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FACULTY OF AGRICULTURE

Cultivation, Monitoring and Application of Microalgae Cultures

(Kultivace, sledování a využití mikrořas)

SUMMARY OF Ph.D. THESIS

Ing. Karolína Ranglová

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Summary of Ph.D. Thesis

Doktoral student: **Ing. Karolína Ranglová**

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Supervisor: prof. Ing. Vladislav Čurn, Ph.D.
University of South Bohemia in České Budějovice, Faculty of Agriculture

Supervisor specialist: prof. RNDr. Jiří Masojídek, CSc.
Institute of Microbiology of the Czech Academy of Sciences, v.v.i., Centre ALGATECH
University of South Bohemia in České Budějovice, Faculty of Science

Opponents:

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Annotation

The subject of this thesis are microalgae cultures grown in laboratory as well as outdoor cultivation systems and a potential use of their biomass. The main research objective was focused to the correlation of growth of microalgae with photosynthetic activity measured by various techniques.

The introduction to microalgae biotechnology is given in Chapter 1. Chlorophyll fluorescence techniques and the changes of fluorescence variables during cultivation of microalgae (e.g. *Chlorella* during the process of obtaining Se-enriched biomass) is given in Chapter 2. The culturing of microalgae in various types of laboratory and outdoor cultivation systems is described in Chapter 3. The first case study is related to the cultivation of microalgae in a novel laboratory photobioreactor with high-irradiance internal illumination simulating outdoor irradiance. The development of thin-layer cascades as highly productive outdoor units is also described. Last case study in this chapter was related to the cultivation of the cyanobacterium *Arthrospira* in thin-layer cascades with the comparison to open mixed pond under optimal and suboptimal temperature to simulate weather changes. In Chapter 4 several applications of microalgae biomass are given focusing mainly on the use of microalgae biomass as a food supplement. One example is the production of selenium enriched biomass of *Chlorella* which is able to metabolize this metalloid into the proteins by sulphur substitution. In this study it was monitored what is the effect of selenium on the photosynthetic apparatus, how much selenium is organically bond in individual aminoacids and their bioaccessibility was also studied.

Anotace

Předmětem této práce jsou mikrořasy pěstované jak v laboratorních, tak i ve venkovních kultivačních systémech a jejich potencionální využití. Hlavní cílem výzkumu bylo korelovat růst mikrořas s fotosyntetickými aktivitami měřenými různými technikami.

Úvod do řasové biotechnologie je popsán v kapitole 1. Techniky monitorování fluorescence chlorofylu a odezva fluorescenčních proměnných při kultivaci kultur mikrořas (např. *Chlorella* za účelem získání biomasy obohacené selenem) jsou uvedeny v kapitole 2. V kapitole 3 je popsáno pěstování mikrořas v různých typech laboratorních i venkovních kultivačních systémů. První případová studie se vztahuje k pěstování mikrořas v unikátním laboratorním fotobioreaktoru s vnitřním osvětlením simulujícím vysokou venkovní ozářenost. Je popsán i vývoj kaskád založených na kultivaci v tenké vrstvě jako vysoce produktivního systému. Poslední případová studie se týká kultivace sinice *Spirulina* při optimální a suboptimální teplotě v kaskádě v porovnání s otevřenou míchanou nádrží při optimální a suboptimální teplotě s cílem simulovat změny počasí. V kapitole 4 je popsáno několik způsobu využití biomasy mikrořas, především jako doplňku výživy. Jedním z příkladů je produkce biomasy obohacené selenem v kultuře mikrořasy *Chlorella*, která je schopna metabolizovat zmíněný metaloid ve svých proteinech záměnou za síru. Cílem této studie bylo sledovat, jaký je vliv selenu na fotosyntetický aparát, kolik selenu je organicky vázaného v aminokyselinách a studovat jejich biodostupnost.

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Partnership

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Introduction

Microalgae (prokaryotic cyanobacteria and eukaryotic algae) are fast growing photosynthetic microorganisms since their doubling time can be as little as a few hours. During the growth they produce various storage or protective compounds such as polyunsaturated fatty acids (PUFAs), lipids, antioxidants and others. They can be grown in various cultivation systems – open units with direct contact with the environment and in closed photobioreactors (PBRs) (Masojídek and Torzillo 2014). The biomass production in open systems is generally cheaper compared to PBRs as the former are easier to clean and requires lower operation costs (Ugwu et al. 2008). A thin-layer systems like sloping cascades or cascade raceways are highly efficient for biomass production due to short light-path (Masojídek et al. 2015, Becker 1994, Richmond 2003, Masojídek et al. 2011a, b). Culturing of microalgae and the production of valuable compounds is affected by various environmental factors and conditions such as irradiance, temperature, nutrition, pH, presence of contaminants (Hu 2013, Ranglová et al. 2019). The composition (quality) of biomass produced by microalgae may be affected by various environmental factors and conditions.

Successful cultivation requires monitoring of physicochemical variables of cultures such as irradiance, temperature, dissolved oxygen concentration, nutrient concentration, and pH as well as the basic controlling method base on the use of biological examination under the microscope to detect morphological changes, mechanical cell damage (caused by mixing) and contamination by other microorganisms. Since the 1990s chlorophyll (Chl) fluorescence techniques have been used to monitor photosynthetic performance and optimise the growth of microalgae mass cultures as to detect various unfavourable effects (Knoppová et al. 1993; Vonshak et al. 1996; Torzillo et al. 1996, 1998; Masojídek et al. 2011a, b; Malapascua et al. 2014). Two basic Chl fluorescence techniques are used for photosynthesis monitoring of microalgae. Rapid fluorescence induction kinetics and the pulse-amplitude modulation method (for recent reviews, see Maxwell and Johnson 2000, Strasser et al. 2004, Schreiber 2004, Baker 2008, Masojídek et al. 2011a). While the rapid fluorescence induction kinetics provides information on the reduction of the photosynthetic electron transport chain, the PAM technique gives information on the balance between photochemistry and non-photochemical energy dissipation. The usefulness of these sensitive and simple methods is indicated in several case studies in this thesis. Changes of photosynthetic performance measured as Chl variables can be correlated with the growth of microalgae (e.g. Masojídek et al. 2011a; Malapascua 2018, Malapascua et al. 2019, Babaei et al. 2017).

Microalgae have been utilized by humans for hundreds of years as food, feed, remedies and fertilizers (Barsanti and Gualtieri 2006). At present, there are several potential applications of microalgae biomass. For example, the microalgae *Chlorella* and *Arthrospira* have been widely used as nutritional food supplements because of the high protein content (Becker 2004, Liu and Hu 2013).

Some microalgae species are able to metabolize chemical elements (e.g., heavy metals) from aqueous solutions (Sandau et al. 1996). In case of metalloid selenium (Se), microalgae are able to incorporate it to organic compounds in Se-AAS by sulphur replacing (Babaei et al. 2017, Vu et al. 2019). A low dosage of organic Se compounds brings numerous health benefits having anti-inflammatory, immunostimulating, antiaging and cytotoxic activities (Vu et al. 2019, Mylenko et al. 2020).

Objectives of this thesis

The main objective of this thesis was to get introduced to the field of microalgae biotechnology, especially monitoring of the growth of microalgae cultures.

The particular objectives of individual chapters are:

(i) To study variables influencing selected microalgae growth (Chapter 1 and 2).
(ii) Culture monitoring and maintenance, namely photosynthesis using chlorophyll fluorescence for monitoring of the physiological status of microalgae cultures in order to estimate suitable growth regimes (Chapter 2).
(iii) The use and comparison of various laboratory and outdoor cultivation systems for growth of selected microalgae in order to optimize growth regimes for production of required biomass and/or bioactive compounds (Chapter 3).
(iv) Examples of the use of microalgae in human and animal nutrition and in agriculture as biostimulants and biopesticides (Chapter 4).

Summary

Microalgae (both prokaryotic cyanobacteria and eukaryotic algae) have developed wide physiological and functional diversity as they inhabit various ecosystems – from arctic areas, through moderate climate regions, to extremely alkaline or saline habitats, hot springs and deserts**.** Therefore, they produce the variety of high-value bioactive substances such as pigments, PUFAs, polysaccharides, antioxidants, as well as antimicrobial, biostimulating, immunologically effective, virostatic and cytostatic compounds. Selected species are being cultured commercially as production strains in various cultivation systems under various environmental conditions. These include irradiance intensity, temperature, supply nutrients, mainly nitrogen and phosphorus (Chapter 1).

The growth of the culture can be estimated by determining the status of the photosynthetic apparatus which can detect various unfavourable effects. Two chlorophyll fluorescence techniques are used for photosynthesis monitoring. Rapid fluorescence induction kinetics and the pulse-amplitude modulation (PAM) method provides information on the reduction of the photosynthetic electron transport chain while the PAM technique gives information on the balance between photochemistry and non-photochemical energy dissipation (Malapascua 2018). In Chapter 2, two case studies are illustrated. In the first one, a fast, one-day preliminary test was developed based on photosynthetic measurements (Chl fluorescence and photosynthetic oxygen evolution and respiration) in order to estimate a suitable growth regime temperature (Publication 1 – Ranglová et al. 2019). Photosynthetic variables (the maximum photochemical yield of PSII, F_v/F_m, $rETR_{max}$, the inflection points V_j and V_i measured by Chl fluorescence techniques) were correlated with microalgae growth and this make it possible to adjust approximate cultivation conditions.
In another case study the fluorescence monitoring techniques were used for examination of *Chlorella* culture grown in the presence of inorganic selenium (Se) as to obtain Se-enriched biomass. This element is incorporated in Se-aminoacids (SeCys, SeMet, MeSeCys) replacing sulphur (Publication 2 – Babaei et al. 2017). The optimum dosage can be estimated from fluorescence variables as they reflect the unfavourable state of the culture. This knowledge is important when producing Se-enriched biomass growing in large-scale experiments.
Microalgae cultures are grown in aquaculture for biomass which can be further used for example as food or feed additives, agriculture applications as biopesticides or biostimulants, or for other purposes. Large-scale cultures are usually grown in open systems where the microalgae are in the direct contact with environment receiving full sunlight. In Chapter 3, one series of laboratory experiments used *Chlorella* strain which was exposed to very high irradiance intensities (up to 3500 µmol photons m^{-2} s^{-1}) in a special type of photobioreactor whit a short light path (about 25 mm) to model the situation when laboratory seed cultures are transferred to outdoor units. Photosynthesis variables were monitored to investigate the process of acclimation. These results are important for the initial phase outdoor cultivation when cultures in thin-layer cascades can be lost due to photoinhibition (Publication 3 – Malapascua et al. 2019).
Thin-layer cascades (TLCs) as production units are characterized by low culture depth (usually <10 mm). They bring together the advantages of open systems (direct sun irradiance, evaporative self-cooling, simple cleaning, low maintenance costs) with the positive features of closed systems (operation at high biomass densities achieving high productivity). The TLC were designed and firstly used in 1960s in Třeboň. In contrast to deep ponds microalgae cultures grow in turbulent thin layer maintaining sufficient mixing to guarantee sufficient cell irradiance, gas exchange. Several generations of TLCs were designed. At present the last generation of TLCs is made of stainless-steel and the biomass productivity of 40 g m^{-2} d^{-1} could

be achieved. The detailed development of thin-layer systems was described recently (Publication 4 – Grivalský et al. 2019)

In the last case study of this chapter, the growth and photosynthetic activity of *Arthrospira platensis* was studied in two different outdoor cultivation units – open circular pond and thin-layer cascade. It was confirmed that the TLCs are more productive than open deeper ponds under both optimal and suboptimal temperature conditions as the weather outdoor can change. The culture in the TLC showed higher productivity than that in the open pond. It was related to higher photosynthetic activity which was ascribed to significantly shorter light path which favourable for photosynthesis due to faster warming of the cultures in the morning as well as for promotion of much faster light/dark cycles, fast turnover of electrons in the photosynthetic apparatus as compared to the open-pond culture (Publication 5 – Benavides et al. 2017).

In Chapter 4, several potential application of microalgae biomass are illustrated. *Chlorella* is a completely natural product with has favourable biomass composition. For example, as mentioned above Se-enriched microalgae biomass is used as important nutrition supplement to ovoid deficiency in some population groups (Mylenko et al. 2020). *Chlorella* can accumulate Se (which is an essential microelement) and metabolize is to Se-proteins. The individual Se-aminoacids (SeCys, SeMet, MeSeCys) in the Se-enriched biomass were analysed using advanced gas (GC-APCI-HRMS) and liquid chromatography (HPLC-ICP-MS) combined with mass detection techniques. In this study, the bioaccessibility of Se-aminoacids from Se-enriched *Chlorella* and from several Se-rich food (salmon, brazil nuts, mustard seed) was compared (Publication 6 – Vu et al. 2019).

Some microalgae can even produce biologically active compounds such as biopesticides and biostimulants and thus the biomass could be used in agriculture for crop treatment to minimize the application of chemicals and also for enhancing of environmental sustainability. By using wastewater (WW) as a growing media (which is rich in nitrogen mainly in the form of ammonium) the cultivation cost can be reduced. The main objective of the second case study in this chapter was to study the growth, physiological performance and bioactivity of *Chlorella* when grown in inorganic medium and compare it with the results obtained during cultivation in WW (Publication 7 – Ranglová et al. 2020)

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Vu DL, Saurav K, Mylenko M, Ranglová K, Kuta J, Ewe D, Masojídek J, Hrouzek P (2019) In vitro bioaccessibility of selenoamino acids from selenium (Se)-enriched *Chlorella vulgaris* biomass in comparison to selenized yeast; a Se-enriched food supplement; and Se-rich foods. Food Chem 279: 12-19

List of author's publications with abstracts upon which this thesis is based

1. **Ranglová K**, Lakatos GE, Manoel JAC, Grivalský T, Masojídek J (2019) Rapid screening test to estimate temperature optima for microalgae growth using photosynthesis activity measurements. *Folia Microbiol* 64:615-625

We have worked out a rapid 1-day test based on photosynthesis measurements to estimate suitable growth temperature of microalgae cultures. To verify the proposed procedure, several microalgae - *Chlorella*, *Nostoc*, *Synechocystis*, *Scenedesmus* and *Cylindrospermum* - were cultured under controlled laboratory conditions (irradiance, temperature, mixing, CO_2, and nutrient supply) to find the optima of photosynthetic activity using the range between 15 and 35 °C. These activitieswere recorded at each temperature step after 2 h of acclimation which should be sufficient as oxygen production and the PQ cycle are regulated by fast processes. Photosynthetic activity was measured using three techniques - oxygen production/respiration, saturating pulse analysis of fluorescence quenching, and fast fluorescence induction kinetics - to estimate the temperature optima which should correspond to high growth rate. We measured all variables that might have been directly related to growth - photosynthetic oxygen evolution, maximum photochemical yield of PSII, F_v/F_m, relative electron transport rate $rETR_{max}$, and the transients V_j and V_i determined by fast fluorescence induction curves. When the temperature optima for photosynthetic activity were verified in growth tests, we found good correlation. For most of tested microalgae strains, temperature around 30 °C was found to be the most suitable at this setting. We concluded that the developed test can be used as a rapid 1-day pre-screening to estimate a suitable growth temperature of microalgae strains before they are cultured in a pilot scale.

2. Babaei A, **Ranglová K**, Malapascua JR, Masojídek J (2017) The synergistic effect of Selenium (selenite, -SeO_3^{2-}) dose and irradiance intensity in *Chlorella* cultures. AMB Express 7 (56):1-14

Microalgae are able to metabolize inorganic selenium (Se) to organic forms (e.g. Se-Cys, Se-Met); nevertheless, at certain Se concentration culture growth is inhibited. The aim of this work was to confirm the hypothesis that the limit of Se tolerance in *Chlorella* cultures is related to photosynthetic performance, i.e. depends on light intensity. We studied the relation between the dose and irradiance to find the range of Se tolerance in laboratory and outdoor cultures. At low irradiance (250 μmol photons m^{-2} s^{-1}), the daily dose of Se below 8.5 mg per g of biomass (<20 μM) partially stimulated the photosynthetic activity (rETR) and growth of *Chlorella* cultures (biomass density of ~1.5 g DW L^{-1}) compared to the control (no Se added). It was accompanied by substantial Se incorporation to microalgae biomass (~0.5 mg Se g^{-1} DW). When the Se daily dose and level of irradiance were doubled (16 mg Se g^{-1} DW; 500 μmol photons m^{-2} s^{-1}), the photosynthetic activity and growth were stimulated for several days and ample incorporation of Se to biomass (7.1 mg g^{-1} DW) was observed. Yet, the same Se daily dose under increased irradiance (750 μmol photons m^{-2} s^{-1}) caused the synergistic effect manifested by significant inhibition of photosynthesis, growth and lowered Se incorporation to biomass. Chl fluorescence techniques were used to monitor photosynthetic activity for determination of optimal Se doses in order to achieve efficient incorporation without substantial inhibition of microalgae growth when producing Se-enriched biomass.

3. Malapascua JR, **Ranglová K**, Masojídek J (2019) Photosynthesis and growth kinetics of *Chlorella vulgaris* R-117 cultured in an internally LED-illuminated photobioreactor. Photosynthetica

The aim was to correlate changes of photosynthesis activity vs. growth in *Chlorella vulgaris* R-117 (CCALA 1107), fast-growing and robust microalga cultured in an internally illuminated 10-litre photobioreactor. The cultures were grown at high output irradiance provided by four LED light sources submerged in the culture when the light path was short, between 25–30 mm. The culture of *Chlorella* R-117 grown under 2,500 μmol photons m^{-2} s^{-1} attained a doubling time of 3.5 days and biomass density of 3.5 g DM L^{-1} after about 10-day period. When grown under 3,500 μmol (photon) m^{-2} s^{-1}, the culture reached a doubling time of 1.7 days, and biomass density of ~5.5 g L^{-1} before entering the stationary phase. Electron transport rate changes correlated well with the culture growth demonstrating the usefulness of chlorophyll fluorescence for photosynthesis monitoring. This can be crucial for potential scale-up to large indoor PBRs to optimize culture growth.

4. Grivalský T, **Ranglová K**, Manoel JAC, Lakatoe GE, Lhotský R, Masojídek J (2019) Development of thin-layer cascades for microalgae cultivation: milestones (review). Folia Microbiologica 64:603-614

In this work, the key moments of the development of the so-called thin-layer cascades (TLC) for microalgae production are described. Development started at the end of the 1950s when the first generation of TLCs was set-up in former Czechoslovakia. Since, similar units for microalgae culturing, which are relatively simple, low-cost and highly productive, have been installed in a number of other countries worldwide. The TLCs are characterized by microalgae growth at a low depth (< 50 mm) and fast flow (0.4–0.5 m/s) of culture compared to mixed ponds or raceways. It guarantees a high ratio of exposed surface to total culture volume (> 100 1/m) and rapid light/dark cycling frequencies of cells which result in high biomass productivity (> 30 g/m^2/day) and operating at high biomass density, > 10 g/L of dry mass (DW). In TLCs, microalgae culture is grown in the system of inclined platforms that combine the advantages of open systems—direct sun irradiance, easy heat derivation, simple cleaning and maintenance, and efficient degassing—with positive features of closed systems—operation at high biomass densities achieving high volumetric productivity. Among significant advantages of thin layer cascades compared to raceway ponds are the operation at much higher cell densities, very high daylight productivities, and the possibility to store the culture in retention tanks at night, or in unfavorable weather conditions. Concerning the limitations of TLCs, one has to consider contaminations by other microalgae that limit cultivation to robust, fast-growing strains, or those cultured in selective environments.

5. Benavides AM, **Ranglová K**, Malapascua JM, Masojídek J, Torzillo G (2017) Diurnal changes of photosynthesis and growth of *Arthrospira platensis* cultured in thin-layer cascade and an open pond. Algal Research 28:48-56

Diel changes in photosynthetic performance and biomass productivity were examined in *Arthrospira platensis* cultures grown outdoors in an open circular pond (OCP) and a thin-layer cascade (TLC). The two cultures were grown at the same areal biomass density, but temperature maxima were adjusted to optimal (33°C) and suboptimal (25°C). At the optimal temperature, the cultures grown in TLC showed about 20% higher photosynthetic activity than those in OCP, while at the suboptimal one photosynthetic activity dropped by 20% and 35% in the TLC and OCP, respectively. Accordingly, the highest biomass productivity over 20 g m^{-2} d^{-1} was attained in the TLC at the optimal temperature, while at the suboptimal temperature the productivity decreased by 20%. In the OCP, the biomass productivity at both temperatures was about one third lower compared to those in the TLC.
The better culture performance in the TLC was mainly ascribed to the shorter light path that promoted much faster light/dark cycles favorable for photosynthesis, as well as the faster warming of the cultures in the morning as compared to the OCP cultures. Monitoring photosynthesis performance of a culture can indicate design improvements, which may capitalize this photochemical advantage, increasing biomass productivity further.

6. Vu DL, Saurav K, Mylenko M, **Ranglová K**, Kuta J, Ewe D, Masojídek J, Hrouzek P (2019) *In vitro* bioaccessibility of selenoamino acids from selenium (Se)-enriched *Chlorella vulgaris* biomass in comparison to selenized yeast; a Se-enriched food supplement; and Se-rich foods. Food Chemistry 279:12-19

Selenium (Se) is an indispensable microelement in our diet and health issues resulting from deficiencies are well documented. Se-containing food supplements are available on the market including Se-enriched *Chlorella vulgaris* (Se-*Chlorella*) which accumulates Se in the form of Se-amino acids (Se-AAs). Despite its popular uses, data about the bioaccessibility of Se-AAs from Se-*Chlorella* are completely missing. In the present study, gastrointestinal digestion times were optimized and the in vitro bioaccessibility of Se-AAs in Se-*Chlorella*, Se-yeast, a commercially available Se-enriched food supplement (Se-supplement) and Se rich foods (Se-foods) were compared. Higher bioaccessibility was found in Se-*Chlorella* (∼49%) as compared to Se-yeast (∼21%), Se-supplement (∼32%) and Se-foods. The methods used in production of Se-*Chlorella* biomass were also investigated. We found that disintegration increased bioaccessibility whereas the drying process had no effect. Similarly, temperature treatment by microwave oven also increased bioaccessibility whereas boiling water did not.

7. **Ranglová** K, Lakatos GE, Câmara Manoel JA, Grivalský T, Suárez Estrella F, Gabriel Acién-Fernándéz FG, Molnár Z, Ördög V, Masojídek J (2020) Growth, biostimulant and biopesticide activity of the MACC-1 *Chlorella* strain cultivated outdoors in inorganic medium and wastewater. Algal Res (*submitted for publication in Algal Research 3 August 2020*)

Growth, physiological performance and bioactivity of the microalga strain *Chlorella vulgaris* MACC-1 was studied outdoors in two pilot-scale units – thin-layer cascade and a novel, thin-layer raceway pond. Two nutrient sources were compared – inorganic BG-11 medium and centrate from municipal wastewater (WW).

The main objective of this work was to study bioactivity of *Chlorella* when grown in inorganic medium and the centrate. The correlation between the bioactivity and photosynthetic activity was also studied. The results revealed a clear interplay among ambient irradiance intensity, growth rate, PSII photochemical efficiency F_v/F_m and oxygen production/respiration. Samples were harvested at the end of trial at different daytimes (0800 h and 1300 h) in the semi-continuous cultivation regime from both units for determination of bioactivity using water extracts of freeze dried biomass. The highest biostimulating activities detected by different bioassays were found in *Chlorella* cultures grown in BG-11, but not in WW. On the other hand, the antibacterial and antifungal activities were significantly higher when grown in WW. We expect that antimicrobial activities can be induced by WW-microbes and biostimulating effects depend on physiological status of the algae cells. Here, we found the certain correlation between photosynthetic activity and bioactivity as significant differences in both activities coincided. Thus, photosynthesis monitoring can be used as an indication of microalgae cultures as to indicate biomass harvesting in large-scale unit for agricultural use.

Curriculum Vitae

Ing. Karolína Ranglová

Contact information:

Phone number: +420384340463

Email address: ranglova@alga.cz

Business address: Institute of Microbiology of the Czech Academy of Sciences, v.v.i.
Center ALGATECH
Novohradská 237 – Opatovický mlýn
379 01 Třeboň, Czech Republic

Research interests:

Microalgae biotechnology, physiology and photosynthesis, cultivation of microalgae, photobioreactors, microalgae for aquaculture and agriculture

Education:

2017 – 2020 **Ph.D.** study
Faculty of Agriculture, University of South Bohemia,
České Budějovice, Czech Republic

2012 – 2014 **M.Sc**. in Chemistry of Natural Compounds
Faculty of Food and Biochemical Technology, University of Chemistry and Technology Prague. Czech Republic

2009 – 2012 **B.Sc.** in Chemistry
Faculty of Science, University of South Bohemia,
České Budějovice, Czech Republic

Work experience:

Research Assistant
Dates: June 2014 – present
Institute of Microbiology of the Czech Academy of Sciences, v.v.i.
Center ALGATECH
Novohradská 237 – Opatovický mlýn
379 01 Třeboň, Czech Republic

Posters/Talks presented:

1. 7th Symposium on "Microalgae and Seaweed Products in Plant/soil Systems", Faculty of Agricultural and Food Sciences, University of West Hungary, 29-30 June 2015, Mosonmagyaróvár, Hungary

Poster presented: Diurnal changes of photosynthesis of Arthrospira platensis grown in inclined thin-layer platform and open pond

Presenters: Ranglová K*, Malapascua JR, Benavídes MS, Masojídek J, Torzillo G

2. 2nd EUAlgae Workshop of Algae Bioproducts for Early Career Investigators COST1408, 6 March 2018, Thessaloniki, Greece

Talk presented: Microalgae Strains with Potencial Biopesticide Activity

3. Symposium on Microalgal Biotechnology in Agriculture, 25-26 September 2018, Třeboň, Czech Republic

Talk presented: Production of Microalgae with Biopesticide Activity

4. Annual meeting of SABANA Project, 16-18 November 2018, Las Palmas, Gran Canaria

Talk presented: European Project of Center ALGATECH

5. EUALGAE Final Conference – European Recent Advances in the Microalgae Field, 26-27 February 2019, Madrid, Spain

Talk presented: Rapid screening test to estimate temperature optima for microalgae growth using photosynthetic activity measurements

6. 9th Symposium on "Microalgae and Seaweed Products in Plant/soil Systems", Faculty of Agricultural and Food Sciences, University of West Hungary, 25-26 June 2019, Mosonmagyaróvár, Hungary

Poster presented: Physiological performance and bioactivity of Chlorella grown outdoors in thin-layer raceway pond
Presenters: Ranglová K*, Lakatos GE, Grivalský T, Manoel JAC, Suárez-Estrella F, Aién-Fernández G, Urajová P, Macho M, Ördög V, Masojídek J

7. Algae Biomass Summit, Orlando, 16-19 September 2019, Florida, USA

Poster presented: Biostimulant and biopesticide activity of *Chlorella* sp. cultured in pilot scale (YOUNG RESEARCH AWARD for 2nd place in Biology division poster)
Talk presented: Biopesticide activity of *Chlorella* sp. strain cultured in pilot scale

* Presenting author

List of all author's publications:

1. **Ranglová K**, Krejčová P, Kubec R (2015) The effect of storage and processing on antimicrobial activity of *Tulbaghia violacea*. S Afr J Bot 97: 159-164
2. Babaei A, **Ranglová K**, Malapascua JR, Masojídek J (2017) The synergistic effect of Selenium (selenite, -SeO_3^{2-}) dose and irradiance intensity in *Chlorella* cultures. AMB Express 7 (56):1-14
3. Benavides AM, **Ranglová K**, Malapascua JM, Masojídek J, Torzillo G (2017) Diurnal changes of photosynthesis and growth of *Arthrospira platensis* cultured in thin-layer cascade and an open pond. Algal Research 28:48-56
4. Bednařík A, Kuta J, Vu DL, **Ranglová K**, Hrouzek P, Kanický V, Preisler, J (2018) Thin-layer chromatography combined with diode laser thermal vaporization inductively coupled plasma mass spectrometry for the determination of selenomethionine and selenocysteine in algae and yeast. J Chromatorg A 1533:199-207
5. Vu DL, **Ranglová K**, Hájek J, Hrouzek, P (2018) Quantification of methionine and selenomethionine in biological samples using multiple reaction monitoring high performance liquid chromatography tandem mass spectrometry (MRM-HPLC-MS/MS). J Chromatogr B 1084: 36-44
6. **Ranglová K**, Lakatos GE, Manoel JAC, Grivalský T, Masojídek J (2019) Rapid screening test to estimate temperature optima for microalgae growth using photosynthesis activity measurements. Folia Microbiol 64:615-625
7. Grivalský T, **Ranglová K**, Manoel JAC, Lakatoe GE, Lhotský R, Masojídek J (2019) Development of thin-layer cascades for microalgae cultivation: milestones (review). Folia Microbiol 64:603-614
8. Lakatos G, Kopecký J, Grivalský T, **Ranglová K**, Camara Manoel JA, Masojídek, J (2019) Bioethanol production from microalgae polysaccharides. Folia Microbiol 64:627-644
9. Malapascua JR, **Ranglová K**, Masojídek J (2019) Photosynthesis and growth kinetics of *Chlorella vulgaris* R-117 cultured in an internally LED-illuminated photobioreactor. Photosynthetica
10. Vu DL, Saurav K, Mylenko M, **Ranglová K**, Kuta J, Ewe D, Masojídek J, Hrouzek P (2019) *In vitro* bioaccessibility of selenoamino acids from selenium (Se)-enriched *Chlorella vulgaris* biomass in comparison to selenized yeast; a Se-enriched food supplement; and Se-rich foods. Food Chemistry 279:12-19
11. Mylenko M, Vu DL, Kuta J, **Ranglová K**, Kubáč D, Lakatos G, Caporgno MP, Manoel JA, Kopecký J, Masojídek J, Hrouzek P (2020) Selenium incorporation to amino acids in Chlorella cultures grown in phototrophic and heterotrophic regimes. J Agric Food Chem 12:1654-1665
12. Babaei, A, **Ranglová K**, Malapascua JR, Torzillo G, Shayegan J, Benavides AMS and Masojídek J (2020) Photobiochemical changes of *Chlorella* g120 culture during trophic conversion (metabolic pathway shift) from heterotrophic to photoautotrophic growth regime. J Appl Phycol
13. **Ranglová K**, Lakatos GE, Manoel JAC, Grivalský T, Suárez Estrella F, Acién Fernández G, Molnár Z, Ördög V, Masojídek J (2020) Growth, biostimulant and biopesticide activity of the MACC-1 *Chlorella* strain cultivated outdoors in inorganic medium and wastewater. (submitted to Algal Research)

14. Rearte A, Celis-Plá P, Neori A, Masojídek J, Torzillo J, Gómez C, Silva Benavídes AM, Álvarez-Gómez F, Abdala Diaz R, **Ranglová K**, Caporgno M, Massocato TF, Silva J, Al Mahrouqi H, Atzmüller R, Figueroa F (2020) Diurnal changes of dissolved oxygen gradients in the highly productive strain *Chlorella vulgaris* R117 cultured in thin-layer cascades: effect on photosynthetic activity. (submitted to Algal Research)

15. Mittermair S, Lakatos G, Nicoletti C, **Ranglová K**, Camara Manoel J, Grivalský T, Malikuly Kozhan D, Masojídek J, Richter J (2020) Molecular biology toolkits for glycogen overflow as the first step of a carbon-neutral production in Synechocystis sp. PCC6803. (submitted to Algal Research)