RIGA TECHNICAL UNIVERSITY

Baiba Ieviņa

INTEGRATION OF MICROALGAE CULTIVATION TECHNOLOGY IN BIOGAS PLANTS

Summary of the Doctoral Thesis

RTU Press
Riga 2024

RIGA TECHNICAL UNIVERSITY
Faculty of Natural Sciences and Technology
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DOCTORAL THESIS PROPOSED TO RIGA TECHNICAL UNIVERSITY FOR 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 defence at the open meeting of the RTU Promotion Council on 20th June 2024, at the Faculty of Natural Sciences and Technology of Riga Technical University, Āzenes str. 12/1, room 116.

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DECLARATION OF ACADEMIC INTEGRITY

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

Baiba Ieviņa ……. (signature)

Date: …….

The Doctoral Thesis has been written in English. It consists of an Introduction, 3 chapters, Conclusions, 60 figures, and 12 tables; the total number of pages is 236. The Bibliography contains 292 titles.

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INTRODUCTION

Relevance of the Doctoral Thesis

The depletion of fossil resources, alongside industrial growth, and a growing global population, set the ground for an energy crisis, forcing us to move to renewable energy alternatives. Additionally, rising levels of carbon dioxide in the air are causing climate change, leading to changes in weather and harming nature's balance. In this context, microalgae are considered a potential solution for both sustainable energy and CO_2 sequestration due to their superior qualities such as fast growth rate, ability to absorb high concentrations of CO_2, and resistance to harsh conditions. In contrast to first-generation biomass such as corn or sugarcane, microalgae do not compete with food production because they do not require arable land for cultivation. Microalgae biomass can be converted to various types of energy, including biogas, biodiesel, and bioethanol. Moreover, they contain high-value compounds with high potential in food, feed, nutraceuticals, cosmetics, and medicine. In addition to already existing applications of microalgae, the potential of microalgae is being explored in other emerging areas, including wastewater treatment, biostimulants and biopesticides, and biochemicals [1].

Despite the vast potential of microalgae biomass, its current use is limited to a few products and applications due to substantial challenges of biomass production, including high capital and operational costs, low biomass productivity, scale-up issues, and high costs of biomass harvesting and downstream processing [2]. Recently, much effort has been focused on promoting the economic feasibility of microalgae cultivation including bioreactor design considerations [3], optimization of cultivation conditions [4]–[6], search for new more productive microalgal strains [7], [8], and new biomass harvesting techniques to decrease the costs and increase the harvesting efficiency [9], [10].

The application of various wastewaters as a low-cost nutrient source for microalgae growth has been studied extensively lately to further lower the cost of biomass production [11]. Digestate, a nutrient-rich by-product of anaerobic digestion, is currently used as fertilizer in agriculture; however, several challenges associated with digestate management limit land application. Moreover, the increasing number of biogas plants in Europe creates an overproduction of digestate resulting in environmental and human health risks. Coupling biogas production with microalgae cultivation can provide various benefits, including nutrient recycling from liquid digestate and CO_2 sequestration from flue gas.

To date, most large-scale microalgae cultivation is located in warm low-latitude regions such as Israel, Australia and the southern USA [12], whereas biomass production in Nordic regions remains a major challenge. Nevertheless, several recent studies prove that year-round microalgae cultivation in a low-temperature environment can be achieved if local strains adapted to the local climate are used [13]. However, studies on microalgae cultivation in high-latitude regions are scarce and no reports could be found on year-round cultivation of microalgae in Latvian climate conditions.

Objective and tasks

The Doctoral Thesis aims to develop a novel microalgae biomass production technology for biogas plants integrating biogas side waste streams. In order to achieve the goal, the following tasks were set:

1. Select potential microalgae species for the Latvian climate.
2. Assess the influence of factors affecting microalgae cultivation.
3. Test agricultural digestate as a low-cost nutrient source for microalgae.
4. Test the potential of increased CO_2 concentrations for enhanced biomass production.
5. Design a novel improved microalgae cultivation system.
6. Test the novel technology integrated into a biogas plant.

Scientific novelty

The scientific novelty of the Doctoral Thesis is related to several aspects linked to digestate management and microalgae biomass production. A new microalgae cultivation system was built to overcome the drawbacks of the existing ones, offering improved light availability to microalgae cells, a reduction in land use, and year-round cultivation. Microalgal species for cultivation in high-latitude climates were selected and tested, offering an opportunity for biomass production and wastewater treatment in the Latvian climate. Lower biomass production costs can be achieved by using waste products from biogas production, namely digestate and flue gases. It was demonstrated that selected microalgae can remove nutrients from agricultural digestate at low temperature with high efficiency, thus offering an alternative digestate management tool to traditional land application. To the author's best knowledge no other cultivation technology for microalgae year-round biomass production in Latvian climate conditions has been developed.

Practical significance

A novel microalgae cultivation system was designed and built, allowing biogas operators to potentially incorporate microalgae cultivation in biogas plant daily operations to increase biomass security, lower biomass transportation costs, and offer an alternative route of digestate management to deal with the overproduction issue.

The designed technology set the ground for a patent from the Patent Office of the Republic of Latvia, which was granted explicitly for developing a novel microalgae cultivation system.

Research framework

The research was framed in two blocks – (1) laboratory tests and (2) pilot-scale race-way ponds – and 7 stages, namely (1.1) microalgae strain selection, (1.2) impact of cultivation conditions, (1.3) digestate as a nutrient source, (1.4) CO_2 as a carbon source, (2.1) design of cultivation technology, (2.2) construction and integration of pilot into a biogas plant, and (2.3) testing of the novel cultivation system. The research framework is shown in Fig. 1.

For each stage, a literature review was performed, and extensive laboratory tests were performed for Stages 1.2, 1.3, and 1.4.

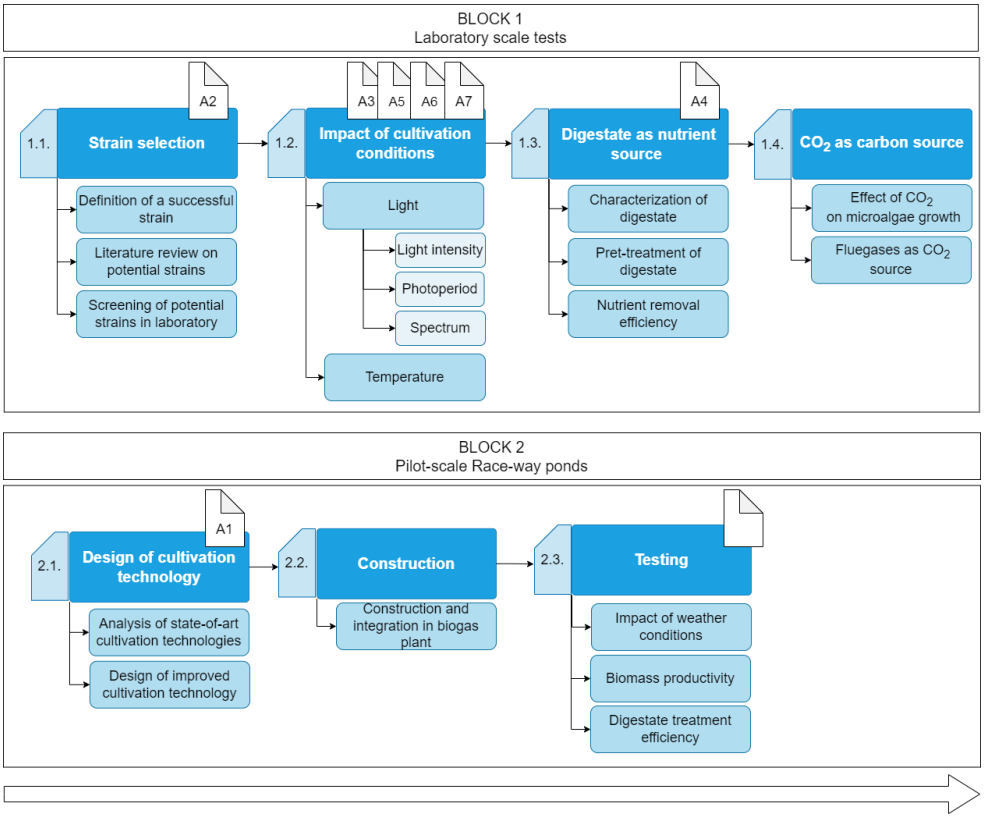


Fig. 1. The research framework of the Doctoral Thesis.

Approbation of the research results

The Thesis is based on seven scientific publications; three other scientific publications arose from the Doctoral Thesis but are not included in the Thesis. Results have been presented at five international scientific conferences. The patent has been granted for the developed novel cultivation system from the Patent Office of the Republic of Latvia.

Scientific publications:

1. Romagnoli, F., Ievina, B., Perera, W. A. A. R. P., Ferrari, D. Novel Stacked Modular Open Raceway Ponds for Microalgae Biomass Cultivation in Biogas Plants: Preliminary Design and Modelling. Environmental and Climate Technologies, 2020, vol. 24, no. 2, pp. 1–19.
2. Ievina, B., Romagnoli, F. The potential of *Chlorella* species as a feedstock for bioenergy production: A review. Environmental and Climate Technologies, 2020, vol. 24, no. 2, pp. 203–220.

3. Ievina, B., Romagnoli, F. Effect of light intensity on the growth of three microalgae in laboratory batch cultures, 2020, European Biomass Conference and Exhibition Proceedings, pp. 169–174.
4. Ievina, B., Mantovani, M., Marazzi, F., Mezzanotte, V., Romagnoli, F. Application of activated carbon treated agricultural digestate for microalgae cultivation, 2021, European Biomass Conference and Exhibition Proceedings, pp. 124–131.
5. Romagnoli, F., Weerasuriya-Arachchige, A. R. P. P., Paoli, R., Feofilovs, M., Ievina, B. Growth Kinetic Model for Microalgae Cultivation in Open Raceway Ponds: A System Dynamics Tool. Environmental and Climate Technologies 2021, vol. 25, no. 1, pp. 1317–1336.
6. Ievina, B., Romagnoli, F. Microalgae *Chlorella vulgaris* 211/11j as a promising strain for low temperature climate. Journal of Applied Phycology, 2024, In press.
7. Ievina, B., Romagnoli, F. Unveiling the underlying factors for light spectrum preference for enhanced microalgae growth. (Algal Research. Under review).

Other scientific publications

1. Mezzanotte, V., Romagnoli, F., Ievina, B., Mantovani, M., Invernizzi, M., Ficara, E., Collina, E. LCA of Zero Valent Iron Nanoparticles Encapsulated in Algal Biomass for Polishing Treated Effluents. Environmental and Climate Technologies 2022, pp. 1196 – 1208.
2. Romagnoli, F., Thedy, A., Ievina, B., Feofilovs, M. Life Cycle Assessment of an Innovative Microalgae Cultivation System in the Baltic Region: Results from SMORP Project. Environmental and Climate Technologies 2023, vol. 27, no. 1, pp. 117–136.
3. Romagnoli, F., Spaccini, F., Boggia, A., Paoli, R., Feofilovs, M., Ievina, B., Rocchi, R. Microalgae cultivation in a biogas plant: Environmental and economic assessment through a life cycle approach. Biomass and Bioenergy 2024, 182: 107116.

Participation in scientific conferences

1. Romagnoli, F., Ievina, B., Perera, W. A. A. R. P., Ferrari, D. Novel Stacked Modular Open Raceway Ponds for Microalgae Biomass Cultivation in Biogas Plants: Preliminary Design and Modelling. CONECT 2019, May 15–17, 2019, Riga, Latvia.
2. Ievina, B., Romagnoli, F. The potential of *Chlorella* species as a feedstock for bioenergy production: A review. CONECT 2020, May 13–15, 2020, Riga, Latvia.
3. Ievina, B., Romagnoli, F. Effect of light intensity on the growth of three microalgae in laboratory batch cultures, 28th European Biomass Conference and Exhibition, July 6–9, 2020, Online.
4. Ievina, B., Mantovani, M., Marazzi, F., Mezzanotte, V., Romagnoli, F. Application of activated carbon treated agricultural digestate for microalgae cultivation, 29th European Biomass Conference and Exhibition, April 26–29, 2021, Online.

5. Romagnoli, F., Weerasuriya-Arachchige, A. R. P. P., Paoli R., Feofilovs, M., Ievina, B. Growth Kinetic Model for Microalgae Cultivation in Open Raceway Ponds: A System Dynamics Tool. CONECT 2021, May 12–14, 2021, Riga, Latvia.

Patents

Romagnoli, F., Dzikēvičs, M., Ieviņa, B. Atvērta tipa modulāra mikroaļģu kultivēšanas baseinu sistēma (Modular open microalgae cultivation pond system). Patent number 15742, 12.06.2023.

1. RESEARCH METHODOLOGY

1.1. Design and construction of a novel cultivation system

An extensive literature review was conducted to uncover the advantages and disadvantages of existing cultivation systems. The design of a novel cultivation system was proposed to overcome the limitations of the existing cultivation technologies.

Planning and design of novel cultivation system included considerations on location and layout of facilities, pond size and configuration, hydraulics, paddle wheel design and materials for the construction. In design consideration, several aspects, including geometrical design in terms of surface area-to-volume ratio, as well as light distribution, nutrient provision and gas transfer, were studied. An understanding of the morphology and physiology of specific microalgae is required for design considerations. Moreover, a knowledge of the complex interaction between biomass production and environmental parameters is essential [14]. The main aim addressed is to provide benefits towards (1) the reduction of land use, (2) the increased light availability, and (3) the lower investment costs of the open systems compared to PBRs.

1.2. Laboratory scale tests

A range of laboratory tests were conducted to assess the influence of diverse environmental and cultivation conditions on the growth rate and productivity of candidate microalgae strains selected during the literature review. The impact of temperature, light intensity, photoperiod, light spectral composition, and level of CO_2 on the microalgae growth rate was tested on a laboratory scale. Moreover, digestate as a nutrient source for microalgae growth was evaluated.

Unless otherwise stated in the corresponding chapter, microalgae were cultivated in 500 mL Erlenmeyer flasks with cotton plugs containing 200 mL BG-11 or TAP medium with an initial pH of 7.5. Tests were performed in a refrigerated incubator (Friocell Eco line, MMM group, Germany) with manually installed natural white (4000 K) linear 10W LED lights (V-TAC, Samsung). Aeration was provided with ambient air using an orbital shaker (Elmi, Latvia) at 150 rpm. The initial concentration of microalgae cultures was approx. 2×10^6 cells mL^{-1}. All the tests were conducted in triplicate. Algae were grown at a constant temperature of 24 °C under a photoperiod of 16 : 8 h (light : dark) for 10 days under batch cultivation mode. Cultures were sampled daily for growth rate evaluation. Daily pH readings were collected manually with a pH meter (Hanna, USA) to monitor the microalgae growth. Biomass production was evaluated based on the cell dry weight at the end of the batch cultivation.

Microalgae strain selection and maintenance

The selection of potential microalgae strains for outdoor cultivation in Latvian climate conditions was based on an extensive literature review of published scientific research. Three microalgae were selected for laboratory tests. Microalgae *Chlorella vulgaris* 211-11j, *Chlorella sorokiniana* 211-8k and *Chlamydomonas reinhardtii* 11-32b were obtained from the SAG Culture collection of algae at Göttingen University, Germany, and The Culture Collection of Algae and Protozoa at Scottish Marine Institute, Scotland, UK.

Growth assessment methods

To consider the effect of various parameters on biomass yield, several growth and productivity assessment methods were used as described below.

1. Cell counts with a hemocytometer and calculation of microalgae cell density (cells mL^{-1}).
2. Optical density measurements with a UV/VIS spectrophotometer at 750 nm.
3. Calculation of specific growth rate (μ).
4. Dry weight measurements (g L^{-1}).
5. Biomass productivity (g L^{-1} d^{-1}).

Evaluation of a low-temperature strain

Chlorella vulgaris 2011-11j was selected as a potential low-temperature strain for outdoor cultivation in Latvian conditions in colder seasons. No comprehensive report on the temperature requirements of this strain could be found; therefore, it was cultivated in a wide temperature range from 8 °C to 32 °C to determine optimum cultivation temperature and lower and upper temperature limits. Cultures were grown in batch mode at 8 °C, 12 °C , 16 °C, 20 °C, 24 °C, 28 °C and 32 °C for 10 days. Illumination was provided with natural white (4000 K) LED lights with light intensity ca. 2800 lux or 50 μmol photons m^{-2} s^{-1} and photoperiod of 16 : 8 h (light : dark).

Light intensity tests

The effect of light intensity on microalgae *C. vulgaris*, *C. sorokiniana* and *C. reinhardtii* growth and biomass production was assessed under various irradiances from 50 μmol to 200 μmol photons m^{-2} s^{-1}. Specific light intensity was achieved by adjusting the number of LED lights and their distance from culturing flasks.

Light spectrum tests

Green microalgae *Chlorella vulgaris*, *Chlorella sorokiniana* and *Chlamydomonas reinhardtii* were used for light spectrum tests. To find the optimal light spectrum for the maximum growth of the selected microalgae, the red + blue LED spectrum was compared to full-spectrum white LED lights. A mix of red and blue lights was selected based on the literature review as a promising spectral combination to enhance microalgae growth. Red + blue 5 W linear LED lamps with a ratio of 3 : 1 (red : blue) were used. Moreover, the impact of light intensity on the preferred spectrum was also tested since light intensity is known to affect the optimal spectral composition. Three different light intensities were tested by adjusting the distance of culturing flasks to the light source. For simplicity, light intensity in results is converted to the level of intensity: Level 1 – 40 cm apart from the light source, Level 2 – 30 cm apart from the light source, and Level 3 – 20 cm apart from the light source.

Carbon dioxide tests

The impact of elevated CO_2 concentration was assessed on the growth of the potential candidate strains of green microalgae *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Chlamydomonas reinhardtii*. Three different settings were used: (1) cultivation without extra CO_2 supply, (2) 5 % CO_2 mix, and (3) 10 % CO_2 mix. Cultivation was carried out at 24 °C and 50 μmol photons m^{-2} s^{-1}.

Digestate as a growth medium

Liquid digestate after separation of solid fraction was obtained from the "Agro Iecava" Ltd biogas plant located in Iecava, Latvia. Due to the very high turbidity created by suspended solids and high OD, raw digestate was not suitable for microalgae cultivation. Various pretreatment methods were employed prior to use for microalgae cultivation for the removal of suspended particles to reduce turbidity and allow light penetration.

Several pretreatment methods were applied and tested to improve digestate properties:

(1) centrifugation at 10'000 rpm;

(2) vacuum-filtration through a 1.6 μm microfiber filter;

(3) filter centrifugation at 10'000 rpm;

(4) adsorption on activated carbon.

Characterization of digestate was done subsequently for each of the pretreatment methods, including the determination of suspended solids, total nitrogen, total phosphorus, ammonia nitrogen, nitrates, and chemical oxygen demand (COD) to assess the pretreatment efficiency. Analyses were purchased as an external service from the Vides audits laboratory.

The pretreated digestate was thereafter used for microalgae growth tests (can be found in the full version of the PhD Thesis).

Liquid digestate pretreatment with activated carbon

The tests described in this chapter were performed at the Department of Earth and Environmental Sciences of the University of Milano-Bicocca, Italy, during the Erasmus^{+} exchange visit. Although the applied pretreatment methods described in the previous chapter resulted in significantly improved properties of digestate in terms of total solids and turbidity, the main issue remained the dark color, which limited digestate application. To reduce the OD, pretreatment with adsorption on activated carbon was performed.

Activated carbon holds great potential as an efficient, low-cost method to reduce turbidity, optical density and harsh chemicals in digestate due to the high capacity of adsorbing various substances. Although activated carbon has been applied for municipal wastewater treatment, it is a novel pretreatment method for digestate, and its actual potential is still unknown. Several activated carbon concentrations and various adsorption durations were tested to find the most effective conditions. Activated carbon (Chemviron, UK) concentrations of 3 g, 10 g, 20 g, and 40 g per liter were tested. Liquid digestate was incubated with activated carbon on a rotary shaker at 200 rpm for 5 min, 10 min, 30 min and 180 min, and then centrifuged at 13'000 rpm to remove activated carbon particles. The OD was measured after the pretreatment and the reduction rate was calculated. The best-performing combination of activated carbon concentration and adsorption time was then selected for digestate pretreatment for microalgae growth tests based on the most efficient OD reduction to evaluate microalgae growth in pretreated digestate (not included in the PhD Thesis summary).

1.3. Growth tests in pilot raceway ponds

Microalgae cultivation was performed to test the novel cultivation technology in a real environment integrated into a working biogas plant. Liquid digestate was collected from the

Iecava biogas plant and used as a nutrient source for microalgae cultivation in the novel algae cultivation system. Digestate for cultivation was prepared by centrifugation using a filtration centrifuge (Hermle, Germany) at 10'000 rpm. Chemical analysis of digestate was performed before the inoculation to assess the level of nutrients and contaminants at the beginning of microalgae cultivation. Total nitrogen, total phosphorus, ammonia nitrogen, nitrates and chemical oxygen demand were determined in an external laboratory.

C. sorokiniana culture for inoculation of the algal pond was grown in a 5 L photobioreactor in the RTU Biosystems laboratory. The microalgal pond was filled with tap water at 20 cm depth, 2 L of pretreated digestate was thereafter applied and pre-cultivated *C. sorokiniana* biomass at the rate of ~ 1.5 % was added (Fig. 2 A and B). CO_2 introduction in the pond was implemented by bubbling of flue gas captured from the motor chimney of cogeneration unit.

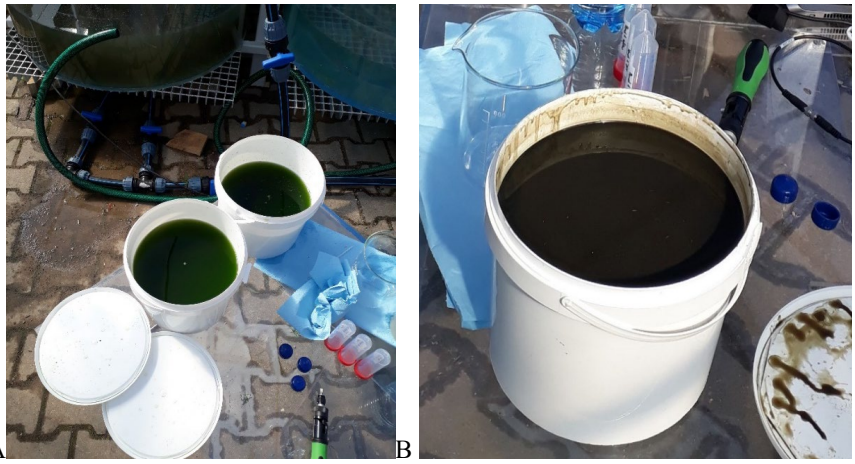


Fig. 2. Prepared inoculum (A) and pretreated liquid digestate (B) for the inoculation of the cultivation pond.

Probes with temperature, pH, and PAR sensors were installed and used to record the cultivation conditions. The temperature in the pond, in the greenhouse and outdoors was recorded. Light intensity in PAR at the water level was recorded. Samples for nutrient removal and biomass analysis were taken every 3 days. Samples were analyzed for nutrient content, suspended solids and optical density. Analysis of total nitrogen, total phosphorus, ammonia nitrogen, nitrates and chemical oxygen demand were performed in an external laboratory, other analyses were done in the RTU Biosystems laboratory. Cultivation was performed in batch cultivation mode; no nutrients were added during the cultivation period, and no biomass was removed. Therefore, it was possible to calculate the nutrient removal of digestate during the cultivation test. Microalgae cultivation was carried out from 21.04.2021–06.05.2021 and lasted for 16 continuous days.

2. RESULTS AND DISCUSSION

2.1. The concept and design of the novel cultivation system

Coupling the anaerobic digestion process with microalgae cultivation may contribute to nutrient bioremediation from liquid digestate as well as CO_2 capture from biogas. The main concept of the created system is shown in Fig. 3. Scenario 1 shows the traditional biogas production and digestate management route, whereas the system with biogas waste streams, namely, digestate and flue gases, integrated into microalgae cultivation, is shown in Scenario 2. The traditional practices involve storage of produced digestate and field application when possible. Flue gases created during the combustion of biogas are commonly released into the atmosphere, and electrical and thermal energy created is sent to the public network. When microalgae cultivation is integrated within a biogas production process, digestate is applied for microalgae cultivation as a source of nutrients, and flue gases as a source of CO_2. Furthermore, the electrical energy produced is used to maintain the operations of microalgae cultivation ponds, whereas the heat can be used for heating microalgal ponds during cold seasons.

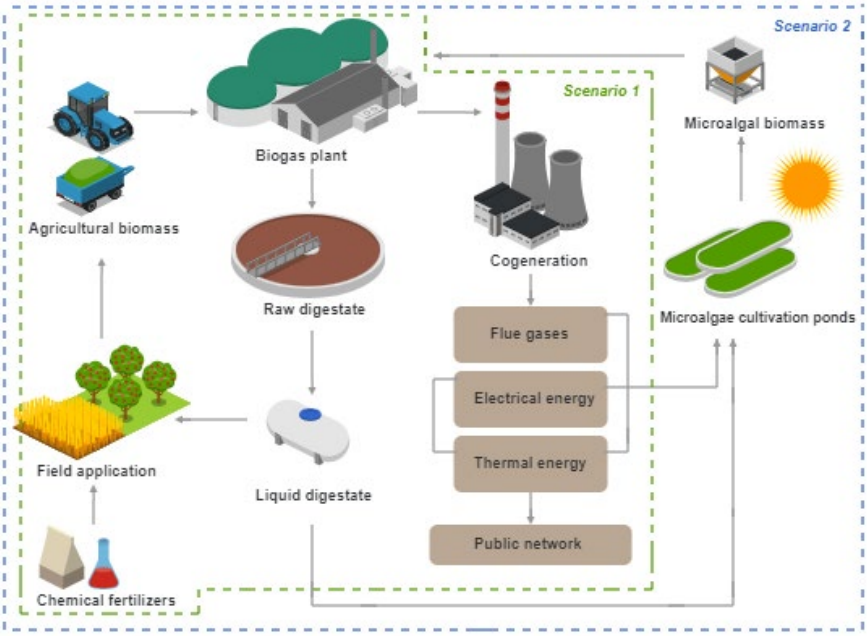


Fig. 3. Simple schematic representation of the concept of integrating microalgae cultivation into biogas plant. Scenario 1 shows a traditional biogas plant; the integration of microalgae cultivation is depicted in Scenario 2.

This cutting-edge technology has been integrated into a biogas plant, utilizing biogas byproducts, namely liquid digestate and flue gases as nutrient sources for growing microalgae. Microalgae uptake nutrients such as phosphorus and nitrogen from digestate for

growth while simultaneously removing other contaminants such as heavy metals, pharmaceuticals, and personal-care products. Consequently, alongside biomass production, wastewater treatment occurs concurrently, thereby reducing costs associated with both microalgae nutrients and wastewater treatment. Moreover, microalgae uptake carbon dioxide from flue gas coming from biogas combustion utilizing it as a carbon source for growth, therefore presenting an opportunity for carbon dioxide biosequestation. The produced microalgal biomass is directed to anaerobic digestion, creating a loop of nutrient use. This integrated approach not only lowers the costs associated with microalgae biomass production by utilizing waste streams as low-cost nutrients but also increases microalgae productivity through improved cultivation conditions. Furthermore, it provides an alternative route for digestate management and facilitates carbon dioxide sequestration. In the novel SMORP system, microalgae acts as a biofilter for the treatment of the liquid digestate and flue gases from the cogeneration unit in a biogas plant, offering an alternative management method of biogas production waste streams. This solution creates a transformation of the main environmental drawbacks from the anaerobic digestion related to the storage and disposal of the digested biomass and high CO_2 emissions in a valuable closed-loop technological system. The overall technological scheme of the SMORP pilot creates a closed loop which enables a biogas operator to produce energy from microalgae biomass creating benefits from the management of waste products and emissions (i.e. digestate and CO_2). At the same time, microalgae biomass production benefits from low-cost nutrients from biogas waste streams. The pilot concept offers a solution for the issue of digestate storage and transport offering an alternative digestate valorization route. This can significantly contribute to reducing the energy cost in the overall plant management and operational system.

The microalgae production unit (SMORP) was integrated into an existing biogas plant and microalgae-based system, and its harvesting can be considered as a side-stream processing module. The main challenge is the development of a mass microalgae cultivation system with high productivity and low energy requirements at the same time. The current research and studies in the field have shown major problems related to the regulation of optimal microalgae growing conditions as well as extensive land use for the open raceway ponds. A novel type of microalgae cultivation system, named Stacked Modular Open Raceway Ponds (SMORP), was created during doctoral studies. The principle of SMORP is to combine the advantages of existing systems creating a hybrid between open and closed cultivation systems. The novel technology is based on a traditional open raceway pond design, but features of closed photobioreactors are added such as artificial lighting, heating, and cooling. Potential limiting factors experienced in open ponds, such as light and temperature limitations were overcome with the novel design. The main concept of SMORP is the stacked design, allowing to save space, which is considered as one of the main limitations of existing designs. Moreover, with a supplemental artificial lighting system, modular design and use of transparent material, the proposed technology has significant advantages over the currently available ones.

Three microalgae cultivation ponds are arranged in a pyramid shape by placing the 3rd tank on top of the two bottom tanks overlapping half of each bottom tank. The use of transparent material and additional LED lighting help to mitigate the shadowing effect. The proposed

concept considers a combined sunlight and artificial lighting system with low power-consuming LEDs and a proper light wavelength to balance the light variation and shadow made by the upper ponds, in turn compensating with a higher biomass yield. Furthermore, technological advances, including the integration of flue gases and digestate as CO_2 and nutrient sources, significantly contribute to the environmental and technological feasibility of microalgae biomass production.

The search for the most appropriate material for ponds was one of the major aspects. Based on the desired characteristics, acrylic was selected for the SMORP pilot. Acrylic material has the capability to be easily shaped for rounded geometry. Using a transparent material, the effect of natural light can be maximized, increasing light penetration through the system, in contrast to conventional open pond designs. Acrylic is a transparent material which allows light to pass through (transparency of 92 %).

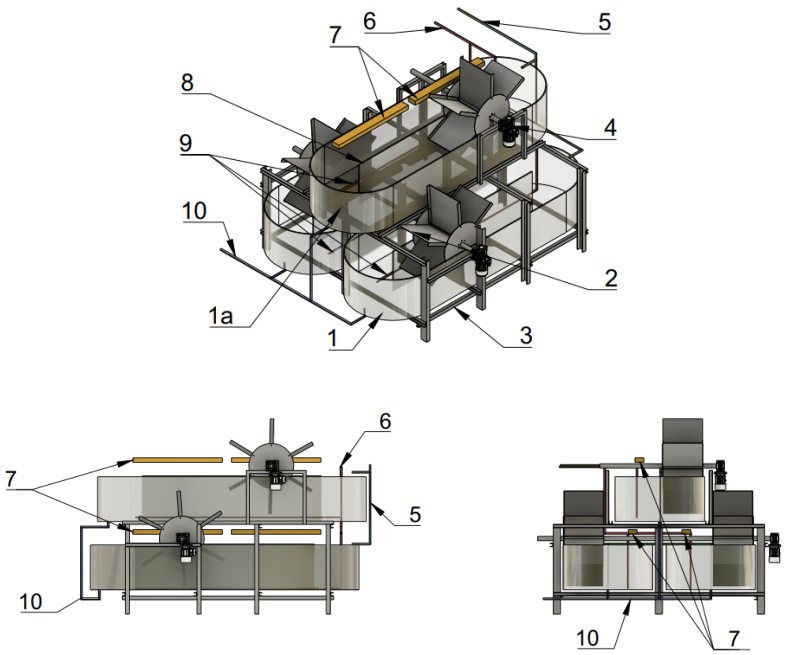


Fig. 4. Schematic representation of novel design open raceway cultivation ponds.

Each pond is one module, which can be arranged in an unlimited number of levels to form a modular microalgae cultivation pond system. Modular design allows ease of construction and flexibility for scaling up by adjusting the number of single modules per construction unit. The single modular pond is an oblong-shaped shallow pond having a length-to-width ratio equal to 3 (i.e. length = 3 m, width = 1 m), an area of 3.6 m^2, and a height of 50 cm. The sides are made from 15 mm acrylic sheets, the bottom is 20 mm thick, and the internal walls are made of 10 mm sheets. The parts are bent and fixed by glueing.

The design of the SMORP pilot is presented in Fig. 4. The cultivation of microalgae takes place in transparent, oval open ponds (1) arranged in a pyramid shape on top of each other. The ponds (1) are arranged on the support structure (3). The support structure is constructed in a way that minimises the shading of the ponds using metal grids to let the light through. The microalgae cultures are continuously mixed with a paddlewheel (2) that is driven by a geared motor (4). Microalgae cultures are fed nutrients automatically or manually using a nutrient supply (5). Flue gases containing carbon dioxide are introduced into the ponds via a carbon dioxide supply (6). LED cultivation lamps (7) are located above each pond. The flow of microalgae cultures is restricted by an acrylic separating wall (8) placed in the middle of each pond. The gas is evenly distributed in each pond using a perforated carbon dioxide diffuser (9) at the bottom of the pond. This also ensures good solubility of carbon dioxide in water. For biomass harvesting, there are openings and inlets (10) for biomass collection at the bottom of the ponds.

The SMORP technological scheme is shown in Fig. 5, and the main components are reported below.

− Liquid digestate as a nutrient source: The digestate can be fed to the pond by an automatically controlled peristaltic pump or manually. Feeding volume depends on the characteristics of digestate. Critical characteristics of digestate, such as pH, oxidation-reduction potential, turbidity, and temperature, are continuously monitored.

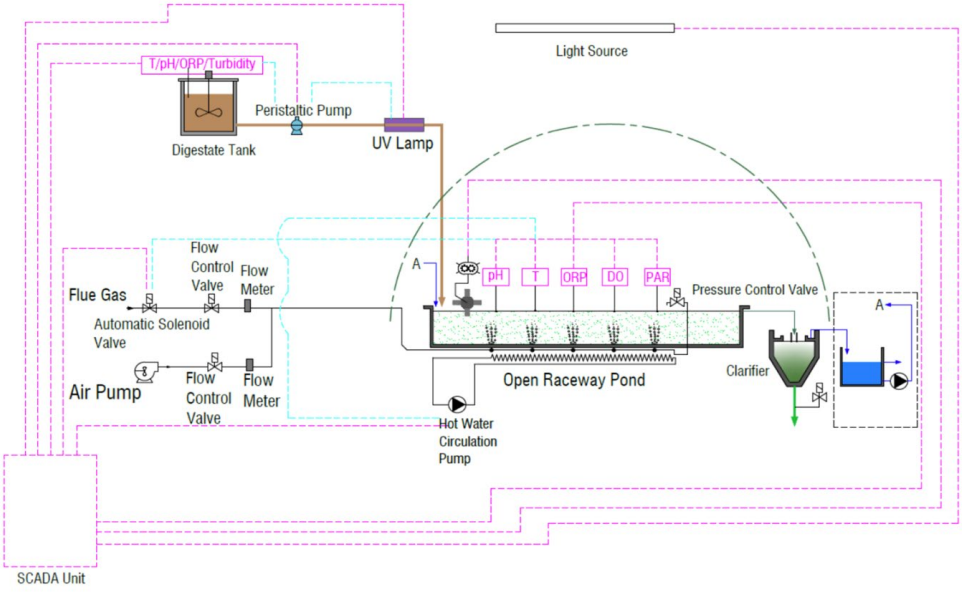


Fig. 5. SMORP technological scheme.

− Flue gas as a carbon source: Flue gas emitted from the biogas cogeneration unit is used as a carbon source for the growth of microalgae biomass. Gas is fed to the system through microporous tubular diffusers installed at the bottom of each pond. The flue-gas, when it exits the engine, has a temperature of 400 °C. The gas cooling is realized by the use of copper tubing

for gas transport and the use of low flow rates which ensure that gas has a lot of time to cool when travelling down the pipe.

− Mixing mechanism of microalgae culture: Adequate mixing is necessary to ensure a suspended state of the microalgae cells, gas exchange between the culture and air, and even light access to the microalgae cells. Mixing in ponds is provided by paddle wheels consisting of flat blades.

− Light Source: Energy efficient LED lights are installed into the pilot, allowing supplemental illumination to ensure year-round optimal light conditions. LED light panels emit blue light at 450 nm, red at 630 nm and 660 nm, and far-red at 735 nm. Due to the combined (sunlight and artificial) lighting system, it is possible to optimize the diurnal and annual lighting cycle. Although the incorporation of artificial lighting adds to the total capital expenditures and cultivation costs, it may be justified by increased biomass productivity. Moreover, supplemental LED lights are used only when necessary, providing optimal light conditions to maximize microalgae growth and maintain consistent biomass production. It can be used to mitigate the natural daily and seasonal fluctuations, e.g. low light intensity on overcast days, short daylight hours during the winter season, or highly dense microalgae cultures.

− Monitoring of key parameters: Sensors are installed in the pond to measure crucial parameters which affect the growth of microalgae, such as pH, PAR, temperature (outside air temperature, air temperature in the greenhouse, and pond water temperature), and dissolved oxygen. For the monitoring of physiochemical parameters and measurement data acquisition, an Aranet remote data logging system with wireless sensors for temperature, light, pH and DO was installed. Additionally, a web camera was installed to provide remote visual observation possibilities.

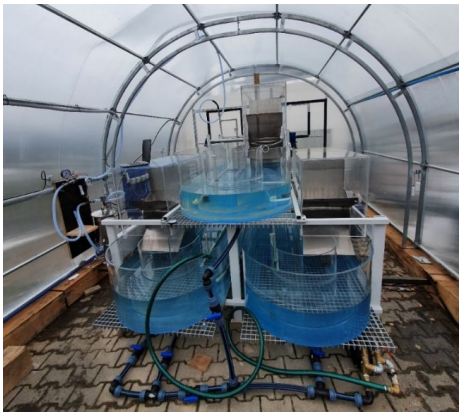


Fig. 6. Construction of SMORP pilot and integration into the Iecava biogas plant.

− Greenhouse: The ponds with the structure are placed inside a greenhouse made from transparent polycarbonate sheets. The greenhouse provides protection from unfavorable weather conditions (wind, rain) and helps to limit the potential biological contamination, such as bacteria, viruses and rotifers, often reported to lead to culture collapse [24]. However, the greatest benefit of the greenhouse in high-latitude regions remains the possibility to heat or cool

down the environment depending on the season. Additional costs in terms of energy can be justified by increased productivity.

− Heating is required during winter months and uses waste heat that comes from cooling cogeneration engines in the biogas plant. Heating is realized by using an air blower heat exchanger. Hot water is pumped by use of a circulating pump to the greenhouse. Then it goes through a heat exchanger equipped with an air blower.

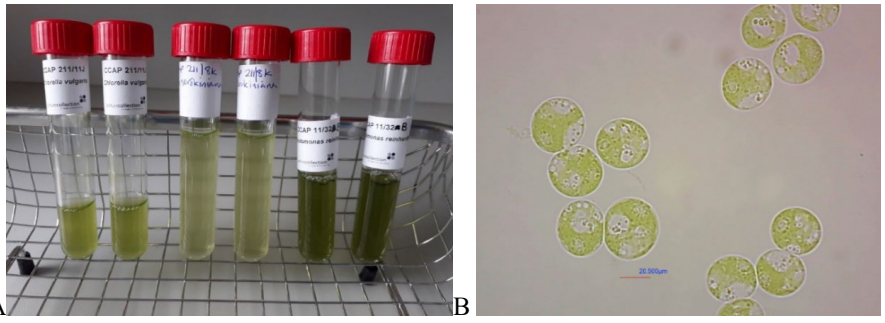
The construction of the SMORP pilot took place during the winter season of 2020/2021. Then the system was tested and adjusted. The final set-up of SMORP pilot ponds and the greenhouse integrated into the Iecava Biogas plant is shown in Fig. 6.

2.2. Microalgae strain selection

After an extensive literature review, three microalgae were selected as candidate strains for mass biomass production at Latvian climate conditions: *Chlorella vulgaris* 211-11j, *Chlorella sorokiniana* 211-8k, and *Chlamydomonas reinhardtii* 11-32b.

Chlorella species are found to be among the predominant strains occurring naturally in wastewater ponds [25], [26] and can survive in various wastewater streams, showing strong flexibility [25], [27]–[29]. Furthermore, it was reported that *Chlorella* is among the top eight pollutant-tolerant genera [26], demonstrating the superiority of this genera over other microalgae and indicating its potential for wastewater treatment. Moreover, *Chlorella* spp. have shown superior resistance to high ammonium concentrations compared to other species [31].

Due to the abovementioned superior qualities of *Chlorella* species, two species were selected from the genus *Chlorella* as potential microalgae for mass culturing using digestate as a nutrient source. Green microalga *C. vulgaris* was selected after an extensive literature review as one of the most promising species for large-scale outdoor cultivation due to its flexibility in cultivation conditions, capability to absorb high CO_2 concentrations and high specific-growth rate. *C. vulgaris* strain 211-11j was selected due to its northern origin in Sweden with a high potential for cultivation at high latitude regions. Very few scientific reports could be found on this strain of *C. vulgaris*, therefore it was necessary to evaluate the optimal cultivation conditions of this species, including both optimum growth temperature and minimum and maximum temperature resistance, to assess its potential for cultivation in Latvia.



A B

Fig. 7. Microalgae cultures from CCAP culture collection (A), *C. reinhardtii* in light microscope (B).

Chlorella sorokiniana has shown outstanding performance in wastewater treatment [32]. Moreover, it has demonstrated better adaptability to physiological stresses than some other green microalgae species [33]. Its usefulness can be particularly appreciated during high-temperature conditions that can be experienced during the summer time as *C. sorokiniana* was shown to be resistant to temperatures up to 42 °C [34]. *C. sorokiniana* was selected for this study due to its resistance to high cultivation temperatures and high irradiation commonly experienced during cultivation in summer.

Chlamydomonas reinhardtii is a photosynthetic biflagellate microalga that has been studied as a model for basic and applied physiology and biochemistry for more than 30 years and is one of the most studied microalgae [35]. Moreover, *C. reinhardtii* was the first green microalga to be sequenced [35], giving the opportunity to use it for genetic manipulations [36]. *Chlamydomonas* species have also been commonly found in wastewaters [30], indicating their suitability to resist harsh conditions and ability to utilize nutrients from wastewaters.

Microalgae strains *Chlorella vulgaris* 211-11j, *Chlorella sorokiniana* 211-8k, and *Chlamydomonas reinhardtii* 11-32b were obtained from reference culture collections in CCAP and SAG (Fig. 7 A and B). Various aspects of cultivation, including optimum and minimum temperatures, light requirements, CO_2 tolerance,, and ability to grow and remove nutrients from liquid agricultural digestate, were evaluated during laboratory tests.

2.3. Evaluation of a low-temperature strain

After an extensive literature review, *C. vulgaris* 211-11j was selected as a potential species for cultivation in low temperatures. The strain was grown in batch cultures at temperatures ranging from 8 °C to 32 °C to evaluate the optimal temperature range as well as both minimum and maximum temperature tolerance. The cultures exhibited good growth at all temperatures tested except at 32 °C (Fig. 8).

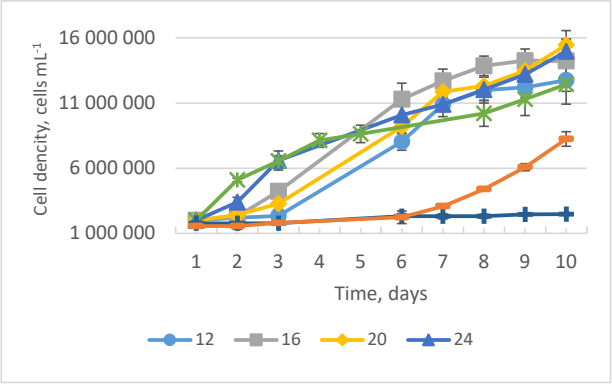


Fig. 8. *C. vulgaris* culture cell density and growth pattern at various temperatures. Error bars indicate standard deviation (n = 3).

Although the highest microalgal cell number was observed at 20 °C and 24 °C, the highest biomass accumulation (dry weight, g L^{-1}) was achieved when cultures were grown at 28 °C,

0.228 g L^{-1} (Fig. 9). Microalgae grown at 8 °C and 32 °C had comparable dry weight, 0.130 g L^{-1} and 0.136 g L^{-1}, respectively, whereas cell density was much higher for cultures under 8 °C, 8.24 x 10^6 cells mL^{-1}. Biomass yield at 24 °C and 20 °C was 92.5 % and 91.1% of the maximum productivity observed at 28 °C. However, the productivity of cultures cultivated at 12 °C and 16 °C reached 80.7 % and 85.4% of the maximum productivity, respectively. A significantly lower biomass yield was observed at 8 °C, reaching 57 % of the maximum productivity.

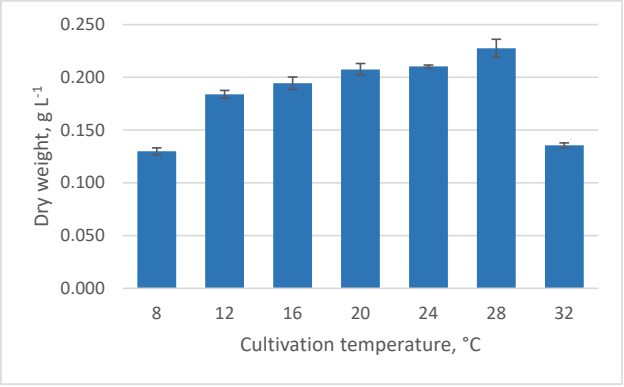


Fig. 9. Biomass yield of *C. vulgaris* at different cultivation temperatures at the end of the cultivation. Error bars indicate standard deviation (n = 3).

Although cell density was higher at 20 °C and 24 °C, higher biomass productivity was observed in cultures cultivated at 28 °C, which might be attributed to the smaller size of the cells at 20 °C and 24 °C. Indeed, the calculation of cell weight of dry biomass showed that cell weight was higher at 28 °C than at 20 °C or 24 °C. The highest cell weight was of microalgae cultivated at 32 °C, whereas the lowest was observed at 16 °C, 20 °C and 24 °C, indicating that the cells of *C. vulgaris* 211-11j were larger at high temperatures compared to average cultivation temperatures. An increase in cell weight was observed again in lower temperatures (12 °C and 8 °C).

The maximum biomass yield of *C. vulgaris* 211/11j was observed at 28 °C, therefore, this temperature is suggested as optimal for cultivation for this strain at the given experimental setup. Furthermore, temperatures from 20 °C to 28 °C can be considered the optimal range for the cultivation of this strain as no significant difference in productivity was observed.

While there are many studies assessing the optimum and maximum growth temperature for *C. vulgaris*, only a few studies considering low temperatures can be found. In the present study, the growth of *C. vulgaris* at low temperatures (16 °C, 12 °C and 8 °C) was studied. While the growth rate decreased by nearly 43 % at 8 °C compared to the maximum productivity at 28 °C, productivity was still near 85 % and 81% of the maximum at 16 °C and 12 °C, respectively, showing the good ability of this strain to grow in moderate temperatures and substantial resistance to low temperature. Although microalgae cultures cultivated at 8 °C did not result in high biomass yield at the end of the cultivation, cells were actively dividing resulting in increasing culture density after the long adaptation phase of 6 days. At the end of the batch cultivation, cultures at 8 °C were still increasing their density; therefore, a longer cultivation

time is needed to fully assess the potential of this strain at very low temperatures. Nevertheless, these results are very promising, showing that cultures cultivated at 8 °C can reach a good growth after the low-temperature acclimation.

The selected strain exhibits a lower optimum cultivation temperature than some other *C. vulgaris.* Moreover, tolerance to low temperatures makes *C. vulgaris* 211-11j a potential candidate for the production of biomass under cooler weather conditions. A wide optimum temperature range is suitable for highly variable outdoor conditions often experienced in higher latitude regions where fluctuations in diurnal temperatures, even during summer, may be high. These properties offer an advantage over other strains for outdoor cultivation in cooler climates, and therefore, this strain could be selected as a candidate strain for biomass production in Latvia. In a study by Gong and Bassi [37] it was demonstrated that the same strain of *C. vulgaris* could be successfully used for lutein production at low temperatures, suggesting the potential application of harvested biomass supporting the biorefinery concept.

2.4. Effect of light intensity on microalgae growth

Since supplemental LED lighting is integrated into the novel cultivation system, it is necessary to understand under what conditions it should be used. To find optimal illumination conditions of three candidate microalgae strains, namely *C. vulgaris* 211-11j, *C. sorokiniana* 211-8k, and *C. reinhardtii* 11-32b, growth rate and biomass production were evaluated at five different light intensities: 50 µmol m^{-2} s^{-1}, 100 µmol m^{-2} s^{-1}, 150 µmol m^{-2} s^{-1}, and 200 µmol m^{-2} s^{-1}.

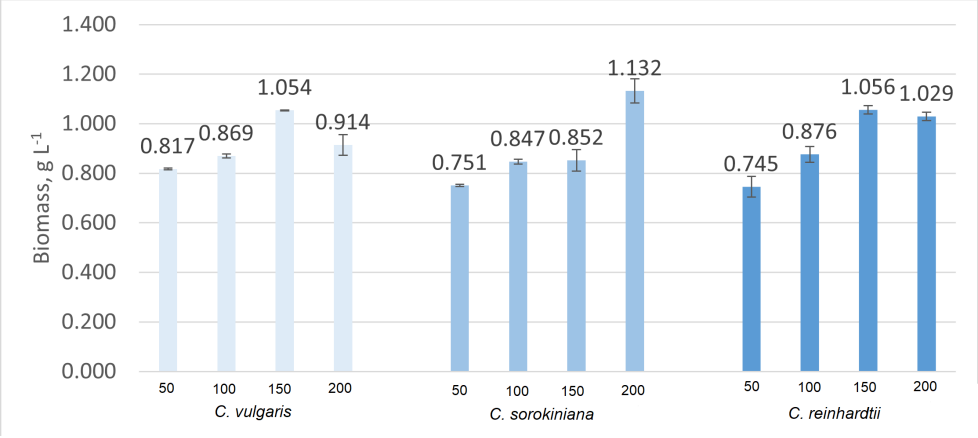


Fig. 10. Maximum biomass yield of *C. vulgaris*, *C. sorokiniana,* and *C. reinhardtii* under various light intensities 50–200 µmol m^{-2} s^{-1}. The number next to the species name indicates light intensity (µmol m^{-2} s^{-1}).

Biomass yield (g L^{-1}) was comparable among all the microalgae strains studied (Fig. 10). Biomass increased with the increasing light intensity up to 150 µmol m^{-2} s^{-1} for *C. vulgaris* and *C. reinhardtii,* and up to 200 µmol m^{-2} s^{-1} for *C. sorokiniana*. The highest biomass yield for *C. sorokiniana* was recorded at a light intensity of 200 µmol m^{-2} s^{-1} (1.13 g L^{-1}). Whereas

C. vulgaris and *C. reinhardtii* produced the most biomass when cultivated at 150 µmol m^{-2} s^{-1}, 1.05 g L^{-1} and 1.06 g L^{-1}, respectively. The lowest biomass yield was recorded at the light intensity of 50 µmol m^{-2} s^{-1} for all three microalgae strains studied, 0.75 g L^{-1} for *C. reinhardtii* and *C. sorokiniana* and 0.82 g L^{-1} for *C. vulgaris*.

Results of the biomass production suggest that the optimal light intensity for *C. vulgaris* and *C. reinhardtii* is around 150 µmol m^{-2} s^{-1}, while a higher light intensity of approximately 200 µmol m^{-2} s^{-1} is more suitable for *C. sorokiniana*. These results confirm other reports as *C. sorokiniana* is known to be a high light intensity tolerant alga [38]. Consequently, optimal light intensity requirements are higher than those of other microalgae. Contrary, *C. vulgaris* biomass decreased at 200 µmol m^{-2} s^{-1}, showing that this light intensity might be too high, and the photo-inhibition process might have been initiated during the cultivation at 200 µmol m^{-2} s^{-1}.

Optimal light intensity for *C. vulgaris* reported in the literature varies widely from 62.5 µmol m^{-2} s^{-1} [39] and 80 µmol m^{-2} s^{-1} [40] to 2000 µmol m^{-2} s^{-1} [41]. However, most often light intensity around 200 µmol m^{-2} s^{-1} is proposed [42], [43]. *C. vulgaris* strain used in the present study exhibits lower light intensity requirements that could be attributed to its Nordic origin and might be well adjusted to lower light intensity conditions as experienced at high latitudes.

The current study was carried out to investigate the effect of light intensity on the growth rate and biomass production of three microalgae strains intended for cultivation in outdoor raceway ponds supplemented with artificial LED illumination. The results show that light intensity plays an important role in determining the productivity of microalgae. All species tested exhibited similar growth rates and biomass productivity under selected light intensities and specific cultivation conditions. It was shown that the light intensity of 50 µmol m^{-2} s^{-1} is too low to maintain the maximum growth rate for microalgae strains studied. Nevertheless, *C. vulgaris* was superior to other strains at low light conditions (50 µmol m^{-2} s^{-1}), exhibiting a potential for cultivation at limited light settings which may be particularly useful in Nordic countries. On the other hand, the results suggest that *C. sorokiniana* has higher light requirements when compared to *C. vulgaris* and *C. reinhardtii,* which offers advantages in high light conditions, e.g. at mid-summer in high latitude regions. The highest biomass yield was produced at a light intensity of 150 µmol m^{-2} s^{-1} for *C. vulgaris* and *C. reinhardtii* and at 200 µmol m^{-2} s^{-1} for *C. sorokiniana*. In case natural illumination does not reach the optimum intensity required for maximum productivity, the supplemental LED illumination might be switched on.

2.5. Effect of light spectrum on microalgae growth

Red and blue spectral ranges of visible light have been frequently reported to enhance biomass production of green microalgae compared to white light. Therefore, red + blue wavelengths were used for microalgae cultivation and compared to full-spectrum white light for the selected candidate strains. Daily optical density measurements were performed to inspect the growth of the cultures at various light intensities under blue + red and full-spectrum LED light. All three green microalgae species, *C. vulgaris*, *C. sorokiniana*, and *C. reinhardtii*

exhibited similar growth trends at the same growth conditions. No significant differences in culture density between blue + red LED illumination and full-spectrum light were observed in any of the species. Regarding biomass production, no significant differences were found between cultivation at blue + red spectrum or fullspectrum LED lights; however, light intensity had a great impact on total biomass yield (Fig. 11). Maximum biomass yield was observed at the highest light intensity for microalgae species tested.

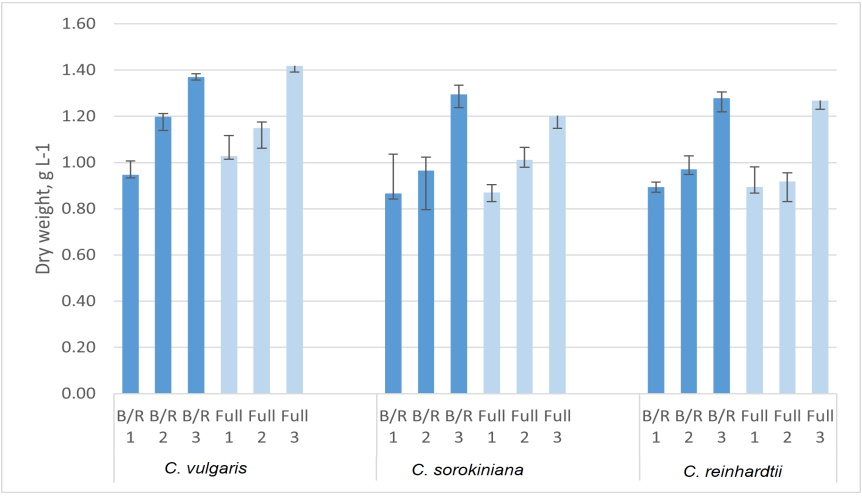


Fig. 11. Maximum biomass production of *C. vulgaris*, *C. sorokiniana*, and *C. reinhardtii* at blue/red (B/R) and full spectrum (Full) at various light intensities.

The current study revealed that the growth rate and biomass production of *C. vulgaris*, *C. sorokiniana*, and *C. reinhardtii* were not influenced significantly by part of the solar spectrum applied but more by the light intensity.

2.6. Effect of carbon dioxide on microalgae growth

Increased CO_2 levels compared to CO_2 content in the atmosphere have been reported to increase the growth rate and productivity of microalgae. In order to test the maximum CO_2 tolerance of selected microalgae strains and the potential of these microalgae for CO_2 sequestration from flue gases, cultivation tests with different CO_2 concentrations were performed in the laboratory. Growth curves of *C. vulgaris*, *C. sorokiniana*, and *C. reinhardtii* at 5 % and 10 % CO_2 are shown in Fig. 12 A and B, respectively. All microalgae species exhibited slower growth at the beginning of cultivation at 10 % CO_2 compared to 5 % CO_2 mix. The observed longer lag phase is most likely due to the need for acclimatization to the new growth conditions with a higher CO_2 concentration.

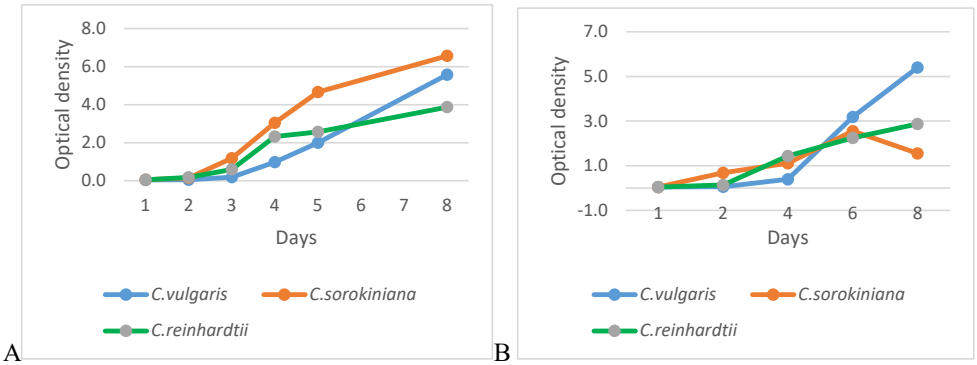


Fig. 12. Growth of *C. vulgaris*, *C. sorokiniana*, and *C. reinhardtii* in 5 % (A) and 10 % (B) CO_2 mix with air measured as optical density at 750 nm.

While all cultures showed similar growth cures at 5 % CO_2, limited growth of *C. sorokiniana* and *C. reinhardtii* was observed at 10 % CO_2 supply. A significant decrease in the culture density of *C. sorokiniana* was observed after day 6.

Biomass productivity was measured at the end of cultivation as a cell dry weight. The maximum biomass yield of *C. vulgaris*, *C. sorokiniana*, and *C. reinhardtii* at a CO_2 supply of 5 % reached 2.0 g L^{-1}, 3.1 g L^{-1} and 3.2 g L^{-1}, respectively (Fig. 13). The most productive species was *C. reinhardtii* with a comparable biomass yield to *C. sorokiniana*; however, *C. vulgaris* showed the lowest biomass productivity among all three species tested.

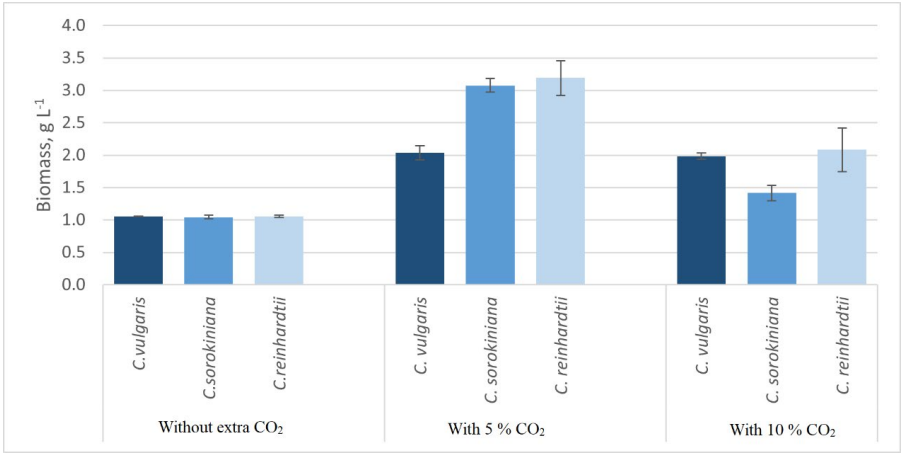


Fig. 13. Comparison of microalgae biomass yield at different CO_2 sparging rates.

CO_2 greatly enhanced biomass productivity compared to cultivation without extra CO_2 bubbling. *C. vulgaris* doubled biomass at 5 % CO_2 supply compared to cultivation with ambient CO_2 level. Moreover, *C. sorokiniana* and *C. reinhardtii* tripled productivity at 5 % CO_2 supply, reaching 3.1 g L^{-1} and 3.2 g L^{-1}, respectively. When the CO_2 level in the CO_2/air mix was increased to 10 %, the biomass yield of *C. sorokiniana* and *C. reinhardtii* decreased

substantially; however, an increase in CO_2 rate did not significantly change the productivity of *C. vulgaris* (Fig. 13). These results indicate that *C. vulgaris* might have higher resistance to high CO_2 concentrations than the other two species tested. At the end of cultivation tests, most cultures were at the exponential growth stage, indicating that the selected cultivation time was too short to reach the stationary phase; therefore, the cultivation time must be extended to assess the real potential of the species and reach the maximum biomass productivity.

The results from cultivation tests with different CO_2 rates show that, indeed, an increase in CO_2 rate to 5 % resulted in significantly higher biomass yield of all three microalgae tested – *C. vulgaris*, *C. sorokiniana*, and *C. reinhardtii*. Furthermore, too high levels of CO_2 can inhibit certain microalgae growth. Air mix with 10 % CO_2 content decreased *C. sorokiniana* and *C. reinhardtii* growth rate and biomass productivity, whereas the productivity of *C. vulgaris* was not affected, indicating the potential of this microalgal strain to tolerate higher CO_2 levels and might be especially useful to uptake CO_2 from flue gases which usually have high CO_2 content.

2.7. Digestate as a growth medium

Pretreatment with centrifugation and filtration

Digestate was obtained from the Agro Iecava biogas plant to determine its suitability as a low-cost nutrient source for microalgae growth. The raw liquid fraction of agricultural digestate was not suitable for microalgae cultivation due to the very high load of total solids and optical density, which visually appeared as black opaque liquid. Dilution of liquid digestate is commonly applied to increase the suitability of digestate for microalgae cultivation; however, other pretreatment methods were tested in the current study to increase the overall feasibility. Centrifugation and filtration were applied as initial pretreatment methods to improve the properties of digestate. The amount of suspended solids in a raw liquid digestate was 9 g L^{-1}. Various pretreatment methods greatly improved digestate's suitability for microalgae cultivation. The amount of suspended solids was greatly reduced in pretreated digestate compared to raw digestate (Table 1). The amount of suspended solids, COD, total nitrogen, total phosphorus, nitrate nitrogen, ammonia nitrogen and turbidity varied based on the pretreatment method applied. Filtration as pretreatment was more effective in the reduction of all parameters tested compared to centrifugation. Filter centrifugation decreased nitrogen and COD more effectively compared to centrifugation and filtration. However, phosphorus and ammonia nitrogen content were higher than with the other two pretreatment methods. Filtration through a 1.6 µm microfiber filter further decreased the solids; however, was considered not a viable option for large-scale digestate pretreatment.

Pretreated digestate was subsequently tested as a growth medium for microalgae cultivation. Although the amount of solids was greatly reduced by the pretreatment, high optical density was still an obstacle, therefore, dilution was used to reduce the optical density. Microalgae *C. vulgaris* was cultivated in (1) centrifuged and (2) filtered digestate diluted to 1 %, 3 % and 5 % with distilled water (Fig. 14).

Table 1

Chemical Composition of Digestate after Various Pretreatment Methods (NA – not available)

Parameter	Unit	Raw liquid digestate	Centrifugation	Filtration	Filter centrifugation
Suspended solids	mg L^{-1}	9080	2450	1700	NA
COD	mg L^{-1}	NA	23210	9580	3630
Total N	mg L^{-1}	NA	11770	6780	6180
Total P	mg L^{-1}	NA	319	157	602
Nitrate N	mg L^{-1}	NA	< 0.07	< 0.07	< 0.3
Ammonia N	mg L^{-1}	NA	3080	2460	3360
Turbidity	mg L^{-1}	NA	NA	7840	NA
Optical density	NA	10.68	NA	NA	NA

The highest biomass productivity was observed with 3 % digestate after the filtration pretreatment (Fig. 15). However, due to the relatively high standard deviation of replicates, this result must be perceived with caution.

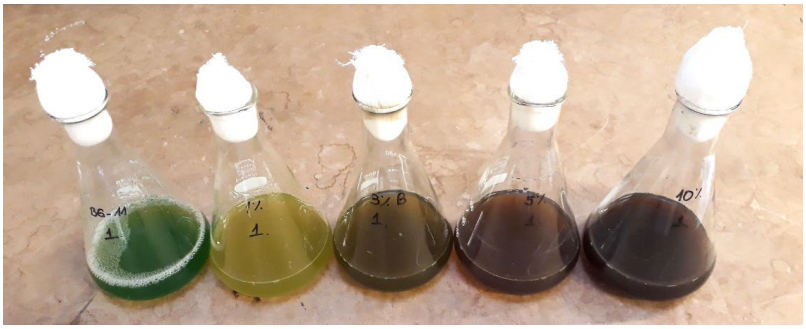


Fig. 14. *C. vulgaris* cultivation in various dilutions of digestate as a growth medium.

The use of liquid digestate as a nutrient source for microalgae cultivation is not straightforward. Some very promising results were achieved at some tests; however, digestate is “alive”, containing a variety of microorganisms which interact with microalgae creating a very complex system. Moreover, both digestate content of nutrients and contaminants and microorganisms are changing based on the feedstock of anaerobic digestion, temperature, inoculum and other anaerobic digestion parameters. The results demonstrate that centrifugation followed by the filtration of digestate was the best method for the digestate pretreatment prior

to microalgae cultivation as it reduced the presence of total solids in the digestate and the highest growth rate of *C. vulgaris* was observed in filtered digestate. However, this solution is not viable when considering large-scale microalgae cultivation. Therefore, filter centrifugation was tested, allowing fast, large-volume digestate filtration and resulting in improved digestate properties.

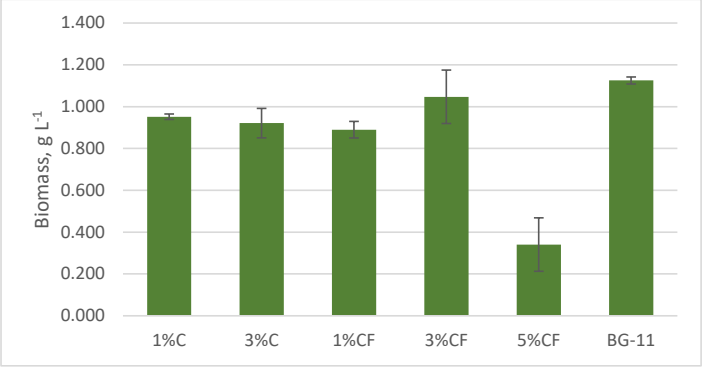


Fig. 15. Biomass yield of *C. vulgaris* cultivated in 1 %, 3 % and 5 % pretreated digestate. The number in the sample name indicates the dilution rate. C – centrifuged, CF – filtration. Error bars represent standard deviation (n = 3).

Although microalgae could grow in pretreated diluted digestate, biomass yield was significantly lower than that of control media. This might suggest that some limiting factors are present in digestate. The high optical density of digestate may imply reduced light availability caused by light-limited conditions. Therefore, a pretreatment method to reduce optical density was tested.

Pretreatment with activated carbon

A new batch of digestate was obtained from the Agro Iecava biogas plant for activated carbon pre-treatment. Chemical characterization of digestate prior to treatment was performed and is reported in Table 2. The total solids content of raw liquid digestate reached 23 g L^{-1} resulting in extremely high turbidity (7840 mg L^{-1}), which suggests that there may be an inhibitory effect on photosynthetic potential and low light availability to microalgae cells. Furthermore, exceptionally high optical density (OD 13) was recorded, resulting in nearly black opaque liquid (Fig. 16 A). Organic material and humic substances present in digestate are most likely responsible for the characteristic dark color.

Nitrogen and phosphorus are primary nutrients required for microalgae growth and usually are abundant in liquid digestate [45]. In particular, agricultural digestate is rich in nitrogen when compared to other wastewater streams [46]. Indeed, 5950 mg L^{-1} and 490 mg L^{-1} total nitrogen and phosphorus, respectively, were detected in the current study. Chemical analysis showed that most of the nitrogen in digestate was in the form of ammonium (NH_4-N), as pointed out in other studies [27], [47]. Although ammonium is a preferred source of nitrogen for most microalgae [48], high total ammonia nitrogen may inhibit microalgae growth [31], [45]. No other reports were found stating such a high value of ammonia nitrogen concentration as in this

study (3600 mg L^{-1}). Phosphorus content (490 mg L^{-1} PO_4-P) was comparable to or higher than that referred to in other studies [48], [49]. The reported values of COD content in anaerobic digestion effluents are commonly higher than found in other wastewater streams [46]; however, exceptionally high COD (6840 mg L^{-1}) was found in the current study indicating excessive load of organic matter.

Table 2

Characterization of the Liquid Fraction of Raw Agricultural Digestate and after Pretreatment with 3 g L^{-1} and 40 g L^{-1} Activated Carbon with Adsorption Time 10 min (TS – total solids, SS – suspended solids, VS – volatile solids, DS – dissolved solids, TN – total nitrogen)

Parameter	Unit	Raw liquid digestate	Pretreated	
			3 g L^{-1}	40 g L^{-1}
TS	g L^{-1}	22.9	NA	NA
SS	g L^{-1}	5.1	NA	NA
VS	g L^{-1}	4.25	NA	NA
DS	-	17.83	NA	NA
OD	-	13.03	3.06	2.81
pH	-	8.17	NA	NA
Turbidity	mg L^{-1}	7840	NA	NA
COD	mg L^{-1}	6840	6540	4960
TN	mg L^{-1}	5950	NA	NA
NH_4-N	mg L^{-1}	3600	3000	2667
NO_3-N	mg L^{-1}	47.5	NA	NA
PO_4-P	mg L^{-1}	490	338	278.4

Uggetti et al. reported COD 210 mg L^{-1} in anaerobic digestate [48], 1980 mg L^{-1} was reported in digestate from livestock waste [50], 2661 mg L^{-1} in anaerobic digested municipal wastewater [51], and 3402 mg L^{-1} in anaerobic digested piggery wastewater [52]. Digestate was slightly alkaline, as commonly reported [45], with a pH of 8.17 being at the optimal range for most freshwater microalgae species [45].

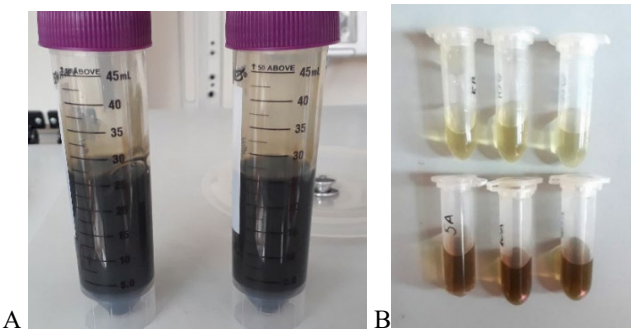


Fig. 16. The appearance of a raw (undiluted, untreated) liquid fraction of digestate (A) and after the pretreatment with activated carbon at two different concentrations (B).

Typical effluent from anaerobic digestion is known to have high nutrient concentrations [47]; however, generally, all parameters measured in this study were higher than those reported in the literature [45], indicating a very dense and highly concentrated digestate. The nutrient content of raw digestate was significantly higher than recommended for microalgae cultivation. Furthermore, dark color and high turbidity make algae cultivation in raw liquid digestate impossible.

Effect of activated carbon adsorption on OD rate of digestate

A novel method for OD reduction of digestate was tested to increase the light penetration to microalgae cultures and to decrease the amount of water required for digestate dilution. The initial OD of raw liquid digestate was 13, indicating that light penetration in a raw liquid digestate is not sufficient for microalgae growth. Activated carbon pretreatment was applied to raw liquid digestate in order to reduce the optical density. Activated carbon concentrations from 3 g L^{-1} to 40 g L^{-1} were applied at various adsorption durations ranging from 5 min to 180 min (Fig. 17). The highest OD reduction rate of 78 % was achieved after 10 minutes of adsorption at 40 g L^{-1} and of 77 % at 40 g L^{-1} with 5 minutes, 3 g L^{-1} with 10 minutes, and 40 g L^{-1} with 30 minutes of adsorption time.

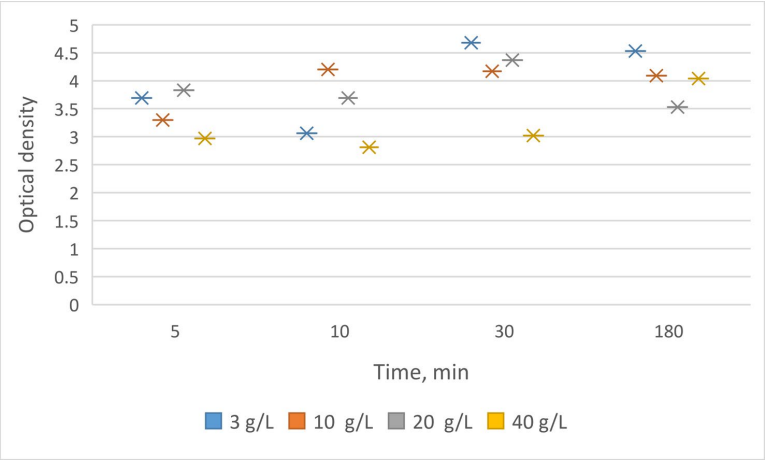


Fig. 17. Optical density of pretreated digestate based on the activated carbon concentration and adsorption time.

Along with the reduction of OD, the concentration of some nutrients and COD was decreased as well (Table 2). A high reduction rate of OD with activated carbon adsorption was achieved in the current study, which could be observed also visually (Fig. 16 B) demonstrating its high potential for improving the properties of digestate for microalgae cultivation.

2.8. Microalgae growth test in pilot raceway ponds

The novel SMORP cultivation system with the greenhouse was constructed and integrated into the Agro Iecava biogas plant using side-products from the biogas plant, namely liquid digestate and flue gases as nutrient and CO_2 sources. Microalgae cultivation was performed to evaluate the technology potential on a pilot scale. The cultivation test was conducted at the end of April 2021 and lasted for 16 consecutive days. Microalgal strain *Chlorella sorokinana* was selected for initial tests in pilot raceways ponds due to its resistance to high light intensity and based on laboratory scale tests, showing its flexibility as the experiments were taking place in springtime when natural light intensity is close to its maximum but temperature is highly variable with a wide range of fluctuations.

Monitoring the cultivation conditions

Outdoor microalgae cultivation is heavily dependent on weather, which, in turn, varies according to location and season. Spring conditions are usually dynamic, with fluctuating temperatures not being ideal for microalgae cultivation. Indeed, the microalgae cultivation test in the SMORP pilot was challenging due to unstable and variable weather conditions that are characteristic of the spring season in Latvian climate conditions. However, it was possible to evaluate the performance of selected microalga in suboptimal conditions. After the inoculation of the raceway pond, probes with temperature, pH, and PAR sensors were used to record the cultivation conditions.

Daytime temperatures can vary greatly from day to day, and the difference between day and night temperatures can be very high. Indeed, the recorded fluctuations in temperature during microalgae cultivation were high. Temperature was monitored continuously inside the cultivation pond, furthermore, air temperature in the greenhouse and outside was also recorded. The water temperature in the microalgae cultivation pond during the biomass cultivation is shown in Fig. 18. The average daytime temperature during the cultivation ranged from around +15 °C to +22 °C. The highest water temperature recorded was around +22 °C during the day, whereas the lowest daytime temperature was recorded on May 3rd when the pond temperature reached only +12 °C. During the nighttime, the water temperature dropped considerably, which was expected due to the low air temperature outside and was generally between +10 °C and +16 °C.

The pond temperature was directly influenced by the outdoor temperature. Fluctuations in the pond water temperature depending on the temperature outdoors and the temperature in the greenhouse are shown in Fig. 18. The lowest air temperature outside, recorded during the growth test, was around +2 °C during the night. Outdoors the nighttime temperature stayed just a couple of degrees above zero for most of the cultivation period. In the last decade of April, the temperature was 3.2 °C lower than average normally (1981–2010) and 4.1 °C lower than normal in 1991–2020 [53], which affected the microalgae growth considerably. During the coldest nights, the pond temperature did not drop lower than +10 °C, showing the contribution of the greenhouse to keep the temperature at a tolerable level for microalgae during cooler

environmental conditions. The greenhouse could ensure around 10 °C higher temperature than the temperature outside.

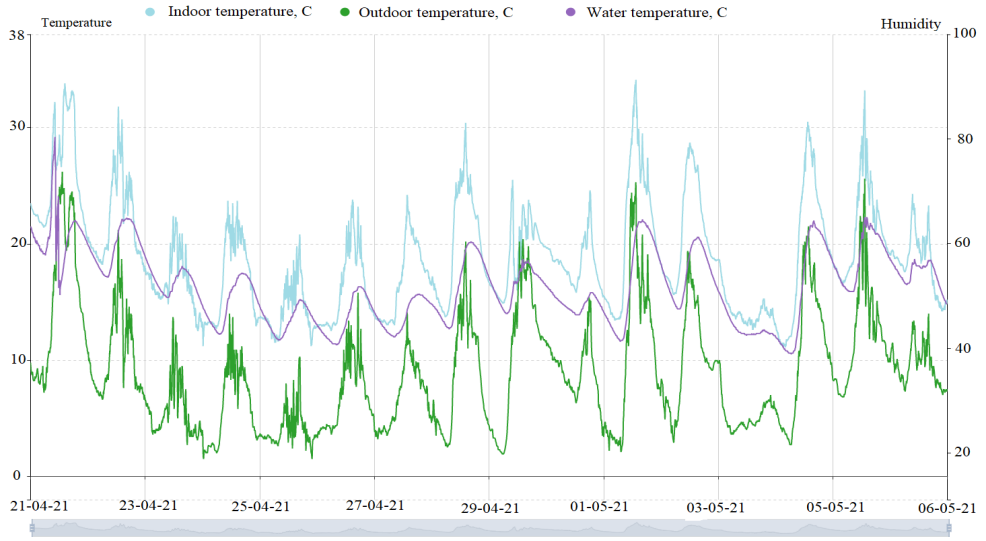


Fig. 18. Outdoor temperature, indoor temperature at the greenhouse, and the pond water temperature during the biomass cultivation test. Green line – outdoor temperature; purple line – temperature inside the pond; blue line – temperature inside the greenhouse.

The contribution of heat of the flue gas was negligible in the present flow rate. The flue gas temperature varied due to the changes in outdoor temperature because the transfer pipes of the flue gas are located outside of the greenhouse with the purpose of cooling down flue gases coming from the biogas motor room. After mixing with air, the temperature of flue gases reaching the pond was a maximum of 45 °C.

Microalgae growth and nutrient removal

A liquid fraction of agricultural digestate collected from the Agro Iecava biogas plant was pretreated by filter centrifugation to remove excess solids prior to application to the microalgae pond. Pretreated digestate was analysed for the content of solids, COD and nutrients. The results of the chemical analysis of digestate are shown in Table 3.7. The total nitrogen content of digestate was high – exceeding 6000 mg L^{-1}. More than half of the total nitrogen was in the form of ammonia nitrogen (3360 mg L^{-1}). The content of nitrates was negligible (< 0.3 mg L^{-1}). COD 36300 mg L^{-1} was observed, indicating a very high load of organic content. Digestate was diluted with tap water in order to decrease the nutrient load and lower the optical density and turbidity. Tap water was also analysed before the inoculation of ponds showing very low levels of nutrients and contaminants (Table 3). The nutrient content in diluted digestate, as used for microalgae cultivation, is shown in the last column (Growth medium) of Table 3.

Table 3

Chemical Analysis of Pretreated Digestate and Water Used for Dilution

Parameter	Units	Tap water	Digestate	Growth medium
Total nitrogen	mg L^{-1}	0.235	6180	12.7
Total phosphorus	mg L^{-1}	0.011	602	1.21
Ammonia nitrogen, $N\text{-}NH_4$	mg L^{-1}	< 0.3	3360	8.4
Nitrates, $N\text{-}NO_3$	mg L^{-1}	< 6	< 0.3	< 0.3
Chemical oxygen demand, COD	mg L^{-1}	0.114	36300	56

Samples from the pond were taken every 3 days to monitor microalgae growth and the removal of nutrients from the growth medium. The cultivation pond was also inspected visually, as shown in Fig. 19.

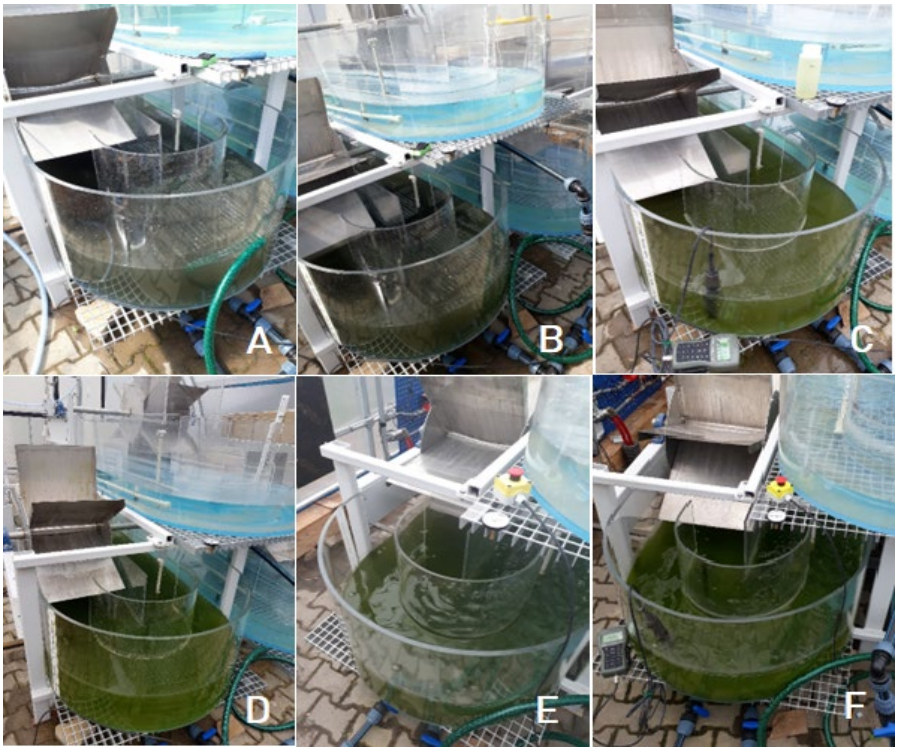


Fig. 19. The cultivation pond: A – with digestate, Day 1 (April 21); B – with digestate + microalgae, Day 1; C – Day 3 (April 23); D – Day 7 (April 27); E – Day 10 (April 30); F – Day 16 (May 6).

After inoculation of the pond with *Chlorella sorokiniana,* the culture exhibited slower growth at the beginning but showed exponential growth from Day 3 to Day 7 (Fig. 20). Culture density started to decrease after Day 7, indicating that some limiting factors were present.

Thereafter culture density continued to decrease till the end of the 16-day cultivation. Several factors might have impacted the culture growth during the cultivation experiment. Some of the environmental conditions were not optimal during the cultivation period. For most of the cultivation, the pond temperature was well below the optimum temperature of the species. The cultivation test was conducted during the springtime when the outside temperature fluctuates greatly. The sun in the springtime can be quite strong, heating the greenhouse during the day, but temperatures can decrease close to zero at night. Microalgae were able to grow in highly changing environmental conditions with fluctuating temperatures. The pond temperature decreased to only +12 °C on Day 7 and can be considered as one of the possible explanations for a decreasing growth on the following days. Exceptionally low productivity of *C. sorokiniana* has been reported in suboptimal temperatures [54]. It is also very likely that suboptimal temperature decreased the light energy requirements, and therefore, the maximum spring irradiance was excessive, leading to photoinhibition.

The optical density of *C. sorokiniana* culture during test cultivation is shown in Fig. 20. The highest density was reached on Day 7, and then a sharp decrease was observed. The same can be seen with biomass yield, which was halved on Day 10 compared to Day 7, and continued to decrease thereafter (Fig. 21).

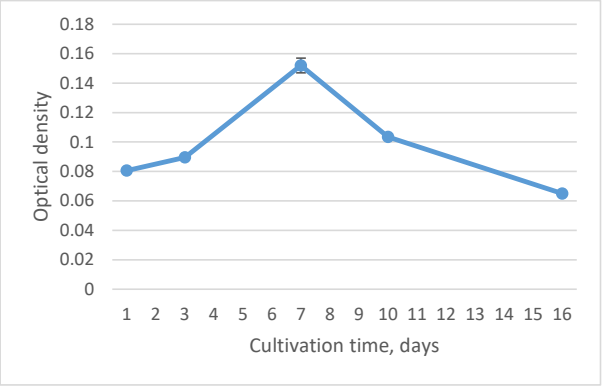


Fig. 20. *C. sorokiniana* culture density during cultivation test in SMORP pilot ponds. The error bars indicate the standard deviation (n = 2).

Although the growth rate of microalgae during the cultivation test was not among the highest reported, it must be considered that cultivation conditions were not optimal for *C. sorokiniana* during the initial trial due to unexpectedly low temperatures. The decrease in growth rate observed after Day 7 might be due to several reasons, including limited nutrients and light availability, relatively high pH, or some other factors. The addition of a higher flow rate of flue gases could contribute to lowering the pH. Additionally, switching on the heating system might be useful when temperatures drop below the optimum, but it was not used in this trial.

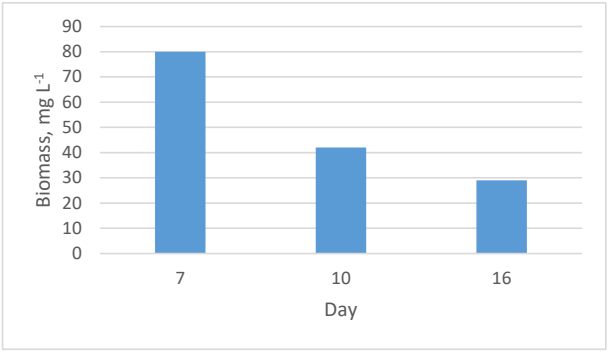


Fig. 21. Biomass production during the cultivation test.

Although a relatively low growth rate was reached during the cultivation test, the nutrient removal rate seems very promising. During the first three days, removal of total nitrogen, total phosphorus and ammonia was negligible most probably due to the adaptation of microalgae to the new growing conditions (Fig. 22.). Nutrient removal increased considerably after the initial lag phase. Ammonia concentration increased slightly again at the last stage of cultivation.

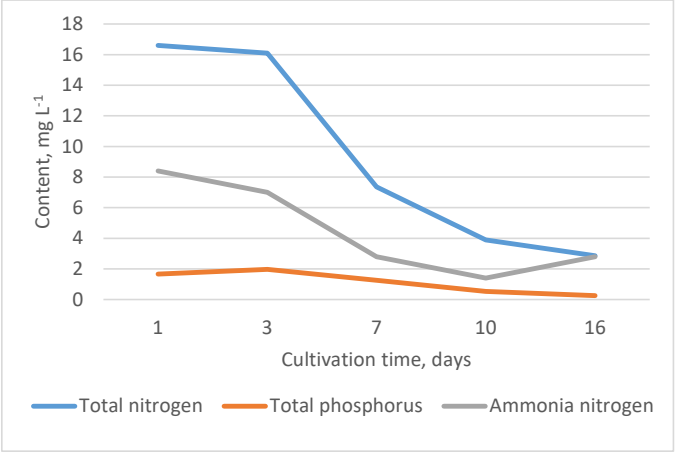


Fig. 22. Removal of total nitrogen, total phosphorus, and ammonia nitrogen by *C. sorokiniana* during 16-day cultivation.

The relationship between COD in wastewater and microalgae growth is complex and influenced by various factors, including the COD concentration and microalgae species [55]. Generally, it is known that microalgae can remove COD from wastewater during growth. However, since microalgae are releasing organic compounds during cultivation, the actual COD in cultivation media might be rising. COD during the cultivation of *C. sorokiniana* was increasing (Fig. 23).

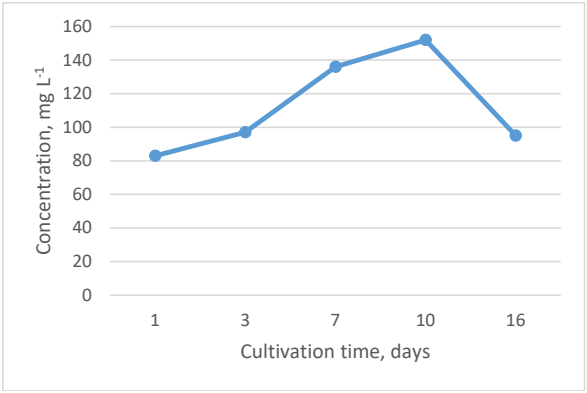


Fig. 23. Chemical oxygen demand during the cultivation of *C. sorokiniana*.

High nutrient removal efficiency was reached at the end of the cultivation (Table 4). In total, 83 % of nitrogen, 85 % of phosphorus, and 83 % of ammonia nitrogen were removed from the growth medium during the cultivation of *C. sorokiniana*. The total nitrogen concentration of 2.86 mg L^{-1} was achieved, corresponding to national legislation regarding requirements for the treatment of wastewaters [56]. In agglomerations with less than 100'000 inhabitants, 15 mg L^{-1} of total nitrogen is the allowance for wastewaters, whereas the allowance of 10 mg L^{-1} of total nitrogen in agglomerations exceeds 100'000 inhabitants. Regarding phosphorus, 2 mg L^{-1} is allowed in agglomerations with less than 100'000 inhabitants, and 1 mg L^{-1} in agglomerations exceeding 100'000 inhabitants. 0.25 mg L^{-1} phosphorus was left in the growth medium after digestate treatment with microalgae. It can be seen that digestate treatment with microalgae could meet the regulations at the present setup.

Table 4

Nutrient Removal from Growth Medium During *C. sorokiniana* Cultivation

Parameter	Initial level in the pond, mg L^{-1}	Removal, mg L^{-1}	Removal rate, %
Total nitrogen	16.6	13.74	82.8
Total phosphorus	1.67	1.416	84.8
Ammonia nitrogen, $N\text{-}NH_4$	8.4	7	83.3
Nitrates, $N\text{-}NO_3$	< 0.3	NA	NA
Chemical oxygen demand, COD	83	–12	–14.5

The developed technology seems promising regarding digestate treatment in the Latvian climate even in suboptimal cultivation conditions. However, it must be taken into account that a high dilution rate of digestate was used for the application as a growth medium due to high optical density. The application of activated carbon adsorption as a digestate pretreatment method was shown to be a very promising technology for OD reduction; however, it must still be developed to be used for digestate treatment at a large scale, therefore it was not used for the

initial trial in the novel cultivation system. A higher microalgae growth rate and, consequently, higher nutrient uptake could be expected in activated carbon pretreated digestate. Furthermore, the selected low-temperature tolerant strain *C. vulgaris* 211-11j must be tested under the current weather conditions, which is likely to lead to higher biomass productivity. Future work includes the cultivation of other selected candidate species in novel raceway ponds, evaluating biomass productivity and digestate treatment efficiency at different seasons. Moreover, the cocultivation of two or more selected microalgal species should be evaluated.

CONCLUSIONS

The Thesis addressed key aspects of energy sustainability and environmental protection challenges. It proposed a novel integration of microalgae cultivation technology within biogas plants, aiming to enhance microalgae biomass production while simultaneously achieving CO_2 sequestration and nutrient recycling. The research successfully developed and patented a microalgae cultivation system optimized for colder climates, identified suitable microalgae strains, and demonstrated the feasibility of coupling the system with existing biogas operations.

The Thesis presents a comprehensive framework for integrating the novel microalgae cultivation system into existing biogas plant operations. This integration has the potential to enhance biomass security, reduce transportation costs, and provide an innovative approach to managing digestate overproduction. The findings offer significant contributions to the fields of renewable energy and circular economy, proposing an innovative approach to leveraging waste streams for energy generation. The study highlights the potential of microalgae as a sustainable resource not only for biogas production but also for the generation of valuable by-products. Despite facing challenges such as scale-up complexity and climate dependency, the research opens up opportunities and viable solutions for enhancing the sustainability of biogas plants. Therefore, the Thesis contributes valuable insights and tools for advancing the bioeconomy towards a more sustainable and circular model.

More specifically in connection to Block 1 and Block 2 of the research framework, the following key results were identified:

1. A novel system designed for microalgae cultivation in colder climates has been developed and patented. This system overcomes the limitations of traditional cultivation systems, offering a promising solution for year-round biomass production in regions with challenging climates, such as Latvia.
2. Microalgae strains suitable for the Latvian climate were identified. *C. vulgaris* 211-11j, *C. sorokiniana* 211-8k, and *C. reinhardtii* 11-32b are promising strains for outdoor cultivation in the Latvian climate conditions. These strains show potential for high biomass production using agricultural digestate, marking a step forward in developing efficient microalgae-based bioenergy solutions.
3. *C. vulgaris* 211-11j was identified as a potential low-temperature strain for winter biomass production in Latvian climate conditions.
4. Various environmental and cultivation conditions were shown to highly affect the microalgae biomass production, the optimal conditions mostly being species-specific.
5. The optimal CO_2 concentration required for maximum growth was shown to be species-specific. An increased CO_2 concentration of 5 % leads to increased biomass of all studied microalgae, offering a potential tool for biosequestration of CO_2 from biogas production flue gas.
6. The research demonstrated the potential effective use of agricultural digestate and flue gases from biogas plants as low-cost nutrient and carbon sources for microalgae

growth. This approach reduces the operational costs associated with microalgae cultivation and contributes to nutrient recycling and greenhouse gas mitigation.

7. *C. sorokiniana* can effectively remove nutrients from digestate in outdoor conditions performing digestate treatment and meeting effluent standards for discharge for nitrogen and phosphorus.

The findings from the Thesis open several avenues for further research, particularly in the areas of optimizing the system for diverse environmental conditions, setting the ground for exploring the economic feasibility of large-scale implementation, and exploring the range of value-added products from microalgae biomass. Additionally, this work lays a foundation for practical applications, encouraging biogas plant operators to consider the integration of microalgae cultivation into their operations as a viable strategy for sustainable growth.

This research contributes significantly to the transition towards a more sustainable and resilient bioeconomy, highlighting the essential role of innovative technologies in transforming waste into wealth.

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