study of extremophilic microorganisms resistant to pesticides

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The Southern Aral Sea region is characterized by extreme natural conditions, which were intensified by the sea drying resulted a number of irreversible changes in soil microbial communities. The common feature of these soil is high salinization, extreme instability of humidity degree, high content of different xenobiotics. Soil-borne microorganisms are one of main factors influencing degradation of xenobiotics in natural ecosystems and are natural bioindicators reflecting changes in soil. Microbial populations may contain strains capable to active degradation of pesticides, which may be applicable for remediation of polluted soils.

In these regards, a study of microbial biota of several pesticide-polluted sites in the Southern Aral Sea region was conducted. Thirty soil samples were selected from this region and studied. It was determined that microbiota of studied soils is diverse, but presence of pesticides impacts microbiological processes undergoing in soil, especially it heavily affects process of nitrification (no nitrifying bacteria were isolated) and a bit less process of ammonification.

This tendency on suppression of indicated groups of microorganisms is characteristic for all studies sites. Detailed analysis of microbial community reveals that organizing role in microbial cenoses belongs to *Bacillus* and *Pseudomonas* bacteria. Filamentous fungi from *Aspergillus*, *Penicillium*, *Cladosporium* genera were isolated as well. The susceptibility of isolated microorganisms to different concentrations of studied pesticides complex (chlorpyrifos+cypermethrin) was determined. It was established that the mixture did not suppress growth of some isolated strains of *Bacillus* and *Pseudomonas*. Majority of isolated bacterial strains were susceptible to small concentrations of pesticides, whereas strains *Bacillus* sp. 1 and sp. 2 *Pseudomonas* revealed considerable resistance to pesticides. It allows to suggest that these strains possess potential to pesticides degradation. Introduction of these microorganisms back to soil may result in remediation of pesticide-polluted soil.

P31

Evaluation of the bile acid-binding capacity of *Rhodella violacea* microalgae

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Hypercholesterolemia is defined as high blood cholesterol level. It is a major risk factor for coronary artery disease. In

the liver, the hepatic microsomal cholesterol 7 α -hydroxylase transform the cholesterol into cholic acid which act as biological surfactants aiding food digestion and absorption of lipids in the gastrointestinal tract.

Bile acid sequestrants have received increasing attention as therapeutic agents for the treatment of hypercholesterolemia. These materials are usually cationic hydrogels, like cholestyramine, that selectively bind and remove bile acid molecules from the gastrointestinal tract, decreasing plasma cholesterol levels. But the drugs may cause serious side effects for some patients. In fact, relatively large amounts of cholestyramin are required to relieve the symptom, which leads to constipation, flatulence, and abdominal pain. Recent data suggest a possible link of cholestyramin to colon cancer in human.

In the present study, it is proposed to evaluate the bile acid binding capacities of the microalgae *Rhodella violacea*.

For that, the cholic acid-binding capacity of the algal samples was determined by measuring the cholic acid-retarding capacity against a dialysis membrane. The bile acid-binding capacity of *Rhodella violacea* was represented by the holding amount of cholic acid inside dialysis sack in this study. The control groups were prepared without containing *Rhodella violacea* sample.

The results indicated that in the presence of *Rhodella violacea* extract, release of cholic acid was retarded by 31.65%, corresponding to 9.95 mmol/L of cholic acid in dialysate.

Rhodella violacea is an attractive target for selective hypocholesterimiant drug

development. In fact, *Rhodella violacea* extract showed a great and specific bile acid retention capacity. We propose the use of the microalgae *Rhodella violacea* as a nutraceutical to lower cholesterol better than medication.

P32

Detection of Crimean-Congo Hemorrhagic Fever Virus CCHFV-Specific IgG Antibodies using Enzyme-Linked Immunosorbent Assay ELISA in Goats, Albania

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Crimean-Congo hemorrhagic fever virus (CCHFV) is a tick-borne virus, belonging to the genus *Nairovirus* of the family *Bunyaviridae*. In certain parts of the world, CCHF constitutes an emerging infectious disease with a severe clinical picture and a marked fatality rate. The case fatality rate (CFR) of the disease ranges between 3% and 30% and usually has a direct relationship with the quality of medical care and

availability of prepotent transfusion therapy. Crimean Congo hemorrhagic fever infection is endemic to Africa, the Balkans, the Middle East, and parts of Asia. The aim of the present study was to reveal the presence of CCHFV among goats in different districts of Albania. This survey was carried out in 2013 and was conducted in a total of 128 randomly selected goats from five localities in Albania respectively: 9 goats in Kolonje-Erseke, 15 goats in Pogradec-Tushemisht, 26 goats in Burrel-Baz, 10 goats in Lezhe-Torovice, and 68 goats in Lezhe-Mamurras. The sera were kept in the Faculty of Veterinary Medicine, Agricultural University of Tirana, at -20°C until analysis. The exposure status to CCHF was determined using enzyme-linked immunosorbent assay (ELISA) for detection of CCHFV-specific IgG antibodies in goats serum samples at Friedrich-Loeffler-Institute (FLI), Greifswald Germany. The seroprevalence of CCHF infection identified in this study was respectively 25% giving evidence for an active circulation of this virus in the country. Further epidemiological studies and improved surveillance are urgently needed to prevent a possible outbreak of CCHF among humans in Albania.

Keywords: Crimean-Congo hemorrhagic fever virus, *Nairovirus*, *Bunyaviridae*, Goats, Indirect ELISA

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P33

Effects of Selenium Supplementation in Wistar and Spontaneously Hypertensive Rats

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Selenium, an essential trace mineral for animals and humans, acts as an antioxidant and is incorporated into many vital proteins. Because antioxidants may prevent or mitigate essential hypertension (HTN), a major risk factor for cardiovascular disease, selenium supplementation at a balanced dose may be an appropriate supportive treatment. However, because selenium is toxic in higher amounts, the correct dosage is crucial. To determine whether a healthy organism responds differently than one suffering from high blood pressure, we measured the effects of selenium supplementation in spontaneously hypertensive rats (SHRs), a common HTN model. The effects were monitored at the enzymatic level and compared with normotensive Wistar rats. Four diets were tested, each of which contained inorganic selenium in a low or high dose, with or without the addition of other antioxidants. The diets were fed to the laboratory rats for 60 days before samples of blood and tissue were collected. The activities of antioxidant enzymes were determined spectrophotomet-

rically, with significant differences found among the groups. Moreover, whereas the Wistar rats showed no visible health problems, some SHR rats from the high-selenium groups died before 60 days and even the surviving animals had internal organ damage. These results suggest that the dose for a hypertensive organism should be even more carefully chosen and probably lower than for a healthy one.

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P34

In vitro determination of active secretion of visfatin by macrophages, adipocytes and hepatocytes

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Visfatin is a protein with multiple functions. It operates in NAD biosynthetic pathway and as a secreted factor, it affects the immune system and cell metabolism. Its upregulation is also associated with certain types of cancer. Visfatin is actively secreted, probably by one of the non-classical secretory pathways, or passively released during cell death. However, the exact mechanism of the secretion remains unknown. Our aim was to verify the

active secretion of visfatin in cell lines of adipocytes, cancer cell lines of hepatocytes and immune cells.

Following model cell lines were chosen: U-937, THP-1 and HL-60 (monocytes and macrophages), HepG2 (hepatocytes) and 3T3-L1 (preadipocytes and adipocytes). The quantification of visfatin in cell lysates and in media was performed by immunoblotting. Visfatin amount was based to the amount of cytosolic GAPDH protein to eliminate visfatin passive release due to cell death.

Visfatin was not detected in the media of preadipocytes 3T3-L1 but it was determined in the media of differentiated 3T3-L1 adipocytes and HepG2 hepatocytes, together with GAPDH. The ratios of the determined proteins in the media and cell lysates were similar, suggesting that these cells passively released visfatin due to cell death. Visfatin level was significantly increased only in the medium of immune cells (U-937, THP-1, HL-60) The highest increase was found in the line of U-937 macrophages.

In this study, the active secretion of visfatin was confirmed only for the cells of the immune system. Due to the presence of visfatin in the blood serum, it is possible to assume that visfatin plays an important role in the development of type 2 diabetes and other diseases (cancer, cardiovascular diseases). Our results provide a new perspective on the complex role of macrophages in the pathogenesis of these diseases.

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P35

The combined effect of pressure and temperature on the kinetics of lactic acid fermentation in yogurt production

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Yogurt has gained popularity over the last years, mainly due to its high nutritional value and health benefits. High pressure (HP) is a novel technology with several applications in different fields, including biotechnology. One of these emerging HP applications is the performance of fermentation under sub-lethal pressures, which has already been reported to influence the fermentative process and/or the final product characteristics^[1,3].

The combined application of HP at sub-lethal pressures and temperature to lactic acid fermentation involved in yogurt production was studied in this work. For that, kinetics of lactic acid fermentation was performed under different conditions of pressure (10, 30, 50 and 100 MPa) and temperature (43 °C, 35 °C and room temperature (≈25 °C)), and the product formation and the substrate consumption were monitored over fermentation time. Fermen-

tation at atmospheric pressure (0.1 MPa) and 43 °C was used as control.

At 43 °C, a gradual inhibition of fermentation was observed as pressure increased, until no fermentation was achieved at 100 MPa. However, despite the decrease in fermentation rate with pressure increase, a pH characteristic of yogurt (≈4.6) was obtained at both 10 and 30 MPa by extension of the fermentation time. On the other hand, the reduction of fermentation temperature down to room temperature further decreased the fermentation rate. Nevertheless, a fermentation profile similar to control samples was observed during fermentation at 10 MPa and a final product with pH 4.6 was also obtained. In addition, activation volumes and activation energies were estimated, varying from 21.8-100.5 cm³ mol⁻¹ and 94.0-156.1 kJ mol⁻¹, respectively.

Therefore, lactic acid bacteria can withstand sub-lethal pressures and changes in the metabolic activities may have been triggered in response to these stressful conditions, which in turn may cause differences in characteristics of the yogurt produced under pressure.

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P36

Biomethane from biogas or waste carbon dioxide using hydrogen and hydrogenotrophic methanogens

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Upgrading biogas to biomethane enables complete utilization of energy directly in place where it is needed by using grid for natural gas. Biogas contains energetically usable methane and unavoidable content of carbon dioxide that is commonly removed by different physico-chemical methods. Another approach can be chemical conversion of CO₂ to methane, but with disadvantage in demand of high pressure and temperature. Biological transformation is a process, which takes place under much milder conditions. Hydrogenotrophic methanogenic bacteria are able to catalyze biological conversion of CO₂ and hydrogen to biomethane at temperatures of 35 or 55 °C and pressure close to atmospheric. Bio-transformation of carbon dioxide from biogas or other resources to biomethane needs an addition of hydrogen. Recent rapid development of electricity production from renewable energy sources from wind and solar energy has caused an imbalance between supply and demand. The impossibility of electricity storage can be solved by the power-to-gas strategy representing the production of storable hydrogen by elect-

rolysis of water. The energy of hydrogen can be converted to the easily utilizable and transportable form by biological methanation of CO₂ to biomethane using the activity of hydrogenotrophic methanogens [1–4]. The main goal of our project is to find the optimal conditions of biomethanation with H₂ in terms of process parameters and device type. Experiments started with hybrid anaerobic reactors (upflow sludge bed reactor with packed bed in the upper part) fed with distillery slops as organic substrate and gaseous hydrogen was introduced to the bottom of reactor. The efficiency of hydrogen utilization was 80.3 % and the main problem in the process was low gas-liquid mass transfer of H₂. Therefore, the method of effective input of hydrogen into the system have to be optimized. Possibilities of implementation of this method of biogas upgrading directly into biogas plants are promising.

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P37

The use of plant materials for the protection of vegetable crops

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In recent years, antimicrobial properties of plant extracts have been reported increasingly more often in different parts of the world. It was demonstrated effectiveness of extracts based on different plant parts such as roots, leaves, seeds and flowers in inhibiting growth of fungi and bacteria causing plant diseases or as an insecticide.

The scope of our research work is to develop and test plant based bioproducts for agrochemical applications in organic systems. The main objective is the development new formulas composed of a mix of plant extracts. The research is built on a conventional methodology for extraction of biologically active compounds from plants, development of applicative aspects and evaluation of the effect of developed formulas, in laboratory conditions. The plant based bioproducts will be obtained by an original association and combination of plant extracts. The plant species taken into consideration are: *Humulus lupulus*, *Equisetum arvense* and *Urtica folium*. The research will focus on extracti-

ons performed by maceration and ultrasonication. The plant extracts will be enriched with a basic substance (as defined by EC regulation 1107/2009).

The crop-disease model couples targeted were taken into account from among the major fungal crop disease in vegetable crops (*Phytophthora infestans*, *Rhizoctonia solani*, *Pythium sp.*, *Botrytis cinerea* and *Fusarium sp.*).

Aiming the development of new plant protection bioproducts, this research will address to organic and low input farming systems highly depending at this moment on limited range of input products. Better knowledge of these preparations will help reduce the number and / or doses of plant protection treatments while maintaining production levels and ensuring adequate protection for crops.

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P38

Novel Aminoketooxime Ligand and its Cu(II) and Mn(II) Complexes: Synthesis, Characterization, Oxidoreductase Enzymatic Activities and Molecular Docking Studies

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Oximes are a large number of bioactive compounds with a broad range of activities. Their derivatives are of great interest owing to their possession of donor sites which increase their coordinating capability. Oximes and their coordination compounds are used extensively due to their biological activities as fungicides, bactericides, analgesic, anti-inflammatory, antioxidant, antitumor and insecticidal^[1,2]. Homo- and heteronuclear metal complexes derived from oxime-type ligands have been reported; the observed IC₅₀ values indicated that they are potential antioxidant^[3].

We synthesized a novel ligand, N,N'- (4-methyl-1,2-phenylene)bis(2-(biphenyl-4-yl)-N'-hydroxy-2-oxoacetimidamide) (H2L) with its Cu(II) and Mn(II) complexes. All compounds synthesized were also characterized by ¹H- and ¹³C-NMR, FT-IR, elemental analysis, ICP-OES, molar conductivity, magnetic susceptibility measurements and TG-DTG, XRD analysis. Synthesized complexes' enzyme-like acti-

vities were tested and molecular docking simulations were performed to illuminate the nature of ligand-protein interactions.

For enzyme-like activity studies, three enzymes from oxidoreductase family were chosen namely, catalase (EC 1.11.1.6), catecholase (catechol oxidase: EC 1.10.3.1) and phenoxazinone synthase (o-aminophenol oxidase: EC 1.10.3.4). Catalase activity was measured volumetrically by following the volume of O₂ formed by the disproportionation reaction of hydrogen peroxide while catecholase enzyme activity was measured spectrophotometrically by the following the increase in absorbance at 400 nm. which indicates the increase in the concentration of 3,5-di-*tert*-butylquinone formed by the oxidation reaction of 3,5-di-*tert*-butylcatechol. Likewise, phenoxazinone synthase activity was measured spectrophotometrically by following the increase in absorbance at 433 nm, which is a typical band for 2-aminophenoxazine-3-one (APX) formation by the oxidation reaction of 2-aminophenol (OAPH).

Selective COX-2 inhibitors are a type of non-steroidal anti-inflammatory drug (NSAID) that directly targets cyclooxygenase-2 (COX-2) an enzyme responsible for inflammation and pain. They also have the potential for use as chemoprophylactic and chemotherapeutic agents after the discovery of their anticancer related activities. COX-2 inhibition is often used as a parameter for being a potent anticancer agent in docking studies. We performed molecular docking studies to show the interaction between COX-2 and the synthesized ligand.

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P39

Influence of sheep manure on the cow dung anaerobic digestion under mesophilic conditions

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Animal wastes constitute a high proportion of agricultural activities and their utilization and recycling is important for economic and environmental aspects. Anaerobic digestion one of the most widely used processes for treating these wastes and represents an attractive method for treating organic wastes for biogas production as alternative energy sources.

In this study, we investigated the effect of sheep manure addition on the digestibility of cow dung under mesophilic

condition in order to determine the biogas potential. Microbial community analysis was performed using real-time qPCR. The results reveal that co-digestion ratio of 50:50 (cow dung and sheep manure) cannot enhance the biogas yield. Current research is underway to optimize the anaerobic co-digestion technology by investigating different process and microbiological parameters.

P40

Influence of poly- γ -glutamic acid on *Candida* drug resistance

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Poly- γ -glutamic acid (γ -PGA) is a biopolymer of glutamic acid, the component of the Japanese food Natto that is produced by *Bacillus subtilis* var. natto via soybean fermentation. γ -PGA is a biodegradable and non-hazardous material of biological origin that has many potential biotechnological and biomedical applications. γ -PGA nanoparticles containing amphotericin B were recently shown to have antifungal activity with reduced cytotoxicity [1].

Candida albicans is a pathogenic yeast-like fungus that causes exogenous and endogenous infections. *C. albicans* strains exhibit multidrug resistance to commonly used antifungal agents, which correlates with overexpression of Cdr and Mdr

efflux pumps that are located in the plasma membrane.

The objective of this study was to test the influence of PGA on *Candida* drug efflux pumps. The ability of four pathogenic *Candida* species (*C. albicans*, *C. glabrata*, *C. krusei*, and *C. parapsilosis*) to grow on γ -PGA (high or low molecular weight) as a sole carbon source was tested. The *Candida* species grew on both γ -PGAs, but at a slower rate than using glucose, especially *C. glabrata*. Growth on γ -PGA induced Mdr1 expression in *C. albicans*, as shown by microscopic observations of a GFP-tagged pump. This was further confirmed by identifying a higher resistance to fluconazole using phenotypic tests of *C. albicans* Mdr1p single strain (with CDR deletions). The level of H⁺-ATPase protein that is crucial for membrane potential and extracellular acidification seemed to be unchanged regardless of the growth conditions.

Research was funded by the Wrocław Centre of Biotechnology programme: The Leading National Research Centre (KNOW) for years 2014-2018. www.know.wroc.pl.

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P41

Is it possible to utilize commonly unused parts of medicinal plants?

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Nowadays the humankind tends to traditional plant drugs in all aspects of life from two main reasons. The first one is the loss of activity of commercially used drugs, the second one is based on actual lifestyle. Plant drugs are common components of cosmetics, teas or dietary supplements. Probably based on historical background we utilize only certain plant parts. In many cases there is not enough of required material or collecting damages plant itself. The possible solution we could find in unused plant parts (waste).

Our work is focused on analysis of biologically active compound content in different parts of medicinal plants such as marigold *Calendula officinalis* and Leuzea *carthamoides*. Used and unused plant parts were separated and extracted with 80% methanol in water. Methanolic extracts were then fractionated using nonpolar solvents and different pH conditions^[1]. Alternatively plant parts were extracted according to traditional medicine procedures – tinctures and infusions. Each extract was screened for presence of biologically active compounds and antimicrobial, antioxidant, anti-inflammatory, hemolytic and cytotoxic activities. Extracts exhibiting positive

results were than separated using HPLC and components were identified using mass spectrometry.

In study with flowers (used) and leaves (waste) of *Calendula officinalis* we found in both parts similar composition of biologically active compounds. Extracts from both parts exhibited antimicrobial activity, extract from leaves were more potent against fungi and inflammation.

With roots (used) and leaves (waste) of *Leuzea carthamoides* we found similar results as in study with marigold and the most active fraction from both parts comprised the same compounds only in different amount.

In conclusion we are able to tell, that waste plant material have interesting potential to be utilized in the same way like requested plant materials.

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P42

Development of a green sustainable technology for biotechnological production and extraction of biodegradable polymers – Polyhydroxyalkanoates (PHAs)

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An imprudent use of environmentally hazardous petrochemical-based plastics and limited availability of fossil fuels have provoked research interests towards production of biodegradable plastics – polyhydroxyalkanoate (PHAs). However, the industrial application of PHAs based products is primarily restricted by their high cost of production. Development of efficient downstream strategies, along with utilization of inexpensive renewable resources, will accelerate their commercialization. In this study, a process was developed for sustainable and cost-effective production of PHAs by *Cupriavidus necator* using crude glycerol obtained from biodiesel plant. Eventually, various extraction strategies were designed for efficient recovery of the PHAs using non-toxic environment friendly solvents viz. 1,2-propylene carbonate, ethyl acetate, butyl acetate and linear alkylbenzene sulfonic acid. The effect of biomass pre-treatments (pH and heat treatments), temperature (60-120°C), incubation time (10-120 min) and precipitation period was evaluated on polymer purity and recovery yields. Ethyl acetate and

1,2-propylene carbonate were observed to be efficient solvents for PHAs extraction from cells with a precipitation period of 24h. The highest recovery yield (90%) and purity (93%) were obtained with 1,2-propylene carbonate under the condition of 120°C temperature and 30 min incubation. 1,2-propylene carbonate can serve as an effective alternative to commonly used highly toxic halogenated solvents.

P43

Production of pectin enriched material by extraction of sugar beet pulp in subcritical water – Use of Doehlert design for optimizing the extraction conditions

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Pectin, a highly complex carbohydrate polymer, traditionally is obtained in the industrial scale by the extraction with hot mineral acid of the cell-wall of raw material, such as citrus peels and apple pomace, followed by alcohol precipitation. Growing demand for pectin as a functional ingredient in the food industry, textile

industry, pharmacy, medicine and cosmetic production, is an incentive to undertake research both on the extraction of pectin from the raw materials used yet commercially insufficient, and the development of new proposals to optimize the process of extraction of pectin^[1].

A new method for the pectin obtaining is proposed in this work. It consists of the hydrothermal extraction^[2] of pectin from the sugar beet pulp (SBP) in subcritical water^[3,4]. This study focuses on the effect of the extraction temperature (100-140 °C) and holding time (0-30 min) on the yield and composition of pectin enriched extraction product. The experimental design methodology (Doehlert matrix) was used for establishing a statistically significant extraction model as well as for optimizing the conditions for pectin enriched extract production.

The obtained model ($R^2=0.907$) predicts an optimal yield of pectin enriched product ($121.9 \text{ g kg}^{-1} \pm 0.5 \text{ g kg}^{-1}$) at a extraction temperature and a holding time of about 117.5 °C and 22 min. The hydrothermal extraction of pectin was accompanied by as well as a partial hydrolytic depolymerization of extracted pectin and solubilization and depolymerization of hemicellulose, leading to the production of the primary hydrothermolysis products (uronic acids, monosaccharides and acetic acid). These products were obtained with the highest yield ($78.4 \text{ g kg}^{-1} \pm 0.1 \text{ g kg}^{-1}$) at 120 °C and 30 min. An further increase of extraction parameters would lead to the subsequent undesired, decomposition transformations of primary products, leading to the production, among others, of carboxylic acids and furfurals, and their

partial carbonization and gasification.

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P44

Production of pectin enriched material by extraction of sugar beet pulp in subcritical water – Use of Doehlert design for optimizing the extraction conditions

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of the primary hydrothermolysis products (uronic acids, monosaccharides and acetic acid). These products were obtained with the highest yield ($78.4 \text{ g kg}^{-1} \pm 0.1 \text{ g kg}^{-1}$) at 120°C and 30 min. An further increase of extraction parameters would lead to the subsequent undesired, decomposition transformations of primary products, leading to the production, among others, of carboxylic acids and furfurals, and their partial carbonization and gasification.

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P45

Screening of Mucoromycota fungi for single cell oil production by high-throughput FTIR Spectroscopy

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Oleaginous filamentous fungi are important organisms for the commercial production of high-value omega-6 polyunsaturated fatty acids (PUFAs), such as gamma-linolenic acid (GLA, C18:3) and arachidonic acid (ARA, C20:4)^[1]. Furthermore, they have been suggested as an alternative source of lipids for biodiesel production because of their ability to accumulate lipids grown on various agro-industrial by-products^[2]. Selection of highly productive oleaginous fungi on low cost substrates requires an efficient screening platform.

We have demonstrated that the Duetz-microtiter plate system combined with rapid, non-invasive FTIR (Fourier-transform Infrared) spectroscopy is a suitable high-throughput screening system for oleaginous fungi as well as for initial optimization of culture conditions^[3]. In the study, 100 different fungal strains from thirty *Mucoromycota* species were screened for lipid production with the developed screening system. Lipid rich fungal biomass was measured by FTIR spectroscopy and fatty acid composition was determined by

reference gas chromatography (GC). As a result, several highly productive strains from the genera *Mucor*, *Umbelopsis*, *Absidia*, *Cunninghamella* and *Mortierella* were identified. It was observed that certain strains produced significant quantities of the high-value long chain PUFAs. In addition, the prediction ability of individual fatty acids in microbial biomass by FTIR spectroscopy was studied from intraspecies to inter-subphyla taxonomic levels.

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The effect of alginate in the protection of probiotics from the harsh conditions of digestion

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Probiotics are live microorganisms that when administered in adequate amounts confer a health benefit to the host. However, to accomplish this positive influence on Human health, probiotics should survive to the passage through the upper digestive tract in large numbers to ensure a desired beneficial effects in the host. Several encapsulation methods have been used to protect probiotics. Alginate is the most used biopolymer in the production of these systems, although its performance is totally dependent of its characteristics. In this work, alginates with different molecular weights and different M/G ratio were used in the encapsulation of *Lactococcus lactis* spp. *cremoris* (LLC) aiming the protection of this probiotic bacteria against the harsh conditions of digestion. Alginate-based be-

ads were produced using an external gelification process (extrusion technique) where variables regarding the processing conditions and alginate chemical characteristics were studied to assess their relevance in this process aiming the most efficient encapsulation system. The most important variables influencing the size of alginate beads were the alginate concentration, alginate type (M/G ratio and molecular weight) and the nozzle diameter. Beads with sizes between 1.8 to 3.6 mm were produced using low, medium and high molecular weight alginate. Fourier transform infrared (FTIR) spectroscopy showed relevant differences between beads produced proving the impact of different M/G ratios in the beads' chemical structure. In general, low molecular weight and low M/G ratio alginate (LFR5/60) proved to produce the most well organized (according to SEM analyses), less permeable (pore diameter of 2.52 mm) and stronger alginate beads, moreover molecular weight and M/G ratio proved to be an important variable on the protection of probiotics against the harsh conditions of digestion. Produced beads proved to be efficient in the protection of probiotics (i.e. high viability), with the best performance presented by the medium and low molecular weight alginates.

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Comparison of harvesting methods for the cyanobacteria *Microcystis aeruginosa*

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Microcystis aeruginosa is a well-known cyanobacterium that has been spreading all over the world due to increased temperatures and eutrophication of water bodies caused by intensive anthropogenic activities. This toxin-producing microorganism is frequently responsible for diminishing water quality and causing intoxication of humans and animals. Due to this, its intracellular cyanotoxin – microcystin (MC) – is commonly used as tool for molecular and cell biology studies or as a standard in human and environmental risk assessment assays. Moreover, MC is a promising anticancer/antitumor drug candidate and a possible antimicrobial, antifungal, antialgal and insecticide agent. Despite MC's potential application in several biotechnological fields, its high production costs significantly contribute for the prohibitive selling prices (28000 e/mg). Thus, improvements in process' cost-effectiveness is needed, especially in terms of downstream processing techniques which are probably the major bottlenecks of cyanobacteria production at large

scale, commonly representing 20-30 % of the total costs.

Bearing this in mind, this study aimed at optimizing harvesting of *M. aeruginosa* induced by pH change and compares the optimal conditions obtained with the use of three different flocculant agents: chitosan, ferric chloride, and aluminium chloride. Harvesting induced by pH was assessed by testing pH values ranging between 2 and 14. Despite the fact that harvesting efficiencies above 90 % were obtained for most pH values, pH 2 was the one where higher sedimentation rate was observed and consequently the chosen method to compare with the three flocculants. Aluminium chloride addition was found to be the most efficient method, reaching 93 % of sedimentation efficiency within the first 2 h. These results are in agreement with zeta potential measurements where cells presented nearly neutral (approx. 0 mV) charge, while positive or negative charges were achieved using the other three methodologies.

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Rational bioprocess design: beneficial associations between data evaluation and process simulation

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In contrast to many other manufacturing approaches, the development of bioprocesses typically requires numerous laborious cultivation experiments. Translation of a product idea into a manufacturing process can, therefore, necessitate development times of 5-10 years. This lengthy period, as well as the high risk of failure, represents a major hurdle in the successful introduction of novel biotechnologies, in particular, for products where chemical synthesis or extraction from natural material are already established in the manufacturing process. In addition, the large quantity and specificity of data generated during bioprocess monitoring is a challenge to biotechnological development as proprietary software solutions and expert knowhow are needed to extract the relevant information. Our aim is to present a comprehensive, systematic approach to bioprocess development based on experimental data and empirical models, exploiting them for specific computations, advanced data analyses and simulations.

In our approach physiological rates such as specific growth rate (μ), specific substrate consumption rate (q_s) and specific product formation rate (q_p) are first calculated, being crucial for any rational, systematic bioprocess development. This enables analysis and comparison of strain performance independent of process-specific settings such as biomass concentration, reactor volume or process time. In general, optimum growth conditions for any new genetically designed system are not predictable *a priori*, and must be determined empirically. Experimentally determined $q_p(\mu)$ -relationships are subsequently captured in a descriptive (mathematical)

model of product formation kinetics, which is then used to perform experimental simulations aimed at identifying the optimum process strategy for substrate/feed addition. Simulations are necessary because the substrate addition strategy to maintain an optimum μ is largely defined by the technical limits of the bioreactor system and therefore q_p, \max and the corresponding μ does not necessarily identify the optimum μ for a production phase aimed at achieving maximum product titre (i.e. the final product concentration).

We will demonstrate our approach using bioprocesses with *Pichia pastoris* secreting recombinant proteins, while working with both a broad variety of data published in the scientific literature and own experimental data (e.g. production of industrial enzymes such as CALB, which are easy to 'benchmark' and thus, to compare with previous process optimisation attempts).

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A Swiss farming enterprise for microalgae: fiction or reality?

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Technologies for the cultivation of microalgae show great promise for future environmentally sustainable manufacturing of specialty and commodity che-

micals. Both research and entrepreneurial activities have already emerged, particularly in the USA but also in countries neighbouring Switzerland, such as Germany, France and Austria, where several commercial production facilities for microalgae are in operation. Within the scope of the NBO-course forming part of the Master's study programme in Pharmaceutical Biotechnology at the ZHAW, the potential for establishing the commercial farming of microalgae in Switzerland has been analysed. Technical and commercial feasibility, regulatory and legal requirements, and the societal impacts of biotechnological manufacturing of microalgal biomass for primary use in foods, feeds and cosmetics have been evaluated.

From this, a business model has been proposed: a Swiss-based SME with cooperative options in the agricultural sector, i.e. re-using available greenhouse space, and with a close connection to a power plant utilising biomass, in particular demolition waste wood. A biorefinery concept would be pursued with using CO₂ for microalgal cultivation provided by ClimeWorks AG (Zürich, Switzerland), which harvests and concentrates CO₂-gas from ambient or polluted air. The customers of this 'microalgae farm' would come from traditional, established Swiss producers in the cosmetics, food or feed sectors. They are interested in compounds with certain biological activities (i.e. bioactives), which can be extracted from microalgal biomass, and could enlarge their portfolio of starting materials with an innovative natural resource. The remaining biomass waste (after the extraction) would be used in biogas production or burned in the power plant, the-

reby recycling energy for the cultivation facility, or as a biodegradable fertilizer. After biomass harvest, process waste water could be reused to irrigate fields on the farm.

Based on a rough estimate, the financial analysis revealed that, in combination with agricultural implementation, a microalgal manufacturing facility in Switzerland is likely to be financially and environmentally sustainable. Under an extendable licencing model, microalgal biomass would be produced by Swiss farmers using their purest resource, clean Swiss mountain water.

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miwelt: the principle of microbial growth and division

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Cellular division is demonstrated through the everyday analogy of converting a child's room into two identically equipped rooms. Two cells, which have divided, are identical since they both need the same organelles to survive.

miwelt is a science communication project initiated under the Agora scheme of the Swiss National Science Foundation (SNSF). Scientists, artists and journalists have jointly developed illustrated materials, thematic excursions, and laboratory ex-

periments on the subject of microbial biotechnology for children from the age of 7 to 11.

As part of the miwelt project, scientists have been encouraged to explain both the content of and methods used in their everyday work to children and their families as well as to teachers who have only a basic grounding in science. For this purpose, they are aided by illustrations and analogies with everyday life, produced by a professional illustrator and journalists engaged in dialogue with the scientists.

For more information visit: www.miwelt.net

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Potencial toxicity of graphene-based materials

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Graphene-based materials are gaining popularity in recent years and should be introduced commercially in the future. They expose unique electronic, thermal, and mechanical properties, and are very promising in potential applications, such as nanoelectronics, nanosensors or nanomedicine. Therefore, their impact on health and environment need to be evaluated before their commercial applications. In this paper, we investigated the impact of graphene-oxide and reduced graphene foils, both modified with selenium, on a model Gram-positive bacterium *Rhodococcus erythropolis* CCM

2595. The antibacterial activity was confirmed by zone of inhibition. Foils placed on agar plates inoculated with *R. erythropolis* exhibited no antibacterial activity against this bacterial species.

P52

Sustainable technology to remove nitrates from agricultural washes

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The aim of the work is introduction of the new system of denitrifying reactor – technology using of natural nitrogen removal from farmland drainage in the place of its origin. This technology has a potential to decrease the impact of growing crops and breeding livestock on the environment thus reduce ecological load from agricultural production. Verification of the technology in laboratory-, pilot- and full-scale and adaptation of results for the use in Central European agriculture will be presented.

Natural materials (tree different kinds of woodchips – a mixture of woodchips from coniferous and deciduous trees, birch woodchips (*Betula pendula*) and plum woodchips (*Prunus domestica*) as carbon sources for low C:N wastewater treatment were evaluated. The amount of organic

matter released from these wood materials to aqueous media was evaluated, including analysis of total amount of nitrogen and ecotoxicity tests. Tests showed that dissolved organic compounds leached were initially at the largest amounts which clearly adversely affect the ecotoxicity of the water flowing from the system. However, as woodchips yielded relatively high amounts of suspended solids and organic compounds, they constitute an adequate exogenous source for the biological treatment of carbon-deficient effluents.

When leaching tests and ecotoxicity tests were conducted, it was observed that the inhibition of aquatic organisms can also be affected by the microorganisms present in the wood (namely fungi). To verify the results, further experiments were carried out which demonstrated the effect of microorganisms on the inhibition of selected aquatic organisms. Thus, new approach was chosen and the results showed that partially degraded wood (stored in outdoor conditions for a period of six months) may have lower adverse effects on the aquatic environment. When leaching tests and ecotoxicity tests were done, it was concluded that the long-term storage of wood material decreased ecotoxicity (except algae). The findings of the tests were used as a basis for the selection of suitable organic carriers to be applied in operational and pilot plant facilities.

Acknowledgement

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P53

Antibacterial Ag-doped titania coatings for orthopaedic implants prepared by sol-gel method

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Postoperative infection, a common complication after orthopaedic surgery, can lead to implant rejection, with first few days being the most critical. Therefore, the development of new antibacterial and, at the same time, cytocompatible biomaterials with suitable elution kinetics is of the utmost importance. We developed and characterised a titanium-based material coated with Ag-doped titania. The coatings were prepared by the sol-gel method. Silver (AgNO_3 or Ag_3PO_4) was added to the sol at a concentration of 0.03 mol/l. The substrates were dip-coated and fired. ICP-OES was used to measure the elution kinetics of the silver in the cultivation medium and to determine the total Ag content after layer dissolution in diluted HF. The distribution of particles (Ag^0 and Ag_3PO_4) in the layer was documented by SEM before and after elution. Antibacterial activity against *E. coli* was tested, as was cytotoxicity towards L929 and U-2 OS cell lines (ISO 10993-5). The elution tests showed that ca. 25 % of the silver was released within the first 4 h of extraction. Ag particles were still observable in the layer after 12 h;

Ag was still detectable in the extract after seven days. The materials showed an antibacterial effect against *E. coli*. The extracts of the tested materials were not toxic towards the tested cell lines. Our results indicate that the tested materials exhibit antibacterial properties that make them potential candidates for orthopaedic implants.

P54

Biogas Purification for Enriched Biomethane: An Overview of Biotechnology and Regenerative Process

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Biomethane, a carbon-neutral fuel produced from Biogas, can be used directly to generate power. Biogas, an energy-rich methane is produced by microbial decomposition of organic matter through anaerobic conditions but Presence of CO_2 and H_2S affects its performance. The microbiological process contains several type of microorganism leading to methane production is complex since the microbial structure is shaped not only but incoming material but also by operating parameters such as process temperature. Biogas purification is attracting increasing attention since it reduce the GHG emissions as well as used for the natural gas grid and other applications. Biogas purification to remove CO_2 and H_2S

is required to use for the different applications whereas a large number of methods are practiced by industry at present includes membrane separation, water scrubbing, chemical absorption, cryogenic separation and physical adsorption. Activated carbon is a universal adsorbent with high porosity, high adsorption capability, rapid kinetics and quite easy regeneration. Whereas the current industry practice is to adsorb gases and subsequently discard as hazard waste.

The current study is concentrated on in-situ regeneration of activated carbon using electric potential for the production of low cost bio-methane. A packed bed regenerative reactor has been designed, installed, and benchmarked against the industry standard activated carbon. Different samples of activated carbon were tested for the adsorption and desorption analysis where absorption / desorption cycles and regeneration efficiency for activated carbons were calculated. The experimental data at various times was evaluated and modelled by different kinetic equations. It is envisioned that this method can transform the production of bio-methane. Since the replacement of activated carbon can be up to 20% of the OPEX of the plant. The results obtained from the current research could be utilized as a guide for the further design and operation of the industrial system.

P55

Identification of PBAT degraders isolated from soil by molecular biology methods

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Introduction

Polybutylene adipate-co-terephthalate (PBAT) is biodegradable random semi-crystalline synthetic co-polyester based on fossil resources. It can be degraded by compost isolates^[1,2], but eventual occurrence of its degraders in environment has not been mapped. Direct visualisation of microbial diversity and identification of members of microbial consortium can be accomplished by denaturing gradient gel electrophoresis (DGGE).

Methods

Fifty samples of soils were collected from different environments (forest, agricultural areas, meadow). Degraders of PBAT were selectively enriched at composting temperature. After 64 days suspended microorganisms from this mixture were incubated on agar plates with PBAT as carbon source at 58 °C. Degraders were identified by clear zone method. Isolated DNA was amplified by nested PCR and DGGE with denaturing gradient 30-70 % was conducted. The results were evaluated by BLAST (National Library of Medicine). Results and conclusions

Co-polyester degraders were present in the majority of soil samples. DNA of different PBAT degraders was separa-

ted into bands in polyacrylamide gel. It can be concluded that co-polyester degraders are abundant in the environment. Selective enrichment was necessary for the isolation of the degraders so their concentration is rather low. It is likely that more bacterial groups are involved in biodegradation of PBAT. Thermophilic actinomycetes and *Bacilli* were identified as predominant groups present in degradation consortia. It has been previously suggested that thermophilic actinomycetes have high potential in depolymerisation of PBAT, but are not able to readily metabolize the monomers and oligomers formed^[1]. It is possible, that actinomycetes poses necessary enzymatic apparatus and can degrade co-polyesters to intermediates which are assimilated by bacteria from *Bacilli* group.

Acknowledgment

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P56

Non-thermal plasma in combination with antibiotics interfering with *Pseudomonas aeruginosa* quorum sensing system

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During the infection process, human opportunistic pathogen *Pseudomonas aeruginosa* uses quorum sensing system (QS) to coordinate the behavior of the whole microbial population. QS is mainly mediated by N-acyl-homoserine lactones (AHLs). Subsequent detection of these signal molecules by cells causes changes in virulence factors expression such as biofilm formation. Because biofilm formation plays an important role in nosocomial infection and antibiotic resistance, tools which are able to interfere with QS are required.

In current study, we evaluated the ability of non-thermal plasma (NTP) to interfere with the QS system of *P. aeruginosa*. Our second aim was to determine the mutual combination with antibiotics to support their anti-biofilm effect. We applied *Agrobacterium tumefaciens* NTL4 (pZLR4) biosensor to detect signal molecules, from spent bacterial culture supernatants, after the treatment.

A. tumefaciens NTL4 (pZLR4) does not produce its own AHLs, but the reporter gene is induced when exogenous

signal molecules are present. Induction of reporter gene, leads to production of β -galactosidase, which can be measured spectrophotometrically by X-Gal usage. This method demonstrated that, combined treatment with NTP and polymyxin has an additive effect in *P. aeruginosa* QS attenuation and associated biofilm formation. Mutual combination of polymyxin (7,5 mg/l) with NTP (30 minutes) was by 30 % more effective in *P. aeruginosa* ATCC 15442 QS attenuation, than the use of polymyxin (7,5 mg/l) alone.

P57

Isolation of 2-ethylhexanol utilizing *Pseudomonas japonica* strain P for potential remediation of contaminated environments

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An isolation of 2-ethylhexanol utilizing strain for potential remediation of plasticizer(s) contaminated environments was the main aim of the work. The active degrading strain designated as EHOL P was isolated from activated sludge originated from wastewater treatment plant treating common municipal wastewater.

The isolated strain was characterized and its degradation ability was examined. Biodegradation tests were performed in liquid media containing 2-ethylhexanol as

the only carbon and energy source; 2-ethylhexanol was added at initial concentration of 500 mg/l and the biodegradation was monitored via 2-ethylhexanol determination by gas chromatography, after performing solid phase extraction. Moreover, concentrations of dissolved organic carbon (DOC) throughout the degradation tests were monitored as well. Identification of the strain was done by 16S rDNA sequence analysis.

The strain EHOL P was revealed to be non-fermenting, catalase positive rods. The study of its growth properties showed that its optimal growth temperature, pH optimum and salt concentration suitable for its growth were 5-45 °C, pH 6-9 and 0-5.5 % NaCl (w/v), respectively. Subsequent identification revealed that the strain is a member of the genus *Pseudomonas* and closely related to *P. japonica* with 99 % similarity. Successful biodegradation of 500 mg/l of 2-ethylhexanol occurred during 36 hours and the substrate determinations as well as DOC monitoring suggest that the strain is capable of complete 2-ethylhexanol degradation.

Our work showed that bacterial strain(s) with 2-ethylhexanol degrading capability occur naturally in common activated sludge; further properties of isolated strain, including verifying anticipated mechanism of 2-ethylhexanol degradation, are being studied.

P58

Functional properties of *Lactobacillus plantarum* strains with potential cholesterol-lowering effects

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A new trend in the application of probiotic bacteria is their use in non-dairy foods in combination with various prebiotic preparations. Species *Lactobacillus plantarum*, which naturally occurs on many plant substrates, could be a suitable candidate for this purpose.

In this work the growth characteristics and metabolic activity on non-traditional fibre source of four strains of *L. plantarum* of different origin were compared with probiotic strains 299v and LP01. Furthermore, their probiotic and functional properties were tested *in vitro*, such as the stability in conditions simulating those in gastrointestinal tract, bile salts hydrolase activity and the ability to grow in the presence of bile, ability to assimilate cholesterol, antimicrobial activity against conditional pathogens and cells surface properties measuring the hydrophobicity and auto-aggregation.

Addition of some plant substrates positively affected the growth of tested lactobacilli strains. Strains isolated from plant sources showed the highest growth activity. Plant substrate moringa (powder from the

leaves of the tree *Moringa oleifera*) and quinoa (powder from plant *Chenopodium quinoa*) support the growth most.

All strains hydrolysed glycocholate better than taurocholate, indicating substrate specificity of the bile salt hydrolase (BSH enzymes). Deconjugation activity was maximal in the late exponential phase of growth. All tested *Lactobacillus plantarum* strains inhibited the growth of three human pathogenic bacteria (*E. coli* CCM 4517, *E. coli* CCM 4787, *S. aureus* CCM 4516). The cell-free supernatant of one strain (*L. plantarum* ATCC 14917) possessed antibacterial activity against mentioned bacteria. Cholesterol was removed from medium both by live and heat-killed lactobacilli cells and activity was proved to be strain dependent. The characteristics of two lactobacilli strains are promising for future non-traditional synbiotic functional food development. Financial support from specific university research (MSMT No 20-SVV/2017).

P59

Biological activity of carotenoids from *Salinispora tropica*

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Currently there is a great development of biotechnologies based on marine organisms. There are numerous marine microorganisms (bacteria, yeast, algae) being

tested for their bioactive substances. An interesting species, which has not yet been completely explored, is the marine bacterium *Salinispora tropica* occurring in marine sediments of tropical and subtropical waters. This taxon produces several secondary metabolites. In the vegetative stage *S. tropica* forms orange colonies containing pigments (carotenoids). Carotenoids are lipid-soluble substances consisting of 40-carbon polyene chains, often with antioxidant activity^[1]. Recently the structure of a carotenoid from *S. tropica* (sioxanthin) was identified^[2]. The biological activity of sioxanthin is not yet known. In this work the cultivation conditions of *S. tropica* were optimized (carbon and nitrogen source) by response surface methodology. There were developed extraction and quantification methods of carotenoids. The carotenoids dissolved in tetrahydrofuran were tested on human cell lines (HEK, HeLa, HepG2, LNCaP and MCF7 Kyse30). The determination of the reactive oxygen species (ROS) was optimized using CM-H2DCFDA fluorescent probe. The antioxidant effects of carotenoid extracts from *S. tropica* were compared with the effects of b-carotene. Positive effect on the induced oxidative stress was detected in cells lines HepG2, LNCaP, MCF7 and Kyse30.

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P60

Functional characterization of recombinant fish aquaporins for the development of biomimetic membrane use in water purification and desalination

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The global demand for clean freshwater resources is ever increasing and its unmet needs pose serious problems that are compounded by urban population growth, industrialization, pollution and climate change. As membrane technology plays a central role in water purification and desalination, and that membrane filtration is an energy costly process, developing novel high-performance and energy efficient membranes for water reuse and desalination is a major thrust in research and development. Inspired by water channel proteins on biological membranes, engineers are working with biologists to use aquaporins to develop biomimetic membrane that is energy efficient and cost-effective for water purification and desalination. The incorporation of aquaporin pro-

tein on biomimetic membrane will enhance water permeability and selectivity hence improve efficiency of membrane performance with lower energy consumption.

We have characterized the expression of several aquaporin isoforms in fish tissues, cloned them into yeast expression system, sequenced confirmed and expressed them in large scale for functional characterization of their water flux and permeability. We have employed stopped-flow light-scattering spectrophotometry on aquaporin-based proteoliposomes and yeast spheroplast expressing aquaporin to determine the water flux or permeability of the fish aquaporin isoforms. Depending on the aquaporin isoforms, about two- to over ten-fold increase in water flux or permeability were observed when compared to the control group without fish aquaporin. Mercury inhibition performed on selected yeast spheroplast expressing aquaporin further confirmed that the increase in water flux or permeability was indeed facilitated by the aquaporins. One of the aquaporin clone that demonstrated high water flux is currently being used to assemble a biomimetic membrane for further characterization and testing under nanofiltration mode. This ongoing project will contribute to the development of aquaporin-based biomimetic membranes that are more energy efficient and intended for water purification and desalination.

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P61

Optimization of surfactin production by *Bacillus subtilis* natto

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As a result of their diversity and properties, lipopeptides are the most promising group of biosurfactants. *Bacillus subtilis* is a microorganism that produces three kinds of lipopeptides with different amino acid composition in the hydrophilic part: 1) iturin; 2) surfactin; and 3) fengycin^[1].

Surfactin (SU) is one of the most well known lipopeptides produced by *B. subtilis*. This biosurfactant has strong emulsifying properties. The aim of this study was to design and produce lipopeptides using submerged (SMF) and solid-state fermentation (SSF) processes. Using *B. subtilis* strain KB1 natto (B/00114)^[2], a mixture of five SU structural analogues with b-hydroxyl fatty acids tails ranging from C12 to C16 were produced.

Optimization of SU production was performed using the flask batch culture of *B. subtilis* and changing one or more of the medium components. The basic medium used in SMF was semisynthetic, Modified Landy's medium with 2% glucose (LM2%)^[3], and in SSF it was rapeseed cake supplemented with 1:1 (w/v) LM2%. The influence of pH, ratio of air to sample, and carbon and nitrogen sources on the production of biosurfactant were also in-

vestigated.

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P62

Lactate as a signaling molecule in the tumor microenvironment

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Malignant transformation of cells is associated with enhanced uptake of glucose and suppression of respiration leading to increased secretion of lactate even under conditions when oxygen is available. This phenomenon is called the Warburg effect. The levels of lactate in tumor tissue are

20 to 40 times higher than normal and it was shown that they correlate negatively with patient survival, stimulate the formation of metastases and increase the likelihood of disease recurrence. The aim of our work was to determine the effect of lactic acidosis on tumor cells radioreistance and sensitivity to oxidative stress. This feature was studied in the previously unexplored context of the lactate's ability to activate a master antioxidant and chemoprotective transcription factor Nrf2. The viability of pancreatic, cervical and liver cancer cells cultured in different concentration of lactate anion and pH and treated with hydrogen peroxide and X-ray was determined. In addition, the nuclear translocation of Nrf2 protein was characterized with western blot and the expression of its target gene heme oxygenase - 1 with real time PCR. Our data show that the presence of lactate anion in culture medium increases the resistance of tumor cells to oxidative stress while acidic pH have the opposite effect. The same trend was found in non-cancer cell line – fibroblasts. The lactate treatment also enhanced the nuclear translocation of Nrf2 protein and the expression of its target gene heme oxygenase-1. This is, to our knowledge, the first demonstration of Nrf2 induction with lactate and of disjunction between the effects of lactate and acidosis on tumor cell resistance to stress.

P63

Isolation of psychrotolerant yeast strains and their ability to N-phosphonomethylglycine utilization

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N-phosphonomethylglycine (PMG, glyphosate) is the active ingredient of Roundup- a broad-spectrum postemergence herbicide. Glyphosate mode of action is based on inhibition of 5-enol-pyruvyl-shikimate-3-phosphate (EPSP) synthase and thereby the biosynthesis of aromatic amino acids in plants.

The rate of PMG utilization depends on the soil type and microbial activity. There are two modes of N-phosphonomethylglycine degradation. Both of them lead to breakage of the carbon-to-phosphorus bond. In the first one, due to glyphosate oxidoreductase action, glyphosate is converted to aminomethylphosphonic acid (AMPA) and glyoxylate. Next, AMPA is directly degraded to methylamine and orthophosphate or undergoes acetylation. In the other way of degradation, the C-P cleavage leads to sarcosine formation, which is further converted to glycine.

The aim of research was isolation of psychrotolerant yeast strains from soil samples and testing their ability to glyphosate utilization as sole phosphorus or nitro-

gen source. Soil samples collected from agricultural areas of Poland. Pure cultures were grown on YPD agar with ampicillin and chloramphenicol in 18°C.

For phosphorus/nitrogen assimilation tests, isolates were cultured on liquid Czapek Dox Medium (CDM) in 2 mM and 4 mM PMG concentrations as a sole phosphorus and nitrogen source respectively in 18°C for 7 days.

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Polish peat bog "Rucianka" as a source of psychrotolerant yeast with ability to biodegradation of phenolic compounds

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Phenolic compounds are used in many industries and can be detected in wastewaters and industrial effluents. Main sources of these toxic compounds are petroleum refineries, chemical industry and textile industry. Phenols are stable and resistant to self-degradation in water solutions. When discharged into water, they can become a danger for fish life, even at very low concentration like 5-25 mg l⁻¹. Biodegradation of phenolic compounds is preferable according to low cost of process and rare possibility of toxic secondary metabolites production. The aim of this study was to

select psychrotolerant yeast strains that degraded both of phenol and catechol or only one of investigated compounds.

In the proposed study 3 strains of psychrotolerant yeast were examined. A total of 39 strains were isolated from soil and water samples collected from Rucianka raised bog. After a preliminary test for screening of yeast capable of phenol biodegradation, 3 strains were chosen for next steps. These 3 strains were cultivated in MSM mineral medium with phenol at concentration 500 mg l^{-1} – 2000 mg l^{-1} or in YNB medium without amino acids supplemented with catechol at concentration 500 mg l^{-1} – 2000 mg l^{-1} . During incubation time, monitoring of OD_{600} values changes and residual phenol concentration (by means of GC-FID) were performed. All strains were cultivated under 18°C .

The studies revealed that three strains exhibited an effective degradation of phenolic compounds at wide range of concentration. In all experiments, complete degradation of phenol or catechol was possible in relatively short period of time.

P65

Effect of high temperature on accumulation of energy reserves in microalgae

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Some stress conditions in microalgae do not affect growth but block cell division. Since cell division is the main consumer of energy reserves such conditions can be used to induce over-accumulation of starch or lipids. Temperature is one of the key components affecting algal growth rate. Temperature increase in a certain range will increase growth rates. Here, we studied the effect of high, sub-lethal, temperature on growth and energy reserve accumulation in green alga *Chlamydomonas reinhardtii*. The algae transferred to high temperature grew similarly or better compared to the control conditions but ceased cell division. The cells were not severely stressed for at least the first 24 hours of the high temperature treatment as judged by similar accumulation of RNA, protein and chlorophyll as well as Fv/Fm ratios when compared to control conditions. However, they accumulated starch faster compared the controls. During the second, stress, phase, cultures showed sharp decrease of Fv/Fm ratios and chlorophyll degradation but significant increase in starch amount so that it represented up to 90% of the cell volume. High temperature treatment seems to be specific for induction of starch over-accumulation since the same treatment in *sta6* mutant unable to produce starch and producing only lipids as energy reserves did not lead to lipid over-accumulation. Currently, treatment with high temperature seems to be the fastest and most effective means of achieving starch hyper-accumulation in algae, leading to a more than 6 fold increase in starch content compared to control conditions, within a day of cultivation.

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A two-plasmid inducible CRISPR/Cas9 genetic tool for genome edition in *Clostridium acetobutylicum*

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CRISPR/Cas-based genetic engineering has revolutionized the molecular biology dedicated to both eukaryotes and prokaryotes. In particular, several tools dedicated to the gram-positive *Clostridium* genus have already been described in the literature, although integration of large DNA fragments still remains relatively limited. In this study, a CRISPR/Cas9 genetic tool based on a two-plasmid strategy was developed for the solventogenic *Clostridium acetobutylicum* ATCC 824. Codon-optimized *cas9* from *Streptococcus pyogenes* was placed under the control of an anhydrotetracycline-inducible promoter on a first plasmid, while gRNA expression cassettes and editing templates were located on a second plasmid. Through sequential introduction of the vectors into the cell, it was possible to achieve highly-accurate genome edition, such as nucleotide substitution, gene deletion and cassette insertion up to 3.6kb without any off-target. To

demonstrate its potential, this genetic tool was used to generate a marker-free mutant of ATCC 824 able to produce an Isopropanol/Butanol/Ethanol mixture. Whole genome sequencing was undertaken to confirm that only the desired modifications were present in the mutants obtained. Such tool is a prerequisite for efficient metabolic engineering in this relevant solvent producer and provides an alternative edition strategy applicable to other *Clostridium* strains.

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Production of levan in solid-state fermentation using rape-seed meal and *Bacillus* strains

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Levan is a polymer consisting of D-fructofuranose units joined by β -(2,6) lin-

kages and it is produced by both plants and bacteria. Biosynthesis of microbial levan is a process that occurs gradually. First the hexose residues are transferred from sucrose to the growing acceptor structure, which becomes a polysaccharide. More fructose units are then connected to the growing polymer chain. This reaction is catalyzed by levansucrase. Microbial levan has a wide range of applications in the food industry, cosmetics, pharmaceuticals, and medicine. Industrial application of levan depends on its molecular weight, which can be modulated using different culture conditions^[1]. The aim of this research was to obtain levan in solid-state fermentation using rapeseed meal as the main substrate and different *Bacillus subtilis* natto strains. Suitable methods for product separation and purification were also investigated. The purification process was performed with low-pressure liquid chromatography, using different types of column filler, and to identify levan, ¹H NMR was used. The molecular weight distribution of levan produced in the solid-state fermentation process was determined using gel permeation chromatography. We observed some differences in the molecular weight range of levan obtained from cultivation of *Bacillus strains* in submerged and solid-state fermentation.

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Inhibition of melanoma B16 tumor growth mediated by biosurfactant pseudofactin II

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Biosurfactants are tested in view of induction of apoptosis or necrosis in cancer cells. Pseudofactin II (PF II) is a novel, cyclic lipopeptide produced by bacterial strain *Pseudomonas fluorescens* BD5. Studies have shown that PF II triggers apoptosis in skin cancer cells (melanoma A375), causing DNA fragmentation, actin condensation, the release of lactate dehydrogenase, and activation of caspase-3, while the same concentrations of PF II did not affect normal human dermal fibroblasts (NHDFs) and normal human epithelial keratinocytes [NHEKs]). These data suggest that PF II is specifically targeting cancer cells^[1].

We investigated the anticancer properties of PF II *in vivo* in nude mice. The experiment was conducted over 17 days on 14 mice (7 as a control group, 7 as investigated group). PF II was applied to the skin of the mice for 10 days. During the 10-day application period, PF II inhibited tumor growth by approximately 50%, relative to the control

rol group. No significant changes of skin or weight of the mice were observed. Blood cells were not affected. During the last seven days of the experiment, when PF II was not applied, the inhibitory effect on tumor growth was decreased. These data confirm *in vivo* the antitumor activity of PF II previously observed *in vitro*. PF II seems to be non-toxic to normal cells (e.g., keratinocytes, fibroblasts, and blood cells). At the end of the experiment, there were no observed differences in blood cells between the control and investigated groups. Any observed changes in blood cells were likely caused by tumor development. The *in vivo* anticancer properties of PF II suggest that it passes skin barriers.

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A novel method to increase methane yield in a full-scale plant that co-ferments pig manure and corn silage was investigated. The process involves increasing the lignocellulosic bioavailability of the additional co-substrate wheat straw (WS), which is pretreated using a combination of Liquid Hot Water (LHW) and Steam Explosion (SE). LHW-SE of WS resulted in hemicellulose hydrolysis, partial cellulose depolymerization, and lignin bond destruction that was evaluated using fourier transform infrared spectroscopy (FTIR) analysis. The quantity of polysaccharides present in the leachate from LHW-SE WS was then measured. There was a significant increase in the low mass polysaccharides of approximately 0.6 Da compared with raw WS. The methanogenic potential of LHW-SE WS was evaluated using an inoculum from two different biogas plants to study the importance of different microorganism consortia. There was a 20-30% higher methane yield as a result of the LHW-SE combined pretreatment process. Based on published data and preliminary experiments, the process was scaled-up to full scale at the biogas plant. The LHW-SE optimized conditions were approximately 165°C and 23 atm for 10 min in LHW, followed by SE in an expansion tank, where conditions were approximately 65°C and 1 atm. Processes did not generate inhibitors that were detectable

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Enhancing methane production in a biogas plant using pig manure and corn silage by adding wheat straw pretreated using the Liquid Hot Water and Steam Explosion combined process

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by GC-MS analysis such as furfural and 5-hydroxymethylfurfural (HMF). Efficiency of the process was calculated and further optimization was proposed.

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P70

Heterotrophic cultivation of *Chlorella vulgaris* on saline wastewater from dairy industry

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The last few decades have seen a growing interest in using microalgae as potential producers of bioactive compounds. In this context, *Chlorella vulgaris* has been a model organism, because of rapid biomass growth and potentially high oil content. One of the major obstacles of their large scale production lies in the

high cost of algal biomass production and harvesting, related to their low biomass concentration achievable during phototrophic cultivation. Solution to increase algal biomass concentration and decrease production costs is offered by heterotrophic cultivation.

Saline waste water (SWW) represents a dairy industry waste that can be obtained at no cost whereas disposing the same according to regulations will incur significant costs^[1]. In this study, different strains of *Chlorella vulgaris* were examined for their ability to grow on saline waste water from demineralization of cheese whey used as the basic component of waste culture medium (WCM), under heterotrophic conditions. The results were evaluated by comparison with cultivations on defined nutrient medium (DNM) in shake flask cultures.

The experiments showed that WCM had positive influence on biomass productivities of *C. vulgaris* in comparison with DNM, under the same conditions. Furthermore, the composition of WCM was optimized (carbon and nitrogen sources) by response surface methodology and evaluated statistically. The results showed that the SWW can be considered as a valuable component of an alternative medium for heterotrophic cultivation of microalgae. Its application has a potential to find a meaningful use for saline wastewater from cheese whey desalination.

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Biodiversity and dynamic of Microalgae and Cyanobacteria from freshwater streams of Djurdjura national park forest of Darna (north Algeria)

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Due to the new emphasis on environmental protection and the development of green chemistry, microalgae were identified as producers of biofuels, specialty chemicals, pigments, biologically active compounds etc. To this end, the large study of identification of indigenous strains of microalgae is carried out through various research laboratories.

In this aim, the biodiversity of microalgae and cyanobacteria, from one of the unexplored habitats of freshwater streams of Djurdjura national park forest of Darna (North Algeria), was studied during 12 months since March 2014 to February 2015. They reveal a high specific diversity of flora examined: a total of 29 species representing 18 genera have been identified. A dominance of Chlorophyceae and Cyanophyceae compared to other classes identified was noted. The appreciation of the dynamics of phytoplankton showed that the

population density is higher in dry season than rainy season.

P72

Study on the tolerance and lipid synthesis of *Rhodotorula glutinis* with acetic acid as substrate

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In spite of the increasingly serious problem of global energy shortage and environmental deterioration, Biodiesel has been developed as a promising renewable energy. Acetic acid, as a main by-product generated in the pretreatment process of lignocellulose hydrolysis, significantly affects cell growth and lipid synthesis of oleaginous microorganisms. In this work, the tolerance of *Rhodotorula glutinis* to acetic acid and the lipid synthesis by *Rhodotorula glutinis* with acetic acid as substrate were investigated. The results indicated that in the mixed sugar medium including 6 g L⁻¹ glucose and 44 g L⁻¹ xylose, with acetic acid addition, the cell growth would not be inhibited with the acetic acid concentration was not higher than 10 g L⁻¹. Compared with the control, the biomass, lipid concentration and lipid content of *Rhodotorula glutinis* increased 21.5%, 171.2% and 121.6%, respectively when acetic acid concentration was 10g/l. Furthermore, *Rhodotorula glutinis* has the ability to accumulate lipid with acetate as sole carbon

source. Lipid concentration and lipid yield reached 3.20 g L^{-1} and 13% respectively with the initial acetic acid concentration of 25 g L^{-1} . The lipid composition was analyzed by gas chromatograph. The main composition of lipid produced with acetic acid was palmitic acid, stearic acid, oleic acid, linoleic acid and Linolenic acid, including 40.9% saturated fatty acids and 59.1% unsaturated fatty acids. The lipid composition was similar to that of plant oil, which indicated that lipid from oleaginous yeast *Rhodotorula glutinis* had potential as the feedstock of biodiesel production. These results demonstrated that a certain concentration of acetic acid needn't to be removed in the detoxification process when using lignocelluloses hydrolysate to produce microbial lipid by *Rhodotorula glutinis*.

P73

AT-rich enhancer greatly improves the expression of *Streptococcus pneumoniae* hyaluronan lyase in *Escherichia coli*

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The *Streptococcus pneumoniae* hyaluronan lyase (SpnHI) possesses extremely high activity and digests hyaluronan (HA) processively to unsaturated disaccharides exclusively. The activity of SpnHI is approx. 60 times higher than the activity

of common wide-spread bacterial hyaluronan lyase HylIP1 (1000 and 16 U/mg respectively). The ability to cleave processively and high activity is determined by the structure of SpnHI. Unlike most bacterial HA lyases the SpnHI has the molecular weight of more than 80 kDa, while 35-45 kDa is more common. The investigation of enzyme structure showed that it is composed of two domains – α and β , each playing its own role in activity^[1].

To elucidate the exact role of each domain all three forms of protein were expressed in *Escherichia coli*, i.e. the native form of SpnHI and each domain separately. The expression from pET-derived expression vector by default was rather inefficient with low yields and stability. The recent work on Shine-Dalgarno sequence influence on protein expression efficiency^[2] suggested the application of non-degenerated Shine-Dalgarno box and specific AT-rich region upstream of it for expression improvement. The modification of pET-derived expression vector by insertion of such AT-rich enhancer and fully functional Shine-Dalgarno sequence between Lac operator and start codon of expressed gene greatly improves both stability of cultivation and protein yield in all expression experiments.

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P74

KEGG pathway and transcriptome analyses under two conditions, sulfur deprivation and high light, accelerating starch and lipid accumulation in *Parachlorella kessleri*

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Parachlorella kessleri, a unicellular green alga belonging to the class Trebouxiophyceae, achieves very high biomass, lipid, and starch productivity levels. We sequenced the whole genome and transcriptome and analyzed the behavior of *P. kessleri* under lipid production-inducing conditions. The assembly includes 13,057 protein-coding genes and the metabolic network was reconstructed based on KEGG pathway analysis^[1]. Lipid content in algae can be increased by nutrient depletion (nitrogen, phosphate, and sulfur). To characterize the phenotype and transcriptome under sulfur deprivation, *P. kessleri* was batch-cultivated under sulfur replete and deplete ($\pm S$) conditions. Although growth was restricted under the sulfur-depleted condition, higher starch contents per cell were observed after 2 days by sulfur deprivation (6.19-6.62 fold changes). Total lipid yield was accelerated under sulfur deprivation, in contrast to cultivation in TAP medium, so that in the 5-day-old, sulfur-deprived culture, the lipid content

represented up to 51% of the dry weight. Transcriptome analysis under $\pm S$ is shown near the pathway as a heat map. Transcriptomic analysis suggested that lipid accumulation under conditions of sulfur depletion is associated not only with the induction of sulfur metabolism but also TAG synthesis, light-harvesting complexes, and autophagy. These deficiencies also limit their growth and productivity. Therefore, the *Chlorella* strains were attempted to increase starch and lipids productivity under high-light-intensity conditions (600- $\mu\text{mol photons m}^2 \text{ s}^{-1}$)^[2]. The high productivity was achieved by reducing the culture time and using a high light intensity. *P. kessleri* achieved the greatest biomass productivity (1.04 g L⁻¹day⁻¹), which was about two-fold that in *C. vulgaris* (0.55 g L⁻¹day⁻¹). Lipids (0.3 g L⁻¹day⁻¹) and starch (0.22 g L⁻¹day⁻¹) productivity was greatest under LL conditions in *P. kessleri*. We performed transcriptome analysis of *P. kessleri* under the high light condition. The gene expression of sulfur metabolism and chlorophyll synthesis decreased and sugar and lipid synthesis increased according

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P75

Utilisation of food waste for lactic acid production

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Food waste is a growing problem related with increasing of the population. Around 1.3 billion tonnes of food produced for the human consumption in the world is every year getting lost or wasted^[1]. Food waste is considered a non-valued material. Food waste are for example vegetable and fruit peel, nut shells and coffee residuals^[2].

Saccharides and other nutrients contained in food waste are suitable for fermentation. Generally, these organic food wastes are composted or used as animal feed. Microorganisms could convert it into profitable products such as biofuels, enzymes, useful chemicals or monomers for production of bioplastics. Conversion of food waste into other valuable products promise different options^[2].

One of these valuable products could be lactic acid. It is organic acid usually used in food and pharmaceutical industries. It has also great potential as monomer for producing the biodegradable plastics. Polylactic acid (PLA) is suitable alternative to traditional plastics. Its large-scale application is limited by high price of lactic acid production. High price of production could be reduced by optimization of fermentation and searching of cheaper raw materials.^[3]

The aim of this study was to use different food wastes as raw material for lactic

acid fermentation.

Keywords: Lactic acid, food waste, fermentation, lactic acid bacteria

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Optimization of Alcalase Immobilization on the Alginate Beads Obtained by Electrostatic Extrusion

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Alcalase (EC 3.4.21.14), as one of the proteolytic enzymes, is widely used in a variety of industries that require hydrolysis of proteins. This study is examining the impact of immobilization conditions on the biocatalysts activity and the enzyme coupling yield of the alcalase covalently immobilized to the Ca-alginate beads, obtained by the electrostatic extrusion, in purpose of determining the most optimal immobilization conditions. For that

purpose, we examined the influences of: enzyme concentration, optimal pH and the molarity of the TRIS immobilization buffer, the mass of activating agent EDAC and the time required for activation of the Calginate beads, as well as the time of the alcalase immobilization process.

It was obtained that the optimal immobilization conditions were: alcalase concentration of 5,32 IU, TRIS buffer pH 8,5, molarity of 50 mM, beads activation with 10 mg of EDAC in 30 minutes time and 20 h for the immobilization process.

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Biosorption of micropollutants onto microalgal biomass

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Municipal wastewaters are contaminated by a wide range of chemicals including pharmaceutical residues, personal care products, various household chemicals and biocides/pesticides. One group of wastewater contaminants is called micropollutants. Micropollutants are occurring in a very low concentration (ng/l to µg/l) often below detectable limits and may generate adverse effects on aquatic organisms. Micropollutants release into the environment depends on their fate in wastewater treatment plants. They have complicated structure resistant to biological degradation (low speed and completeness of degradation – often only

biotransformation). This makes them difficult to remove from wastewater in the primary and secondary level of conventional wastewater treatment plants. Currently used methods, such as absorption on activated carbon, are operationally intensive investments, with the necessity of regeneration or even destruction of activated charcoal as waste. For these reasons, novel specific methods for tertiary treatment are necessary. Biosorption onto microalgal biomass offers a potential alternative for removing micropollutants from the environment. In this study, *Chlorella vulgaris* was used as a model organism. Their cell wall contains large number of variable sites, which can adsorb different chemical compounds. For biosorption tests, several steroid hormones (testosterone, estrone, estradiol etc.) were selected. Various sorption efficiencies of biomass were observed. The highest degree of sorption was observed for progesterone (5 µg algal biomass).

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Competence Center for Biocatalysis (CCBIO)

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The Competence Center for Biocatalysis (CCBIO) promotes biocatalysis as a complementary method to classic organic synthesis and aims to help bridge the gap between academic laboratories and the production plant.

Biocatalysis enables the sustainable production of chiral and highly functionalized compounds for the chemical and pharmaceutical industry. Recent key advances in DNA sequencing, gene synthesis and data analysis are now enabling scientists to tailor biocatalyst according to industrial needs and to reorganize enzymes into new biosynthetic pathways. This progress fuels the so-called «third wave of biocatalysis» and promises to unlock hitherto inaccessible enzyme activities for industrial biocatalysis and engineer microbial and fungal strains for the production of novel asset molecules.

The Competence Center Biocatalysis (CCBIO) at the Zurich University of Applied Sciences (ZHAW) was founded in January 2016 and is led by Dr. Rebecca Buller. By connecting relevant research competences CCBIO strives to develop a comprehensive biocatalytic toolbox consisting of enzyme libraries and methods (e.g. enzyme engineering, design of experiments), which will facilitate the development of biocatalytic and biosynthetic processes for the chemical and pharmaceutical industry. CCBIO is embedded in the ZHAW and heads the platform "Biocatalysis and Biosynthesis" within the frame of the National Thematic Network (NTN) Swiss Biotech. Additionally, CCBIO leads the network project "Innovation in Biocatalysis: A toolbox for sustainable bio-based production", a program supported by project contributions from the Swiss Higher Education Council.

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A Novel Immobilization Method for Hydroxynitrile Lyase: Protein-Coated Microcrystals

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The use of enzymes in asymmetric carbon-carbon bond formation reactions is steadily increasing in industry. Hydroxynitrile lyases (HNLs, EC 4.2.1.10) catalyze the addition of hydrocyanic acid (HCN) to prochiral aldehydes/ketones leading to formation of enantiopure cyanohydrins. Enantiopure cyanohydrins are key intermediates in the production of important chemicals used in pharmaceutical and agrochemical industries, including α -hydroxycarboxylic acids, α -hydroxyaldehydes, α -hydroxyketones, β -amino alcohols, α -fluoro cyanides. HNLs obtained from various plant resources have been used for the synthesis of enantiopure cyanohydrins in pure or partially pure form. However, the reusability is critical feature for most biocatalysts as well as HNLs which can be achieved with the immobilization of HNLs. In recent years, the carrier-based and carrier-free immobilizations of HNLs have been described. Protein-coated microcrystals (PCMCs) have emerged as

a new, cheap and effective immobilization method for enzymes used in organic media. PCMCs are simply prepared by simultaneous precipitation of enzyme and a carrier such as inorganic salt and may be an attractive immobilization way for HNLs.

In this study, the immobilization of partially purified HNL from *Prunus armeniaca* (Pars HNL) as protein-coated microcrystals was prepared by simultaneous precipitation of K₂SO₄ and Pars HNL in pre-chilled acetone. The prepared PCMCs of ParsHNL was used the enantioselective transcyanation of benzaldehyde in buffer saturated methyl tert-butyl ether (MTBE) at pH 4.0. The results showed that (R)-mandelonitrile was obtained with 100% yield and 99% enantiopurity after 96 h reaction time.

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Bioremediation of permethrin contaminated soils using *Acinetobacter baumannii* ZH-14

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Abstract: Persistent use of the pesticide permethrin has resulted in serious environmental contamination problems, yet little is known about microbial remediation in contaminated regions. In our previous study, a novel bacterial strain named ZH-14 belonging to *Acinetobacter baumannii* was isolated from an aerobic wastewater

treatment system using an enrichment procedure. Strain ZH-14 was capable of rapidly degrading permethrin over a wide range of temperature and pH. In this study, bioremediation efficiency of permethrin contaminated soils was investigated based on the results of laboratory experiment. Strain ZH-14 was used to degrade permethrin in sterile and non-sterile soils.

Keywords: Bioremediation, Permethrin, *Acinetobacter baumannii*, Kinetics

MW3

miwelt: the foolproof yeast's experiment

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The illustrations show an experimental set-up and the conditions that enable yeast to grow, divide and produce carbon dioxide while utilising sugar (glucose). The experiment has been adapted for miwelt with children performing the experiment in a plastic bottle.

miwelt is a science communication project initiated under the Agora scheme of the Swiss National Science Foundation (SNSF). Scientists, artists and journalists have jointly developed illustrated materials, thematic excursions, and laboratory experiments on the subject of microbial biotechnology for children from the age of 7 to 11.

As part of the miwelt project, scientists have been encouraged to explain both the content of and methods used in their everyday work to children and their families as well as to teachers who have only a basic grounding in science. For this purpose, they are aided by illustrations and analogies with everyday life, produced by a professional illustrator and journalists engaged in dialogue with the scientists.

For more information visit:
www.miwelt.net

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Chemometric approach in optimization of fermented cabbage production: Antioxidant activity, vitamin C content and organic acids profile

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Cabbage heads (*Brassica oleracea* var. *capitata*) were fermented in agricultural holding conditions. Experiments (13 runs) were set using Box-Behnken experimental design on three levels with fermentation temperature (18, 22 and 26 °C), starter culture volume (0, 0.5 and 1 dL) and NaCl concentration in water (6, 7 and 8%) as independent variables. Samples of fermented cabbage were analyzed after 5, 12, 27

and 62 days. Antioxidant activity determined by DPPH assay, vitamin C content and organic acids profile (oxalic, formic, lactic, acetic and succinic acid) were investigated as response variables. Experimentally obtained values were not successfully fitted to a second-order polynomial model, therefore, chemometric procedures were used as alternative approach. Hierarchical cluster analysis (HCA) and sum of ranking differences (SRD) were applied for that purpose. Samples obtained at different fermentation conditions were compared with sample obtained at 22 °C, 6% NaCl and without addition of starter culture, which is commonly used procedure for cabbage fermentation. Comparison was based on SRD and comparison of ranks by random numbers (CRNN). Chemometric approach was able to group samples obtained at different conditions and to distinguish samples with the best properties (antioxidant activity and vitamin C content).

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Production of lipid compounds by red yeast grown on animal fat waste substrate

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Characterization of metabolic activity of yeasts in biotechnological applications is an important issue both for routine analysis and trouble-shooting incidences. In this

study we present results of cultivation, metabolic and production activity and single cell analysis of industrial red yeasts. As a substrate waste animal fat and its hydrolysis products were used according to the biorefinery concept.

20 strains of red yeasts (*Rhodotricula*, *Sporobolomyces*, *Cystofilobasidium*) were used for analyses. As the substrate hydrolyzed and crude animal was used. Yeasts were cultivated in medium with particular C/N ratio. Simultaneously with growth characteristics total metabolic activity and production of lipids and fatty acids (GC/FID), sterols, carotenoids and CoQ (LC/MS) were followed. Glycerol, lipase and lipids were measured by enzyme methods. Cells were monitored by advanced fluorescence techniques on single-cell level.

All strains were able to utilize fat hydrolyzate, some of them produce lipase and utilize crude fat. Amount of lipid metabolites depended on glycerol content in medium and C/N ratio. Using time-differentiated fluorescence and Nile red staining localization and trafficking of pigments and lipids in yeast cells was determined. The results could contribute to better understanding and control of biotechnological process.

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Using of Raman spectroscopy to focus on production of metabolites by *Metschnikowia* strains

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The yeasts genus *Metschnikowia* have been studied using Raman spectroscopy due to its potential applications in the field of biofuel generation and food industry applications. In order to utilize yeast biomass for efficient industrial/biotechnological production, the optimal cultivation parameters have to be determined.

Firstly, there is an ability to form large amounts of same lipids under certain conditions (60-80%) secondly, there is a strong ability of producing different pigment, which cause different antifungal antagonism effect as well.

Therefore, we focused on how different cultivation conditions (the effects of temperature regime and medium composition) influence microorganism growth. The cells can be monitored using our dedicated instrumentation. Raman spectroscopy can be exploited in instances where fast and accurate monitoring/determination of samples is required.

In our preliminary experiments we successfully monitored information about the degree of saturation of fatty acids (io-

dine number) in cells during the yeast cultivation (one or two weeks) in medium containing different ratios of C/N. Samples were cultivated under different temperatures below 15 °C. The whole procedure – from sample preparation to advanced chemometric methods – can take several minutes. Thus, Raman spectroscopy proved to be a very efficient tool for the rapid quantitative/qualitative analyses of oil produced by yeast. Moreover, spectroscopic identification of the red pigment (pulcherrimin) of *Metschnikowia* that accumulates in the cells and in the medium near a colony is possible.

Individual fatty acid profile was determined by gas chromatography. The studied cells at different phases of cultivation were imaged by scanning electron microscopy which visualized their response on the cultivation procedure.

Acknowledgments

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P84

Physico-chemical properties of fermented cabbage obtained at different fermentation conditions

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Cabbage heads (*Brassica oleracea* var. *capitata*) were fermented in agricultural holding conditions. Experiments (13 runs) were set using Box-Behnken experimental design on three levels with fermentation temperature (18, 22 and 26 °C), starter culture volume (0, 0.5 and 1 dL) and NaCl concentration in water (6, 7 and 8%) as independent variables. Samples of fermented cabbage were analyzed after 62 days. Characterization of the fermented cabbage was based on their physical (moisture content, firmness, a_w value, total colour change and pH) and chemical (antioxidant activity, vitamin C, organic acids, NaCl, sugars and biogenic amines content) properties.

P85

Microbial lipid production using whey permeate

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Increased use of fossil fuels has released toxic contaminants to the environment such as CO₂, a major greenhouse gas responsible for global warming. In order to reduce the usage of fossil fuels and protect the environment, a more sustainable alternative energy source is demanded. One of the promising sources is biodiesel. Currently, the major source of biodiesel is vegetable oil, with 95% of biodiesel produced from edible plant oils. This creates a competition with the food manufacturing industry for arable land, although currently a variety of non-edible oils are being used. Hence, there is a need for innovative approaches of oil production, among which microbial oils provide a potential solution. An ideal scenario for the cost-effective cultivation of oleaginous microorganisms would be the utilization of abundant industrial by-product streams that have limited competing applications.

One such by-product stream is the whey permeate from the dairy industry. Whey permeate is obtained by ultrafiltration and removal of protein from whey that is generated during cheese manufacturing. Whey permeate displays an overall composition of mostly lactose along with

salts and non-protein nitrogen.

Glucose and galactose from the pre-hydrolyzed whey permeate were used as main carbon sources for the cultivation of *Metschnikowia* yeast strains. Those cultivations resulted in 8.7 ± 0.3 g/l of biomass production with a total lipid accumulation of $32.0 \pm 4.9\%$ (dry weight basis). To maximize the lipid production various carbon/nitrogen ratios has been used.

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P86

Membrane adaptation profile of *Geobacillus stearothermophilus* PS11 in presence of petroleum hydrocarbons

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The increased generation of petroleum hydrocarbon waste has been stated as one of the most critical environmental problems. Though microbial bioremediation has been widely used for waste treatment but their application in petroleum hydrocarbon waste treatment is limited since they have toxic effects on the microbial cells.

In the present work, soil samples from the petroleum contaminated sites were screened for petroleum hydrocarbon degrading microbes. Among 32 petroleum

degrading bacteria, PS11 strain was selected for further work on the basis of highest zone of petrol utilization. Biochemical characterization and phylogenetic analysis confirmed strain PS11 to be *Geobacillus stearothermophilus*. When grown in presence of petrol, PS11 strain showed a delayed pattern of growth compared to that of control but it utilized crude petroleum-oil hydrocarbons as sole source of carbon and energy. The strain was also capable of growing in presence of wide range of other hydrophobic solvents with log P-values between 1-4. Transmission electron micrograph of PS11 cells in the presence of 10% crude petrol showed convoluted cell membrane and accumulation of petrol in the cytoplasm, indicating the adaptation of the bacterial strain to the petrol after 48h of incubation. The solvent adaptation property of *G. stearothermophilus* PS11 seems to be related to both restoration of membrane fluidity and metabolic transformation of hydrophobic solvent to less toxic products. GC analysis showed that after 15 days incubation PS11 strain could degrade almost all aromatic compounds compared to that of alkanes. The membrane phospholipids composition of *G. stearothermophilus* PS11 was altered in the presence of 10% (v/v) petrol. Decrease in phosphatidylethanolamine (PE-11%) and phosphatidylglycerol (PG-14%) with parallel increase in cardiolipin (DPG-15%) and diphosphatidylglycerol (PGL-128%) was observed in presence of petrol. An increase (22.4%) of the iso-acids was noted in cell membranes adapted to 10% (v/v) petrol with concomitant decrease of the anteiso-acids (17.6%) and straight-chain saturated fatty acids (17.8%).

P87

Down-regulation of lignin biosynthesis pathway genes in rice (*Oryza sativa* L.) using RNAi

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Rice straw is one of the largest biomass in the world that can potentially be exploited for bio-fuel. Nevertheless, the association of lignin with cellulose and hemicellulose has hindered the efficient utilization of rice straw for cellulosic bio-fuel. The objective of this study was, therefore, to down-regulate genes involved in lignin biosynthesis pathway, such as hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase (HCT), cinnamoyl CoA reductase (CCR), and cinnamyl alcohol dehydrogenase (CAD) through terminatorless construct to reduce lignin in transgenic rice straw for its use in cellulosic bio-fuel. Real-time qPCR analyses of the selected T₁ transgenic rice plants indicated at least 36-87% transcript reduction in HCT lines, 75-94% in CCR lines, and 14-85% in CAD lines. Of the nine down-regulated lines (three lines from each gene) analyzed for lignin, total lignin content was sig-

nificantly reduced in seven lines (HCT-4, HCT-7, CAD-1, CAD-7, CCR-3, CCR-7, and CCR-12) with lignin reduction ranged from 4.6% to 10.8%. The results from this study indicated that the simple binary vector without termination sequence can be used for down-regulation of lignin genes in rice; and that the rice straw from transgenic lines containing reduced lignin could be used as feedstock for cellulosic biofuel.

P88

Prevalence of Mastitis pathogens and its molecular identification in Milk collected from clinically healthy cows

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Study was conducted on lactating dairy cows of Bundelkhand to determine the prevalence, risk factors and molecular epidemiology of different mastitis pathogens. A total of 685 milk samples collected from 3 districts during March 2016 to March 2017. Study revealed, the overall prevalence of mastitis was found to be 29.20 % (20.10% clinical and 9.12% sub-clinical cases). Among the total (685) quarters examined, 3.21% had blind teats. The prevalence of mastitis showed statistically significant difference between, season, number of parity, lactation stage, body condition of animals, hygiene and udder washing procedures ($P < 0.05$). Pathogens *Staphylococcus aureus* (*S.aureus*) (22.12%) followed by *Streptococcus agalactiae* (*S.agalactiae*)

(18.30%) and *Coagulase negative staphylococci* (CNS) (11.27%) and the lowest isolation rate was for *Micrococcus species* (2.82%) were isolated from positively screened milk samples. The Other species which isolated include *Klebsiella species* and *Streptococcus uberis* (*S.uberis*) (4.23% each), *Streptococcus dysgalactiae* (*S. dysgalactiae*) (5.63%), and *Escherichia coli* (*E.coli*) (7.04% each), *Streptococcus pyogenes* (*S.pyogenes*) (8.45%) and *Bacillus species* (5.63%). Further Coagulase negative *Staphylococci* were confirmed by Restriction Fragment Length Polymorphism (RFLP)-PCR using the gap gene.

Study concluded the predominance of CoNS in subclinical mastitis infections in Bundelkhand region which ultimately lead to the deterioration of milk quality. By eliminating these pathogens, the recurrence of mastitis may be controlled in this region. Further study may be designed to develop methods based on PCR can be developed for rapid identification and differentiation of mastitis pathogens.

P89

Phytic Acid Degrading Probiotic Bacteria Having Bacteriocinogenic Activity, isolated from Kaladi, A Fermented Milk Product

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Total of 100 LAB isolates were obtained from thirty sources, thirty two of these strains were isolated from Kaladi. These isolates were identified as potential strains with probiotic properties on the basis of their colony morphology, biochemical tests viz. bile tolerance, low pH tolerance, high survival rate in simulated gastric conditions. It was observed that these isolated lactic acid producing bacteria were having inhibitory activity against *E. coli* MTCC 1652, *Bacillus subtilis* MTCC 121, *Proteus vulgaris* MTCC 426, *Staphylococcus aureus* MTCC 3160, *Mycobacterium smegmatis* MTCC 994. The strains had the ability to degrade phytate, a prominent source of phosphorus in cereals and legumes constituting 90 % of its content, associated with anti-nutritional properties due to chelation of divalent cations. These isolates were tested for their survival in simulated gastric conditions. LAB isolates had tolerance to NaCl concentrations (6.5 %, 3.5 %) with an optimum growth at 37 °C and an ability to survive at 45 °C. In conclusion, almost all the isolates from Kaladi had a survival rate of above 90% under simulated gastric condition and the phytase

activity observed was at pH 4.0 which was near to pH range 2.0- 4.0 observed in the intestine. Fermented milk product used for isolation of LAB was a potential source of LAB having all the probiotic characteristics which can help them to serve as a dietary adjunct for all food and feed supplements. It can also be considered in food industry, fit for human consumption. LAB isolates which had a high phytate degrading ability did not show high lactic acid production.

P90

Investigations on Anticancer Drug Potential of a Lectin Isolated from *Aloe vera*

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Lectins are glycoproteins from plant or animal origin that binds to specific carbohydrates on cell surfaces and that are thus promising as antitumour agents or markers. Drugs of plant origin are promising strategy for cancer therapy because they might be harmless or less toxic than synthetic chemotherapeutic agents on normal cells. Several studies were conducted with *A. vera* leaf extracts regarding its antitumour effects. However, studies on the anticancer effect of lectin purified from *A. vera* are scarce. In the present study, we aimed

to compare the concentration dependent effects of a lectin (Aloctin) isolated from *A. vera* in our laboratory, on the *in vitro* growth of some human carcinoma cells.

The gel portion of leaves of *A. vera* was separated from the leaf skin and Aloctin was isolated by ammonium sulphate precipitation and affinity chromatography. The purity was checked by PAGE. Specific ligands were detected as fetuin and avidin by haemagglutination inhibition test. The cytotoxic effect of Aloctin was tested using MTT assay. Imatinib and aloe emodin were tested as standard positive controls. Apoptosis and necrosis were detected by Annexin V-FITC/ propidium iodide assay.

The most sensitive cells to Aloctin treatment, in terms of cytotoxic activity, were identified as AGS₂Saos-2₂HEP3B₂K562₂HL-60₂HCT116. It was shown that this effect does not occur by apoptosis or necrosis. It is expected that the results of this study will reveal important findings for the future use of *A. vera* lectin in glycoprotein targeting and other applications of lectins in biotechnology.

P91

Effect of glucose/xylose ratio on xylose consumption and butanol production using different *Clostridium* species on defined media

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The production of butanol using *Clostridium sp.* has been widely studied and significant progresses have been made towards an economically competitive process. However, the fermentation of lignocellulosic hydrolyzates by these microorganisms has shown to be problematic due to glucose-mediated catabolic repression on xylose degradation^[1]. This study attempts to understand the limitation of growing, xylose consumption and butanol production under different glucose/xylose ratios (0, 0.5, 1, and 2) using two wild *Clostridium sp.*: *C. acetobutylicum* ATCC824 and *C. beijerinckii* NCIMB8052; and a butanol-tolerant mutant strain: *C. beijerinckii* PTA-1550 (BA101). All tested strains preferred glucose over xylose as carbon and energy source. However, the consumption of xylose by the mutant strain was not dependent on the proportion of this sugar in the media. Even when only xylose was formulated in the culture medium, this sugar was consumed at the same proportion. The higher total sugar consumption was reached at a ratio of 0.5 for all strains. Sugars consumption for the native strains decreased progressively with the increase of the xylose proportion. The wild and mutant strains of *C. beijerinckii* consumed the same proportion of sugars at glucose/xylose ratios of 0.5, 1 and 2. But, the mutant strain showed higher (3 times) xylose consumption than native when only xylose was formulated. On the other hand, *C. acetobutylicum* showed the worst xylose consumption at all ratios. The higher buta-

nol yield was obtained with *C. beijerinckii* PTA-1550 (0.32 g butanol/g sugar) when only xylose was formulated. On the other hand, *C. beijerinckii* NCIMB8052 showed the higher butanol yield (0.29 g butanol/g sugar) for sugar mixtures at a ratio of 0.5, but this strain produced only 1.9 g/L of butanol when only xylose was used.

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P92

Novel photoactivable colchicine-BODIPY conjugates as a tool for phototherapy

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Colchicine is a well-known inhibitor of microtubule polymerization that acts by binding to tubulin. Microtubules are responsible for cell shape maintenance and movement of cell organelles and their destabilization leads to cell cycle arrest in

the G2/M phase followed by apoptosis. So far, broader use of colchicine in clinical practice has been limited by its high systemic toxicity. Recent findings opened new possibilities for a colchicine application, such as cancer treatment.

Iodo-BODIPYs have been lately proven to be very potent for photodynamic therapy (PDT), which is approved for treatment of both cancerous and noncancerous diseases. PDT principle lies in photoactivation of a photosensitive compound (PS) in presence of molecular oxygen. After light activation, PSs generate toxic, highly reactive O₂ species, which results in cell death.

The aim of this work was to develop PDT active conjugates with enhanced biological availability, cell uptake, and specific targeting. As a carrier of a photoactivable molecule, we chose colchicine, the therapeutic potential of which was maintained, and thus a multimodal agent was developed. Specifically, we synthesized two colchicine-BODIPY derivatives, where a BODIPY core was or not substituted by iodine.

Either of the conjugates did not exhibit significant toxicity (without illumination) in cancer cell lines of HeLa, U-2 OS and PC-3 up to 500 nM concentration. Nevertheless, after photoactivation, an iodo-BODIPY conjugate resulted in a 50% decrease in cell proliferation already at 5 nM concentration for HeLa cells and 10 nM for U-2 OS and PC-3 cells. This derivative was also able to arrest the cell cycle in G2/M phase in HeLa cells. Using live-cell fluorescence microscopy, we found that the conjugates localized inside cells in 2 h. The high phototoxicity and low dark toxicity

make the newly prepared colchicine-iodo-BODIPY conjugate a suitable candidate for use in PDT and an effective tool for multimodal therapy.

P93

Determination of alpha-lactalbumin using modified silver nanoparticles

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Alpha-Lactalbumin (α -LA) is a small and acidic milk protein. The major role of α -LA in mammary secretory cells is to participate in lactose synthesis^[1]. Also, α -LA and its hydrolysate have some physiological functions such as antimicrobial activity and regulation of cells growth^[2]. Another important feature of α -LA is that its cation-binding ability. The α -LA is the second most abundant protein in bovine milk whey and has been widely used as food ingredients due to its emulsifying and gelling properties^[3,4]. In this study, a colorimetric α -LA detection method was developed based on the cation-binding ability of α -LA. In this method, α -LA was determined by glutathione-modified silver nanoparticles in the presence of metal ions based on the cooperative metal-ligand interactions. The experiments were carried out with Cd^{2+} , Fe^{3+} , Cu^{2+} and Zn^{2+} metal ions. The best results were obtained using Cu^{2+} ions. The formed com-

plexes between glutathione-modified silver nanoparticles-metal ions- α -LA were analyzed by ultraviolet-visible (UV-Vis) spectroscopy. The shifted absorption peak intensities were increased depending on the α -LA concentrations. The obtained linear determination ranges were 50-400 $\mu\text{g/mL}$, 5-300 $\mu\text{g/mL}$, 5-200 $\mu\text{g/mL}$ and 5-200 $\mu\text{g/mL}$ in the presence of Cd^{2+} , Fe^{3+} , Cu^{2+} and Zn^{2+} ions, respectively. IR spectroscopy and atomic force microscope were also used for characterization of modified nanoparticles and complexes.

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P94

Isolation, structural analysis, and expression characteristics of the maize nuclear factor Y gene families

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NUCLEAR FACTOR-Y (NF-Y) has been shown to play an important role in growth, development, and response to environmental stress. A NF-Y complex, which consists of three subunits, NF-YA, NF-YB, and, NF-YC, binds to CCAAT sequences in a promoter to control the expression of target genes. Although NF-Y proteins have been reported in Arabidopsis and rice, a comprehensive and systematic analysis of ZmNF-Y genes has not yet been performed. To examine the functions of ZmNF-Y genes in this family, we isolated and characterized 50 ZmNF-Y (14 ZmNF-YA, 18 ZmNF-YB, and 18 ZmNF-YC) genes in an analysis of the maize genome. The 50 ZmNF-Y genes were distributed on all 10 maize chromosomes, and 12 paralogs were identified. Multiple alignments showed that maize ZmNF-Y family proteins had conserved regions and relatively variable N-terminal or C-terminal domains. The comparative syntenic map illustrated 40 paralogous NF-Y gene pairs among the 10 maize chromosomes. Microarray data showed that the ZmNF-Y genes had tissue-specific expression patterns in various maize developmental stages and in response to biotic

and abiotic stresses. The results suggested that ZmNF-YB2, 4, 8, 10, 13, and 16 and ZmNF-YC6, 8, and 15 were induced, while ZmNF-YA1, 3, 4, 6, 7, 10, 12, and 13, ZmNF-YB15, and ZmNF-YC3 and 9 were suppressed by drought stress. ZmNF-YA3, ZmNF-YA8, and ZmNF-YA12 were upregulated after infection by the three pathogens, while ZmNF-YA1 and ZmNF-YB2 were suppressed. These results indicate that the ZmNF-Ys may have significant roles in the response to abiotic and biotic stresses.

P95

Identification and function analysis of PtoPLC1, which is highly expressed in the roots of *Populus tomentosa*

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Phosphoinositide-specific PLC (PI-PLC) is a conserved key enzyme in phosphoinositide signaling both in animal and plant. It plays an important role in growth and development, different abiotic and biotic stresses. On the basis of *Populus trichocarpa* genome database and bioinformatics method, The results showed that 7 PI-PLC genes existed in the *P.trichocarpa* genome and located on 4 chromosomes. Multiple alignment and motif display results indicated that all PI-PLC proteins

contain four conserved domains. According to the corresponding gene sequence from *P. trichocarpa*, PtoPLC1 PtoPLC2 PtoPLC6 and PtoPLC7 were isolated from *P. tomentosa* by PCR amplification. Among them the recombinant PtoPLC1 protein expressed in vitro was able to hydrolyze phosphatidylinositol 4,5-bisphosphate (PIP2) to generate inositol 1,4,5-trisphosphate (IP3) and 1,2-diacylglycerol (DAG). The catalysis of PIP2 by PtoPLC1 is Ca^{2+} dependent and the optimum concentration of Ca^{2+} is $1 \mu\text{M}$. The qReal-time PCR analysis revealed strong expression of PtoPLC1 in roots, but weak expression in stems and leaves. The gene is strongly induced under high salt, dehydration and ABA. These results suggest that PtoPLC1 might be involved in the signal-transduction pathways of high salt, dehydration and ABA responses in *P. tomentosa*. Analysis of transgenic poplars in which the PtoPLC1 is overexpressed and knockout should give us some ideas as to the function of PtoPLC1. Characterization of the PtoPLC1 and its products should contribute to further understanding of plant signal transduction. And is supposed to be a potential candidate gene to improve stress tolerance by genetic engineering in poplar and other trees.

Key words: stress-tolerance; PtoPLC1; *Populus tomentosa*; PI-PLC

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Concise stereoselective synthesis of beta-secondary alkenols

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The pure enantiomers show more specific biological property compared to the racemic forms. Therefore these molecules have increased demands for the development of synthesis methods. The chiral catalysts and biocatalysts are two of the used methods for the synthesis of chiral complex molecules with high enantiomeric purity and reaction yield. The use of enzymes as biocatalysts is the most successful industrial method. The new enzymes variants are rising by increasing research and development in the field of modern biotechnology. These new catalysts provide the conditions of industrial process, more stable property and can be applied without changing the existing process conditions. Also the use of biocatalysts is supported by some control mechanism on chemical processes that support/mandating environmental approach. The allenes show versatile product feature of important asymmetric molecules at the last 10-15 years. Also many important medicines and natural product structure is described with allene groups^[1,2]

In this study, beta-secondary allenols is synthesis from aromatic ring fused ring aldehydes via very efficeint enviromentally friendly 3 step procedures. First, corresponding aldehyde is subjected to grignard reaction to yield secondary homo propargylic alcohols. Then alcohols are transformed to allenes by using Crabbe reaction. In both reaction, extra prufication step is not necessary. The NMR spectrum of crude reaction mixture represent the total yield between 85 and 95 %. To get the chiral target allenols having around 93 % enantiomeric excess value, enzymatic kinetic resolution strategy was applied on racemic alcohols^[3].

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P97

Overexpression of the Gene Encoding for Subunit A of the Vacuolar H⁺-ATPase from Cotton Enhances Drought Tolerance in Tobacco

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In plant cells, V-ATPases are responsible for deacidification of the cytosol and the energization of the secondary transport processes across the tonoplast. So they may be involved in regulation of the response to abiotic stressors, particularly drought. In this study, a cotton vacuolar H⁺-ATPase subunit A (*GhVHA-A*) gene was functionally characterized, especially with regard to its role in drought stress tolerance. The expression analysis revealed that *GhVHA-A* was differentially expressed in various cotton tissues, and was induced by drought, high salinity, low temperature, and abscisic acid treatment in leaves. Transgenic tobacco expressing *GhVHA-A* exhibited enhanced drought resistance, a lower leaf water loss rate, higher average root length, higher proline content, higher superoxide dismutase (SOD) and peroxidase (POD) activities, and lower malonaldehyde levels under drought stress. These results suggest that the overexpression of *GhVHA-A* in tobacco conferred drought-stress tolerance by enhancing osmotic adjustment (proline) and the activities of anti-

oxidant enzymes (SOD and POD), thereby enhancing ROS detoxification. Our results have identified *GhVHA-A* as a candidate gene for improving drought tolerance in plants.

Keywords: Cotton (*G.hirsutum*), Vacuolar H⁺-ATPase gene, Gene expression, Drought tolerance

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Relationship between flavonoid metabolism pathway genes and resistance to *Fusarium wilt* of *Gossypium barbadense* L

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The flavonoid pathway plays an important role in plant growth and disease resistance. This study selected resistant and susceptible parent strains, resistant and susceptible lines of transcriptome sequencing and DEG for a total of 6 materials; using real-time fluorescence quantitative (qRT-PCR) method to analysis the expression feature of the enrichment of flavonoid metabolism pathway genes between resistant materials (06-146, Egyptian cotton2, DJ-07-136-5917) and susceptible materials (Xinhai14, Hai92-3, Egyptian cotton 424). The results showed : RNA-seq analysis found sample resistance differences of ultra high resistance, high resistance, susceptible, susceptible and flavonoid metabolism pathway were signifi-

cantly related, and significantly associated with the resistance of cotton is consistent; DEG analysis showed that the resistance of flavonoids metabolism pathway is related genes expression ,which is significantly higher than that of susceptible varieties. The expression characteristic of 8 key genes: ANR (BAN), CHI (TT5), ANS (TT18), CHS (TT4), DFR (TT3), TT7-1 (F3'H), C4H FLS in flavonoid metabolism pathway were analyzed using qRT-PCR, found in 40 h after inoculation, the expression level of 8 genes were significantly higher than that of susceptible materials in resistant materials. In addition to 4h, the expression of CHI gene in resistant materials was significantly higher than that of susceptible materials, in 18 h after inoculation, the expression level of 44 and 35 was the highest. The DFR gene expression of 4 h, 10 h, 28 h, 40 h in the resistant materials was significantly higher than that of the susceptible materials. The expression of TT7 gene was not significantly changed in the susceptible materials, and the expression of the latter was significantly increased in the resistant materials. It is speculated that CHI, DFR and TT7 may be related to resistance to *Fusarium Wilt* of *Gossypium barbadense*.

Key words: *Gossypium barbadense*, *Fusarium wilt*, Flavonoids, Expression analysis, RNA-seq

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Quality of raw materials is strategic to increase sustainability of craft beer production

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Craft beer is perceived by consumers as a drink that differentiates itself from industrial beers in function of its quality (genuineness) and for the absence of preservative treatments such as filtration or pasteurization. Thus, it appears strategic for craft brewers to utilize high quality raw materials selected for increased wholesomeness and sustainability. In this context, the main aim of our recent works was the production of a zero-mile craft beer by using local yeasts and local-cultivated cereals. In particular, the objectives of this specific work were related to the evaluation of the quality of cereals and malts cultivated and produced in Tertenia (Sardinia, Italy) by a local brewery in 2012 and 2013. To do this, the microbial composition (fungi, bacteria and yeasts) of kernels and malt of Senatore Cappelli durum wheat and Scarlett barley cultivars was determined. Subsequently, mycotoxins contamination was evaluated in both kernels and malted cereals. In both the years, reduced contamination by filamentous fungi of the genus *Fusarium*, able to produce mycotoxins of the trichothecene group, was observed in all the analyzed samples with the exception of barley kernels in 2012. In agreement,

small quantity of deoxynivalenol (DON) and T2-HT2 were identified in all the samples. Besides the filamentous fungi, 35 different bacterial isolates and 33 yeast isolates were identified by classical and molecular methods. Among bacteria, not harmful and pathogenic bacteria was detected in all analyzed samples. Gram+ bacteria were isolated among which *Bacillus pumilus* is of particular interest because it has been already used in a biofungicide active against

P100

Validation of Reference Genes for Gene Expression by Quantitative Real-Time RT-PCR in Stem Segments Spanning Primary to Secondary Growth in *Populus tomentosa*

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The vertical segments of *Populus* stems are an ideal experimental system for analyzing the gene expression patterns involved in primary and secondary growth during wood formation. Suitable internal control genes are indispensable to quantitative real time PCR (qRT-PCR) assays of gene expression. In this study, the expression stability of eight candidate reference genes was evaluated in a series of vertical

stem segments of *Populus tomentosa*. Analysis through software packages geNorm, NormFinder and BestKeeper showed that genes ribosomal protein (RP) and tubulin beta (TUBB) were the most unstable across the developmental stages of *P. tomentosa* stems, and the combination of the three reference genes, eukaryotic translation initiation factor 5A (eIF5A), Actin (ACT6) and elongation factor 1-beta (EF1-beta) can provide accurate and reliable normalization of qRT-PCR analysis for target gene expression in stem segments undergoing primary and secondary growth in *P. tomentosa*. These results provide crucial information for transcriptional analysis in the *P. tomentosa* stem, which may help to improve the quality of gene expression data in these vertical stem segments, which constitute an excellent plant system for the study of wood formation.

P101

Valorisation of olive stones used to produce an extract that inhibit the growth of *Botrytis cinerea* and *Fusarium oxysporum*

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As part of the development of valorisation systems for olive mill waste generated in Chile, which allows the use of alperujo, leaves, process waters, and

olive stone, in biological and thermochemical conversion processes, the present work shows the results of extracts obtained from the olive stones. Some of these extracts showed an inhibitory effect on *Botrytis cinerea* and *Fusarium oxysporum*, which represents an alternative to recover compounds of interest prior to the energy utilization of this residue.

The olive stones were collected in olive mills located in the Maule region, and this samples were subjected to a process of aqueous extraction with ethanol, and where different temperatures and extraction times were evaluated, to separate solids and concentrate in rotavapor, and to filter to 8 μm and 0.22 μm . The obtained extracts were evaluated by an in vitro technique, using Petri plates with PDA medium inoculated with strains of *Botrytis cinerea* and *Fusarium oxysporum*. These strains were selected because of their negative effect over crops in the region. Growth of the halo was observed for 72 hours and where antifungal extracts were identified over the evaluated strains. An important finding was the detection of *Trichoderma asperellum* and which acted as a complement to the antifungal effect of the extracts evaluated.

Based on the experiments, an extract was obtained with a capacity to totally inhibit the growth of *Botrytis cinerea* and *Fusarium oxysporum*, with a null growth after 72 hours of incubation compared to the control without addition of extract. An important aspect of the extract preparation process was to work at temperatures below 45°C, since at higher temperatures the antifungal effect was significantly decreased. The research work continues to progress through evaluation in tomato seedlings for

in vivo evaluation of the extract.

P102

A cottodrought-induced heat shock proteins70 geneGhHSP70confers drought stress tolerance in tobacco

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Plants are exposed to various abiotic stresses, such as drought, high salinity and freezing, which disturb the water balance of cells, affecting plant growth and, thereby influencing crop productivity. Genes functioning in plant responses to abiotic stress are essential for the development of abiotic tolerant crops. The 70-kD heat shock proteins (HSP70s) are highly conserved molecular chaperones that play essential roles in cellular processes including abiotic stress responses. GhHSP70 was isolated and characterized from a strong drought-resistant cotton variety 'KK1543'. Real time RT-PCR analysis indicated that GhHSP70 was induced by abscisic acid (ABA) and abiotic stresses, such as PEG, 4°C

P103

Targeted mutagenesis in cotton (*Gossypium barbadense* L.) using the CRISPR/Cas9 system based on GbU6 promoters

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Cotton play crucial roles in our daily life and the world economy. The widely cultivated cotton cultivars are allotetraploid species, including upland cotton and sea island cotton, which consist of two set of subgenomes, "A subgenome"and "D subgenome". The complex genome feature of allotetraploid cotton presents a big challenge for cotton genes functional analyses.

Extensive studies have shown that high-frequent creating DNA double-strand breaks (DSBs) in desired gene sites is a reliable approach to induce gene mutations. The type II RNA-guided CRISPR/Cas9 system has been recently proven to be effective for targeted gene editing in a wildly range of organisms, including human cells, mice, zebrafish, *Arabidopsis thaliana*, *Nicotiana benthamiana*, maize, wheat, rice and cotton (*Gossypium hirsutum* L.). Here, we report the application of CRISPR/Cas9-mediated targeted mutagenesis in cotton (*Gossypium barbadense* L.).

Firsly we cloned four U6 promoters with high-level transcription activity from

sea island cotton and then the Cas9-GbU6-sgRNA vectors specific for GbGGB and GbERA1 were done. The activity of the CRISPR/Cas9 system based on two GbU6 promoters were verified in cotton protoplasts respectively. The vectors were transformed into cotton protoplasts, and the genomic DNA of transformed protoplasts was extracted after 16 h of incubation in darkness at room temperature. A restriction enzyme PCR (RE-PCR) assay was used to detect mutations in the targeted genes. The results confirmed that the two Cas9-GbU6-sgRNA vectors could both induce targeted mutagenesis. Sequence analysis revealed that most of the mutations were nucleotide substitutions, with one nucleotide insertion found in GbERA1 and one deletion found in GbGGB.

P104

Impact of zerovalent noble metal nanoparticles on plants

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Engineered nanoparticles have become components of a wide range of commercial products. Besides indisputable beneficial properties of nanomaterials, their environmental impact is of great concern since they may accumulate in the environment almost unchanged for many years. Thus,

nanoparticles may trigger so far unknown processes upon their interaction with animal and plant cells. The family of noble metal nanoparticles (NMNPs) has been poorly studied so far. Some of the NMNPs (e.g. Ag) revealed toxicity at relatively low concentrations towards plants. Regarding phytotoxicity, some studies also indicate, that the particle size and specific surface area are more important attributes determining phytotoxicity than the nominal NPs concentration. Our study focuses on these properties of NMNPs in relation to plant growth and development, as well as defence capacity to phytopathogens.

Metal zerovalent NMNPs synthesized by unique preparation method were used in experiments. NMNPs differed in size, shape, and concentrations and their effects on *Arabidopsis thaliana* and *Brassica napus* were studied on both cellular and whole plant levels. Preliminary results of the effect of NMNPs on plant physiological parameters, cytological changes, and defence responses are presented. In addition, the influence of NMNPs on phytopathogens will be reported as well.

P105

Is there a role for dedicated PAT-concepts for microalgae?

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High-value added compounds from microalgae are rapidly gaining importance for a wide range of applications, including the pharmaceutical, nutraceutical and cosmetic sector. Similar to other fields with high demands to quality and safety, the associated production methods need to be well controllable, robust, and, in line with the emerging PAT and QbD initiative, should be based upon a sound understanding of the critical process attributes.

Microalgae bioprocesses at this come with unique demands not only to process design but also to on-line/at-line monitoring and post-hoc data analysis routines. Light-dependent pigmentation, substantial variations in cell morphology as well as remarkable changes in the intracellular structural/compositional configuration during the process are among the factors that differentiate microalgae cultures from classic microbial systems, and render the application of conventional monitoring concepts difficult.

Here, the role of microalgae-dedicated PAT concepts is discussed, with special emphasis on on-line-capable biomass sensors. Currently, the impact of several factors with influence on the measurement sig-

nal (chlorophyll and lipid content, morphology, synchronized cell division, cell viability, culture aeration) is only poorly described, and requires further elucidation for meaningful interpretation. Only in combination with orthogonal methods can the actual status of the culture be reliably assessed, and used as a basis for feedback-guided control operations.

P106

Structure elucidation of active metabolites of the marine-derived endophytic *Streptomyces* sp. GSBNT10

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The secondary metabolites study of the selected marine strain *Streptomyces* sp.GSBNT10 started by chromatographic analysis (PLC and HPLC) of the ethyl acetate crude extracts followed by molecular structures characterization by mass spectrometry and NMR spectroscopy.

Using agar state fermentation, three pure bioactive molecules were obtained from the cultured strain GSBNT10, chemically characterized as members of the actinomycins family (reported to be produced by different species of the genus *Streptomyces*) and identified as: Actinomycin D,

Actinomycin X2 and an Actinomycin D analogue.

The isolated compounds exhibited bioactivities against almost all the tested organisms including Gram negative and positive bacteria. The studied strain GSBNT10 was able to produce actinomycins on all the tested carbon sources.

In conclusion, these studies suggest that marine algae-actinobacteria associations are a particularly promising group from which novel metabolites can be elicited. The isolation of strains with antimicrobial activity indicated that marine seaweeds may represent an ecological niche, which harbors a largely untapped microbial diversity and a yet unexploited potential for new secondary metabolites.

Key words: Streptomyces, agar state fermentation, structure elucidation, marine algae-actinobacteria association

MW4

miwelt: the principle of exponential growth

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'Exponential growth' of microorganisms implies that a single mother cell divides into two new daughter cells, these two cells into four, four into eight etc. The system accelerates due to the constant

doubling, so that biomass increase becomes quicker and quicker over time.

miwelt is a science communication project initiated under the Agora scheme of the Swiss National Science Foundation (SNSF). Scientists, artists and journalists have jointly developed illustrated materials, thematic excursions, and laboratory experiments on the subject of microbial biotechnology for children from the age of 7 to 11.

As part of the miwelt project, scientists have been encouraged to explain both the content of and methods used in their everyday work to children and their families as well as to teachers who have only a basic grounding in science. For this purpose, they are aided by illustrations and analogies with everyday life, produced by a professional illustrator and journalists engaged in dialogue with the scientists.

For more information visit: www.miwelt.net

P107

Production of butanol using lignocellulosic material: selection of high tolerant strains; development of mutagenesis protocol

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Butanol can be produced by different solventogenic clostridia from renewable material during the process of acetone-

butanol-ethanol (ABE) fermentation. Lignocellulose complex containing material for example different kinds of food, agricultural or municipal waste can be used as a feedstock after digestion of cellulose and hemicellulose in such materials into fermentable saccharides. Obtaining saccharides from lignocellulose complex is usually performed in two steps including physico-chemical pre-treatment and enzyme hydrolysis of cellulose and hemicellulose. Main advantage of the use of clostridia is their capability to ferment pentoses, hexoses or disaccharides which may be present in the hydrolysate. On contrary, the main disadvantage of their application is their sensitivity to different types of inhibitors which may be generated from cellulose, hemicellulose and lignin during the hydrolysis. To obtain clostridial strains with high tolerance toward fermentation inhibitors, various adaptation techniques and chemical mutagenesis will be tested. For chemical mutagenesis, ethan methylsulphonate will be used as mutagen; protocol of its use for *Clostridium beijerinckii* strains will be developed and mutants will be screened based on the tolerance toward selected inhibitors.

P108

Zero-waste biorefinery of microalgae for production of biodiesel, bioethanol, bio-char and bio-oil

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Microalgae are one of the most promising biomass for production of biofuels and chemicals compared to other biomass including lignocellulosic biomass, microalgae have many advantages as a feedstock for biorefinery due to their high growth rate and lignin-free characteristics. In order to utilize microalgae biomass for commercial production of chemicals and fuels, all components of microalgae biomass should be completely utilized to enhance technological feasibility. Herein, we developed integrated biorefinery process. The lipid of microalgae was converted to biodiesel, in dimethyl with 89% yield. The carbohydrate after lipid extraction was saccharified and fermented for bioethanol fermentation with 80% yield. The residual biomass after removal of lipid and carbohydrate was converted to bio-oil and bio-char. Microalgae lipid was also transformed into bio-insulating oil.

P109

Flow cytometry as a convenient tool for process control during utilization of lignocellulosic biomass to butanol

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Flow cytometry offers a unique tool for evaluation of microbial culture condition. It can be applied in a wide range of applications from general microbiological studies to biotechnological processes control. One of the most frequent applications is determination of cell viability; but more detailed insight into cell physiology is possible as well. The main obstacle of employing flow cytometry in microbial cultures analysis lies in enormous variability of microbial physiology and thus nearly impossible generalization of given protocols. Such an example represent solventogenic clostridia that for a long period resisted to an effort to set suitable staining protocol for distinction of live and dead cells. Clostridia, promising producers of biofuels from lignocellulosic waste, undergo during acetone-butanol-ethanol fermentation various morphological as well as physiological stages, each with distinct staining pattern. We have developed rapid, simple and reliable protocol for *Clostridium beijerinckii* viability measurement, based on combined carboxyfluorescein diacetate and propidium iodide staining [1]. Moreover, this fluorescent probes combination can provide information about

number of spores in culture when used in connection with light scatter characteristics. This methodology was used for a routine control of culture status during acetone-butanol-ethanol fermentation.

References:

[1] Kolek J, Branska B, Drahokoupil M, Patakova P, Melzoch K. Evaluation of viability, metabolic activity and spore quantity in clostridial cultures during ABE fermentation. *FEMS Microbiol Lett* 2016; **363**(6): fnw031. doi: 10.1093/femsle/fnw031

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P110

Scale up of bio/copolymers production using *Cupriavidus necator* (DSM 545)

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Petroleum derived polymers are commonly being used in society due to their low cost and durability. However these are invariably non degradable in nature and are produced from disappearing non renewable resources (fossil fuels). It has been indicated the bacteria has a distinct capability to accumulate polymers (Poly hydroxyl butyrate) which are biodegradable and also have similar properties as petroleum

derived polymers. However the cost of production is higher than the petroleum derived plastics and therefore vigorous research investigations are being directed to cut down the production cost by using cheaper substrates, such as glycerol.

Successful attempt was made to scale up the "PHB production process" from 7L to 300L bioreactor for the batch cultivation of *C. necator*. Several scale up criteria (P/V, Tip speed, Reynold's no, mixing time etc) were used to successfully translate the results obtained in 7L bioreactor and P/V turned out to be suitable criteria for successful scale up to obtain same yields & productivity in two scales of bioreactors.

A maximum biomass (10.98 g/L) and PHB (8.12 g/L) was obtained at the end 54h during the batch cultivation in 300L bioreactor which was approximately equal to the biomass / PHB obtained in 7L and 70L bioreactors during batch cultivation.

However PHB as such is brittle in nature, therefore the derivatives of PHB (co-polymers) are produced by the addition of different electron acceptors (acetic, butyric, valeric acids) at different feeding intervals during batch cultivation for improvement of physico-chemical properties such as molecular weight, elasticity, hardness etc. To address above problem, valeric acid was added after adequate growth of *C. necator* in the bioreactor (22h) was achieved. Addition of valeric acid concentration (0.5-10g/L) its feeding interval (6-12h) was thereafter statistically optimized in batch cultivation, which featured (predicted) a biomass PHA & maximum HV concentration 8.03g/L, 4.76 g/L & 0.448g/L with optimum feed of 0.665g/L valeric acid initiated at 22h followed by subsequent addition

at an interval of 6.24h.

The result of above statistical optimization was validated in batch cultivation (3.5L bioreactor) which exhibited a biomass, PHA and HV concentration of 10.18g/L, 6.32g/L and 0.655g/L at 57h. Successful attempt was then made to scale up the "PHB copolymer production" from 3.5L to 70L bioreactor (using P/V as scale up criteria) for the batch cultivation of *C. necator*. It was possible to reproduce the results of 3.5 L bioreactor with respect to biomass & PHB-V production in 70 L bioreactor.

Key words: PHB, PHB-V, *C.necator* DSM 545, batch cultivation, scale up

EB5

Separate carbon and nutrients removal from sewage: Making the concept reality

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Should our societies develop in a sustainable way, the concept of water management in urban environment needs to be substantially revised. This is true both for developing societies, where the building of traditional water management systems is prohibitively expensive, as well as for more developed societies, where operation and maintenance of the current systems is becoming uneconomical. Moreover, tradi-

onal water management implies high water and energy consumption which in turn causes overexploitation of natural resources in many countries all over the world.

It has been shown in many municipal wastewater treatment plants (WWTP) that the cost of energy is by far the highest operational cost in municipal wastewater treatment. Of all energy consumed at conventional WWTP, approximately 50% is consumed by the aeration in activated sludge tank with nitrogen removal (i.e. nitrification) being the major oxygen sink. Therefore, the main effort is focused on the minimal use of aerobic processes (activated sludge process, aerobic biofilters), maximal utilization of anaerobic digestion and application of low energy demanding methods for nutrients (N and P) removal. As anaerobic processes do not provide opportunity for nitrogen and phosphorus removal, carbon and nutrient removal must be separated in the new wastewater treatment concepts.

This presentation will review the most advanced concepts for separate carbon and nutrient removal. Special attention will be paid to energy recovery from wastewater through direct anaerobic digestion and bioflocculation. As traditional nitrogen removal implies high consumption of organics and energy, we will describe current achievements in energy efficient autotrophic processes such as autotrophic denitrification and nitritation-anammox. We will also discuss the most important challenges and explain, how these challenges can be approached.

MAB9

Screening cyanobacteria for novel compounds with potential anticancer activity

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Cyanobacteria are a well-established source of potential pharmaceuticals. Due to the involvement of non-ribosomal peptide synthetases and polyketide synthetases they are capable to produce thousands of variants of diverse chemical scaffolds. Except for compounds with antibacterial, antiviral and antifungal activities, compounds with cytotoxic and cytostatic effects are frequently found in cyanobacteria and are of special interest because of their anticancer potential. Some of these chemicals have already found their way on market, some of them are in clinical trials. An advantageous feature of a candidate anticancer compounds is their ability to induce apoptosis, a regulated cell death. We have performed a screening for novel pro-apoptotic inducers in crude cyanobacterial extracts via the detection of activities of caspases 3 and 7, the key apoptotic enzymes. Additionally to this main screening parameter, we have monitored cell morphology alteration and cell division rate by time-lapse microscopy as an auxiliary criterion. We have found out that the combination of these methods can bring high rate of positive hits for future work in characterization of novel bioactive compounds. Finally, we have isolated a novel chemical scaffold, nocuolin A, which is a

natural oxadiazine with promising anti-cancer activity *in vitro*. In the first part of this talk examples of pharmaceutically important cytotoxic/cytostatic compound originating from cyanobacteria will be given. Secondly, the pitfalls of the screening for novel pro-apoptotic inducers from crude natural extracts will be discussed. Finally, examples on novel compounds with anti-cancer activity will be introduced.

Pilot screening of natural samples may be intricate due to sample complexity. We tested cyanobacterial extracts for their ability to enhance Casp3/7 activity, inhibit proliferation, and cell metabolism in human pancreatic tumor cells PaTu 8902. The majority of extracts inhibited cell division, but this was only partly reflected by a concurrent MTT viability measurement. The time elapsed by the end of the measurement affects the cell number differently in

treated and control cells. The resulting cell counts greatly influence the evaluation of the Casp3/7 assay, since we obtained substantially different results when evaluating primary luminescence data (3 hits) as opposed to when the actual cell number was taken into account (23 hits). Based on the fact that crude extracts manifest miscellaneous effect including cytostatic activity, it is necessary to couple Casp3/7 assay with cell count measurement. The crude extract of *Nostoc* sp. CCAP1453/38 and its active fraction (proapoptotic metabolite nocuolin A) were used to demonstrate the validity of our approach since its effects was detectable only when normalized to cell number. We demonstrate that the Casp3/7 luminescence assay is useful for apoptotic inducer screening from cyanobacterial extracts and present amendments which help deal with the drawbacks of the method.



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

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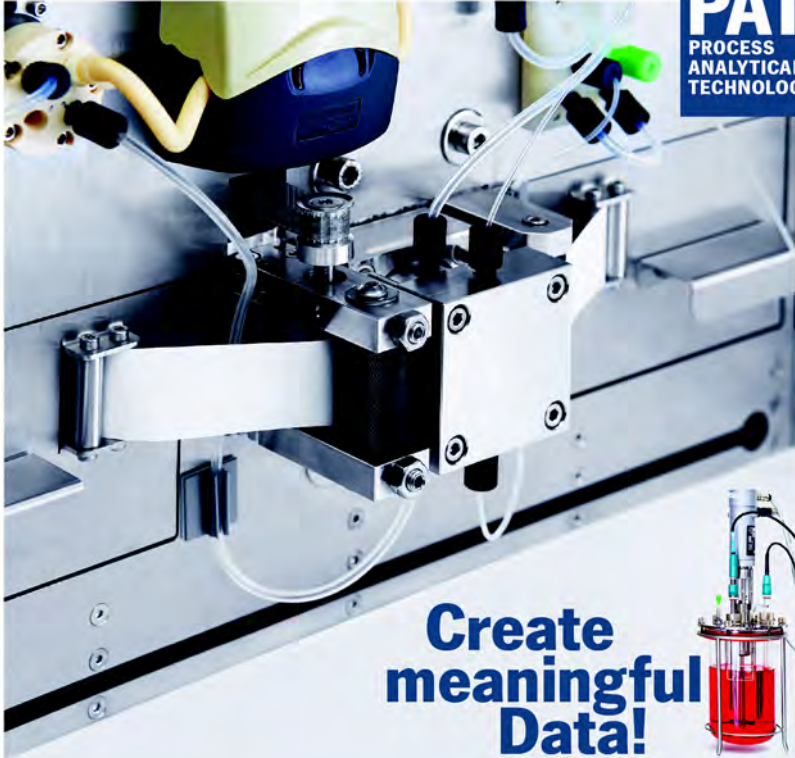
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