Algatech Centre Třeboň
Institute of Microbiology, Czech Academy of Science
R&D in Microalgae Biotechnology

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Outline

- Centre Algatech - Laboratory of Algal Biotechnology
- Microalgae research in Třeboň - Historical entré
- Laboratory of Algal Biotechnology - R&D topics
- Case studies – monitoring microalgae performance
- Current projects & contracts
About the Algatech Centre

Established in 2011, previously Department of Phototrophic Microorganisms since 1960s
~100 employees

Basic & Applied Microalgae R&D
Physiology / Photosynthesis / Molecular Biology / Biophysics
Biochemistry / Cell Biology / Biotechnology

Laboratories:
Photosynthesis / Algal Biotechnology
Cell Cycles / Photosynthetic Bacteria
1950s-1960s – Outdoor cascade units and indoor tubular PBRs for cultivation of microalgae, – designed in early 1960s by Prof. Šetlík & co-workers in Třeboň, Czech Republic

1st generation of TLC
Cascade – unique thin-layer cultivation system

2nd generation of TLC - 1970s

1990s – highly-productive thin-layer outdoor cascades
Similar units work in CR, Bulgaria, Italy, Spain, Portugal, Switzerland

3rd generation of TLCs

1990s
1990s – highly-productive thin-layer outdoor cascades
One of the most efficient systems for microalgae cultivation and biomass production

4th generation of TLCs
2013
Laboratory of Algal Biotechnology

R&D topics

• Screening and selection of microalgae strains
• Design and construction of various cultivation units
• Optimisation of culturing regimes for microalgae
• Phototrophic & heterotrophic cultivation of microalgae
• Production of biomass as food and feed additives, for cosmetics, etc.
• Identification and characterisation of bioactive compounds with potential pharmacological use
Scale-up of Microalgae Production

1\textsuperscript{st} – collection of strains on agar

2\textsuperscript{nd} step – 400 mL

3\textsuperscript{rd} step – 10 L

4\textsuperscript{th} step - 100 L

5\textsuperscript{th} step – 25, 100 and 650 m\textsuperscript{2}/ 200 up to 6,500 L
Flat-panel PBRs, Inst. of Microbiology, Třeboň
100-150 L
Column and tank photobioreactors with internal LED illumination - 100 & 1,000 litres
Biotechnology hall, IMIC Třeboň
Fermenters for heterotrophic microalgae production
and processing line
R&D - Case studies

- Bioactive compounds isolated from soil cyanobacteria
- Monitoring of microalgae growth – directed production of biomass enriched in some elements (e.g. Se) in mass cultures – nutraceutical purposes
- Performance of *Chlorella* strains with small vs. large antenna size in outdoor mass cultures
Bioactive compounds isolated from soil cyanobacteria

► Cyanobacteria are rich source of potentially bioactive substances

► Aim: isolation of novel chemical entities by activity guided fractionation

► Focus on compounds interacting with human cells

Muscotoxin A (Desmonostoc)
► cytotoxic - cyclic peptide

Aeruginosin 865 (Nostoc)
► anti-inflammatory - peptide

Puwainaphycin F (Cylindrospermmum)
► cytotoxic - cyclic peptide
Analytical laboratory for characterisation of bioactive compounds
Early 1990s in Třeboň
Introduction of Chl fluorescence in monitoring of microalgal mass cultures

In the 1990s many operations were often carried out semi-empirically - discussions between biotechnologists vs. physiologists & photosynthetists

Based on photosynthetic studies in crops, we’ve pioneered the use of chlorophyll fluorescence to monitor changes of photosynthesis and physiology of microalgal mass cultures in large-scale units (Láďa Nedbal, Ondřej Prášil, Johan Grobbellaar, Giuseppe Torzillo, Avigad Vonshak,)

AlgaEurope 2016, Madrid, Spain
Use of Chl Fluorescence

• Chlorophyll (Chl) fluorescence techniques have been developed as one of crucial tools to monitor physiological status of microalgae cultures - strain selection and characterisation
• Monitoring photosynthetic performance makes it possible to control and optimise cultivation regime of a selected strain culture in a suitable cultivation system.
• We can also estimate growth and biomass yield of microalgae in large-scale outdoor installations, or even production of selected bioactive compounds.

Chlorophyll Fluorescence can be used as a unique microalgae signature.
We intend to work ut simple Chl fluorescence manuals for microalgae biotechnologists to examine culture physiological status which reflects microalgae growth and productivity.
Chl fluorescence monitoring – in-situ/on-line

Induction kinetics fluorometers – handheld AquaPen AP-100, P.S.I. Ltd. Brno

Rapid fluorescence induction (OJIP-test)

Pulse-Amplitude-Modulation Technique (PAM)

Junior-PAM fluorimeter. PAM-2500 (H. Walz, Germany)
Semi-empiric & photochemical culture control
Effect of Selenium on growth & PS activity in *Chlorella*
Growth control - effect of Selenium on growth & PS activity in *Chlorella* cultures
Incorporation of Se to biomass

(a) *Chlorella* 250 µmol m⁻² s⁻¹
- 2.5 mg Se/g DW
- 8.5 mg Se/g DW
- 25 mg Se/g DW

(b) *Chlorella* 16 mg Se/g DW
- 250 µmol.m⁻².s⁻¹
- 500 µmol.m⁻².s⁻¹
- 750 µmol.m⁻².s⁻¹

Se in biomass [10³ mg/kg DW]

Time of experiment (h):
- Control
- 24 h
- 48 h
- 72 h
The Small vs. large antenna size strains
The Small vs. large antenna size strains - CPs

Small antenna strain

- PSI
- RCC(1)
- LHCII Tri
- LHCII Mon

Large antenna strain

- RCC(2)-LHCsuper
- PSI-LHCl
- RCC(2)
- RCC(1)-LHCl
- RCC(1)
- LHCII Tri
- LHCII Mon
Research projects

- National Sustainability Program I: ALGATECH PLUS - Centre of Algal Biotechnology (2016-2019)
- Interreg Austria-Czech Republic cross-border project: ALGENETICS - Joint Czech-Austrian Centre of Algal Biotechnology (2017-2019)
- Conversion from heterotrophic to phototrophic growth regime to enhance carotenoid production in microalgae cultures. CNR-AVČR – bilateral mobility project (2016-2018)
Acknowledgements

Karolína Ranglová, Richard Lhotsky, Pavel Hrouzek, Jiří Kopecký, Soňa Pekařová, Petr Novotný, Tomáš Budín, Eduard Pareis, Algatech Centre, Institute of Microbiology, Academy of Sciences, Třeboň

Jose R. Malapascua (PhD student), Faculty of Science, University of South Bohemia, České Budějovice, now Sarawak Biodiversity Center, Malaysia

Giuseppe Torzillo, Istituto per lo Studio degli Ecosistemi del CNR, Sesto Fiorentino, Italy

Celia G. Jerez, Félix L. Figueroa, Faculty of Sciences, University of Málaga, Spain
Thank you for attention ...

Questions, remarks?

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Laboratory cultivation of various microalgae strains