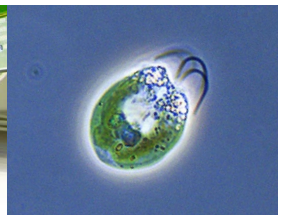

The 2nd Nordic Algae Symposium 2019 – NAS19

27th February 2019
in Thon Hotel Bristol in Oslo, Norway

Collected abstracts
of presentations and posters
before the meeting

To be published

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ABSTRACTS OF ORAL PRESENTATIONS

Folvengaard as – NIBIO's microalgae pilot farm

Dag Hjelle
Folvengaard AS, Norway
dag.hjelle@folvengaard.com

Folvengaard as (Folven farms as) in Stryn is NIBIO's pilotfarm for integrating microalgae technologies into the agricultural sector in Norway as documented in the Co-operation Agreement signed by the two parties 2 November 2017.

Folvengaard as cooperates with neighbouring farms and research institutions to develop new technologies and production lines based on local resources. The main resource in this area is large quantities of clean fresh water in glaciers, rivers and mountain lakes. So far one local owned hydroelectric powerplant is built together with a high capacity water distribution system. There are plans for one more hydropower station. If that is built, the local hydroelectric production will reach 25 to 30 GWh per year. Furthermore, one biogas plant is being built at the neighbouring farm. Based on grants from the Regional Research fund VEST, County Agriculture authorities and the maritime research fund in Sogn og Fjordane County, Folvengaard now participates in development plans for:

- fresh water fish farms based on arctic char
- biogas production based on manure from milk and meat production lines and
- pilot scale bioreactors for fresh water microalgae biomass for production of proteins to be opened 13 June this year at an event back-to back with the A2F mid-term meeting

Folvengaard have for many hundred years produced milk and meat based on cattle, sheep and goats. The last century, the main products have been milk and meat from cows and cattle. All activities are done in concert with a continuously updated development plan where all local resources are mapped and possible new ways to use these resources are analysed.

Based on this work Folvengaard as has developed a plan and a system to link these production lines together in such a manner that the overall production in the system is increased in a sustainable way caused by less loss of nutrients, more efficient energy use and less emissions of CO_2.

In the Folvengaard system, the different production lines are linked together in a grid of underground pipes to transport, water, gas, electricity, heat energy and manure / nutrients, as well as data. To make the system work efficiently, Folvengaard as has developed an information architecture that will create a virtual representation of the physical production system. Datasets from sensors and effectors will be used to monitor and operate the system. A pilot microalgae production line is integrated in the physical system, and in the virtual system and is part of the Algae to Future research project.

The development plan is divided into four phases of which phase I and II are under construction and are planned to be operative in May 2019. The pipeline grid will be completed in October 2019 and planning of phase III will start this year. The information system is under construction and will be based in a large secure datacentre (Lefdal Mine Datacentre) and made available as a commercial service for other biological production pipelines in the near future.

From lab scale to pilot factory: How to navigate through an ocean of bioreactors?

Maren Gagnat
C-Feed AS
maren@cfeed.no

CFeed AS has since 2014 taken the step from being a laboratory scale, start-up company into building a 900 m^2 pilot factory in Vanvikan, from where we supply copepods (*Acartia tonsa*) to the global aquaculture market. Microalgae play a vital role in our production as the main source of food for the copepods, and at the moment we have an algal biomass production of more than 2 kg dry weight per day. With plans of substantially increasing the production further in 2019 and 2020, finding the perfect bioreactor solution has been of high priority the last couple of years. Different designs on the market is plentiful and the investment costs are often high, so deciding what solution to go for can be difficult. The performance of *Rhodomonas baltica*, the most sensitive of the algal species in our production, has therefore been tested on a range of different air lift columns, flat panelreactors and tube reactors the last few years, with the aim to determine which would give the highest total volumetric productivity in a continuous harvesting system while at the same time being easy to operate and clean.

Challenges in macroalgae production - from gametophyte to product

Nikolai Buer
Lofoten Blue Harvest
nikolai@lofotenblueharvest.com

Seaweed seedlings have a tough start in life, not only are there upwards of 200 of them pr. meter rope, but in Lofoten there is little light and very cold water. Visible growth doesn't show until early March. Later in the season the conditions change radically with huge amounts of fresh water runoff from the mountains and 24-hour daylight. Harvesting the seaweed crops require methods and equipment for handling the seaweed efficiently yet carefully so that quality is preserved. Once the seaweed is harvested there is a race against time to get it transported to land and processed as quickly as possible.

For us, and most other producers, there are only two options, freeze it or dry it, and both are expensive and require further product refinement. Every step of the value chain introduces new challenges and every season is different as we develop our production, which makes seaweed cultivation one of the most exciting businesses to be in.

Microalgae – turning a new page in aquafeed development

Kiron Viswanath and Mette Sørensen
Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway
kiron.viswanath@nord.no

Microalgae can be cultivated to produce biomass for aquafeeds. The continued growth of the aquaculture sector offers vast opportunities for employing microalgae-derived ingredients – either whole algae or their co-products can be replacers of currently employed marine/plant aquafeed ingredients. As biomass from different biorefinery streams become available, their nutrient profile and bioavailability should be evaluated prior to their commercial adoption. Microalgae are rich sources of macro- and micro-nutrients as well as functional compounds, and various microalgae species are currently being evaluated as aquafeed components. These include *Nannochloropsis, Tetraselmis, Scenedesmus* and *Phaeodactylum*. Market adoption of microalgae-containing feeds requires demonstrable improvement in fish growth and health. A recent industry report claimed algal-oil fed Atlantic salmon as a premium food product. Microalgae rich in protein can serve as substitute to other protein ingredients in aquafeeds, including fish meal. The global aquafeed production at present is about 40 million tons and even at a modest inclusion level of 5% in salmon and shrimp feeds, the estimated demand for microalgae meal would be 500,000 tons. Industrial production of microalgae has to grow to meet the demands of the aquafeed sector that is willing to welcome the new feed ingredient.

Algae – cancer medicine of tomorrow?

Bjørn Klem
Oslo Cancer Cluster Incubator, Oslo, Norway
bk@occincubator.com

Algae ingredients are utilized for a number of indications in the nutrition and pharmaceutical industry, including use as drug carrier and anticancer agents. The NorAqua consortium will explore the biotechnological potential of high-value fine chemicals and encourage establishing spin-offs from this research. Researchers and start-ups need support to get access to facilities, funding and advice to move start-ups forward.

Oslo Cancer Cluster Incubator facilitates a cancer innovation ecosystem in Oslo Cancer Cluster Innovation Park, closely connected to Cancer Research Institute, OUS Department of Cancer Genomics and Informatics, Cancer Registry and OUS Radium hospital.

This compact innovation ecosystem including academia, education and commercialization has shown promise in accelerating innovations in oncology and generate value for the patients, start-ups and society. Oslo Cancer Cluster Incubator provides infrastructure (e.g. laboratories and offices), funding and mentoring to entrepreneurs and start-ups. Through a national and international network and media platforms, the incubator draws attention to our members from an international audience and can provide them with a range of services. In providing such services Oslo Cancer Cluster Incubator works closely together with local tech-transfer-offices and incubators.

From research to innovation

Kaia Kjølbo Rød
Ard Innovation, Norway
kaia.rod@ardinnovation.no

Ard Innovation is a Technology Transfer Office (TTO) owned by The Norwegian Institute of Bioeconomy Research (NIBIO) end The Norwegian University of Life Science (NMBU). We assist both employees and students with commercial development of research-based ideas, in addition to promote innovation and entrepreneurship in general. This includes a wide range of services like idea-development, project planning, market-analysis, assisting with getting external grants, protection of intellectual property and more. The goal for innovative ideas is to reach the marked, and thereby create impact and values.

Micro- and macroalgae is a resource that has potential to solve nearly all of our future challenges; from the climate crisis and the growing demand for food and animal feed; to novel hydrocarbons and medicines; or the more eccentrics ideas like seaweed furniture and eatable water bottles. The potential for innovations is endless. However, the commercialization path from ideas to market is long and rocky, and most innovative ideas die along the way. What are the common challenges along this road, seen through the eye of a TTO?

A knowledge and technology platform for the establishment of a viable microalgae industry as a part of the new norwegian bioeconomy

Stig A. Borgvang, Hanne Skomedal and Kari Skjånes
NIBIO - The Norwegian Institute of Bioeconomy Research, Ås, Norway
stig.borgvang@nibio.no

With regard to the rapidly growing world population, microalgae is regarded as one of the most promising resources for the sustainable supply of biomass for food and feed applications. Although the use of commercial microalgae for food has been mainly limited to dietary supplements, the recent development of more cost-effective production technology makes it feasible to explore various other food applications. In the project ALGAE TO FUTURE, funded by the Norwegian Research Council, we has established a Consortium of 20 Norwegian and international research and industry partners to approach this topic from multiple angles, merging multiple research fields.

The Vision is to contribute towards a viable Norwegian microalgae industry within 10 years.

The focus of the research is on bioprocess developments linked to lipids, carbohydrates and proteins, where cultivation conditions are used to obtain microalgae biomass with specific nutrient composition targeting specific products. We have chosen to target the development of 3 example products, namely bread, beer and aquaculture feed, that will be produced in a commercial context towards the end of the project. These case studies have been chosen in order to demonstrate the use of algal biomass from various algae species with highly different nutrient composition suitable for different products. The project combines expertise on algae cultivation and optimisation at lab and pilot scales, fish feeding technology, biorefining, bioeconomy, baking technology, broadcast journalism and animation, food quality and safety with the experience of innovative farmer entrepreneurs, professional bakers, brewers and fish-feed producers in a cross-disciplinary manner.

Research in itself will not be sufficient to achieve the said goals of a viable Norwegian algae industry. We need good interactions between researchers, decision-makers, societal organisations/interest groups, commercial interests and consumers, as well as good communication and dissemination channels also to young generations, for together to pave the way for the future microalgae based food- and feed products.

To that end A2F has established:

- A multi-actor Consortium
- A Student-network of Master- and PhD students connected to the project, with natural-science, socio-economic and/or dissemination related activities/tasks
- Webpages:
  - One showing general information about the project: www.nibio.no/prosjekter/algae-to-future-a2f
  - One for the youth who would like to learn more about algae: www.A2F.no
- A Pilot farm for microalgae biomass production in Stryn – Folvengaard as
- A Reference Group (ALGAE TO FUTURE Multi Actor Team, A2F-MAT) consisting of people with varying «green» interests/preferences other people with connections to alternative food and beverages.

Cultivation of *Scenedesmus acuminatus* in an outdoor raceway pond for nutrient recovery from source-separated urine

Aino-Maija Lakaniemi, Sonja Saarnio, Ilmari Laaksonen, Marianna Granatier, Pritha Chatterjee, Praveen Ramasamy, Marika Kokko and Jukka Rintala
Tampere University, Faculty of Engineering and Natural Sciences, Tampere, Finland
aino-maija.lakaniemi@tuni.fi

Approximately 80% of nitrogen and 50% of phosphorous in domestic wastewaters originate from human urine. With the current centralized sanitation systems, the estimated volume of municipal wastewater generated is 150-200 L per capita per day, while the estimated production volume of urine is only 1-1.5 L per capita per day. Utilization of microalgae for the recovery of nutrients from source-separated urine near the source could enable economical and sustainable means for nutrient recycling. Source separation of urine could be a relevant option especially in areas without existing sanitation infrastructure, such as Hiedanranta district in Tampere, Finland, where the city of Tampere is planning a new residential area with up to 25,000 inhabitants. During the last two years, cultivation of *Scenedesmus acuminatus* for nutrient recovery from diluted source-separate urine has been studied in an open raceway pond (2000 L) located in a green house in the Hiedanranta area. Growth of *S. acuminatus* in the urine has been successfully demonstrated in fed-batch and continuous operation modes at pond temperatures ranging from 30 °C (July 2018) to below 5 °C (November-December 2017). The microalgae have removed on average approximately 50% of the nitrogen and 37-38% of the phosphorous present in the urine.

Growth and photo-physiological responses of the green marine macroalga *Ulva lactuca* to light-emitting diode (LED) and fluorescence light

Ralf Rautenberger
Department of Algae Production, NIBIO, Bodø, Norway
ralf.rautenberger@nibio.no

Ulva lactuca, also known as sea lettuce or 'havsalat' in Norway, is an ecologically important marine macroalga, which is rich in amino acids, proteins, carbohydrates and minerals. Under optimal environmental conditions, morphological and physiological traits allow it to grow rapidly with daily rates of 20-30%. Traditionally, fluorescence tubes, which emit light throughout the wavelength-range of the photosynthetically active radiation range (PAR: 400-700 nm), have been used to drive photosynthesis for the formation of biomass. This light source, however, could be soon replaced by more economic, environmentally friendly and flexible light-emitting diodes (LEDs), which are characterised by distinct wavelength ranges in the PAR range.

In this presentation, a study on the photo-physiological responses and growth of a strain of *U. lactuca* from North Norway to four different LEDs in comparison to fluorescence light will be presented. The objective of the study was to understand the potential of LEDs as alternatives to the traditionally-used fluorescence light for the growth of *U. lactuca* and how they influence the photosynthetic performance. This knowledge could be useful for optimised biomass production of *U. lactuc*a as foundation for a bio-based economy in Norway.

Microalgal carbohydrate production and manipulation

Youmiko Sakuragi
University of Copenhagen, Denmark
ysa@plen.ku.dk

Microalgae and cyanobacteria are aquatic photosynthetic microorganisms and produce a large array of carbohydrates as storage (e.g., glycogen and starch) and non-storage carbohydrates (i.e., sucrose and extracellular polysaccharides). Some of these "green" carbohydrates are used as feedstock and health-promoting ingredients in foods, cosmetics, and medicines. We are interested in understanding how the photosynthesis process affects the carbohydrate production, so that we can in the future improve their productivity. We found that the overexpression of a Calvin Benson cycle enzyme, fructose1,6-/sedoheptulose-1,7-bisphosphatase, increased accumulation of non-storage carbohydrates while decreasing that of the storage polysaccharide in the cyanobacterium *Synechococcus* sp. PCC 7002. We also found that a putative histidine kinase (PmgA), involved in the regulation of photosynthetic light acclimation, regulates the glycogen amount through a signal transduction cascade involving a non-coding RNA (PmgR1). These and other findings underscore the existence of yet uncharacterized metabolic and molecular mechanisms that are closely coupling photosynthesis and carbohydrate accumulation. We are also interested in carbohydrate biorefinery using bioengineering of microalgae. We showed that transgenic microalga *Chlamydomonas reinhardtii* secreting a fungal acetylxylan esterase can utilize lignocellulosic biomass as a carbon source for growth. This also led to removal of acetyl esters, well-recognized inhibitors, from the plant biomass, paving the path towards establishing an eco-friendly method to remove acetyl esters and, hence, to improve lignocellulosic biofuel productions.

Energy-efficient cultivation of microalgae

Johan Engelbrektsson, Susanne Ekendahl and Niklas Strömberg
RISE Research Institutes of Sweden
johan.engelbrektsson@ri.se

Microalgae cultivation as a source of biomass or sink for carbon dioxide promises high productivity and limited land use, but attractive as those features are, they come at the cost of energy efficiency. Most problematic is that even with selected high productivity strains and carefully optimized conditions, energy use scales faster than increases in productivity.

We have focused on development of an algal cultivation system for production of bulk chemicals, in which the mixing and harvesting procedures are minimized, thus aiming for energy efficiency first, rather than high productivity.

Several pilot trials in the field show that it is possible to achieve energy efficiency and gain biomass of good enough quality for bulk chemical production by using CO_2 flue gas from the industry. Although relatively large areas are needed, the system is still several times more productive than any other crop and in addition, cultivation can be made on non-arable land as well as in the sea. Thus, energy efficient algae cultivation might become the carbon sink we are looking for to reduce CO_2 emissions from the industry.

Microalgae as true biocatalysts for chemicals production

Yagut Allahverdiyeva and S. Kosourov
Molecular Plant Biology, Department of Biochemistry, University of Turku, Finland
allahve@utu.fi

The photosynthetic machinery of algae and cyanobacteria uses sunlight as energy source and raw materials such as CO_2, water and some mineral nutrients for building up energy-rich organic molecules and O_2. Unfortunately, solar-based suspension production systems have inherent problems: (i) self-shading leading to a low light utilisation; (ii) excessive water consumption; (iii) requirements for energy intensive mixing, (iv) diluted cultures (and product) with short catalytic life-time.

These bottlenecks drastically decrease the economic feasibility of the algal biotechnology and restrict applicability of suspension cultures for the large-scale chemicals production. We are aiming to mimic nature and develop a solid-state production platform consisting of immobilized algal thin films, which act as 'artificial leaf' stimulating efficient conversion of solar energy into valuable bioindustrial compounds.

The system overcomes the bottlenecks of suspension cultures, thus changing a paradigm in algal biotechnology. Strong limitation of cell growth due to entrapment in the rigid matrix allows application of phototrophs as true biocatalysts funneling light energy into the desired products, instead of wasting it to cell growth and metabolism. In other words, in proposed solid-state cell factories energy and carbon flow to product is maximized by uncoupling growth from product synthesis.

Value chain optimization for macro- and microalgae products

Matilde S. Chauton, Inger B. Standal, Rasa Slizyte, Revilija Mozuraityte, Shraddha Mehta and Jorunn Skjermo
SINTEF Ocean AS
matilde.chauton@sintef.no

Aquatic environments are treasure troves of new resources and potentially high-value materials or bioactives, and low-trophic resources such as algae are playing an important role here. With increasing exploitation and development of circular bioeconomy, we focus on developing sustainable industrial production to complement resource harvesting where possible. We see a great potential in products from macro- and microalgae and although there are examples of products on the market, there is a huge potential in finding new applications and developing more products. On the way we not only encounter scientific challenges related to the biology and production technology, but also topics in the other end of the chain, e.g. legislation around use of waste streams and potential applications of the biomass, marketing and consumer acceptance, sustainability and environmental impact, and so on.

The NordAqua consortium cover many topics along the value chain, from academic deep-mining into photosynthesis or bioactivity via biomass production and processing, and further onto industrial production, Life Cycle Analysis and entrepreneurship. It is challenging to cover many topics and scientific fields in one project, but it is also a good way to learn from other areas and identify where we need to focus in order to develop the value chain. We also cross between single cell organisms (microalgae and cyanobacteria) and macroalgae, and this may serve to show where we can exploit synergies and where we must understand the differences between the organisms. As an example, the pigment fucoxanthin is found in many microalgae and in the brown seaweeds, but availability in terms of biomass and processing to get to the fucoxanthin may be different. However, once the pure product is obtained from different sources, it can be used in the same applications.

Here we present some examples of SINTEFs contributions to the NordAqua activities, to illustrate the overall goal of advancing Nordic aquatic photoautotrophs for industrial use in biorefineries and novel cell factories, and hopefully, catalyse processes towards a sustainable and circular bioeconomy.

Optimizing cyanobacteria for bioproduction

Karin Stensjö
Uppsala University
karin.stensjo@kemi.uu.se

One of the major challenges for the future is to develop sustainable methods for producing biofuels. Biofuels will help fight climate change as liquid and gaseous fuels will be needed for ground transportation but also for ships and aviation for many years to come.

Our approach is to use cyanobacteria as a production platform for chemicals. We work towards a direct process-chain from solar energy to designed fuel by using engineered cyanobacteria, thus producing 4th generation of biofuels. One benefit of this approach is to avoid some of the energy consuming steps of traditional biofuels; such as biomass formation, harvesting and extraction. In the lecture I will present advancements and ongoing research activities in metabolic engineering, cell physiology, and synthetic biology; all critical fields to improve cyanobacterial biotechnology.

Our focus has for many years been on the engineering of biosynthetic pathways for metabolites such as alcohols, terpenoids, and molecular hydrogen. Current work also includes bioproduction of alkenes as raw material for jet fuel. Another area in our research portfolio is the improvement of the production host. The ability of the host to sense and respond to variations in the environment is important to avoid accumulation of reactive oxygen species. We have identified a group of ferritin-like proteins that are involved in acclimation to light and reactive oxygen species. The development of well-defined synthetic biology tools for controlled transcription and translation are critical research in many of our projects. Recently we have developed a minimal synthetic promoter for expression of oxygen intolerant enzymes in cyanobacteria.

ABSTRACTS OF POSTERS

Poster 01

Bioprocess development for microalgae with high starch content: potential use in fermented beverages

Giorgia Carnovale[1,2], Maria Barbosa[3] and Kari Skjånes[1]
[1] Norwegian Institute for Bioeconomy (NIBIO), Ås, Norway, [2] Norwegian University of Life Sciences (NMBU), Ås, Norway, [3] Wageningen University & Research, The Netherlands
giorgia.carnovale@nibio.no

Research on microalgae has thrived in the last 30 years and many ideas for sustainable products have been developed but only few of those made it to the market. This is imputable mostly to cost-effectiveness but also to a lack of connection between research and industry.

This PhD project aims to bring together algal biotechnology and industry during the phase of research and development, so that biomass with characteristics specifically tailored to the needs of industrial processes will be produced. Our industrial partner in this task has requested algae pellets with high starch content combined with starch degrading enzymes (temporarily inactivated) and low lipid content.

In this frame, algae would be an active ingredient with properties to be defined as algae malt, used to partially substitute barley malt including starch and its degrading enzymes during the "mashing" process: the moment of brewing in which milled malt is heated in water to activate enzymatic activity and degrade starch into simple sugars available to yeast.

To achieve the requested biomass, algae are subjected to multiple stressors that induce starch accumulation such as high light intensity, nutrient deprivation and temperature. As the maximum starch/biomass ratio is reached, optimal conditions are restored inducing production of starch-degrading enzymes. Degradation of starch happens quickly, thus the correct timing and optimal method for harvesting will have to be optimized.

Experiments on optimal stressor combination are ongoing and will be scaled up to 250L tubular reactors. The species used are the food approved *Tetraselmis chuii* and *Chlorella vulgaris*.

Poster 02

Microalgae for whole-year biomass production on the Swedish west coast

Otilia Cheregi[1], Mats X. Andersson[1], Anna Godhe[2] and Cornelia Spetea[1]
[1] Department of Biological and Environmental Sciences, University of Gothenburg, Sweden, [2] Department of Marine Sciences, University of Gothenburg, Sweden
otilia.cheregi@bioenv.gu.se

Biomass from microalgae can become a sustainable source of energy, food, feed and raw materials. Large scale microalgae cultivation has been considered a practical option in dry and hot regions of the globe. However, ecological records of algae growth show that algal blooms happen in Northern hemisphere, at low temperatures and in low light. In the case of the Swedish west coast, different species/strains of microalgae dominate the cold and the warm season blooms. The seasonal succession of microalgae strains prompts us to propose a rotation culture strategy in which local strains best adapted to each season would replace each other during a whole year cultivation. To reach our aim we developed a screening pipeline targeting 180 species/strains of marine microalgae from the Swedish west coast.

Twenty-one strains of *Skeletonema marinoi* and one strain of *Nannochloropsis* had the best growth in laboratory standard conditions and are under further selection for photosynthetic performance and best biomass/lipid production under simulated environmental conditions for the Swedish west coast.

(Supported by grant 45907-1 from the Swedish Energy Agency.)

Poster 03

Breeding of kelp

Franz Goecke[1], Diogo Raposo[2], Jon Funderud[2], Claire Gachon[3], Gunnar Klemetsdal[1], Jorunn Skjermo[4], Alejandro Buschmann[5], Carolina Camus[5], Trina Galloway[6], Liv Torunn Mydland[1], Margareth Øverland[1] and Åshild Ergon[1].
[1]Norwegian University of life Sciences, [2]Seeweed Energy Solutions A/S, [3]Scottish Association for Marine Sciences, [4]Sintef Ocean, [5]University of Los Lagos, [6]AquaGen A/S
franz.goecke@nmbu.no

So far, seaweed farming in Europe is in its infancy and lacks structured and scientifically sound breeding programs, in spite of the growing demand for seaweed biomass. Under the NFR-project Breed4Kelp2Feed, which aims to initiate kelp breeding in Norway, we established *Saccharina latissima* gametophyte cultures at NMBU, Ås. This species, "sukkertare", is the most commonly farmed kelp in Norway.

In order to run a breeding program, it is vital to be able to cultivate, store, cross and reproduce the organism, as well as to define and evaluate different breeding strategies, including crossing and selection schemes founded on definition ofbreeding goals and relevant phenotyping and genotyping methods. *S. latissima* has a diplohaplontic life cycle, with a large diploid (sporophyte) and a microscopic haploid (gametophyte) stage. In a controlled environment, we are now able to release spores from sporophytes, isolate, clone, and maintain male and female gametophytes, for further crossing experiments.

Poster 04

Cultivation and characterization of several microalgae – from laboratory to pilot scale cultivation

Tomáš Grivalský[1], Karolína Ranglová[1,2], João A. Câmara Manoel[1,3], Gergely E. Lakatos[1], Kumar Saurav[1] and Jiří Masojídek[1,3]
[1]Centre Algatech, Laboratory of Algal Biotechnology, Institute of Microbiology, Třeboň, Czech Republic
[2]Faculty of Agriculture, University of South Bohemia, České Budějovice, Czech Republic
[3]Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic
grivalsky@alga.cz

Microalgae (Cyanobacteria and Eukaryots) grow in diverse environments that often determines production of various bioactive, high-value compounds with potential biotechnological use. Culturing conditions can affect production of these compounds.

Firstly, we aimed to characterize three microalgae strains (filamentous *Cylindrospermum* sp. CCALA 988, and *Nostoc* sp. MACC-612 and single-celled *Chlorella vulgaris* MACC-1) to find out the suitable growth regime in laboratory scale. Thereafter, we cultivated these strains in pilot scale in two open cultivators – thin-layer cascade (TLC) with pump circulation and raceway pond (RWP) with paddle wheel mixing – which were placed in greenhouses to avoid cross-contamination. Practical cultivation feasibility of both units for selected strains were compared. Physiological changes, growth and photosynthetic activity were monitored and evaluated.

All strains showed cytotoxic activity with potential use in pharmacology. For example the cytotoxicity of lipopeptides puwainaphycins and minutissamides produced by *Cylindrospermum* sp. was tested against several bacteria and fungi strains.

These results are important from a biotechnological point of view for potential production of bioactive compounds for agricultural or pharmacological use.

Acknowledgement
This research was funded by cross-border InterReg programme between the Czech Republic and Bavaria (CZ-BV 41) and in part by EU programme Horizon 2020 (project SABANA, grant no.727874).

Poster 05

The Norwegian Culture Collection of Algae, NORCCA

Vladyslava Hostyeva
Norwegian Institute of Water Research, Norway
vho@niva.no

The Norwegian Culture Collection of Algae, NORCCA, maintained and owned by the Norwegian Institute for Water Research (NIVA) and the University of Oslo (UiO), merges algal strains from the two institutes and the former Danish algal culture collection SCCAP.

Nearly established NORCCA comprises more than 2000 strains of prokaryotic and eukaryotic microalgae and gives access to 1078 marine and 996 freshwater strains mainly isolated from Scandinavian lakes, rivers and coastal waters. Algae cultures are increasingly used in studies of algal toxins that have negative impacts on water quality that relate to health issues. In freshwater, this applies primarily to cyanobacteria. Our culture collection includes more than 70 strains of known toxigenic cyanobacteria.

These organisms are suitable for the chemical and biological studies of secondary metabolites such as hepatotoxins, neurotoxins, and substances that produce protracted symptoms of intoxication.

In consideration that microalgae can be used as an alternative source for biobased raw material for various applications within pharmaceutical, cosmetic, food/feed, and biofuels, NORCCA represents a potential resource for researchers around the globe to find out substances of specific relevance and exploit them for the benefit of the growing human population.

Poster 06

Functional genomics of coldwater microalgae

Chris J. Hulatt[1,2], Kiron Viswanath[1], René H. Wijffels[1,3] and Matthew C. Posewitz[2]
[1]Bioscience and Aquaculture, Nord University, Bodø, Nordland, Norway
[2]Dept. Chemistry, Colorado School of Mines, Golden CO, USA
[3]Bioprocess Engineering, Wageningen University, Wageningen, Netherlands

Microalgae are a valuable source of food and feed ingredients with a wide range of possible applications in human health and animal nutrition. Our research investigates the potential of novel cold-adapted microalgae, primarily toward the production of food and feeds. In early experiments we found that by selecting specialized cold-adapted microalgae strains, we could achieve higher growth rates than expected in cooler temperatures. In addition to their growth performance, some coldwater strains can also synthesize valuable metabolites. These products include long-chain polyunsaturated fatty acids (PUFAs) such as eicosapentanoic acid (EPA), carotenoids (pigments and antioxidants), proteins and functional carbohydrates. Here we present our current research that uses molecular methods to explore the metabolism of non-model microalgae. Our approach includes de-novo genome assembly and examination of metabolic pathways. Our genome-scale analysis is complemented by investigation of gene expression and metabolite biosynthesis. We anticipate that this will create new opportunities to investigate a greater diversity of industrially-relevant strains with molecular tools. We will use our data to gain understanding of the evolutionary biology of selected microalgae, and to optimize non-model strains for bioprocesses.

Poster 07

Bioprospecting of microalgae strains as a tool for identification of health protective compounds

Kamila Hurkova[1], Monika Jiru[1], Milena Stranska-Zachariasova[1], Petr Kastanek[2], Jana Hajslova[1]
[1]University of Chemistry and Technology, Department of Food Analysis and Nutrition, Prague, Czech Republic
[2]Ecofuel Laboratories, Ocelarska 9, 190 00 Prague 9, Czech Republic
kamila.hurkova@vscht.cz

Microalgae represent an extremely diverse, yet highly specialized group of micro-organisms. During their long existence under adverse conditions (UV radiation, oxygen deficit, etc.) they have created new effective defense systems and metabolic pathways. Secondary metabolites of microalgae showing various antioxidant, antimicrobial, antitumor and anti-inflammatory effects can therefore be of great benefit in the field of nutrition or food / feed technology and biotechnology. Nowadays, several strains are being industrially used, but unexplored microalgae still exist in nature.

Presented study is focused on bioprospecting of 41 samples of selected microalgae strains (Chromophyta, Chlorophyta, Rhodophyta, Heterokonta and Cyanobacteria). For this purpose, simple and high-throughput extraction strategy based on consecutive extraction with water, aqueous methanol (80:20, v/v) and hexane/isopropanol (50:50, v/v) was developed. Both the biological activity tests (antioxidant activity, enzyme inhibitory assay), as well as the metabolomic fingerprinting using ultra-high performance liquid chromatography coupled to high resolution tandem mass spectrometry (U-HPLC–HRMS/MS), were realized. Specialized software was employed for HRMS/MS data mining allowing sample clustering according to strains and lipid species characterization. Among analyzed samples, seven showed high antioxidant activity (scavenging activity > 50%), two had high elastase inhibitory activity, and one sample highlighted α-glucosidase inhibitory activity (inhibition > 50%).

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Poster 08

Microalgae as a potential source of organic selenium form

Monika Jírů, Diomid Revenco, Richard Koplík, Jana Hajšlová, Milena Stránská-Zachariášová
University of Chemical Technology, Prague, Department of Food Analysis and Nutrition, Czech Republic
jirum@vscht.cz

Microalgae, which are exposed to selenium in the form of selenite dissolved in cultivation medium, are able to incorporate this element to their cells thus organo-selenium compounds (selenocysteine, selenomethionine etc.) with higher biological availability for humans are formed. The aim of the presented study was to evaluate the potential of various strains of microscopic algae (*Chloridella simplex, Vischeria helvetica, Eustigmatos vischeri, Chlorella vulgaris*) to accumulate better available forms of selenium (under conditions simulating human gastrointestinal tract) in a biomass.

To monitor this process, analytical methods based on liquid chromatography coupled with (i) high-resolution mass spectrometry (HRMS) and (ii) inductively coupled plasma mass spectrometry (ICP-MS), were developed. Because majority of the organically bound selenium is present in the form of seleno-peptides and proteins, the enzymatic hydrolysis (*Streptomyces griseus* protease) representing a critical step ensuring cell walls disintegration was used.

To achieve the best recovery (hydrolysis and extraction), three methods of physical cell disintegration were tested utilizing (i) glass beads, (ii) shock freezing and (iii) ultrasonic needle of which the third option provided the best results.

In *Chlorella vulgaris*, higher amount of selenium bounded in amino acids (69 %) was determined. The bioavailability under conditions simulating human gastrointestinal tract was 89 %.

Acknowledgement

This work was supported by the "Operational Programme Prague – Competitiveness" (CZ.2.16/3.1.00/21537 and CZ.2.16/3.1.00/24503) and the "National Programme of Sustainability I" - NPU I (LO1601 - No.: MSMT-43760/2015) and by the Ministry of Education, Youth and Sports, project No LTC17089.

Poster 09

Enhancement of photoautotrophic, efficient, and sustainable H2 production in a *Chlamydomonas reinhardtii flv* mutant

Martina Jokel[1], Valéria Nagy[1], Sergey Kosourov[1], Anna Podmaniczki[2], Szilvia Tóth[2] and Yagut Allahverdiyeva-Rinne[1]
[1] University of Turku, Molecular Plant Biology, Turku, Finland
[2] Biological Research Centre of the Hungarian Academy of Sciences, Institute of Plant Biology, Szeged, Hungary
martjok@utu.fi

One of today's huge global challenges is the development of renewable and sustainable biofuels to cover the future energy supply. Biohydrogen, produced by photosynthetic microorganisms, has the potential to become one of the fuels of the future, since it provides energy without CO2 emission.

The green alga *Chlamydomonas reinhardtii* has the capability to produce H2 via hydrogenases that protect photosynthesis against excess electron pressure following hypoxia. Once the Calvin-Benson cycle is activated, the O2 evolved by photosystem II inhibits the hydrogenases. Other key players of this protective alternative electron transport are flavodiiron proteins (FDPs) which have a similar role as hydrogenases but in the presence of O2 (1).

To date, the most efficient method to sustain H2 production in *Chlamydomonas* is based on nutrient deprivation, which results in the degradation of photosynthetic complexes and reduced O2 evolution enabling H2 production. We recently developed a method in which no nutrient deprivation is involved and a sustained photoautotrophic H2 production is achieved: a train of strong white light pulses is applied interrupted by longer dark phases preventing the activation of the Calvin-Benson cycle (2).

In the present work, we applied this protocol to an *flv* knock-out mutant strain achieving a higher H2 production as compared to wildtype. The H2 production efficiency was compared to another novel H2 production protocol involving continuous illumination and the additional application of an O2 absorbent (3). Our data provide novel fundamental knowledge on the hydrogen metabolism of algae and new opportunities for constructing highly efficient cell factories to produce biohydrogen.

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Poster 10

Engineering of cyanobacteria for production of terpenes

Pia Lindberg
Dept of Chemistry - Ångström, Uppsala University, Sweden
pia.lindberg@kemi.uu.se

Cyanobacteria are ideal host organisms for biotechnological applications converting solar energy into useful energy-rich compounds. Using genetic engineering, we can make directed changes in the genome of model cyanobacteria, introduce new capabilities into the cells, and thereby engineer their metabolism to produce a vast array of compounds suitable for use as fuels or as feedstock for synthetic chemistry.

Terpenes are a large family of hydrocarbon molecules with a wide range of potential applications. We have engineered strains of the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 to accumulate several different terpenes and terpenoids.[1-4] To develop cyanobacterial production of terpenes and other interesting molecules further, we are currently investigating ways to direct substrate in the cells towards product formation. Recent results from pathway engineering guided by metabolic modelling and development of tools for efficient expression in *Synechocystis* will be presented.

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Poster 11

The H2020-project Photofuel: Biocatalytic solar fuels for sustainable mobility in Europe

Peter Lindblad
Uppsala University, Sweden
Peter.Lindblad@kemi.uu.se

Photofuel studies and advances the biocatalytic production of alternative liquid transportation fuels, which require only sunlight, CO_2 and water. Microbial cells directly excrete fuel compounds like 1-butanol, iso-butanol, octanol or bisabolene to the medium from which they are separated, without the need to harvest biomass. This significantly improves the costs and energy balances as only a minimum of nutrients is required for self-replication of the biocatalyst, whilst cell harvesting, drying and lipid extraction is omitted. Such minimum-input systems are compatible with operation on degraded or desert land, which avoids the pitfalls of most of the currently available biofuel technologies. The products are drop-in fuels that fully or partially replace their fossil counterparts without the need for new infrastructure.

Three research groups collaborate in the advancement of the biocatalysts and increased the productivity benchmark to over 100 mg/L solar fuel per day. The best biocatalytic system(s) are up-scaled and operated outdoors in photobioreactors modified for direct fuel separation at a scale of cubic meters. The identification of optimal future fuel blends with a fossil fuel base and Photofuel biofuels as additives, as well as the analysis of performance and emissions in car or truck engines, are evaluated by the oil- and automotive-industry partners. The entire pathway is assessed for environmental and economic performance as well as social acceptance of large-scale production in rural communities and by the consumer.

All results will be combined to a business development plan, which clearly identifies the opportunities but also the challenges prior to an economic fuel production in compliance to the EC Fuel Quality Directive. Photofuel partners are Volkswagen, University Uppsala, University Bielefeld, Imperial College, University Florence, A4F, IFP Energies nouvelles, Neste, Karlsruhe Insitute of Technology, Fiat Research Centre, Volvo and SYNCOM.

Further info: www.photofuel.eu

Poster 12

Creating value from waste nutrients by integrating algal and anaerobic digestion technology

Carole Llewellyn
Swansea University, United Kingdom
c.a.llewellyn@swansea.ac.uk

This talk will give an overview of the INTERREG North West Europe project called ALG-AD. The project involves 11 partners across academia, industrial and policy makers and runs for three years until March 2021. In the project technology is being developed to take excess waste nutrients produced from anaerobic digestion of food and farm waste to cultivate algal biomass. The biomass is being assessed for use as animal feed and for other products of value. Biomass is being screened for niche products using a metabolomics approach for the eventual development of a biorefinery.

Poster 13

Nutrient recovery and improved effluent quality of anaerobically treated blackwater by microalgae biomass production

Melesse Eshetu Moges[1,2], Arve Heistad[1] and Thorsten Heidorn[3]
[1] Faculty of Science and Technology, Norwegian University of Life Sciences (NMBU)
[2] Ecomotive AS, Norway
[3] Norwegian Institute of Bioeconomy Research, Ås, Norway
melesse.eshetu@nmbu.no, arve.heistad@nmbu.no, thorsten.heidorn@nibio.no

The blackwater stream of domestic wastewater contains the majority of nutrients that could be recycled within a circular economy. The integration of microalgae into the treatment of source-separated blackwater (BW) can effectively assimilate and recover phosphorus (P) and nitrogen (N), as well as macro- and micronutrients, convert these nutrients into valuable products (e.g., biofertilizer), and hence close the nutrient cycle. With this objective, a flat panel photobioreactor was used to grow the unicellular green microalga *Chlorella sorokiniana* strain NIVA-CHL176 in a continuous culture at a dilution rate of 1.5 d^{-1}, a temperature of 37 °C and a pH of 7. The results indicate that *C. sorokiniana* can assimilate and recover N and P from a treated source-separated blackwater. The N and P removal rates were 99 mg N $L^{-1}d^{-1}$ and 8 mg P $L^{-1}d^{-1}$ for 10% treated BW and reached 212 mg N $L^{-1}d^{-1}$ and 35 mg P $L^{-1}d^{-1}$, respectively, when using 20% treated BW as a substrate. The corresponding biomass yields on light, N and P with 20% treated BW were 0.37 g $(mol\ photon)^{-1}$, 9.1 g g^{-1} and 54.1 g g^{-1}, respectively.

Poster 14

Towards photobiological isobutene production in *Synechocystis* PCC 6803

Henna Mustila, Peter Lindblad, Pia Lindberg, Karin Stensjö
Uppsala University, Sweden
henna.mustila@kemi.uu.se

Cyanobacteria can be utilized as a platform to produce several types of biofuels. One promising compound to be produced photobiologically in cyanobacteria is isobutene, which could be used as a raw material for renewable jet fuels. In our setup, photobiological production is followed by a subsequent photochemical step in which isobutene will be assembled into longer hydrocarbons. As a gaseous compound isobutene will quickly escape out of the cells without building up as toxic levels in growth medium. Unlike liquid biofuels, volatile isobutene may be collected from the headspace and thus avoid the costly extraction of a chemical from culture medium or from cells.

Our approach is to introduce in *Synechocystis* PCC 6803 an engineered pathway to isobutene via isobutanol. A strain synthetizing isobutanol is already established and used as a host. To convert isobutanol to isobutene, we are testing oleate hydratase (Ohy) activity towards isobutene synthesis. In addition to hydrating fatty acids, OhyA has been reported to be able to dehydrate short alcohols to alkenes such as isobutene and isoprene. We are testing and characterizing four OhyA variants encoded by genes derived from distinct bacterial phyla. In addition, two other putative heterologous pathways for isobutene production are studied.

Poster 15

Nutrient recovery from wastewater by microalgae – Leväsieppari project

Jussi Huotari[1], Lauri Arvola[1], Tiina Tulonen[1], Aino-Maija Lakaniemi[2], Jukka Rintala[2], Jarkko Nummela[3], Annakaisa Elo[3], Jonna Piiparinen[4], Kristian Spilling[4] and Sanni Manninen-Johansen[5]
[1]University of Helsinki, Finland (co-ordinator), [2] Tampere University, Finland, [3] Häme University of Applied Sciences HAMK, [4] Finnish Environment Institute SYKE, [5] Vanajavesi Centre
jonna.piiparinen@ymparisto.fi

Leväsieppari is a 2-year project, which aims to improve the use of waste nutrients by incorporating them into microalgal biomass and applying the collected algal biomass as fertilizer. By taking up the nutrients, algae reduce the treatment costs of wastewater and runoff of nutrients to waterways. Leväsieppari is a part of the Finnish government's key programme Bioeconomy and clean solutions: Breakthrough to a circular economy and adoption of clean solutions funded by the Ministry of Environment.

In this project, the algal cultivation is demonstrated with four different waste streams: treated municipal wastewater (UH), source-separated urine (TAU), landfill leachate (HAMK) and reject water from centralized biogas plant (SYKE). The applicability of the harvested algal biomass as biofertilizer is tested in controlled experiments with pot plants (HAMK). Based on the contents of nutrients and harmful substances (e.g. heavy metals, pharmaceuticals, PCB), analyzed both from the algae biomass and plants, also the potential of algae as stripper of harmful substances is evaluated.

Poster 16

Efficient secretion of a lytic polysaccharide monooxygenase in a fast-growing cyanobacterium

David A. Russo and P.E. Jensen
Department of Plant and Environmental Sciences, University of Copenhagen, Denmark
russo@plen.ku.dk

Cyanobacteria have great biotechnological potential due to their ability to use water as an electron donor, CO2 as a carbon source and light as a primary energy source to express heterologous enzymes. In particular, *Synechococcus elongatus* UTEX 2973 is a novel fast-growing cyanobacterium that has gained attention as a new biotechnological chassis. To establish this strain as a host for heterologous protein production we demonstrate the expression and secretion of industrially relevant *Tf*AA10A, a lytic polysaccharide monooxygenase (LPMO) from the gram-positive bacterium *Thermobifida fusca*.

We generated two cyanobacterial transformants expressing the *Tf*AA10A – one with the native, Sec-targeted, signal peptide (*Tf*AA10A) and a second with a heterologous, Tat-targeted, signal peptide (TorA-*Tf*AA10A) replacing the native signal peptide.

Results showed that the native signal peptide was recognized by the cyanobacteria secretion machinery and virtually all the protein was directed to the culture medium, in an active state, with a secretion yield estimated at 779 ± 40 μg L^{-1}. This proof-of-concept study constitutes the first demonstration of the full secretion of an industrially relevant enzyme in *S. elongatus* UTEX 2973. Cyanobacterial secretion may have applications in whole cell biocatalysis, bioremediation and carbon capture.

Poster 17

Effects of flashing light on microalgal growth

Peter S.C. Schulze[1], Jose M. Fernandez[2], René H. Wijffels[1,3] and Viswanath Kiron[1]
[1]Nord University, Faculty of Biosciences and Aquaculture, Bodø, Norway; [2]University of Almeria, Department of Engineering, Almería, Spain; [3]Wageningen University, Bioprocess Engineering, AlgaePARC, Netherlands
peter.schulze@nord.no

Self-shading of cells within a photobioreactor is a major bottleneck in microalgal production. Recent studies suggested that flashing light could mitigate self-shading as high- intense-light flashes could penetrate deep into a culture and drive photosynthesis of cells inside a photobioreactor. We compared growth responses of *Tetraselmis chuii* and *Chlorella stigmatophora* to flashing light conditions, including frequencies from 1 Hz-1 MHz, light on-off ratios (duty cycles) of 0.005-0.7 and average light intensities of 50, 500 and 1000 µmol s^{-1} m^{-2}. We also compared the oxygen evolution of diluted and concentrated *T. chuii* cultures to the different flashing light conditions.

Photosynthetic rates and growth rates were always similar or less than under continuous light. At frequencies >400 Hz, no differences to continuous light were found regardless of the adjusted duty cycle, light intensity or culture concentrations. Notably, concentrated cultures exposed to low-frequent flashing light (f<10 Hz) had higher rates of respiration than photosynthetic rates. Contrary to our expectation, flashing light did not improve oxygen evolution or growth of microalgal cultures. However, it may be a promising tool to induce intracellular biomolecules that are related to high light stresses (e.g., pigments or fatty acids).

Poster 18

Growth and LC-PUFA production of the cold-adapted microalga *Koliella antarctica* in photobioreactors

Hirono Suzuki[1], Chris J. Hulatt[1,2] , René H. Wijffels[1,3] and Viswanath Kiron[1]
[1.] Faculty of Biosciences and Aquaculture, Nord University, Bodø, Norway
[2.] Department of Chemistry, Colorado School of Mines, Golden, CO 80401, USA
[3.] Bioprocess Engineering, AlgaePARC, Wageningen University, The Netherlands
hirono.suzuki@nord.no

Microalgae are excellent source of long-chain polyunsaturated fatty acids (LC-PUFAs), yet bioprospecting and optimization of strains that are able to grow at low temperatures has only recently been initiated. In this work, the cold-adapted microalga *Koliella antarctica* (Trebouxiophyceae) was cultivated at 15 °C to optimize growth and PUFA production in bubble-tube and flat-plate photobioreactors. The effect of nitrogen starvation, phosphorus starvation, salinity, and light intensity on the growth, fatty acids, and protein content was studied. *Koliella antarctica* exhibited a maximum biomass productivity of 2.37 g L^{-1} day^{-1} after culture optimization, and tolerated a broad range of salinities. Nitrogen and phosphorus starvation strongly induced neutral lipid accumulation, up to 90.3% of total fatty acid, which mostly consisted of the monounsaturated fatty acid C18:1n−9. PUFAs were also abundant and together accounted for 30.3–45.8% of total neutral lipids. Phosphorus starvation was an effective strategy to obtain high total fatty acid yields (mg L-1) while maintaining the protein, total PUFA, and omega-3 fatty acid contents. The strong growth of *K. antarctica* concurrent with its favorable biochemical composition, containing LCPUFAs, could make this strain efficient PUFA producers and possibly offer the opportunity for cultivation in cooler climates or during winter in temperate regions.

Poster 19

Multi-step valorisation of *Aphanizomenon flos-aquae* biomass isolated from the Curonian Lagoon

Michail Syrpas[1], Jolita Bukauskaitė[1], Ričardas Paškauskas[2], Loreta Bašinskienė[1] and Petras Rimantas Venskutonis[1]
[1]Department of Food Science & Technology, Kaunas University of Technology, Lithuania
[2] Laboratory of Algology and Microbial Ecology, Nature Research Centre, Vilnius, Lithuania
michail.syrpas@ktu.lt

Mass proliferation of cyanobacteria can lead to bloom formation with strong financial and environmental impacts. According to recent reports, removal of wild cyanobacterial blooms from the Curonian Lagoon, as a management measure, should be prioritized. Herein, we envisioned the utilization of wild cyanobacteria biomass as a potential source of high-added value products. Towards this end, multistage biorefining schemes based on high pressure techniques were developed.

Recovery of lipophilic products by means of supercritical CO2 extraction (SFE-CO2) was optimized. Central composite design (CCD) and response surface methodology (RSM) indicated 42.5 MPa, 55 °C and 120 min of extraction as optimal conditions, under which SFE-CO2 yielded 4.43 g/100 g DW of non-polar extract characterized by the presence of α-linoleic acid and α-tocopherol. Phycobiliprotein extraction was performed based on several conventional techniques assisted with ultrasound extraction optimized with CCD and RSM. Homogenization followed by application of 8.75 min of ultrasounds at 84% amplitude showed the highest phycobiliprotein yield. The remaining biomass was further subjected to pressurized liquid extraction with increasing polarity solvents. Acetone, ethanol and water extracts were collected and their *in vitro* antioxidant activity was evaluated by various assays. Finally, preliminary phytochemical composition of obtained extracts was assessed by means of UPLC-ESI-TOF-MS.

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Poster 20

Synthetic *in vivo* activation of heterologous [FeFe]-hydrogenase in engineered cyanobacteria

Adam Wegelius, Namita Khanna, Charléne Esmieu, Gustav Berggren and Peter Lindblad
Department of Chemistry- Ångstöm, Uppsala University, Sweden
adam.wegelius@kemi.uu.se

Hydrogenases are a broad class of enzymes that catalyze reversible formation of hydrogen. Among hydrogenases, the [FeFe]-hydrogenase (HydA) is the most active hydrogen producer (1). The active site of [FeFe]-hydrogenases consists of a [4Fe-4S] cluster connected to a [2Fe] subcluster via a cysteine. In nature, biosynthesis of the [2Fe] subcluster requires three hydrogenase specific maturases which assemble the subcluster and transfer it to the apo-HydA protein, already containing the pre-assembled [4Fe-4S] cluster. This maturation process is not fully characterised and has long been an inconvenience for heterologous expression of HydA in cyanobacteria. Synthetic chemistry has been used to create precise mimics of the [2Fe] subcluster (2) and these clusters are able to activate the apo-HydA enzyme *in vitro* (3,4) and also *in vivo* in cells of *Escherichia coli* (5). We have successfully activated an apo-HydA from *Chlamydomonas reinhardtii,* heterologously expressed in the cyanobacterium *Synechocystis* PCC 6803, using a synthetic [2Fe] subcluster mimic. The activated cells exhibit hydrogen production from HydA under a number of different conditions, including both light and darkness, with the highest production recorded under organic nitrogen deprivation.

This provides interesting and valuable insight to the usage of metabolically engineered cyanobacteria as platforms for hydrogen production. Also, it provides a convenient new tool to evaluate recombinant [FeFe]-hydrogenases expressed in metabolically engineered cyanobacteria.

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Poster 21

Protein scaffolding for next-generation cyanobacterial cell factories

Julie Zedler and Poul Erik Jensen
Copenhagen Plant Science Centre, Section for Plant Molecular Biology, Department for Plant and Environmental Sciences, University of Copenhagen, Denmark
jz@plen.ku.dk

Cyanobacteria are versatile photosynthetic microorganisms with great potential as sustainable cell factories. However, there are several major limitations that will have to be overcome to make cyanobacteria a competitive alternative to existing biotechnological chassis. The Cynthetica project, funded by the Horizon 2020 research and innovation programme of the European Union, uses a synthetic biology approach to tackle one of the principal limitations in cyanobacteria: low product yields.

Our goal is to engineer designated locations and compartments for product synthesis within the cell. To achieve this, we are engineering cytoplasmic protein-based structures serving as a scaffold for enzymes of a biosynthetic pathway. We have expressed a structural scaffold protein in two different cyanobacterial species, the widely used model organism *Synechocystis* sp. PCC 6803 and a recently described, fast growing cyanobacterium *Synechococcus elongatus* UTEX 2973. Our preliminary results are promising showing intracellular, *de novo* structures formed *in vivo*. In this presentation we will discuss implications of this technology for the next generation of cyanobacterial cell factories.