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Efficient Low-Temperature Nutrient Removal from Agricultural Digestate using Microalgae

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***Abstract* – In the face of energy crises and climate change, microalgae present a promising solution for sustainable energy production and carbon dioxide sequestration. Recently, digestate has been considered a cost-effective nutrient source for microalgae cultivation. Utilizing digestate not only enhances the sustainability and economic feasibility of microalgal biofuels but also offers a method for wastewater treatment. Nevertheless, the application of digestate is limited by its high optical density and a substantial amount of total solids. In the current study, several pretreatment methods were tested to increase the feasibility of digestate application for microalgae cultivation. Our findings show that various centrifugation methods and filtration decrease total solids' content but are ineffective in reducing optical density. Although the use of microalgae in treating various wastewaters has shown promising outcomes, the effectiveness of nutrient removal at low temperatures remains largely unexplored. To fill this gap, green microalga *Chlorella sorokiniana* was cultivated in pretreated diluted liquid digestate in dynamic springtime weather at high-latitude conditions. An innovative pilot-scale open race-way pond system was integrated into a biogas plant using its side streams, namely liquid digestate and flue gases as nutrient and CO_2 sources for microalgae cultivation. Coupling biogas production with microalgae cultivation can provide various benefits, including nutrient recycling from liquid digestate and CO_2 sequestration from flue gas. During the cultivation, high solar irradiance and low temperatures were recorded resulting in suboptimal conditions for *C. sorokiniana* growth. Despite the low productivity of *C. sorokiniana*, its nutrient removal efficiency was notably high. *C. sorokiniana* effectively removed 83 % of nitrogen and 85 % of phosphorus, demonstrating the promising potential of microalgae for wastewater treatment in high-latitude regions.**

***Keywords* – Bioeconomy; biogas; biomass; *Chlorella sorokiniana*; circular economy; microalgae cultivation; wastewater treatment.**

1. INTRODUCTION

The depletion of fossil resources, alongside industrial growth and a growing global population, set the ground for an energy crisis, forcing humanity to move to renewable energy alternatives. Additionally, rising levels of carbon dioxide in the atmosphere are causing climate change, leading to altered 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

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they do not require arable land for cultivation. Microalgae biomass can be converted to various types of energy, including biogas, biodiesel, and bioethanol. Moreover, it contains value-added compounds, such as proteins, fatty acids, pigments, vitamins with high market potential in food, feed, nutraceuticals, cosmetics, and medicine [1], [2]. In addition to already existing applications, the potential of microalgae biomass is being explored in other emerging areas, including wastewater treatment, biostimulants, biopesticides, and biochemicals [3].

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, high costs of biomass harvesting and downstream processing, and scale-up issues [4]. Recently much effort has been focused on promoting the economic feasibility of microalgae cultivation including bioreactor design considerations [5], optimization of cultivation conditions [6]–[8], a search for new more productive microalgal strains [9]–[11] and the development of new cost-effective and efficient biomass harvesting techniques [12], [13].

The application of various wastewaters as a low-cost nutrient source for microalgae growth has been studied extensively lately as a promising strategy to lower the costs linked to biomass production [14]. Digestate, a nutrient-rich by-product of anaerobic digestion, is currently used as a biofertilizer in agriculture; however, several challenges associated with storage and land application limit its use and impose environmental and human health risks. The number of biogas plants across Europe has steadily increased over the past decade, reaching 18 774 by the end of 2020 [15]. This growth has led to an oversupply of digestate. Overproduction and improper land application of digestate can pose significant risks to human health and the ecosystem through pathways such as water and soil contamination, air pollution, and the introduction of harmful substances into the food chain. Major concerns are related to nutrient runoff and water pollution, variations in the digestate nutrient content leading to nutrient imbalance, odor issues due to high ammonia content in the digestate, and air-quality issues due to the release of methane. Nevertheless, digestate is a valuable resource and its overproduction has driven the need for more sustainable management practices and diverse applications to prevent environmental and health challenges. In this context, digestate as a source of nutrients for microalgae growth has been considered, offering a novel digestate valorization route. Microalgae can use pollutants in various wastewaters, such as inorganic phosphorus and nitrogen, heavy metals, and pesticides, as nutrients for their growth. The main mechanism to remove pollutants includes the absorption of nutrients in cells to build biomass and support cell functions, reducing the nutrient concentration in water.

An innovative stacked modular microalgae cultivation system was designed and constructed as described in [16], [17] to overcome the limitations of existing technologies. This novel approach employs transparent acrylic material for pond construction, offering an alternative to traditional ground-level concrete ponds. The system's stacked design reduces land requirements and enhances light penetration in algal cultures due to the transparency of the material. Cultivation ponds are placed in a greenhouse to mitigate the impact of unfavorable weather conditions and to promote year-round biomass production in high-latitude climate conditions. Integrated into a biogas plant, the system utilizes waste products, namely digestate and flue gas, as nutrient and CO_2 sources. The cost of biomass production can be greatly reduced by avoiding the expenses of synthetic fertilizers, freshwater, and clean CO_2 [18]. In this setup, microalgae serve as a treatment interface for the liquid digestate and flue gases produced by the biogas cogeneration unit simultaneously generating valuable biomass, which can be reintroduced into anaerobic digestion as a feedstock or utilized in a biorefinery. Consequently, converting waste products and emissions into valuable biomass establishes a closed-loop system, advancing bioeconomy goals.

The digestate as a nutrient source is associated with certain drawbacks including an excessive amount of solids and high optical density (OD) thus limiting its application as a growing media for microalgae. Due to the high turbidity created by suspended solids and dark color due to the high optical density, raw liquid digestate is unsuitable for microalgae cultivation. Dilution of digestate is commonly applied to overcome these limitations, however, the substantial amount of freshwater required limits its sustainability. Freshwater scarcity is a serious problem in many parts of the world [19], therefore other pretreatment methods were tested in the current study to decrease the required dilution rate.

To date, most large-scale microalgae cultivation is located in warm low-latitude regions such as Israel, Australia, and the southern USA [20] 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 [21]. 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.

Here we report the initial results of the cultivation of microalgae *Chlorella sorokiniana* at high latitude in innovative outdoor pilot-scale race-way ponds integrated into a biogas plant using its waste streams, namely, liquid digestate and flue gases. Digestate treatment efficiency by microalgae is evaluated by nutrient removal.

2. METHODOLOGY

2.1. Pretreatment of Liquid Digestate

Liquid agricultural digestate after separation of the solid fraction was obtained from the Ltd "Agro Iecava" biogas plant located in Iecava, Latvia to assess its suitability as a low-cost nutrient source for microalgae growth. Several pretreatment methods were tested to decrease the required dilution rate with freshwater. Centrifugation, vacuum filtration, and filter centrifugation were applied to remove suspended particles to reduce turbidity and allow better light penetration into microalgal cultures.

Centrifugation was performed in a laboratory centrifuge (MegaFuge 16R, Thermo Scientific) in 50 mL flasks at 10 000 rpm for 10 min. The liquid fraction was then transferred to a new tube and centrifugation was repeated. The final supernatant was collected and stored in a fridge until the start of the growth tests. For vacuum-filtration pretreatment, centrifuged digestate was vacuum filtered through a 1.6 µm glass microfiber filter (Whatman) to further reduce the total solids content. Filter centrifugation was performed at 10 000 rpm with Hermle centrifuge (Hermle sieva, Germany) as a more viable option for large-scale pretreatment of digestate.

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.

2.2. Microalage Growth Tests in Pretreated Digestate

The pretreated digestate was subsequently used as a growth medium to evaluate its potential as a low-cost nutrient source. Green microalga *Chlorella vulgaris* 211-11j was cultivated in sterilized centrifuged and vacuum-filtered digestate diluted to 1 %, 3 %, and 5 % with distilled water. A 5 % dilution was applied only to the vacuum-filtered digestate, as the centrifugation-pretreated digestate at the same concentration exhibited excessively high OD,

rendering it unsuitable for microalgae growth. Standard BG-11 medium [22] was used as a control. Cultivation was carried out at 28 °C and 150 µmol photons m^{-2} s^{-1} white LED illumination under a photoperiod of 16:8 h (light: dark) in 500 mL flasks with a working volume of 200 mL. Aeration was provided with ambient air using an orbital shaker at 150 rpm. Microalgae were cultivated in batch conditions for 10 days. The daily growth rate was assessed by optical density measurements at 750 nm. All tests were conducted in triplicate. At the end of cultivation, biomass yield was determined for all cultures based on a dry weight. 50 mL of homogeneous microalgae culture was collected from a culture flask in a pre-weighted 50 mL tube and centrifuged at 10 000 rpm for 10 minutes at room temperature. The liquid fraction was removed and the tube with biomass was dried at 80 °C in the oven until constant weight and weighed. Dry weight was calculated and biomass yield was expressed as grams of biomass per liter of growth medium (g L^{-1}).

2.3. Growth Tests in Pilot-Scale Raceway Ponds

Digestate pretreated with filter centrifugation was used for growth and nutrient removal tests in the novel pilot-scale cultivation system (Fig. 1). Chemical analysis of digestate was performed before the inoculation of algal ponds 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.

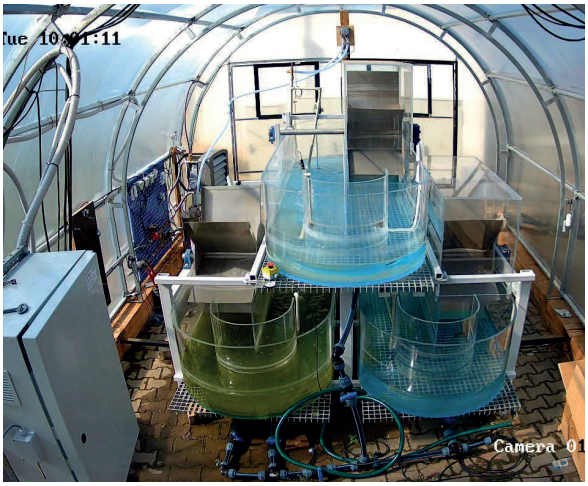


Fig. 1. Novel microalgae biomass production unit integrated into a biogas plant.

Although a low-temperature resistant strain has recently been identified for potential cultivation in high-latitude regions [23], the microalgae *Chlorella sorokiniana* 211-8k was chosen for the initial system trial. This species was selected due to its resistance to high solar irradiation, which was anticipated during the late spring test period (end of April to early May). The *C. sorokiniana* biomass for inoculation of the algal pond was cultivated in a 5 L photobioreactor (Bio4, Biotehniskais centrs) with TAP medium as a nutrient source. Microalgae were cultivated at 26 °C, rotation was set to 150 rpm, bubbling with air at the rate of 50 L per minute. pH was automatically controlled at the optimum level by the addition of 2 M HCl acid when the pH exceeded pH 7.9. The light was provided with 3 pieces of 10 W

linear white LED lamps attached to the outside of the reactor. The light-dark cycle was set to 16 h light and 8 h dark.

The pilot-scale microalgal pond was filled with tap water at 20 cm depth, 2 L of pretreated digestate (0.5 %) and pre-cultivated *C. sorokiniana* biomass (~ 1.5 %) was added. CO_2 introduction in the pond was implemented by bubbling flue gas captured from the biogas plant motor chimney. Mixing was provided with an electrical motor-driven paddlewheel at the frequency of 10 Hz, resulting in a paddlewheel speed of approximately 2.6 rpm and water speed of 10 cm s^{-1}. Probes with temperature, pH, and Photosynthetically active radiation (PAR) sensors were installed and used to record the cultivation conditions. The temperature inside the pond, in the greenhouse, and outdoors was recorded. Light intensity at the water level and the temperature of flue gas pumped inside the pond were also recorded. Samples for nutrient removal and biomass analysis were taken every 3 days. Samples were analysed for total nitrogen, total phosphorus, ammonia nitrogen, nitrates, chemical oxygen demand, suspended solids, and optical density.

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 assess the nutrient removal efficiency and biomass yield. The cultivation test lasted for 16 continuous days.

3. Results and Discussion

3.1. Pretreatment of Digestate

Centrifugation, vacuum filtration, and filter centrifugation were applied as initial pretreatment methods to improve the properties of digestate. Various pretreatment methods greatly improved digestate suitability for microalgae cultivation. The amount of suspended solids, COD, total nitrogen, total phosphorus, nitrate nitrogen, and ammonia nitrogen varied based on the pretreatment method applied. The concentration of suspended solids in the raw liquid digestate initially was 9080 mg L^{-1}. Pretreatments significantly reduced the suspended solids content. After centrifugation, the concentration dropped to 2450 mg L^{-1} and further decreased to 1700 mg L^{-1} following filtration (Table 1). Although filtration through a 1.6 µm microfiber filter decreased the solids more efficiently than centrifugation, it was considered not a viable option for large-scale digestate pretreatment. Accordingly, filter centrifugation was tested as an alternative and showed a good ability to reduce the amount of suspended solids (2135 mg L^{-1}). Filter centrifugation was selected as the preferred method for large-scale digestate pretreatment due to its suitability for high-volume processing, offering greater efficiency than traditional centrifugation and higher throughput than standard filtration.

Table 1. Chemical Composition of Digestate after Various Pretreatment Methods

Parameter	Unit	Centrifugation	Filtration	Filter centrifugation
Suspended solids	mg L^{-1}	2450	1700	2135
COD	mg L^{-1}	23 210	9580	36 300
Total N	mg L^{-1}	11 770	6780	6180
Total P	mg L^{-1}	319	157	602
Nitrate N	mg L^{-1}	<0.07	<0.07	<0.3
Ammonia N	mg L^{-1}	3080	2460	3360

3.2. Microalgae Cultivation in Pretreated Digestate

Pretreated digestate was subsequently tested as a growth medium for microalgae cultivation in laboratory conditions. Although the pretreatment greatly reduced the amount of solids, high optical density was still an obstacle, therefore dilution with water was used to reduce the optical density. During the first round of experiments, microalgae *C. vulgaris* was cultivated in (1) centrifuged and (2) filtered digestate diluted to 1 %, 3 %, and 5 % with distilled water. *C. vulgaris* exhibited better growth in filtered than in centrifuged only digestate at a concentration of 3 %; however, no significant difference in growth rate was observed in the case of 1 % digestate (Fig. 2).

The highest culture density was reached in 1 % digestate regardless of the pretreatment method and was close to that obtained in the control BG-11 medium showing very promising results. When 3 % digestate was applied, cultivation of *C. vulgaris* in digestate after centrifugation pretreatment resulted in a very slow growth. Whereas 3 % digestate after the pretreatment with filtration provided a good growth medium for *C. vulgaris*.

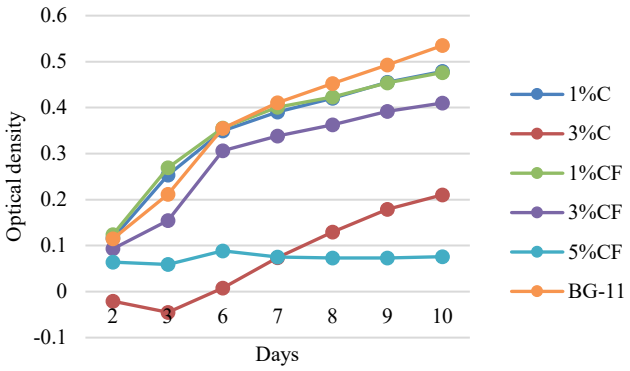


Fig. 2. Cultivation of *C. vulgaris* in 1 %, 3 %, and 5 % digestate processed using various pre-treatment methods (C = centrifugation, CF = filtration).

No growth of microalgae was observed when a 5 % dilution was applied. This could be attributed to the high optical density of the more concentrated digestate, which is visually apparent (Fig. 3). As a result, light access to the microalgae cells is limited.

Fig. 3. *C. vulgaris* cultivation in various dilutions of digestate. Flasks from the left to right: control, 1 %, 3 %, 5 %, and 10 % digestate as a growth medium.

At the end of the growth test biomass yield was calculated. Although the highest cell density was achieved in the 1 % digestate, the 3 % digestate, after filtration pretreatment, resulted in the highest biomass production (1.05 g L^{-1}). Cultivation in 1 % digestate after centrifugation yielded 0.95 g L^{-1} (Fig. 4). The lowest biomass yield was observed in 5 % digestate (0.34 g L^{-1}), likely due to the increased solids content and high optical density, which limited light access. Nevertheless, due to the relatively high standard deviation of some samples, this result must be perceived with caution. The increase in standard deviation with rising digestate concentration can be observed and may be attributed to the microalgae adaptation to suboptimal growth conditions, such as elevated turbidity and optical density, limiting the availability of light. Some microalgal colonies may have been more successful in establishing healthy populations than others, resulting in greater variability in biomass yield across replicates.

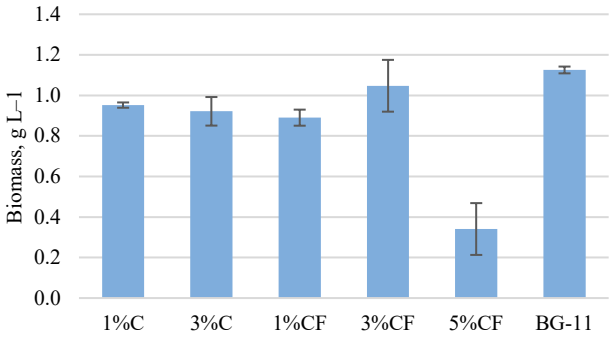


Fig. 4. Final biomass yield of *C. vulgaris* cultivated in 1 %, 3 %, and 5 % pretreated digestate. Pretreatment: C – centrifugation, CF – filtration. BG-11 – control in standard growth medium. Error bars represent Standard deviation (n = 3).

3.3. Cultivation in Pilot-Scale Raceway Pond

3.3.1. Monitoring the Cultivation Conditions

Outdoor microalgae cultivation is heavily dependent on weather, which in turn varies according to location and season. Spring conditions are typically dynamic in high-latitude regions, with vastly fluctuating temperatures that are not ideal for microalgae cultivation. Indeed, the microalgae cultivation test in the novel pilot-scale system was challenging due to unstable and variable weather conditions. Nevertheless, 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.

In spring, daytime temperatures can fluctuate significantly from one day to the next, with substantial diurnal variation between day and night. Indeed, during the microalgae cultivation trial, recorded temperature fluctuations were notably high. Temperature was continuously monitored inside the cultivation pond, and air temperature was recorded both in the greenhouse and outdoors. The average daytime pond temperature ranged from approximately 15 to 22 °C (Fig. 5), with the highest water temperature reaching around 22 °C. The lowest daytime temperature was recorded on May 3rd when the pond temperature dropped to 12 °C. At night, water temperature fell considerably, as expected due to the low outside air temperature, ranging generally between 10 °C and 16 °C.

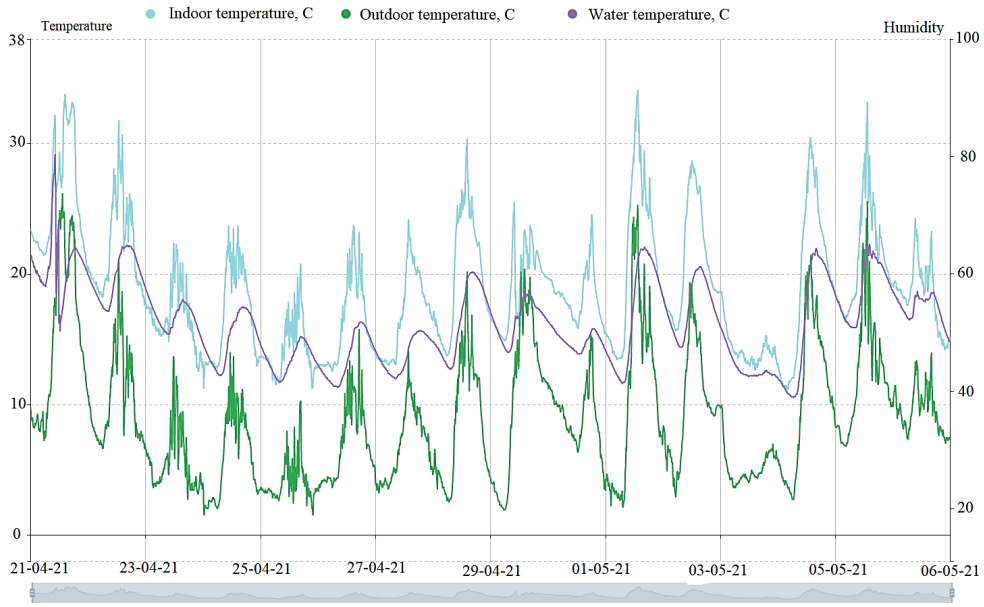


Fig. 5. Outdoor temperature, indoor temperature at the greenhouse, and pond water temperature during the biomass cultivation test.

Fluctuations in pond water temperature, influenced by both outdoor and greenhouse conditions, are depicted in Fig. 5. The lowest recorded nighttime air temperature outside during the growth trial was around 2 °C, with outdoor nighttime temperatures hovering a couple of degrees above zero for most of the cultivation period. In the last decade of April, temperatures were 3.2 °C lower than the 1981–2010 average and 4.1 °C lower than the 1991–2020 norm [24], impacting microalgae growth. Nevertheless, even during the coldest nights, the pond temperature did not drop below 10 °C, demonstrating the greenhouse's effectiveness in maintaining higher temperatures under unfavourable conditions. In present conditions, the greenhouse maintained a temperature approximately 10 degrees higher than the outside temperature.

During the initial trial, the temperature in the pond was significantly below the optimum range reported for *C. sorokiniana*, which is between 30 °C and 35 °C [25]. The contribution of heat from flue gas was negligible in the present flow rate. Flue gas temperature varied due to the changes in outdoor temperature because 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. The temperature of pure flue gases was 522 °C; however, while travelling up the chimney and through the pipes, the temperature decreased considerably. Moreover, after mixing with air the temperature of flue gases reaching the pond was a maximum of 45 °C.

Natural light intensity fluctuation recorded during the biomass cultivation test is shown in Fig. 6. Maximum surface irradiance generally was in a range from 600 to 900 µmol photons m^{-2} s^{-1} in the middle of the day on mostly clear days with no or very low cloud cover. Significantly lower light intensity was observed on overcast days, for example on April 30th maximum light intensity reached just 300 µmol m^{-2} s^{-1} but did not exceed 130 µmol photons m^{-2} s^{-1} on May 3rd. The received light might be excessive for the selected species in low temperature conditions causing photodamage and affecting the growth rate.

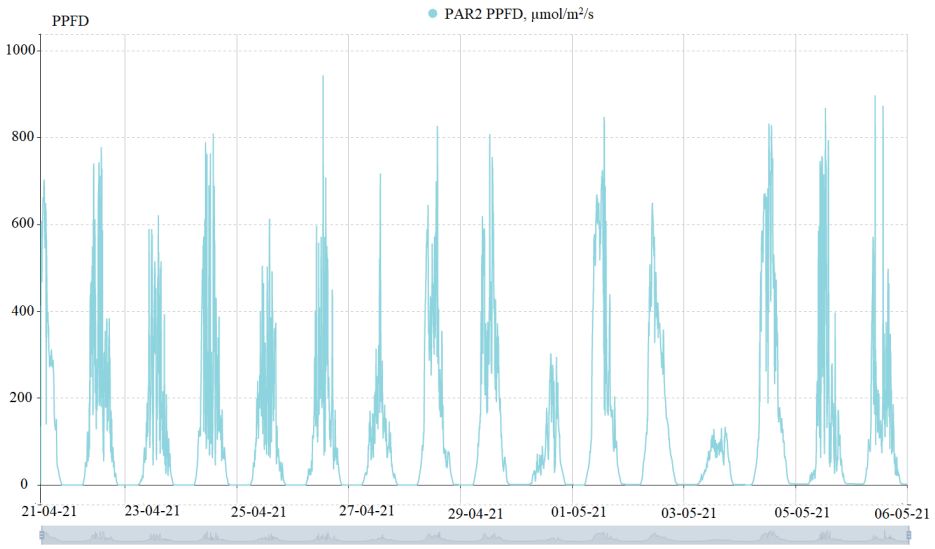


Fig. 6. Light intensity (PPFD – Photosynthetic Photon Flux Density) at the water level during the biomass cultivation test.

The pH of the microalgae pond fluctuated according to the day/night cycle and increased during the cultivation from around pH 7.5 at the beginning of cultivation to pH 9.2 on day 7 (not shown). The pH rises gradually during the day due to the algae growth and uptake of carbon and decreases again during the nighttime. The contribution of CO_2 from flue gases was difficult to evaluate because it was not possible to measure the actual amount of CO_2 entering the ponds in the present setup conditions. The amount of CO_2 in pure flue gases before the mixing with air was 14 %, however, the actual volume of CO_2 entering the microalgae pond should be measured. Moreover, a higher input of flue gas might be required to lower the pH and increase microalgae productivity.

3.3.2. 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 before application to the microalgae pond. Pretreated digestate was analysed for the content of solids, COD, and nutrients. The results of the chemical analyses of digestate and tap water used for dilution are shown in Table 2.

TABLE 2. CHEMICAL ANALYSIS OF PRETREATED DIGESTATE AND WATER USED FOR DILUTION

Parameter	**Unit**	**Tap water**	**Pretreated 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-NH4	mg L^{-1}	<0.3	3360	8.4
Nitrates, N-NO3	mg L^{-1}	<6	<0.3	<0.3
Chemical oxygen demand, COD	mg L^{-1}	0.114	36 300	56

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 of 36 300 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 showing very low levels of nutrients and contaminants. The nutrient content of diluted digestate used as a growth medium is shown in the last column of Table 2.

Samples from the pond were taken every 3 days to monitor microalgae growth and removal of nutrients from the growth medium. After inoculation, *Chlorella sorokiniana* initially exhibited slower growth but then showed exponential growth from day 3 to day 7 (Fig. 7).

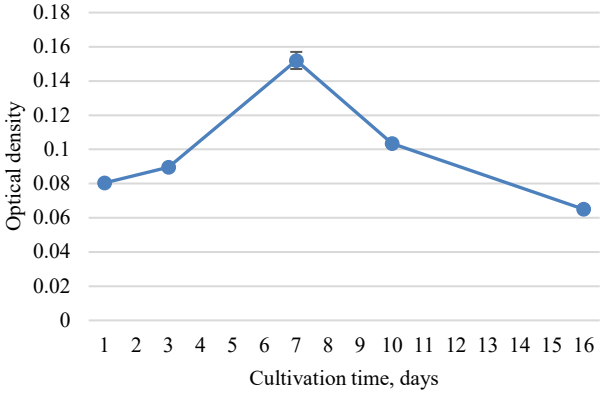


Fig. 7. *C. sorokiniana* culture density during cultivation test in pilot ponds. Error bars indicate Standard deviation (n = 2).

The culture density began to decrease after day 7, suggesting the presence of limiting factors. Thereafter culture density continued to decrease till the end of the 16-day cultivation. The microalgae growth is similarly reflected in the biomass yield. Maximum biomass yield was recorded on day 7, reaching 80 mg L^{-1} dry weight, as seen in Fig. 8.

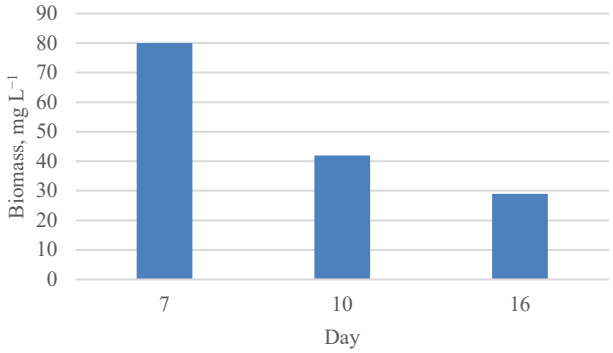


Fig. 8. Final microalgal biomass yield (mg L^{-1}) during the trial cultivation test of 16 days in the novel microalgal cultivation system.

By day 10, biomass had decreased by 50 % compared to day 7 and continued to decline thereafter. In another study, *C. sorokiniana* UTEX 1230 biomass reached 280 mg L^{-1} when cultivated in 10 % anaerobic digestate effluent derived from cattle manure for 25 days [26]. Another study reported a *C. sorokiniana* biomass yield of 1.73 g L^{-1} when cultivated in 5 % high-nitrogen anaerobic digestion effluent as a nutrient source using an air-lift tubular photobioreactor [27].

Although the biomass productivity of microalgae during the trial cultivation test was not high, it must be considered that cultivation conditions were not optimal for *C. sorokiniana*. Several factors might have impacted the culture growth including limited nutrients, high solar irradiance, and bacterial contamination. Though most likely suboptimal temperature had a profound effect on microalgae growth and biomass yield. Throughout most of the cultivation period, the pond temperature was unexpectedly low, as previously described, and well below the optimal range for the species. The cultivation trial was conducted during the springtime when the outside temperature fluctuates greatly. Moreover, 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 one of the possible explanations for the observed sharp decrease in culture density on the following days. Low temperature is known to slow down metabolic rates, enzyme activity, photosynthetic efficiency, and other cellular processes in microalgae [28]. Consequently, microalgae experience a reduction in cell division rates and slow growth. Exceptionally low productivity of *C. sorokiniana* has been reported in the scientific literature in suboptimal temperatures [29]. It is also plausible that the light intensity requirements were reduced due to the low temperature, making the maximum spring irradiance excessive. Temperature and light are major factors determining the photosynthetic efficiency and consequently growth rate. Low temperature and high light intensity may disrupt the balance between energy absorption during photosynthesis and the ability to utilize this energy [30]. High light intensity under low temperature conditions can cause photoinhibition when Photosystem II becomes damaged by excess light energy. This is because microalgae tend to absorb more light energy in high light conditions than they can utilize for photosynthesis in low temperature conditions due to slow metabolic reactions. Consequently, photoinhibition reduces growth rates and biomass production. To address these issues, in future studies temperature and light intensity should be adjusted to optimal levels. This was not implemented in the current study, which served as the initial trial of the novel cultivation system. Temperature control within the novel system can be achieved in multiple ways, including increasing the flue gas temperature used as a CO_2 source for microalgae and utilizing the waste heat from a biogas cogeneration unit to raise the temperature in cultivation ponds. These adjustments would help maintain an optimal balance between light and temperature, enhancing the system's efficiency.

Although a relatively low biomass yield was reached during the cultivation trial, the nutrient removal rate seems 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. 9(a)). Nutrient removal increased considerably after the initial lag phase. Ammonia concentration increased slightly again at the last stage of cultivation.

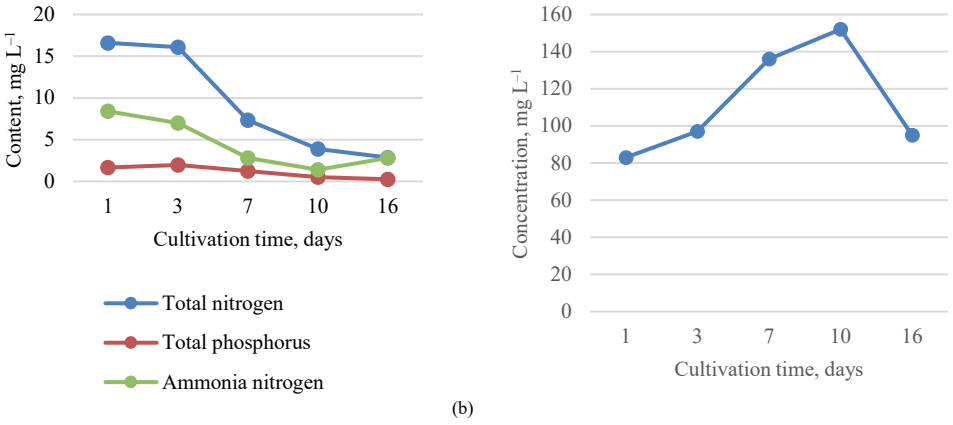
High nutrient removal efficiency was reached at the end of the cultivation (Table 3). In total, 83 % of nitrogen, 85 % of phosphorus, and 83 % of ammonia nitrogen (till day 10) were removed from the growth medium during the cultivation of *C. sorokiniana*.

TABLE 3. 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 (day 10)	83
Nitrates, $N\text{-}NO_3$	<0.3	NA*	NA*
Chemical oxygen demand, COD	83	–12	–14.5

Notes: *The level of nitrates was outside the Method detection limit (<0.3 mg L^{-1}) for all samples taken during the cultivation trial, therefore calculation of nitrates removal rate was not possible.

The concentration of 2.86 mg L^{-1} and 0.25 mg L^{-1} of total nitrogen and phosphorus, respectively were reached at the end of the cultivation corresponding to national legislation regarding requirements for wastewater treatment for discharge [31]. In agglomerations with fewer than 100 000 inhabitants, the permitted total nitrogen concentration in wastewater is 15 mg L^{-1}, whereas in agglomerations exceeding 100 000 inhabitants, the allowance is 10 mg L^{-1}. Regarding phosphorus, 2 mg L^{-1} is allowed in agglomerations with fewer than 100 000 inhabitants, and 1 mg L^{-1} in agglomerations exceeding 100 000 inhabitants. Accordingly, the current setup for digestate treatment with microalgae could meet regulatory requirements.



(a) (b)

Fig. 9. Removal of total nitrogen, total phosphorus, and ammonia nitrogen (A) and COD (B) by *C. sorokiniana* during a 16-day cultivation trial.

COD during the cultivation of *C. sorokiniana* was increasing (Fig. 9(b)). The relationship between COD in wastewater and microalgae growth is complex and influenced by various factors, including initial COD concentration, microalgae species and density [32]. Some studies have demonstrated that microalgae can remove COD from wastewater. However, since microalgae release extracellular polymeric compounds during growth, the COD removal might not be effective, and an increase in COD during cultivation could be observed. It is generally assumed that COD removal is not efficient in wastewater treatment using microalgae due to the release of organic compounds [33]. Consortium systems where microalgae are co-cultivated with bacteria or fungi creating a symbiosis between microorganisms are more efficient [34]. In such systems, bacteria mainly remove organic

carbon compounds from wastewater while microalgae reduce inorganic nitrogen and phosphorus compounds more effectively [33]. Accordingly, it is recommended that the microalgae-bacteria consortium be tested in future studies for efficient COD removal from digestate.

The developed technology seems promising regarding digestate treatment with microalgae in the Nordic climate even in suboptimal cultivation conditions. However, it must be taken into account that a high dilution rate was used to apply digestate as a growth medium due to its high optical density. The digestate pretreatment methods tested in the current study increased digestate suitability in terms of the amount of suspended solids but were not effective in optical density reduction. The use of activated carbon adsorption as a digestate pretreatment method was shown to be a very promising novel technology for OD reduction [35]. It must still be developed for large-volume digestate treatment, 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.

4. CONCLUSION

The current research explored integrating microalgae cultivation systems within biogas plants, aiming to enhance microalgae biomass production at high-latitude regions, while simultaneously achieving CO_2 sequestration and nutrient recycling. A novel microalgae cultivation system optimized for colder climates was utilized in this research and the feasibility of coupling the microalgae cultivation with existing biogas operations was demonstrated. The research revealed the potential effective use of agricultural digestate from biogas plants as a low-cost nutrient source for microalgae growth. It was demonstrated that *C. sorokiniana* can effectively remove nutrients from digestate in an open race-way pond meeting effluent standards for discharge for nitrogen and phosphorus. Although centrifugation and filtration effectively reduced the solids content of digestate, high optical density was still a major challenge for efficient digestate valorization as a microalgae growth medium. Further research is needed regarding digestate treatment to decrease optical density.

The findings from the current study open several avenues for further research, particularly in the areas of optimizing the system for diverse environmental conditions, testing other potential microalgae species, 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.

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