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ADVANCING AGRICULTURAL SUSTAINABILITY THROUGH MICROALGAE BIOTECHNOLOGY INNOVATIONS

João Rocha Salazar

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SARJA – SER. AI OSA – TOM. 729 | ASTRONOMICA – CHEMICA – PHYSICA – MATHEMATICA | TURKU 2024

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University of Turku

Faculty of Technology
Department of Life Technologies
Molecular Plant Biology
Doctoral Programme in Technology (DPT)

Supervised by

Professor Yagut Allahverdiyeva-Rinne Photosynthetic Microbes Molecular Plant Biology unit Department of Life Technologies Faculty of Technology University of Turku Turku, Finland	PhD Sema Sirin Photosynthetic Microbes Molecular Plant Biology unit Department of Life Technologies Faculty of Technology University of Turku Turku, Finland

Reviewed by

Professor, Gabriel Acién Department of Chemical Engineer CIESOL Solar Energy Research Centre University of Almería Almería, Spain	Professor, Raúl Muñoz Torre Institute of Sustainable Processes School of Industrial Engineering University of Valladolid Valladolid, Spain

Opponent

Assoc. Prof., PhD, Dorinde Kleinegris
University of Bergen
Principal Investigator Microalgae
Norce Norwegian Research Centre
Bergen, Norway

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“If I have seen further, it is by standing on the shoulders of giants”
Isaac Newton

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ABSTRACT

Technological advancements in agriculture have significantly contributed to the rise of soilless techniques such as hydroponic farming, claiming to be a more sustainable approach when compared to soil-based agriculture. Despite the efforts to be more sustainable, nutrient concentrations are far from optimized and hydroponic wastewaters are overloaded with nutrients when discharged into nature. In the present study, the principles of circular economy were applied to explore the potential of microalgae biotechnology for purifying hydroponic wastewaters from a commercial greenhouse. Initially, the quality of the hydroponic wastewater was evaluated at laboratory scale, and screening trials were performed to identify promising microalgae species. Afterwards, the process was scaled-up to a glass greenhouse using an indoor photobioreactor (PBR) equipped with artificial light. Finally, several microalgae species were tested for their potential as novel plant biostimulants and biopesticide agents. The laboratory results confirmed the quality of the hydroponic wastewaters and identified several photosynthetic microorganisms capable of growing in it. Two promising species were selected for the experiments using the indoor PBR. In our first attempt, the microalgae *Scenedesmus obliquus* was cultivated in continuous mode, recirculating over 1000L of hydroponic wastewater for more than a month of operation. In the following attempt, a batch mode cultivation was performed using the microalgae *Tetradesmus obliquus*. The species achieved a density of over 6 g L^{-1} and a maximum N uptake of 132.5 ± 14.0 mg g^{-1}. The experiments regarding the bioactivities of microalgae biomass revealed that extracts from *Chlorella sorokiniana* can inhibit fungal growth by as much as 50%. In addition to that, extracts from *T. obliquus* increased the fresh weight of lettuce by more than 15%. Overall, this work reveals the potential of microalgae biotechnology to accelerate sustainable solutions in agriculture.

KEYWORDS: hydroponic, wastewater, microalgae, biopesticides, biostimulants

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TIIVISTELMÄ

Uudenlaiset teknologiat maatalouden alalla ovat mahdollistaneet viljelyn ilman maaperän käyttöä. Näihin teknologioihin kuuluu esimerkiksi vesiviljely, jota pidetään ympäristöystävällisempänä kuin perinteinen multaa hyödyntävä viljely. Vaikka vesiviljelyä voidaan pitää kestävämpänä menetelmänä, siitä tulevat jätevedet sisältävät vielä runsaasti hyödyntämättä jääneitä ravinteita, jotka päätyvät vesistöihin. Tässä väitöskirjatutkimuksessa tutkittiin tapoja vähentää kaupallisen vesiviljelylaitoksen ravinnekuormaa kiertotalousajattelun näkökulmasta käyttämällä mikroleviin perustuvaa bioteknologiaa. Tutkimuksen alussa vesiviljelylaitoksen jäteveden laatu karakterisoitiin, jonka jälkeen seulottiin vedenpuhdistukseen parhaiten sopivimmat mikrolevälajit. Tämän jälkeen pudistusprosessi skaalattiin kasvihuonetasolle, jossa levä kasvatettiin käyttäen fotobioreaktoria ja keinovalaistusta. Tutkimuksen viimeisessä osassa tutkittiin useiden mikrolevälajien käyttöä biostimulantteina ja biologisina torjunta-aineina. Laboratoriotulosten perusteella löydettiin useita mikrolevälajeja, joita pystyttiin kasvattamaan vesikasvatuksesta tulevassa jätevedessä. Kaksi lupaavinta mikrolevälajia valittiin fotobioreaktorikokeisiin: *S. obliquus* -mikrolevää kasvatettiin onnistuneesti jatkuvana kasvatuksena 1000 litran jätevesimäärässä yli kuukauden ajan, jonka jälkeen *T. obliquus* -mikrolevää kasvatettiin panoskasvatuksen avulla. Molemmat lajit kasvoivat eri tutkimusasetelmissa yli 6 g L^{-1} tiheyteen ja ne sitoivat typpeä enimmillään 132,5 ± 14.0 mg g^{-1}. Tutkimus mikrolevien bioaktiivisuuksista osoitti, että *C. sorokiniana* ehkäisi sieniperäisten organismien kasvua jopa 50 %. Tämän lisäksi *T. obliquus* lisäsi salaatin kasvua tuorepainona mitattuna yli 15 %. Tämä väitöskirjatutkimus osoittaa, että mikroleviin perustuvaa bioteknologiaa voidaan hyödyntää kestävän maatalouden edistämiseksi.

ASIASANAT: vesiviljely, jätevesi, mikrolevä, biologinen torjunta-aine, biostimulantti

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Abbreviations

BOD	Biological Oxygen Demand
CAPEX	Capital Expenditures
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
DW	Dry Weight
EABA	European Algae Biomass Association
EC	European Commission
EEA	European Environmental Agency
EU	European Union
FAME	Fatty Acid Methyl Ester
GC-MS	Gas Chromatography - Mass Spectrometry
HPLC-DAD	High Performance Liquid Chromatography - Diode Array Detection
LED	Light-Emitting Diode
MUFA	Monounsaturated Fatty Acids
N:P	Nitrogen:Phosphorus
$N\text{-}NH_4$	Nitrogen in the form of Ammonium
$N\text{-}NO_3^-$	Nitrogen in the form of Nitrate
$[N\text{-}NO_3^-]$	Concentration of Nitrogen in the form of Nitrate
$[P\text{-}PO_4^{3-}]$	Concentration of Phophorus in the form of Phosphate
O_2	Oxygen
OD	Optical Density
OPEX	Operating Expenditures
PAR	Photosynthetic Active Radiation
PBR	Photobioreactor
PPFD	Photosynthetic Photo Flux Density
$P\text{-}PO_4^{3-}$	Phosphorus in the form of Phosphate
PUFA	Polyunsaturanted Fatty Acids
SFA	Saturated Fatty Acids
UWWTD	Urban Wastewater Treatment Directive

List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Salazar, J., Valev, D., Näkkilä, J., Tyystjärvi, E., Sirin, S., & Allahverdiyeva, Y. Nutrient removal from hydroponic effluent by Nordic microalgae: from screening to a greenhouse photobioreactor operation. *Algal Research*, 2021; 55, 102247.

II Salazar, J., Santana-Sánchez,A., Näkkilä, J., Sirin, S., Allahverdiyeva, Y. Complete N and P removal from hydroponic greenhouse wastewater by *Tetradesmus obliquus*: a strategy for algal bioremediation and cultivation in Nordic countries. *Algal Research*, 2023; 70, 102988.

III Chovancek, E., Salazar, J., Sirin, S., Allahverdiyeva, Y. Microalgae from Nordic collections demonstrate biostimulant effect by enhancing plant growth and photosynthetic performance. *Physiologia Plantarum*, 2023; e13911

IV Jokel, M., Salazar, J., Chovancek, E., Sirin, S., Allahverdiyeva, Y. Screening of several microalgae revealed biopesticide properties of *Chlorella sorokiniana* against the strawberry pathogen *Phytophthora cactorum*. *Journal of Applied Phycology*, 2023; 1-13

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Author's Contribution

I	Salazar, J. planned the experimental design in collaboration with the coauthors and was responsible for the laboratory experiments, the trials with the PBR in the greenhouse and the data curation. Salazar, J., wrote the manuscript with the contributions of the coauthors.
II	Salazar, J. planned the experimental design in collaboration with the coauthors and performed the trials with the PBR, in addition to the biochemical characterization of the biomass and the data curation. Salazar, J., wrote the manuscript with the contributions of the coauthors.
III	Salazar, J. conducted part of the experiments related with the preparation of microalgae extracts and contributed to the discussion and revision of the manuscript.
IV	Salazar, J. performed the biomass extractions and fractionated the extracts according to the experimental design. Salazar, J. contributed to the writing of the manuscript.

1 Introduction

1.1 Wastewater pollution and the state of the Baltic Sea

Contaminated water streams, often referred to as wastewaters, are defined as any used water body resulting from anthropogenic activities and containing high levels of pollutants. Depending on their origin, wastewaters are known to possess a wide variety of physicochemical properties that can include the presence of micropollutants (e.g. microplastics), mineral nutrients (e.g., nitrogen and phosphorus), dissolved organic matter, as well as biological and chemical contaminants (e.g., pathogenic bacteria, heavy metals) (Admirasari et al., 2022; Perera et al., 2022). Regardless of major progress made in the last decades, numerous conventional wastewater treatment facilities are equipped with outdated technologies and infrastructure that were not initially designed to effectively purify such a wide range of contaminants and meet the needs of densely populated regions (Kehrein et al., 2020; Qadir et al., 2020).

The Urban Wastewater Treatment Directive (UWWTD) is the legal European framework for the correct collection and treatment of urban and industrial wastewater. Despite the significant improvements in water quality since its implementation in 1991, the European Commission and the European Environmental Agency recently recognized that approximately 15% of the wastewater collected is still poorly treated and in some Member States more than 10% of the population is still not connected to adequate wastewater treatment plants (European Environment Agency., 2022; Eurostat, 2019). As a result, a significant portion of urban and industrial wastewater across Europe is frequently released to nature with insufficient treatment. This problematic trend is expected to become increasingly worse in the next decades because of the growth of population and the linear economic model that supports it. For all these reasons, the European Commission estimates that an investment of more than 250 billion € will be needed until 2030, to update wastewater treatment plants across all Member States and initiate the transition towards the zero-pollution goals of the European Green Deal (European Commission, 2019) .

The Baltic Sea region is a particularly sensitive area for the discharge of nutrient rich wastewater. Its natural semi-enclosed shape and relatively shallow topography reduce water exchanges with the Nordic Sea and significantly increase the residence time of wastewaters in the area (Orio et al., 2022). Consequently, an overload of nitrogen, phosphorus and other persistent pollutants promotes the eutrophication of the water and induces the growth of harmful algal blooms (HELCOM, 2022). As the biomass decomposes, the consumption of oxygen from the water drastically increases and transforms the landscape in what is now considered to be the largest man-made hypoxia area in the world (Carstensen and Conley, 2019). This outcome is the result of decades of continuous wastewater discharges and river runoffs with impacts on the ecosystem and devastating consequences, not only on biodiversity, but also on local services and coastal communities.

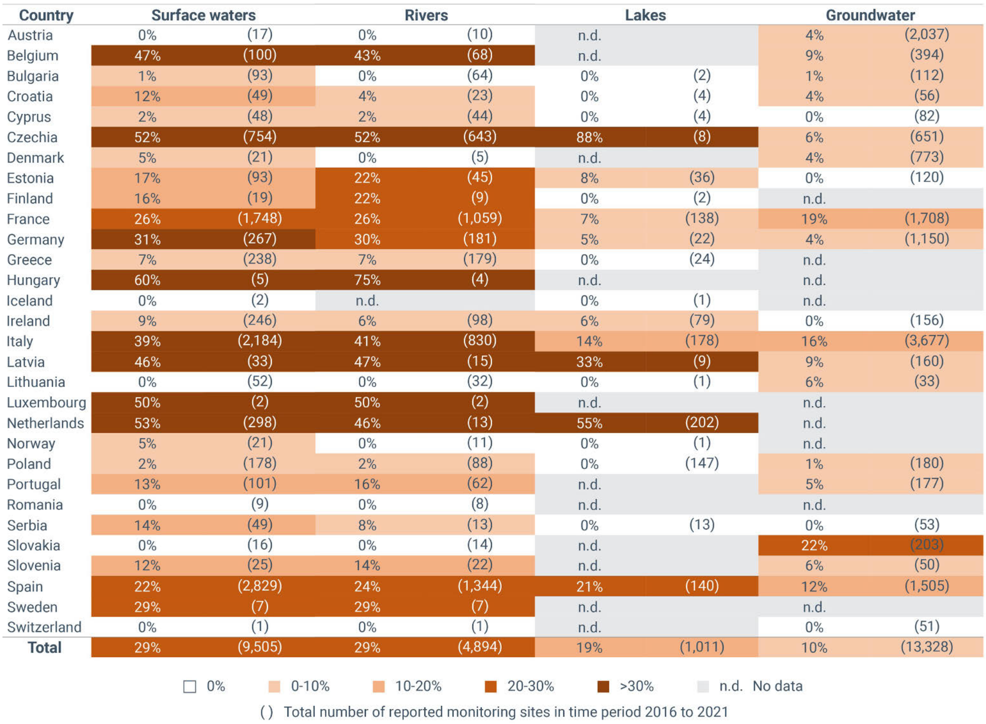
Figure 1. Satellite images of harmful algal blooms on the southwest coastline of Finland. Source: SYKE, Finnish Environmental Institute.

1.2 Agricultural wastewaters

The pollution caused by wastewater discharges in the Baltic Sea is a complex problem influenced by anthropogenic activities such as the ever-growing need to produce food and the presence of outdated wastewater treatment facilities (Kehrein et al., 2020). In the EU, agriculture is responsible for 60% of the total water consumption, and it is often referred to as the main human activity contributing to such an unsustainable use of natural resources and nutrient inputs into rivers, lakes and seas (EEA- European Environment Agency, 2021). The agricultural industry is the primary sector of any society, and it comprehends a broad array of services that cultivate, produce and transform biomass. Typical activities include livestock and dairy production, crop cultivation, horticulture and forestry management. These economic activities generate different types of wastewaters that represent a real challenge for the current wastewater treatment infrastructure.

Wastewater from livestock and dairy industries usually contains high concentrations of nitrogen and phosphorus, organic matter, as well as antibiotics and hormones that are used in the process of producing meat, milk and its derivatives. Likewise, the wastewater generated from forestry activities and pulp industry also contain high concentrations of turbidity, in addition to, resins and other dissolved chemicals used in the process of transforming wood into paper. This wastewater often presents altered pH and acidity making it a real threat for aquatic life, soil and groundwater. As a result, the purification of these waste streams often requires the combination of different high energy-consuming techniques to deal with the high biological and chemical oxygen demands (BOD, COD). The cleaning process is often inefficient as the conventional wastewater treatment facilities were not originally designed to deal with such a spectrum of veterinary pharmaceuticals and synthetic chemicals (Delgado et al., 2023; Vaishnav et al., 2023).

Regarding crop production activities, farmers often use a wide array of agrochemical solutions to maximize crop yields and prevent the contamination of plants. These products, often classified as pesticides, herbicides or fungicides include a diverse group of compounds with different chemical structures, water stability and solubility. They are toxic to a wide variety of organisms and are frequently found in water bodies in concentrations that exceed the maximum acceptable detection limit determined by the European Food Safety Authority (Fig. 2). On top of the challenges related to the physicochemical nature of the wastewaters, each one of these agricultural activities has its own seasonality and production schedules. Production peaks often result in increased volumes of wastewater containing even higher amounts of nitrogen, phosphorus and other chemical pollutants. Moreover, several of these industries coexist in the same area creating a compound pressure on the wastewater treatment network of any given municipality. For all these reasons, the agricultural sector faces a sustainability crisis as traditional practices and technology are responsible for a severe degradation of the environment. Therefore, the journey towards a more sustainable agriculture sector will require a collective effort to update the whole value chain and develop innovative solutions that prioritize efficient resource usage and water management.



Country	Surface waters		Rivers		Lakes		Groundwater	
Austria	0%	(17)	0%	(10)	n.d.		4%	(2,037)
Belgium	47%	(100)	43%	(68)	n.d.		9%	(394)
Bulgaria	1%	(93)	0%	(64)	0%	(2)	1%	(112)
Croatia	12%	(49)	4%	(23)	0%	(4)	4%	(56)
Cyprus	2%	(48)	2%	(44)	0%	(4)	0%	(82)
Czechia	52%	(754)	52%	(643)	88%	(8)	6%	(651)
Denmark	5%	(21)	0%	(5)	n.d.		4%	(773)
Estonia	17%	(93)	22%	(45)	8%	(36)	0%	(120)
Finland	16%	(19)	22%	(9)	0%	(2)	n.d.	
France	26%	(1,748)	26%	(1,059)	7%	(138)	19%	(1,708)
Germany	31%	(267)	30%	(181)	5%	(22)	4%	(1,150)
Greece	7%	(238)	7%	(179)	0%	(24)	n.d.	
Hungary	60%	(5)	75%	(4)	n.d.		n.d.	
Iceland	0%	(2)	n.d.		0%	(1)	n.d.	
Ireland	9%	(246)	6%	(98)	6%	(79)	0%	(156)
Italy	39%	(2,184)	41%	(830)	14%	(178)	16%	(3,677)
Latvia	46%	(33)	47%	(15)	33%	(9)	9%	(160)
Lithuania	0%	(52)	0%	(32)	0%	(1)	6%	(33)
Luxembourg	50%	(2)	50%	(2)	n.d.		n.d.	
Netherlands	53%	(298)	46%	(13)	55%	(202)	n.d.	
Norway	5%	(21)	0%	(11)	0%	(1)	n.d.	
Poland	2%	(178)	2%	(88)	0%	(147)	1%	(180)
Portugal	13%	(101)	16%	(62)	n.d.		5%	(177)
Romania	0%	(9)	0%	(8)	n.d.		n.d.	
Serbia	14%	(49)	8%	(13)	0%	(13)	0%	(53)
Slovakia	0%	(16)	0%	(14)	n.d.		22%	(203)
Slovenia	12%	(25)	14%	(22)	n.d.		6%	(50)
Spain	22%	(2,829)	24%	(1,344)	21%	(140)	12%	(1,505)
Sweden	29%	(7)	29%	(7)	n.d.		n.d.	
Switzerland	0%	(1)	0%	(1)	n.d.		0%	(51)
Total	29%	(9,505)	29%	(4,894)	19%	(1,011)	10%	(13,328)

☐ 0% ▨ 0-10% ▨ 10-20% ■ 20-30% ■ >30% ▨ n.d. No data

( ) Total number of reported monitoring sites in time period 2016 to 2021

Figure 2. Percentage of monitored sites with pesticide levels exceeding legal thresholds in surface waters, rivers, lakes and groundwater in European Countries, 2016-2021. Adapted from the European Environment Agency (EEA).

1.2.1 Soilless agriculture: Hydroponic farming

In recent decades, different techniques have been proposed to address the environmental concerns regarding agriculture. Special focus has been given to finding alternatives to mitigate soil degradation, water pollution and scarcity. The recent technological advancements in the fields of artificial light and process automatization have significantly contributed to the rise of soilless agriculture techniques. These methods, commonly referred to as hydroponics, eliminate the need to use soil for cultivating plants and are generally recognized for their sustainability advantages. In hydroponic systems, plants are grown preferably in a fully controlled environment, such as greenhouses, where all the cultivation parameters are closely monitored. A nutrient rich solution containing all the ingredients necessary for the development of the plants is provided directly to the roots or the substrate supporting them. This novel cultivation technique offers optimized growth conditions to plants, resulting in higher yields in comparison with traditional methods (Barbosa et al., 2015). For these reasons, hydroponic farming has become increasingly popular across the globe. It not only offers a solution to farmers maximizing their yields but also enables the year-round production of crops, protecting farmers from climate change events such as unstable precipitation patterns or heatwaves. In North America, USA and Canada, soilless farming has modernized cucumber and tomato agriculture and is now responsible for more than 90 % of the yearly production (Gruda, 2019; Incrocci et al., 2020; Walters et al., 2020). In Europe, soilless agriculture is estimated to occupy more than 20 000 ha and for countries like The Netherlands, Belgium or Poland, hydroponic systems cover more than 80% of the total greenhouse area. These systems are utilized to produce a wide variety of fruits and vegetables, including strawberries, lettuce, and peppers (Incrocci et al., 2020).

Conventional hydroponic systems cultivate vegetables and fruits using two distinct approaches: open or closed cultivation systems. The open cultivation systems do not reuse or recirculate the nutrient solution provided to the plants. After the nutrients are delivered to the roots of the crops, any excess solution is drained away. This approach offers several advantages regarding simple daily operation and equipment maintenance. In addition to those, open cultivation systems also offer reduced risk of nutrient imbalances and pathogen contamination. On the other hand, the constant need to prepare fresh nutrient solutions increases the water consumption of the facility and decreases the sustainability of the process. Despite the disadvantages, the open cultivation systems are the most common hydroponic farming method in Europe (Massa et al., 2020).

Contrasting with this approach, in the semi-closed and closed cultivation systems, the same nutrient solution can be recirculated several times through the hydroponic facility before being discharged. This method offers better water and nutrient efficiency in comparison with the open cultivation technique, but also requires a bigger initial investment and has an increased complexity in daily operations. Closed systems often require constant monitoring of nutrients to prevent plant stress due to increased salinity or nutrient imbalances. In addition to that, these cultivation systems also require state of the art equipment, such as proper water filtration units to deal with the elevated risk of contaminations, or ion selective probes to monitor nutrient concentrations in the water. Despite the elevated costs in comparison with open systems, the close cultivation methods offer a better cost efficiency regarding nutrient recycling and improve the overall sustainability of the process.

1.2.2 Limitations of hydroponic farming: Wastewater discharges

Soilless cultivation systems were designed to reduce the misuse of natural resources in comparison with soil-based agriculture. Despite the technological progress during the cultivation stage, much is still to be done regarding optimal nutrient dosage and water management (Sambo et al., 2019). Farmers frequently encounter challenges when it comes to monitoring nutrient levels or implementing proper irrigation strategies because they lack reliable technology to selectively monitor ion concentrations in the nutrient solution, or to accurately measure plant transpiration rates on industrial scale (Cáceres et al., 2021). These technological limitations result in water and nutrients being supplied in excess of crop requirements. On top of that, recirculating water that can contain pathogenic contaminants, as proposed in the closed cultivation systems, is seen as a real threat that can compromise the yields of the greenhouses. For all these reasons, the farmers are not keen on recycling nutrient solutions, and the majority prefer to operate their greenhouses in open cultivation systems (Massa et al., 2020). Depending on the irrigation strategy of each greenhouse and the water consumption requirements of each crop, millions of litters of water are discharged into nature because of these decisions. As nutrient dosing is far from optimized, hydroponic wastewaters can contain as much as 30% of the original nutrient concentration when released into nature (Savvas and Gruda, 2018).

Given the fact that hydroponic systems are becoming increasingly popular and that the wastewaters generated by this activity contain all the necessary nutrients to support photosynthetic life, these wastewaters possess a significant threat to the environment when discharged without treatment (Cifuentes-Torres et al., 2020). Thus, despite the progress on water management in comparison with soil-based agriculture, the sustainability of the hydroponic greenhouses needs to be further improved to ensure a continued commitment to sustainable practices.

1.3 Bioeconomy and the transition to circular value chains

The current economic model that supports all activities worldwide, including agriculture, relies on a linear supply chain based on resource extraction, product manufacturing and waste disposal. This economic model prioritizes production and consumption to generate economic growth, relying heavily on the use of natural resources to meet market demands. As the final price to the consumers does not account for the environmental costs, there is an urgent need to increase consumers' awareness and establish new low-carbon value chains that encourage sustainable consumption patterns. The European Commission regards these topics as instrumental to the vision of the European bioeconomy, particularly within the context of the EU Farm to Fork strategy, the EU Green Deal, and the new framework for the Common Agricultural Policy 2023-2027 (European Commission, 2023a).

Bioeconomy is widely recognized as the economic system that fosters the production of goods and services while embracing sustainable industrial practices. Within this system, a strong emphasis is given to the natural environment, aiming to minimize pollution through the application of a resource efficient supply chain. This vision aligns perfectly with the concept of circular economy where the byproducts and waste produced during manufacturing can be reused and recycled continuously. This concept has the potential to improve the life cycle assessment of any given industrial pipeline by optimizing solid waste and water management, as well as reducing greenhouse gas emissions.

Alongside the changes in the traditional supply chain, world leaders also see in bioeconomy an opportunity to stimulate innovation and accelerate the transfer of knowledge from academia to industry. A new generation of industrial practices can be achieved by fostering new upstream and downstream processes that prioritize green chemistry principles and zero-waste solutions. Likewise, the commercialization of new sources of biomass offers promising benefits to consumers as it can facilitate a shift to a low-carbon economy and promotes the restoration of the ecosystems.

1.4 Photosynthetic microbes and their potential in the bioeconomy

Microalgae and cyanobacteria are a diverse group of autotrophic microorganisms present in a wide range of aquatic environments and equipped with a cellular mechanism known as photosynthesis. In this process, the cells can harvest natural sunlight and atmospheric CO_2 to synthesize carbohydrates and biomass with O_2 being produced as a byproduct. These biological entities exhibit an immense variety of morphological traits and metabolisms and are commonly found in nature living as individual cells or colonies. Microalgae represent a broad range of eukaryotic microorganisms present in both marine and freshwater habitats and including groups such as chlorophytes, dinoflagellates and diatoms. Cyanobacteria are a diverse phylum of photosynthetic prokaryotic microorganisms that include single celled individuals as well as filamentous species. This group is considered to be the ancestors of oxygenic photosynthesis due to their evolutionary role in the development of chloroplasts.

The biomass of both microalgae and cyanobacteria is extensively studied either to advance our understanding of photosynthesis or due to its biochemical composition that is of great commercial interest for several industrial applications. Numerous species have been granted approval for consumption in human food and supplement markets, as well as for use in animal feed and cosmetic industries. This recognition highlights the immense potential of microalgae and cyanobacteria biomass as a versatile feedstock for various activities worldwide. Moreover, the technology of this emerging sector can be combined with existing infrastructure from other industries to optimize natural resources, improve greenhouse gas emissions and accelerate innovation. Despite the inherent differences between eukaryotic microalgae and prokaryotic cyanobacteria, from this point on, the term “microalgae” will be used to collectively refer to both groups, in the interest of enhancing readability.

1.4.1 Cultivation systems for photoautotrophic microalgae

In recent decades, the large-scale cultivation of microalgae biomass has gathered a lot of interest due to its promising applications. Numerous approaches have been proposed, considering factors such as the species to be cultivated, the geographic location, as well as the available initial investment. Over the years, extensive research has been carried out to understand the most important limiting factors of large-scale production of microalgae. Comprehensive knowledge of parameters such as light intensity and quality, nutrient uptake or gas-liquid mass transfer, helped establish the open raceway ponds, the closed tubular photobioreactors and the flat panel reactors as the most common cultivation methods. These systems have been continuously improved, allowing the industry to operate year-round and ensuring uninterrupted production cycles.

Closed cultivation systems such as the flat panels (Fig. 3A) or the tubular photobioreactors (Fig. 3 B, C) are the most common method for large-scale cultivation of eukaryotic microalgae in Europe (Araujo 2021). The tubular PBR system is typically built of acrylic or glass, and the tubes are vertically stacked to maximize light penetration inside the reactor. These systems require a high investment (CAPEX) and have high operation costs (OPEX) mostly because of the materials use to build the reactor and the electricity consumption of the circulation pumps needed to mix the microalgae culture inside the system. In return, they provide a fully controllable closed environment for the cultivation of different species. As the closed cultivation systems protect the microalgae cultures from the risk of contamination and from sharp weather fluctuations, the biomass productivity in these systems is often higher than the one recorded for open cultivation systems. The most common genera of microalgae cultivated in tubular PBR are *Chlorella*, *Haematococcus* and *Nannochloropsis*.

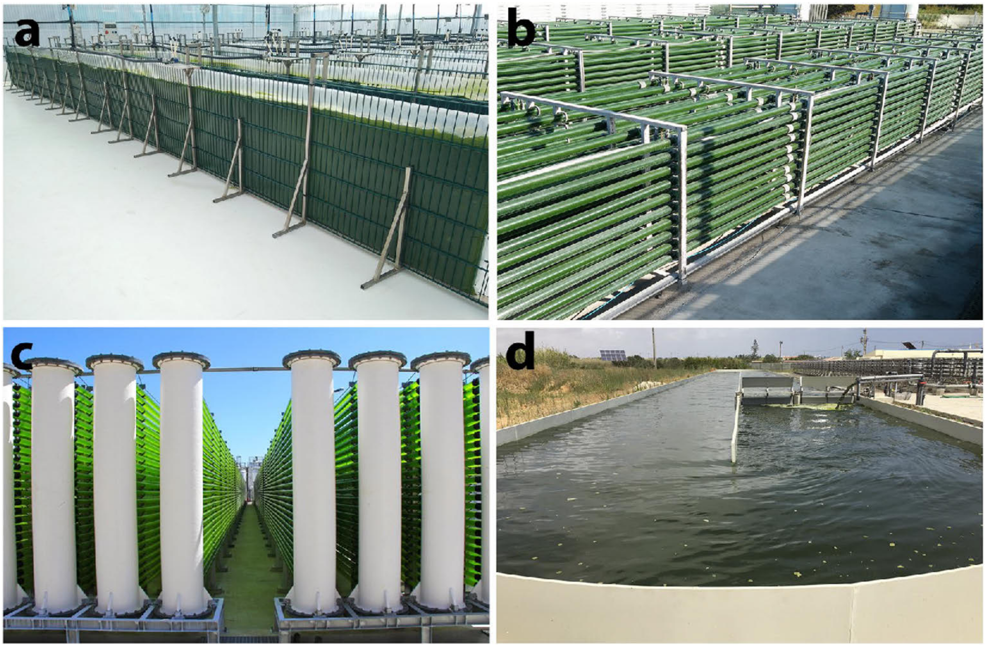


Figure 3. Examples of cultivation systems used for the industrial production of microalgae biomass. **(A)** 1 m3 flat panel reactor; **(B)** 2.5 m3 tubular photobioreactor; **(C)** 100 m3 tubular photobioreactor; **(D)** 200 m3 open raceway pond with paddelwheel. The images were kindly provided by Allmicroalgae Natural Products, SA (**A**-**C**) and by Necton S.A. (**D**) and were originally published by Pereira et al., 2018.

Open raceway ponds (Fig. 3D) consist of a shallow open-air pond equipped with a paddlewheel to mix the culture and facilitate gas exchanges. These systems have low maintenance and energy costs, but typically require large surface areas to maximize light absorption and the photosynthetic efficiency of the cells. Raceway ponds are a cost-effective method for the cultivation of robust species that can survive in uncontrolled environments exposed to outdoor conditions. The most common species cultivated this way is *Arthrospira platensis*, mostly known as Spirulina.

1.4.2 Biochemical composition of microalgae biomass

The biochemical composition of microalgae biomass can vary significantly depending on the species, the cultivation conditions provided during growth and the downstream processes upon harvesting and processing. Nonetheless, the biomass from the most common commercially available species contains a well-balanced nutritional composition that is comparable to that of traditional and other emerging sources of biomass (Fig.4, (Pereira et al., 2019). Lipids, proteins and carbohydrates are vital components of microalgae cells. These molecular groups act as energy storage reservoirs and structural membrane complexes, regulating intracellular metabolic pathways and participating in interactions with the extracellular space of the cells.

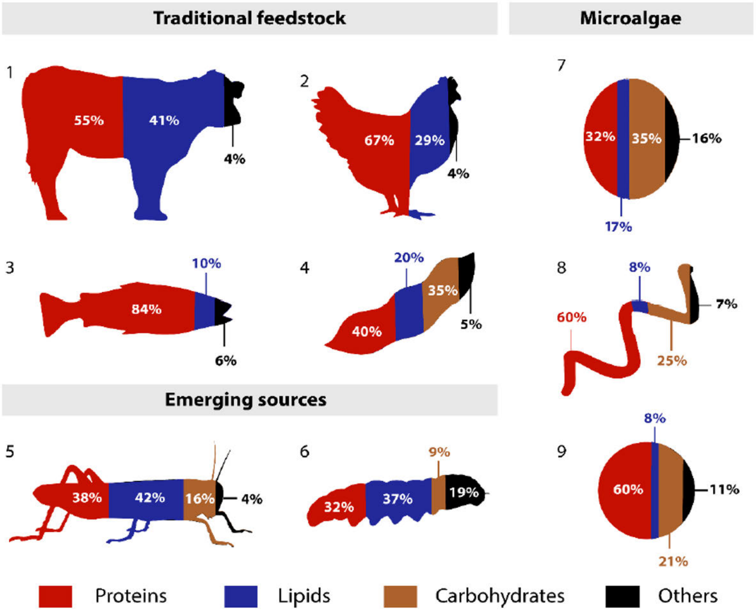


Figure 4. A comparison of the biochemical composition of traditional feedstocks, emerging biomass sources and microalgae biomass. Traditional feedstock: (1) Beef; (2) Chicken; (3) Bass; (4) Soybean. Emerging feedstock sources: (5) Grasshopper; (6) Black soldier fly prepupae. Microalgae biomass: (7) *Haematococcus* sp.; (8) Spirulina; (9) *Chlorella vulgaris*. Adapted from Pereira et al., 2019.

A wide variety of species can accumulate as much as 60% of its dry weight (DW) as protein, containing all the essential and non-essential amino acids and vitamins required for a healthy diet (Akter et al., 2023; Russell et al., 2022). Moreover, species such as *Chlorella vulgaris* or *Nannochloropsis* sp. also exhibit a lipid content that is notably abundant in polyunsaturated fatty acids (PUFAs) such as omega-3 (Cunha et al., 2020; Yun et al., 2020). These fatty acids play a crucial role in the diets of both humans and animals. The carbohydrate content of microalgae biomass can vary according to factors such as nutrient limitation or light availability, ranging from 10-60% of the dry weight (Debnath et al., 2021; Silvello et al., 2022). The predominant monosaccharides are usually glucose, xylose or galactose and the most common polysaccharides are cellulose or starch (Silvello et al., 2022). This molecular class has gained a lot of attention due to several microalgae species being able to produce exopolysaccharides that have been shown to display a broad array of bioactive activities in humans, animals and plants (Tounsi et al., 2022).

As the industrial production of microalgae relies mostly on nutrient solutions using mineral compounds dissolved in water, the biomass of several commercially available microalgae is also a rich source of micronutrients such as iron, zinc and magnesium. The total ash content can represent as much as 5-30% of the dry weight (Hawrot-Paw et al., 2020; Schüler et al., 2020). Several species of microalgae are also known for producing high-value compounds in their biomass. Species such as *Haematococcus pluvialis*, *Dunaliella salina* or *Arthrospira platensis* are notorious for accumulating a significant amount of pigments such as astaxanthin, β-carotene or phycocyanin, respectively. These pigments act as scavengers of reactive oxygen species in the cells, protecting the photosynthetic apparatus from excessive light. Once extracted, they are powerful natural antioxidants that can be used in food supplements or in the cosmetic industry.

1.4.3 Market applications for microalgae biomass

The European Algae Biomass Association (EABA) estimates that the microalgae industry worldwide can produce as much as 130 000 tonnes of dry biomass each year (European Commission, 2023b). In the European market, microalgae biomass is mostly present in the human food and animal feed sectors, where it is used as a functional ingredient to enhance the nutritional content of both human and animal diets. Apart from these, the biomass is also present in the nutraceutical and cosmetic industries as a source of bioactive and high-value compounds such as fatty acids, vitamins and antioxidants (Araújo et al., 2021). Historically, species belonging to the genera *Chlorella* and *Arthrospira* (Spirulina) have been extensively used by humans in food applications. For that reason, several species (e.g., *Chlorella vulgaris*, *Chlorella sorokiniana*, *Arthrospira platensis*) are not subjected to the Novel Food EU classification and can be freely commercialized by Member States. Apart from those, species such as *Tetraselmis chuii* or *Haematococcus pluvialis* have been recently approved for human consumption and are mostly found as multivitamin supplements or as high-value products.

In the food and nutraceutical markets, most of the microalgae biomass is sold as a dry powder to be added in drinks or to be consumed in addition to a healthy diet. Prices for dry powder whole biomass can range from 25 € kg^{-1} for Spirulina up to 120 € kg^{-1} for *Chlorella vulgaris*. In the nutraceutical market, multivitamin tablets containing the same species can cost as much as 140 € kg^{-1} and high-value products such as astaxanthin from *Haematococcus pluvialis* are sold with a retail price of over 35 € g^{-1}.

In the aquaculture industry, several species of microalgae biomass are used as animal feed for new-born larvae of fish, shrimp and oyster. In hatchery facilities, microalgae biomass serves as a source of essential amino acids, fatty acids and vitamins ensuring a balanced diet and promoting the healthy development of offspring. The biomass is commercialized either in liquid form containing up to 25% of dry weight or as dry powder to be added directly to the aquariums. Depending on the species, prices for wet biomass can vary between 40 - 100 € kg^{-1} while the prices for dry biomass can vary between 200-400 € kg^{-1} for species such as *Isochrysis galbana*, *Phaeodactylum tricornutum* or *Skeletonema costatum*.

In the cosmetic sector, the use of microalgae extracts enables the development of formulations using natural, sustainable and organic sources of fatty acids, antioxidants and vitamins. By doing so, manufacturers create a high-value product that can be used in face creams or serums and can be purchased by consumers that are environmentally conscious or with diet restrictions such as vegetarians or vegans.

1.4.4 Emerging applications for microalgae biomass in agriculture: Biopesticides and plant biostimulants

Pesticides are a class of agricultural products extensively used by farmers to help control all sorts of pests. This class of plant protection products includes different chemical compounds and active substances designed for eliminating unwanted herbs, repelling insects and other animals, as well as pathogenic fungi and bacteria. The chemical formulations are intentionally designed to ensure stability and low degradation rates, providing long lasting effects on pests, soils and groundwater. Their approval in Europe is dependent on its toxicity to humans as well as on its impacts on the environment regarding non-target species and biodiversity. Despite the extensive amount of regulation on the safety and commercialization of pesticides (Regulations EC: 1107/2009 and 1272/2008), recent reports from non-profit organizations such as the Pesticide Action Network (PAN) have shown that fruit and vegetables produced in Europe are sold to consumers containing hazardous pesticides above the maximum residue levels (PAN, 2022). In addition to that, recent studies estimated that on a yearly basis, as much as 730 tonnes of pesticides leach directly into nature and some pesticides are detected in groundwater long after being banned from circulation in EU markets (Alexandrino et al., 2022; Maggi et al., 2023). For all those reasons, the “Farm to Fork” strategy of the European Green Deal aims at reducing by 50% the use of hazardous pesticides in the EU until 2030 (EEA, 2021). To achieve this goal, the European Commission is determined to find alternative products that improve the sustainability of the agriculture sector. Within the EU fertilizer legislation, plant biostimulants are defined as plant enhancing products capable of improving the tolerance to abiotic stresses, quality traits or nutrient use efficiency (EU Regulation No 1107/2009). In addition to those, plant biostimulants can also be used as soil amenders, improving the availability of nutrients in the soil. The composition of this category of products includes substances such as humic acids and protein hydrolysates, as well as non-pathogenic bacteria and fungi that are known to form symbiotic relationships with the plant root system, enhancing defences and water uptake (Albrecht, 2019).

One of the promising alternatives to traditional plant protection products and plant enhancing substances, relies on the use of microalgae biomass as a novel source of biopesticides and plant biostimulants. Aqueous extracts of microalgae biomass are a rich source of bioactive compounds such as phytohormones (e.g. auxins, cytokinins, gibberellins), humic substances (e.g. fulvic acids, polyphenolic compounds), vitamins, amino acids and polysaccharides (Ferreira et al., 2023; Gemin et al., 2019; Calvo et al., 2014). Given the correct dosage, these extracts are known to improve several aspects of the plant physiology such as seed germination rate, root development, nutrient uptake and tolerance to abiotic stresses (Parmar et al., 2023; Rupawalla et al., 2022). Moreover, they are also able to inhibit the growth of common plant pathogenic bacteria and fungi (Ranglová et al., 2021). The effectiveness of the extracts is often dependent on parameters such as the concentration of the algae biomass, the type of administration and the crop (Gonçalves, 2021; González-Pérez et al., 2022).

1.4.5 Microalgae technologies in circular economy: The biorefinery concept

The services provided by microalgae biotechnology offer a broad range of solutions that can be merged with traditional industries to establish new value chains, improve carbon footprints and optimize life cycle assessments. This industrial symbiosis is often referred to as the biorefinery concept, a circular economy vision that fosters sustainability and promotes innovation. Microalgae cultivation systems are often at the centre of this strategy. Not only because they can be part of a new approach to deal with industrial pollution, but also because the biomass resulting from those processes can be used as a raw ingredient for a new generation of low-emission supply chains. The most common biorefinery designs include the use of microalgae cultivation systems for the purification of wastewaters from municipal, agricultural or industrial sources, and the conversion of the resulting biomass into animal feed, biofertilizers or biogas (e.g. EU projects: All-gas, Sabana, Magnificent). Besides the environmental benefits regarding nutrient recycling and the establishment of low-pollution value chains, this approach also has the potential to significantly reduce the production costs of microalgae biomass, since freshwater, nutrients and CO_2 can all be recycled from nearby industries.

2 Aim of the Study

Microalgae biotechnology holds great potential to develop new value chains that support sustainability and promote low-carbon footprint practices. To succeed, these solutions need to be not only meaningful for the society, but also cost-efficient, offering viable alternatives to the current linear economic model. To do so, it is imperative to identify the most promising microalgae species that can thrive with minimum requirements while producing a high-quality biomass. Equally important is identifying the most promising industries and products that can be enhanced using microalgae-based solutions. In the present study, the principles of circular economy were applied to explore the potential of microalgae solutions in agriculture. Nordic culture collections were screened in search of promising microalgae species, capable of growing in agricultural wastewaters and exhibiting promising results as plant biostimulants and biopesticides.

To do so, the study prioritized the following tasks:

I Screen and identify the most promising microalgae species capable of growing in agricultural wastewaters from a hydroponic greenhouse.

II Establish a suitable method to purify the hydroponic wastewater.

III Scale-up microalgae cultivation in a 65L PBR and understand the dynamics of nutrient consumption during batch and continuous mode experiments using hydroponic wastewater.

IV Biomass valorization as plant biostimulant and biopesticides on plants and fungi.

3 Materials and Methods

3.1 Hydroponic wastewater characterization

The hydroponic wastewater used in papers I and II was collected from a commercial greenhouse, Puutarha Timo Juntti Oy, specialized in the production of cucumbers and located in Kaarina, Finland. For a comprehensive understanding of the chemical composition of the wastewater, a detailed analysis was performed by an independent and certified laboratory (Lounais-Suomen vesi-ja ympäristötutkimus Oy). The values presented in Table 1 represent the chemical composition of one of the samples used in the experimental studies.

Table 1. The chemical composition of the hydroponic wastewater.

Parameter	Value	Unit
Conductivity	280	mS m^{-1}
TSS	5.5	mg L^{-1}
COD (Mn)	28	mg L^{-1}
BOD7	3.0	mg L^{-1}
P-PO_4^{3-}	32	mg L^{-1}
N-NO_3^-	270	mg L^{-1}
N-NO_2^-	0.07	mg L^{-1}
N-NH_4^+	0.13	mg L^{-1}
SO_4	220	mg L^{-1}
Fe	2.3	mg L^{-1}
K	320	mg L^{-1}
Mg	61	mg L^{-1}
Ca	180	mg L^{-1}
Zn	0.44	mg L^{-1}
pH	6.8	

3.2 Microalgae strians and inoculum maintenance

All the microalgae and cyanobacteria species used in this study belong to the NordAqua Culture Collection database and are listed in Table 2. Stock cultures of each species were periodically prepared and maintained in the corresponding growth media, following the specifications of each culture collection.

Table 2. The list of microalgae and cyanobacteria species applied in papers I-IV and the corresponding culture collection.

Species	Culture Collection	Code	Paper
Nostoc sp.	HAMBI/UHCC	UHCC 0252	I
Nostoc sp.	HAMBI/UHCC	UHCC 0268	I
Microcystis sp.	HAMBI/UHCC	UHCC 0582	I
Synechococcus sp.	HAMBI/UHCC	UHCC 0524	I
Synechococcus sp.	HAMBI/UHCC	UHCC 0527	I
Scenedesmus sp.	HAMBI/UHCC	UHCC 0027	I, III
Unknown	HAMBI/UHCC	UHCC 0492	I
Scenedesmus sp.	NORCCA	NIVA-CHL 99	IV
Selenastrum sp.	NORCCA	NIVA K-1877	I, IV
Chlorococcum sp.	NORCCA	NIVA-CHL 131	I
Scenedesmus sp.	NORCCA	NIVA-CHL 99	I
Tetradesmus obliquus	NORCCA	NIVA-CHL107	I, II, III, IV
Monoraphidium contortum	NORCCA	NIVA-CHL100	I, IV
Apatococcus lobatus	NORCCA	NIVA-CHL144/1	I
Klebsormidium flaccidum	NORCCA	NIVA-CHL 80	IV
Tribonema sp.	NORCCA	NIVA-1/84	IV
Haematococcus lacustris	NORCCA	K-0084	IV
Porphyridium purpureum	NORCCA	NIVA-1/92	III, IV
Chlorella Vulgaris	NORCCA	NIVA-CHL 19	IV
Chlorella sp.	NORCCA	NIVA-CHL 69	IV
Chlorella sp.	NORCCA	NIVA-CHL 125	IV
Chrlorella sorokiniana	NORCCA	NIVA-CHL 176	III, IV
Tetraselmis subcordiformis	NORCCA	NIVA-2/94	III, IV
Coelastrum sp.	NORCCA	K-0559	III, IV
Chlamydomonas reinhardtii	CRC	CC-124	IV

The species from the University of Helsinki Culture Collection (HAMBI/UHCC) were kept in Z8X (Kotai, 1972) or BG11 (Rippka et al., 1979) growth media and the species from the Norwegian Culture Collection of Algae

(NORCCA) and Chlamydomonas Resource Centre (CRC) were kept in Z8 medium (Kotai, 1972). All stock cultures were maintained in continuous low-intensity light of 5–10 µmol m^{-2} s^{-1} and at a room temperature of 20 °C.

3.3 Screening: Identification of promising species

In paper I, the promising microalgae and cyanobacteria species were identified by performing screening trials in 24 well plates with hydroponic wastewater as growth media. The hydroponic wastewater was collected fresh from the greenhouse and pre-treated before the experiments with a coarse filtration (4-7 µm) to remove small, suspended particles and contaminants. The experiments were performed inside a growth chamber at a constant light intensity of 50 µmol m^{-2} s^{-1} and a temperature of 22 °C. The 24 well plates were constantly agitated throughout the experiment at 120 rpm using an orbital shaker (Sanyo, Japan). The growth was monitored daily by measuring OD_{750nm} with a microplate reader (Infinite 200 PRO, Tecan, Switzerland).

3.4 Pre-treatment effect on wastewater purification

To establish the most efficient pre-treatment method to purify the hydroponic wastewater, three different methods were evaluated both for the laboratory and the greenhouse tubular PBR operations (paper I). These methods were evaluated based on the ability to reduce particles or contaminants present in the wastewater and considering their ability to be scaled-up to the greenhouse PBR system. To this end, three different methods were tested: coarse filtration (4–7 µm); vacuum microfiltration (0.7 µm); and bleach followed by neutralization with sodium thiosulphate. The pre-treated hydroponic wastewaters were inoculated with a promising microalgae strain (Scenedesmus UHCC 0027) selected from the screening trials (section 3.3) and cultivated inside a growth chamber. The system was programmed to provide a 3% CO_2, continuous light intensity of 50 µmol m^{-2} s^{-1} and a temperature of 22 °C. Blank flasks with just the pre-treated hydroponic wastewater were also incubated to evaluate the effectiveness of each pre-treatment method. The experiments were made in duplicate and the growth was assessed daily by measuring OD_{750nm} (Genesys 10S UV–VIS, Thermo Fisher, USA) and the total chlorophyll content (Porra et al., 1989). The removal efficiencies of [$N\text{-}NO_3^-$] and [$P\text{-}PO_4^{3-}$] were evaluated for each condition tested.

3.5 Greenhouse pilot-scale cultivation

In papers I and II, the pilot-scale cultivation of microalgae using wastewater as growth media was performed in a PBR operated inside a controlled glass greenhouse used for research studies. The greenhouse infrastructure was equipped with temperature control systems and artificial light, which allowed recreating optimal conditions for microalgae growth. A meteorological weather station was also present in the premises of the greenhouse to record the total solar radiation and outside temperature.

The reactor used in papers I and II is a 65L airlifted vertical tubular PBR made of acrylic and equipped with inline sensors for constant monitoring of several cultivation parameters (Fig. 5). The PBR is equipped with an electronic solenoid valve for CO_2 injection, as well as, a turbidity sensor (OD_{880nm}), a relative chlorophyll sensor (OD_{680nm}), a temperature, a pH and a flow sensors. The pilot-scale operation also includes a scale to measure the overflow of harvested biomass during the continuous mode operation. In addition to that, a filtration system was installed to purify the hydroponic wastewater. All the sensors and peripheral equipment are controlled via NI LabView software which allows for real-time monitoring of the whole operation.

In paper I, the PBR was illuminated by high-pressure sodium bulb lights (Philips Green Power 400 W) that were arranged to provide a light intensity of 200 μmol m^{-2} s^{-1} at the top surface of the PBR. In paper II, the PBR was illuminated by red and blue LED lights, installed in a frame and placed 1.3 meters apart from one side of the PBR. The average intensity was near 320 μmol m^{-2} s^{-1}. In both papers, I and II, the artificial light systems were programmed to operate in a photoperiod of 17:7 h (light:dark) cycle following the same conditions that farmers use for cucumber production.

Figure 5. The tubular photobioreactor operation installed in the greenhouse.

3.5.1 Batch and continuous mode operations

The pilot-scale cultivation trials were initiated by collecting the hydroponic wastewater from the commercial greenhouse and filtering it through the two-step filtration system described in section 3.5. The concentrations of $N\text{-}NO_3^-$ and $P\text{-}PO_4^{3-}$ in the wastewater were quantified after the filtration to remove possible interferences and debris. In paper I, the PBR was operated in continuous mode to evaluate the effect of different cultivation parameters on the productivity of the strain and its ability to remove nutrients efficiently (Table 3). A calibration curve was established between the dry weight of the culture and the turbidity sensor (OD_{880nm}) of the PBR to achieve a stable continuous mode operation. In paper II, the experimental design evaluated the daily uptake of nitrogen in batch mode cultivations by following the $N\text{-}NO_3^-$ consumption in different periods of cellular growth.

Table 3. The experimental parameters and the macronutrient composition of the hydroponic wastewater during the continuous PBR operation.

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
$N\text{-}NO_3^-$ (mg L^{-1})	240 - 263	247 - 269	275 - 293	276 - 277
$P\text{-}PO_4^{3-}$ (mg L^{-1})	29 - 33	22 - 28	16 - 20	22 – 23
N:P	16 - 19	20 - 27	32 - 37	26 - 27
Duration (days)	10	10	5	4

3.6 Analytical Measurements

3.6.1 Nutrient consumption

In papers I and II, the macronutrient composition of the hydroponic wastewater was analysed by spectrophotometric methods. To determine the $[N\text{-}NO_3^-]$ and $[P\text{-}PO_4^{3-}]$ in the hydroponic wastewater, calibration curves were prepared with $NaNO_3$ (>99%, Merck) and K_2HPO_4 (99%, VWR), respectively. The samples were filtered and diluted prior to the analysis. Nitrate concentration was determined using the method 4500-NO_3-B (APHA, 1992) and phosphate concentretion was determined using a laboratory-grade commercial kit (Merck Spectroquant 1.14543).

3.6.2 Cellular growth

At laboratory scale, in papers I, III and IV, the growth of the microalgae and cyanobacteria cultures was monitored by measuring OD_{750nm} using a spectrophotometer (Genesys 10S UV–VIS, Thermo Fisher, USA). At the greenhouse pilot-scale cultivation with the PBR, in papers I and II, the growth of cultures was determined by measuring the DW from daily samples collected from the PBR. The algal samples were filtered through a glass microfiber (Whatman GF/F, 0.7 µm) using a vacuum pump. The filters were dried overnight at 95 °C and cooled down in before being weighed.

3.6.3 PBR operated in batch and continuous modes

The daily measurements of the culture's dry weight and the volume of the harvested biomass were used to calculate the areal and volumetric productivities of the PBR during the batch and continuous mode operations (papers I and II), according to the calculations described in paper I.

3.7 Biomass Valorization

3.7.1 Biomass characterization

In paper II, the characterization of the biomass was performed for the samples collected at the exponential and stationary phases of growth. The biochemical composition of the biomass was evaluated regarding its protein and carbohydrate content, as well as the fatty acid methyl ester (FAME) and pigment composition. The total protein content of the biomass was determined according to the Lowry method (Lowry et al., 1951). The cellular disruption of the freeze-dried biomass sample was achieved with a bead beater followed by centrifugation to precipitate debris. The protein content in the supernatant was quantified using a laboratory-grade commercial kit (Bio-Rad DC Protein assay kit) and absorbance was measured at OD_{750nm}. The protein concentration was calculated from a bovine serum albumin (BSA) calibration curve ($R^2 = 0.998$). The carbohydrate fraction of the biomass was determined according to the method described in DuBois et al., 1956. The cellular extraction was achieved by resuspending the biomass sample in 2.5M HCl and incubating the mixture in a dry heating block at 120 °C for 1 hour. After cooling down, the mixture was neutralized with 2.5M NaOH and centrifuged to sediment debris. The supernatant was collected and mixed with 5% Phenol:H2O (v/v) and 5mL of H_2SO_4. A standard curve was prepared using glucose ($R^2 = 0.997$) and absorbance was measured at OD_{488nm}.

In a dark and cold room, the cellular extraction of pigments was achieved by resuspending a freeze-dried sample in cold acetone and placing the mixture in a bead beater. Later, the samples were centrifuged, and the supernatant was collected. Under a gentle stream of N_2, the supernatant was dried and resuspended in methanol. The samples were filtered into vials and analysed with HPLC-DAD according to the protocol described in paper II. The composition of the FAME was determined using a single-step extraction and in situ transesterification. A sample of freeze-dried biomass was diluted in a mixture of Chloroform:Methanol (2:1) and 0.6 M HCl:Methanol, and placed in a sand bath at 85 °C for an hour. After cooling down, the FAME fraction was extracted by adding Hexane to the mixture. The samples were analysed by GC-MS according to the protocol described in paper II.

3.7.2 Biomass applications: plant biostimulants and biopesticide activity

The promising applications of microalgae biomass were explored in papers III and IV. The research aimed to uncover the potential of microalgae as a novel source of plant biostimulants, as well as assessing its potential against prevalent plant pathogens commonly found in Finland. In both papers, the methodology focused primarily on the identification of promising species from Nordic culture collections, followed by an evaluation of the effect of different extraction and dosage protocols on the desired bioactivity. In paper III, the biomass of 6 different microalgae species (Table 2) was cultivated under controlled conditions at laboratory scale and lyophilized prior to extraction. The impact of the extraction protocol on the plant biostimulant effect was evaluated by submitting the freeze-dried biomass of each species to mechanical or chemical procedures using bead-milling and acid hydrolysis techniques. The bioactivity of the extracts was assessed at different concentrations in two plant bioassays. At laboratory scale, rapid screening trials were performed using plant seedlings of *Arabidopsis thaliana* to evaluate the effect of the extracts on the root development of the plants. At the greenhouse, the extracts were applied on lettuce (*Lactuca sativa*) to evaluate their potential for enhancing plant growth and photosynthetic performance.

In paper IV, a preliminary screening trial was performed to evaluate the biopesticide activity of 15 different microalgae species (Table 2) against 7 plant pathogens known to be common contaminants of fruits and vegetables (*Fusarium oxysporum*, *Fusarium graminearum*, *Alternaria solani*, *Verticillium alboatrum*, *Botrytis cinerea*, *Phytophthora cactorum*, *Pythium ultimum*). Following the selection of a promising interaction between a microalgae species and plant pathogen, a comprehensive protocol was developed to assess the effects of various factors on the extraction process. These factors included: (i) utilizing wet or dry biomass during extraction; (ii) employing different solvents, differentiating between polar and non-polar solvents; and (iii) determining the ideal dosage of each extract to attain the highest possible antifungal activity. The best extraction route was determined in vitro by plating the different extracts on agar plates and applying the plant pathogen. Once the best extraction protocol was established, the antifungal trials were performed in strawberry leaves (*Fragaria* × *ananassa)*.

4 Overview of the Results

4.1 Screening of microalgae grown in hydroponic wastewater

In paper I, the screening of microalgae and cyanobacteria species from Nordic culture collections was performed in 24 well-plates using hydroponic wastewater as growth media (Fig. 6). The experiments evaluated the use of the agricultural waste stream as an alternative source of nutrients and water for the cultivation of photosynthetic microorganisms.

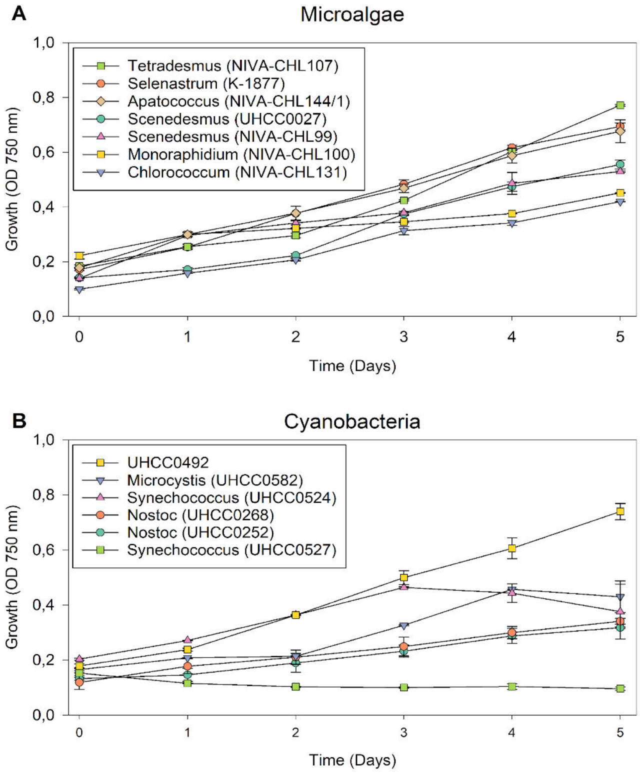


Figure 6. The screening of microalgae (A) and cyanobacteria (B) using hydroponic wastewater as growth media. The data are presented as mean ± SE (n = 2).

The majority of the dissolved nitrogen present in the hydroponic wastewater was in the form of nitrate, whereas both [N-NH_4] and [N-NO_2] were negligible (Table 1). The wastewater presented a N:P ratio of 19 and the concentration of several micronutrients (e.g., Fe, Mg, Zn) was at least 10-fold higher in comparison with traditional growth medium recipes (e.g. BG11 or Z8). The hydroponic wastewater also presented a low COD and BOD values in comparison with other sources of agricultural greenhouse effluents (Patrinou et al., 2020; Seo et al., 2008). In addition to testing the suitability of the greenhouse wastewater as an alternative source of nutrients, this experiment also highlighted the importance of the screening trials as a tool to select the most promising species.

The eukaryotic species belonging to the genus *Tetradesmus* showed the best performance with a maximum OD_{750nm} = 0.77 on the fifth day, followed by *Selenastrum* and *Apatococcus* genera which demonstrated a slightly lower growth capacity (Fig. 5A). The species from the *Scenedesmus* genus showed a similar performance achieving a maximum value of OD_{750nm} = 0.5 at the end of the experiment. In contrast, the genera *Monoraphidium* and *Chlorococcum* showed a slow growth during the whole experiment, achieving a final OD_{750nm} of 0.45 and 0.42, respectively. Within the cyanobacterial strains used in paper I, the UHCC0492 showed the most rapid growth reaching a maximum OD_{750nm} similar to the best eukaryotic strains (Fig. 5B). The *Microcystis* genus and the two filamentous N_2-fixing *Nostoc* strains showed a similar performance with a relatively low growth rate. The screening trials revealed that several microalgae species are promising candidates for bioremediation experiments, and the hydroponic wastewater is a promising alternative source of nutrients. In paper I, the species *Scenedesmus* UHCC0027 was selected for the following steps, given the empirical knowledge gathered at that time within the research group in laboratory and pilot scale experiments. In paper II, the species *Tetradesmus obliquus* (NIVA-CHL107) was selected since it had outperformed all the other species tested (Fig. 6).

4.2 Pre-treatment of hydroponic wastewater

The use of waste streams for the large-scale cultivation of photosynthetic microalgae presents different challenges regarding the control of some physicochemical parameters such as the excess of turbidity or nutrient imbalances, in addition to the presence of microbial competitors that can outcompete the desired microalgae culture. For those reasons, it is important that the proper pre-treatment method is applied to purify the waste stream before inoculating it with the target species. Thus, in paper I, the pre-treated methods tested were selected based on their ability to remove the native microbial community and the feasibility of integration into a pilot-scale cultivation. The three different methods tested to purify the hydroponic wastewater were: Coarse filtration (CF), microfiltration (MF) and Bleach followed by neutralization with Sodium Thiosulfate (BST) (Fig. 7). The wastewater of each method was seeded with *Scenedesmus* UHCC0027 and incubated in a growth chamber. Blank flasks containing only the pre-treated wastewater were also incubated to monitor the growth of the native microbial community and evaluate the ability of each method in removing it.

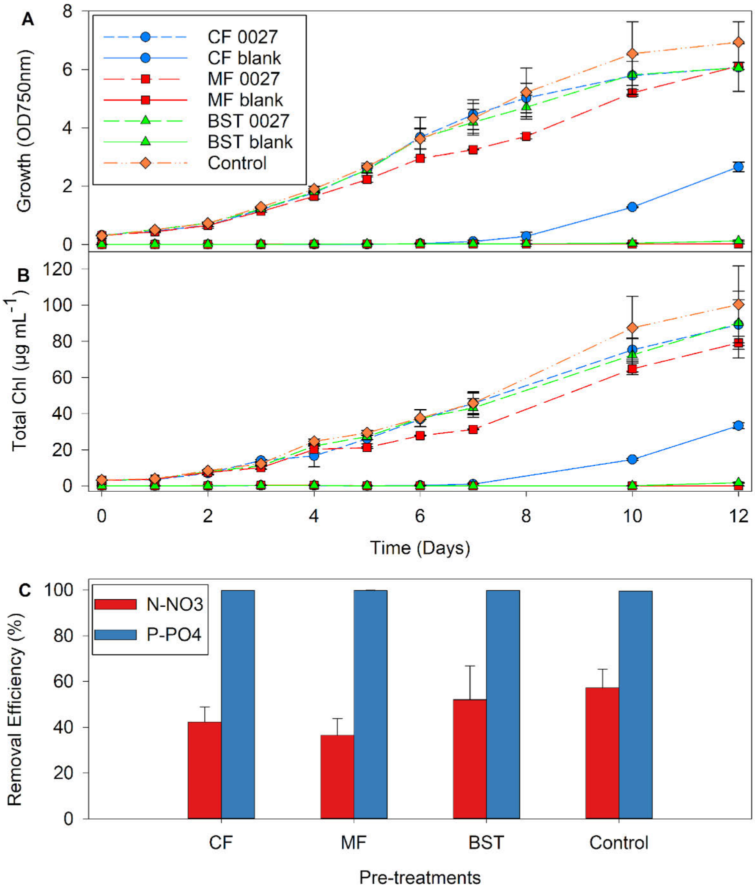


Figure 7. Effect of different pre-treatments on the growth (A), chlorophyll content (B) and nutrient removal efficiency (C) of the species *Scenedesmus* UHCC0027. The flasks CF 0027, MF 0027, BST 0027 correspond to the pre-treated wastewater seeded with target microalgae. The control flask was inoculated using BG11 growth medium. The data is represented as mean ± SE (n = 2).

The results showed that *Scenedesmus* UHCC0027 was able to perform without significant differences between the different pre-treatments (Fig. 7A, B). In addition to that, the results also showed there were no significant differences in the nutrient consumption between the pre-treated wastewaters with the *Scenedesmus* UHCC0027 being able to remove up to 50 % of N-NO_3^- and 99% of P-PO_4^{3-}. The results also indicate that the coarse filtration method was the least effective in eliminating the native microbial community. At day 7 of cultivation, the coarse filtration blank flasks recorded an OD_{750nm} = 0.1 ± 0.03 which gradually increased until the end of the experiment, achieving an average maximum OD_{750nm} = 2.66 ± 0.1. An increase in the total chlorophyll content was also observed, suggesting the presence of photoautotrophic microorganisms in the native microbial community. Assuming this microbial community was also present in the coarse filtration 0027 flasks, the results indicate that the species *Scenedesmus* UHCC0027 was able to outcompete the native microbial community and establish itself as the dominant culture. Since the different pretreatments did not significantly affect the growth or the nutrient removal efficiencies of *Scenedesmus* UHCC0027, and the culture was able to outperform the natural biome of the hydroponic wastewater, a decision was made to combine the bleach method with the coarse and microfiltration in the pilot-scale PBR system.

4.3 Pilot-scale bioremediation of hydroponic wastewaters

The screening trials and the pre-treatment experiments were fundamental to identify the most promising microalgae species and the suitable pre-treatment method for the hydroponic wastewater. In papers I and II, the pilot-scale bioremediation trials occurred inside a greenhouse using a tubular PBR illuminated with artificial light. The glass greenhouse was equipped with temperature systems that allowed it to create a favourable atmosphere for the growth of photosynthetic microorganisms.

4.3.1 Continuous mode PBR operation

In paper I, the microalga *Scenedesmus* UHCC0027 was cultivated in the PBR using the hydroponic wastewater as an alternative source of water and nutrients. The PBR was illuminated by a mixture of natural sunlight and artificial lights. The productivity of the strain and its ability to remove nitrogen and phosphorus were evaluated in a continuous cultivation where 4 different setups of parameters were tested (Fig. 8). In setups 1 and 2, the effect of the cellular concentration inside the PBR was tested by keeping a constant DW of 1 g L^{-1} or 0.5 g L^{-1} for 10 consecutive days, respectively. The following setups, 3 and 4, were planned to evaluate the performance of the culture under varying pH (Table 3). The trial was performed during the early spring season when the temperatures outside the greenhouse varied between -11 and 8 °C. To prevent light limitation due to lack of natural sunlight, the PBR was illuminated with artificial light using high-pressure sodium bulb lights that provided an average intensity of 200 μmol photons m^{-2} s^{-1} at the top surface of the PBR. The greenhouse infrastructure provided a constant temperature inside the PBR of 23.8 ± 2.9 °C. Under these abiotic conditions, the productivity of the strain was monitored daily.

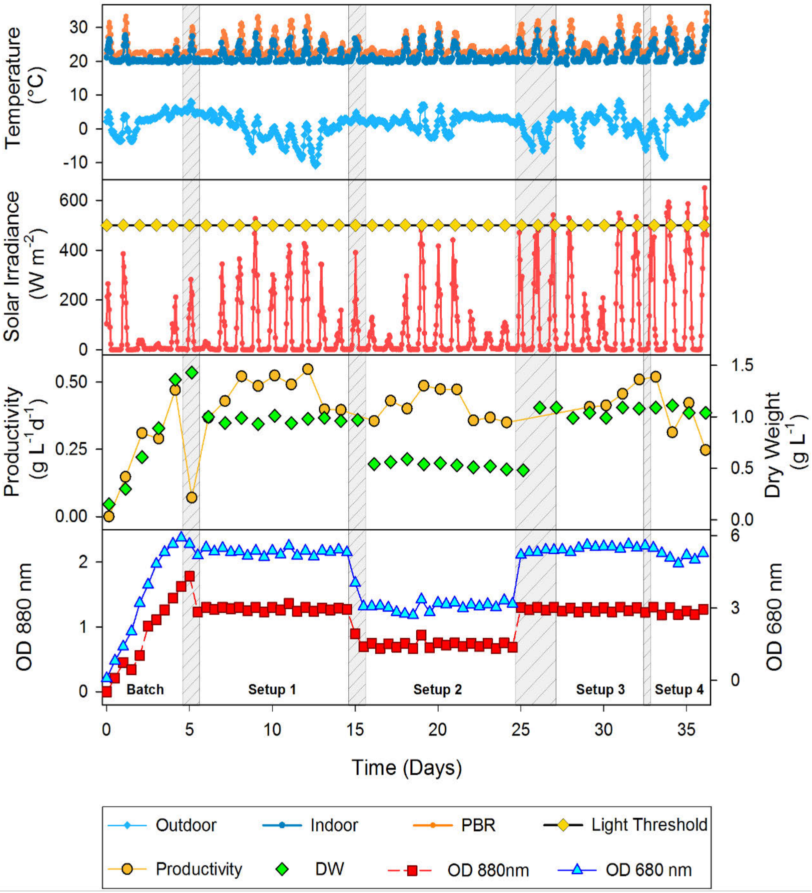


Figure 8. The abiotic conditions and the performance of the microalgae *Scenedesmus* UHCC0027 during the continuous mode cultivation. The grey bars show the transition periods between setups.

The culture was able to proliferate efficiently during the 35 days of continuous operation, exhibiting a steady volumetric productivity ranging from 0.38 to 0.46 g L^{-1} d^{-1} (Table 4). Depending on the growth rate of the culture in each set of parameters tested, the PBR recirculated 22 - 48 L d^{-1} of hydroponic wastewater, to a total of over 1000 L during the experiment (Table 4).

Table 4. The performance of Scenedesmus 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).

Parameter	Setup 1	Setup 2	Setup 3	Setup 4
Growth rate (d^{-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
Recirculated wastewater ($L\ d^{-1}$)	30.0 ± 4.04	48.07 ± 5.57	27.65 ± 2.67	22.54 ± 6.62

The hydroponic wastewater was regularly collected from the greenhouse and its nutrient composition varied significantly throughout the course of the experiment, presumably because of the greenhouse cultivation schedule. These variations, taken together with the daily fluctuations of natural sunlight and temperature, plus the pH and the cellular concentration inside the system, help explain the variations recorded for nutrient uptake across all stages of the trial (Fig. 9). During the continuous experiment, the culture achieved its maximum N uptake of 92.80 ± 11.3 mg g^{-1} biomass under the conditions of setup 2, when the cultivation pH was 7.5 and the dry weight was established at 0.5 g L^{-1}. In contrast, the minimum N uptake of 75.52 ± 6.9 mg g^{-1} biomass was recorded during setup 4 when the cultivation pH was 9 and the dry weight was set at 1 g L^{-1}. Despite the stable values of N uptake, the removal efficiencies were low across all the setups tested due to the high amount of nitrates present in the hydroponic wastewater.

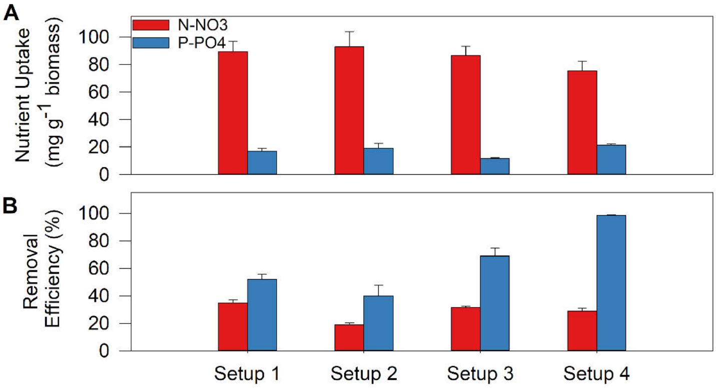


Figure 9. The nutrient uptake (A) and removal efficiencies (B) of *Scenedesmus* UHCC0027 culture during the different setups of the continuous cultivation.

The P uptake was stable across all stages of the cultivation except during setup 3 when the culture achieved its lowest value of 11.8 ± 0.4 mg g^{-1} biomass. During this period, the pH of the PBR was lowered to 6.8 and the culture also received a lower amount of natural sunlight. Nonetheless, the concentrations of $P\text{-}PO_4^{3-}$ were relatively low in the hydroponic wastewater which resulted in promising removal efficiencies. In fact, during the last period of the cultivation, the microalgae *Scenedesmus* UHCC0027 was able to remove 98% of the $P\text{-}PO_4^{3-}$ present in the hydroponic wastewater which allowed the system to comply with the requirements of the EU directive (91/271/EEC) for wastewater discharge.

4.3.2 Batch mode PBR operation

In paper 2, the experiments with the PBR were conducted using a batch mode approach to study the dynamic behaviour of nitrogen consumption. The research focused on understanding the utilization of nitrogen, which was the most prevalent nutrient found in hydroponic wastewater, throughout various stages of cell growth. In addition to that, the molecular composition of the biomass was determined at exponential and stationary phases of the growth to evaluate the best possible industrial applications. To this end, the microalgae *Tetradesmus obliquus* was cultivated in the PBR, and the system was illuminated with a combination of natural sunlight and artificial light using red and blue LEDs (Fig. 10).

During Trial 1, the maximum solar radiation was 187 W m^{-2} and the average duration of natural sunlight exposure was 7.7 ± 0.5 h day^{-1}. The temperature inside the PBR was consistently maintained at 22.0 ± 1.1°C, thanks to the greenhouse setup. At the start, the hydroponic wastewater contained 284.84 ± 0.18 mg L^{-1} of $N\text{-}NO_3^-$ and a 15.33 ± 0.01 g L^{-1} of $P\text{-}PO_4^{3-}$. Under this set of conditions, no lag phase was observed after inoculation and by the third day of cultivation, the system had already achieved 100 % removal efficiency of $P\text{-}PO_4^{3-}$. By day 13, the nitrate removal reached 97.5 %, meeting the standards outlined by the EU wastewater directive (91/271/EEC). By day 15, nitrate was fully removed (100%), and the culture continued growing until the end of the experiment (day 20), achieving a maximum dry weight of 4.8 ± 0.02 g L^{-1}.

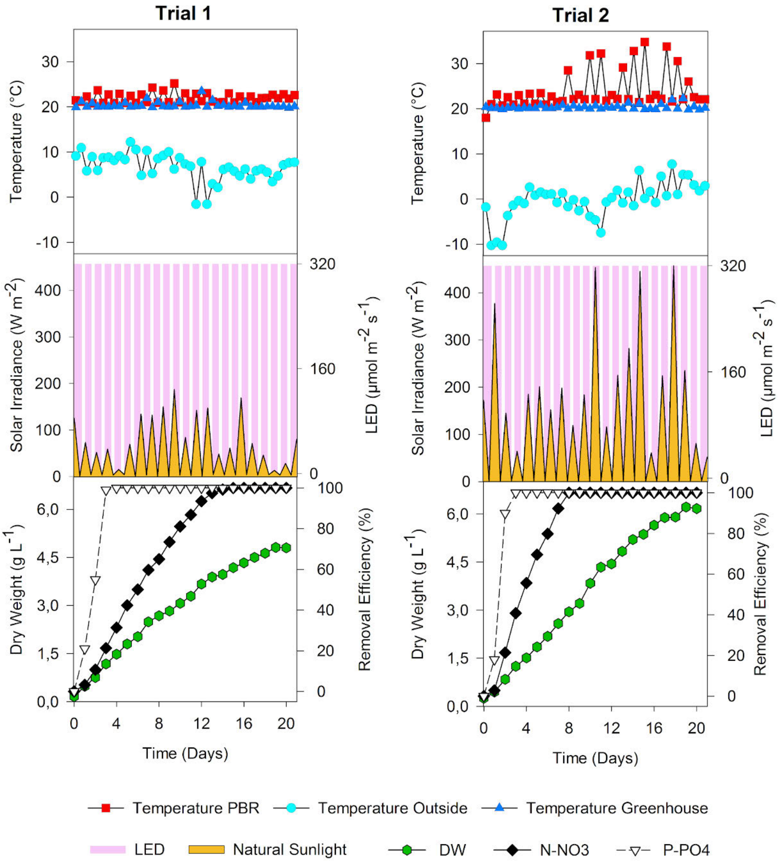


Figure 10. The performance of the microalga *Tetradesmus obliquus* was evaluated during its cultivation in hydroponic wastewater. Temperature and light measurements were recorded every 12 hours. The data for removal efficiency and dry weight are reported as the mean ± standard deviation, based on three technical replicates (n = 3). The highest standard deviation observed for removal efficiency was 0.8%, while for dry weight it was 0.15 g L^{-1}.

In the second trial, the average temperature within the PBR was higher, at 24.0 ± 4.1°C, and the maximum solar radiation increased to 457 W m^{-2}. The culture received an average of 11.9 ± 0.55 hours of natural sunlight each day, which was about four more hours of light per day than in Trial 1. The initial wastewater composition showed lower nutrient levels, with 235.02 ± 0.29 mg L^{-1} of nitrate and 8.79 ± 0.04 mg L^{-1} of phosphate. Similar to Trial 1, the culture removed all phosphate within just three days. It also continued to grow, achieving full nitrate removal seven days earlier than in the first trial. At the stationary phase, the system reached a maximum dry weight of 6.2 ± 0.03 g L^{-1}.

By the conclusion of Trial 2, the biomass concentration in the PBR exceeded that of Trial 1, even though the nutrient concentrations in the wastewater were lower.

This improved performance can be attributed to more favorable environmental conditions, which enhanced nitrogen uptake in Trial 2 by almost twofold compared to Trial 1 (Figure 11). Despite this increase, the maximum nitrogen uptake for both trials corresponded to a dry weight of approximately 1.5 g L^{-1}.

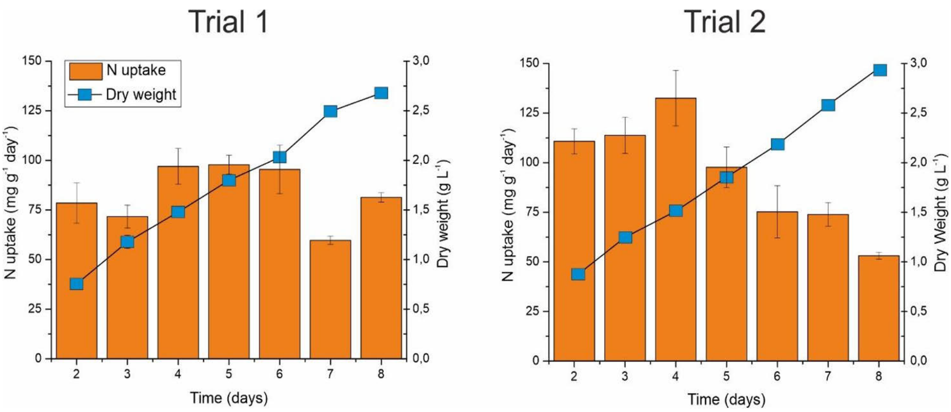


Figure 11. The daily nitrogen uptake dynamics of the microalga *Tetradesmus obliquus*, cultivated in an indoor photobioreactor (PBR), were analysed. The results are expressed as the mean ± standard deviation, based on three technical replicates (n = 3)

4.4 Biomass characterization and valorisation

In paper II, the biomass composition was comprehensively examined with respect to its protein and carbohydrate content, as well as its pigments and FAME composition. This groups were analysed, both qualitatively and quantitatively, to assess the potential industrial applications of the biomass. The results demonstrate that protein and carbohydrates concentration at different stages of growth are significantly influenced by nitrogen availability in the growth medium. When nitrogen is abundant during the early stages of growth, there is a higher allocation of carbon and nitrogen towards the synthesis of protein. However, as the cells approach the later stages of cultivation and nitrogen becomes depleted, their metabolism shifts towards the accumulation of carbohydrates (Fig. 12).

Regarding the pigment composition, the total amount of the primary pigments, chlorophyll a and b, was significantly higher at the exponential phase of growth in comparison with the stationary phase. Likewise, the presence of secondary pigments such as Violaxanthin and Lutein was also higher during the early phase of growth. These results suggest a strong relationship between high cell division and the need to efficiently preserve the photosynthetic machinery as a direct supply of energy to the cells. During this cellular stage, light penetration inside the PBR was higher due to a lower cell concentration, which promoted the biological synthesis of carotenoids as a photoprotection mechanism of the cells against excessive light.

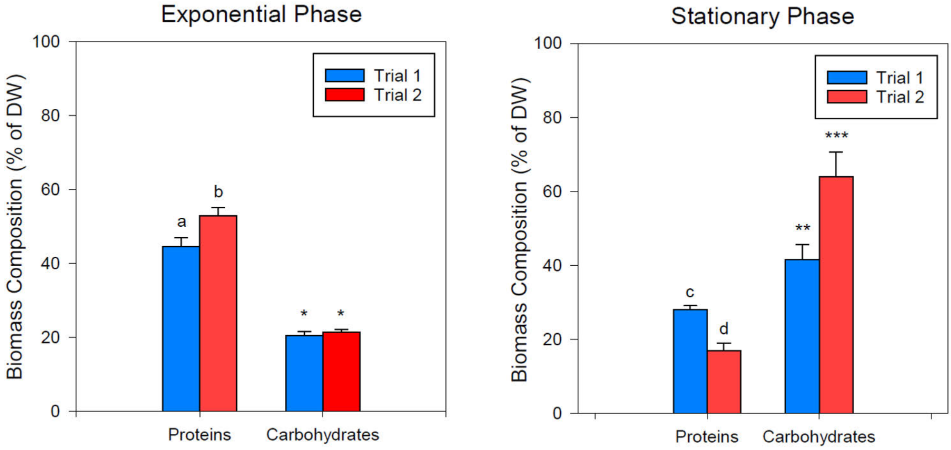


Figure 12. The protein and carbohydrate content in the biomass of *Tetradesmus obliquus* harvested at various growth stages was measured. The data is presented as the mean ± standard deviation (n = 3). Statistically significant differences across growth stages are indicated by different letters.

The results from the fatty acid composition of the biomass also demonstrated that across all stages of the growth, the biomass of *Tetradesmus obliquus* contained high levels of polyunsaturated fatty acids (PUFAs), a moderate amount of saturated fatty acids (SFAs) and a low content of monounsaturated fatty acids (MUFAs). In both trials, the PUFAs were the most abundant fatty acids in the biomass, ranging from 56 to 73 %. Overall, these findings indicate that the success and scaling up bioremediation project using microalgae is highly dependent on the physicochemical characteristics of the waste streams and the algae cultivation system, as both significantly influence the composition and potential uses of the resulting biomass.

4.4.1 Valorisation of algal biomass as plant biostimulants

Paper III assessed the potential of photosynthetic microorganisms as a new source of plant biostimulants. The biomass of six different microalgae species was used and the study employed two distinct extraction methods for each species, tested through laboratory and pilot-scale trials. These extraction methods, namely acidic hydrolysis and aqueous bead-milling were considered based on their potential effectiveness and the ability of being easily used in industry. To this end, at laboratory scale, the bioactive effect of the different extracts was evaluated using the plant model species *Arabidopsis thaliana*. At pilot-scale, the trials were conducted in a greenhouse using lettuce (*Lactuca sativa*) as a plant model species. The effectiveness of the extracts was evaluated based on plant morphological traits (e.g., root size, fresh weight) and photosynthetic performance indicators. The laboratory results from the *Arabidopsis* *thaliana* trials revealed significant differences between species and the extraction method applied. The bioactivity of the acidic hydrolysis extracts was unique to each species and the most effective concentration of the extracts ranged from 0.1 to 0.5 g L^{-1}, depending on the microalgae biomass (Fig. 13).

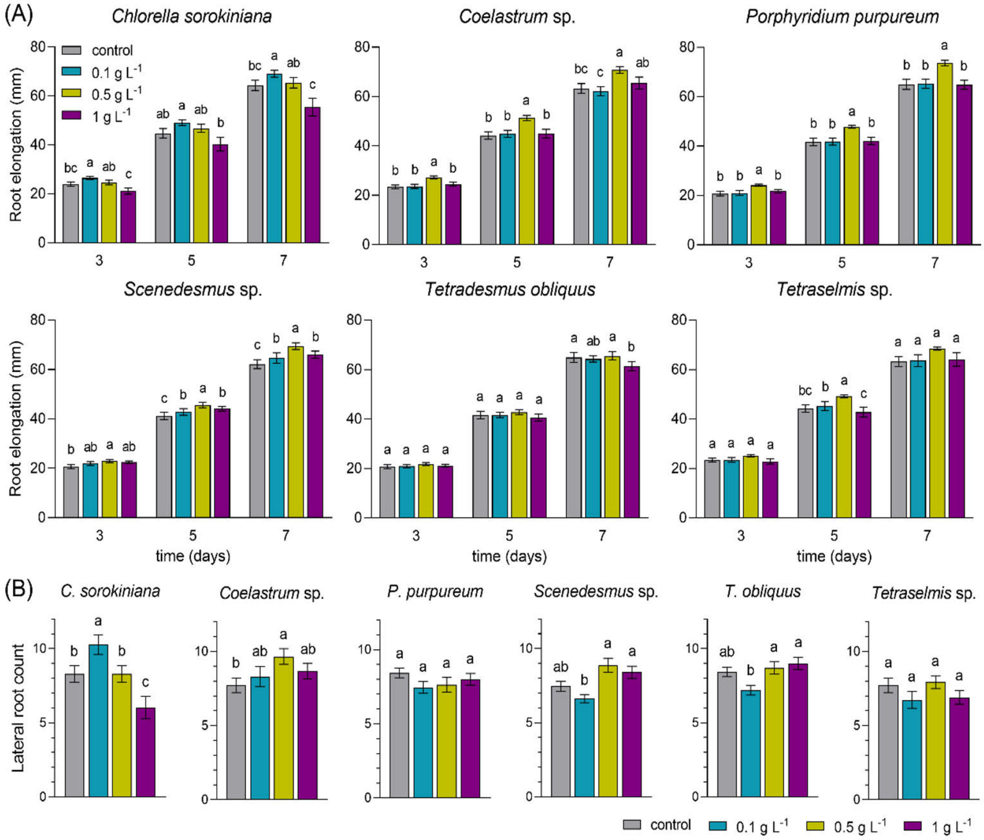


Figure 13. The effect of different acidic hydrolysis extracts on the root elongation (A) and on the lateral root count (B) of *Arabidopsis thaliana*. Bars represent mean ± SE (n= 28-30). Different letters above bars indicate statistically significant differences ($p < 0.05$).

The most promising species tested were *Porphyridium purpureum*, *Coelastrum* sp. and *Scenedesmus* sp. which showed significant differences in comparison with the control groups. The extract derived from *P. purpureum*, at a concentration of 0.5 g L^{-1}, demonstrated the most advantageous effects on the plants, resulting in a 13.4 % increase in root elongation compared to the control group. Despite the promising outcome of the experiment, the results also indicate that for some species, the extract concentrations of 1 g L^{-1} can have detrimental effects on root development. The lateral root count is a plant parameter of the surface area of the roots and a proxy for the measurement of the health of the rooting system. The microalgae extracts obtained from acid hydrolysis did not significantly affect the development of the lateral roots, with the exception being made to the *Chlorella sorokiniana* extracts at the concentration of 1 g L^{-1} which revealed to be toxic for the roots.

The results obtained from the aqueous bead-milling extracts exhibited a higher level of consistency, as the plants responded uniformly to all microalgae species (Fig. 14). Most of the plants displayed no significant enhanced root elongation when exposed to the lowest concentration, 0.05 g L^{-1}. In contrast, the plants exposed to concentrations equal or greater than 0.1 g L^{-1} exhibited smaller roots in comparison with the control groups. Despite the negative effects on the root elongation, the extracts of all the microalgae species tested did not significantly affect the lateral root count at the end of the experiment.

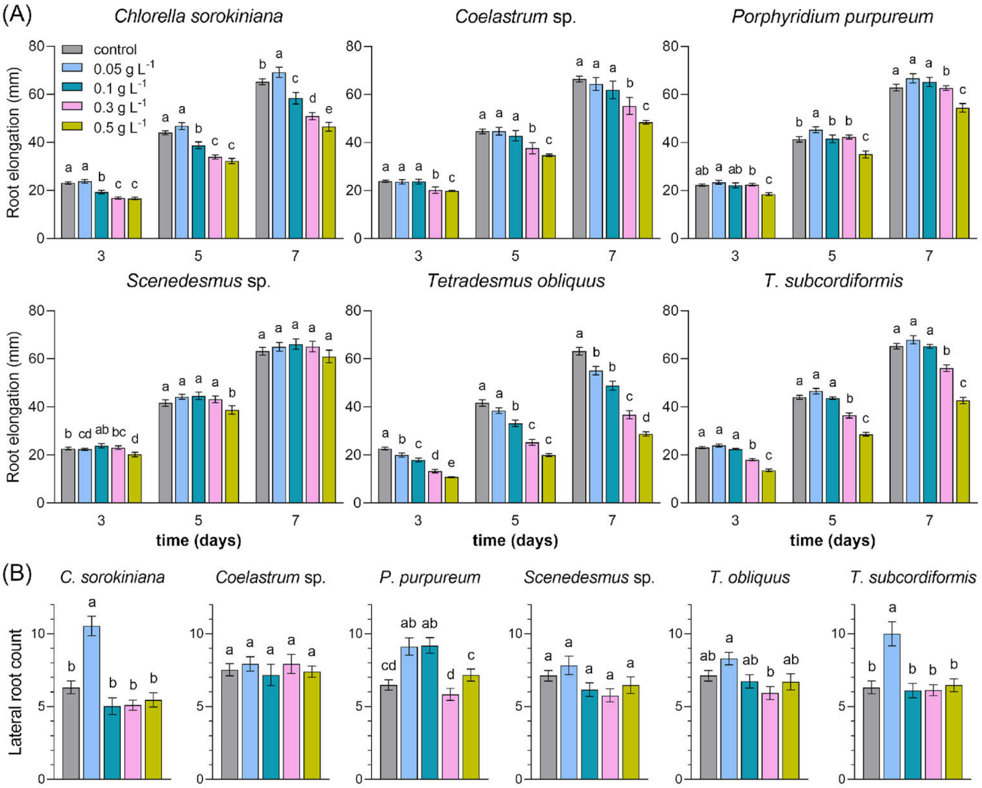


Figure 14. The effect of different aqueous bead-milling extracts on the root elongation (A) and on the lateral root count (B) of *Arabidopsis thaliana*. Bars represent mean ± SE (n= 28-30). Different letters above bars indicate statistically significant differences ($p < 0.05$).

The pilot-scale trials using lettuce as a crop model species revealed that the plants exhibited a more favourable response to the acid hydrolysis extracts compared to the aqueous bead-milling extracts (Fig. 15). The results also emphasize the importance of testing different extraction protocols, as the extracts obtained from the same biomass demonstrated significantly different outcomes. The plants treated with the aqueous bead-milling extracts from the microalgae *Coelastrum* sp. and *P. purpureum* had a fresh weight that was 11.6 – 13.2 % lower when compared with the control group, respectively. In contrast, the acidic hydrolysis extracts made from the same species and using the same concentration, 1 g L^{-1}, resulted in fresh weight increments of up to 13.2 %. The most promising species tested was *Tetradesmus obliquus* which was able to increment the fresh weight of the lettuce plants by almost 16% compared with the control group. Taken together, these findings emphasize the importance of conducting phenotyping screening assays at small scale to determine the optimal dosage of the extracts, the optimal extraction protocol and to differentiate potentially harmful microalgae species from promising candidates.

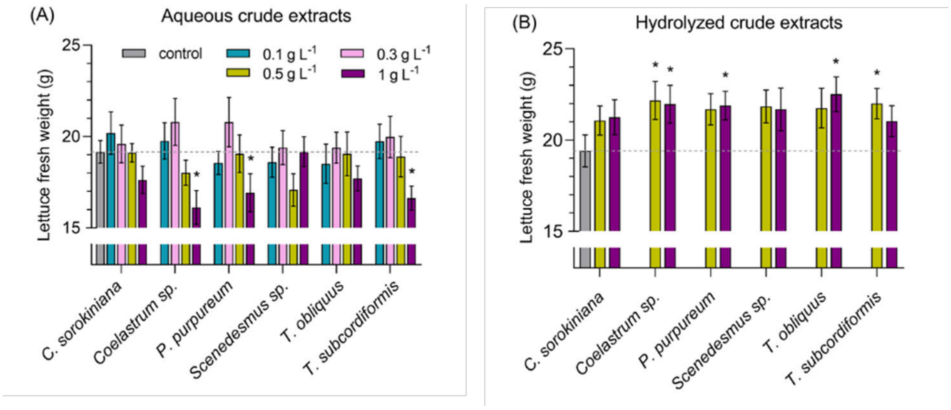


Figure 15. The effect of different aqueous crude extracts (A) and hydrolyzed crude extracts (B) on the fresh weight of lettuce plants. The extracts were applied three times during the vegetative cycle and plants were harvested after 35 days of germination. Bars represent mean ± SE (n = 15).

4.4.2 Valorisation of algal biomass as biopesticide

In paper IV, the antifungal activity of 15 microalgae species was evaluated against a selection of 7 plant pathogens commonly present in Finnish agriculture. The preliminary screening trials yielded promising results, indicating that the extract from *C. sorokiniana* was highly effective in inhibiting most of the plant pathogens. Among the plant pathogens tested, the fungus *P. cactorum* exhibited a particularly high sensitivity to the extract. To better assess the physicochemical properties of *C. sorokiniana* extracts and determine the optimal extraction protocol, the microalga was cultivated in 5L bottles to generate sufficient biomass. The harvested biomass was divided into two equal parts: one portion was used in its fresh state (wet biomass), while the other portion was submitted to freeze-drying (dry biomass). During the cell disruption stage, two distinct solvents were employed to examine the impact of utilizing both polar and non-polar solvents. The extracts were fractionated according to crude, supernatant or pellet to evaluate the strength of the bioactivity. The wet biomass extracts had a final concentration of 200, 100, and 50 g L^{-1}, which correspond to a final concentration of 16, 8, and 4 g L^{-1} for the dry biomass extracts, respectively (Fig. 16).

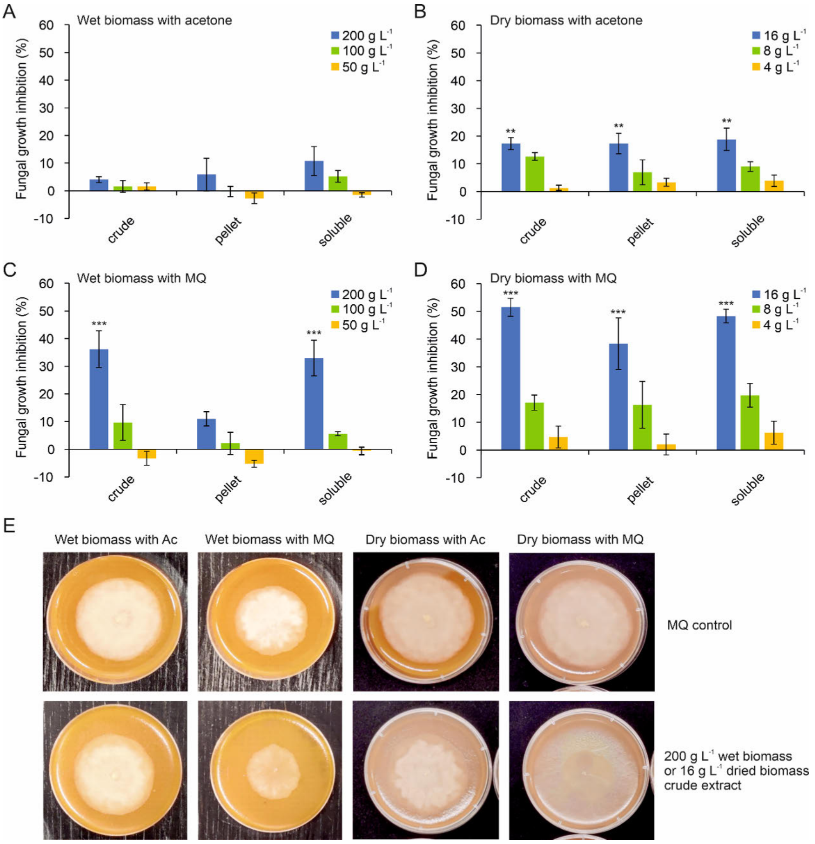


Figure 16. Growth inhibition of *P. cactorum* exposed to different *C. sorokiniana* extracts. Wet biomass tests with acetone (A) and MQ water (C); dry biomass tests with acetone (B) and MQ water (D); (E) PDA + tomato plates were coated with 100 µl of acetone extracts (Ac) or MQ as negative control and the fungal growth was observed for 7 days (mean ± SE, n = 3).

The results demonstrated that the bioactivity of the *C.sorokiniana* extracts is strongly dependent on the downstream processes chosen, particularly the solvent used during cellular extraction as well as the final concentration of the extracts. The aqueous extracts exhibited the highest bioactivity when used at a concentration of 16 g L^{-1}, effectively inhibiting the growth of *P.cactorum* by a minimum of 38% and up to 51% depending on the fractions tested. On the contrary, when acetone was used as the extraction solvent for dry biomass, the bioactivity of the extracts was considerably lower, with an ability to inhibit fungal growth by less than 20%. No significant effect was recorded when using wet biomass and acetone as the extraction solvent. Therefore, the experimental results show that the extracts obtained from freeze-dried biomass using MQ water exhibited the highest levels of antifungal activity. To further evaluate the antifungal effect of the extracts, the best performing extract was tested in detached leaves from strawberry plants (*Fragaria* × *ananassa*). The leaf damage index was used as a proxy to measure the antifungal activity directly in plants. The strawberry leaves were dipped into the extract and infected with *P.cactorum* at the base of the petiole. The control group was dipped in MQ water (Fig. 17).

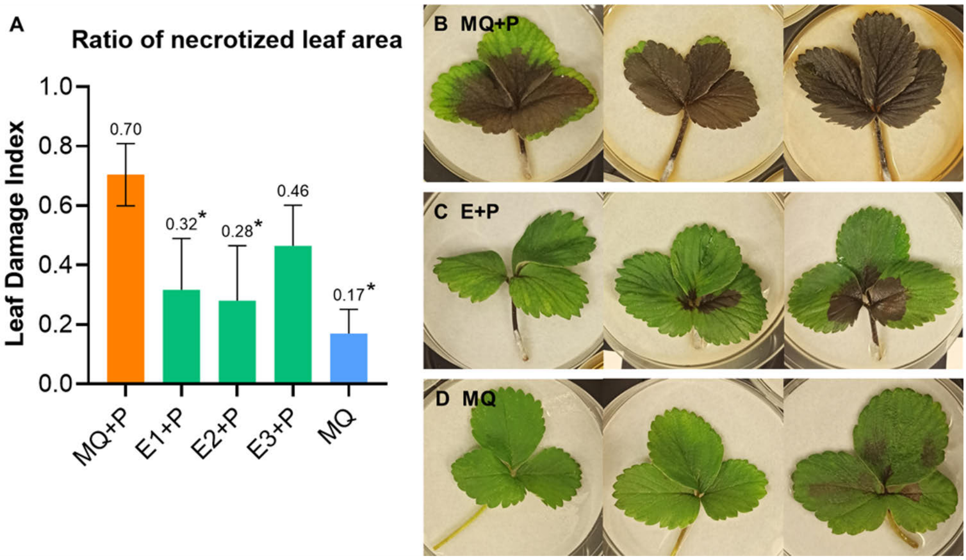


Figure 17. Effect of the aqueous extract of *C. sorokiniana* on strawberry leaves infected with *P. cactorum*. (A) The Leaf Damage Index of control leaves (MQ), non-treated infected leaves (MQ+P), and the infected leaves treated with extracts of 3 microalgal extract replicates (E1, E2, E3 +P). (B) Non-treated leaves infected with *P. cactorum*, (C) leaves treated with extracts and infected with *P. cactorum*, (D) control leaves (dipped in MQ-water). Data points represent ± SE of 5 technical replicates (n = 5).

The results demonstrated that in the absence of the microalgae extract, the fungus *P.cactorum* is highly effective in infecting strawberry leaves. The majority of the untreated leaves showed extensive contamination by *P.cactorum*, characterized by necrotic tissues near the stem and inner areas of the leaf (Figure 16 B). The untreated leaves presented an overall average Leaf Damage Index of 0.71 ± 0.11, contrasting with the value 0.28 ± 0.19 for the group of leaves treated with the *C.sorokiniana* extract. This outcome reveals that the aqueous microalgae extracts were able to decrease the infection rate by 60% (Fig. 17 C).

5 Discussion

Microalgae-based technologies offer a promising solution to recycle a significant volume of industrial side streams that are currently being discharged into nature. The integration of this green solution into traditional manufacturing processes not only promotes the mitigation of environmental pollution at its origin, but also represents a promising step towards the development of a new framework of sustainable industrial practices. In the present study, the feasibility of using microalgae cultivation methods for the bioremediation of hydroponic wastewaters was evaluated. Additionally, we explored the potential of microalgae biomass as a new source of biopesticides and plant biostimulants. We identified several species that can thrive and effectively purify the hydroponic wastewaters, selecting two for the pilot-scale tests using the PBR. Several microalgae species were screened for biopesticide and plant stimulant effects however, only a small fraction displayed promising bioactivities. Furthermore, we designed and optimized a series of rapid screening protocols, demonstrating the importance of applying the correct downstream processes to the biomass.

5.1 Microalgae technologies for wastewater treatment in greenhouses

The use of photosynthetic microorganisms to purify wastewaters from hydroponic greenhouses remains a promising solution to mitigate diffuse pollution caused by wastewater discharges. The deployment of this technology inside the greenhouses represents an industrial symbiosis that stimulates innovation and promotes sustainability. On one hand, the greenhouse infrastructure offers the optimal environment for microalgae to grow. On the other hand, the microalgae cultivation systems can clean the wastewaters produced by the greenhouse operation, improving the resource efficiency and water management of the whole facility. In addition to these, the microalgae biomass can be used as an effective biopesticide or plant biostimulant, reducing even more the environmental footprint of the greenhouses. Despite the promising concept, the future success and scalability of this solution is dependent on a complex multiparametric interaction between biological, technical and economic factors. From a biological perspective, it is crucial to identify robust microalgae species that can grow fast in agricultural wastewaters while tolerating different nutrient loads, due to varying irrigation regimes and dynamics at different growth stages of crops. Besides the fitness of the species, it is equally important to identify the proper pre-treatment for the wastewaters to reduce turbidity, minimize contaminants and remove suspended organic matter.

In parallel to these constraints, the use of indoor facilities for hydroponic wastewater treatment in the Nordic countries possesses a great challenge to the conventional microalgae cultivation systems. The spatial requirements of this technology and the need for high hydraulic retention times due to nutrient-rich wastewaters, are simply not compatible with the volume of water to be remediated. To overcome these challenges, new reactor designs and processes must be created, considering the architecture of the greenhouses and making the most of the available space. A great example of this new generation of cultivation systems is the recently designed facilities of the Swedish Algae Factory, located in Gothenburg, where an artificially illuminated vertical stacked raceway pound was built to maximize the cultivation volume inside the greenhouse. Another example is the recent study published by Pechsiri et al., 2023, proposing a new generation of vertical bubble columns that rely on internal illumination. The authors estimated that the new design can outperform conventional cultivation systems with the advantage of requiring a fraction of the footprint. Taken together, these examples represent what the promising near future of indoor microalgae systems can look like, where the combination of tailored designs with artificial light can enable the industrial cultivation of phototrophic microorganisms regardless of space limitations and outdoor conditions. If used for the purpose of bioremediating hydroponic wastewaters, these novel cultivation systems can theoretically outcompete the conventional cultivation systems because of their enhanced ability to transfer light to the cells, resulting in higher productivity, more nutrient consumption and lower hydraulic retention times. These advantages combined with a spatially optimized design would allow the purification of a larger volume of wastewater, resulting in a more meaningful improvement of the resource efficiency and water management of the greenhouses.

Besides the biological and technical challenges, the business concept around microalgae technologies for wastewater treatment remains to be fully explored. As the consumer's price for clean water and wastewater treatment in Finland does not exceed 4€ / m^3, microalgae-based solutions that rely on artificial light are not expected to compete with conventional treatment methods. Operating artificial lights for several days to effectively treat hydroponic wastewater would result in expenses multiple times higher than those of traditional methods. For that reason, microalgae-based business models must emphasize the multi-platform that they can offer, including sustainable products and the valorisation of sidestreams. To that end, the use of microalgae biomass as an alternative source of biopesticides and plant biostimulants promotes a holistic approach towards the development of circular economy models in agriculture.

5.2 Microalgae biomass as a new source of plant protection products

Biopesticides and plant biostimulant products are currently one of the most promising fields of applied research for microalgae biomass. In the current study, the biopesticide results clearly identified several eukaryotic microalgae capable of inhibiting the growth of fungi and oomycetes that are commonly found in Finnish agriculture. Likewise, the plant biostimulant results identified several species capable of effectively stimulating plant root development in laboratory trials and increasing the fresh weight of lettuces grown in a greenhouse. In both applications, the results highlight a strong species-specific interaction between the microalgae extracts and the hosting organisms. Particularly in the biopesticide effects, where the screening trials revealed very significant differences between the bioactivities of each extract. Similarly, in the plant biostimulant trials, the results showed the importance of the extraction protocol, with several microalgae displaying significant differences depending on the methods. For this reason, in the near future it would be of great value to explore the molecular composition of the extracts and its interactions with the host organisms.

Notably, the results published in papers III and IV also suggest that depending on the dosage, some microalgae species can be used as multipurpose plant protection products, showing both biopesticide and plant biostimulant effects. A microalgae-based multipurpose plant protection product would be commercially and environmentally relevant. From a business perspective, several other solutions already exist in the category of multipurpose plant products, therefore it would be easy to advertise and place a product in the market without the need to re-educate consumers. From an environmental standpoint, the use of biological derived solutions to mitigate pests or stimulate the growth of plants is often considered to be less harmful to the surrounding nature in comparison with the synthetic alternatives (Fenibo et al., 2021).

5.3 Microalgae-based technology as a catalyser for sustainability

Circular economy projects, such as the one proposed in this study, are expected to become increasingly popular in the upcoming years, reflecting a growing focus on implementing zero-waste policies and redesigning traditional manufacturing processes. Despite the obvious need for alternative industrial standards, the transition towards a more sustainable use and conversion of natural resources is happening slowly. Numerous factors underpin this gradual progression, including the readiness level of the new technologies, a lack of harmony between policymakers and a general feeling of uncertainty across stakeholders due to the socio-economic disruptions it may cause (Kovacic et al., 2020). Microalgae-based products and services can be advertised as one of the missing links in this discussion because they enable an alternative route that is complementary to the current industrial practices, rather than being disruptive. This study demonstrated that microalgae cultivation systems can be coupled with existing infrastructures to purify wastewaters and help tackle the environmental burden caused by wastewater pollution. Moreover, we demonstrated that it is possible to create a new generation of plant protection solutions that can help reduce and replace traditional products. By giving the necessary incentives to replicate this model in other industries, both public and private stakeholders are placing the microalgae industry in a privileged position to become a fundamental sector in the transition to a more sustainable use of natural resources.

6 Conclusion and Future Perspectives

The present study demonstrated that microalgae-based technologies can play a meaningful role in addressing the environmental challenges faced by the agriculture sector. Here, it has been shown that several microalgae species are able to proliferate in wastewaters from a hydroponic greenhouse (Paper I, II). In addition to that, it has been demonstrated that numerous species produce a biomass that can be used as an organic biopesticide or as a plant biostimulant (Paper III, IV). Despite the many progresses made in recent years, the technological readiness level of the microalgae sector remains a work in progress. Whilst the raceway ponds or the tubular reactors showed the world the potential of photosynthetic microalgae biomass, they also represent a bottleneck by being highly dependent on variables such as natural light, weather or available land. The development of a growth system that favours vertical cultivation and integrates artificial light would be of great value to the sector.

In addition to the strategy proposed in this study for managing hydroponic wastewaters, future efforts should also consider using heterotrophic microalgae. On the many occasions working in the greenhouse, the amount of plant biomass wasted was too excessive to be overlooked. In the scope of circular economy and sustainability, such biomass could be used as a low-carbon source of sugars to be used during the fermentation of the microalgae. A closed-loop system would be created in this way, where the wastewater and waste biomass of the greenhouse operation could be reused and recycled. The CO_2 created during the process, could be released in the greenhouse atmosphere and it would be consumed by the crops, reducing the need to enrich the air with external sources of CO_2.

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