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Nutrient removal from hydroponic effluent by Nordic microalgae: From screening to a greenhouse photobioreactor operation

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ABSTRACT

To guarantee year-round horticultural production, Nordic countries rely on greenhouses to cope with the harsh winter conditions. Despite substantial progress in greenhouse operation, greenhouse effluents are still overloaded with nutrients and are known to be a source of eutrophication that decreases the quality of natural waters worldwide. Here we investigate the possibility of recirculating hydroponic effluents from a commercial cucumber greenhouse to cultivate photoautotrophic microalgal biomass. A stepwise methodology was applied to scale-up procedures from laboratory to a pilot scale photobioreactor (PBR) which was operated inside a greenhouse. The screening results identified that 12 (of 13) strains were capable of proliferating in the hydroponic effluent without any adjustment or dilution. A Nordic microalga from the *Scenedesmaceae* family was selected for a PBR operation which lasted 36 days. This continuous cultivation was undertaken under four different test conditions and removal efficiencies between 18 and 35% for N-NO_3^- and 40–98% for P-PO_4^{3-} were achieved. Over 1000 L of hydroponic effluent was recirculated, with a steady N-NO_3^- uptake of 86–92 mg g^{-1} and a P-PO_4^{3-} uptake of 11–21 mg g^{-1} biomass. These results demonstrated that a hydroponic effluent N:P of 15–26 and pH of 7.5 were the most appropriate conditions for nutrient uptake, although increasing the pH to 9 may promote P-PO_4^{3-} uptake. Overall, this work indicates the feasibility of microalgal bioremediation of greenhouse effluents in the Nordic countries.

1. Introduction

The climate of the Nordic countries presents several challenges to agriculture practices. In Finland, the winter season can last from 4 to 6 months and is often characterized by freezing temperatures, a lack of natural sunlight and heavy snowfall. To cope with this harsh environment, a significant part of horticulture production occurs in greenhouses equipped with artificial light and regulated temperature systems, guaranteeing year-round food availability [1]. Hydroponic farming is often preferred to improve water management compared to traditional practices, and to reduce the need for mineral fertilizers [2]. Nonetheless, hydroponic greenhouse effluents are still overloaded with nutrients, containing up to 1000 mg L^{-1} of nitrate and over 200 mg L^{-1} of phosphate [3,4], with much room for improvement in dosage procedures [5]. Recently, novel strategies have been proposed to mitigate pollution caused by agriculture wastewaters. These involve using photosynthetic microalgae to simultaneously: recover nutrients, purify water streams, and generate biomass for industrial application [6,7].

Photosynthetic microalgae and cyanobacteria are a diverse group of microorganisms able to convert sunlight into chemical energy. Through this process, the cells assimilate inorganic carbon and nutrients while generating biomass rich in organic molecules such as proteins, lipids, carbohydrates and pigments [8]. Despite the industrial potential of these bioactive molecules, the large-scale production of microalgae is still limited by high operation costs. This currently limits the potential use of microalgal biomass, which has been proposed as alternative sources of food or nutrition, animal feed, commodity chemicals or biofuels [9,10]. Several life cycle assessment (LCA) studies have identified the costs associated with the supply of nutrients, fresh water and energy as some of the most important constraints to fiscal viability [11,12]. These parameters, together with the scale of production and market applications, can drastically increase the cost of production to values ranging from 69€ to over 400€ kg^{-1} DW [13–15]. In order to reduce production costs, liquid waste streams containing a suitable nutrient composition (and

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lacking toxic components) could be used as substitute for mineral fertilizers and fresh water. When combined with optimized cultivation and a suitable production scale, this alternative approach can decrease the cost of production to just 1.4€ kg^{-1} DW [16]. Biologically, the demonstrated resilience of photoautotrophs to different wastewater streams indicates their aptitude to nutrient reclamation and environmental remediation processes [17,18]. Therefore, the use of microalgae and cyanobacteria to recirculate agricultural effluents presents an opportunity to mitigate environmental pollution, while simultaneously decreasing the costs of biomass production [19]. Under harsh Nordic conditions, the existing greenhouse infrastructure, already targeted to optimal food production, likely provides advantageous conditions for the concomitant farming of microalgae and cyanobacteria. Use of artificial light for the production of microalgae enables optimized productivity and can limit the biochemical variability of the biomass product which might otherwise result from natural climatic fluctuations [20]. Thus, the use of greenhouse infrastructure to improve the yield of a cultivation system presents an opportunity to decrease production costs and ensure the reproducibility and reliability of biomass supply [21]. Indeed, overall, it appears that the sustainability of horticulture cultivation can be greatly improved by integrating microalgae cultivation into greenhouses for bioremediation of hydroponic effluents.

In this study, the feasibility of using Nordic cyanobacteria and microalgae for bioremediation of hydroponic effluents from a commercial cucumber greenhouse was evaluated. A laboratory scale rapid screening of the Nordic culture collection was performed to prove the general suitability of a variety of Nordic strains to growth in hydroponic effluent. Pretreatments were then investigated to identify suitable options to deal with microbial communities that are commonly found in waste streams [22]. Finally, a pilot-scale cultivation in a real greenhouse environment was used as a proof of concept to demonstrate the feasibility of integrated microalgal wastewater reclamation and greenhouse cultivation in a Nordic climate.

2. Materials and methods

2.1. Characterization of hydroponic greenhouse effluent

The effluent was obtained from a commercial hydroponic cucumber cultivation greenhouse, Puutarha Timo Juntti Oy, located in Kaarina, Finland. The samples were collected prior to the greenhouse filtration system and stored at 4 °C in the dark. The chemical composition of the effluent was characterized by a certified laboratory – Lounais-Suomen vesi- ja ympäristötutkimus Oy – and it is summarized in Table 1. The COD was quantified by permanganate oxidation, while BOD was determined with a modified method for undiluted samples using allyl thiourea (ATU).

Table 1
Chemical characterization of greenhouse effluent collected from a hydroponic cucumber cultivation prior to filtration.

Parameter	Value	Unit	Method
Conductivity	280	mS m^{-1}	SFS-EN 27888
TSS[a]	5.5	mg L^{-1}	Nuclepore 0.4 μm (internal method)
COD (Mn)[b]	28	mg L^{-1}	SFS 3036
BOD_7[c]	3.0	mg L^{-1}	SFS-EN 1899–2
P-PO_4^{3-}	32	mg L^{-1}	SFS-EN ISO 15681-2
N-NO_3^-	270	mg L^{-1}	Hach Lange LCK 339 (internal method)
N-NO_2^-	0.07	mg L^{-1}	SFS-EN ISO 13395:1997
N-NH_4^+	0.13	mg L^{-1}	Fluorometric (CFA, internal method)
SO_4	220	mg L^{-1}	SFS-EN ISO 10304-1
Fe	2.3	mg L^{-1}	SFS-EN ISO 11885, SFS-EN ISO 15587-2
K	320	mg L^{-1}	
Mg	61	mg L^{-1}	
Ca	180	mg L^{-1}	
Zn	0.44	mg L^{-1}	
pH	6.8		pH meter

[a] Total suspended solids.
[b] Chemical oxygen demand.
[c] Biological oxygen demand.

2.2. Maintenance and pre-cultivation of microalgae and cyanobacteria

A total of 13 strains of cyanobacteria and eukaryotic microalgae from NordAqua Nordic Culture Collection database (https://nordaqua.fi/our-culture-collection) were selected for a screening trial (Table 2). These strains belong to the University of Helsinki Culture Collection (HAMBI) and the Norwegian Culture Collection of Algae (NORCCA). Strains were maintained in 100 mL Erlenmeyer flasks with 50 mL of media. Z8 medium [23] was used for NORCCA strains, while HAMBI strains were kept in Z8X (without combined nitrogen) or BG11 medium [24]. The cultures were maintained and pre-cultivated in continuous low-intensity light (photosynthetic photon flux density (PPFD) 5–10 μmol m^{-2} s^{-1}) at room temperature (20 °C) before the experimental screening. The strains were pre-cultivated in the respective culture media to guarantee good proliferation rates. The medium Z8 has a N:P ratio of 24 while Z8x contains only residual amounts of N-NO_3^- from its trace metal stock solution. Additionally, the nitrogen composition of the hydroponic effluent was mainly dominated by the same N-source and the pH was similar to the culture media. For those reasons, an adaption period before the experiment was not performed, as the purpose of the trial was to identify robust strains that could survive and proliferate in unadjusted hydroponic effluent.

2.3. Rapid screening trial

The rapid screening trial was carried out in 24 well plates using hydroponic effluent as growth medium. The effluent sample was pretreated with a coarse filtration (4–7 μm) for retention of small particles. The cells were inoculated at an initial OD750 of ~0.15 with a working volume of 2 mL. The rapid screening trial was performed in duplicates and growth was monitored daily by OD750 using a microplate reader (Infinite 200 PRO, Tecan). The experiments were performed under PPFD 50 μmol m^{-2} s^{-1} at 22 °C and atmospheric CO_2, with constant shaking at 120 rpm (orbital shaker, Sanyo, Japan).

2.4. Comparison of different pre-treatment methods

Three different pre-treatment methods were tested: coarse filtration (4–7 μm, **CF**); microfiltration (0.7 μm glass microfiber filters, **MF**); and bleach (sodium hypochlorite, 14% at a final concentration of 10 ppm for 1 h with mixing, followed by neutralization with 12 ppm of sodium thiosulphate, **BST**). These methods were evaluated to combat any potential challenges regarding the elimination of competitors or the content of organic matter and considering their effectiveness and feasibility

Table 2
List of microalgae and cyanobacteria employed in this study.

Culture collection	ID code	Taxonomy	Growth medium
HAMBI	UHCC 0252	*Nostoc* sp.	Z8x
	UHCC 0268	*Nostoc* sp.	
	UHCC 0582	*Microcystis* sp.	BG11
	UHCC 0524	*Synechococcus* sp.	
	UHCC 0527	*Synechococcus* sp.	
	UHCC 0027	*Scenedesmus* sp.	
	UHCC 0492	Unknown	
NORCCA	K-1877	*Selenastrum* sp.	Z8
	NIVA-CHL 131	*Chlorococcum* sp.	
	NIVA-CHL 99	*Scenedesmus* sp.	
	NIVA-CHL107	*Tetradesmus obliquus*	
	NIVA-CHL100	*Monoraphidium contortum*	
	NIVA-CHL144/1	*Apatococcus lobatus*	

of being scaled-up and integrated in the PBR operation in the greenhouse. The pre-treated effluents were seeded with UHCC0027 in 500 mL Erlenmeyer flasks with a working volume of 250 mL and cultivated in a growth chamber (Sanyo, Japan) providing 3% CO_2, continuous PPFD 50 μmol m^{-2} s^{-1} and a constant temperature of 22 °C. The experiments were performed in duplicate and growth of the cultures was monitored daily by measuring OD750 (Genesys 10S UV–VIS, Thermo Fisher, USA) and total chlorophyll content [25]. At the end of the experiment, the removal efficiencies for [N-NO_3] and [P-PO_4^{3-}] were quantified for each pre-treatment method.

2.5. Pilot scale cultivation

2.5.1. Photobioreactor operation in a greenhouse infrastructure

A tubular PBR was constructed in a research greenhouse belonging to the Natural Resources Institute of Finland (LUKE) in Kaarina, Finland. A detailed description of the PBR can be found elsewhere [26]. Briefly, the tubular PBR made of PLEXIGLAS-XT acrylic holds a volume of 65 L and is equipped with electronic CO_2 solenoid valve injection and inline sensors that allow an effective real-time monitoring of OD at 880 nm (home-made turbidity sensor), OD at 680 nm (home-made relative chlorophyll content sensor), temperature, pH and flow. All sensors are controlled via NI LabView based software which records data points at 10 s intervals and displays a real-time graphic representation of each parameter. The footprint of the PBR is 2 m by 0.5 m.

The LUKE glass greenhouse is equipped with artificial light, heating and cooling systems that are programmed to maintain constant abiotic conditions. The heating system circulates hot water through a pipeline spread across the facilities. Cooling is achieved either by roof vents that allow air circulation, or fan cooling with ethylene glycol circulation. These systems maintained a constant temperature of 20–25 °C. The greenhouse compartment is also equipped with 15 high pressure sodium bulb lights (Philips Green Power 400 W) evenly distributed in three rows. The height of the light bulbs was adjusted to provide an average PPFD of 200 μmol m^{-2} s^{-1} at the top surface of the PBR. The PPFD was adjusted at night, using a PPFD meter (LI-1000, LI-COR, Lincoln, USA) and a quantum sensor (LI-190R LICOR, Lincoln, USA). The system was programmed to provide a 17 h photoperiod, following the conditions of cucumber cultivation. The greenhouse has a dedicated meteorological station which monitors and records the outside weather throughout the year. In order to prevent photoinhibition due to excessive light, a threshold of 500 W m^{-2} of solar irradiance was established upon which the high-pressure sodium bulb lights would automatically shut down.

2.5.2. PBR operating modes

The PBR was inoculated with a 5 L culture of UHCC0027 corresponding to a dry weight (DW) of 0.15 g L^{-1} and an OD750 of 0.18 (see Section 2.4 for growth conditions). After inoculation, the PBR was operated in batch mode and when the culture approached stationary phase, the operation was manually set to turbidostat mode. During the batch phase, samples were collected daily for quantification of DW and establishment of a calibration curve with the turbidity sensor. Throughout turbidostat operation, NI LabView software determined turbidity relative to the set threshold. When this was exceeded, the culture was diluted with fresh hydroponic effluent via a peristaltic pump (323, Watson Marlow, USA). The overflow was collected in a vessel positioned on top of a scale (GFK 150H, Adam DU, UK) which allowed for the quantification of growth rate and system productivity. Daily samples were analyzed in triplicate for DW and nutrient consumption. Additionally, the PBR was equipped with a two-step filtration system to purify the hydroponic effluent. For the batch phase operation, the effluent was purified with bleach plus sodium thiosulphate (BST) before being submitted to a two-step filtration system consisting of a primary coarse filtration followed by a 0.1 μm polyvinylidene fluoride (PVDF) membrane filter (Polymem, Waterpia GOLD, South Korea) to remove all particles and pathogenic microorganisms. For turbidostat mode, the effluent was only channeled through the two-step filtration system to ensure the feasibility of daily operation and facilitate dealing with large volumes of effluent. Turbidostat operation was used to evaluate the performance of UHCC0027 over four different abiotic setups, described in Table 3. The transitions between setups were achieved by loading the new values of turbidity and pH thresholds into the LabView software and allowing the culture to stabilize. The pH threshold was used as a set point for automatic CO_2 injection into the system.

2.6. Analytical procedures

At laboratory scale, nutrient concentrations were quantified using Merck Spectroquant test kits (1.14773 for N-NO_3^- and 1.14543 for P-PO_4^{3-}). In the greenhouse PBR experiment, samples were collected daily and N-NO_3^- was quantified according to the method 4500-NO_3-B [27]. Phosphate was quantified as described above. Calibration standards were prepared using $NaNO_3$ (>99%, Merck) and K_2HPO_4 (99%, VWR). Prior to analysis, all samples were filtered through Whatman 0.45 μm syringe filters and samples with high concentrations were diluted as necessary.

2.6.1. Calculations

Algal growth was monitored daily by DW (PBR experiments). Aliquots of the algal suspension were filtered through a pre-dried, pre-weighed glass microfiber filter (Whatman GF/F, 0.7 μm) using a vacuum pump. The filters were dried at 95 °C and cooled down in a desiccator before being weighed. DW (g L^{-1}) was calculated according to Eq. (1):

$$DW = (Final - Initial\ weight)/(Samplevolume) \quad (1)$$

The removal efficiencies (RE in %) for [N-NO_3^-] and [P-PO_4^{3-}] were calculated according to Eq. (2):

$$RE = (C_i - C_t)/C_i{*}100 \quad (2)$$

where C_i and C_t are the nutrient concentrations measured at the beginning and time t (day) of the experiment, respectively. In the case of batch studies, C_i and C_t were measured from the hydroponic media after the inoculation of algae. For continuous experiments, C_i was measured from the influent – hydroponic media, while C_t was measured from the effluent – harvested algal suspension.

2.6.2. PBR operated in batch mode

The specific growth rate (μ in d^{-1}) was calculated using Eq. (3):

$$\mu = ln\left(N_f - N_i\right)/T_{(f-i)} \quad (3)$$

where N_f and N_i are the dry weight at the beginning and end of a time interval (T_{f-i}) in the logarithmic growth phase.

Volumetric productivity (P_{vol} in g L^{-1} d^{-1}) was calculated according to Eq. (4):

$$P_{vol} = DW_{(f-i)}/T_{(f-i)} \quad (4)$$

Table 3
Experimental parameters and macronutrient composition of the hydroponic effluent during continuous PBR operation. The setups were operated sequentially, with transitions achieved by adjusting dry weight via dilution or growing of cells, and/or adjusting pH by the injection of CO_2 (see methods for details).

Experimental parameters	Setup 1	Setup 2	Setup 3	Setup 4
Dry weight (g L^{-1})	1.0 ± 0.03	0.5 ± 0.03	1.05 ± 0.04	1.07 ± 0.03
pH	7.5	7.5	6.8	9
N:P	16–19	20–27	32–37	26–27
N-NO_3^- (mg L^{-1})	240–263	247–269	275–293	276–277
P-PO_4^{3-} (mg L^{-1})	29–33	22–28	16–20	22–23
Number of days	10	10	5	4

where P_{vol} is the ratio of ratio of the DW difference (DW_{f-i}) and the time interval (T_{f-i}).

Areal productivity (P_{area} in g m^{-2} d^{-1}) was calculated from Eq. (5):

$$P_{area} = (P_{vol}{*}V)/\text{Area} \quad (5)$$

where V is the total volume of the PBR and area (m^2) represents the area occupied by the footprint of the PBR.

2.6.3. PBR operated in continuous mode

In continuous turbidostat operation the growth rate (μ in d^{-1}) is equal to the dilution rate (D) as described by Eq. (6):

$$\mu = D = Q/V \quad (6)$$

where the dilution rate is determined as (Q) the ratio of the increase of mass in the outflow vessel as a consequence of inflow of fresh medium and the total volume (V) of the PBR.

The daily values for areal productivity (P_{area} in g m^{-2} d^{-1}) were calculated from Eq. (7):

$$P_{area} = (Q_{harvested}{*}DW_{PBR})/\text{Area} \quad (7)$$

where $Q_{harvested}$ (L day^{-1}) is the harvested volume per day; DW_{PBR} is the culture dry weight in the PBR.

The daily values for volumetric biomass productivity (P_{vol} in g L^{-1} d^{-1}) were calculated from Eq. (8):

$$P_{vol} = P_{area}{*}(\text{Area}/V) \quad (8)$$

where P_{area} is the areal productivity; and V (L) is the total volume of the PBR.

Hydraulic retention time (HRT in d^{-1}) was calculated with Eq. (9):

$$HRT = V/Q \quad (9)$$

2.7. Statistical analysis

The effects of the different pre-treatment methods on growth, total chlorophyll and removal efficiency were compared using Student's *t*-tests. For the pilot scale operation, a one way ANOVA followed by Tukey's post-hoc test was applied to test the impacts of continuous variables (temperature, solar radiation) on the different setups of the cultivation. The Games-Howell's post-hoc test was applied to investigate significant differences between the N:P ratio of the effluent. Significant differences in the uptake of N and P were identified using paired *t*-tests. All tests were performed with a 95% confidence interval using SPSS software (version 23).

3. Results and discussion

3.1. Screening of cyanobacteria and microalgae

Six cyanobacterial and seven microalgal strains from HAMBI and NORCCA were screened to evaluate the growth performance in the hydroponic effluent. The effluent was freshly collected prior to each experiment, and therefore nutrient concentrations varied during the study. Table 1 shows the chemical composition of the greenhouse hydroponic effluent used in the screening trial. The N:P ratio of the effluent (19) was slightly above the general optimum Redfield ratio for phytoplankton growth [28]. The nitrogen composition of the effluent was mainly dominated by a single N-source, nitrate, whereas both $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$ concentrations were negligible. The effluent used in this study had a lower content of organic matter (Table 1) than other reported sources of agricultural greenhouse effluents [29]. The screening trial demonstrated that the hydroponic effluent from cucumber cultivation is suitable for the growth of eukaryotic microalgae and cyanobacteria (Fig. 1).

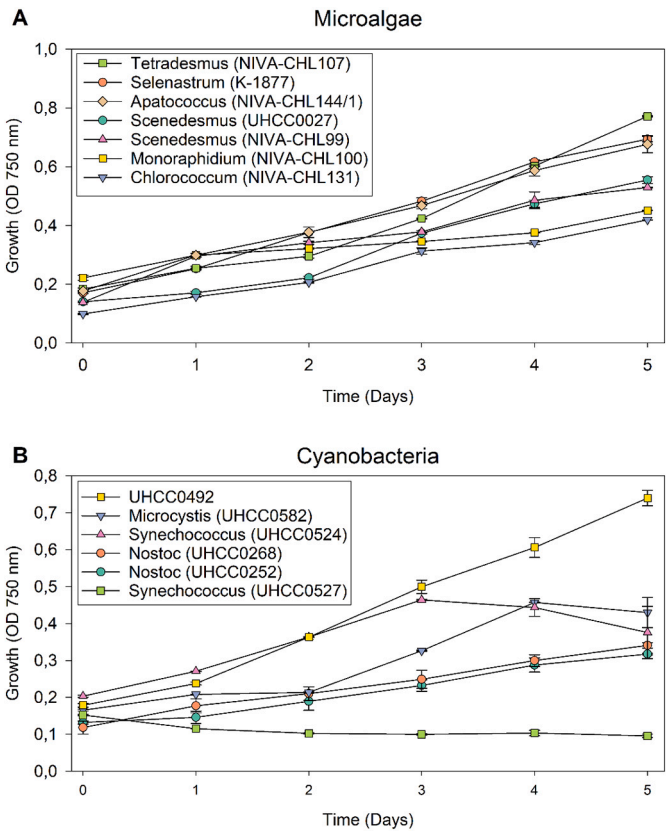

Fig. 1. Screening of microalgae (A) and cyanobacteria (B) grown in hydroponic greenhouse effluent. The cells were inoculated in 24 well plates. The data are represented as mean ± SE (n = 2).

The genus *Tetradesmus* demonstrated the best growth with OD750 = 0.77 on the fifth day followed by *Selenastrum* and *Apatococcus* genera which demonstrated steady growth but reached a slightly lower OD (Fig. 1A). The strains of *Scenedesmus* genus showed a similar performance achieving a maximum value of OD750 of 0.53 and 0.55 respectively by the end of the fifth day. The genera *Monoraphidium* and *Chlorococcum* displayed slow growth throughout the course of the experiment reaching a final OD750 of 0.45 and 0.42, respectively.

Among the studied cyanobacterial strains, UHCC0492 showed the most rapid growth reaching an OD750 similar to the best eukaryotic strains by the fifth day (Fig. 1B). The *Microcystis* genus had a lag phase of two days followed by a short phase of linear growth, and reached a stationary phase before the end of the trial. The two filamentous, heterocystous N_2-fixing *Nostoc* strains both displayed slow growth, although no lag phase was observed. This outcome will be further investigated as it would be expected that a sudden availability of macronutrients from the hydroponic effluent would improve the growth performance of these strains. The two *Synechococcus* strains were distinct in that UHCC0524 reached stationary phase on the third day, while UHCC0527 did not display any growth during the entire experiment. Microalgal strains belonging to the genera *Selenastrum*, *Scenedesmus* or *Tetradesmus* have been extensively used in municipal and industrial wastewater research [30–32]. Compared to other waste streams such as municipal or poultry wastewater, the hydroponic effluent presents a significantly lower COD and BOD content but higher amounts of nitrogen and phosphorous [33]. Lower COD and BOD content generally allows better light penetration which, combined with rich nutrient concentrations, demonstrates the suitability of hydroponic effluent as a growth medium. The genus *Apatococcus* represents a highly versatile group of mixotrophic photobionts often establishing symbiotic relationships with lichens and having the ability to survive in terrestrial

habitats as free-living cells or in biofilm colonies [34]. To the best of our knowledge, this was the first time an *Apatococcus* strain was applied for agricultural wastewater remediation, although the presence of this genus in wastewater streams has been previously reported [35]. The *Chlorococcum* genus has received significant attention due to its growth performance in wastewater streams and ability to synthesize high lipid content potentially suitable for biodiesel [36]. Likewise, the genus *Monoraphidium* has been known for the ability to proliferate in industrial wastewaters and accumulate saturated fatty acids appropriate for biodiesel [37]. The *Microcystis* genus is commonly identified as a dominant species in harmful algal blooms; however, it is worth noting that not all strains produce microcystins, and in this study a non-toxic strain was used. Its moderate growth in hydroponic effluent suggests that this isolate may not be as robust as previously reported strains. The *Nostoc* genus represents a group of N_2-fixing heterocystous filamentous cyanobacteria that have demonstrated ability to grow in sewage and synthetic wastewaters [38]. The unicellular *Synechococcus* strains are used in wastewater remediation either as immobilized or free-living organisms [39]. In this study, the inoculum of both *Synechococcus* strains were maintained as free-living cells in low-light intensity, and therefore light stress might have inhibited the growth of the UHCC0527 strain.

The results of the screening experiment confirmed that the hydroponic effluent is a promising alternative source of nutrients for photosynthetic microorganisms. Several microalgal and cyanobacterial strains can effectively proliferate in the effluent without any adjustment (e.g. pH, macro/micronutrients). The results also showed the potential of the NordAqua Nordic Culture Collection by uncovering several isolates suitable for bioremediation of hydroponic effluents. Considering our extensive knowledge of the performance of UHCC0027 in synthetic and municipal wastewaters and previous scale up experience [40,41] we continued to work with this Finnish isolate of the Scenedesmaceae family in the following upscaling experiments. Besides, the micronutrient concentrations of the hydroponic effluent (Table 1) were within growth promoting ranges for the *Scenedesmus* genus [42].

3.2. Comparison of different pre-treatment methods

The application of waste streams for the large scale cultivation of microalgae presents different challenges regarding the elimination of competitors, nutrient composition, and the content of organic matter. Employing suitable pre-treatment methodologies to the source and characteristics of any given effluent can aid the efficient proliferation of the seeded culture [43]. To this end, three different pre-treatments were compared for their potential impacts on the growth of UHCC0027: Coarse filtration (CF), microfiltration (MF), and bleach (BST). The selection criteria for the applied methods were the presence of native microorganisms in the hydroponic effluent and the feasibility of integration into a pilot scale PBR. A control group was grown in autoclaved BG11 medium. Non-seeded flasks containing only pre-treated effluent were also included to evaluate the impacts of each method on the indigenous microbial community.

The results demonstrated the ability of UHCC0027 to proliferate without significant differences between the tested pre-treatments (Fig. 2A, B). Additionally, there were no significant differences in the nutrient removal efficiencies with UHCC0027, demonstrating an ability to remove 36–52% and 99% for $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$, respectively (Fig. 2C). Interestingly, the non-seeded CF-treated medium demonstrated an increase in OD750 after 7 days of incubation (Fig. 2A). At day 12, the CF non-seeded medium displayed a significant increase in turbidity (OD750 = 2.66) followed by an increase of the total chlorophyll content, suggesting the presence of an indigenous photosynthetic microbial community (Fig. 2B). Assuming that indigenous microbes were also present in the CF-treated flasks seeded with UHCC0027 but its growth did not significantly differ from the control (UHCC0027 seeded in autoclaved BG11), it can be concluded that the chosen microalga was able to outcompete the indigenous microbial community. A negligible

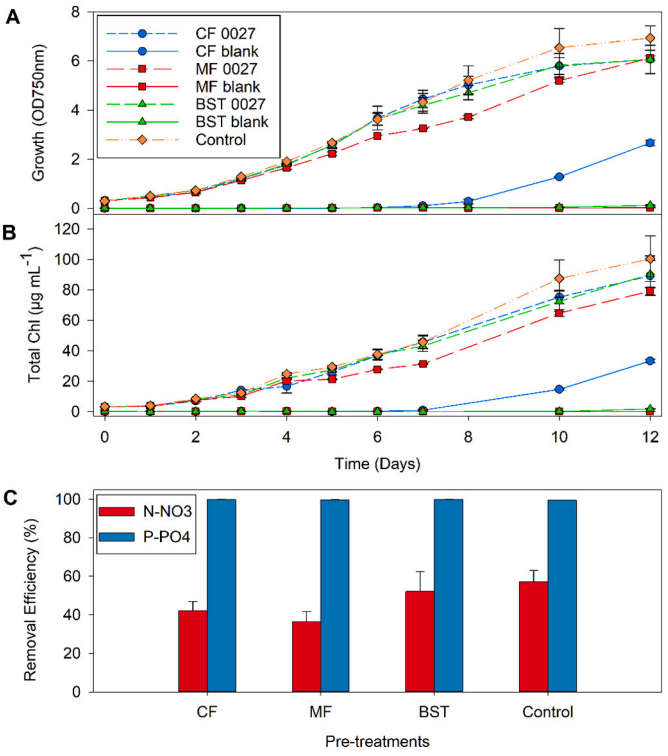

Fig. 2. Effect of different pre-treatments on the growth and nutrient removal efficiency of the UHCC0027 strain. Cell density at OD750 nm (A), total chlorophyll content (B) and nutrient removal efficiency (C). CF = coarse filtered effluent; CF 0027 = coarse filtered effluent seeded with UHCC0027; MF = micro-filtered effluent; MF 0027 = micro-filtered effluent seeded with UHCC0027; BST = bleach and sodium thiosulfate effluent; BST 0027 = bleach and sodium thiosulfate effluent seeded with UHCC0027; control = UHCC0027 seeded in autoclaved BG11. The data are represented as mean ± SE (n = 2).

increase in OD750 in non-seeded MF and BST flasks by the end of the experiment indicates that these methods are effective in reducing indigenous microbial activity.

The effluent used for pre-treatment experiments had an average concentration of 186.17 ± 2.94 mg L^{-1} for $N\text{-}NO_3^-$ and 11.77 ± 0.02 mg L^{-1} for $P\text{-}PO_4^{3-}$. The N:P ratio of 35 indicates an unbalanced nutrient ratio that can negatively affect the growth of *Scenedesmus* species with implications in lipid and protein content [44]. At 22 °C, over 90% of the $P\text{-}PO_4^{3-}$ was consumed in the beginning of our experiment, similar to what has been observed previously [45], although cellular adsorption has been reported to partially contribute to this process [46]. This may have enabled the growth of UHCC0027 for 2 weeks with only residual amounts of $P\text{-}PO_4^{3-}$ in the medium. Thus, uptake of $N\text{-}NO_3^-$ continued until all $P\text{-}PO_4^{3-}$ was consumed. The constant supply of 3% CO_2 also favored nutrient uptake by improving the C:N:P ratios during the experimental period until the nutrients were depleted from the medium [41].

At the end of the experiment, microscopic observations of the CF blank samples showed the complex food webs of the indigenous microbial community which were characterized by the presence of several taxonomic entities. In contrast, the microscopic observations of the seeded CF samples demonstrated the dominance of the microalga UHCC0027 (data not shown). Therefore, since the different pre-treatments did not significantly affect the growth or the nutrient removal efficiencies of UHCC0027, and, the culture was able to overcome the presence of indigenous microorganisms in lab scale experiments, a decision was made to combine the BST method with the two-step filtration system (CF followed by membrane filtration) for the PBR inoculation. However, the BST method was not applied during the

turbidostat mode to ensure the feasibility of daily operation and to facilitate handling large volumes of effluent.

3.3. Pilot scale PBR operation in a greenhouse

Pilot scale cultivation of UHCC0027 was undertaken between February and March of 2020 in a 65 L tubular PBR operated in a real greenhouse infrastructure. Prior to the inoculation of algae, the greenhouse illumination and heating systems were programmed and fresh hydroponic effluent was collected. The hydroponic effluent was sampled at a point before the slow-sand filtration system of the commercial cucumber greenhouse and channeled through the two-step filtration system before entering the PBR. Four different setups were evaluated for nutrient removal efficiency and strain performance. The different setups (Table 3) were tested sequentially and the impact on the performance of the UHCC0027 is listed in Table 4.

The UHCC0027 culture was inoculated in the PBR which was primarily operated in batch mode with a pH setpoint of 7.5. The culture grew exponentially until the fourth day, with a specific growth rate of 0.55 d^{-1}. The dynamics of the system showed a positive correlation between nutrient removal and daily biomass productivity (R^2 = 0.84–0.98, Fig. 3). The culture approached a stationary phase by the fifth day, at which point maximum removal efficiency was achieved.

For the batch cultivation, the hydroponic effluent had an N:P ratio of 18 with a concentration of 238.60 ± 0.48 mg L^{-1} of $N\text{-}NO_3^-$ and 29.72 ± 0.07 mg L^{-1} of $P\text{-}PO_4^{3-}$. The maximum removal efficiencies were 55.9% and 98.7% for $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$, respectively. The maximum DW was 1.43 ± 0.02 g L^{-1}, corresponding to OD880 = 1.75. The highest volumetric productivity of 0.47 g L^{-1} d^{-1} and areal productivity of 29.40 g m^{-2} d^{-1} were achieved during the late exponential phase.

To prevent the culture from reaching a stationary phase under continuous operation, the DW threshold was set to 1 g L^{-1} (Fig. 4). For ten consecutive days, UHCC0027 was cultivated under the conditions of Setup 1 (Table 3) with an average growth rate of 0.48 ± 0.06 d^{-1} and volumetric productivity of 0.46 ± 0.06 g L^{-1} d^{-1} (Table 4). Under the conditions of Setup 1, a daily average of 30 ± 4.04 L of hydroponic effluent was pumped into the PBR. The effluent had an average macronutrient composition of 249.12 ± 6.75 mg L^{-1} $N\text{-}NO_3^-$ and 31.53 ± 1.90 mg L^{-1} $P\text{-}PO_4^{3-}$, resulting in molar N:P ratios ranging from 16 to 19. During this period, UHCC0027 demonstrated an average N uptake of 89.55 ± 7.46 mg g^{-1} DW of algal biomass, and P uptake of 16.89 ± 2.02 mg g^{-1} DW, corresponding to average removal efficiencies of 35 ± 2.2% and 52 ± 4%, respectively (Fig. 5A, B). The values achieved for N uptake are in accordance with previous reports where *Scenedesmus* was cultivated in semi-continuous mode in outdoor flat-panel PBRs or open raceway ponds [47].

In Setup 2, the DW was lowered to one half, 0.5 g L^{-1}, which increased the average growth rate to 0.76 ± 0.09 d^{-1} and consequently the daily demand for hydroponic effluent to 48.07 ± 5.57 L d^{-1}. For this setup, the N:P ratio was substantially higher (20–27) than in Setup 1 (Table 3). Despite this, N and P uptakes did not differ significantly, with values of 92.81 ± 11.27 mg g^{-1} and 19.13 ± 3.55 mg g^{-1}, respectively (Fig. 5A). The removal efficiencies during this period were 18.8 ± 1.6% for $N\text{-}NO_3^-$ and 40 ± 7.6% for $P\text{-}PO_4^{3-}$. This decline in comparison with the previous setup can be explained by a combination of effects such as:

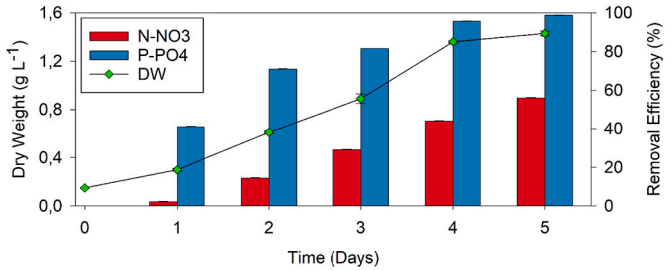

Fig. 3. The dynamics of nutrient removal efficiency and biomass production of UHCC0027 during the PBR batch mode. Data are represented as the average of three technical replicates of single daily sample measurements with a maximum SD for the removal efficiencies of 0.3%.

decreasing the biomass concentration inside the system; the significant differences in the effluent's N:P ratio between during setup 1 and setup 2; and the lower solar irradiance which could have affected the

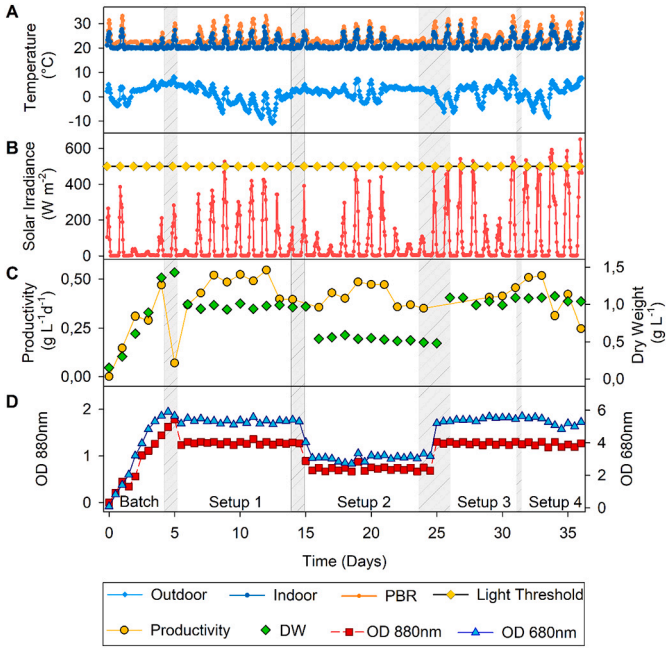

Fig. 4. The PBR operated in batch and turbidostat modes under continuous cultivation inside a greenhouse. Temperature (A) and solar irradiance (B) recorded for the greenhouse area during the PBR operation. The light threshold corresponds to the reference value upon which the greenhouse light system would automatically switch off. The productivity and dry weight of UHCC0027, data represents triplicate measurements of daily samples (C). The readings of the PBR sensors OD880 nm (red) and OD680 nm (blue) (D). The correlation coefficient of the calibration curve between the DW and the turbidity sensor was R^2 = 0.995. Grey bars show the transition periods between setups. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 4
The performance of UHCC0027 culture during the different setups of the continuous cultivation. Data show average values obtained over each setup with standard deviation (n = 10 for Setups 1 and 2; n = 5 for Setup 3; n = 4 for Setup 4).

Turbidostat operation	Setup 1	Setup 2	Setup 3	Setup 4
Growth rate (day^{-1})	0.48 ± 0.06	0.76 ± 0.09	0.44 ± 0.04	0.36 ± 0.11
Volumetric productivity (g L^{-1} d^{-1})	0.46 ± 0.06	0.41 ± 0.05	0.46 ± 0.05	0.38 ± 0.11
Areal productivity (g m^{-2} d^{-1})	29.10 ± 3.89	25.84 ± 3.33	29.12 ± 3.55	24.12 ± 7.23
HRT (days)	2.14 ± 0.31	1.33 ± 0.15	2.30 ± 0.21	3.05 ± 0.87
Total biomass (g DW)	61.14 ± 1.69	33.49 ± 1.97	66.22 ± 2.39	67.32 ± 1.85

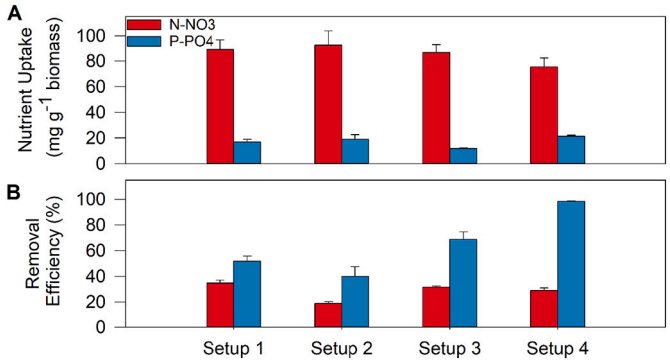

Fig. 5. The nutrient uptake (A) and removal efficiencies (B) of N-NO_3^- and P-PO_4^{3-} for each setup of the continuous cultivation. Data represent average values obtained over each setup with SD (n = 10 for Setup 1 and 2; n = 5 for Setup 3; n = 4 for Setup 4).

performance of UHCC0027 (Table 3, Fig. 5B). Nonetheless, when compared with the previous setup, the outcome of Setup 2 demonstrated no significant impact in terms of nutrient uptake when the DW of the culture was reduced to one half. Additionally, this result shows that Setup 1 was operated without limitation, presenting an opportunity for increased DW in future experiments. This could improve the removal efficiencies of the system.

The following setups, 3 and 4, were planned to evaluate the performance of the culture under varying pH. The transition period between Setups 2 and 3 was the longest undertaken, due to changes in both biomass concentration and pH (Table 3, Fig. 4). A two-step approach was used, whereby the culture was grown to a DW of 1 g L^{-1} before the pH was changed. In Setup 3, the pH was lowered to 6.8 and the hydroponic effluent had the highest N:P ratio, oscillating between 32 and 37 (Table 3). This variability did not compromise N uptake which remained stable at 86.68 ± 6.5 mg g^{-1}, but led to a significant reduction of P uptake to 11.84 ± 0.42 mg g^{-1} ($p < 0.05$, Fig. 5A). The removal efficiencies were 31.5 ± 0.9% for N-NO_3^- and 68.9 ± 5.9% for P-PO_4^{3-}. The volumetric productivity remained stable within the same ranges of previous setups (Fig. 4, Table 4). By this time, the P-PO_4^{3-} in the hydroponic effluent was at its lowest concentration which explains the increase in the removal efficiency (Table 3, Fig. 5B). The stress induced by different setups, particularly from this transition period onwards, was observed as a gradual biofouling effect.

In Setup 4, pH was increased from 6.8 to 9 and the N:P ratio varied between 26 and 27. These factors, in combination with several days of high solar radiation (Fig. 4) led to a significant increase of P uptake to its maximum value of 21.30 ± 0.96 mg per 1 g DW ($p < 0.05$). During this period, the system met the requirements of the EU directive (91/271/EEC) for wastewater maximum nutrient discharge for P-PO_4^{3-}. However, the growth rate was lower than in the previous setups, which contributed to a longer HRT (Table 4). The contribution of biofouling to this outcome should also be considered, since it continued to accumulate throughout Setup 4. Shifting the pH from 6.8 to 9 led to changes in dissolved inorganic carbon species from CO_2 to HCO_3. The supply of CO_2 on demand through setpoint injection may have created a scenario where different inorganic carbon species were present simultaneously. This detail may have caused extra stress to the culture if the cellular metabolism was activated to favor the absorption of one inorganic carbon source to the detriment of the other. Nonetheless, loss of growth rate due to a pH as high as 9 has previously been reported for *Scenedesmus* strains [48].

The N:P ratio of the hydroponic effluent varied significantly between each setup of the PBR ($p < 0.05$). During continuous cultivation, the N:P ratio of the hydroponic effluent varied between 16 and 37, which did not affect the biomass productivity to the extent observed for the chlorophyte *C. vulgaris* grown in municipal wastewater [49]. Our data suggest no strong correlation between the N:P ratio and the volumetric productivity throughout the course of the experiment. This outcome may result from the high concentrations and constant availability of N-NO_3^- and P-PO_4^{3-} in the media as well as the cellular physiological saturation of nutrient uptake. The scenario of nutrient repletion throughout the course of continuous operation is a likely factor in the moderate removal efficiencies observed for N-NO_3^- and P-PO_4^{3-}. This, combined with cultivation in a PBR and the steady abiotic conditions provided by the greenhouse infrastructure may also explain the higher biomass productivity observed compared to other studies on *Scenedesmus* species [50]. Additionally, due to the nutrient replete conditions, it can be hypothesized that the rate of assimilation did not follow a linear relationship with the rate of supply. This trend is described by the theoretical kinetics model of Michaelis-Menten and has been observed by n batch experiments [49,51]. Despite this, luxury uptake of nutrients may have occurred during the last setup of the PBR run, since the growth rate was at its lowest, but the nutrient uptake was not substantially suppressed (Fig. 5, Table 4).

In a continuous cultivation system, the HRT is negatively correlated with the growth rate of the species. If the growth rate decreases, the inflow of media is reduced, which increases the retention time. This trend was verified throughout the course of the experiment, indicating that the transition periods were crucial to stabilize the culture between each setup (Fig. 4, Table 4). Longer retention times yielded higher amounts of biomass to a total maximum of 90.2 ± 0.02 g of DW obtained at the 5th day of the batch operation. During the turbidostat operation the maximum value of total biomass was 67.32 ± 1.85 g of DW, observed in the last period of the PBR run (Table 4). In our system, the coefficient of correlation between the turbidity sensor and the DW was obtained with a suspended algal culture. Therefore, the gradual accumulation of biofilm presented a threat to the sensor light path and turbidostat operation. For that reason, the PBR was stopped after 36 days of continuous operation and over 1 m^3 of hydroponic effluent circulated.

3.4. Greenhouse cultivation conditions

The outdoor weather conditions during the pilot-scale experiment fluctuated greatly, with temperatures ranging from −11 to +8 °C and periods of low solar intensity (Fig. 4). During the experiment, the day length increased from ≈9 h in mid-February to ≈13 h in late March. The heating and light systems of the greenhouse infrastructure were essential to mitigate such harsh Nordic conditions. The temperature systems were able to keep a constant atmosphere of 21 ± 2.18 °C throughout the course of the experiment; the average temperature inside the PBR was maintained at 23.80 ± 2.86 °C. However, on cloudless sunny days, the temperatures inside the greenhouse and the PBR exceeded 30 °C to a maximum record of 34 °C registered during Setup 4. Nonetheless, this range is within the reported temperatures associated with maximum biological productivity for *Scenedesmus* strains [52]. A substantial increase in productivity was recorded whenever temperatures were above 25 °C and solar irradiance averaged or exceeded 400 W m^{-2} (Fig. 4A, B, C). In contrast, on low solar radiation days, the greenhouse light system prevented any sharp declines in productivity and possibly prevented a collapse of the culture. The rapid response of the culture to such stimulus suggests that light intensity, which in this study results from the combined effect of the greenhouse light system and the solar radiation that penetrated through the greenhouse infrastructure, did not trigger light-induced stress. This is also supported by the steadiness of the relative chlorophyll content sensor during the entire experiment (Fig. 3). However, long exposures to solar radiation levels above the threshold level of 400 W m^{-2} coupled with temperatures above 30 °C may have negatively interfered with UHCC0027 productivity at the end of the experiment (Fig. 3, Table 4). Even so, the tolerance to light intensity and the efficient conversion of light into biomass are known to be strain specific characteristics and intrinsically linked to parameters such as the angle of incidence, composition and quality of light, the cultivation system and

nutrient availability [53].

4. Conclusion

This work proved the potential of the biodiversity of Nordic culture collections in providing sustainable solutions to treat hydroponic effluents, and the possibility of using existing greenhouse infrastructure to cultivate Nordic microalgae, thus allowing cultivation under harsh climatic conditions. Several promising species were identified and a robust microalga was able to proliferate in a pilot-scale PBR in unadjusted hydroponic effluent. The cultivation process achieved EU discharge values for $P\text{-}PO_4^{3-}$ but needs further optimization regarding the removal efficiencies of $N\text{-}NO_3^-$. Future strategies can focus on adjusting the N:P ratio of the effluent to achieve better nutrient removal efficiencies for a fully sustainable operation. The effect of different artificial light sources such as LED's might also be evaluated. The use of a greenhouse infrastructure to provide artificial light and temperature for the cultivation of microalgae creates an exciting opportunity for microalgae bioremediation in Nordic countries.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

CRediT authorship contribution statement

João Salazar: Conceptualization, Methodology, Formal analysis, Data curation, Writing – original draft. **Dimitar Valev:** Methodology, Writing – review & editing. **Juha Näkkilä:** Resources. **Esa Tyystjärvi:** Writing – review & editing. **Sema Sirin:** Conceptualization, Methodology, Data curation, Supervision, Writing – original draft. **Yagut Allahverdiyeva:** Conceptualization, Supervision, Writing – original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] E-yearbook of Food and Natural Resource Statistics for 2019: Statistical Facts on Agriculture, Forestry, Fisheries and Hunting in Finland, Natural Resources Institute Finland (Luke).
[2] L. Cifuentes-Torres, L.G. Mendoza-Espinosa, G. Correa-Reyes, L.W. Daesslé, Hydroponics with wastewater: a review of trends and opportunities, Water Environ. J. (2020), wej.12617, https://doi.org/10.1111/wej.12617.
[3] C. Lee, D.S. Kim, Y. Kwack, C. Chun, Waste nutrient solution as an alternative fertilizer in curled mallow cultivation, JAS. 12 (2020) 55, https://doi.org/10.5539/jas.v12n3p55.
[4] M. Rufí-Salís, M.J. Calvo, A. Petit-Boix, G. Villalba, X. Gabarrell, Exploring nutrient recovery from hydroponics in urban agriculture: an environmental assessment, Resour. Conserv. Recycl. 155 (2020), 104683, https://doi.org/10.1016/j.resconrec.2020.104683.
[5] P. Sambo, C. Nicoletto, A. Giro, Y. Pii, F. Valentinuzzi, T. Mimmo, P. Lugli, G. Orzes, F. Mazzetto, S. Astolfi, R. Terzano, S. Cesco, Hydroponic solutions for soilless production systems: issues and opportunities in a smart agriculture perspective, Front. Plant Sci. 10 (2019) 923, https://doi.org/10.3389/fpls.2019.00923.
[6] F.G. Acién Fernández, C. Gómez-Serrano, J.M. Fernández-Sevilla, Recovery of nutrients from wastewaters using microalgae, Front. Sustain. Food Syst. 2 (2018) 59, https://doi.org/10.3389/fsufs.2018.00059.
[7] D. Nagarajan, D.-J. Lee, C.-Y. Chen, J.-S. Chang, Resource recovery from wastewaters using microalgae-based approaches: a circular bioeconomy perspective, Bioresour. Technol. 302 (2020), 122817, https://doi.org/10.1016/j.biortech.2020.122817.
[8] P.S.C. Schulze, C.F.M. Carvalho, H. Pereira, K.N. Gangadhar, L.M. Schüler, T. F. Santos, J.C.S. Varela, L. Barreira, Urban wastewater treatment by *Tetraselmis* sp. CTP4 (Chlorophyta), Bioresour. Technol. 223 (2017) 175–183, https://doi.org/10.1016/j.biortech.2016.10.027.
[9] D. De Francisci, Y. Su, A. Iital, I. Angelidaki, Evaluation of microalgae production coupled with wastewater treatment, Environ. Technol. 39 (2018) 581–592, https://doi.org/10.1080/09593330.2017.1308441.
[10] J. Hoffman, R.C. Pate, T. Drennen, J.C. Quinn, Techno-economic assessment of open microalgae production systems, Algal Res. 23 (2017) 51–57, https://doi.org/10.1016/j.algal.2017.01.005.
[11] M. Singh, K.C. Das, Low cost nutrients for algae cultivation, in: R. Bajpai, A. Prokop, M. Zappi (Eds.), Algal Biorefineries, Springer Netherlands, Dordrecht, 2014, pp. 69–82, https://doi.org/10.1007/978-94-007-7494-0_3.
[12] M.S. Chauton, K.I. Reitan, N.H. Norsker, R. Tveterås, H.T. Kleivdal, A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: research challenges and possibilities, Aquaculture. 436 (2015) 95–103, https://doi.org/10.1016/j.aquaculture.2014.10.038.
[13] P.C. Oostlander, J. van Houcke, R.H. Wijffels, M.J. Barbosa, Microalgae production cost in aquaculture hatcheries, Aquaculture. 525 (2020), 735310, https://doi.org/10.1016/j.aquaculture.2020.735310.
[14] F.G. Acién, J.M. Fernández, J.J. Magán, E. Molina, Production cost of a real microalgae production plant and strategies to reduce it, Biotechnol. Adv. 30 (2012) 1344–1353, https://doi.org/10.1016/j.biotechadv.2012.02.005.
[15] F.G.A. Fernández, A. Reis, R.H. Wijffels, M. Barbosa, V. Verdelho, B. Llamas, The role of microalgae in the bioeconomy, New Biotechnol. 61 (2021) 99–107, https://doi.org/10.1016/j.nbt.2020.11.011.
[16] F.G. Acién Fernández, J.M. Fernández Sevilla, E. Molina Grima, Costs analysis of microalgae production, in: Biofuels from Algae, Elsevier, 2019, pp. 551–566, https://doi.org/10.1016/B978-0-444-64192-2.00021-4.
[17] K. Li, Q. Liu, F. Fang, R. Luo, Q. Lu, W. Zhou, S. Huo, P. Cheng, J. Liu, M. Addy, P. Chen, D. Chen, R. Ruan, Microalgae-based wastewater treatment for nutrients recovery: a review, Bioresour. Technol. 291 (2019), 121934, https://doi.org/10.1016/j.biortech.2019.121934.
[18] F.G. Gentili, J. Fick, Algal cultivation in urban wastewater: an efficient way to reduce pharmaceutical pollutants, J. Appl. Phycol. 29 (2017) 255–262, https://doi.org/10.1007/s10811-016-0950-0.
[19] J. Lowrey, M.S. Brooks, P.J. McGinn, Heterotrophic and mixotrophic cultivation of microalgae for biodiesel production in agricultural wastewaters and associated challenges—a critical review, J. Appl. Phycol. 27 (2015) 1485–1498, https://doi.org/10.1007/s10811-014-0459-3.
[20] M.N. Metsoviti, G. Papapolymerou, I.T. Karapanagiotidis, N. Katsoulas, Comparison of growth rate and nutrient content of five microalgae species cultivated in greenhouses, Plants. 8 (2019) 279, https://doi.org/10.3390/plants8080279.
[21] A. Millán-Oropeza, L. Fernández-Linares, Biomass and lipid production from *Nannochloropsis oculata* growth in raceway ponds operated in sequential batch mode under greenhouse conditions, Environ. Sci. Pollut. Res. 24 (2017) 25618–25626, https://doi.org/10.1007/s11356-016-7013-6.
[22] L. Ferro, Y.O.O. Hu, F.G. Gentili, A.F. Andersson, C. Funk, DNA metabarcoding reveals microbial community dynamics in a microalgae-based municipal wastewater treatment open photobioreactor, Algal Res. 51 (2020), 102043, https://doi.org/10.1016/j.algal.2020.102043.
[23] J. Kotai, Instructions for Preparation of Modified Nutrient Solution Z8 for Algae vol. 11, Norwegian Institute for Water Research, Oslo, 1972, p. 5 (69).
[24] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, Microbiology. 111 (1979) 1–61, https://doi.org/10.1099/00221287-111-1-1.
[25] R.J. Porra, W.A. Thompson, P.E. Kriedemann, Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy, Biochim. Biophys. Acta (BBA) Bioenerg. 975 (1989) 384–394, https://doi.org/10.1016/S0005-2728(89)80347-0.
[26] D. Valev, H. Silva Santos, E. Tyystjärvi, Stable wastewater treatment with *Neochloris oleoabundans* in a tubular photobioreactor, J. Appl. Phycol. 32 (2020) 399–410, https://doi.org/10.1007/s10811-019-01890-x.

[27] American Public Health Association, American Water Works Association, Water Pollution Control Federation, Water Environment Federation, Standard Methods for the Examination of Water and Wastewater 1992, 1992.
[28] A.C. Redfield, The biologial control of chemical factors in the environment, Am. Sci. 46 (1958), 230A–221.
[29] D.C. Seo, S.H. Hwang, H.J. Kim, J.S. Cho, H.J. Lee, R.D. DeLaune, A. Jugsujinda, S. T. Lee, J.Y. Seo, J.S. Heo, Evaluation of 2- and 3-stage combinations of vertical and horizontal flow constructed wetlands for treating greenhouse wastewater, Ecol. Eng. 32 (2008) 121–132, https://doi.org/10.1016/j.ecoleng.2007.10.007.
[30] J. Sun, H. Simsek, Bioavailability of wastewater derived dissolved organic nitrogen to green microalgae *Selenastrum capricornutum*, *Chlamydomonas reinhardtii*, and *Chlorella vulgaris* with/without presence of bacteria, J. Environ. Sci. 57 (2017) 346–355, https://doi.org/10.1016/j.jes.2016.12.017.
[31] I. Ambat, S. Bec, E. Peltomaa, V. Srivastava, A. Ojala, M. Sillanpää, A synergic approach for nutrient recovery and biodiesel production by the cultivation of microalga species in the fertilizer plant wastewater, Sci. Rep. 9 (2019), 19073, https://doi.org/10.1038/s41598-019-55748-w.
[32] S. Ma, Y. Yu, H. Cui, R.S. Yadav, J. Li, Y. Feng, Unsterilized sewage treatment and carbohydrate accumulation in *Tetradesmus obliquus* PF3 with CO_2 supplementation, Algal Res. 45 (2020), 101741, https://doi.org/10.1016/j.algal.2019.101741.
[33] V. Patrinou, O.N. Tsolcha, T.I. Tatoulis, N. Stefanidou, M. Dourou, M. Moustaka-Gouni, G. Aggelis, A.G. Tekerlekopoulou, Biotreatment of poultry waste coupled with biodiesel production using suspended and attached growth microalgal-based systems, Sustainability. 12 (2020) 5024, https://doi.org/10.3390/su12125024.
[34] M. Zahradníková, H.L. Andersen, T. Tønsberg, A. Beck, Molecular evidence of *Apatococcus*, including *A. fuscideae* sp. nov., as photobiont in the genus *Fuscidea*, Protist. 168 (2017) 425–438, https://doi.org/10.1016/j.protis.2017.06.002.
[35] W.M. Kirumba, D.D. Shushu, H. Masundire, N. Oyaro, Diversity of Algae and Potentially Toxic Cyanobacteria in a River Receiving Treated Sewage Effluent: A Case of Notwane River (Gaborone, Botswana), 2014.
[36] V.D. Tsavatopoulou, A.F. Aravantinou, I.D. Manariotis, Biofuel conversion of *Chlorococcum* sp. and *Scenedesmus* sp. biomass by one- and two-step transesterification, Biomass Conv. Bioref. (2019), https://doi.org/10.1007/s13399-019-00541-y.
[37] G. Pineda-Camacho, F. de M. Guillén-Jiménez, A. Pérez-Sánchez, L.M. Raymundo-Núñez, G. Mendoza-Trinidad, Effect of CO_2 on the generation of biomass and lipids by *Monoraphidium contortum*: a promising microalga for the production of biodiesel, Bioresour. Technol. Rep. 8 (2019), 100313, https://doi.org/10.1016/j.biteb.2019.100313.
[38] M.M. El-Sheekh, W.A. El-Shouny, M.E. Osman, E.W. El-Gammal, Treatment of sewage and industrial wastewater effluents by the cyanobacteria *Nostoc muscorum* and Anabaena subcylinderica, J. Water Chem. Technol. 36 (2014) 190–197, https://doi.org/10.3103/S1063455X14040079.
[39] B. Aguilar-May, M. del Pilar Sánchez-Saavedra, Growth and removal of nitrogen and phosphorus by free-living and chitosan-immobilized cells of the marine cyanobacterium *Synechococcus elongatus*, J. Appl. Phycol. 21 (2009) 353–360, https://doi.org/10.1007/s10811-008-9376-7.
[40] M. Jämsä, F. Lynch, A. Santana-Sánchez, P. Laaksonen, G. Zaitsev, A. Solovchenko, Y. Allahverdiyeva, Nutrient removal and biodiesel feedstock potential of green alga UHCC00027 grown in municipal wastewater under Nordic conditions, Algal Res. 26 (2017) 65–73, https://doi.org/10.1016/j.algal.2017.06.019.
[41] F. Lynch, A. Santana-Sánchez, M. Jämsä, K. Sivonen, E.-M. Aro, Y. Allahverdiyeva, Screening native isolates of cyanobacteria and a green alga for integrated wastewater treatment, biomass accumulation and neutral lipid production, Algal Res. 11 (2015) 411–420, https://doi.org/10.1016/j.algal.2015.05.015.
[42] J. Liu, K. Tan, L. He, Y. Qiu, W. Tan, Y. Guo, Z. Wang, W. Sun, Effect of limitation of iron and manganese on microalgae growth in fresh water, Microbiology. 164 (2018) 1514–1521, https://doi.org/10.1099/mic.0.000735.
[43] A. Guldhe, S. Kumari, L. Ramanna, P. Ramsundar, P. Singh, I. Rawat, F. Bux, Prospects, recent advancements and challenges of different wastewater streams for microalgal cultivation, J. Environ. Manag. 203 (2017) 299–315, https://doi.org/10.1016/j.jenvman.2017.08.012.
[44] Z. Arbib, J. Ruiz, P. Álvarez-Díaz, C. Garrido-Pérez, J. Barragan, J.A. Perales, Photobiotreatment: influence of nitrogen and phosphorus ratio in wastewater on growth kinetics of *Scenedesmus Obliquus*, Int. J. Phytoremediat. 15 (2013) 774–788, https://doi.org/10.1080/15226514.2012.735291.
[45] J. Ruiz, P.D. Álvarez-Díaz, Z. Arbib, C. Garrido-Pérez, J. Barragán, J.A. Perales, Performance of a flat panel reactor in the continuous culture of microalgae in urban wastewater: prediction from a batch experiment, Bioresour. Technol. 127 (2013) 456–463, https://doi.org/10.1016/j.biortech.2012.09.103.
[46] M. Martínez, Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*, Bioresour. Technol. 73 (2000) 263–272, https://doi.org/10.1016/S0960-8524(99)00121-2.
[47] E. Eustance, S. Badvipour, J.T. Wray, M.R. Sommerfeld, Biomass productivity of two *Scenedesmus* strains cultivated semi-continuously in outdoor raceway ponds and flat-panel photobioreactors, J. Appl. Phycol. 28 (2016) 1471–1483, https://doi.org/10.1007/s10811-015-0710-6.
[48] G. Hodaifa, S. Sánchez Ma.E. Martínez, Influence of pH on the culture of *Scenedesmus obliquus* in olive-mill wastewater, Biotechnol. Bioproc. E. 14 (2009) 854–860, https://doi.org/10.1007/s12257-009-0119-7.
[49] H.J. Choi, S.M. Lee, Effect of the N/P ratio on biomass productivity and nutrient removal from municipal wastewater, Bioprocess Biosyst. Eng. 38 (2015) 761–766, https://doi.org/10.1007/s00449-014-1317-z.
[50] S.-H. Ho, C.-Y. Chen, J.-S. Chang, Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N, Bioresour. Technol. 113 (2012) 244–252, https://doi.org/10.1016/j.biortech.2011.11.133.
[51] S. Aslan, I.K. Kapdan, Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae, Ecol. Eng. 28 (2006) 64–70, https://doi.org/10.1016/j.ecoleng.2006.04.003.
[52] J.F. Sánchez, J.M. Fernández-Sevilla, F.G. Acién, M.C. Cerón, J. Pérez-Parra, E. Molina-Grima, Biomass and lutein productivity of *Scenedesmus almeriensis*: influence of irradiance, dilution rate and temperature, Appl. Microbiol. Biotechnol. 79 (2008) 719–729, https://doi.org/10.1007/s00253-008-1494-2.
[53] P.M. Slegers, P.J.M. van Beveren, R.H. Wijffels, G. van Straten, A.J.B. van Boxtel, Scenario analysis of large scale algae production in tubular photobioreactors, Appl. Energy 105 (2013) 395–406, https://doi.org/10.1016/j.apenergy.2012.12.068.