BIOTECHNOLOGY

opportunity for intersectoral cooperation and technology transfer

Date: 19th September, 2018 ■ Venue: Vodňany, MEVPIS – Na Valše 207

Interdisciplinary Approach to Microalgae Biotechnology

ALGATECH

Přírodovědecká fakulta
Faculty of Science

JiŚ MASOJÍDEK

Institute of Microbiology of the CAS, Centre ALGATECH, TŚeboŔ

University of South Bohemia, Faculty of Science,

Ļeské BudĲovice

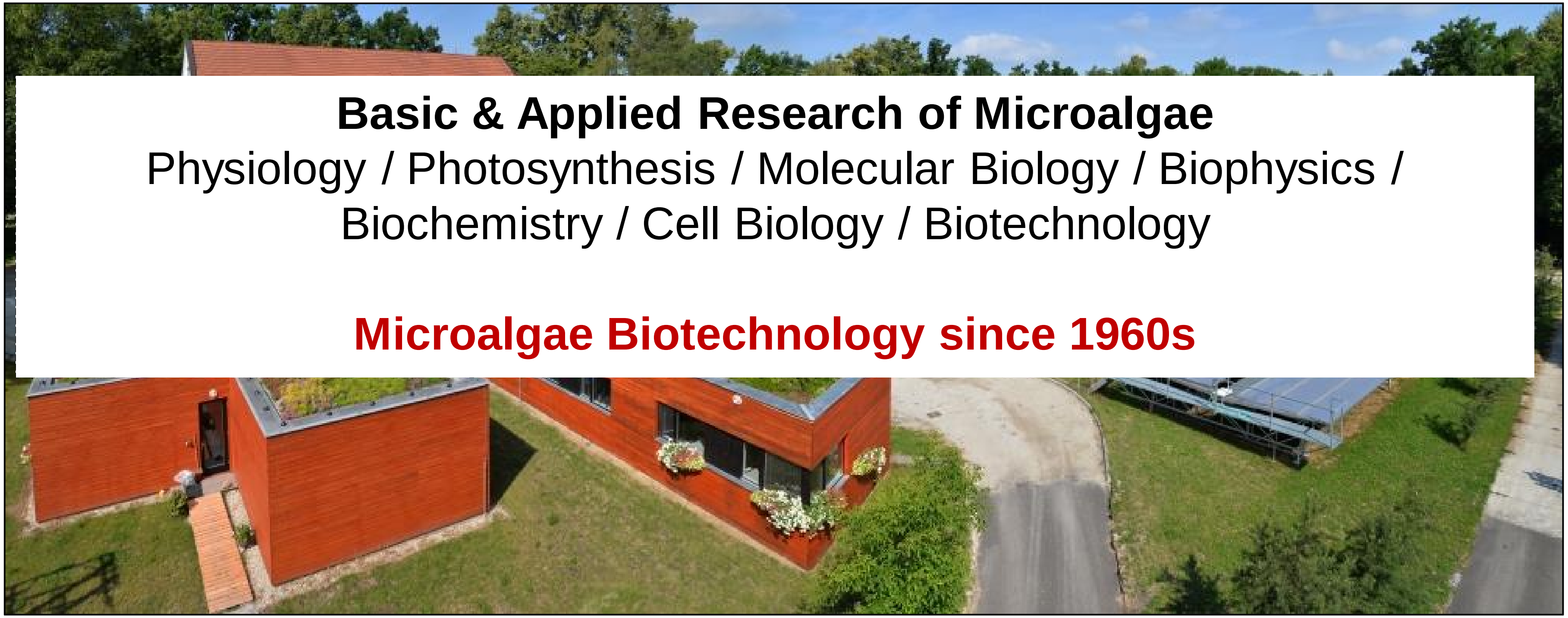
Outline

- Algatech Centre in TŚeboŔ
- Historical entrée to Microalgae Biotechnology
- **European projects – Interdisciplinary Approach to Microalgae Biotechnology**

Czech Academy of Sciences
Institute of Microbiology
Algatech Centre ï since 2011

ALGATECH

>100 employees



Basic & Applied Research of Microalgae

Physiology / Photosynthesis / Molecular Biology / Biophysics / Biochemistry / Cell Biology / Biotechnology

Microalgae Biotechnology since 1960s

LABORATORY OF PHOTOSYNTHESIS

LABORATORY OF CELL CYCLES OF ALGAE

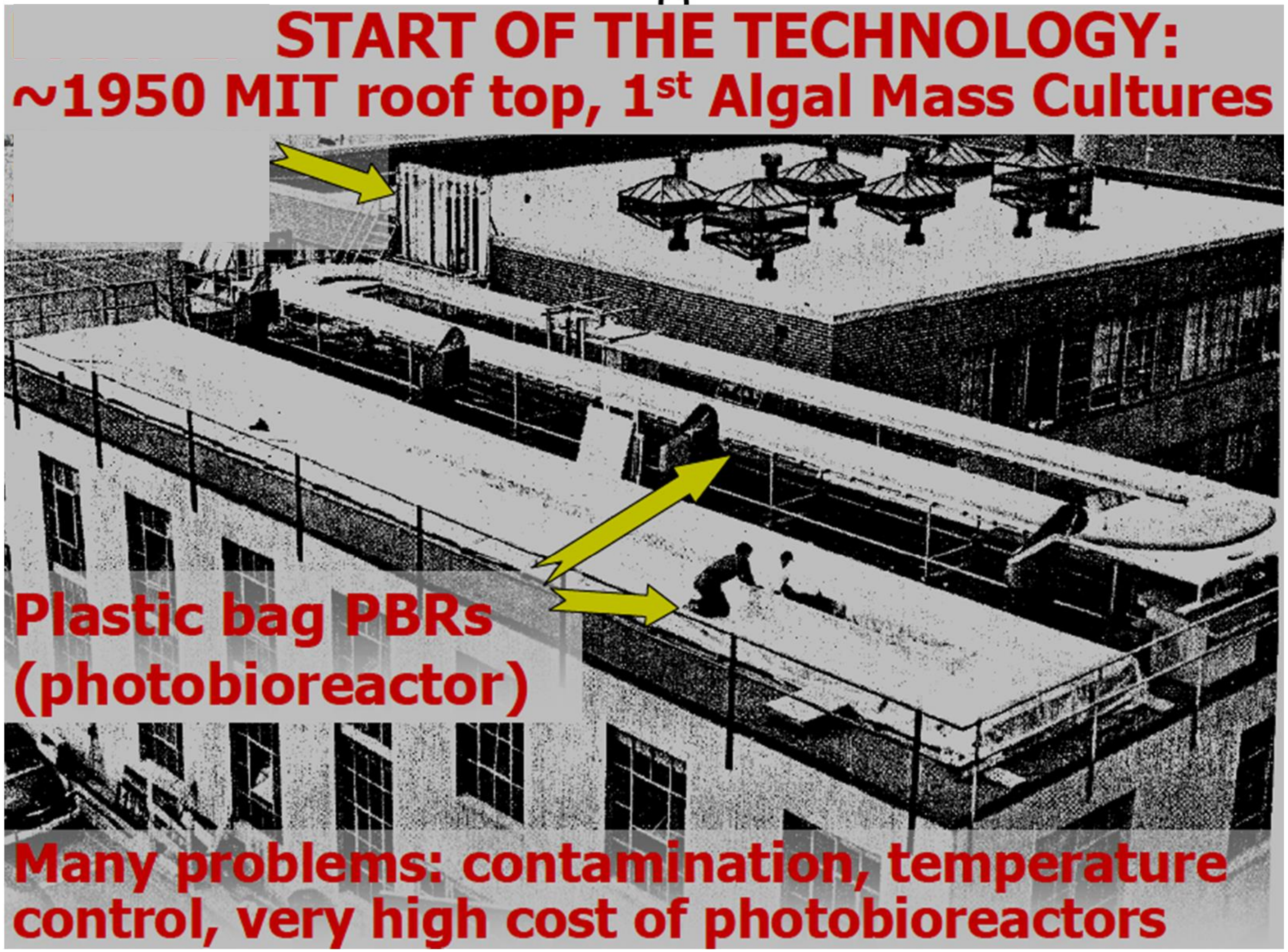
LABORATORY OF ANOXYGENIC PHOTOTROPHS

LABORATORY OF ALGAL BIOTECHNOLOGY

ALGATECH

The Bible of Microalgae Biotechnology

John S. Burlew (editor) 1953 Algal culture: from laboratory to pilot plant



Past and present: a timeline
Microalgae Biotechnology

Development in Czechoslovakia (Czech Republic)

Large scale cultivation of microalgae Institute of Microbiology Třeboň, Czechoslovakia

Russian Army invasion to Czechoslovakia

Czechoslovak astronaut Remek carried out experiment 'Chlorella 1' on board of aircraft Salyut 6

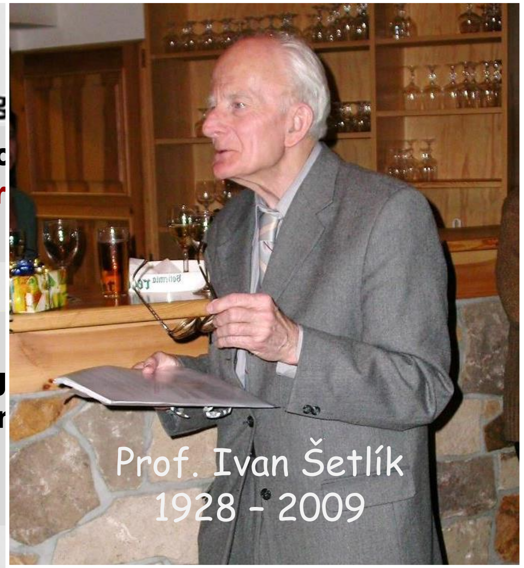
Revival of microalgal biotechnology in Czechoslovakia

1940 1950 1960 1970 1980 1990 2000 2010

Proposal to produce oil from alg

Pilot plants for mass cultivation c started as a source of protein for Germany, Japan,

Massive development of microalg Taiwan, Japan, USA, Mexico, Chir



New strains – *Dunaliella, Haematococcus* – Australia, USA

Development of microalgae cultivation worldwide – biofuels?

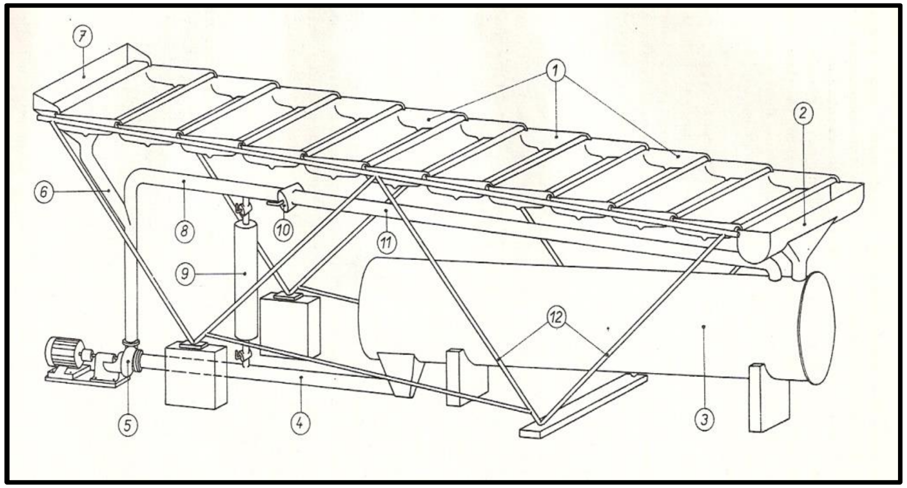
Search for bioactive compounds

Biorafinery

M.Borowitzka 2011, O.Pulz 2013

First cultivation system built in 1958 at Koġice
for the short popular-science movie

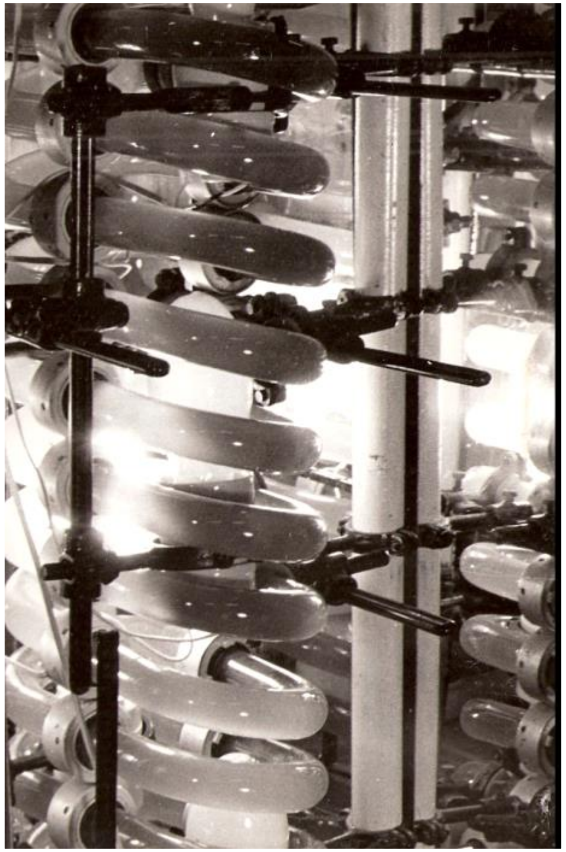
óSOLAR LABORATORYô



Schematic diagram of cascade cultivation unit of 12 m^2 -- cultivation surface was set up as shallow troughs made of reinforced polyester resin and arranged stepwise (1960)

Thin-layer cascade ï unique cultivation system

First large-scale cultivation system in Europe in 1962



Šetlík, Málek et al. (1970) Dual purpose open cultivation units for large scale culture of algae in temperate zones. Algological Studies 1: 111-164.

mid 1970s

Space programme Intercosmos - first Czechoslovak cosmonaut Remek carried out experiment óChlorella 1ôon board of aircraft Salyut 6 - March 1978

Experiment was prepared by the team from TŚeboŔ - study of microalgae growth under microgravity conditions

1978

TLCs ï further development

DEMO unit 90 m^2
Highly productive system - 2013

ChlFluo use in microalgae mass culture

Early 1990s in TŚeboŔ ï introduction of Chl fluorescence into microalgal mass culture monitoring

- In the 1990s - operations were often carried out semi-empirically ï disputes/discussions between biotechnologists vs. physiologists & photosynthetists
- Based on photosynthetic studies in crops, weǒve pioneered the use of chlorophyll fluorescence to monitor changes of photosynthesis and physiology of microalgae mass cultures in large-scale units (Práǵil, Nedbal, Grobbellaar, Torzillo, Vonshak).
- Classical approach - semi-empirical growth optimisation vs. photosynthetic activity monitoring

Microalgae Biotechnology ï interdisciplinary topic

Biology
- Strain selection and characterization
- Determination of growth conditions

Culture Activity Monitoring
- Physiology
- Photosynthesis
- Growth

Engineering
- Construction of cultivation units (photostage, pumps & electronics, sensors)

Culturing
- Optimisation of growth regimes
- Pilot trials
- Scale-up

Down-stream processing
-Biorefinary
- High value products Bioactive compounds
- Low value products

Evropská unie
Evropský fond
pro regionální rozvoj

European projects in Microalgae Biotechnology

- **EU H2020 óSABANAô project** (2016-2020)
Sustainable integrated Algae Biorefinery for the production of bioactive compounds for Agriculture aNd Aquaculture

- **Interreg project CR-Austria óALGENETICS**
Czech-Austrian Centre for Algal Biotechnology (2017-2019)

- **Interreg project Ba-Cz**
Joint research of natural substances from cyanobacteria as a example of crossboarder partnership in science

Cíl EÚS
Česká republika –
Svobodný stát Bavorsko
2014–2020

Funded by
the European Union

SABANA project

ALGATECH

Sustainable Algae Biorefinery for Agriculture and Aquaculture

EU Horizon 2020 (2016-2020) Research and Innovation Programme

- Aims to demonstrate a microalgae-based sustainable technology for production of biostimulants and biopesticides for agriculture and feed for aquaculture recovering nutrients from wastewaters (sewage, centrate and pig manure)
- Scale-up to area of DEMO plant of tens of hectares thin-layer cascades, raceway ponds

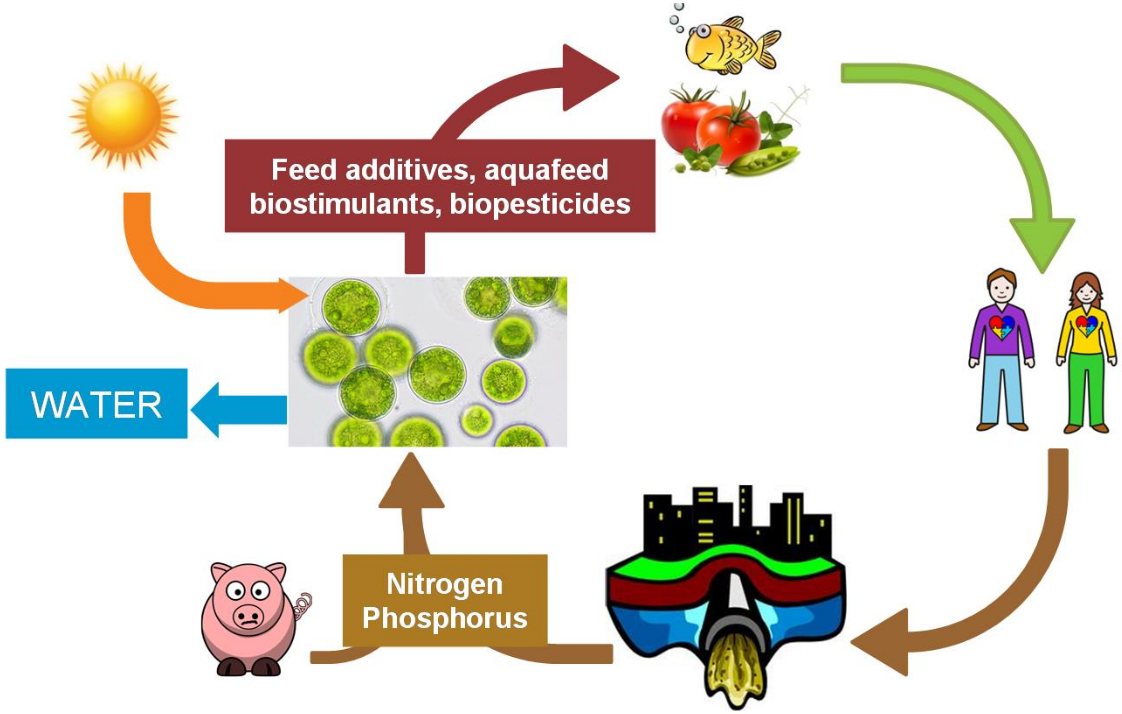
Partners of the project

Cordinator: F. Gabriel Acién

Chemical Engineering Department, University of Almeria, SPAIN

Schematic diagram of SABANA

Almeria, Andalusia, Spain ï 26 000 ha of greenhouses



Å **Large scale production**: Develop robust and scalable microalgae production and biomass processing, in continuous mode all year around

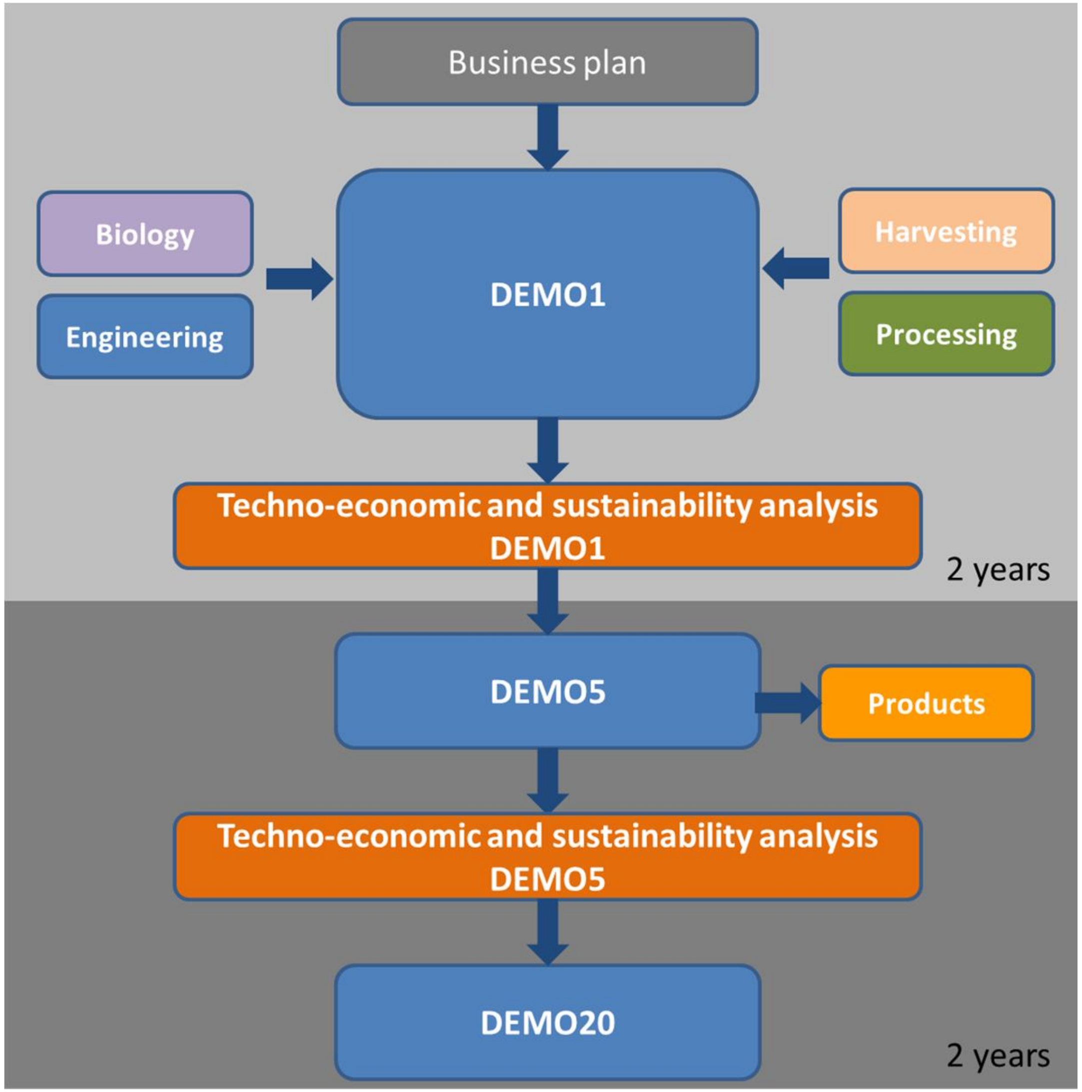
Å **Sustainable production:** To integrate microalgae biotechnology and the treatment of wastes in order to increase the sustainability of the process

Å **Markets/commercialization:** Only products demanded by the market and legally accepted are considered

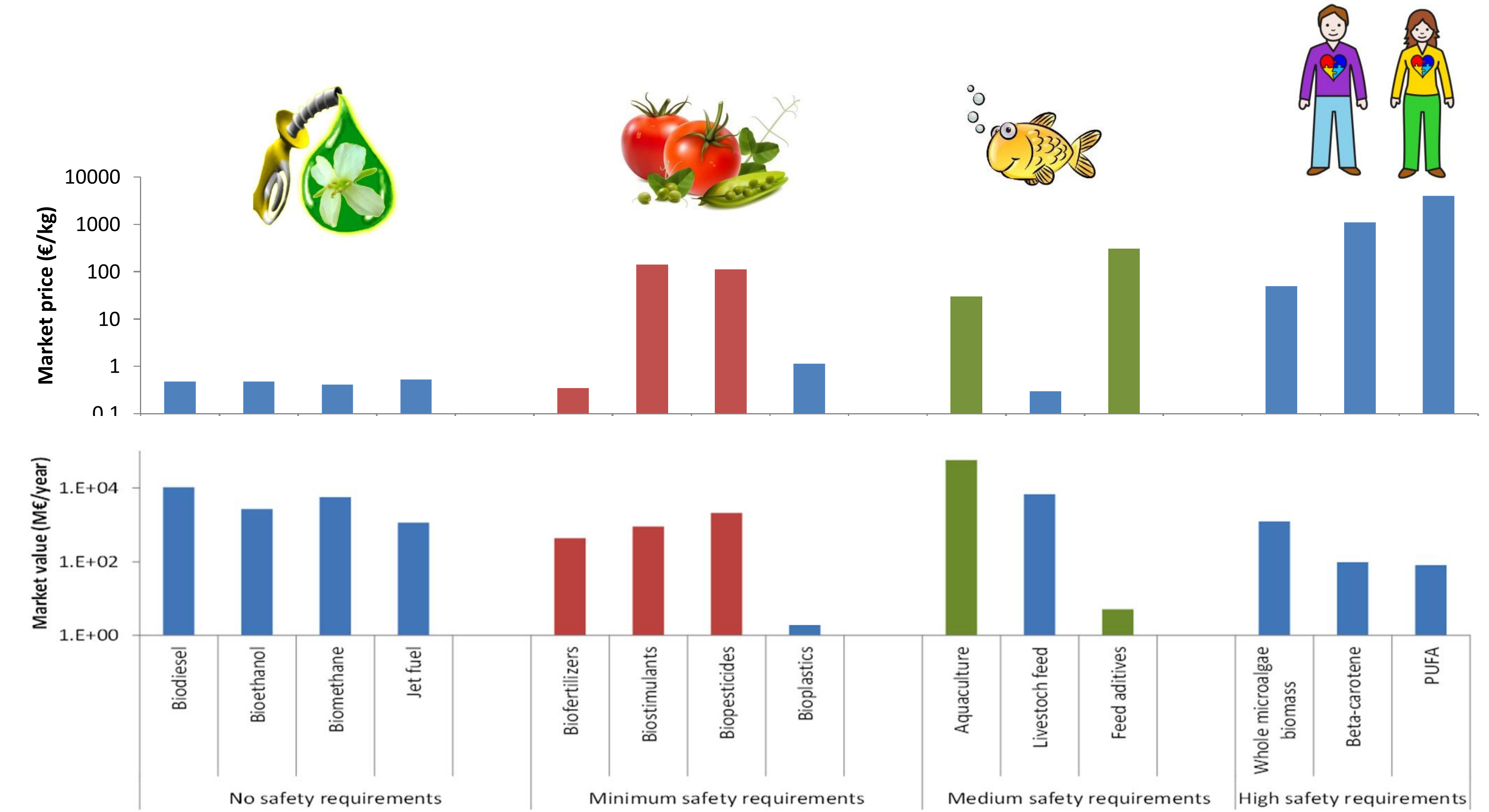
Block diagram of the project

Overal objective of SABANA - to develop and demonstrate an integrated microalgae-based sustainable biorefinery to produce a range of value-added products (biostimulants, biopesticides and aquafeed additives) and low-value products (biofertilizers, aquafeed) for agriculture and aquaculture recovering nutrients from wastewaters (sewage, centrate and pig manure)

Schedule of the project multidisciplinary approach

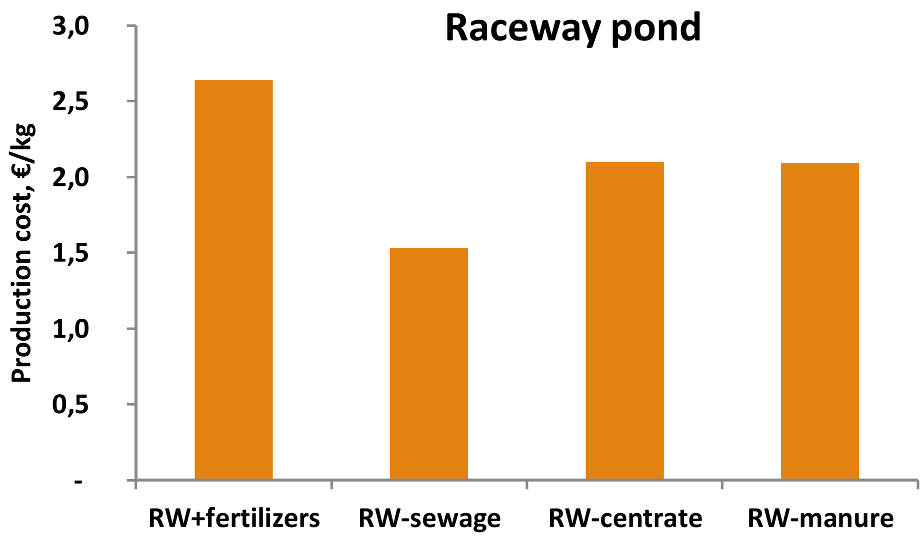


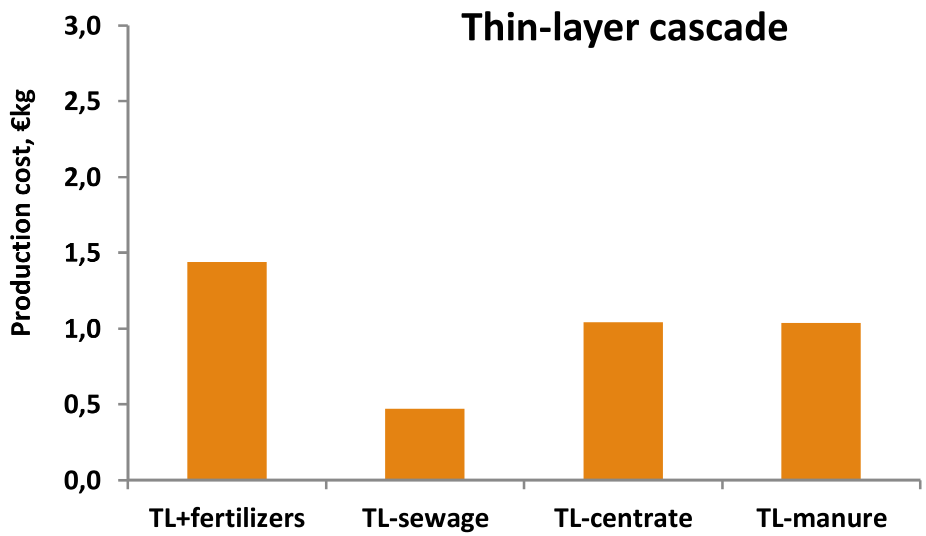
Start point: market study



Agriculture and aquaculture are large markets demanding new products from microalgae with lower safety requirements

Analysis of Biomass Production Costs

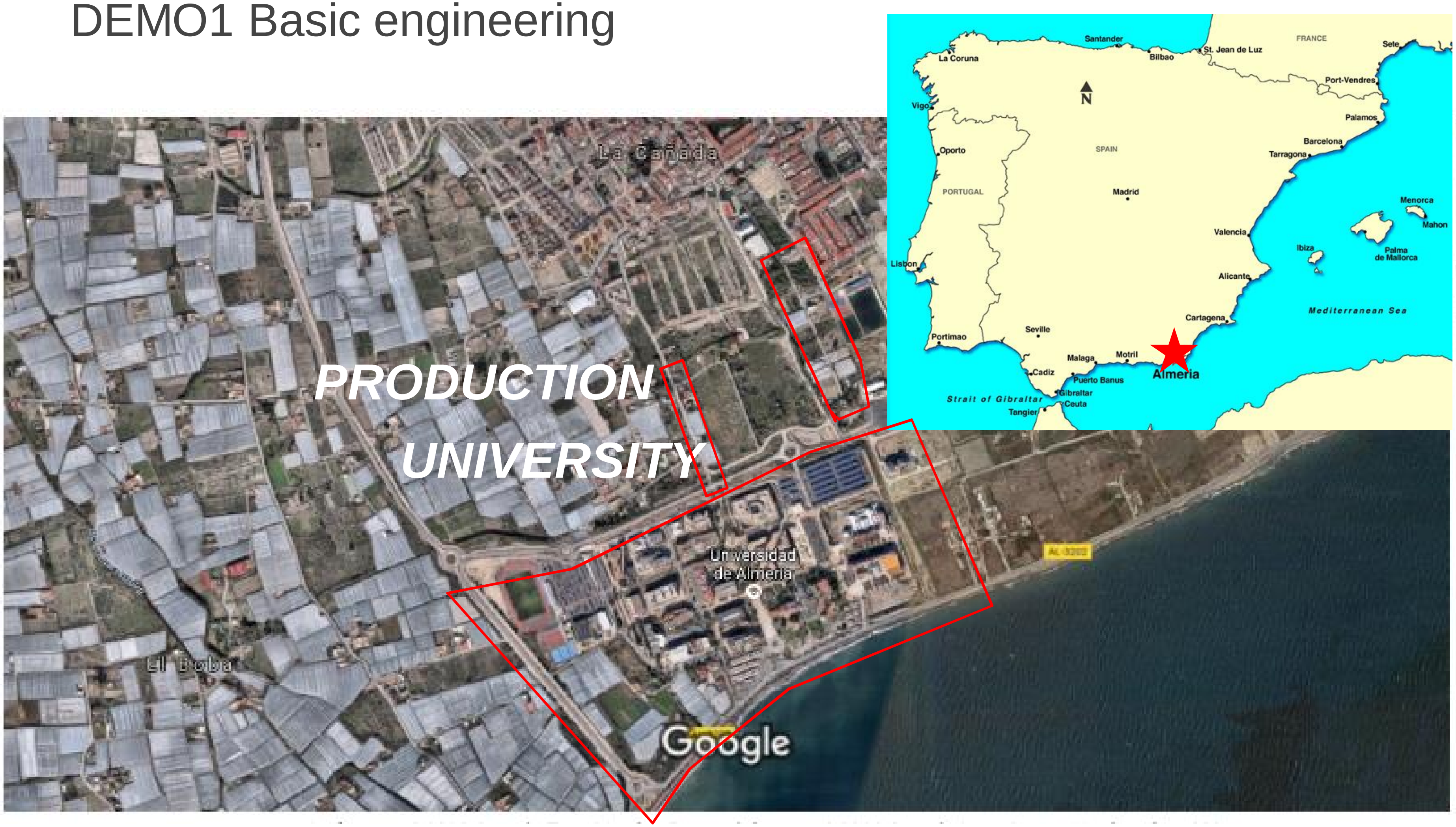




- Market of agriculture products is more interesting that aquaculture
- Production costs below 2 ú/kg are feasible only when using nutrients from wastewaters
- Production costs are lower when using Thin-layer cascades due to the higher productivity of these systems

Location of production plant

DEMO1 Basic engineering



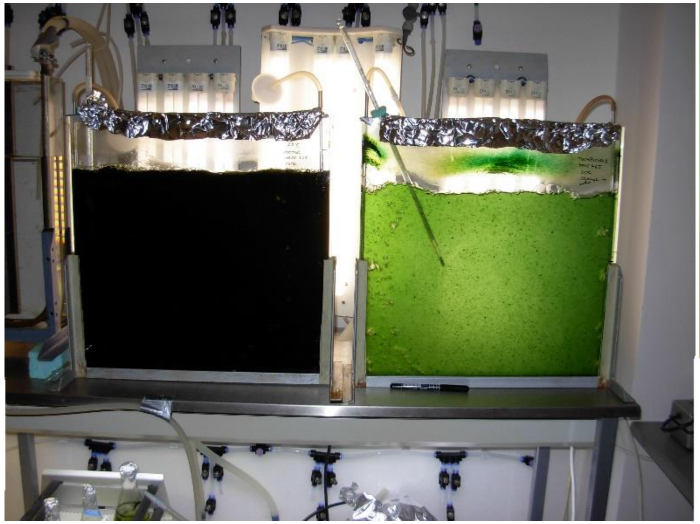
Tasks of ALGATECH team

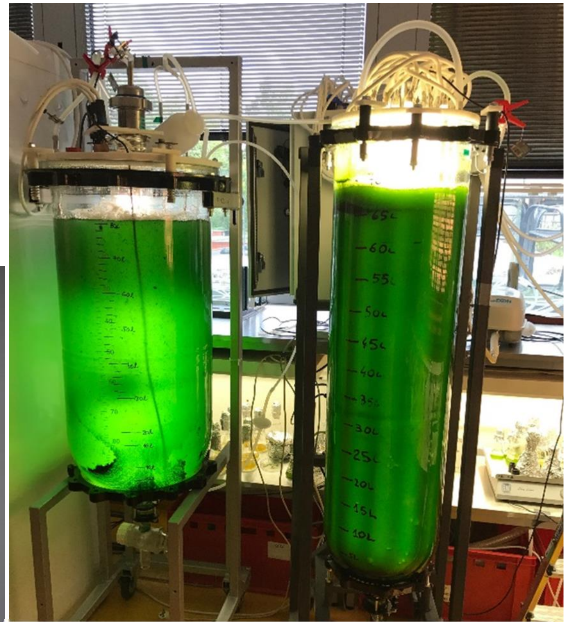
- **Design and construction of cultivation units** (Algatech, University of Almeria)
- **Characterisation of selected microalgae strains** for agricultural purposes - freshwater cyanobacteria and greens producing biostimulants and biopesticides (supplied from Culture collection of Szechenyi Istvan University, Hungary)
- **Selection and verification of monitoring techniques** to optimise the culture growth in large-scale units (Algatech, University of Almería, University of Málaga, ISE-CNR in Sesto Fiorentino)

Lab Cultivation - Photobioreactors

Cultivation in 300mL glass cylinders

Chlorella strains
in 3-L flat-plate PBRs





Nostoc MACC-612 and *Chlamydopodium* MACC-430 in 100L bubble column PBRs

ALGATECH TŚeboŔ

Outdoor Pilot Units

Thin-layer cascade and raceway pond mounted in a greenhouse (area 5 m^2, culture depth 5-20 mm) → a hybrid technology between raceway pond and thin-layer cascade

After one year in Almeríaé

ALGENETICS

Czech - Austrian Centre for Algal Biotechnology

Cross-boarder collaborative research between

- FH OÖ Forschungs & Entwicklungs GmbH in Wels
- Centre Algatech, Institute of Microbiology in Třeboň

Strategic Partner:

FH OÖ Studienbetriebs GmbH
University of South Bohemia in České Budějovice

ALGENETICS

- Main objective – to set-up joint Czech-Austrian Centre for Algae Biotechnology
- Research objective - characterisation of genetically improved/modified cyanobacteria strains as a potential producers of high-value compounds
- Construction, selection and characterisation of genetically modified cyanobacteria (over)producing glycogen/starch
- Optimising cultivation regimes of mutants from laboratory to pilot scale cultivation

Hypothesis of the project

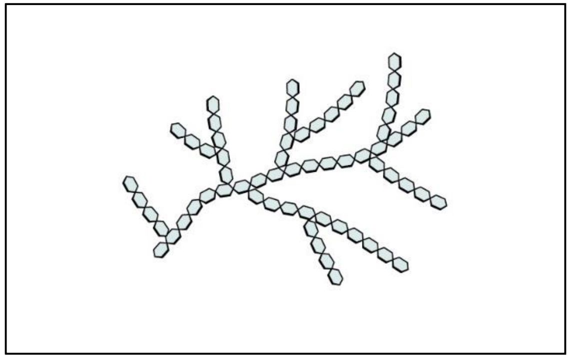
Å **Starch** is one of the **basic industrial feedstock**

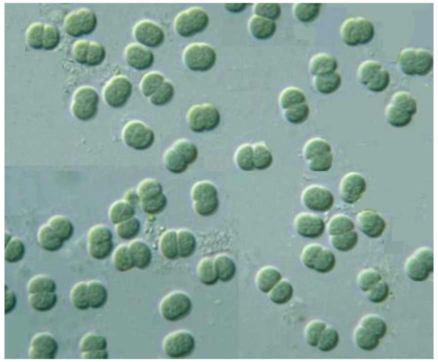
Å Cyanobacteria are **easy to grow** using just solar energy and waste nutrients

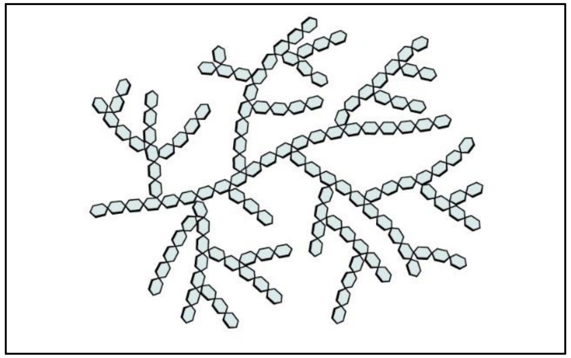
Å Cyanobacteria produce **glycogen** (storage sugar) in contrast to higher plants (starch)

Å **Glycogen is more complicated to proces** than starch

Å Lets change **cyanobacteria to produce starch** and/or ethanol







Construction of Synechocystis mutants for ethanol and starch production

Molecular Biology

FH ÖO Wels

- Contruction of Mutants

Cultivation

ALGATECH Třeboň

- Characterisation of strains
- Optimisation of culturing regimes

Processing

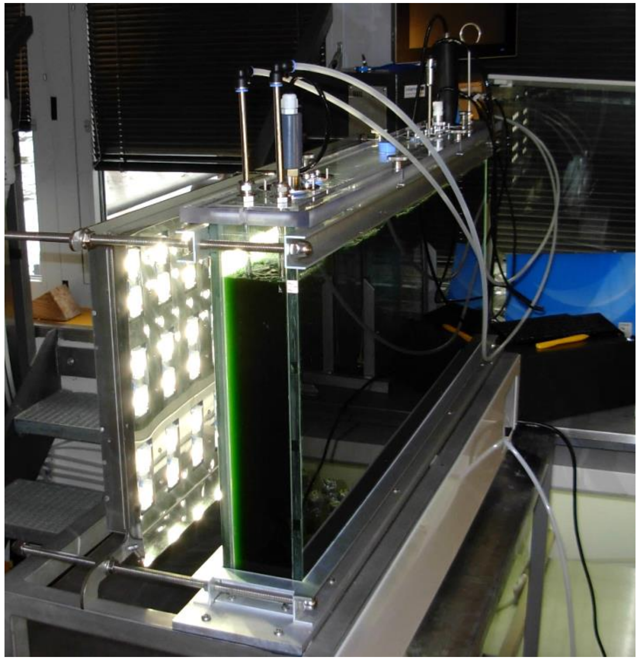
Both partners

- Isolation of products – ethanol, starch

Photobioreactors

Laboratory cultivation of *Synechocystis* PCC6803

10-L PBR

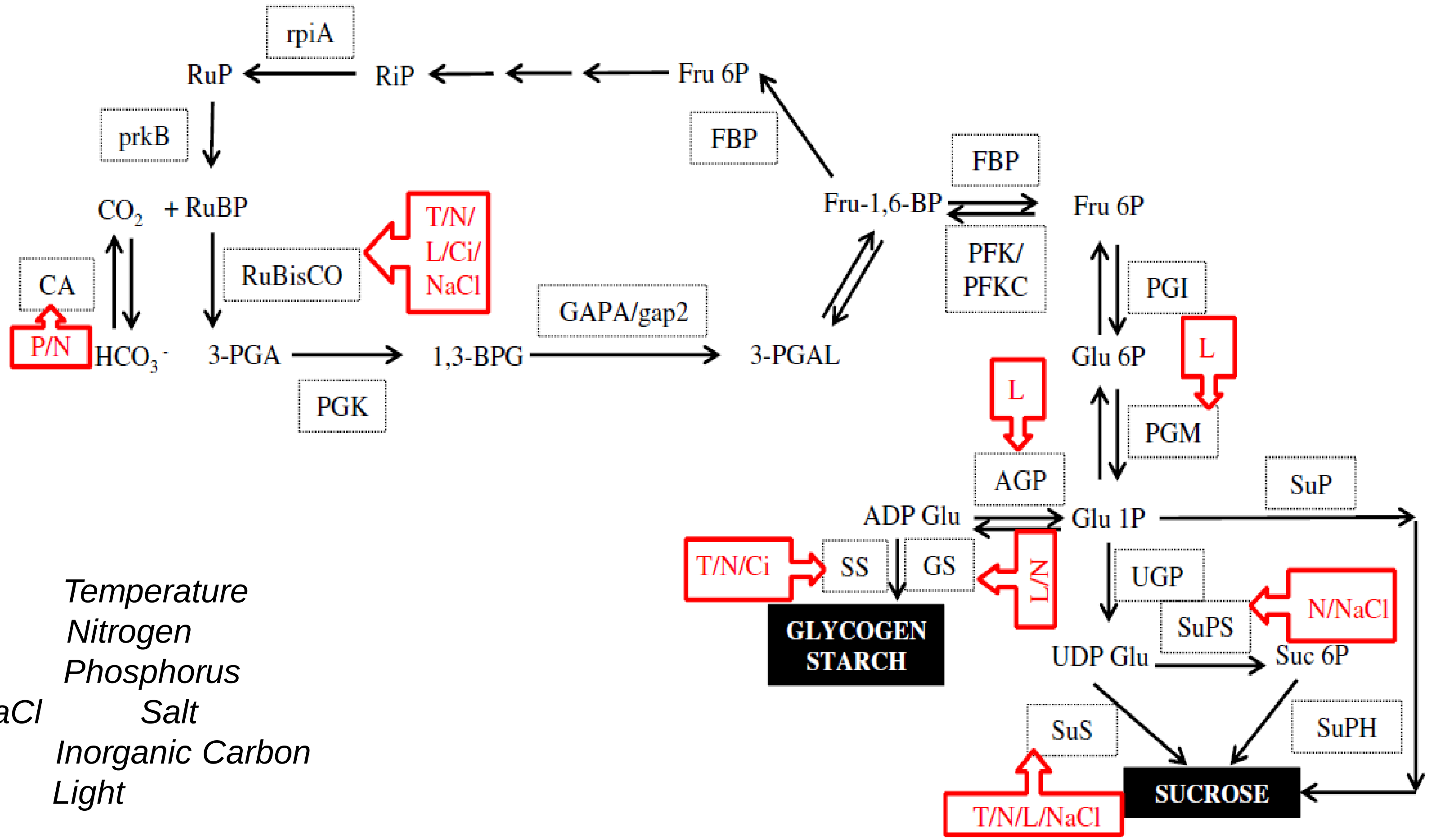


25-L fully controlled
Flat Panel PBR

100-L PBR
with internal illumination

Natural metabolic pathways

Schematic diagram of metabolic pathways
of the accumulation of starch, sucrose and glycogen in microalgae

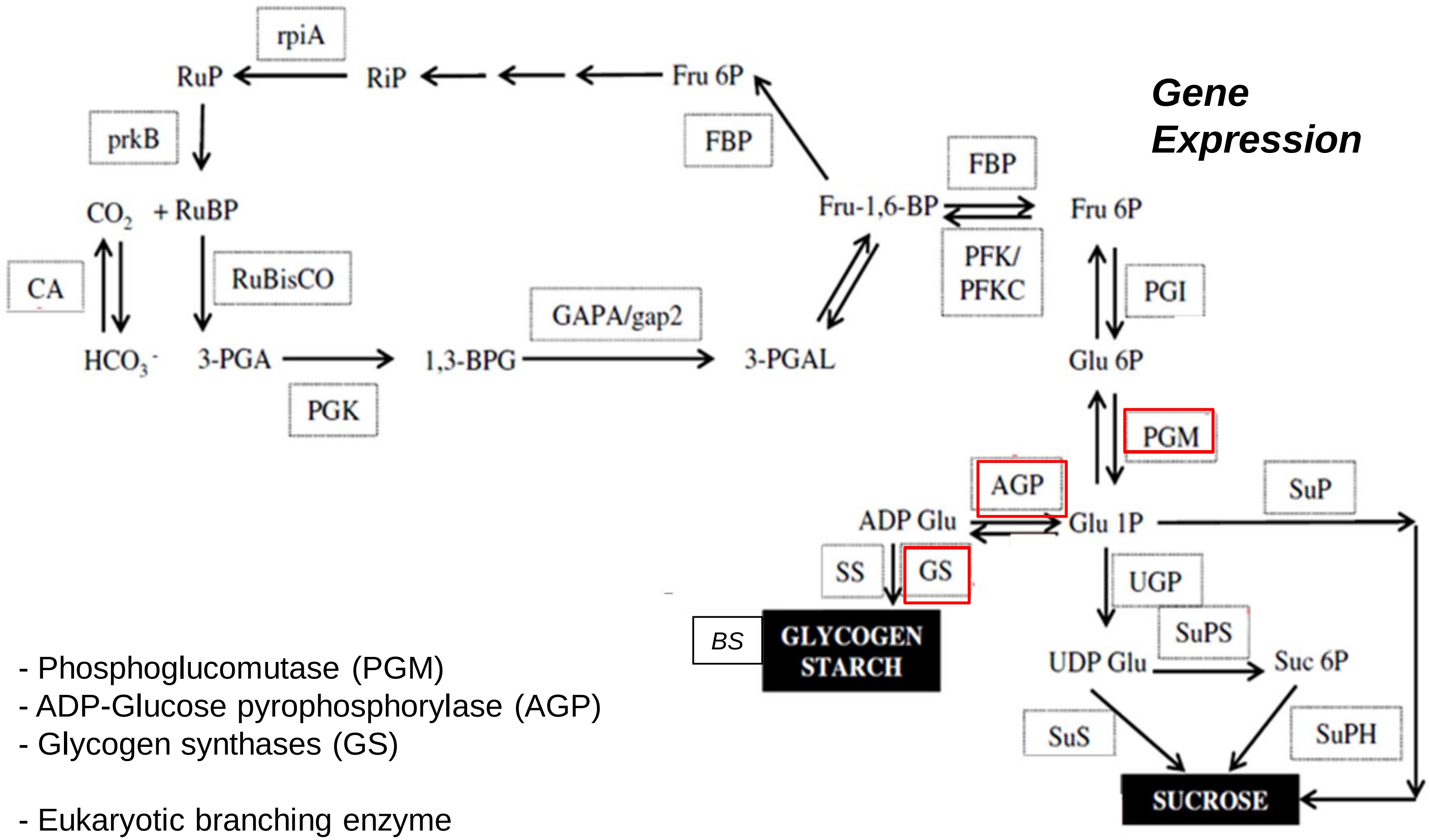


T Temperature
N Nitrogen
P Phosphorus
NaCl Salt
Ci Inorganic Carbon
L Light

Gonzáles-Fernandez and Ballesteros, 2012

Natural metabolic pathways

Schematic diagram of metabolic pathways
of the accumulation of starch, sucrose and glycogen in microalgae



Gene Expression

- Phosphoglucomutase (PGM)
- ADP-Glucose pyrophosphorylase (AGP)
- Glycogen synthases (GS)

- Eukaryotic branching enzyme

Gonzáles-Fernandez and Ballesteros, 2012

Construction and selection genetic modified cyanobacteria - Starch/Glycogen

Potential production of Starch by Cyanobacteria Can Supplement Production of EtOH from wheat Starch

- Endogenous
- Glycogen deg
  - Glycogenp
  - Glycogen
  - Glycogen
- Alternative sto
  - Sucrose sy
  - Polyhydrox
  - Fatty acid

InterReg project Ba-Cz

Joint Research of Natural Substances from Cyanobacteria as an Example of Cross-boarder Partnership in Science

TUM
UR
Universität Regensburg
MBÚ
ALGATECH

WP 1 Screening of strains affecting neural stem cell differenciation and antifungal compounds

WP 2

Cultivation and characterisation of cyanobacteria strains

WP 3 – Genetic modification of cyanobacteria

WP 4 - Transcriptomics and metabolomics

WP 5 Development of various lipopeptides using synthetic modification

WP 6 Biomass residues and their utilisation

WP 7 Sustainability and EIA
LCA, social impact on regional development

LABORATORY OF ALGAL BIOTECHNOLOGY

Further prospects

ALGATECH

Å Optimisation of cultivation regimes in photobioreactors

Å Characterisation and production of novel bioactive substances - secondary metabolites of microalgae - testing of their applications.

Å Development of new technological procedures leading to production of microalgae biomass/products in phototrophic or heterotrophic growth regimes

Å Innovations of downstream processes in the production of microalgae biomass

Å Development of new methods for the extraction of bioactive compounds from biomass

Acknowledgements

- **Karolína Ranglová, Gergély Lakatos, SoŔa PekaŚová, Tomáġ Grivalský, Joao Manoel, Richard Lhotský, Kumar Saurav, Pavel Hrouzek -** Centre Algatech, Institute of Microbiology, Academy of Sciences, TŚeboŔ
- **Felix Figueroa -** University of Málaga, Spain
- **Gabriel Acién, Cintia Gómez, Francesca Suarez , Marta Barceló -** University of Alméria, Spain
- **Vince Ördög -** Széchenyi István University in Mosonmagyaróvár, Hungary (strains)
- **Juliane Richter, Richard Gundolf -** FH OÖ Forschungs & Entwicklungs GmbH in Wels

Funding programmes

- **Algatech Plus - Ministry of Education, Youth and Sports, CR**
- **EU H2020 project SABANA**
- **Interreg At-Cz**
- **Interreg Cz-Ba**
- **Bilateral scientific agreement CNR-AVĻR**

Thank you for attention

Questions,

remarks?

masojidekj@seznam.cz

High-energy products – Starch/Glycogen

Starch

- In plants and eukaryotic algae
- Consists of
  - Å 20-30 % Amylose: ***Ŭ***-1.4 Glucose (Glc)
  - Å 70-80 % Amylopectin: ***Ŭ***-1.4 and ***Ŭ***-1.6 Glc (every 30th ***Ŭ***-1.4 Glc)
- Granules up to 100 µm (diameter)

Glycogen

- In Prokaryotes, e.g. Cyanobacteria
- Consists of
  - Å ***ȷ*** -1.4 and ***Ŭ***-1.6 Glc (every 8-12 ***Ŭ***-1.4 Glc)
- Particles up to 42 nm (diameter)

LABORATORY OF ALGAL BIOTECHNOLOGY

Fields of expertise

ALGATECH

- Design and construction of various cultivation units
- Screening and selection of microalgae strains
- Optimisation of culturing regimes for selected microalgae strains ï monitoring techniques
- Production of biomass enriched in bioactive compounds
- Identification and characterisation of bioactive compounds

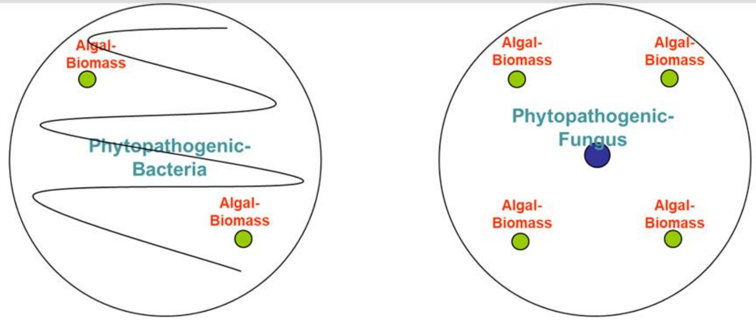
Characterization of microalgae for agriculture purposes ï Biopesticides & Biostimulants

Strains - freshwater, marine

- *Nostoc,Tolypothrix*
- *Chlorella,Scenedesmus,Nostoc*

Microalgae Collection of Institute of Microbiol
Microalgae Collection of Szeczenyi Istvan Ur

Bioassays: Biopesticide effect



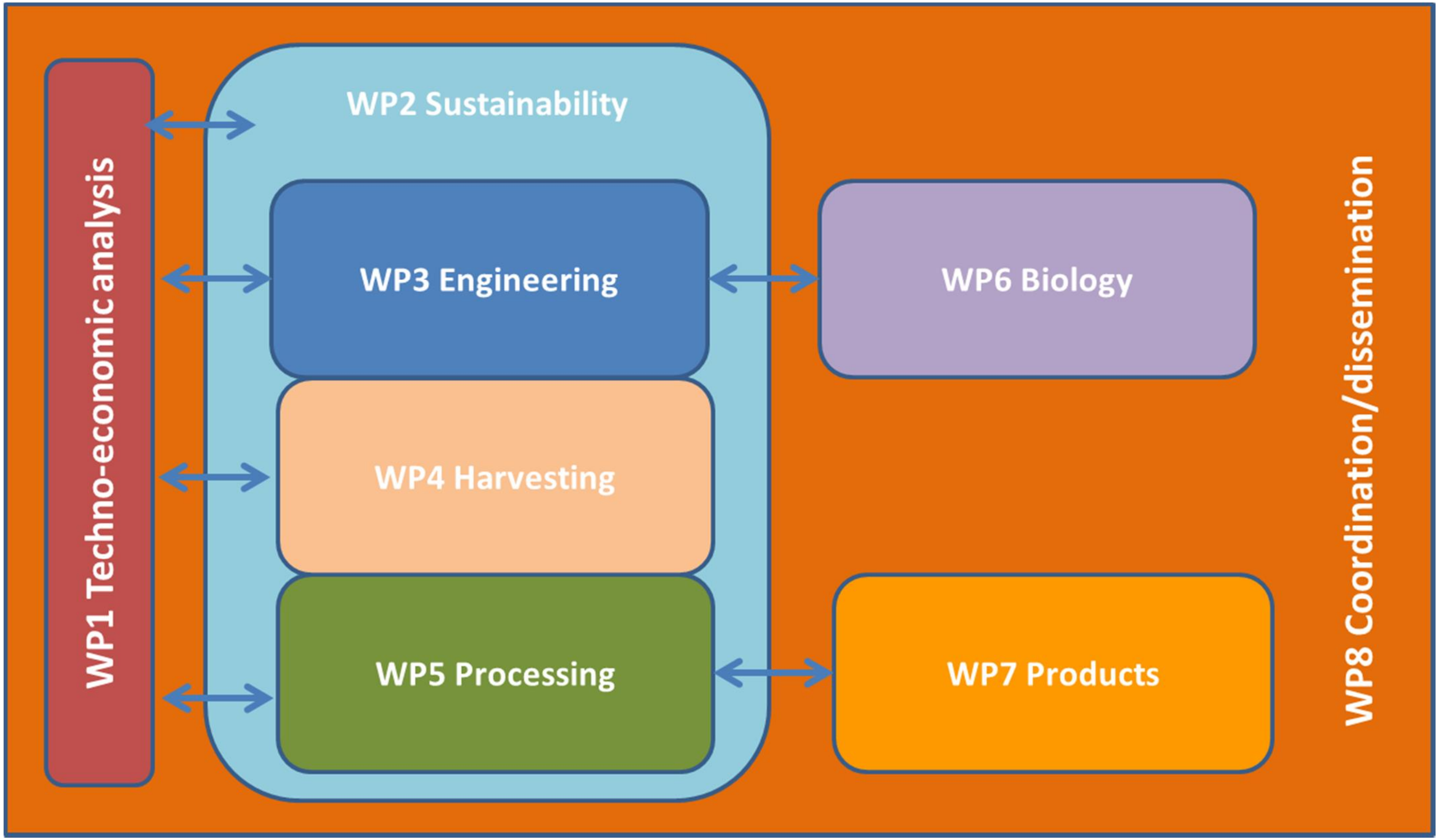
Dual culture PhyB-Algal Extract

Dual culture PhyF-Algal Extract

Effect against different plant pathogens measured *in-vitro:* extracts of freeze-dried biomass ï University of Almería and Szeczenyi Istvan University,

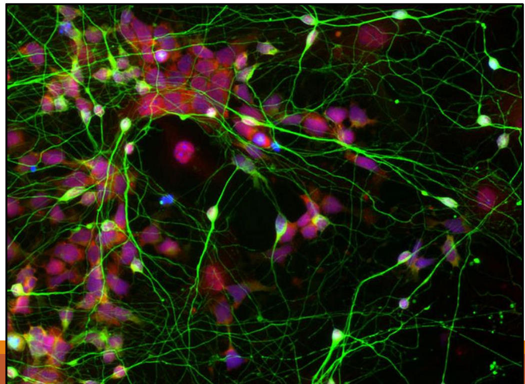
Phytopathogenic Fungi:	***Phytopathogenic Bacteria:***
PU: Pythium ultimum	*XC: Xanthomonas campestris*
FOM: Fusarium oxysporum f.sp. melonis	*PCC: Pectobacterium carotovorum*
RS: Rhizoctonia solani	*PST: Pseudomonas syringae*
PCAP: Phytophthora capsici	*CMM: Clavibacter michiganensis*

Workpackages



WP 1 ï Screening of valuable compounds that influence neural stem cells differenciation, cytotoxic compounds and compounds with antifungal activity

- *Tests of 80-100 cyanobacterial extracts*
- *Fractionation of extracts to identify the compound*
- *Detailed study of clean substances nad structure determination*

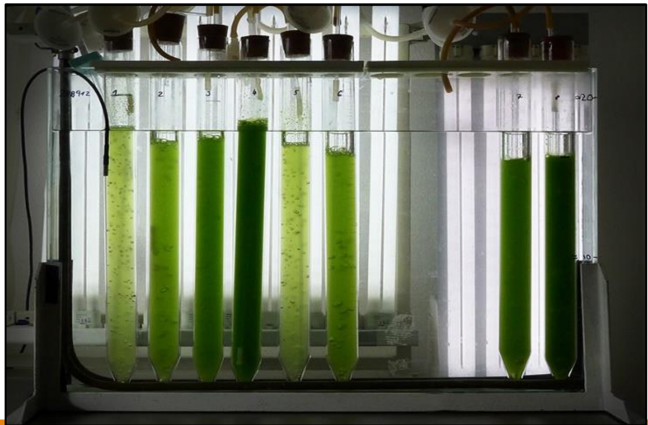


WP 2 ï Cultivation and characterisation of cyanobacterial species and strains

Å *Growth condition optimisation*

Å *Phototrophic cultivation from lab scale to pilot scale*

Å *Outdoor cultivation systems ï TLC or RW*



WP 3 ï Genetic modification of cyanobacteria

Å *Introduce microalgae as a safe, cheap, variable and sustainable platform for biopharmaceutical production*

Å *Develop fast methods for approvement of genetical transformation*

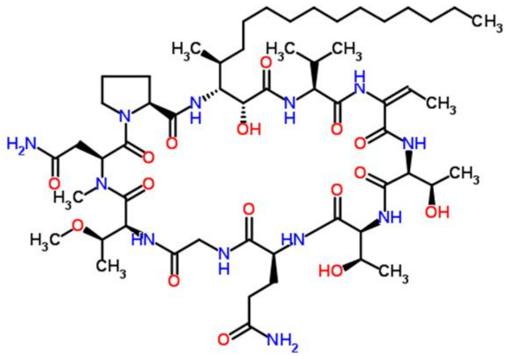
Å *Preparation of transformed strains for scale up*

WP 4 - Transcriptomics and metabolomics

- *Study of genetical metabolic changes after changes in the environment (e.g. light or nutrient stress)*

WP 5 ï Development of various lipopeptides using synthetic modification

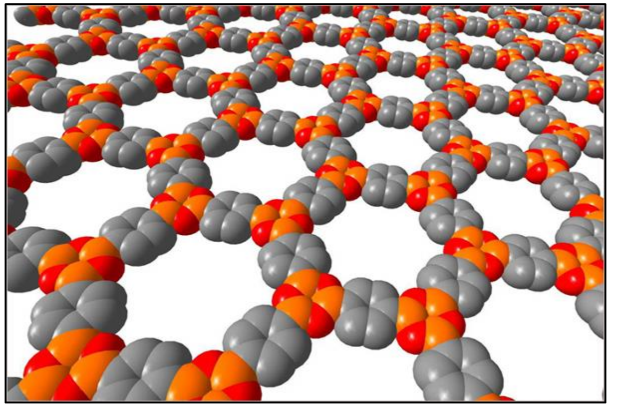
- *Chemical modification of lipid chains in puwainaphyc F and lyngbyatoxin A*
- *Efficiency verification of changed chemical structure*



WP 6 ï Biomass residues and their utilisation

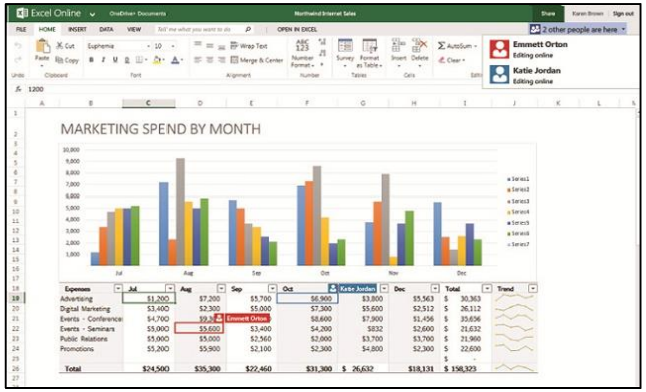
Å *Biomass use*

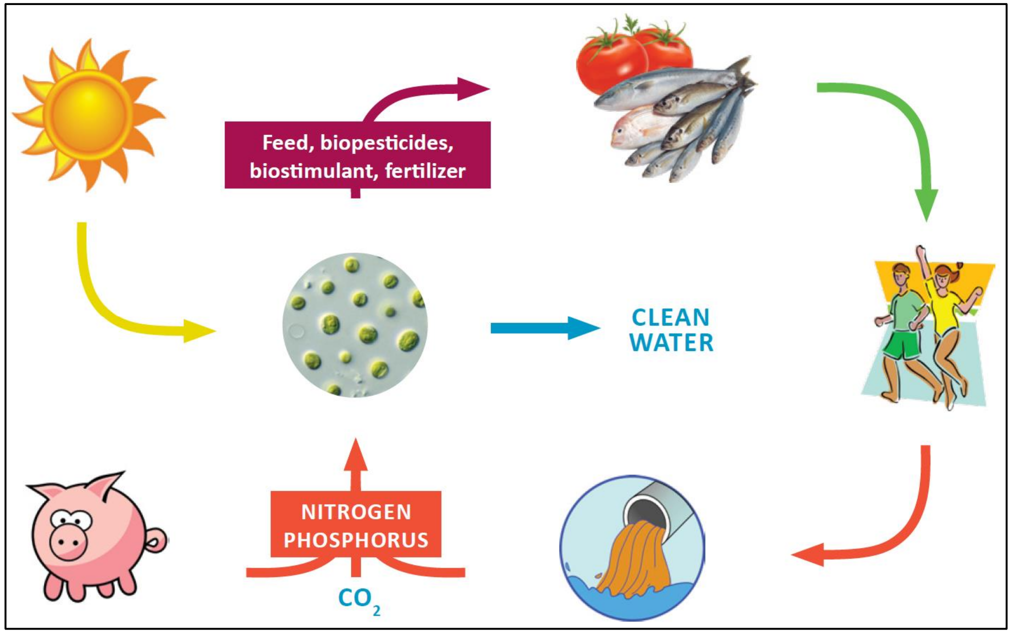
Å *Biopolymers from residue biomass*



WP 7 ï Sustainability and EIA

Å *LCA, TEA, social impact on regional development*





***Mass cultivation**: Development of a robust, economical and modular system of a mass cultivation and down stream processing of microalgae.*

***Sustainabe production:** Integrate algae technology to the waste water treatment systém to decrease the price of final biomass.*

***Market and commercialisation:** Project considers only products demanded by the makret.*

Technical challenges

LARGE SCALE BIOMASS PRODUCTION

Biology

- Strains: pure/mixture cultures
- Growth promoters: bacteria/biostimulants
- Photosynthetic efficiency
- Characterization: PCR-HRM

Engineering

- Bioreactors: thin-layer cascade, improved raceway
- Efficiency: power consumption, mass transfer
- Modeling and advanced control
- Scale-up: 1000 m^2, 5000 m^2

Sustainability

- Nutrients recovery: C, N, P.
- Reduction of GHG emission
- CO_2 supply from biomass
- Zero waste processes

Technical challenges

INTEGRAL UTILIZATION OF THE BIOMASS

Harvesting

- Conventional: flocculation, sedimentation, flotation, centrifugation
- Novel methods: electro flocculation, membranes.

Processing

- Cell disruption: PEF, mechanical, enzymatic
- Extraction: biocompatible solvents.
- Fractionation/purification: selective solvents, chromatography

Products

- High value products: biostimulants/biopesticides for crops, antioxidants/health enhancer for aquaculture
- Low value products: biofertilizers for crops, feed for aquaculture

Participants

SPAIN

- **Coordinator: Universidad de Almería, España (prof. Gabriel Acién)**
- AQUALIA SA
- BIORIZON BIOTECH
- Spanish Bank of Algae, Universidad de Las Palmas, Gran Canaria

GERMANY

- GEA WESTFALIA GROUP Gmbh
- Karlsruher Institut fűr Technologie

ITALY

- Universita degli Studi di Milano
- CIB - Consorzio Italiano Biogas e Gassificazione

HUNGARY

- Szechenyi István University

CZECH REPUBLIC

- Institute of Microbiology AVČR, v.v.i.

masojidek@alga.cz

Chl fluorescence ï O_2 production

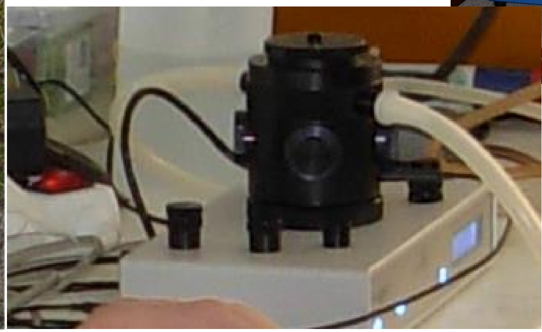
ALGATECH

Various approaches – on-line/in situ & off-line

Fast Fluorescence Induction Kinetics
(OJIP-test)

ï handheld fluorometers

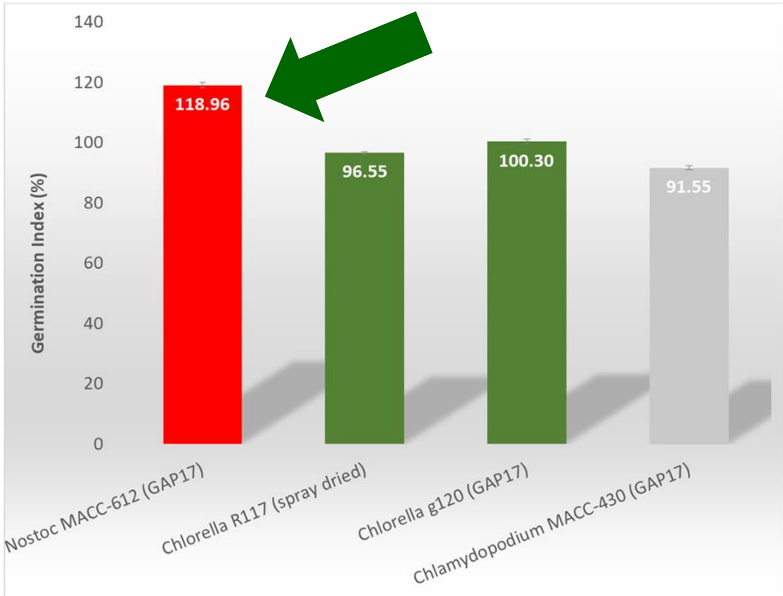
Pulse-Amplitude-Modulation technique (PAM)
Saturation pulse analysis of fluo quenching



Tasks in WP6 for UAL ï Biostimulant

Task 6.1. **Characterization of microalgae for agriculture purposes**

Biostimulant effect: optimzation of growth conditions
Bioassay ï Germination Index (%)



Chlorella R-117, *Nostoc* MACC-612, *Chlamydopodium* MACC-430

(microalgae collection of Institute of Microbiology, Třeboň, Czech Republic and
microalgae collection of Szeczenyi Istvan University, Mosonmagyarovár, Hungary)

High-productivity Microalgae Cultures

ALGATECH

Photosynthetic productivity of microalgae mass cultures is influenced by „average" cell irradiance

→ the interplay among irradiance intensity, cell-layer thickness (light path), biomass density & turbulence → optimisation of growth

- Flashing light effect – short light/dark cycles → match the turnover of the photosynthetic apparatus ~ 10-100 msec

Advantage of thin layer systems

Case Studies - ALGENETICS

WP 1: Construction and selection genetic modified cyanobacteria - Starch/Glycogen