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Introduction to Microalgae Biotechnology

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Algatech Třeboň - Centre for Algal Biotechnology - since 2011
Laboratory for Algal Research - est. 1960 in Třeboň

LABORATORY OF PHOTOSYNTHESIS

LABORATORY OF ALGAL BIOTECHNOLOGY

LABORATORY OF ANOXYGENIC PHOTOTROPHS

LABORATORY OF CELL CYCLES OF ALGAE

R&D Topics in Laboratory of Algal Biotechnology

- Screening and selection of microalgae strains
- Design and construction of various cultivation units
- Optimisation of culturing regimes for microalgae
- Production of biomass as food and feed additives
- Isolation and characterisation of bioactive compounds – analytical techniques

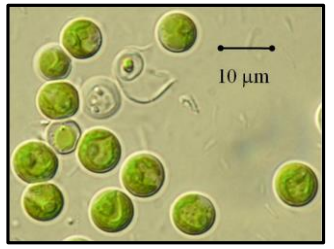
Keywords: Macroalgae vs. Microalgae,

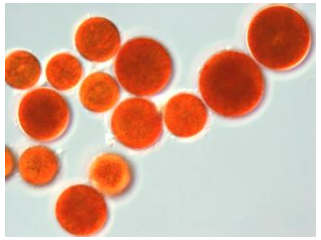
Mass culture vs. Phytoplankton population

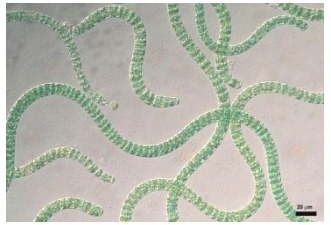
Macroalgae vs. Microalgae

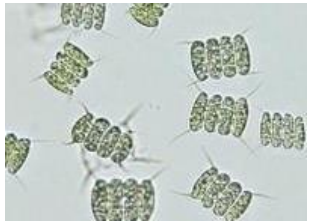
Kelps, seaweeds – dimensions of thallus in cm or m (Ulva, Porphyra, Gracilaria)

Photosynthetic microorganisms - prokaryotic cyanobacteria & eukaryotic algae)
Dimensions of cells in ~1-30 µm

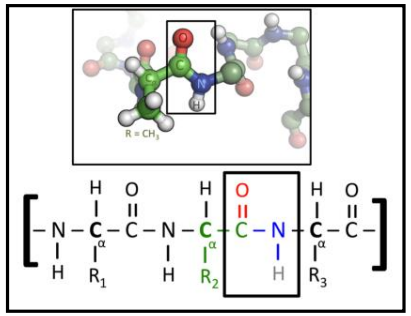


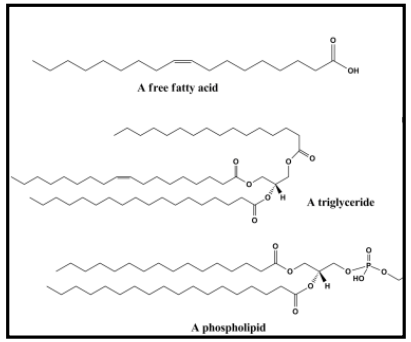


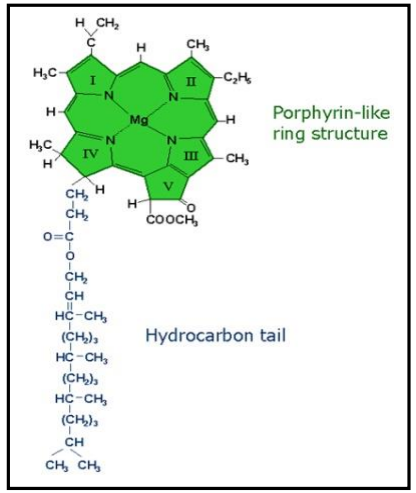




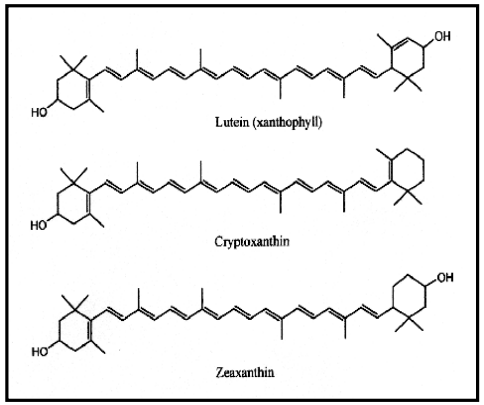
Microalgae – Single-Cell Solar Factory

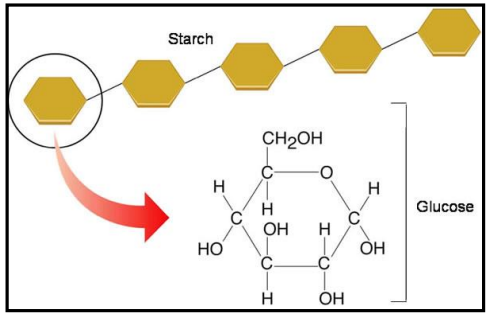


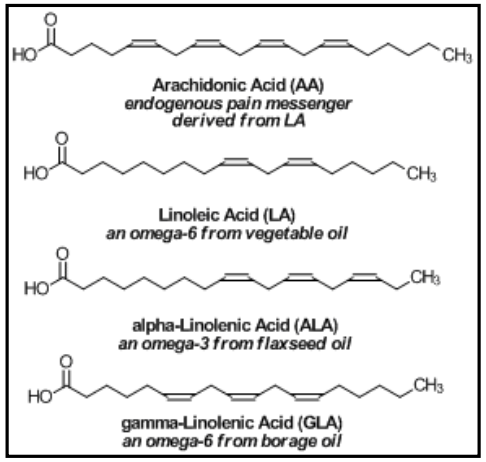


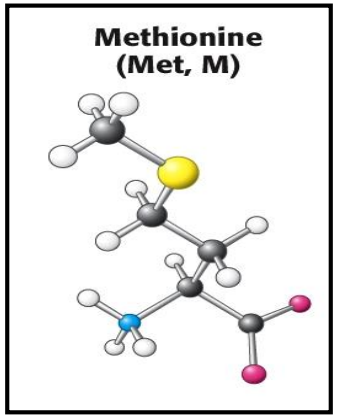


- Proteins and essential AAs
- Polysaccharides
- Lipids and fatty acids (PUFA)
- Pigments (carotenoids)
- Antioxidants
- Minerals and vitamins
- Fiber
- Enrichment by various elements - Se, I, Cr, Zn, Fe







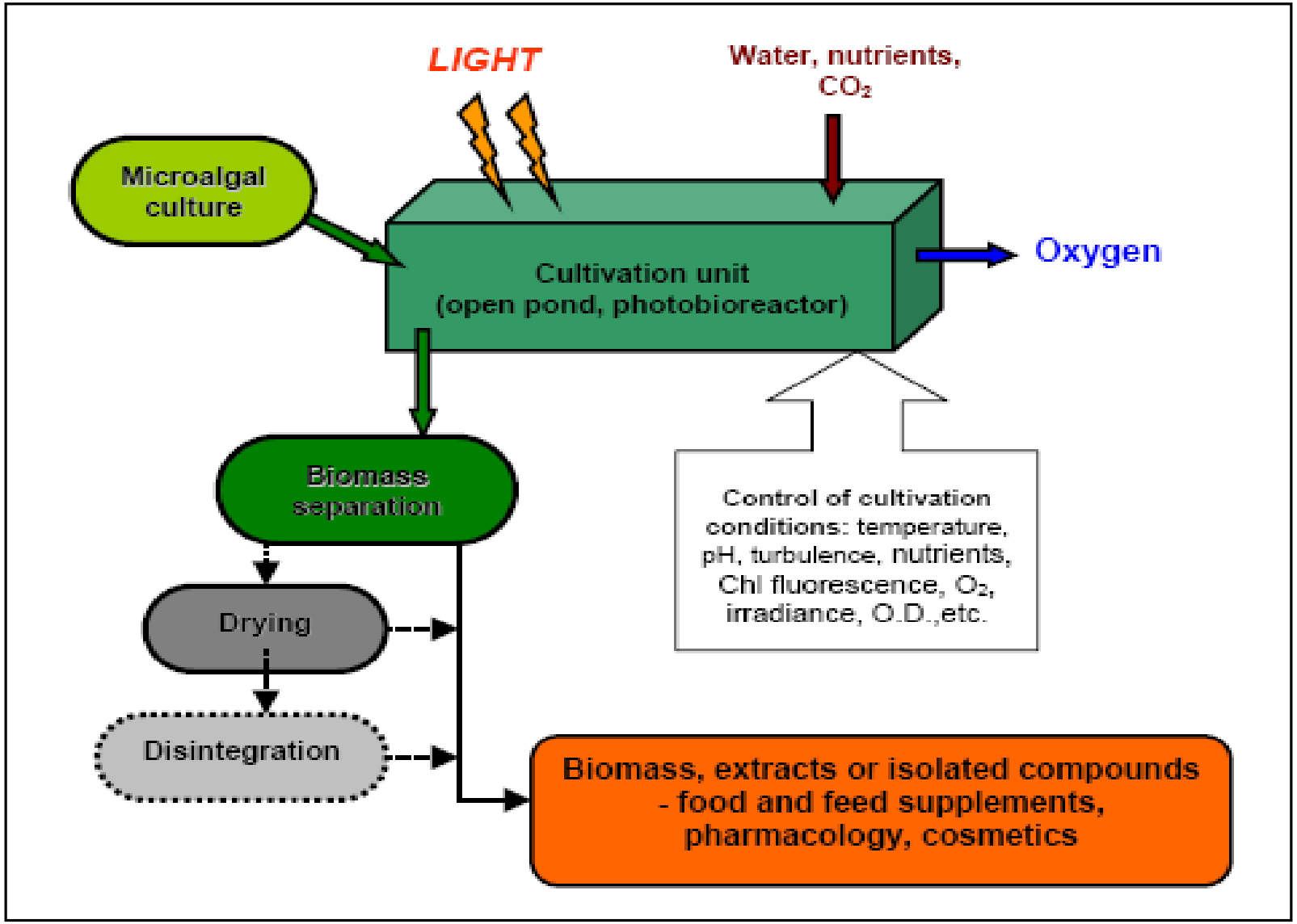


Advantages of Microalgae

Thousands of strains in collections

- Fast reproductive cycles - fast growth (doubling time of several hours)
- Single-celled micro-organisms – minimum internally competitive metabolic functions (as compared to crops) – high photosynthetic efficiency
- Grow in aquaculture in man-made cultivation systems - **cultivation process can be well controlled and manipulated**
- **Mass cultures of microalgae in photobioreactors** – dense, well-mixed, homogenous suspensions of cells in man-made cultivation systems with sufficient supply of light and nutrients - represent artificial production system
- Very **different from natural phytoplankton populations**

Schematic Diagram of Mass Production



The scale-up of Microalgae Production:
starting with agar and flask culture up to pilot and production units

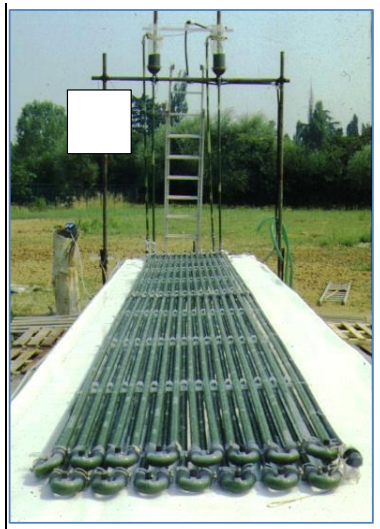
1st step – strains on agar

2nd step - Erlenmayer flasks

Cultivation Systems for Microalgae Growth

Open Outdoor Systems

Closed and Semiclosed Outdoor Photobioreactors



Goal of Microalgae Biotechnology

The major goal of microalgal biotechnology:

to achieve higher production of biomass, or valuable compounds per illuminated surface or volume of culture, i.e. to optimise/maximise the culture growth and productivity

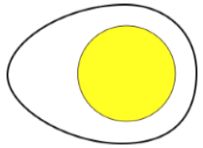
Cultivation Areas of Microalgae

Production >30,000 metric tons, large-scale outdoor systems
Major producers Asia (Japan, China, Taiwan, Thailand, South Korea, India), North Americas (Mexico, USA), Europe, South Africa, Australia

Use of Microalgae

Food & feed additives

- Health food, supplements
- Feed
  - ✓ Chicken, eggs
  - ✓ Ornamentals – fish, birds
- Cosmetics
- Pharmacology



Technology & Environment

- Wastewater treatment (removal of N, P, heavy metals, etc.)
- Agriculture use – biostimulants, biopesticides, biofertilisers
- Production of bio-fuels and sources of chemicals

The Current Industrial-scale Applications of Most Exploited Microalgae

Product and Application	Status	Microalga
Health food, food and feed supplements	Established	Arthrospira (Spirulina), Chlorella
β-Carotene	Established	Dunaliella
Astaxanthin	Established	Haematococcus
Live food and feed supplements in aquaculture	Established	Nannochloropsis, Isochrysis, Chaetoceros, Pavlova, Tetraselmis, Phaeodactylum, Skeletonema, etc.
PUFAs	Established	Phaeodactylum, Nannochloropsis
Xanthophylls (lutein, zeaxanthin)	Developing	Scenedesmus, Chlorella
Polysaccharides	Developing	Porphyridium
Oils, biofuels	Developing	Botryococcus, Nannochloropsis, Phaeodactylum, mutants of Chlamydomonas & Synechocystis
Biopharmaceuticals	Developing	Nostoc, Cylindrospermum, Anabaena
Biostimulants, biopesticides	Developing	Chlorella, Scenedesmus, Nostoc

Downstream Processing

Downstream processing is any treatment of culture after cultivation to concentrate and purify products.

It follows a general sequence of steps:

1. **Harvesting** - cell removal (centrifugation, floculation, filtration, cross-flow filtr.)
2. **Desintegration** - destroying of the cell walls to release intracellular compounds
3. **Dewatering** - drying, lyophilization, precipitation
4. **Primary isolation** - to remove components with properties significantly different from those of the products (adsorption, extraction, precipitation, hydrolysis).
5. **Purification**. Highly selective (chromatography, ultrafiltration, fractionation)
6. **Finalisation** (crystallization, followed by centrifugation or filtration and drying, tableting, packing). Typical for high-quality products such as pharmaceuticals.

ALGATECH projects

ALGENETICS

Czech - Austrian Centre for Algal Biotechnology (2017-2019)

Research objective: Construction and characterisation of genetically improved /modified strains of the cyanobacterium *Synechocystis* PCC 6803 as a potential producers of valuable energetic compounds – bioethanol, glycogen and starch

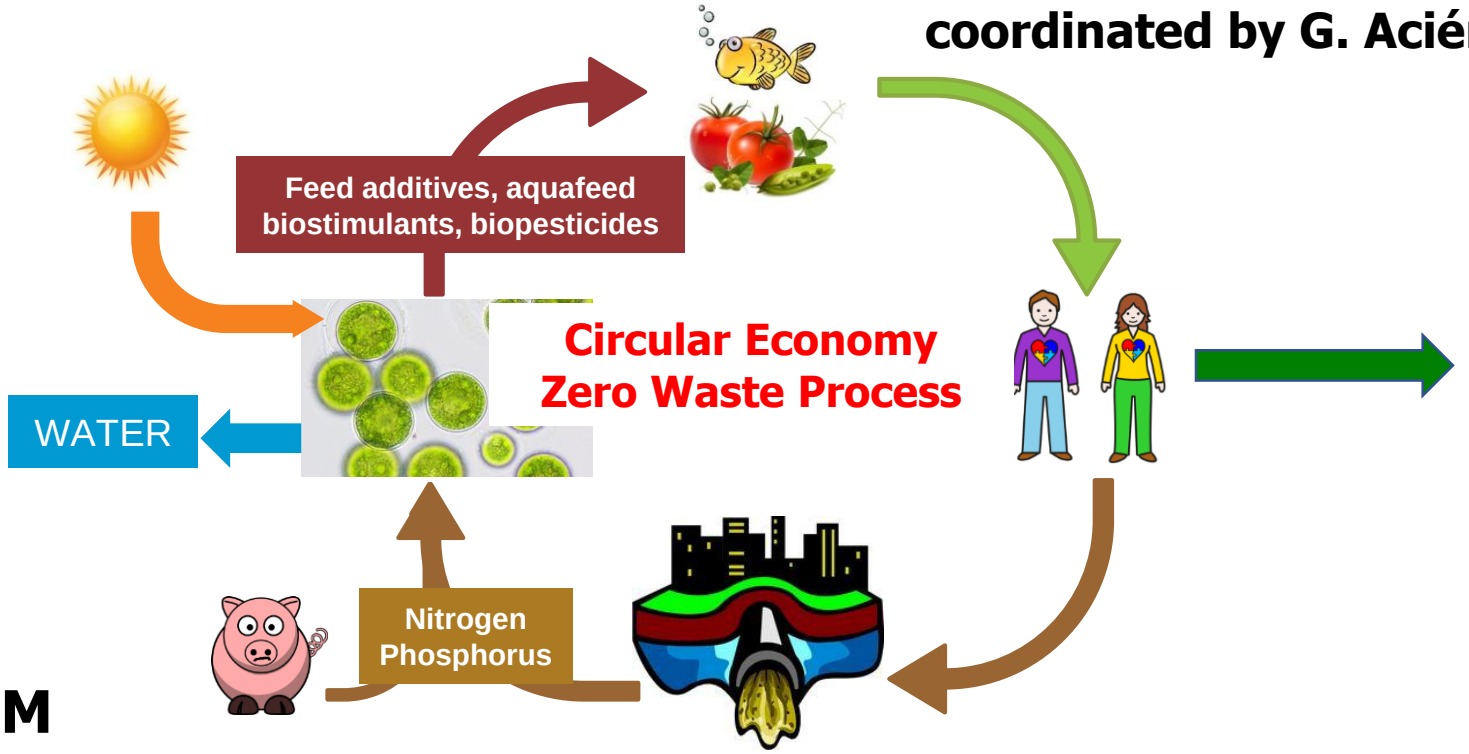
Cross-boarder collaborative research between

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

Strategic Partners:
FH OÖ Studienbetriebs GmbH
University of South Bohemia in České Budějovice

EU H2020 SABANA = Sustainable Algae Biorefinery for Agriculture aNd Aquaculture (2016-2021)

coordinated by G. Acién (University of Almería, Spain)



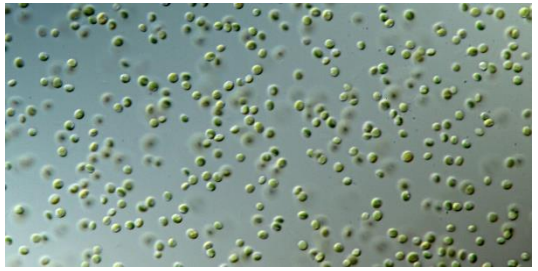
Andalusia, Spain – 25 000 ha of greenhouses

AIM

- To demonstrate a large-scale microalgae-based biorefinery for production of ***high-value products*** (**biostimulants, biopesticides, aquafeed additives**) **as well as** ***low-value products*** (**biofertilizers, fish feed**) recovering nutrients from **wastewaters** (sewage, centrate and pig manure) in continuous mode all year around (Spain)
- DEMO scale plants – 1 and 5 ha for biomass production and processing

Scheme of the project

MICROALGAE PHOTOBIOREACTOR → ROTIFER → FISH LARVAE → FISH (with added value)



Microalgae in inorganic media, or wastewater

Brachionus

Day 4-12
Pikeperch

Horizon 2020 Bio Based Industry project MULTISTR3EAM (2020-2024)

A sustainable multi-strain, multi-method, multi-product microalgae biorefinery integrating industrial side streams to create high-value products for food, feed and fragrance

Coordinator: A4F Algafuel SA, (Portugal)

- Sustainable products from microalgae for food, feed and cosmetics
- Dedicated to multi-strain, multi-process and multi-product biorefinery
- In-demand products: lipids including omega-3 and omega-6 fatty acids for feed and food applications
- Pigments such as carotenoids and phycocyanin for food and feed applications
- Low molecular weight organic compounds for fragrance applications

IMIC role: heterotrophic and phototrophic cultivation, mutagenesis, countercurrent chromatography

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- **Vince Ördög**, Széchenyi István University in Mosonmagyaróvár, Hungary
- **Giuseppe Torzillo**, **Margarita Da Silva,** Istituto per lo Studio degli Ecosistemi CNR, Sesto Fiorentino, Italy

Funding

- **EU H2020**
- **Interreg At-Cz**

POWER4BIO website and social media

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Thank you for attention

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POWER4BIO

REGIONS FOR BIOECONOMY

ALGATECH

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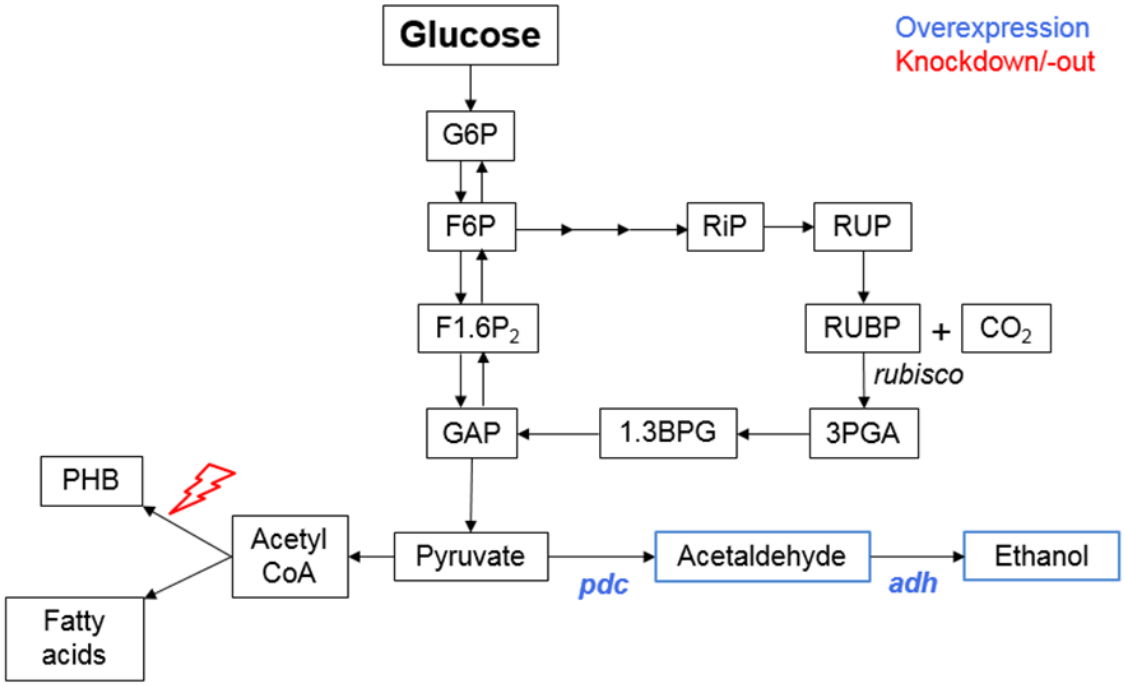
Genetically Modified *Synechocystis* PCC 6803 Strains for Bioethanol and Starch Production

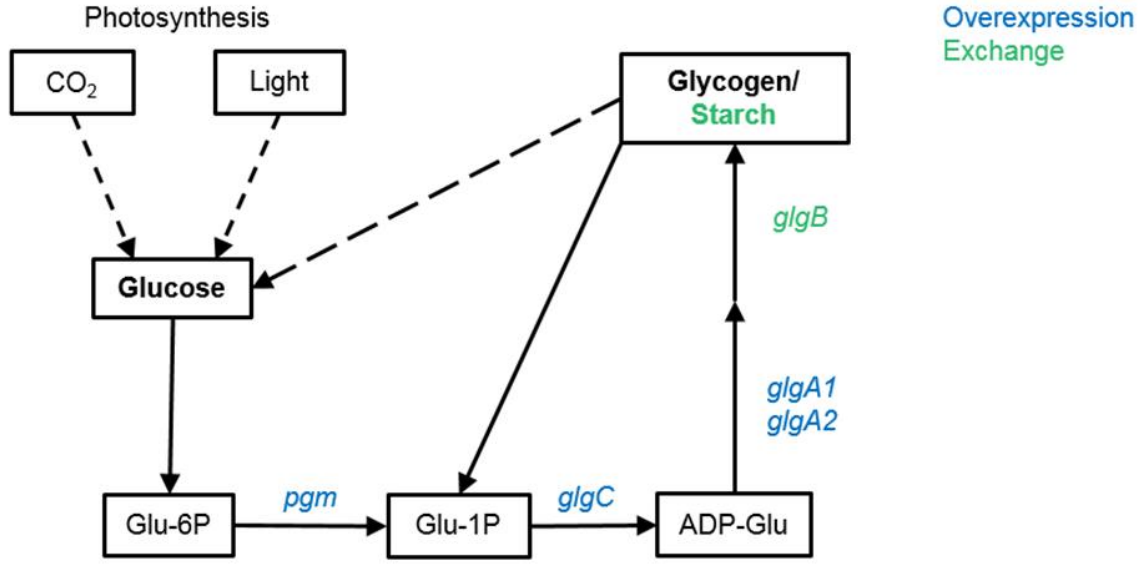
Tomáš GRIVALSKÝ, Gergely LAKATOS and co-workers

a) Ethanol Production



- Pyruvate decarboxylase → *pdc (Zymomonas mobilis)*
- Alcohol dehydrogenase → *adh (Synechocystis PCC6803)*
- **Polyhydroxybutyrate (PHB)**

b) Starch Production



- Phosphoglucomutase (PGM) → *pgm*
- ADP-Glucose pyrophosphorylase (AGP)→ *glgC*
- Glycogen synthases (GS) → *glgA1, glgA2*
- Eukaryotic branching enzyme → *glgB*