

Centre **ALGATECH**

Institute of Microbiology of the Czech Academy of Sciences





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**ALGATECH** – The Centre of algal biotechnology evolved from the Laboratory of Algal Research, founded in 1960 in Třeboň. Throughout its history, the Třeboň's site of the Institute of Microbiology of the AS CR has focused on microscopic algae and their use in food and feed industries and in human and veterinary medicine. At present, the ALGATECH Centre is an internationally recognised centre for basic and applied research of microalgae, cyanobacteria and photosynthetic bacteria, including the development of algal biotechnology. It is the largest centre of microalgae research in the Czech Republic. The ALGATECH Centre is housed in the historic building of Opatovický Mill from the 18th century, which has been recently renovated. The Centre also operates unique thin – layer cultivation units for autotrophic cultivation and a biotechnological hall for heterotrophic cultivation of microalgae, including the equipment for processing algal biomass. Today, algal biotechnology focuses on the use of algae in food and feed or as a source of renewable energy and fuels. Practical significance can be found in various valuable substances isolated from algae and cyanobacteria. These substances, which are isolated, characterised and tested in tissue cultures in the laboratory of the ALGATECH Centre, have potential uses in medicine. The Centre's activities also include the development of new instruments and methodologies. The Centre collaborates with laboratories the world over, and hosts students and researchers from abroad. The Centre is known for its excellent research in photosynthesis and algal cell cycles.

#### LABORATORY OF PHOTOSYNTHESIS

- Biosynthesis and repair of photosystem II, ecophysiology of algae, biosynthesis and function of photosynthetic pigments, dynamics of membrane complexes.
- Research in regulation of photosynthetic metabolism in microscopic algae.
- Development of new methods and instruments used for research and applications in agriculture, environmental monitoring and water management.

#### LABORATORY OF ALGAL BIOTECHNOLOGY

- Development of new technological processes leading to increased productivity of microalgae in phototrophic and heterotrophic modes.
- Innovation of downstream processes in the production of algal biomass.
- Prospecting, characterization and production of new active substances from secondary metabolism of microalgae, testing of their applications.
- Development of new methods for the extraction of bioactive compounds from microalgae.

#### LABORATORY OF CELL CYCLES OF ALGAE

- Regulation of cell cycle of algae, characterised by multiple division.
- Study of accumulation and hyper-accumulation of energy reserves (starch, lipids, polyphosphates) in algae.
- Bioremediation and metal recycling using microalgae.

#### LABORATORY OF ANOXYGENIC PHOTOTROPHS

- Development of new optical devices for detecting anoxygenic phototrophs.
- Isolation of bioactive substances and bioaccumulation studies in anoxygenic microorganisms.
- Study of phototrophic microorganisms as potential sources of biofuels, especially hydrogen.



Other activities of the Centre include the organisation of scientific meetings, conferences, symposia, seminars and workshops, both at national and international levels, and scientifically oriented lectures intended to present science to the public.

We are committed to collaborative applied research, especially in the area of algal biotechnology.

We provide facilities and analysis for research.

The researchers of the Centre are significantly involved in the teaching and training of master and doctoral students, especially at the University of South Bohemia in České Budějovice, at the Charles University in Prague and the Masaryk University in Brno.

The aim of the ALGATECH Centre is to further serve as centre of excellence in basic and applied research of phototrophic microorganisms. Our strength is in highly skilled personnel, accumulated know-how and modern facilities.



# LABORATORY OF PHOTOSYNTHESIS



## GROUP LEADERS

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## THEMATIC RESEARCH FOCUS

### Basic research topics

- Regulation of the chlorophyll biosynthesis pathway and its coordination with protein biosynthesis during biogenesis of cyanobacterial pigment-proteins.
- Assembly of cyanobacterial photosystems from individual components and the localization of the individual steps.
- Quality control of photosynthetic membrane proteins including the mechanisms underlying repair of Photosystem II and the role of FtsH proteases in this and other cellular processes.
- Molecular mechanisms regulating microbial photosynthesis in newly discovered and ecologically important microorganisms under variable light and nutrient conditions.
- Mechanisms regulating photosynthesis and nitrogen fixation in diazotrophic cyanobacteria and the effect of increased CO<sub>2</sub> concentration on nitrogen fixation.
- Regulation and molecular mechanisms of photoprotection in pigment-proteins of algae and cyanobacteria.
- Thylakoid membrane dynamics, protein mobility and the role of membrane microdomains in photosynthesis.

### Applied research topics

- New design and improvement of instrumentation for photosynthesis research including cultivation facilities, microscopic techniques and spectroscopic devices.
- Identification of microalgal and cyanobacterial strains appropriate for production of biomass and valuable compounds.

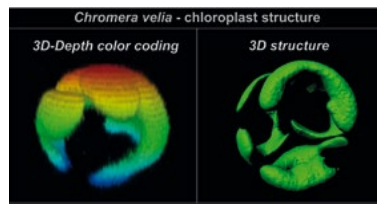


Schematic model of the Cyanobacterial Photosystem II. Green color indicates chlorophylls, purple color polypeptide chains (modified figure from Franck Michoux, Imperial College London)

## KEY RESEARCH EQUIPMENT

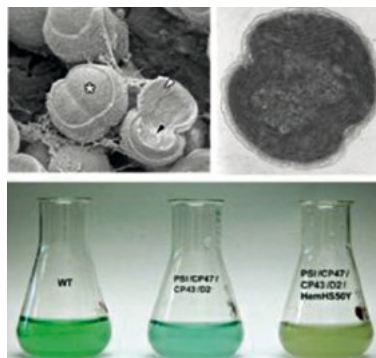
### Services Offered

- HPLC for analysis of pigments and their biosynthesis precursors including radioactive flow detector.
- Facility for analysis of membrane protein complexes using two-dimensional native/denaturing electrophoresis combined with their immunochemical and autoradiographic detection.




Application of confocal microscopy methods – structure of alga chloroplast.

- Absorption spectrophotometers for the characterization of various samples under a variety of conditions.
- Various instruments for kinetic, microscopic and spectral fluorescence measurements for the characterisation of photosynthetic parameters (e.g. light-harvesting efficiency, photosystem function), from single cells or cell suspensions.
- Analysers for assessing carbon assimilation and oxygen evolution.
- Mass spectrometric facility for isotopic measurements of gas exchange, Fourier transform infrared spectrometer for characterization of the biomass composition.
- Confocal Microscopy Facility for highly sensitive, small photosynthetic cell analysis, time-lapse imaging, 3D imaging and spectral imaging. Advanced confocal microscopy methods (FRAP, FCS).



The upper pictures show cells of the *Cyanobacterium Synechocystis* PCC 6803 under a scanning (L) and transmission (R) electron microscope (van de Meene et al. Arch. Microbiol, 2006). Below is the wild type strain together with two genetically modified mutants exhibiting changed content of photosynthetic pigments.





# LABORATORY OF CELL CYCLES OF ALGAE



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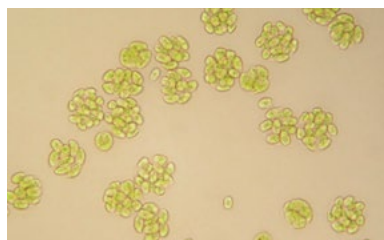
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## THEMATIC RESEARCH FOCUS

### Basic research

- Molecular mechanisms of cell cycle regulation. Cell cycle block in response to DNA damage in green algae dividing by multiple fission.
- Coordination of growth and cell cycle; relationship between growth, critical cell size and cell cycle progression.
- Production and consumption of energy reserves within the cell cycle; coordination among growth, cell cycle progression and production/consumption of starch, oil and polyphosphates.
- Influence of external factors (light, temperature) and metals (e.g. rare earth elements (REEs), selenium and cadmium) on the cell cycle progression, DNA damage and physiology of unicellular algae.
- Interaction of metals with the nutrients; uptake by cells; mode of action.



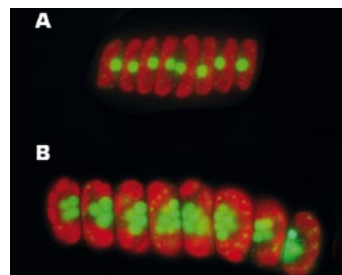
Synchronization of algae. Light micrograph of synchronized culture of green alga *Chlamydomonas reinhardtii* undergoing cell division. Undivided mother cells and mother cells dividing into 4, and 8 daughter cells can be seen.

### Applied research

- Testing of algal strains; development of conditions for production of algal biomass enriched in starch or lipids.
- Production and testing of algal strains capable of metal (selenium, REEs) accumulation; use for remediation and/or recycling.
- Testing of metal-enriched algae for feed supplement.

## MISSION

Understanding the regulation of cell cycle in green algae dividing by multiple fission and its coordination with different environmental factors. Application of the knowledge in directed condition design for stress resistance or for production of different compounds.

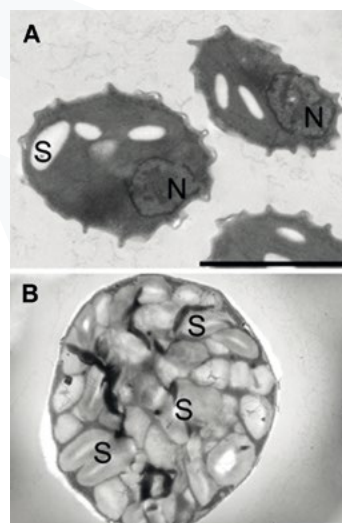


Multiple fission cell cycle. Fluorescence micrographs of cells of green alga *Scenedesmus quadricauda* stained with nuclear dye SYBR Green I.

**A** daughter coenobium containing cells with single nucleus, **B** coenobium with tetra-nuclear and octo-nuclear cells.

## EXPERTISE AND OFFERS

- Synchronization of algae for production of defined cultures to study various environmental effects.
- Directed over-production of starch or oil by algae in laboratory and semi-industrial conditions.
- Algae mediated recycling and remediation of metals from the environment.
- Testing of metal-enriched algae for feed supplement.
- Improvement of algal growth and biomass production by manipulation of cell cycle.
- Expertise in cultivation of diverse algal strains at different growth conditions; optimization of growth conditions.



Over-production of starch in algae. Electron micrographs of daughter cells of green alga *Chlorella* grown in complete mineral medium (**A**) and in the presence of cycloheximide (1 mg/L) (**B**). N, nucleus; S, starch granules. Bar 2  $\mu$ m. After Brányiková et al., 2011, *Biotechnol Bioeng* 108: 766-776.



# LABORATORY OF ALGAL BIOTECHNOLOGY



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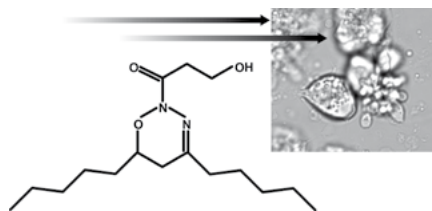


## RESEARCH TOPICS

The laboratory of algal biotechnology is generally engaged in basic and applied research in the field of autotrophic and heterotrophic cultivation of microalgae, finding biologically active and valuable substances of microalgae and then complex processing of algal biomass. The theoretical knowledge is used for the development of mass culture devices for biomass production and its subsequent use in dietary supplements, animal feeds, aquaculture, pharmacy and medicine.

### Research activities

Design, construction and operation of various cultivation units based on natural and artificial light sources (raceways and cascades, panel and column photobioreactors).



Novel cyanobacterial metabolite nocuolin A discovered in our laboratory is causing programmed cell death (apoptosis) in human cancer cells. Its unusual structure can serve for future drug development.

Photosynthesis monitoring to characterize microalgae strains and optimize cultivation regimes and photobioreactors design.

The development of the whole hit-to-lead process including cultivation, extraction, screening, purification, in vitro testing of biological activities and subsequent structure elucidation of the bioactive secondary metabolites. Heterotrophic cultivation of microalgae to produce biomass containing bioactive compounds for food and feed supplementation.

### Excellence

More than a half-century long experience in most aspects of microalgae research and biotechnology, which is supported by basic knowledge of microalgae photosynthesis and life cycles.

## DEVELOPED TECHNOLOGIES

### Layer Cascade

Thin layer cascade unit is made of stainless steel and designed as a pilot production module with an area of 100 m<sup>2</sup> and total working volume of 550-1200 litres. Microalgae culture is delivered to the highest point by a pump and flows along inclined surface in a uniformly thin layer where it is exposed to sun radiation.

### Column Photobioreactor with Internal Illumination

The pilot unit (volume of 100 litres) consists of a closed vessel internally illuminated by strips of light-emitting diodes. The lighting can be regulated and depends on cell density to avoid photoinhibition in diluted culture or photolimitation.



Final product of the green microalgae Chlorella cultivation – dry disintegrated biomass. Disintegration of the cell walls is necessary for better digestibility and use of active compounds of the microalgae in a human body.



Patented outdoor thin-layer cascade for the autotrophic cultivation of freshwater microalgae. A thin layer up to 1 cm ensures full sun illumination of the microalgae culture and high biomass concentration as high as 30 g/L.

### Downstream Processing

We develop technologies for economical extraction, purification, and stabilization of target products. As these compounds may also occur as isomers, the fractionation and isomer separation present significant challenges. Diverse approaches are employed, including selective crystallization, the extraction with selective solvents, preparative liquid chromatographic and counter current chromatographic separations.

### Bioactive Compounds

We have characterized novel bioactive secondary metabolites produced mainly by cyanobacteria using a number of approaches that include in vitro biochemical screening as well as the application of analytical techniques (GC/MS, HPLC/MS, HRMS, FTIR). Nostotrebins 6 polyphenolic compound with antibiotic activities, a novel structural variant aeruginosin 865, possessing with antiinflammatory activity, cytotoxic cyclic lipopeptides muscotoxin A and Puwainaphycin F/G and heterocyclic 1,2,3-oxadiazine containing compound nocuolin A with pro-apoptotic and autophagy activity.

# LABORATORY OF ANOXYGENIC PHOTOTROPHS



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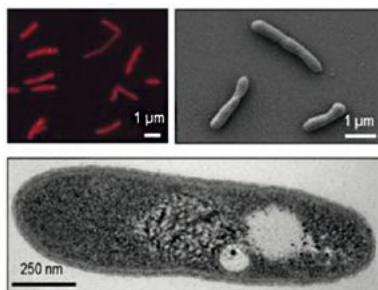
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## RESEARCH FOCUS

- Ecology of anoxygenic phototrophs in natural environments.
- Genomics and evolution of phototrophic organisms.
- Physiology and metabolism of anoxygenic phototrophs.
- Architecture of anoxygenic light harvesting complexes.
- Biotechnology application of anoxygenic phototrophs.

We study phototrophic microorganisms both in the laboratory and in the field using biophysical, biochemical and molecular techniques. The diversity of phototrophic species in their natural habitats is investigated using Next Generation Sequencing tools. Phototrophic cells are enumerated using infra-red epifluorescence microscopy. In collaboration with Photon Systems Instruments Ltd. we developed an ultrasensitive kinetic fluorometer for the detection of phototrophic microorganisms in aquatic environments.



Microscopic images of our isolate *Gemmatimonas phototrophica*. For details see Zeng et al., PNAS 111: 7795-7800, 2014.

We isolated over 100 novel strains of phototrophic organisms using a custom made infra-red screening system. The novel isolates are characterized regarding their taxonomy, growth requirements and pigment composition. We performed whole genome sequencing for 30 of our isolates. The photo-synthetic apparatus of selected strains is studied with advanced biochemical and spectroscopic methods.

In collaboration with the University of South Bohemia in České Budějovice we train MSc and PhD students in the study programs of Hydrobiology, Biochemistry and Molecular Biology. The team established several international collaborations in Europe, America and Asia, and hosted over 20 scientists and students from collaborating teams.

## INSTRUMENTS

- We operate a flowcytometry and cell sorting facility. We offer cytometric analyses and cell sorting for both academic and commercial partners.
- BD Influx cell sorter, 150 mW blue laser, 4 fluorescence detectors.
- Apogee A50 Micro flowcytometer.
- Zeiss Axio Imager Z2 epifluorescence microscope + Hamamatsu EM CCD camera.
- Custom made infra-red system for colony screening and micro-spectroscopy.
- Shimadzu Prominence-i, HPLC system.



Field measurements of phototrophic bacteria in Svalbard.

## MAIN COLLABORATORS

### Academic partners

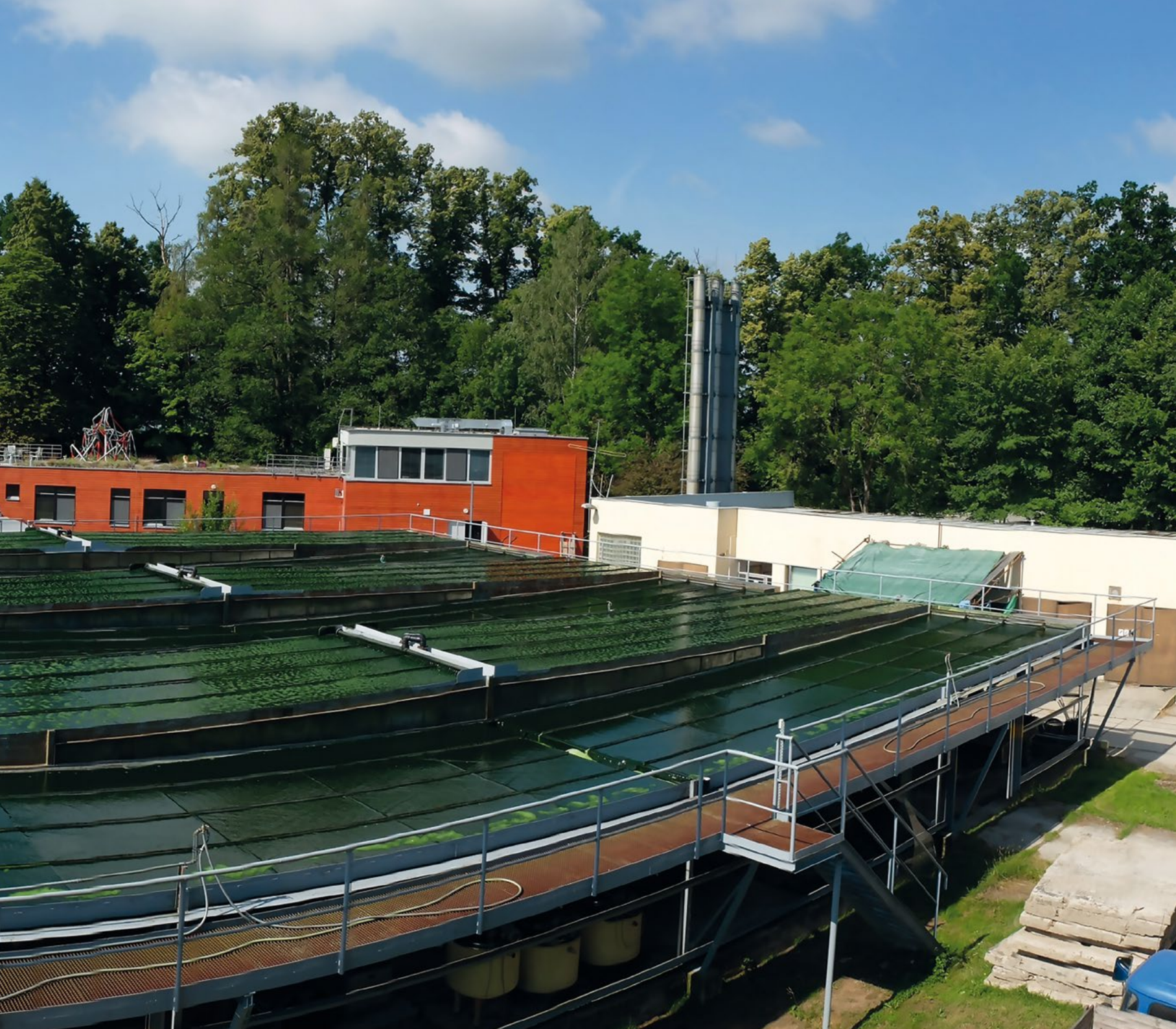
- Helmholtz Institute for Infection Research, Braunschweig, Germany.
- Leibnitz-Institute of Freshwater Ecology and Inland Fisheries, Germany.
- Institut de Ciències del Mar-CSIC, Barcelona, Spain.
- Woods Hole Institute of Oceanology, Massachusetts, USA.
- Metropolitan University of Tokyo, Japan.
- Technion, Haifa, Israel.
- Biology Center, České Budějovice, Czech Republic.

### Industry

- Photon Systems Instruments Ltd., Brno, Czech Republic.
- BioMa proNatur GmbH, Gmünd, Austria.









# HISTORY OF OPATOVICKÝ MLÝN (OPATOVICE MILL)





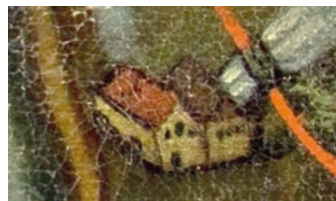
## OPATOVICKÝ MLÝN (Opatovice mill)

The first written report of Opatovický mlýn and the water canal that leads to it from the Lužnice River dates back to 1367 to a deed in which the Rožmberk (Rosenberg – significant and influential Bohemian noble family) brothers sold the mill to their neighbour Merkl “the lender” in Třeboň.

However, the origin of the Opatovický mlýn and the Golden Canal that provides water is older. According to sources, it is linked to the emergence of the so-called Landštejn Canal, which later formed the basis of the Golden Canal. Research places its origin around the middle of the 13th century. The origin of the mill is directly linked to the Cistercian monks of Světlá (Zwettl, Austria), to whom Vítek of Klokot donated the village of Presnik. It is assumed this village was located on the site of Opatovice.

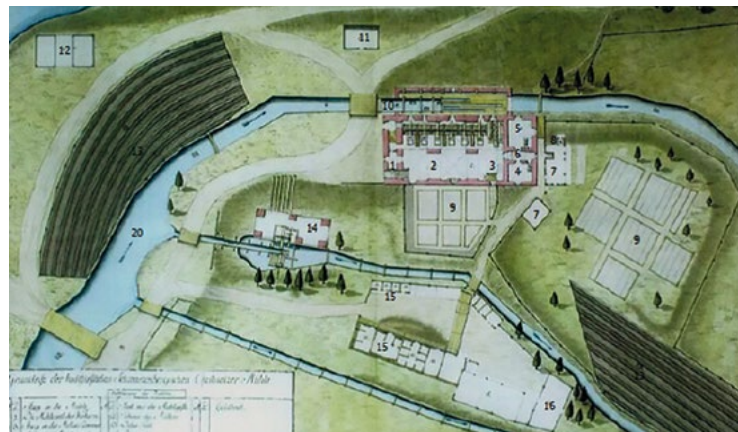
### Further highlights from the history of the building

- 1270 Presnik sold by the monastery in Světlá.
- 1367 Augustine monastery was established in Třeboň, obtaining half of Opatovice prior to 1374 as a gift from the founding Rožmberk family. The monastery bought the remaining half of the settlement in 1376.
- 1439 the mill was outfitted with seven wheels; the mill canal was used for transporting wood to the town.
- 1566 the Augustine monastery was closed and ownership passed to the Rožmberks. At that time the mill had seven flour wheels and eighth oil wheel, a draper's fulling machine and a stamping mill for hulled grain.
- 1568 the Rožmberks have the mill reconstructed: “The Opatovický mlýn and courtyard is built. In this year Jan Vlach built the Opatovický mlýn and courtyard. The walls of which were three cubits wide (V. Březan “Life of the Last Rožmberks”).
- 1579 Václav Březan notes: “... the mill in Opatovice has sixteen wheels and is most thoroughly rebuilt from the ground up, also the sawmill at the same mill has been rebuilt, in which a large and excellent amount of planks can be cut.” Its sixteen wheels ranked it among the three largest mills in Bohemia.



Opatovický mlýn on a section of the Map of Třeboň from 1684

photo: The State Chateau of Třeboň



Map of Opatovický mlýn, 1807

photo: State Regional Archives in Třeboň

Opatovický mlýn from the southwest.

photo: Archive of Š. Kubín



The grounds of the Opatovický mlýn, 1918–1938.

photo: Jan Přibil

The interior of the living quarters  
of the mill.

photo: Břeský family archive



Room in the mill with arches and  
columns in their original baroque  
form, uncovered during recon-  
struction of the building in the  
2012.

photo: Ondřej Prášil



Guardian angel, sculpture from  
1721, located in the grounds of  
the mill.

photo: Markéta Stefanová



Institute of Microbiology in 1980.

photo: MBÚ Třeboň

- 1590 the mill has 15 flour wheels, 5 stamping mills and 2 sawmills.
- 1603 Opatovice gully was put to use as a water pipeline for Třeboň.
- 1625 four years prior, at the beginning of the Bohemian Revolt, the mill was burned to the ground by Imperial troops, and renewed at great expense.
- 1631 the Třeboň monastery was restored and as part of the “restitution” received the mill. The prelate installed a permanent beer taproom in the mill in 1642 (which of course, was the subject of a long-standing dispute with the city up until 1720).
- 1727 the mill burned down, but the abbot, Prechtel, rebuilt it to its current form. (On the façade of the mill is a well-preserved stone cartouche documenting the reconstruction). 6 grinders, 5 stamping mills and one sawmill were built. Třeboň’s abbots spent the summer months here, which is why a sacramental chapel was built on the first floor. F. J. Prokyš painted the altar recess and a picture of St. Ubald on the altar in 1777.
- 1785 the monastery was closed. Jan Nepomuk of Schwarzenberk purchased the mill.
- 1788 he sold the mill with six mill water mechanisms, one to produce millet grain with five stampers and one sawmill to Václav Wolf, a miller, the mill then passed on to his son Václav in 1810.
- 1816 upon Václav’s death, the mill was taken over by his widow Terezie Wolfová. Shortly before her death, she married the head miller Václav Břeský (descended from an old milling family) and willed him the mill. Three generations of the Břeský family managed the Opatovický mlýn until the 1950s.
- 1912 a turbine was set up, replacing the existing seven waterwheels (the turbine covered the electric energy needs of the whole mill, agricultural operations, household, the nearby so-called Prátr, a toll house and also a large farm nearby). At the same time, the mill was rebuilt and modernized into an “artificial”, cylindrical, modern milling system (a grinding cylindrical workbench, lifts, a screening machine and a bank of flour bags...).

After the death of the widow of the last miller in the 1950s, the state took over the mill. The Institute of Microbiology of the Czechoslovak Academy of Sciences was located here in 1962. During the last reconstruction (2009–2014) the arches and columns were re-exposed, the decorations restored in the chapel recess, and the rafters were exposed in the attic. The Guardian Angel statue was returned to the compound after renovation and the Chapel of Jan Nepomuk was restored near the mill. The Chapel was added to the List of cultural monuments of the Czech Republic in 2010.

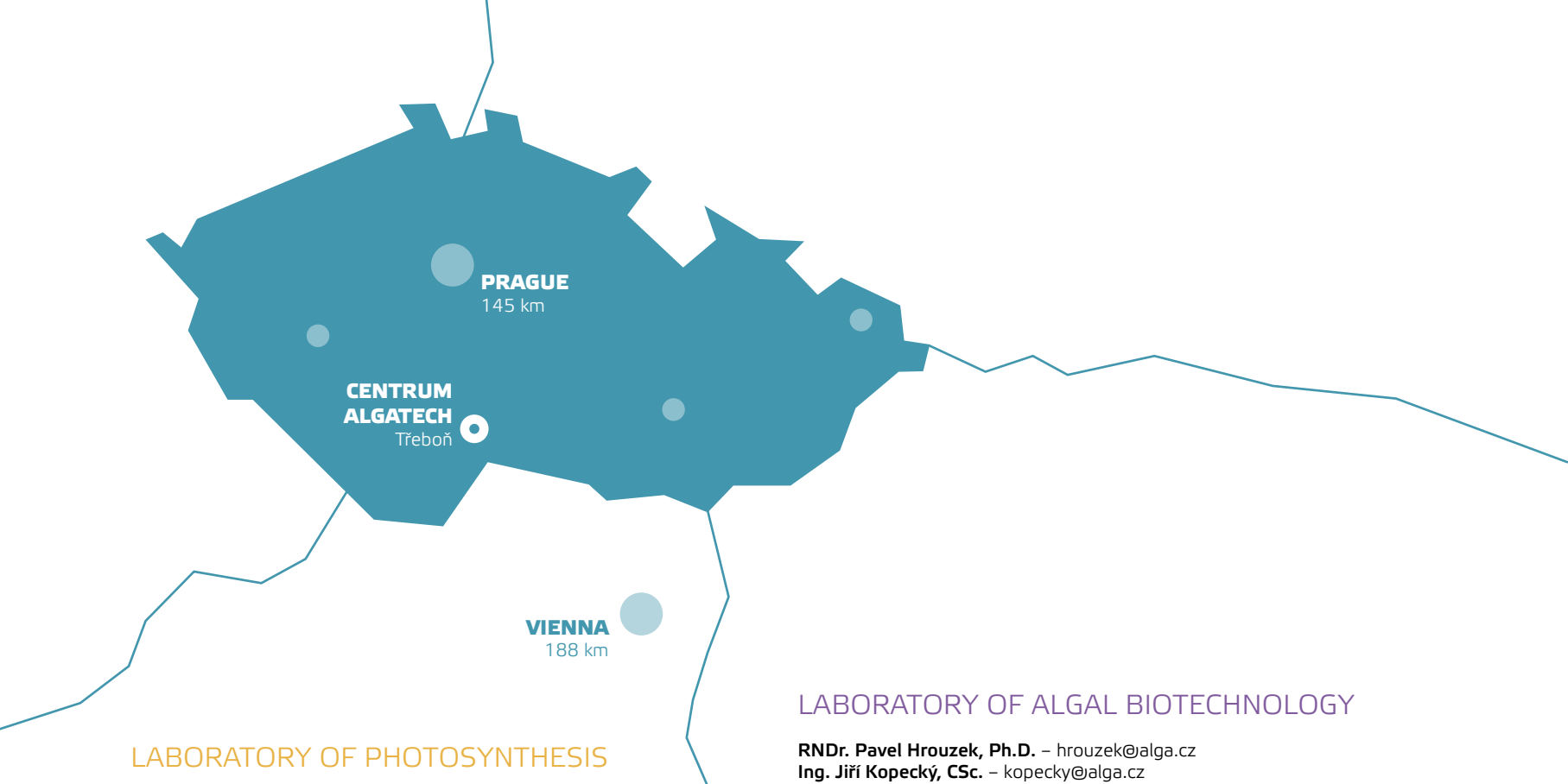
*Adapted according to Renata Nováková  
“Opatovický mlýn at the Golden Canal near Třeboň”, Prague 2014*



# CENTRE ALGATECH 2016







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- HPLC for analysis of pigments and their biosynthesis precursors including radioactive flow detector
- Development of various techniques and instrumentation applied in basic research, agriculture, environment monitoring, and water management.

## LABORATORY OF CELL CYCLES OF ALGAE

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- Study of accumulation and hyper-accumulation of energy reserves (starch, lipids, polyphosphates) in algae.
- Bioremediation and recycling of metals using microalgae.

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- Development of new technological processes to increase microalgal productivity in phototrophic and heterotrophic cultivation regimes.
- Metabolism studies to produce and characterise new metabolites and bioactive compounds and their commercial applications.
- Technological innovation in downstream processing of microalgal biomass.
- New approaches to bioactive component extraction.

## LABORATORY OF ANOXYGENIC PHOTOTROPHS

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- Development of new optical instruments for the detection of anoxygenic phototrophs.
- Isolation of bioactive substances from phototrophic microorganisms, and bioaccumulation studies of anoxygenic microorganisms.
- Phototrophic organisms as a source of biofuels (namely hydrogen).



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