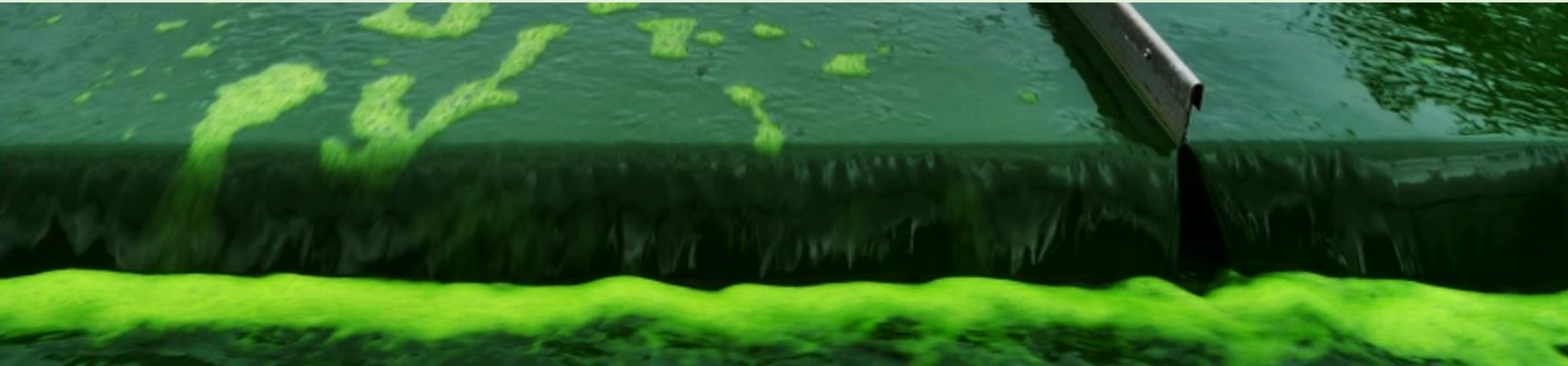


Algal Biotechnology in Bioeconomy

Good Examples from Applied Research in Algatech



Richard Lhotský, Ph.D.
Technology Transfer
Institute of Microbiology CAS
Centre Algatech, Třeboň



Institute of Microbiology CAS – Centre Algatech



LABORATORY
OF ALGAL
BIOTECHNOLOGY



LABORATORY
OF CELL CYCLES
OF ALGAE



LABORATORY OF
PHOTOSYNTHESIS



LABORATORY OF
ANOXYGENIC
PHOTOTROPHS

What is bioeconomy?

The production of renewable **biological resources** and the conversion of these resources and **waste streams** into **value added products**, such as food, feed, bio-based products and bioenergy...



This principle is very old.

In ancient times was well known in „Fertile Crescent“ – Egypt, Palestine, Mesopotamia.

Very often ended with dead land – Indus, Middle East.

What is bioeconomy?

... Its sectors and industries have strong **innovation** potential due to their use of a wide range of **sciences and industrial technologies**.

It is cool – bioproducts, natural products, eco-friendly technologies.

Lot of technologies fell down immediately when grant sources finished.

In many cases we reinvent the wheel.

- Algae for food – WW1
- Algae for fuel - WW2
- Algae to save the hungry world – never happened

So what? Algae?

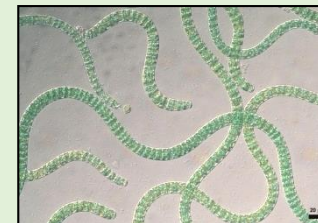
Macroalgae

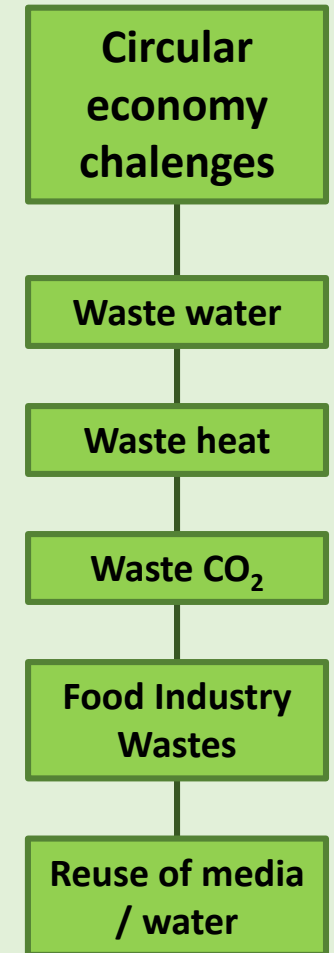
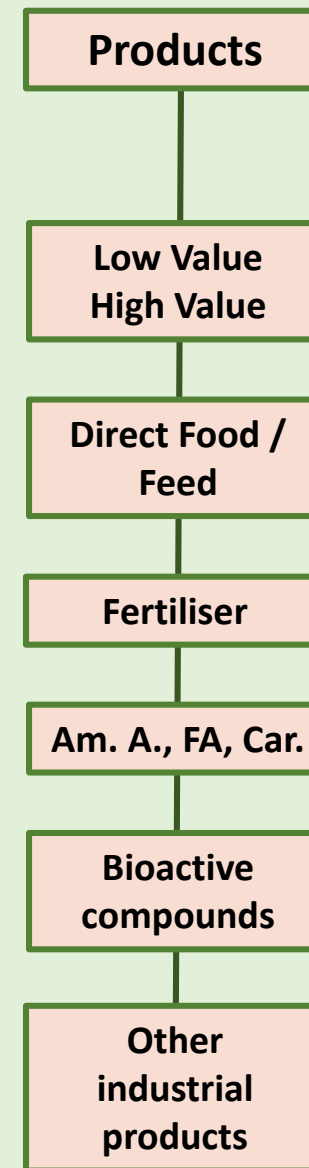
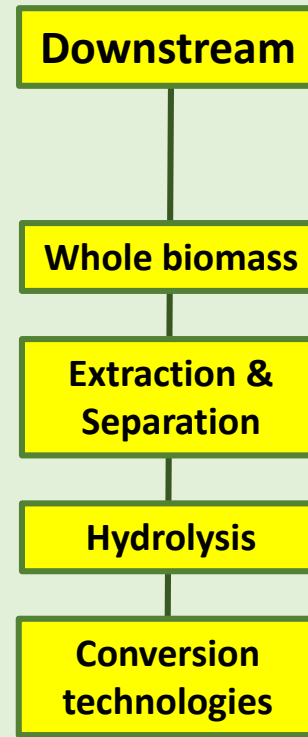
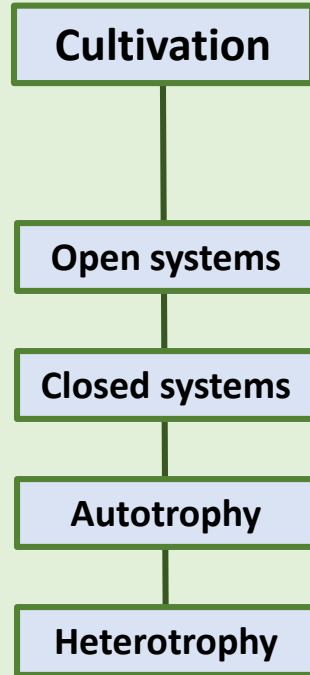
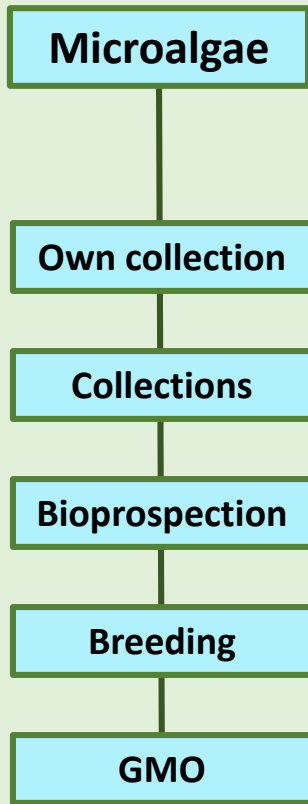
Kelps, seaweeds – sea water
dimension from cm to m
(*Ulva*, *Porphyra*, *Gracilaria*)



Microalgae

Fresh, sea water, soil, walls
dimension in μm
(*Chlorella*, *Spirulina*, *Dunaliella*)

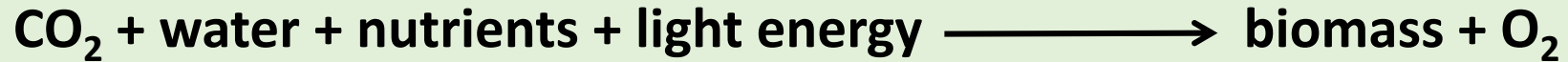




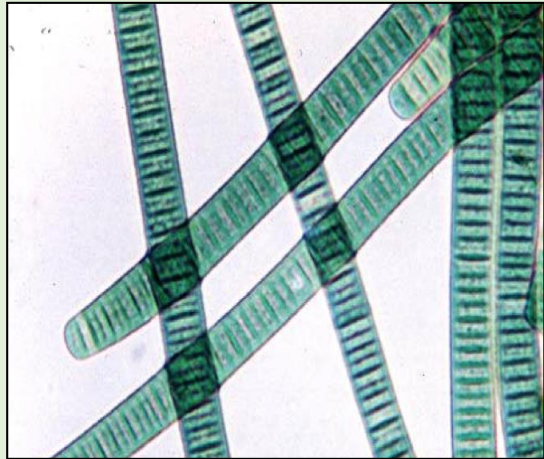




Photosynthesis is the key of green life



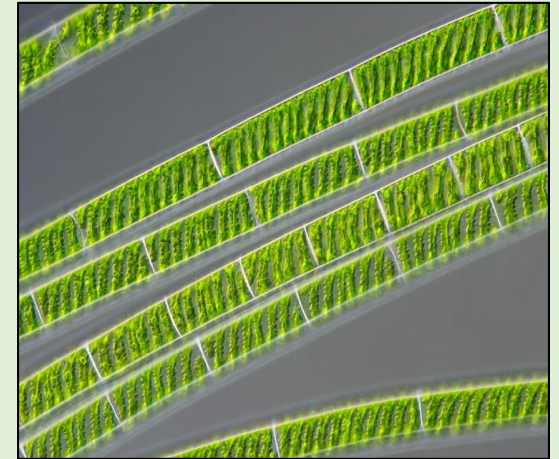
Photosynthesis = transformation of light energy into the chemical energy



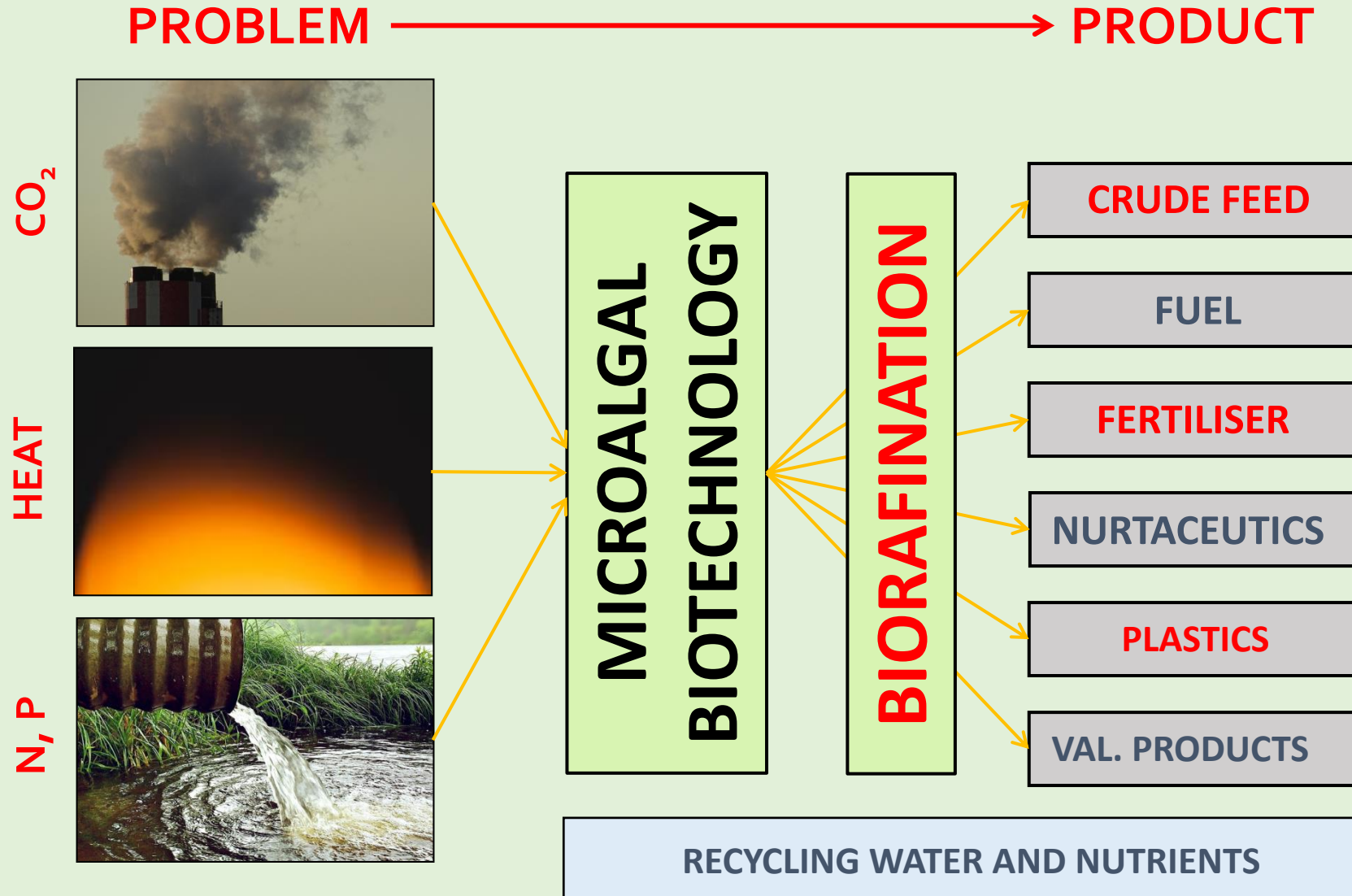
Nutrients for growth in inorganic form
macronutrients N, P, K, Mg,
micronutrients B, Zn, Co, V, etc.
Salts or waste

CO₂ - atmospheric, pure, waste

Water, temperature, pH...



Ideal scheme



Comparing productivity

The average production & market prices

2017 Czech Republic (26,33 CZK/EUR)

	Production (tDW/ha/y)	Price (EUR/tDW)
Rape seeds	3.1 (+ 3 t straw)	444
Potatoes	8.8	680
Peas dried	2.2 (+ 3 t straw)	222
Wheat	4.7 (+5 t straw)	170
Maize silage	10.0	90
Chlorella (CZ)	15 - 30 (season)	10 – 15 Th. € Food grade



Low value products

Algae as a source of protein, starch or oil are not **cost** competitive with traditional plants

How to decrease production cost of algae?

- Decrease price of inputs – nutrients, waste utility.
- Decrease price of technology – simple and durable outdoor cultivation.
 - New more productive strains – bioprospection, breeding, GMO
 - Recycling residual nutrients and water after cultivation.
 - Switch to **HIGH VALUE PRODUCTS**

The advantages of mineral fertilizers (over wastewaters)

- High conc. of nutrients (urea = 46 % N)
- No problem with delivery. (In place in time)
 - Small storage capacities needed
 - Stable nutrient content
- **They are certified** - composition, content of impurities (heavy metals)
- HMs are critical mainly P fertilizers which are produced from phosphate rock,
(40 % Pb, 15 % Cd, 4 % Hg, 15 % As, 3 % Cr
contamination in algal biomass from fertilizers)

When to use other nutrient sources

- When it does not contain compounds toxic for algae growth or compounds accumulating in them and devaluating a product (HM, PCB, medicaments, etc).
- If they have the same ratio and concentrations of nutrients as required by growing algae.
- Waste water source should be close to the production site and should be producing at least during the cultivation season.
 - Stable source of waste all over the year or storage.
 - Ideally should be connected with CO₂ production.
 - Always is required very basic economic assessment.

$\text{CO}_2 - \text{CO}_2 - \text{CO}_2$

- Atmospheric CO_2 **is not sufficient.**
 - Theoretical algal consumption is cca 2 t CO_2 /t dw
but in practice is up to 5 t CO_2 /t dw = **release to atmosphere 3 t.**
 - Direct emission from steel production = **2 t CO_2 /t steel**
- 1 MW el. = 12 000 m³ biogas/day = 5000 m³ of CO_2 = 10 000 kg CO_2 /day. Enough for cca 3 t /day of algae production.
- Middle European weather conditions allow 3 g/ m² / d
= **300 ha of open cultivation space.**
- Legal consequences same as with waste water.

Source: M. Kajan (2018)

$\text{CO}_2 - \text{CO}_2 - \text{CO}_2$

Another example:

Source: J. Kopecký (2021)

500 MW coal-fired power plant produces 9×10^6 kg CO_2 /day.
Our outdoor unit (1000 m^2 , productivity $30 \text{ g/m}^2/\text{d}$, spend 54 kg/d of CO_2).

This represents **0,0006 %** of daily CO_2 production of average Power plant.



Innovative role of Science

Bioeconomy ... Its sectors and industries have strong **innovation** potential due to their use of a wide range of **sciences and industrial technologies**.

HISTORICAL CHALLENGES

- Feed the hungry world (1950s)

-Fuel (1970s and 1990s)

- Plastics

- Lack of strains



RESULTS

- Principles of mass cultivation
- Spirulina for starving children

- Production of Valuable substances under stress conditions
- Genetic engineering (GMO)

- New separation techniques
- Chemical engineering as part of algal biotechnology

- Bioprospection
- Mixotrophy and heterotrophy.
- Mutagenesis

REEgain

Sustainable biological recycling of Rare Earth Elements from electronic waste and water

Metals of the future



La-Ni-H batteries
Fluorescent lamps
Generators
Communication technologies
Hybrid vehicles

Environmental impact



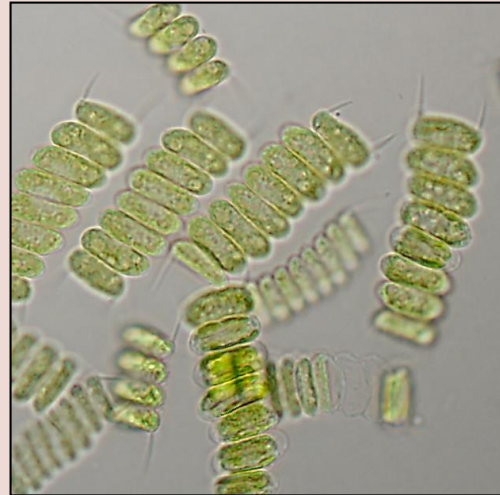
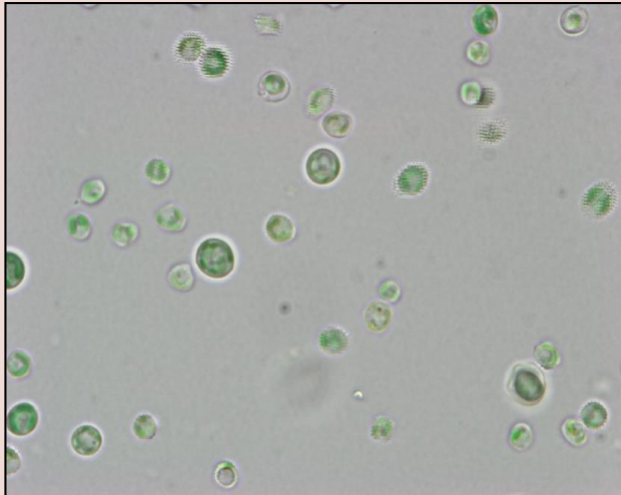
120 Mt of Red Mud
Waste Water
Toxicity
Need to Recovery

Geopolitics



China produces 97 % REE
Dependence on import
Important for technological development

REEgain



Galdieria sulphuraria – extrem. red alga / low pH

Desmodesmus quadricauda – green algae

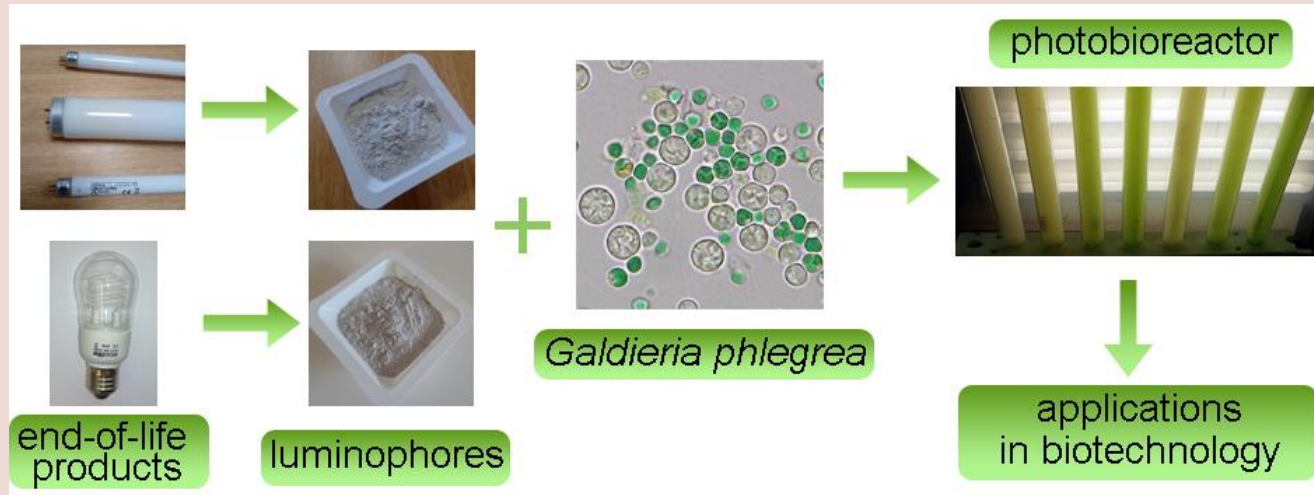


Project Aims

- Recovery of REEs from industrial waste using microorganisms
- Selection of appropriate microorganisms
- Optimization of co-cultures
- E-waste (luminophores)



REEgain



For more information:

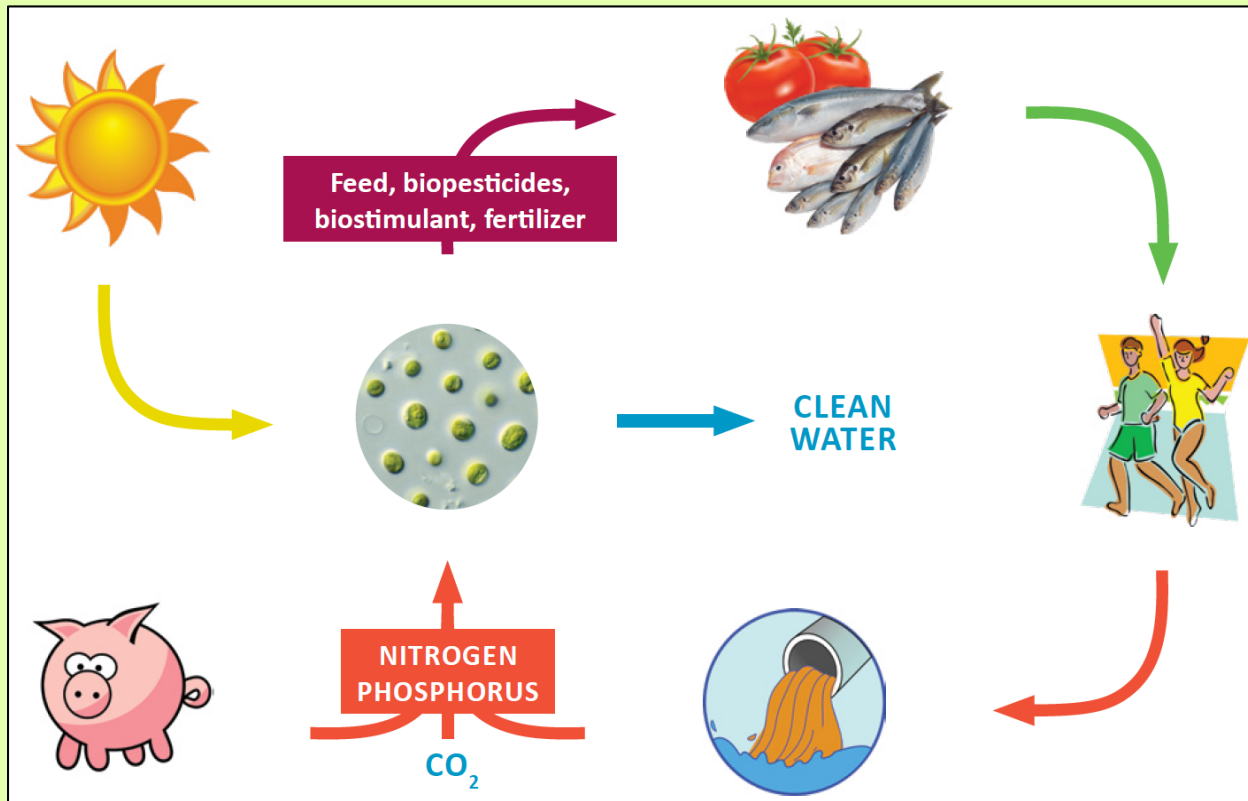
Milada Vítová, Ph.D.
Lab. of cell cycles
www.alga.cz

- Accumulation of metals
cca 10% of biomass
- Selective accumulation using mutants
probably more
- Endproducts – metal oxides, fertilisers
(not in Europe)
- Lanthanum, Cerium, Europium,
Gadolinium, Neodymium, ...



SABANA

Sustainable Algae Production for Agriculture and Aquaculture



Aims

- Development of robust, modular and economically viable cultivation system .
- Sustainable production of microalgae – integration of WW from svine farms.
- Focus on High value product – biopesticides and stimulats of vegetable and flowers grow.
- Rest of biomass is feeded in aquaculture or hydrolysed and transformed into fertilisers



SABANA

Sustainable Algae Production for Agriculture and Aquaculture

Results

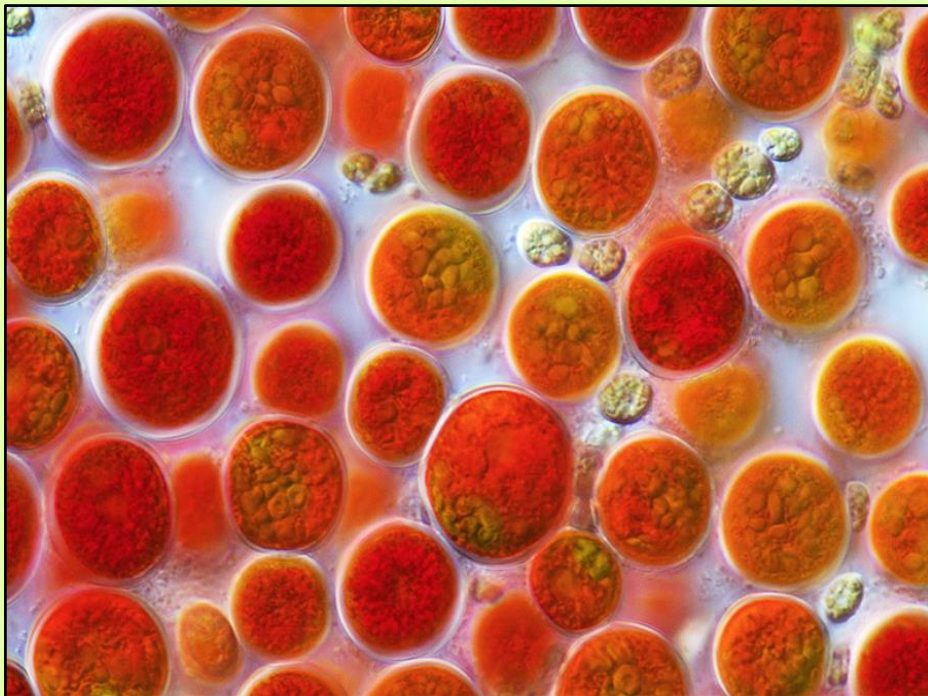
- 1, 2 and 5 ha demonstration units in Spain.
- Special algal strains producing biopesticides and antifungal substances were identified and tested on crop
- Using wastes the final price of biomass was decreased to 2 € / kg.



Prof. Jiří Masojídek
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PHYTOCOMPLEX

Development of CCC separation techniques for isolation of monoesters of Astaxantin



- Astaxantin is one of the most powerful antioxidants produced by *Haematococcus*.
- Use for Salmon and Trout feeding.
- Final product for human consumption is „oleoresin“ – mixture of different forms of astaxantins and plant oil.
- Monoesters of astaxantin – Phytocomplex - can be used in functional food.
- Bioactivity was already tested *in vitro* with promising results

PHYTOCOMPLEX & BIOCIIRTECH



© Algamo, Ltd.

- Technology was patented and have promising collaboration with industrial project partner to place phytocomplex on the market in EU.
- Another TAČR project „**BIOCIIRTECH**“ is focused on improving production of Astaxantin using breeding techniques.
 - Algae should grow under lower temperature and lower light – improve economy of producing plant.

Algenetics

Use GM Cyanobacterias for ethanol production

Challenges

- GMO can improve production of fuel
- How to obtain fuel without harvesting biomass
- Cyanobacteria produces glycogene
 - Starch is much easier to handle

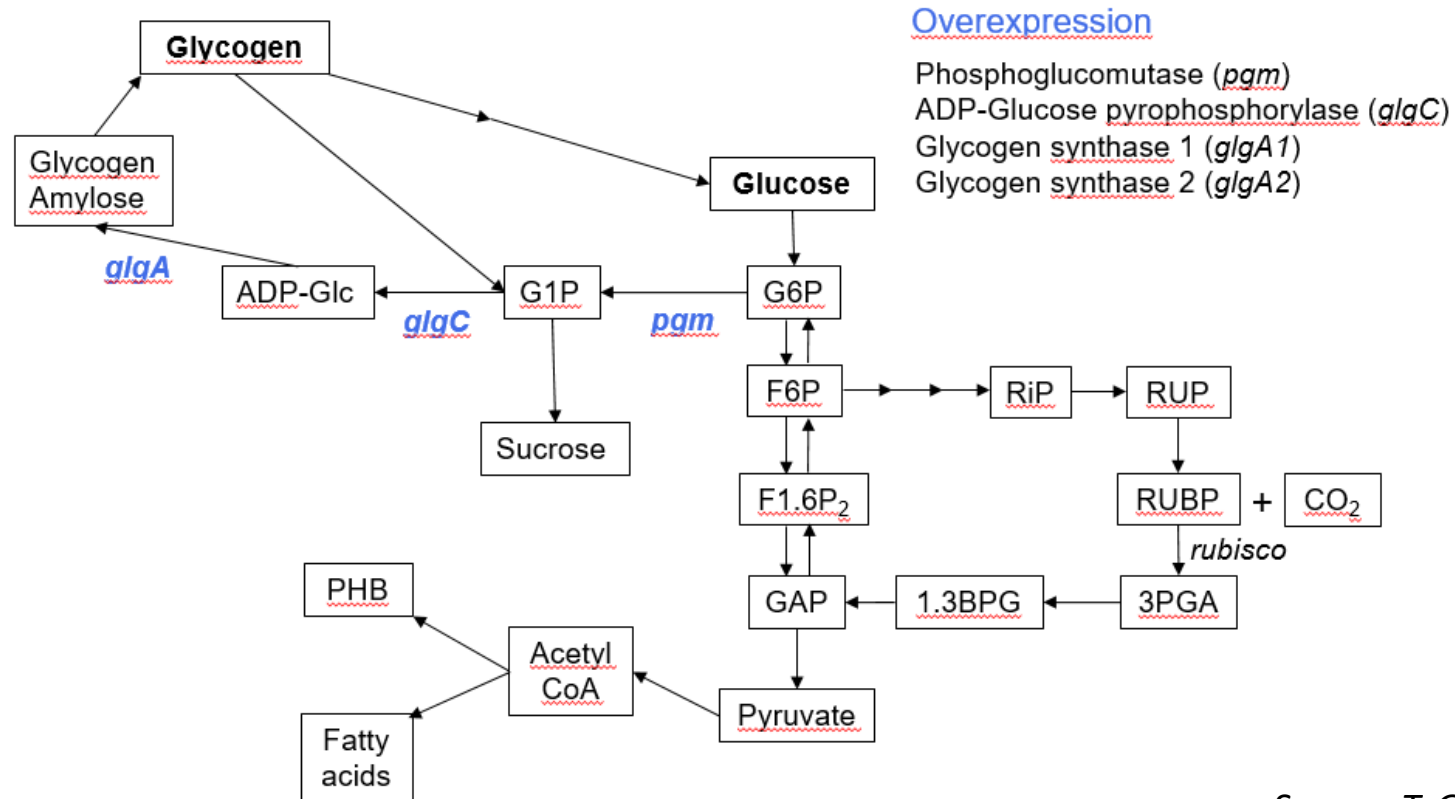


Aims

- Switch (Gene knockout) metabolism of Cyanobacteria (glycogene to starch)
- Modify cyanobacteria to direct production of ethanol.

Algenetics

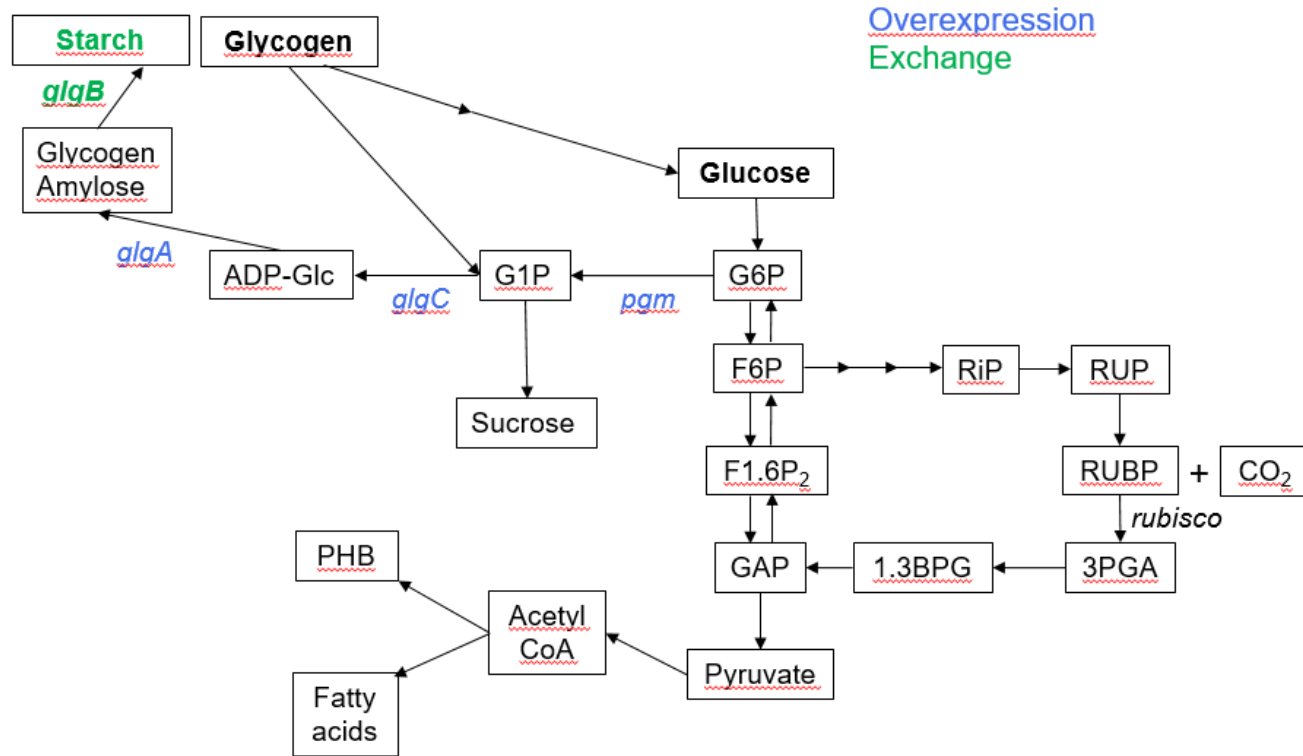
Overexpression of Genes



Source: T. Grivalský, J. Richter (2019)

Algenetics

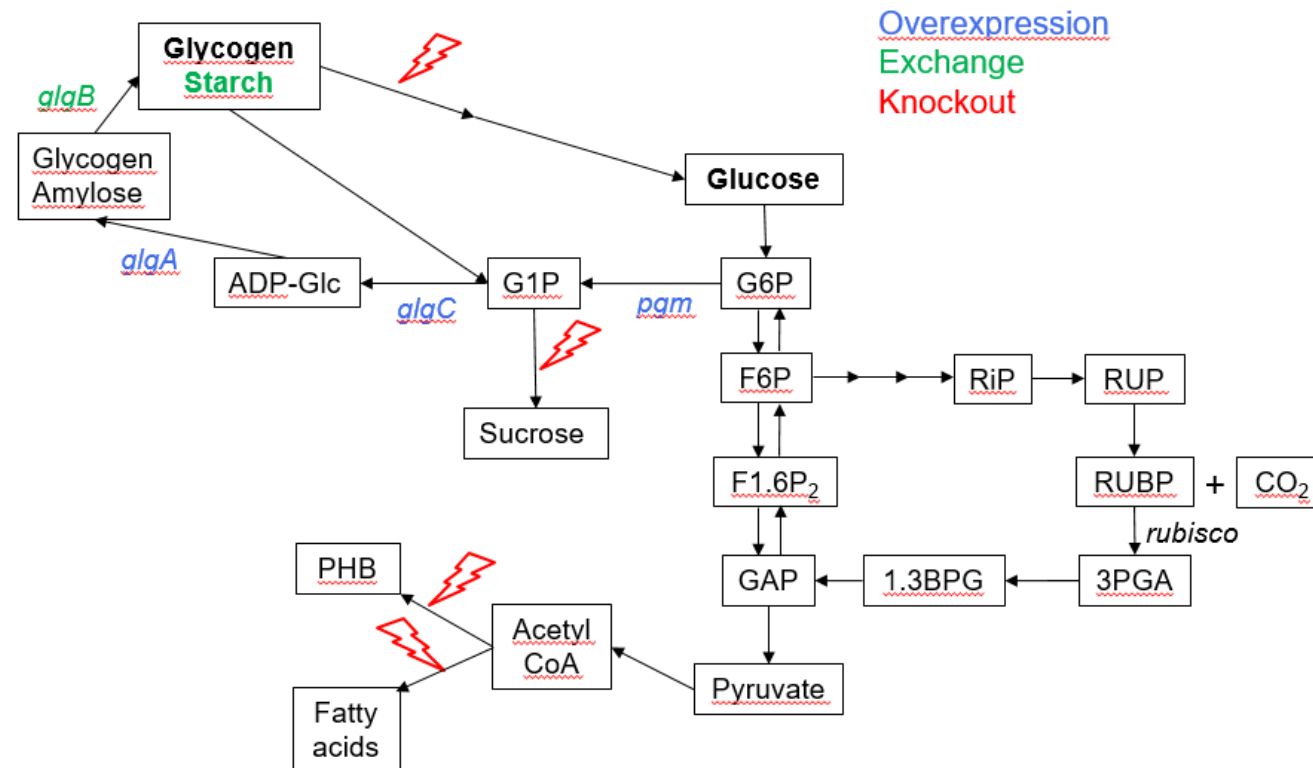
Exchange of Branching Enzyme



Source: T. Grivalský, J. Richter (2019)

Algenetics

KO of Alternative Storage Pathways



Source: T. Grivalský, J. Richter (2019)

Algenetics

RESULTS

- Cyanobacterium *Synechocystis* sp. PCC6803. This organism doesn't produce neither starch nor ethanol.
- Genes needed for starch and Et-ol were introduced to the DNA. Substances using the same building blocks as starch and Et-ol were eliminated.
- Metabolism was genetically improved by increasing the capability to utilise CO₂.
- Except GM natural strains were grown in Et-ol rich to select these with high Et-ol tolerance. Mutants were able to growth in 3 % Et-ol while natural one was inhibited in 1,5 % Et-ol.
- UV mutation was done and we received mutants with high light tolerance = increase in photochemical activities.

Algae4Fish

Fatty acid changes throughout the food chain of Pikeperch fry

Challenges

- How to utilise biogas WW?
- How to utilise Intensive recycling aquaculture WW and CO₂?
- Pikeperch is valuable fish.
- Can we increase survival rate of fry using appropriate algae – zooplankton – fish feeding strategy?



Aims

- Testing different zooplankton.
 - Find FA rich microalgae.
 - Developing continual PHBR.
- Test if are able to improve FA spectrum in fish fry using living feed?

Algae4Fish

- Rotifers are the best food for a fish fry. (Based on previous knowledge of FROV JCU).
- When 7 different diets were compared – most suitable for larvae prosperity seemed to be the microalgae **without EPA** and high LA and ALA content.
- When rotifers have EPA source, they degrade EPA to essential fatty acids (LA and ALA).
- Final EPA content in larvae comparable when fed by rotifers fed by microalgae with or without EPA – EPA is important for a brain development.

Algae4Fish

- Digestate can be used as nutrient source – tested on several microalgae.
- Solid parts removal is needed to increase light permeability.
- Auto-, hetero- and mixotrophic regimes are possible.
- Legal and market limitations.



Review

Digestate as Sustainable Nutrient Source for Microalgae—Challenges and Prospects

Lisa Bauer ¹, Karolína Ranglová ^{2,3}, Jiří Masojedek ^{2,4}, Bernhard Drosig ^{1,5} and Katharina Meixner ^{1,5,*}

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Featured Application: This review provides insight into the origin of digestate and how it can be processed to meet requirements for microalgae cultivation as well as challenges that occur in cultivation, in downstream processing, and in terms of products when using this nutrient source.

Abstract: The interest in microalgae products has been increasing, and therefore the cultivation industry is growing steadily. To reduce the environmental impact and production costs arising from nutrients, research needs to find alternatives to the currently used artificial nutrients. Microalgae cultivation in anaerobic effluents (more specifically, digestate) represents a promising strategy for increasing sustainability and obtaining valuable products. However, digestate must be processed prior to its use as nutrient source. Depending on its composition, different methods are suitable for removing solids (e.g., centrifugation) and adjusting nutrient concentrations and ratios (e.g., dilution, ammonia stripping). Moreover, the resulting cultivation medium must be light-permeable. Various studies show that growth rates comparable to those in artificial media can be achieved when proper digestate treatment is used. The necessary steps for obtaining a suitable cultivation medium also depend on the microalgae species to be cultivated. Concerning the application of the biomass, legal aspects and impurities originating from digestate must be considered. Furthermore, microalgae species and their application fields are essential criteria when selecting downstream processing methods (harvest, disintegration, dehydration, product purification). Microalgae grown on digestate can be used to produce various products (e.g., bioenergy, animal feed, bioplastics, and biofertilizers). This review gives insight into the origin and composition of digestate, processing options to meet requirements for microalgae cultivation and challenges regarding downstream processing and products.

Keywords: sustainable nutrients; digestate treatment; nutrient requirements; microalgae cultivation; downstream processing; biorefinery



Citation: Bauer, L.; Ranglová, K.; Masojedek, J.; Drosig, B.; Meixner, K. Digestate as Sustainable Nutrient Source for Microalgae—Challenges and Prospects. *Appl. Sci.* **2021**, *11*, 1056. <https://doi.org/10.3390/app11031056>

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Another large projects

MULTISTR3AM (H2020 BBI) - New strains for novel high value products for large scale food and feed industry and cosmetics. Non GMO. Breeding of strains.

Contact person: dr. J. Kopecky, dr. R. Lhotský, www.alga.cz

Algae4IBD (H2020) – Searching new microalgal substances – antioxidant, anti inflammation and analgetic for promoting treatment of autoimmune Inflammatory Bowel Disease (IBD) e.g. Crohn's disease.

Contact person: dr. P. Hrouzek, www.alga.cz

PhotoReDesign (ERC Synergy, EU) – A way how to rebuild photosynthetic light reactions – increase photosynthesis efficiency. Synthetic biology.

Contact person: Prof. J. Komenda, www.alga.cz

Food For Future (Strategy AV 21) – New approaches in sustainable food production including Natural Science, Climatology and Social studies.

Contact person: Prof. O. Prášil, dr. Lhotský, www.alga.cz

CONCLUSION

Scientific and industrial world are very different – in expectations, approaches, objectives, private vs. public financing, legal limitations – hard to understand each other.

Collaboration step by step and understanding each other is the only way how to move forward and **DO** something and not only TALK about it.



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M. Kajan

Richard Lhotský, Ph.D.

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