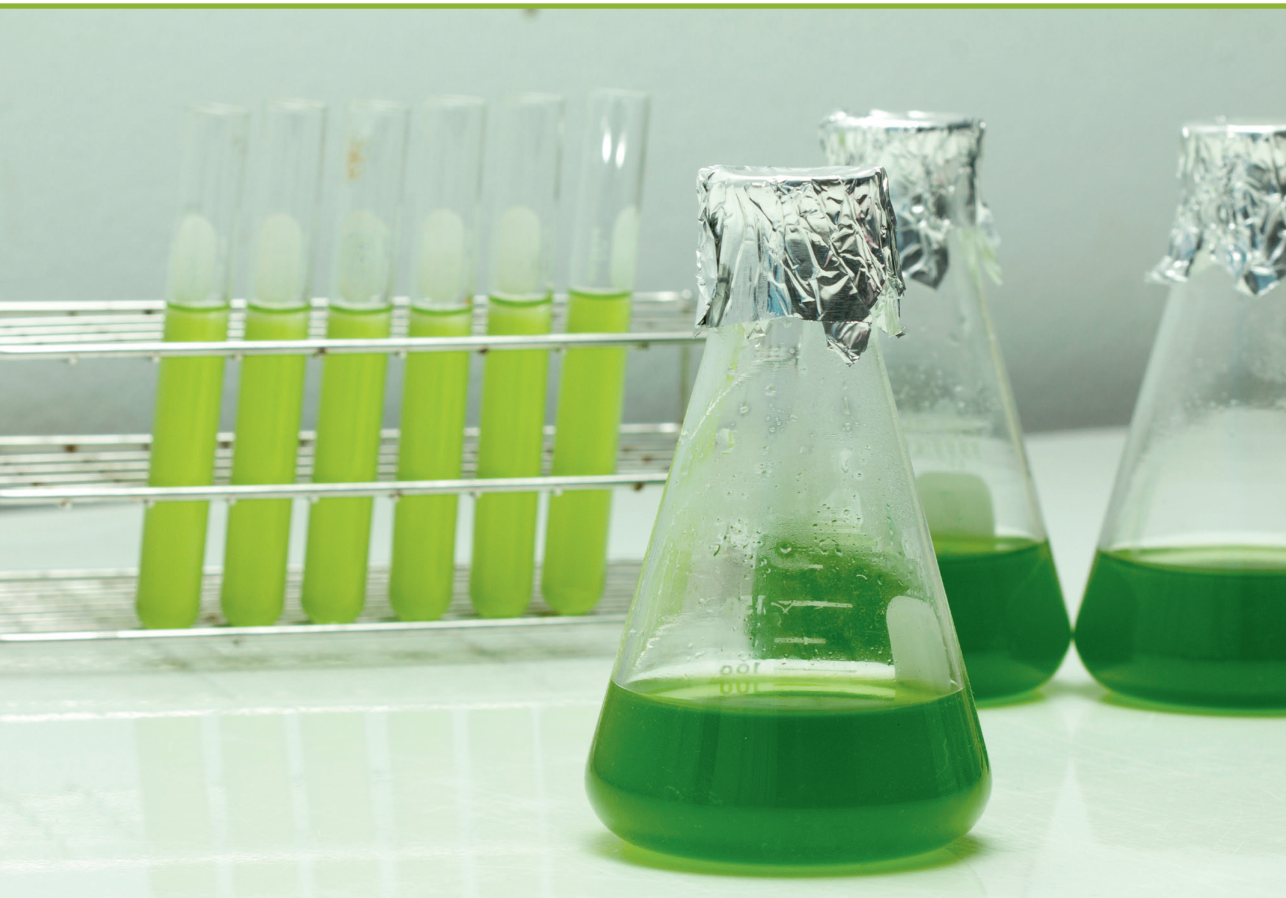
RIGA TECHNICAL UNIVERSITY

Aigars Lavrinovičs

MICROALGAL TECHNOLOGY FOR MUNICIPAL WASTEWATER POST-TREATMENT

Summary of the Doctoral Thesis



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RIGA TECHNICAL UNIVERSITY
Faculty of Civil Engineering
Institute of Heat, Gas and Water Technology

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DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR THE PROMOTION TO THE SCIENTIFIC DEGREE OF DOCTOR OF SCIENCE

To be granted the scientific degree of Doctor of Science (Ph. D.), the present Doctoral Thesis has been submitted for the defence at the open meeting of RTU Promotion Council on March 13, 2023, at 13.00 at the Faculty of Civil Engineering of Riga Technical University, Ķīpsalas street 6a, Room 127.

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DECLARATION OF ACADEMIC INTEGRITY

I hereby declare that the Doctoral Thesis submitted for the review to Riga Technical University for the promotion to the scientific degree of Doctor of Science (Ph. D.) is my own. I confirm that this Doctoral Thesis had not been submitted to any other university for the promotion to a scientific degree.

Aigars Lavrinovičs ……………………………. (signature)
Date: ………………………

The Doctoral Thesis has been written in English. It consists of an Introduction, 3 chapters, Conclusions, 19 figures, 3 tables, 4 appendices; the total number of pages is 58, not including appendices. The Bibliography contains 104 titles.

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GENERAL OVERVIEW OF THE THESIS

Relevance of the study

Globally the aquatic ecosystems are central to the identities of cultures and societies. A healthy and functional aquatic ecosystem provides resources for food, living space, and welfare. However, growing areas of surface waters receive elevated loads of nutrients, mainly phosphorus and nitrogen, originating from various sources, including wastewater treatment plants (WWTP). Eventually, the eutrophication status evolves and leads to an overall degradation of the aquatic ecosystems (Fig. 1). Therefore, wastewater treatment has always been among the main concerns for the conservation of the aquatic environment. This has challenged the scientific community to develop efficient technologies for wastewater purification and even change its perception as resource recovery rather than waste disposal.

Fig. 1. Algal bloom in the central Baltic Sea (© European Space Agency).

Microalgae-based systems are viewed as a promising alternative to the traditional methods for wastewater phosphorus removal and recovery (Borowitzka, 2013; Whitton et al., 2015). It is often shown that various microalgal strains or mixed cultures are capable of near-complete removal of phosphorus when produced in wastewater (Cai et al., 2013). Moreover, enhanced algal phosphorus uptake and storage can be achieved by manipulation with external phosphorus availability. The manipulation approach includes phosphorus starvation (Hernandez et al., 2006), which is initiated by phosphorus limited conditions. Such a biomass manipulation can increase the phosphorus uptake rate multiple times (Brown and Shilton, 2014) when compared to phosphorus consumption rates by regular biomass. In addition, the manipulation with phosphorus availability can enhance high value molecule synthesis by algal cells (Levasseur et al., 2020), thus adding value to the produced biomass and providing an opportunity for financial return.

The main limitations for microalgae manipulation with phosphorus availability include a reduced biomass growth rate (Kamalanathan, 2016) followed by lower total yields of high value molecules. Also, it remains unknown what is the margin for optimum biomass P-starvation period. Insufficient P-stress can attenuate the desired enhancement of P-uptake, while overexposure to P-deficiency can damage the algal cell physiological processes and disrupt the whole wastewater treatment process. Also, there are no clear indicators for P-deficiency quantification and tools for biomass P-starvation control. Thus, the reported phosphorus removal rates after microalgal biomass exposure to P-deficiency are variable and the effects on similar biomass P-starvation conditions can be antipodal (Hernandez et al., 2006; Van Moorleghem et al., 2013; Wu et al., 2012). Considering these knowledge gaps, more in-depth studies on the microalgal biomass P-starvation approach are required for its successful integration in engineered systems.

Goal of the study

The main goal of this study was to investigate the potential of microalgae for enhanced phosphorus removal from municipal wastewater during the post-treatment step. Therefore, the main scientific question addressed in this study was: *Can microalgae rapidly reduce phosphorus content to ultra-low level in municipal wastewater after its biomass exposure to phosphorus deficiency?* According to this question, the following tasks were set to complete this study:

- To evaluate the present status of microalgae-based wastewater treatment and the associated challenges for phosphorus removal from municipal wastewater.
- To identify microalgal species that can grow in wastewaters, efficiently remove nutrients and provide a biomass for high value product extraction.
- To evaluate the biomass growth and nutrient removal rates of various microalgal species in different types of municipal wastewaters under batch conditions.
- To assess the microalgal biomass exposure to phosphorus deficiency as a technique for enhanced phosphorus uptake from wastewater.
- To identify indicators for quantification of the microalgal phosphorus deficiency status.
- To study the metabolic pathways of phosphorus for microalgal cells after their exposure to phosphorus deficiency.
- To identify the optimum conditions for municipal wastewater post-treatment in sequenced batch photobioreactor.
- To evaluate the prerequisites for scale-up of microalgae-based wastewater post-treatment with integrated biomass phosphorus starvation technique.

Scientific novelty and application

The novelty behind this study is the demonstration of microalgae biomass P-starvation with its target application for municipal wastewater post-treatment with cost recovery option. To the author's best knowledge, no currently operating microalgae-based wastewater treatment system is supplemented with biomass manipulation with P-stress to achieve an enhanced phosphorus removal. The author sees the presented approach for wastewater post-treatment with P-starved microalgal biomass as a potential process improvement to reduce the reaction time for phosphate removal without notable biomass productivity loss and maintain high yields of valuable molecules synthesized by the algal cells.

Description of the main chapters and methodology

This Thesis is based on four separate journal papers, further referred to by Roman numbers. The main objective of **Paper I** was to review the status of microalgae-based wastewater treatment and its major limitations for full scale use. Although there are several operational full-scale microalgae-based wastewater treatment plants in the world, many drawbacks and challenges exist that hold back a major shift towards this technology. The main obstacles for microalgae-based wastewater treatment are its limitation to certain climatic conditions to ensure high operational performance and high costs for biomass harvesting. In this paper the current solutions and possible strategies are reviewed to overcome the main challenges for microalgae-based wastewater treatment, which include biomass harvest, biomass exposure to pathogens, removal of micropollutants and operation in cold climate.

In **Paper II** the main task was to test and approbate the approach of microalgae biomass phosphorus starvation to enhance phosphorus removal from wastewater. To achieve this task, three microalgal species *Desmodesmus communis*, *Tetradesmus obliquus* and *Chlorella protothecoides* were first exposed to phosphorus deficiency conditions for 7 and 14 days and then cultivated in municipal wastewater to study the biomass growth and nutrient removal (Fig. 2). Also, the phosphorus removal rates, and polyphosphate accumulation were compared between various phosphorus starvation periods. Finally, the main drivers behind the obtained results were discussed.

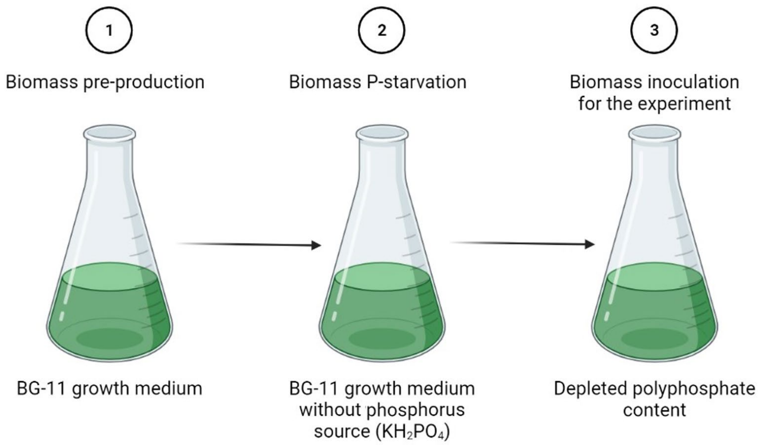

Fig. 2. Process scheme for microalgal biomass phosphorus starvation in phosphate-free medium.

In **Paper III** the effect of 3- and 5-day phosphorus starvation periods was tested on biomass growth, nutrient uptake rates and polyphosphate accumulation by microalgal species *Botrycoccus braunii*, *Chlorella vulgaris*, *Ankistrodesmus falcatus* and *Tetradesmus obliquus.* Unlike in **Paper II**, this experiment was done under batch conditions with aeration and CO_2 supply and the illumination of blue and red spectrum (Fig. 3). Finally, data on alkaline phosphatase activity was collected to study this enzyme as a possible indicator for microalgal biomass phosphorus deficiency status quantification.

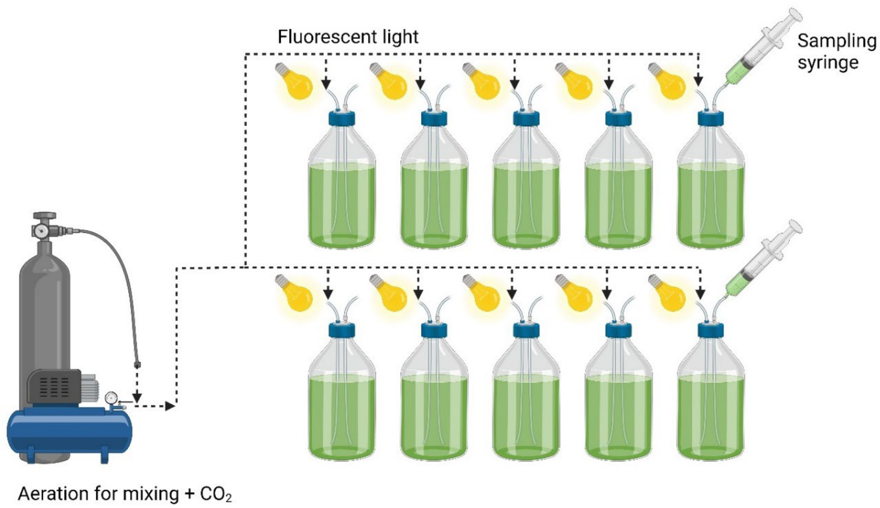

Fig. 3. Batch experiment setup with aeration and CO_2 supply.

The aim of **Paper IV** was to identify the optimum initial conditions for highest biomass growth and phosphorus removal rates at the shortest phosphorus starvation period. The study consisted of two phases. First, the microalgal species *Chlorella vulgaris* was cultivated in a synthetic wastewater using the batch setup from **Paper III**. The obtained data on biomass growth, specific phosphate removal, polyphosphate accumulation and protein productivity were analyzed using an optimization model to determine the optimum initial conditions for highest possible gain. In the second experiment phase, the obtained results for initial conditions optimization were verified in a sequencing batch photobioreactor, comparing the performance between regular and phosphorus-starved biomass (Fig. 4). A different biomass starvation technique was used than in **Papers II** and **III**, focusing on biomass polyphosphate content reduction.

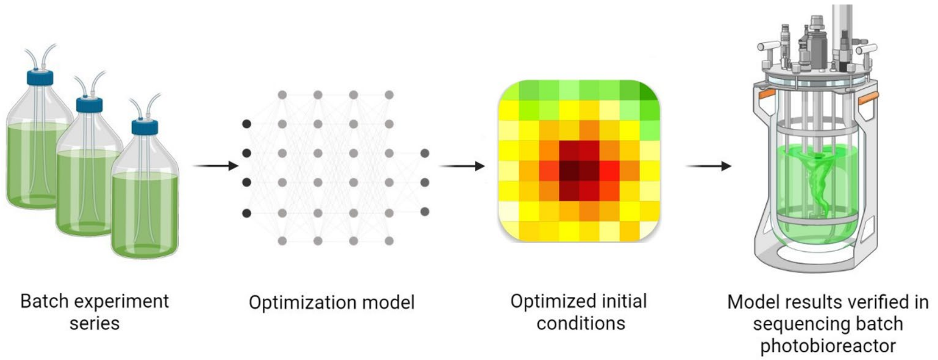

Fig. 4. Experiment sequence for the optimization study in Paper IV.

RESULTS AND DISCUSSION

Nutrient removal from wastewater under batch conditions

In **Paper II** the removal of dissolved inorganic phosphorus and nitrogen from primary and secondary wastewater after biomass P-starvation for 7 and 14 days was studied. *D. communis, T. obliquus* and *C. protothecoides* showed high phosphate removal at the end of experiment. At the reference conditions *D. communis* and *T. obliquus* removed >99.0 % phosphate from secondary wastewater within 5 and 3 days, respectively (Fig. 5). At the same conditions *C. protothecoides* could remove 93.9 % in 10 days. Biomass exposure to P-starvation for 7 days boosted the phosphate removal by *D. communis* and *T. obliquus*, which reduced the phosphate content in secondary wastewater by 88.7 and 89.5 %, respectively, within the first 24 h after inoculation and by >99.0 % after two days. This result is likely to be related to luxury P uptake. This phosphorus uptake and storage mechanism can be triggered by biomass P-starvation, which decreases the cellular phosphorus reserves. When re-supplied with high external phosphate, algal cells rapidly accumulate more phosphorus than needed for their growth.

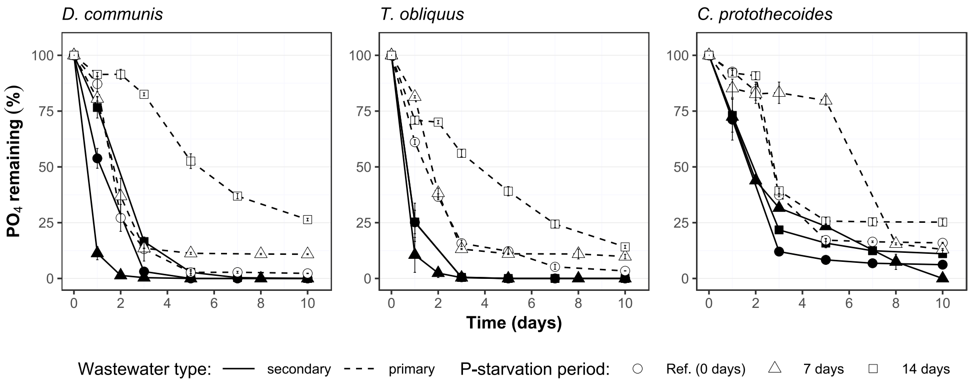

Fig. 5. Phosphate removal rate (means ± SD, *n* = 3) for *D. communis, T. obliquus* and *C. protothecoides* obtained in primary and secondary wastewaters after 7- and 14-day P-starvation and at the reference conditions with no biomass P-starvation.

The removal of dissolved inorganic nitrogen (DIN) for all three strains at all treatments was low. It was observed for *D. communis* that had a prior 7-day P-starvation period, removing 49.0 and 46.5 % from secondary and primary wastewater, respectively. 7-day P-starved *T. obliquus* could remove only 41.1 % of DIN from primary wastewater, other treatments removal was below 28.0 %. *C. protothecoides* could remove only between 0 and 17.1 % of DIN.

In **Paper III** the removal of phosphates and nitrates by *T. obliquus, C. vulgaris, A. falcatus* and *B. braunii* from secondary wastewater after biomass exposure to P-deficiency periods of 3 and 5 days were studied. All species, except *T. obliquus*, showed high nutrient removal rates, reaching more than 97.0 and 91.0 % reduction of nitrate and phosphate, respectively. Higher phosphate removal rates were observed for *C. vulgaris, B. braunii* after biomass exposure to P-deficiency for 3 and 5 days, reaching >99.0 % PO_4 reduction. Also *A. falcatus* showed >99.0 % PO_4 reduction after 3-day phosphorus starvation. In general, the highest phosphate removal rate was recorded after 10 days of growth, while 3-day starved *C. vulgaris* and *A. falcatus* reduced phosphate by 99.2 % on day 7 (Fig. 6). Nitrate removal was not affected by prior biomass exposure to P-deficiency conditions. The maximum nitrate removal for *C. vulgaris*, *B. braunii*, and *A. falcatus* was obtained on days 5 to 6.

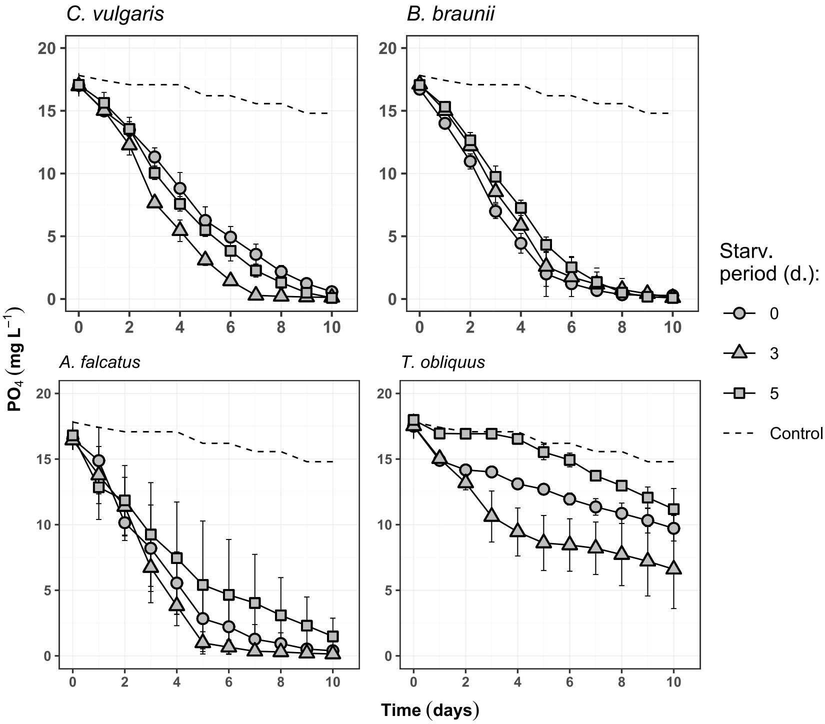

Fig. 6. Phosphorus removal (means ± SD, $n = 3$) for *C. vulgaris, B. braunii, A. falcatus* and *T. obliquus* in secondary wastewater after 3- and 5-day P-starvation and at the reference conditions with no biomass P-starvation. A batch without biomass was used as a negative control.

The obtained results highlight two possible obstacles to enhance phosphate uptake by P-starved biomass. Firstly, the low N/P ratio in the wastewater as well as faster removal of nitrogen indicate the importance of nitrogen limitation. This condition makes nitrate the preferred nutrient over phosphate, omitting the possible biomass status of P-deficiency. Secondly, rapid phosphate removal by P-starved biomass in other studies was achieved at conditions where cellular phosphorus was depleted. However, in this study to mimic practical conditions, internal biomass phosphorus reserve in the form of polyphosphate was still available after biomass exposure to P-deficiency. This condition emphasizes the prospective complexity of P-starvation implementation in pilot-scale wastewater post-treatment.

Phosphorus uptake and metabolic pathways under microalgal phosphorus deficiency conditions

In **Paper II** the polyphosphate accumulation in microalgal cells and its dynamics were studied as the response to biomass P-starvation and the possible involvement of luxury P uptake mechanism. *D. communis, T. obliquus* and *C. protothecoides* could accumulate polyphosphates while growing in wastewater. Higher polyphosphate accumulation for *D. communis* and *T. obliquus* was observed when biomass was growing in primary wastewater after 7 days of biomass P-starvation (Fig. 7). For *C. protothecoides* peak value was obtained in primary wastewater without prior P-starvation. In this case the higher polyphosphate accumulation in primary wastewater is related to lower pH during the initial phase of experiment. Due to the excess organic carbon present in primary wastewater and its oxidation to CO_2 the pH remained low. This prevented pH increase and phosphate precipitation. Instead, it could be consumed directly by biomass and accumulated as polyphosphate.

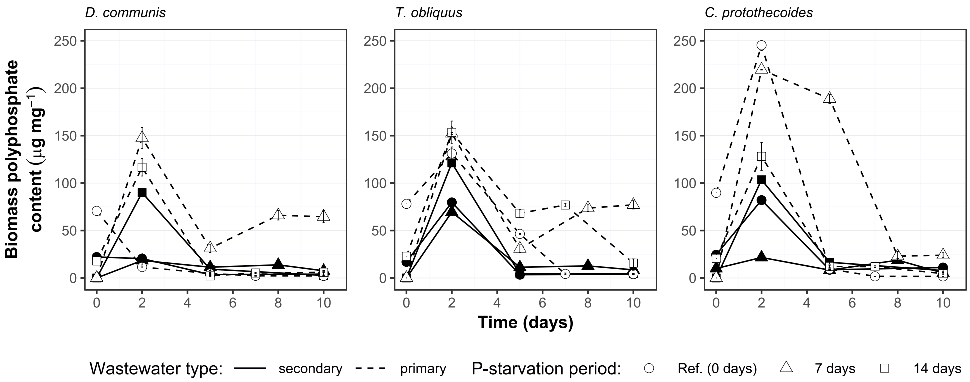

Fig. 7. Polyphosphate accumulation (means ± SD, $n = 3$) for *D. communis, T. obliquus* and *C. protothecoides* obtained in primary and secondary wastewaters after 7- and 14-day P-starvation and at the reference conditions with no biomass P-starvation.

In **Paper III** it was observed that biomass growth phase is a major factor for successful biomass P-starvation and subsequent enhanced phosphate uptake. The result from this study indicates that before the P-starvation *C. vulgaris* and *B. braunii* were at an early stationary growth phase when poly-P was accumulated to overcome extended periods without external orthophosphate availability. Contrary, *A. falcatus* and *T. obliquus* were at the exponential growth phase, when they actively used the acquired internal P reserves for biomass production (Sanz-Luque et al., 2020).

In **Paper IV** the microalgal biomass P-starvation was started after its growth entered a stationary phase. During the experiment, biomass polyphosphate content change showed a rapid increase during the initial six hours, indicating that that majority of the inorganic phosphorus was directly incorporated into the cells instead of its adsorption on the cell surface (Yao et al., 2011). Further, the biomass polyphosphate content gradually decreased during the next days of experiment, especially for biomass with longer P-starvation period. Such dynamics indicate, that after longer biomass exposure to P-deficiency the cell incorporated inorganic phosphorus was transformed into acid soluble polyphosphate which is further used for the synthesis of cell constituents, such as protein, DNA or RNA (Su, 2021). Accordingly, a lesser portion of cellular inorganic phosphorus was transformed into acid insoluble polyphosphate which provides the storage P reserves for conditions of external P limitation.

In **Paper IV** the protein productivity was evaluated as a response to biomass P-deficiency. The obtained result demonstrated the ability of *C. vulgaris* to synthesize protein at substantially higher rate after its biomass long-term exposure to phosphorus stress. On one hand, this observation contradicts the known metabolic pathway for cellular protein synthesis, which strongly depends on nitrogen assimilation and the available light intensity (Huang et al., 2021). On the other hand, it is suggested that phosphorus availability had an indirect impact on the protein synthesis. Enhanced phosphorus assimilation caused by prior P-stress condition accelerated synthesis of adenosine triphosphate (ATP), which is required as an energy source for protein synthesis. Such an involvement of phosphorus in protein synthesis has also been discussed by Perez-Garcia et al. (2010) and Wu et al. (2021) and further supports the previously discussed phosphorus assimilation pathway into acid-soluble polyphosphate.

Initial conditions for optimum performance in PBR

In **Paper IV** the microalgal species *C. vulgaris* was used to identify the optimum biomass P-starvation period as well as the initial concentrations for biomass and phosphate to obtain a rapid phosphate removal, polyphosphate accumulation, protein productivity and biomass growth at the shortest possible period of biomass exposure to P-deficiency period.

The phosphate removal was normalized against the initial biomass concentration and presented as the specific phosphate removal rate R_{xi}. It was found that the specific phosphate removal rate increased with longer biomass P-starvation period. In seven out of nine batches the highest R_{xi} value was obtained after 10-day P-starvation period, reaching 7.74 mg g DW h^{-1}. Such a result was obtained for batch with mid-P/low-biomass concentration (Fig. 8). The maximum specific P removal rates in every batch were on average 62.0 % higher than for the reference biomass in the same batch and reaching even 175.0 % increase of the R_{xi} in conditions with low-P/high-biomass concentration after 10-day P-starvation period. The effect of biomass P-starvation on the biomass growth rate was insignificant.

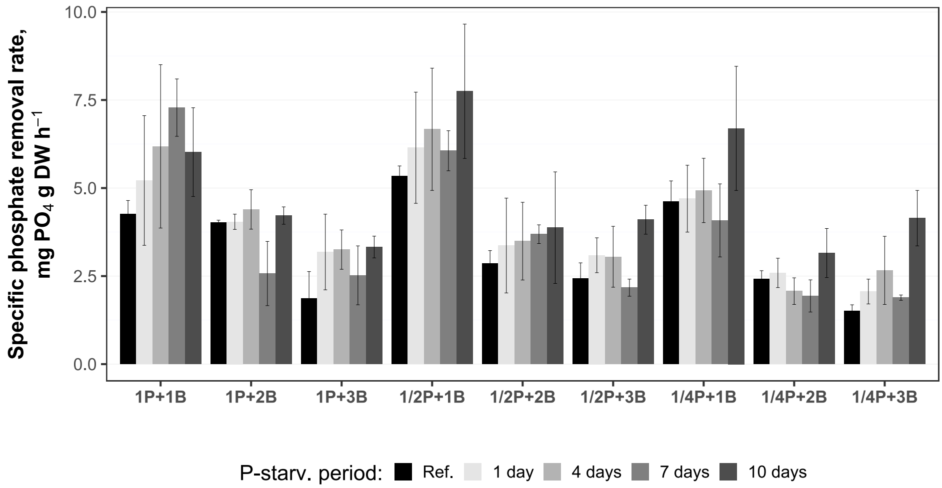

Fig. 8. The specific phosphate removal rate by *C. vulgaris* during first 5 hours of the experiment at various initial biomass and phosphate concentrations and after different biomass P-starvation periods (means ± SD, $n = 3$). The number and letter combinations on the x-axis refers to the initial phosphorus (P) and biomass (B) content in each batch. 1P – 22 mg P L^{-1}, 1/2P – 12 mg P L^{-1}, 1/4P – 5.5 mg P L^{-1}; 1B – 0.2 g DW L^{-1}, 2B – 0.6 g DW L^{-1}, 3B – 1.5 g DW L^{-1}.

The biomass P-starvation period had a substantial effect on the pathways and dynamics of phosphate cellular accumulation and transformation. Biomass polyphosphate accumulation rate gradually increased with longer biomass exposure to P-deficiency conditions (Fig. 9). In all batches the 10-day starved biomass accumulated poly-P at 3.7–40.0 times higher rate, comparing to the reference conditions, and showing, that the biomass poly-P content was restored significantly faster after the microalgal cells have been exposed to more extensive P-deficiency status. From the accumulated polyphosphate, the lesser fraction was transformed into acid insoluble polyphosphate which provides the storage P reserves for conditions of external P limitation. Polyphosphate content decrease over time indicated that it was mainly used for the synthesis of high value products, including protein.

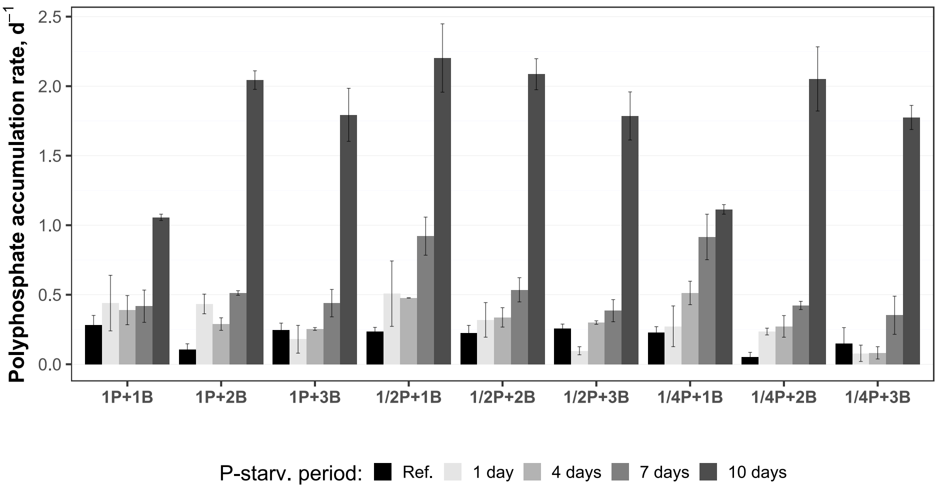

Fig. 9. The polyphosphate accumulation rate for *C. vulgaris* at various initial biomass and phosphate concentrations and after different biomass P-starvation periods (means ± SD, $n = 3$). The number and letter combinations on the x-axis refers to the initial phosphorus (P) and biomass (B) content in each batch. 1P – 22 mg P L^{-1},1/2P – 12 mg P L^{-1}, 1/4P – 5.5 mg P L^{-1}; 1B – 0.2 g DW L^{-1}, 2B – 0.6 g DW L^{-1}, 3B – 1.5 g DW L^{-1}.

The observed biomass protein productivity for a 3-day period was increasing with longer P-starvation periods. However, significantly higher protein productivity was obtained in batches with 10-day P-starved biomass, compared to other P-starvation periods, reaching 9.0 g DW d^{-1} for the high-P/mid-biomass batch. In all batches the 10-day biomass P-starvation resulted in 5.7–46.8 times higher protein productivity than at the reference conditions with no prior biomass P-starvation (Fig. 10).

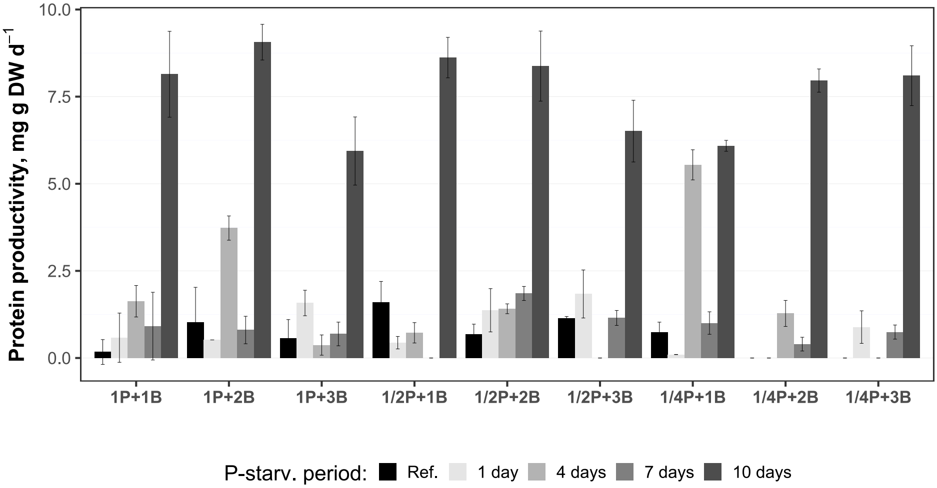

Fig. 10. Protein productivity for *C. vulgaris* at various initial biomass and phosphate concentrations and after different biomass P-starvation periods (means ± SD, $n = 3$). The number and letter combinations on the x-axis refers to the initial phosphorus (P) and biomass (B) content in each batch. 1P – 22 mg P L^{-1},1/2P – 12 mg P L^{-1}, 1/4P – 5.5 mg P L^{-1}; 1B – 0.2 g DW L^{-1}, 2B – 0.6 g DW L^{-1}, 3B – 1.5 g DW L^{-1}.

The results obtained from batch experiment series were used as input data for an optimization model aiming to find the most favorable conditions for efficient wastewater post-treatment. The model results suggested biomass P-starvation period of 1 day as the most favorable to achieve rapid phosphate removal, maintain a high biomass growth rate and ensure high polyphosphate accumulation rate and protein synthesis. To facilitate such a performance, the biomass with short P-starvation period should be inoculated at initial concentration of ~0.2 g DW L^{-1}. The initial phosphate concentration seems to have less influence on maximizing the output of the key variables and is suggested to be initially set within the margin of 10 to 20 mg L^{-1}.

Biomass growth, metabolic activity and nutrient removal from wastewater in a sequenced batch PBR

The initial values for P-starvation period and concentrations for biomass and phosphate obtained during batch experiment in **Paper IV** were verified in a sequencing batch photobioreactor operating for 60 days. The obtained biomass growth rate was 52.7 % higher for the P-starved biomass than at the reference conditions (Table 1). Moreover, the P-starved biomass showed 101.7 % higher specific phosphorus removal rate than at the reference conditions, indicating that one day long biomass P-starvation was sufficient to establish a phosphorus deficiency status and promote enhanced phosphate uptake. Also, the polyphosphate accumulation rate was 138.0 % higher for P-starved biomass than at the reference conditions. The dynamics of biomass polyphosphate content as well as the alkaline phosphatase activity further verified the P-deficiency status. The observed protein productivity for P-starved biomass was 38.7 % lower than at the reference conditions. Such an outcome agrees with the result obtained in batch experiment series where protein productivity after 1-day P-starvation did not show any significant increase comparing to the reference conditions.

Table 1

Kinetic parameter values for the reference and 1-day P-starved *C. vulgaris* biomass in sequencing batch photobioreactor (means ± SD)

	Biomass growth rate, d^{-1}	**Specific PO_4 removal rate, mg g DW h^{-1}**	**Polyphosphate accumulation rate, d^{-1}**	**Protein productivity, mg g DW d^{-1}**
Reference (n = 4)	0.072 ± 0.016	0.279 ± 0.134	0.280 ± 0.066	1.682 ± 0.210
P-starved (n = 5)	0.110 ± 0.024	0.563 ± 0.082	0.666 ± 0.063	1.031 ± 0.365

Cost evaluation of microalgae-based wastewater post-treatment

In **Papers II** and **IV** it was demonstrated that microalgae biomass exposure to phosphorus deficiency can promote rapid and near-complete phosphorus content reduction in wastewater. However, such a performance comes at expenses that are often higher than the conventional post treatment methods (Table 2). Molinos-Senante et al. (2010) have estimated that the chemical precipitation for phosphorus removal can cost starting from 0.215 EUR m^{-3}. The cost for membrane filtration varies greatly, depending on the technology used and location-specific electricity cost. Moreover, the model estimations by Hernandez-Sancho et al. (2011) show that scale factor is important, as the wastewater treatment cost increases with larger operational scale.

On the other hand, comparing the conventional systems for wastewater post-treatment and nature-based systems such as constructed wetlands and high-rate algal ponds shows that not only the conventional system is 2 to 3 times more expensive than nature-based technologies, but also the potential environmental impacts were 2- to 5-fold higher for the conventional systems due to high chemicals and electricity consumption (Garfí et al., 2017).

Table 2

Price range comparison between different wastewater post-treatment methods

Wastewater post-treatment technology	Cost, EUR m^{-3}	Reference
Chemical precipitation	from 0.215	(Molinos-Senante et al., 2010)
Membrane filtration	from 0.859 to 11.828	(Clem and Mendonça, 2022) (Ozturk et al., 2003) (Hernandez-Sancho et al., 2011)
Constructed wetland	from 0.120 to 0.250	(Gikika et al., 2014)
High-rate algal pond	from 0.120 to 0.420	(Kohlheb et al., 2020) (Kit et al., 2021) (Garfí et al., 2017)
Photobioreactor	from 1.520	(Norsker et al., 2011) This study

Using microalgae for wastewater treatment becomes more expensive with the photobioreactor technology, as it requires higher capital and operational costs. From the cost estimation by Norsker et al. (2011) and microalgae growth and phosphorus removal rate data obtained in this study, the projected cost for treatment of 1 m^3 wastewater would be starting from 1.52 EUR. Moreover, the operational costs for PBR can significantly differ between configuration and working environment. A study by Sarker and Salam (2019) shows that indoor PBRs consumed 232 to 270 times more energy, required 25 to 57 times more capital cost, and 3.8 to 16.8 times more operating cost than for PBRs operating outdoors.

Although the cost of microalgal photobioreactor technology currently is higher than other methods, it has a greater financial return potential due to higher control over the process and the resulting variety of options for produced biomass application. The estimations by Arshiro et al. (2018) that microalgae based wastewater post treatment could cover the operational costs or even provide a profit if biomass production and its sales is integrated in the wastewater treatment process. Therefore, when putting the PBR technology for wastewater post-treatment into perspective of resource recovery and circular economy, it can become a cost-positive wastewater treatment technology.

CONCLUSIONS

The objective of this Thesis was to investigate the potential of microalgae-based municipal wastewater post-treatment with integrated biomass phosphorus starvation for enhanced phosphate removal. To achieve the goal of the Thesis, the literature study was done and P-starved microalgae potential for municipal wastewater post-treatment with a focus on dissolved inorganic phosphorus removal was examined.

In **Paper I** the current status of microalgae-based wastewater treatment was reviewed. It was emphasized that the major limitations for a successful microalgae-based wastewater treatment in full scale are the process exposure to certain climatic conditions, expenses for microalgal biomass harvest and the presence of hazardous substances and pathogens in municipal wastewater that has a potentially harmful effect on microalgal productivity and its downstream applications. Considering that the limitations are primarily related to financial problems, microalgae biomass exposure to phosphorus deficiency was identified as a possible means to overcome the bioremediation problem at various environmental conditions and increase the biochemical value of produced biomass.

In **Paper II** it is demonstrated that microalgae biomass phosphorus starvation can enhance phosphorus removal from wastewater. The microalgal species *Desmodesmus communis*, *Tetradesmus obliquus* and *Chlorella protothecoides* could grow in both filtered incoming sewage and the effluent after biological treatment. After the exposure to phosphorus deficiency conditions for 7 days, *D. communis* and *T. obliquus* could reduce the PO_4 content in secondary wastewater by 89 % within 24 hours. *C. protothecoides* showed the highest polyphosphate accumulation when produced in primary wastewater. This study also emphasizes the importance of pH control to avoid phosphorus precipitation and ensure its direct uptake by biomass to ensure its biochemical quality.

In **Paper III** the microalgal species *Botrycoccus braunii*, *Chlorella vulgaris*, *Ankistrodesmus falcatus* and *Tetradesmus obliquus* were exposed to 3- and 5-day phosphorus starvation periods to study the bioremediation of effluent from a small wastewater treatment plant. Unlike in **Paper II**, in this study the batch conditions were supplemented with aeration and CO_2 influx for pH control. The obtained results showed that the nitrogen limitation disrupts the effect on biomass P-starvation for enhanced phosphorus removal. Moreover, the results of polyphosphate accumulation emphasized the importance of biomass growth phase for efficient phosphorus starvation procedure. The accumulated polyphosphate depletion starts at the stationary phase and the phosphorus starvation period should be measured from there. In addition, the alkaline phosphatase activity was identified as a potential indicator for phosphorus deficiency status development for microalgal biomass. *Chlorella vulgaris* was identified as the most appropriate species for further study.

In **Paper IV** *Chlorella vulgaris* was produced in a synthetic wastewater to find the optimum combination of initial values for parameters such as the concentrations of biomass and phosphate as well as the P-starvation period to obtain the most rapid phosphate removal, highest rates for biomass growth and polyphosphate accumulation and protein productivity at the shortest phosphorus starvation period. It was found that the studied parameter values improved with longer biomass P-starvation period. Also, lower initial biomass concentration was favorable for phosphate removal due to higher photosynthetic activity. An optimization model was used to obtain the most efficient combination of initial values, and it suggested 1 day of P-starvation and initial biomass and phosphorus concentrations of 0.25 mg DW L^{-1} and 10 to 15 mg L^{-1}, respectively, for efficient performance. The optimization model results were verified in a sequencing batch reactor, where 101.7 % higher phosphate removal rate (0.563 mg g^{-1} DW h^{-1}), 52.7 % higher biomass growth rate (0.110 d^{-1}) and 138.0 % higher polyphosphate accumulation rate (0.666 d^{-1}) was obtained with P-starved biomass comparing its performance with reference conditions.

The main benefit of microalgae biomass P-starvation is the reduced reaction time for phosphorus removal to ultra-low level. Such an approach can be beneficial for municipal wastewater treatment at the additional treatment stage for targeted dissolved inorganic phosphate removal, especially for cases when standards for low PO_4 content must be met. As demonstrated in **Paper IV**, even a relatively short period of P-deficiency can double the specific phosphorus removal rate, compared to regular biomass. Sustaining an enhanced phosphorus removal rate would reduce the size of PBR hosting the reaction. Thus, the capital costs can be lowered, which is identified as one of the drawbacks for full scale operation. On the other hand, results from **Papers II** and **III** showed possible limitations and drawbacks that could hamper the efficiency of phosphorus removal by P-starved biomass. As the chemical content in municipal wastewater is variable, it might be complicated to maintain high N/P ratio which is complementary for phosphorus limiting conditions and enhanced phosphate removal. Moreover, in all experiments the biomass P-starvation effect was studied in a laboratory scale under sterile and controlled conditions, possibly excluding any organic and chemical factors that might have a negative impact on the microalgal biomass. Micro-filtered and sterile growth medium excluded presence of any other microorganisms that could outcompete the algal cells for nutrients. The content of heavy metals was at trace level, therefore no impact as hazardous substance could be assessed. The presence of certain metals can be an additional stress factor that impacts the biomass growth and high value molecule synthesis (Karcheva et al., 2022). On the other hand, the controlled conditions and exclusion of possible stressors allowed to focus this study on the mechanisms for enhanced phosphorus uptake.

Operating a microalgae-based municipal wastewater post-treatment system at a full scale is primarily regulated by light availability and temperature. However, it was not assessed during this study how major shifts from optimum temperatures and light regimes would affect the phosphate removal after biomass exposure to P-deficiency. In previous studies it has been demonstrated that high nutrient removal rates can be sustained at temperatures as low as 4 °C (Craggs et al., 1997), which potentially allows to extend the system application to higher latitudes.

Overall, the results obtained in this study approve the scientific hypothesis of this Thesis – microalgae biomass exposure to phosphorus deficiency conditions can enhance the uptake rate of dissolved inorganic phosphorus from an aquatic environment, including municipal wastewater. The obtained results also supplement the existing knowledge on microalgae-based wastewater treatment. For instance, it was demonstrated that the phosphorus deficiency status in microalgae can be assessed using the alkaline phosphatase activity as an indicator. Such indicator would allow more control over the operating of P-starvation in full-scale wastewater post-treatment. Also, it was shown how phosphorus deficiency conditions affect its accumulation and transformation pathways within the microalgal cell, allowing to better understand the P-starvation impact on high value molecule synthesis. Ultimately, this study shows, that the proposed technology for municipal wastewater post-treatment can reduce phosphate concentration below 0.1 mg L^{-1} and possibly diminish the impact of municipal wastewaters on eutrophication status development in surface waters.

The future research for microalgae phosphorus starvation for enhanced phosphorus removal from wastewater should focus on its operation in full scale systems treating real wastewater of various origins. It is recommended to include studies on pathogen and hazardous substance presence in high value compounds derived from microalgae grown in wastewater. Such knowledge is crucial for communicating the safety and applicability of such microalgal products with the general public. Also, a better understanding of microalgal cell phosphorus deficiency status at the genetic level would not only benefit the microalgal application in controlled engineered systems but also increase the understanding of the phosphorus cycle in natural aquatic ecosystems.

APROBATION

List of publications

1. Lavrinovičs, A., Juhna, T. Review on Challenges and Limitations for Algae-Based Wastewater Treatment. *Construction Science*, 2017, 20, 17–25.
2. Lavrinovičs, A., Mežule, L., Juhna, T. Microalgae Starvation for Enhanced Phosphorus Uptake from Municipal Wastewater. *Algal Research*, 2020, 52, 102090.
3. Lavrinovičs, A., Murby, F., Zīverte, E., Mežule, L., Juhna, T. Increasing Phosphorus Uptake Efficiency by Phosphorus-Starved Microalgae for Municipal Wastewater Post-Treatment. *Microorganisms*, 2021, 9 (8), 1598.
4. Lavrinovičs, A., Mežule, L., Cacivkins, P., Juhna, T. Optimizing Phosphorus Removal for Municipal Wastewater Post-Treatment with *Chlorella vulgaris*. *Journal of Environmental Management*, 2022, 324, 116313.

List of conferences

1. MELiSSA conference 2022. Current and Future Ways to Closed Life Support System. Presentation: Optimizing Phosphorus Removal for Municipal Wastewater Post-Treatment with *Chlorella vulgaris*. November 10, 2022, Toulouse, France.
2. 10th International Conference on Algal Biomass, Biofuels and Bioproducts. Presentation: Microalgae exposure to phosphate deficiency for enhanced nutrient uptake and biochemical value improvement. June 15, 2021, on-line.
3. IWA 12th Eastern European Young Water Professionals Conference. Presentation: Microalgae starvation for enhanced phosphorus uptake from municipal wastewater. April 2, 2021, on-line.
4. MELiSSA conference 2020. Current and Future Ways to Closed Life Support System. Presentation: The effect of phosphate starvation on nutrient uptake and cellular content of the microalgae *Desmodesmus communis* and *Chlorella protothecoides*. November 3, 2020, on-line.
5. RTU 61st international scientific conference, section "Bioenergy Technologies and Biotechnologies". Presentation: Benefits and challenges of phosphorus-starved microalgae use for municipal wastewater treatment. October 20, 2020, on-line.
6. RTU 58th international scientific conference, section "Bioenergy Technologies". Presentation: Microalgae for wastewater treatment: prospects and shortcomings. October 16, 2017. Riga, Latvia.
7. RTU 58th international scientific conference, section “Heat, gas and water technologies". Presentation: Use of freshwater food-web for phosphorus removal from wastewater in cold climate conditions. October 12, 2017. Riga, Latvia.

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