Master's Thesis Report

Production of natural pigments from *A. platensis* grown on wastewater from local treatment facility.

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Degree Project in Biotechnology (30 ECTS)

Preface

This master thesis was performed in collaboration between the Division of Water Resources Engineering (TVRL) and the Division of Applied Microbiology (TMB), LTH Lund University in Sweden. The project spanned 20 weeks, between January to June 2023, with 12 weeks of laboratory work, and serves as part of the Master of Science in Biotechnology program.

I would like to thank my supervisor, Dr. Jing Li, for the continuous support as well as my examiner, Associate Prof. Catherine Paul, for the helpful comments, suggestions, and guidance throughout this project. Additional thanks to those working at TMB for their help and assistance with laboratory procedures throughout this process. Finally, I would like to express my gratitude towards those working at RecoLab treatment facility in Helsingborg, Sweden, for the preparation of wastewater samples and helpful tips.

Abstract

Although microalgae have been considered for the biological treatment of wastewater since the 1950's, their potential to efficiently remove pollutants from various streams remains largely untapped and is yet to be utilized at large scale. The production of algal biomass and extraction of high-value compounds has, however, become increasingly popular with large scale farms and biorefineries around the world producing many promising alternatives to petroleum-based products and processes. While microalgae systems rival traditional production in terms of environmental sustainability and health benefits, the economic viability remains a challenge. This study aimed to investigate the potential of a biorefinery concept using wastewater as growth medium for the production of natural pigments by cyanobacterium, *Arthrospira platensis.* Using effluent streams as a growth media drastically cuts costs of production and with this circular approach, we are able to address challenges facing the wastewater treatment industry while providing a sustainable alternative to petroleum-based products.

During phase one, the growth of *A. platensis* was monitored on different amounts of wastewater supplemented with standard growth media. Biomass productivity and growth rate ranged from 233,12 – 2000 $mg.L^{-1}.d^{-1}$ and 0,19 – 0,37 d^{-1}, with 50% FWW supplemented with 50% Zarrouk media resulting in the highest values and thus proving to be the preferred growth media. However, cultures grown on 75% FWW supplemented with 25% Zarrouk media resulted in the highest PC content and thus was chosen as the growth media for further investigation. Phase two investigated the effect of different photoperiods (14h:10h and 16h:8h) on the production of PC, while assessing the scale-up potential of the system and monitoring nutrient removal. Photoperiod had no significant effect on PC content, however, a decrease in total PC production was seen between phases, suggesting further optimization for scale-up to be viable. In all cases, NH_4^+, PO_4^{3-}, and TN in the WW media were successfully reduced to levels below the lowest range of detection. While further optimization is necessary, the proposed process proved successful in reducing harmful nutrients from WW, maintaining *A. platensis* growth, and producing sufficient levels of PC.

Keywords: Cyanobacteria, pigments, microalgae, wastewater treatment.

Popular Science Summary

Harnessing Microalgae: Transforming Wastewater into Valuable Pigments

Innovative solutions are constantly sought to address the challenges of wastewater treatment and the increasing demand for sustainable products. One promising approach involves utilizing microalgae in a biorefinery system that not only cleans wastewater but also extracts valuable pigments from the biomass. This concept presents an intriguing solution that combines environmental remediation with the production of high-value products.

Microalgae are microscopic organisms that harness the power of photosynthesis to convert sunlight and carbon dioxide into organic matter. They are incredibly efficient at this process, exhibiting high growth rates and accumulating various valuable compounds, including pigments. Their ability to thrive in diverse environments, including wastewater, makes them an ideal candidate for treatment and resource recovery. Traditional wastewater treatment processes consume large amounts of energy and are environmentally taxing. However, by integrating microalgae cultivation with wastewater treatment systems, we create a symbiotic relationship where the nutrients and organic matter present in the wastewater are used as a nutrient source for growth, effectively purifying the water in the process. One particularly exciting aspect is the extraction of valuable pigments from the microalgae biomass. These pigments not only provide color to the microalgae but also possess significant commercial value in various industries such as food, cosmetics, and pharmaceuticals.

This study aims to investigate the potential of this biorefinery concept on local wastewater sources from the treatment facility RecoLab located in Helsingborg, Sweden. By establishing growth of a popular microalgae, *Arthrospira platensis*, on a food wastewater stream which is high in nutrients, we can optimize the biomass growth and assess the pigment content produced throughout cultivation. Additionally, we investigate the scale-up potential of this system by increasing the working volume to assess the effects of a larger scale implementation. Through two phases of experiments, *A. platensis* successfully reduced the high levels of nutrients to below the lowest detection range while maintaining impressive biomass productivity and producing a sufficient amount of pigment. With further research and testing, this process offers a promising solution which may contribute to building a more circular bioeconomy both locally, here in Sweden, and on a global scale.

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Abbreviations

ANOVA	Analysis of variance
APC	Allophycocyanin
CAGR	Compound Annual Growth Rate
CO_2	Carbon dioxide
COD	Chemical oxygen demand
DW	Dry weight
FWW	Food wastewater
HCl	Hydrochloric acid
NaOH	Sodium hydroxide
NH_4^+	Ammonium
OD	Optical density
PBPs	Phycobiliproteins
PC	Phycocyanin
PE	Phycoerythrin
PO_4^{3-}	Phosphate
PSF	Photosynthetic unit model
TN	Total nitrogen
TOC	Total
USA	United States of America
USD	United States Dollar
WW	Wastewater

1. Introduction

Algae have been exploited for thousands of years and recent decades have seen a significant increase of large-scale cultivation of various species for commercial use in global industries (Chapman, 2010). However, due to the complexity of processing and the high cost of production, many valuable compounds produced by microalgae systems are economically unfeasible and remain outcompeted by their petroleum-based counterparts (Bastiaens, et al., 2017). By employing a biorefinery concept, using effluents as a nutrient source for cultivation, the economic and energy balances can be turned toward a more favorable outcome. This thesis will investigate the potential of a biorefinery process, producing natural pigments from microalgae grown on local municipal wastewater. Although the largest market use for natural pigments is in pharmaceuticals or as a food colorant, this project focuses on producing pigments for use in paint, ink, and dye, addressing sustainability in the paint, printing and textile industries which still rely heavily on synthetic petroleum-based pigments like carbon black. This eliminates the need for the biomass produced to meet food safety requirements and thus allows for growth on wastewater.

Currently, there are only a small handful of companies producing microalgae biomass in Sweden and wider Scandinavia, with even fewer cultivating cyanobacteria and none which focus on pigment production for uses other than pharmaceutical or nutraceutical applications. The field of wastewater treatment research, however, is very prominent in Sweden with a major focus on creating a more circular system through nutrient recovery and reuse of clean water. The current project contributes to this research in offering a hopeful new valorization method for wastewater treatment while producing a high-value product that is not currently produced locally.

1.1. Aim and Objectives

The aim of this project was to establish growth of the microalgae, *Arthrospira platensis*, on a local wastewater stream, determine nutrient removal efficiency and outline the potential of further biomass valorization by extraction of the natural pigment, phycocyanin.

1.2. Delimitations

The following delimitations applied to this research project:

- Only one species and strain of microalgae was included in the investigation.
- Bioreactor design was not optimized due to time limitations and lack or prior resources.
- Hygienic, but not aseptic, conditions were maintained throughout experiments due to lack of necessary laboratory equipment.
- Optimization of aeration system, light intensity, temperature, and downstream processing was outside the scope of this project.
- Monitoring and characterizing the bacterial population present in the cultivation was outside the scope of this project.
- Comparing different separation and purification methods for phycocyanin extraction was out of the scope of this project.
- Comparing abiotic factors; light intensity, temperature, pH, and salinity, for their effect on PC production was out of the project scope.
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2. Theoretical Background

2.1. Arthrospira platensis

2.1.1. Characteristics and classification

Ancestors to the eukaryotic algae, cyanobacteria are among the most ancient organisms on Earth, and were first to utilize oxygen photosynthesis as a metabolism (Jacob-Lopes, et al., 2020). Bounds of evidence point to these ancient algae as the major role player in the oxygenation of Earth 2 billion years ago and thus the transformation of the planet's geochemistry. Not only are they still the major oxygen producers but their nitrogen and carbon fixation capabilities remain unmatched (Soule & Garcia-Pichel, 2019). Additionally, algae, in general, produce up to five times the biomass per area and unit time compared to terrestrial energy crops (Heinsoo, 2014). This superior carbon fixation coupled with high productivity yields, makes microalgae an extremely attractive commercial crop.

What is commonly known as Spirulina, *Arthrospira* is a distinct genus composed of up to 30 species, including *A. platensis*, the most well-known and important cyanobacteria currently

grown commercially (Jacob-Lopes, et al., 2020). It is a multicellular, filamentous, photosynthetic microorganism recognized for its spiral or helical trichomes and is found in freshwater and subtropical alkaline environments with optimum temperatures in the range of 30-35°C and with a preference for higher pH values ranging 9-11 (Jacob-Lopes, et al., 2020). Thus, with the ability to grow in extreme conditions, cultivation can take place with limited sterility. Morphologically, this species is simple with no distinct organelles or nuclei, and with cell walls composed of peptidoglycan (Borowitzka, 2013). It's blue-green color is owed to the presence of phycocyanin and chlorophyll pigments, that are core to its photosystem as essential light-harvesting molecules. Several Spirulina species are naturally occurring in alkaline lakes across Asia, Africa, Mexico, and Burma, where it has been harvested for human consumption for over a thousand years (Chisti, 2018). Since it's rediscovery in 1966, Spirulina has been extensively studied and noted for its high nutritional value with high protein content of up to 65%, antioxidants, vitamin A, iron and gamma-linoleic acid, denoting it a "super food" (Heinsoo, 2014). Today, modern commercial production of *Arthrospira*, mostly for the food industry, is widespread with the largest production in the USA and China (Gershwin & Belay, 2008). Besides its use as food, *A. platensis* is cultivated for the production of natural pigment for various applications, as will be discussed in the following sections.

2.1.2. Algae growth kinetics

While photosynthesis is naturally the main metabolic model, microalgae are extremely versatile in their means of obtaining energy. In the case of some *Arthrospira* species, growth can occur either heterotrophically using organic carbon sources or phototrophically using carbon dioxide and light (Vonshak, 1997). Phototrophic systems prove slightly more complicated due to light being an additional limiting factor, whereas, in heterotrophic cultivation, only one nutrient is considered a limiting growth factor (Heinsoo, 2014). In this project, however, only phototrophic cultivation was considered.

In 1913, Michaelis and Menten proposed one of the most popular mathematical models describing microbial growth kinetics, which was followed by Monod proposing a new non-linear model with high accuracy for simple substrates and pure culture (Islam, et al., 2021). While these models are the preferred and more commonly used systems for describing microbial growth, Monod-kinetics fails to incorporate the complexity of illumination, not taking in to account self-shading and the influence of fluid dynamics. When focusing on the

production of biomass in a photobioreactor system, light effects are the most important factors to consider as light is very different from other substrates because the intensity changes with the geometric position in the reactor, or in simpler terms, the light decays with distance from the source (Jacob-Lopes, et al., 2020). The Lambert-Beer equation is often taken as a first approximation of illuminance at Z, a specific distance from the light source. As indicated in figure 1, where the exponential decrease in light intensity is seen as the distance from the light source increases. This figure is a simplified scheme showing the illuminance profile with distance from the illuminated surface of the photobioreactor.

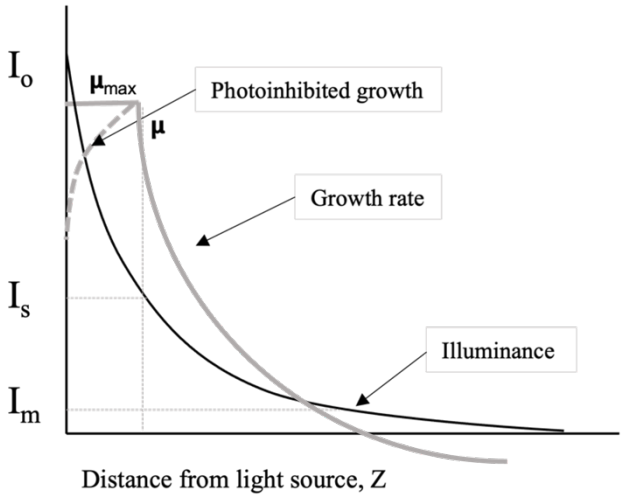

Figure 1. Illumination zones in a photobioreactor. Light saturation zone near the illuminated wall: maximum growth rate. Illuminance between I_s and I_m: μ is linear on I. $I < I_m$: the culture dwindles. Figure adapted from (Jacob-Lopes, et al., 2020).

Therefore, it can be understood that in a dense culture the growth pattern changes from exponential to linear as only the cells close to the surface are able to photosynthesize and thus the growth rate is set by the size of the illuminated surface and not by the number of cells (Fogg & Thake, 1987). The standard growth curve, thus, is slightly different from that of other microorganisms, given this extra linear phase of growth after the exponential phase.

Describing the kinetics of photosynthetic growth is further complicated by the consideration of fluid dynamics, mixing and variation between systems. This has led to numerous models that range in complexity, with the so-called P-I chart or saturation curve being among the most basic (Chiong, et al., 2016). This model outlines the dependence of photosynthesis rate on illuminance, using easily measurable parameters. Other models include more Monod-type equations and the photosynthetic unit model or PSF (Jacob-Lopes, et al., 2020).

2.2. Natural pigments

Microalgal pigments are essential to photosynthesis as they are the molecules responsible for light harvesting and energy transfer. Pigments are classified into three main classes: chlorophylls (chl), carotenoids, and phycobiliproteins (PBPs) (Arashiro, et al., 2020). While all photosynthetic organisms have chl *a* as part of their core reaction center, they differ in the remaining accessory pigment composition, with PBPs exclusive to the cyanobacteria, red algae, and the Cryptophyta and Glaucophyta (Arashiro, et al., 2020). The present work will focus only on PBPs present in *A. platensis* and thus this is the only class of pigments that will be discussed here. The PBPs are water soluble, highly fluorescent proteins that organize to form complexes called phycobilisomes attached to the surface of the thylakoid membrane and absorb light in the visible spectrum between 500 and 650nm (Pagels, et al., 2019). The PBPs are further subdivided into four classes; phycocyanin (PC), allophycocyanin (APC), phycoerytherin (PE), and phycoerythrocyanin, with phycocyanin being the main class produced in cyanobacteria and which has the greatest commercial value (Jacob-Lopes, et al., 2020). In addition to the essential biological role in microalgae metabolism, these pigments possess properties that have been found to be antioxidative, anticarcinogenic, anti-inflammatory, antiangiogenic, and neuro and hepatoprotective (Arashiro, et al., 2020).

2.2.1. Industrial applications of microalgal pigments

The industrial use of microalgal pigments has already been extensive and of great variety. Phycobiliproteins have high commercial value in the pharmaceutical, nutraceutical, aquaculture, feed, and cosmetic industry mostly as a natural colorant with the added health benefits as listed previously. Additionally, the autofluorescent properties of PBPs allow them to be used as labels for antibodies and receptors in important clinical and molecular biology research (Chiong, et al., 2016). PBPs have become particularly popular for use in textile dyes with recent studies showing red and blue pigments, extracted from *Gracilaria vermiculophylla* and *Arthrospira platensis* respectively, had an even distribution on various fabrics tested and thus proved to be of high viability and quality (S Moldovan, et al., 2017). Recent growth in the algal pigments market has been due to this increased demand for natural dyes in the textile and paint industries. It was previously estimated that the market value of algal pigments will grow at a Compound Annual Growth Rate (CAGR) of 4% between 2019 and 2025 when it will reach a value of 452.4 million USD (Patel, et al., 2022). While the market is increasing, there are still very few companies solely using phycocyanin for natural dyes and inks. Among these is the

Colorado based biomaterials company, Living Ink, who create carbon negative pigment and ink products from algae, and have recently gained traction for their ecofriendly printing ink in partnership with Patagonia, the globally well-known sportswear brand. Locally, a startup called Mounid is currently in pilot scale testing and aims to produce various textile dye products made from several macro- and microalgae species.

Figure 3: Colour testing for the first production of phycocyanin pigment at Mounid (left), Patagonia Boulder Guide Book printed with algae ink from Living Ink (right).

2.2.2. Phycocyanin extraction and purification

Phycocyanin is largely extracted at industrial scale from *Arthrospira platensis*, *Anabaena* and *Nostoc* species through a multistep bioprocess consisting of biomass production, harvesting, cell disruption, extraction, and finally purification and further downstream processing (Jacob-Lopes, et al., 2020). In most cases, the purification represents the largest cost of production and most literature only reports on lab-scale processes as large-scale applications are protected by patents. PBPs are water-soluble pigments and thus do not require the use of organic solvents or supercritical carbon dioxide for recovery, however, due to the thick and robust cell wall of *A. platensis*, a mechanical pretreatment or cell disruption step is often necessary to rupture the cyanobacterial cell wall and release the pigment into solution (Gogate & Joshi, 2020). In phycocyanin processing, common extraction solvents include sodium phosphate buffer, hydrochloric acid, ionic liquids, and distilled water, depending on the cell disruption method, and the intended downstream processing and application of the extracted pigment (Furuki, et al., 2003). Abiotic factors such as light, nutrients, temperature, and salinity, are the main influencers of phycocyanin production, with light being the most important factor to consider due to its essential role in the photosynthesis apparatus. Therefore, optimizing light conditions

by investigating growth under various light intensities, photoperiods, and light sources is fundamental to all pigment production processing (Singh, et al., 2015). Further downstream processing and purification can increase the biological benefit and quality of pigment. This involves a precipitation step using ammonium sulfate which separates PC from other pigments, followed by a chromatography step based on both polarity and size for separation (Estrada, et al., 2001). The present study followed findings from Moraes et al., 2011, which suggested phycocyanin extraction by ultrasonication followed by solvent extraction using concentrated hydrochloric acid resulted in the highest yield of PC per unit biomass. However, there was no ultrasonication bath in the lab used during this project and freeze-thawing was used as an alternative cell disruption step following Moraes' findings (Moraes, et al., 2011).

2.2.3. Economic interests, challenges, and solutions

The increasing demand for natural colorants has increased the production of microalgae worldwide. However, there is now a need for further optimization of the bioprocess to cut costs and ensure that the production process is, in fact, a more sustainable practice than previous solutions. While the global market for microalgal pigments is well established, this is tailored to use in specific industries and mainly for human consumption or animal feed, however, the rising demand for natural dyes in the textile and paint industry has added to the overall market value of phycocyanin which now sits at an estimated $100 million USD and is predicted to reach $200 million by 2028 (Jacob-Lopes, et al., 2020). The current economic constraints mainly involve the production cost, as mentioned previously. Microalgae production involves an extremely costly installation, usually requiring large areas of land, specialized equipment, and trained engineers. Once installed, the production is costly both in terms of energy and nutrient requirements. Establishing a process with a biorefinery concept and circular production system may be key to economic feasibility of algae production. In addition, biological advances, genetic engineering, and a deeper understanding of microalgae metabolism may pave the way for increased pigment production.

2.3. Microalgae for wastewater treatment

Municipal wastewater treatment plants generally implement a three-step process, beginning with primary treatment to remove larger particles using a mechanical mechanism such as settling tanks or filters (Abdel-Raouf, et al., 2012). This is followed by a secondary biological treatment using aeration to promote degradation of organic compounds by microorganisms

present in the water. Bacteria are most commonly used during biological treatment; however, fungi and microalgae are suitable and often more efficient. At this stage, residual amounts of nutrients still remain high enough to cause significant ecological damage if released into natural water streams, thus a tertiary step is often employed in order to prevent eutrophication (Kilbane, 2022). It is at this stage that microalgae are sometimes used as a polishing step to remove nitrogen and phosphorous still present in the effluent, although this is not yet common practice. Despite their efficient removal of both inorganic and organic contaminants, use of microalgae in wastewater treatment is still limited and where they are implemented, there is little focus on using the biomass produced for valuable products (Arashiro, et al., 2020). As the economic opportunities of microalgal biomass are realized, so too will their use in wastewater treatment increase. The future of wastewater treatment plants lies within a circular system of nutrient and water recycling coupled with the valorization of biomass produced, creating a more sustainable and economically feasible process.

2.3.1. Removal of nitrogen and phosphorous

As mentioned, microalgae are capable of eliminating diverse pollutants from wastewater, which is proven in current literature. Some of the most notable being nitrogen and phosphorous, heavy metals, colorants, and various emerging pollutants (Arashiro, et al., 2020). In the present study, only the removal of nutrients, particularly nitrogen and phosphorous, will be monitored and discussed. The main source of excess nitrogen is the over-use of inorganic nitrogenous fertilizers in agriculture and the production of wastes from human and animal populations, which both continue to increase today (Abdel-Raouf, et al., 2012). The main source of phosphorous which accounts for approximately 50% present in wastewater is from synthetic detergents and although phosphate in water does not seem to present a significant hazard to human health, the removal of this nutrient is still required to prevent harmful eutrophication and associated environmental harm (Comeau, et al., 1987). Over 60 years ago, Oswald and Gotaas (1957) proposed microalgae as a bio-treatment for the removal of nutrients from wastewater and we have seen great advances since then with recent findings indicating between 85-100% removal efficiency by various species (Cañizares-Villanueva, et al., 1995). The microalgal system for removing nutrients has the following advantages over bacterial systems; higher removal efficiencies up to 40mg N L^{-1} d^{-1} and 4mg P L^{-1} d^{-1} for nitrogen and phosphorous respectively, lower operation as it does not require an organic carbon source, no sludge generation, and the added economic potential of obtaining high-value products (Zhu,

2015). Not only does the use of microalgae offer an interesting step for the treatment of wastewater and the recycling of otherwise lost valuable nutrients but provides the potential to transform waste treatment into a circular system with an added economic potential.

2.3.2. RecoLab

The wastewater used during this study was collected from the wastewater treatment facility RecoLab, located in Helsingborg. RecoLab is a development facility that manages and sorts sewage from the Oceanhamnen area in a so-called "three pipes out" model. This model separates wastewater by source; gray water (kitchen, dishwasher water), black water (toilet water), and food waste are all collected into separate tanks, with the aim (Anon., 2021)of better recycling nutrients and more efficiently cleaning and reusing water. Through this revolutionary system, RecoLab has proven increased biogas production up to 60% from the black water stream, increased water recycling (50% of gray water stream), and an overall reduced climate impact of 25-50 kg of CO_2 per person per year (Anon., 2021). These three streams of sewage have unique pollutants from each other and thus by keeping them separate it allows for better handling of the valuable nutrients in some streams and the harmful chemicals in others (Anon., 2021). RecoLab is also equipped with a test bed and invites academics and collaborators to conduct research on samples from their development facility, these collaborations often lead to novel techniques and findings that may accelerate RecoLab's goal to update global understanding of municipal waste and transform the wastewater treatment industry worldwide. The wastewater used during this study was collected from the food waste stream with the average nutrient values displayed in table 1 below.

Table 1: Average nutrient content of the municipal food wastewater stream received at the RecoLab treatment facility in Helsingborg, measured over a 6 month period.

Physicochemical Characteristics	**Value**
d-COD (mg/L)	2393 ± 1459.82
TKN (mg/L)	413 ± 134.18
NH_4^+-N (mg/L)	309 ± 170.92
NO_3^+-N (mg/L)	ND[1]
P-total (mg/L)	37 ± 15.79
PO_4^{3-}-P (mg/L)	ND[1]
VSS (mg/L)	1204 ± 726.98
pH	7 ± 0.21
TSS (mg/L)	1384 ± 896.07

[1]ND: not detected.

3. Materials and Methods

This study was conducted in two main phases. Firstly, to test the photobioreactor system and to determine growth rate and suitable media composition, an initial growth experiment was done, followed by a scale up and photoperiod optimization. Prior to the main investigation, a thorough literature analysis was conducted to compare the suitability of different wastewater streams (black, grey and food -wastewater) for algal biomass growth as well as a comparison between species suitable for phycocyanin production. The cultivation set-up is graphically outlined in supplementary figure 3.

3.1. Materials

3.1.1 Arthrospira platensis *stock culture and inoculum preparation*

A starter culture of *A. platensis* was acquired from the culture collection of a local microalgae producer and supplier, Health Algae. Upon strain arrival, the 250 mL culture was placed under a white-fluorescent light for the culture to start regrowing after degradation may have occurred during shipping. The following day, the culture was split between two 2 L Erlenmyer flasks each containing 1 L of Zarrouk's medium and placed in a climate chamber (Pol-EKO Aparatura) with the following parameter set-up; temperature: 25°C, photoperiod: 14:10hours light:dark cycle, and light intensity: 100%. On the third day, aeration tubes with a gas stone on the tube outlet were placed in the flasks to provide stirring and 5000 mL air per minute from an aquarium air pump (IREENUO Q7 HQ-602). These cultures were maintained under the listed conditions with 10% culture removed and replaced with fresh media every 2-3 days until inoculation.

3.1.2. Media formulation

The Zarrouk medium prepared was an adaptation from the work of Baker et al., 2021 and it contains three separate solutions and was not autoclaved after mixing to prevent the precipitation of metals. Solutions 1 and 2 were autoclaved separately and Solution 3 was sterilely filtered; all three solutions were then combined. Medium composition was as follows; 500 mL/L Solution 1 (16.8 g $NaHCO_3$, 0.5 g K_2HPO_4 (per L)), 500 mL/L Solution 2 (2.5 g $NaNO_3$, 1 g K_2SO_4, 1 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.04 g $CaCl_2$, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.08 g

NaEDTA (per L)), 1 mL/L Solution 3 (2.86 g H_3BO_3, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.222 g $ZnSO_4 \cdot 7H_2O$, 0.079 g $CuSO_4 \cdot 4H_2O$, 0.015 g MoO_3 (per L)).

Wastewater samples: Wastewater was collected from digested influent of the food-waste stream at the wastewater treatment facility, RecoLab. This stream collects food-waste from Oceanhamnen in the H+ district and is kept separate from black and grey -water streams. The food-waste stream was chosen for this study due to its properties being more suitable for microalgae growth with a higher concentration of nutrients compared to that of the grey water stream and more transparent liquid than the black water. Upon arrival at the treatment facility, food waste is pasteurized at 70°C for one hour to kill off any pathogens present and then enters the anaerobic digester to be kept at 37°C for several days to allow for sedimentation. The liquid phase that settles above the sediment was collected for use in this study and henceforth referred to as FWW (Food Waste Water). The samples received were immediately autoclaved at 120°C for 30 minutes to further kill any remaining microorganisms present in the water according to lab safety protocol. After autoclaving, the wastewater was filtered through standard Whatman filter paper to eliminate the larger suspended particles and increase the transparency of the liquid for improved light exposure. The samples were kept at 4°C until use to prevent any biological activity and degradation of nutrients.

Zarrouk medium and wastewater mixtures: Different compositions of wastewater to medium were tested in duplicates: 100% FWW, 75% FWW/25% Zarrouk and 50% FWW/50% Zarrouk, to compare the growth dynamics of *Arthrospira platensis* on increasing concentrations of wastewater. Phase I had a total working volume of 600 mL and phase II was scaled up to 4 L each.

3.1.3. Equipment and photobioreactor set-up

A makeshift photobioreactor system was set up for both the growth experiment and the scale-up phase. During the growth experiment, 1 L glass laboratory bottles (DWK Life Science DURAN™) were set up in sequence with inlet and outlet tubing to allow for aeration and air transfer. Both inlet and outlets were fitted with 0,22µm filters to minimize risk of contamination. Each glass bottle was placed equidistant and sequentially from the light source, ensuring no shadowing effects would prevent light exposure between duplicates. For the scale-

up phase, 5 L glass bottles were used instead, with the same lighting, tubing, and temperature systems.

3.2. Methods

3.2.1. Experimental Design

The first experiment was performed to examine *A. platensis* cultivation on three different wastewater-based growth media: (1) 100% FWW, (2) 75% FWW supplemented with 25% Zarrouk medium, and (3) 50% FWW supplemented with 50% Zarrouk medium. The cultures had a total working volume of 600 mL and were incubated for 21 days under the same conditions used for the inoculum cultures and the optimum ratio of wastewater to media was identified. The second experiment was performed using only 75% FWW and 25% Zarrouk medium. During this experiment, the effect of photoperiod was investigated, and the working volume was scaled up to 4 L to assess the effect of increased illumination on pollutant removal to biomass production and assess the scale-up potential of the cultivation system. During both experiments, the pH was adjusted to 9 with 5N NaOH solution and was kept between 9-11 during cultivation by using 5N HCl or 5N NaOH solutions, the light intensity was set to 100% as per the climate chamber settings provided by white fluorescent lights. The temperature was kept constant at 25 ± 3°C for the first experiment and at 28 ± 3°C during the second. All experiments were performed in duplicates under non-aseptic conditions and with continuous aeration provided by an air pump at a rate of 5 L air/min.

3.3. Analysis

3.3.1. pH and conductivity

During both experiments, pH and conductivity were monitored using the HANNA Combo® HI98129 instrument. This instrument was also used to check pH of wastewater samples and other growth media used.

3.3.2. Dry weight, optical density, and standard curve

For ease of sampling, a calibration curve was made to assess the correlation between dry weight and optical density. A stock culture of *Arthrospira platensis* and five serial dilutions, each with a volume of 20 mL, were prepared. The optical density for each dilution was then read using the spectrophotometer (Ultrospec 2100pro, Amersham Biosciences) at a wavelength of 565nm.

Dry weight was determined by standard protocol, 0.45µm membrane filters were dried in a microwave oven for 4 minutes and left in a desiccator overnight, after which their initial weight was recorded. A volume of 10 mL of each dilution was filtered and washed three times with 10 mL of cold distilled water to collect the algal cells, dried in the microwave for 8 minutes and left to dry in the desiccator overnight. The final weight of the filters was recorded, and the dry weight was calculated according to the equation below.

$$Dry\ weight\ (mg/mL) = \frac{initial\ weight\ of\ filter - final\ weight\ of\ filter}{volume\ filtered\ (mL)}$$

A standard curve was plotted in figure 6 for the data within the range of 0 to 1,4 g/L dry weight of culture and a linear regression was performed to obtain the linear equation and the R-squared value of the curve.

3.3.3. Phycobiliprotein content

The production and concentration of PBPs in the biomass grown during both experiments was quantified according to similar methods outlined by Arashiro et al, (2020), with relevant adaptations. Briefly, 5 mL samples that were taken every 2-4 days, were immediately centrifuged at 1,160 g for 15 minutes, washed twice with cold distilled water and the biomass pellet was frozen at -21°C until further quantification. The pellets were resuspended in 4 mL concentrated 37% HCl and placed in dark at 4°C for 24 hours. The optical densities were then measured at 280nm, 562nm, 615nm, 652nm and 620nm. The PC, APC and PE concentrations and yield were calculated using the equations listed below as proposed by Bennett and Bogobad, (1973).

$$PC\ (mg/mL) = [A_{615} - (0.474 * A_{652})]/5.34$$

$$APC\ (mg/mL) = [A_{652} - (0.208 * A_{615})]/5.09$$

$$PE\ (mg/mL) = [A_{562} - (2.41 * PC) - (0.849 * APC]/9.62$$

Purity of phycocyanin was determined according to the ratio A_{620}/A_{280} defined by Cuellar-Bermudez et al., (2015), where A_{615}, A_{652}, A_{280}, A_{620} and A_{562} correspond to the absorbance measured at the respective wavelengths.

3.3.4. Nutrient removal

Nutrient removal was measured by monitoring concentrations of ammonium (NH_4-N), phosphorous (PO_4^{3-}P), total nitrogen (TN), and total organic carbon (TOC) using the following Hach test kits; LCK303 Ammonium (2-47 mg/L), LCK302 Ammonium (47-130 mg/L), LCK350 Phosphorous total (2-20 mg/L), LCK348 Phosphorous total (0.5-5 mg/L), LCK438 LATON Total Nitrogen (100-250 mg/L), and LCK386 Total Organic Carbon (30-300 mg/L), following the test protocols provided using a portable spectrophotometer (Hach DR3900).

3.3.5. Calculations

For simplicity, the specific growth rate (d^{-1}) of *A. platensis* during this project was estimated using the equation below, where X is cell concentration (g/L) and t is time (days).

Eq.1. $$X\,(t) = X_0 * e^{\mu t}$$

Biomass productivity ($mg \cdot L^{-1} \cdot d^{-1}$) was determined by Eq. 2, where X_{max} ($g \cdot L^{-1}$) was the maximal biomass concentration, X_0 ($g \cdot L^{-1}$) was the initial biomass concentration, T_{max} was the time for biomass to reach maximum (days).

Eq.2. $$\textit{Biomass productivity}\ (mg.L^{-1}.d^{-1}) = \left[X_{max} - \frac{X_0}{T_{max}}\right] * 1000$$

The removal efficiency (%) of pollutants from wastewater was evaluated by Eq. 3, where C_{in} ($mg \cdot L^{-1}$) was the initial concentration of the pollutant (NH_4^+, PO_4^{3-}, and TN), C_{out} ($mg \cdot L^{-1}$) was the concentration of residual pollutant when biomass concentration reached the maximum.

Eq.3. $$\textit{Removal efficiency (\%)} = \frac{C_{in} - C_{out}}{C_{in}}\ x\ 100$$

The average removal rate ($mg \cdot L^{-1} \cdot d^{-1}$) of each pollutant was calculated by Eq. 4, where the T_{max} was the time (days) for biomass to reach maximum.

Eq.4. $$\textit{Av. Removal rate}\ (mg.L^{-1}.d^{-1}) = \frac{C_{in} - C_{out}}{T_{max}}$$

3.3.6. Statistical analysis

All statistical analysis was done using JASP (JASP-stats, Amsterdam, Netherlands). The average values measured during both experiments were analyzed using multi-factor analysis of variance (ANOVA) and the significant differences amongst growth media variations and phycocyanin production were determined using two-tailed T-test at 95% confidence interval.

4. Results and discussion

4.1. General observations

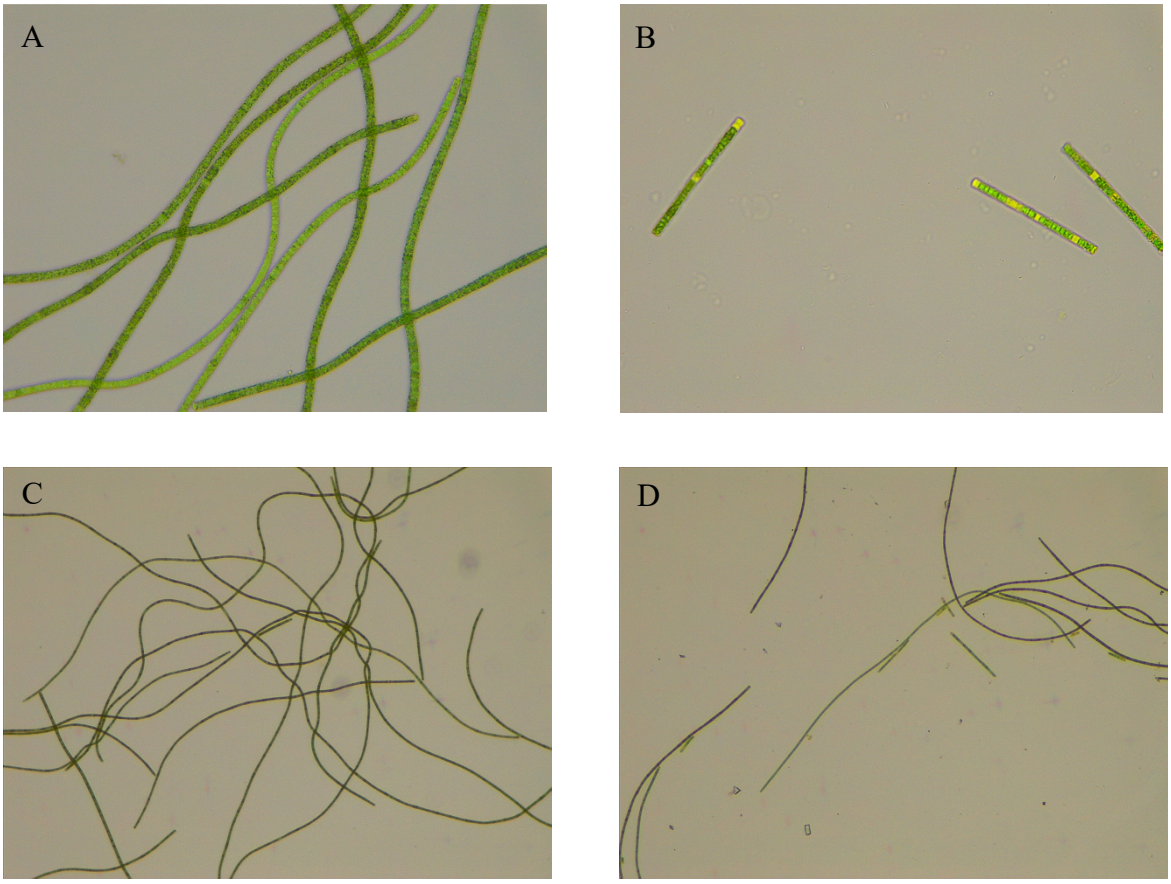

Figure 4: A. platensis cells grown with different photoperiods applied; (A) and (C) 14h:10h photoperiod, (B) and (D) 16h:8h applied. Viewed under a Leica DM750 Light microscope at 40X (A and B) and 20X (C and D).

During sampling, cells were viewed under the microscope to observe morphological changes throughout cultivation. Interestingly, some morphological differences were seen when comparing the cultivations grown under different light-dark cycles. In samples taken from cultures grown at photoperiod 14h:10h, as pictured in figure 4A and 4C, cells appeared long and had a uniform green color. While in samples from the 16h:8h cultures, pictured in figure

4B and 4D, cells often appeared broken and in small pieces with lighter green segments. Previous literature has reported light-induced lysis and abnormally small, clumped cells when cultures are grown either under extreme light intensity or at extended light cycles or continuous illumination (George, et al., 2014).

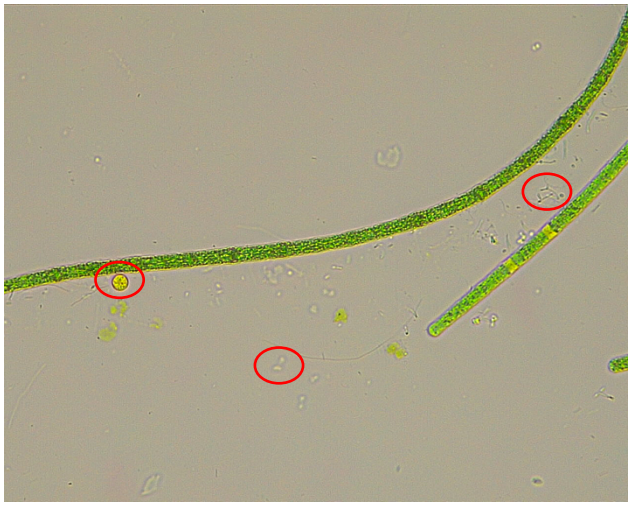

Figure 5: Contamination in cultures grown during the second phase of experiments. Viewed under a Leica DM750 Light microscope at 40X.

Throughout all experiments, samples were observed for any visible contamination throughout cultivation. It was not possible to identify fungal contamination as this requires a fluorescent microscope, which was not available. However, under a standard light microscope, as seen in figure 5, contamination by a different algae species and other unidentified cells suspected to be bacteria, could sometimes be observed. While this provides some insight into possible contamination sources, the necessary equipment was not available to be able to better assess and observe these contaminants.

4.2. Determining biomass concentration

Although OD readings can depend on cell composition and morphology and are thus not as accurate as dry weight measurements, OD was used throughout cultivation to minimize the volume removed during sampling. After measuring the dry weight of various dilutions along with the corresponding OD reading, an initial standard curve was prepared to understand the correlation between dry weight and OD, as seen in figure 6. A linear equation set to intercept at the origin was used to relate DW to OD, using the conversion factor from the resulting equation; DW (g/L) = OD/1,9286. The correlation was found to be strong (R^2 = 0,99) and thus OD was confirmed as a suitable alternative to dry weight measurements.

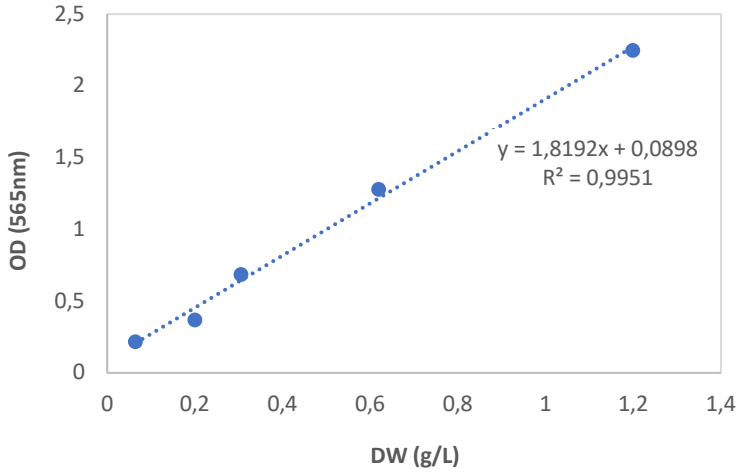

Figure 6: Linear correlation between OD 565nm and DW measurements (g/L).

4.3. Optimization of *A. platensis* cultivation on municipal food wastewater

The initial growth experiment was carried out to define the best growth media composition, in terms of ratio between wastewater to Zarrouk media, that would yield maximal biomass growth and phycobiliprotein production. Zarrouk media was chosen as a standard growth medium to supplement the wastewater with sodium chloride and sodium bicarbonate to enhance microalgae growth. Since all experiments were conducted under non-aseptic conditions, the pH was maintained between 9-11 as these conditions allow for *A. platensis* growth while reducing chances of bacterial contamination.

4.3.1. Biomass growth

The growth curve of *A. platensis* cultivated in media of varying compositions; 100% FWW, 75% FWW, and 50% FWW, is shown in figure 7. The most notable result was that no significant growth was seen in cultivations grown in 100% FWW, indicating that the wastewater alone was not sufficient to support growth of *A. platensis*. The alkalinity, salinity, and additional nutrient components such as potassium and trace elements are thus essential for the growth rate and to sustain the health of the cultivation. The main components of Zarrouk medium; sodium nitrate, sodium chloride, and sodium bicarbonate, all contribute to the optimal salinity for growth of cyanobacteria, thus the lack thereof may result in an unfavorable environment as seen for the 100% FWW cultivation. While microalgae have great capacity to utilize high concentrations of nutrients, with *A. platensis* being particularly well-known as the most resistant to ammonium-inhibition, previous literature has still reported over 50% growth

inhibition when ammonia concentration is close to 140 mg/L (Belkin & Boussiba, 1991). The initial concentration of ammonium in the 100% FWW media was found to be 105±4 mg/L, which is below what has been found to be inhibitory to *A. platensis* growth, however, Belkin and Boussiba further specified in their findings that the concentration at which ammonium is inhibitory is also pH and strain dependent, leaving ammonium-inhibition as a likely culprit for this lack of growth.

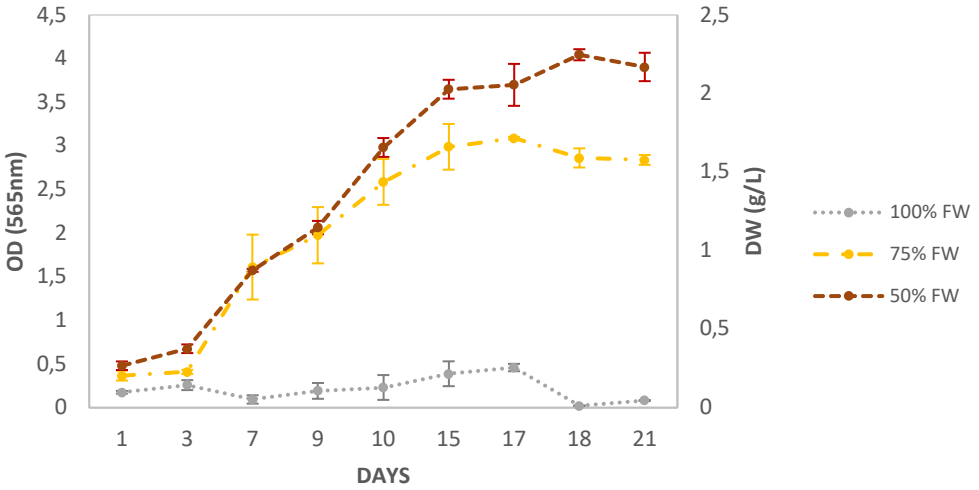

Figure 7: Biomass growth of A. platensis during the optimization experiment on growth media containing different amounts of FWW (100%, 75%, 50%) supplemented with Zarrouk media. Measured as OD 565nm and converted to Dry Weight (g/L).

At the end of the cultivation, *A. platensis* biomass production with 50% FWW was significantly ($p < 0.05$) higher than that in the 75% FWW group. However, the cultivation grown with 75% FWW reached its maximum biomass concentration of 1,6 $g.L^{-1}$ earlier (17 days) than that of the 50% FWW group reaching maximum biomass concentration (2,09 $g.L^{-1}$) after 18 days. The most favorable growth medium from this experiment in terms of final biomass concentration and growth rate was 50% FWW supplemented with 50% Zarrouk media, indicating that *A. platensis* favored the relatively lower nitrogen and phosphate nutrient profile with a higher concentration of salts. Similarly, Papadopoulos et al. (2022) reported maximal growth and higher biomass concentrations, reaching higher than 1,5 $g.L^{-1}$, when supplementing brewery wastewater with Zarrouk medium compared to cohorts without.

4.3.2. Nutrient removal

Ammonium and orthophosphate were used to assess the efficiency of nutrient removal by *A. platensis* on the given wastewater stream as these were the only available test kits during

investigation. However, monitoring the removal of COD, heavy metals, and additional forms of nitrogen during cultivation may provide a more in-depth assessment of this strains potential to treat wastewater. The nutrient removal during this initial growth experiment was determined by testing the growth medium for ammonium (NH_4^+) and phosphate, measured as orthophosphate (PO_4^{3-}), before and after 21 days of cultivation. In all cultivations across all three medium composition groups, ammonium removal was more than 95% and the concentration of ammonium at the end of the cultivation period was below the lower limit of the detection range of 2 mg/L in all media composition groups, as seen in figure 8B.

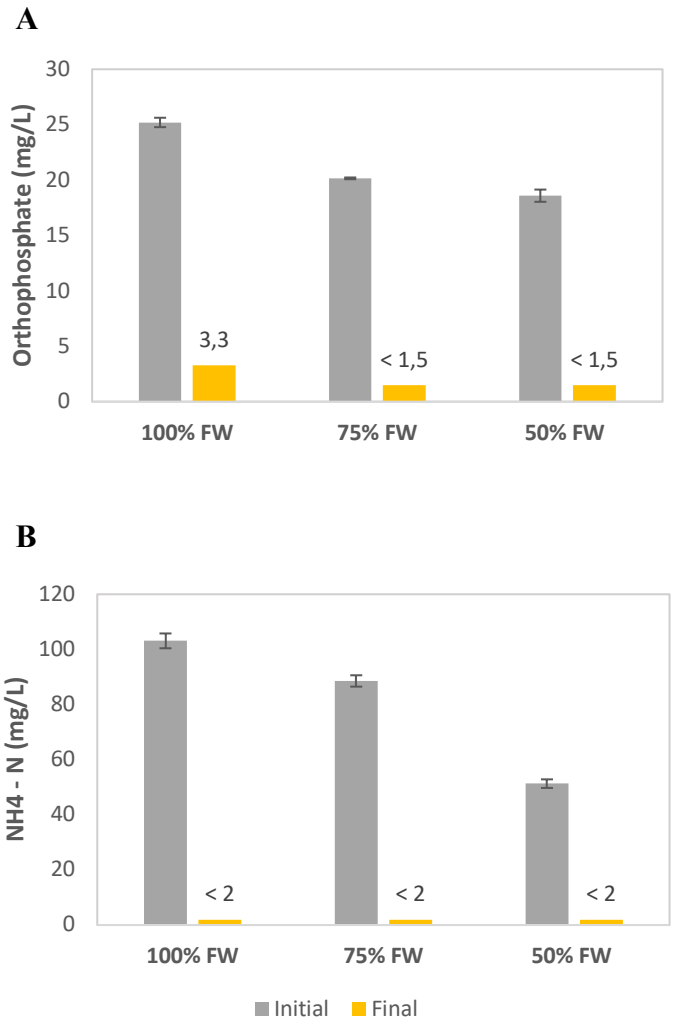

Figure 8: Initial and final concentrations of (A) Orthophosphate (PO_4^{3-}) and (B) Ammonium (NH_4^+), during the optimisation experiment of cultivations grown on different amounts of FWW (100%, 75%, 50%) suplemented with Zarrouk media.

The removal of phosphate was similarly impressive across all groups, showing significant ($p < 0.05$) decreases in concentration with 86%, 92% and 91% removal for the 100%, 75% and 50% FWW composition groups respectively. Despite, no significant growth observed in the cultures grown on 100% FWW, there was a drastic reduction in nutrients evident. This may be due to other microorganisms present in the culture and without the supplementation of Zarrouk media to increase salinity and provide suitable conditions for photosynthetic organisms, it is entirely possible that bacteria dominated this culture and may be the reason for the reduction in nutrients. This would be an interesting area for further investigation as it may give insight into the comparison between bacterial and microalgal systems for the biological treatment of WW.

In both cultivations grown on 75% and 50% FWW, the orthophosphate concentration was reduced to below the lowest range of detection (1,5 mg/L). This experiment was conducted to provide initial insight to whether *A. platensis* would sufficiently remove sources of nitrogen and phosphate from the wastewater stream, thus only initial and final concentrations were taken, and a more thorough analysis of removal rate and efficiency was conducted in the second phase of this project. Given that there was no significant difference between media composition (wastewater percentage) and nutrient removal, this factor did not influence the choice of preferred media composition for the following cultivation.

4.3.3. Phycocyanin concentration and purity

The biomass phycobiliprotein content was also examined in these experiments as it is known to positively correlate with cyanobacterial biomass concentration (Papadopoulos, et al., 2022). Only the cultivations grown in 75% and 50% FWW were analyzed for PC concentration as the 100% FWW cultivation did not yield high enough biomass concentrations for pigment extraction. The concentration and purity of PC extracted from the biomass grown during the optimization experiment can be seen in figure 9, where the concentration represents the amount of PC per gram dry weight and the purity defines the relationship between the presence of phycocyanin and other contaminating proteins in the sample extracted (Cuellar-Bermudez, et al., 2014). The biomass PC content varied according to the media composition, with a significantly ($p < 0.05$) higher final PC concentration seen in the 75% FWW cultivations compared to that of the 50% FWW groups (252,8 ±1,55 and 195,2 ±5,53 mg/g DW respectively).

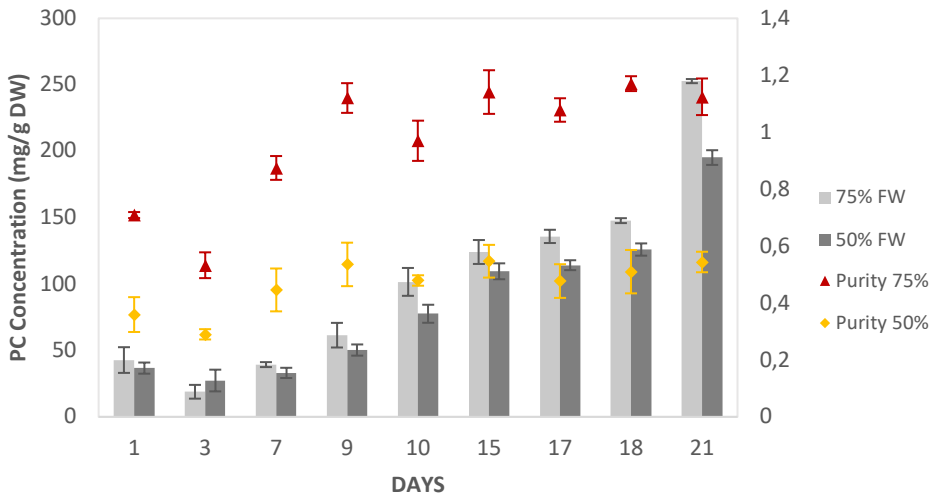

Figure 9: Phycocyanin content into total biomass and respective purity during A. platensis cultivation on 100%, 75% and 50% FWW supplemented with Zarrouk media.

Additionally, the purity of PC extracted from the 75% FWW was significantly ($p < 0.05$) higher than in the 50% FWW samples, maintaining a purity ratio between 0,8 to 1,2. The purity ratio is determined by the A_{620}/A_{280} ratio and high purity correlates to high purity ratio with values above or equal to 0,7 indicating food grade pigment and values above or equal to 4,0 correspond to reagent and analytical grade pigment (Borowitzka, 2013). Previous research has reported similar trends in the effect of wastewater-based media composition on pigment concentration and found that the cultivations containing higher amounts of $NaHCO_3$ and NaCl resulted in the maximum PC concentration observed (Papadopoulos, et al., 2022). Another study by Arashiro et al. (2020), investigating natural pigments from microalgae in industrial wastewater also reported maximum PC concentrations (approx. 200 mg/g DW) when grown with medium amounts of standard growth medium combined with wastewater. Among other things, nitrogen concentration, bacterial populations, salinity, and pH can all effect phycobiliprotein concentration throughout a cultivation and thus further analysis of these factors may provide more in-depth knowledge into the PC production in the given study design. In summary, the best conditions, among those tested, for phycobiliprotein production was at a media composition of 75% FWW and 25% Zarrouk media, and thus the following growth experiments were conducted only on this media composition despite the higher biomass concentrations observed in the 50% FWW groups.

4.4. Effect of photoperiod on *A. platensis* growth and phycobiliprotein production

Light is often considered the most important abiotic factor that effects biomass growth and production of photosynthetic pigments in cyanobacteria, with both light intensity and photoperiod of light exposure playing crucial roles in energy efficiency and metabolism (Niangoran, et al., 2021). Previous studies have suggested a light period of 16 hours and dark period of 8 hours to be ideal for phycocyanin production in *A. platensis* (Keithellakpam, et al., 2015). Although different light intensities were not tested during this study, the second phase of experiments, which was scaled up from phase one, compared the previous photoperiod of light:dark cycle 14h:10h to the suggested cycle of 16h:8h. Biomass concentration as dry weight is outlined in figure 10 showing that the growth of *A. platensis* was significantly ($p < 0.05$) higher when grown under the 14h:10h photoperiod compared to 16h:8h. This comparison was made in terms of growth rate, 0,34 d^{-1} and 0,30 d^{-1}, and maximum biomass concentration, 1,64 g/L and 1,35 g/L, for 14h:10h and 16h:8h respectively. This result was contrary to that of Niangoran et al., (2021), who found that biomass productivity and growth rate decrease with the illumination time, however, the process energy efficiency seems to increase. However, during the aforementioned study, 16h:8h photoperiod was the lowest light cycle tested and was compared to higher light exposure of 20h:4h and 24h. Additionally, these contradicting results may be due to differences in light intensity, other abiotic factors, and possible variations in bacterial population present in the cultures, thus further variations and replicates of would need to be conducted to confirm this finding.

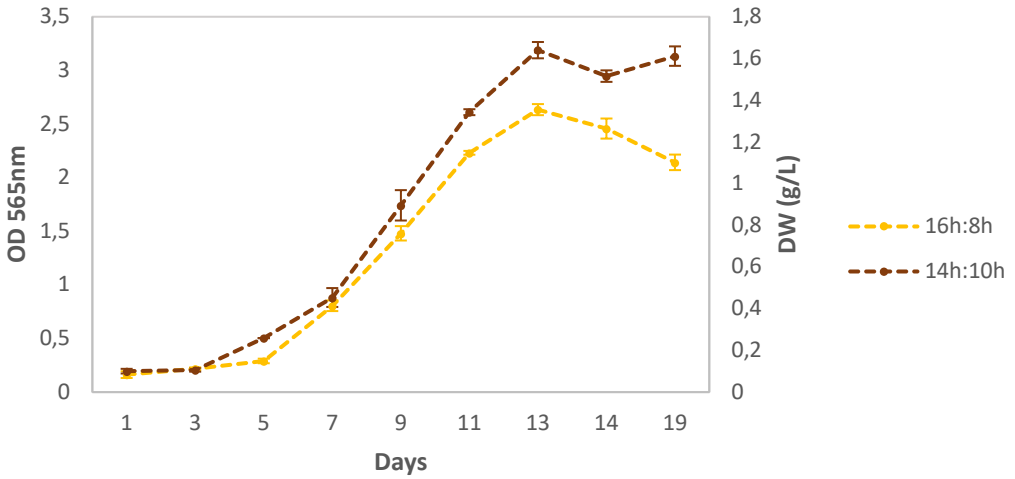

Figure 10: Biomass growth of A. platensis during the scale-up experiment for cultivations grown under a light dark cycle of 16h:8h or 14h:10h. Measured as OD 565nm and converted to Dry Weight (g/L).

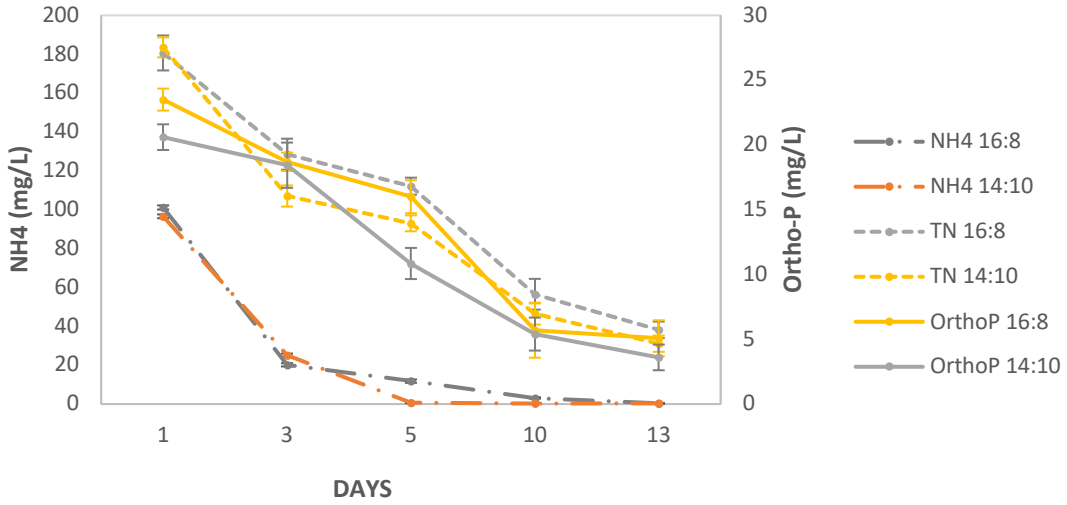

Figure 11: Nutrient removal as shown by NH_4^+, TN, and PO_4^{3-} consumption by A. platensis *throughout cultivation when applying photoperiod 16h:8h or 14h:10h.*

Nutrient removal was monitored more closely during the second phase of experiments. Samples were taken at various time points throughout cultivation and the media was tested for concentration of ammonium (NH_4^+), phosphate (PO_4^{3-}), and total nitrogen (TN), with initial values of 99,8 mg/L, 22,5 mg/L, and 181,45 mg/L respectively. Removal trends for all nutrients in question were similar between photoperiods, as seen in figure 11, and the average removal rate and removal efficiency for cultivations at each photoperiod are outlined in table 2. In all cases, nutrient concentrations were below the accurate detection range by day 10 of cultivation and both nitrogen and phosphate sources were nearing depletion by day 14 of cultivation. This rapid removal of nutrients is consistent with the lack of biomass growth seen after day 14 when the cultivations seem to reach stationary phase. It is interesting that different biomass growth was observed between groups while all groups showed similar nutrient removal trends. Previous literature has observed the consumption of phycobiliproteins by *A. platensis* during nitrogen starvation (Markou, et al., 2014), which could explain this phenomenon.

The removal rate and efficiency of NH_4^+ was similar between photoperiods with no statistical significance ($p > 0.10$). However, for total nitrogen, the 14h:10h photoperiod had a greater removal efficiency of 83% and removal rate that was up to 1 $mg.L^{-1}.d^{-1}$ higher than that of the photoperiod with longer light exposure, as was the removal efficiency of PO_4^{3-}, but the average removal rate was not significantly different between groups. The results of the present study are consistent with previous findings by Arashiro et al. (2020), who reported removal

efficiencies of total nitrogen ranging from 52 to 100%, and between 73 to 82% phosphate removal during cultivation on industrial wastewater with no significant effect from varied light conditions.

Table 2: Respective removal efficiency and average removal rate of NH_4^+, PO_4^{3-}, and TN from cultivation media when applying photoperiod 16h:8h or 14h:10h.

	NH_4^+	PO_4^{3-}	TN
Removal efficiency (%)			
16h:8h	99.62	78.38	78.85
14h:10h	99.65	82.52	83.08
Average removal rate ($mg.L^{-1}.d^{-1}$)			
16h:8h	7.75	1.42	10.96
14h:10h	7.41	1.31	11.73

Concerning phycocyanin content, a decrease in pigment occurred during the cultivation between days 9 and 13, followed by a drastic increase at the end of the cultivation (figure 12). This trend can be linked to any bacterial population present during cultivation or to the decreasing nitrogen content as it is known that phycocyanin can be utilized as a nitrogen source by *A. platensis* to sustain growth under nitrogen limitation conditions (Verma & Mohanty, 2014). A similar trend was reported by Papadopoulos et al. (2022), however, the highest concentration of pigment was reported under continuous illumination when compared to cultivations under 16h:8h photoperiod, indicating higher pigment production under longer times of light exposure. The results of the present study contradict this finding, showing a higher final phycocyanin concentration in cultivations grown under shorter light exposure times (14 hours) compared to the cultures grown under a 16-hour light cycle. Although, during days 9 to 13 when a decrease in phycocyanin content is seen, a significantly higher phycocyanin concentration is maintained in cultures grown under the 16h:8h photoperiod. This could be due to different bacterial populations present between cultivation groups or due to the slower biomass growth and subsequent higher energy efficiency seen in cultivations under the 16h:8h photoperiod. However, phycocyanin content between photoperiods at most time points showed no significant difference and the purity values were barely distinguishable. Therefore, photoperiod was not considered to have a significant impact on phycocyanin concentration when grown at the same light intensities, further analysis of varying light intensities and

additional photoperiods may provide more information on the possible effects of light on phycobiliprotein production.

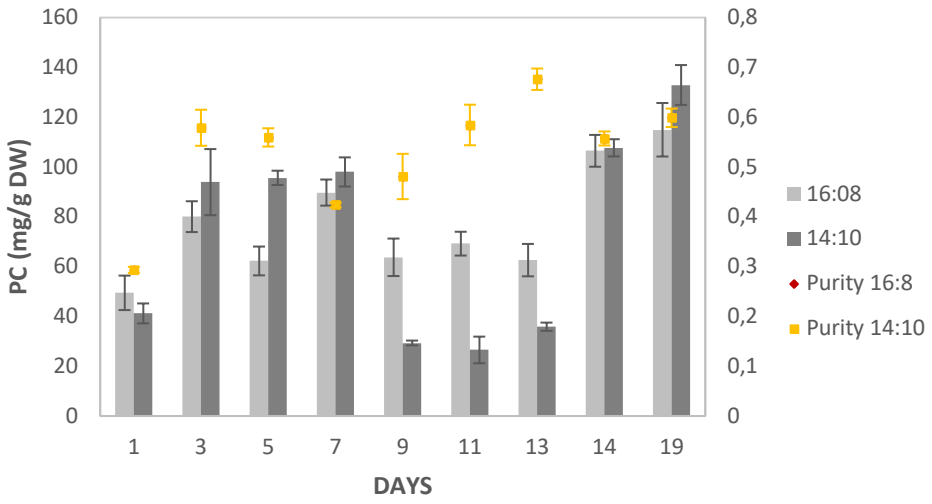

Figure 12: Phycocyanin content into total biomass and respective purity during A. platensis cultivation when applying photoperiod 16h:8h or 14h:10h.

4.5. Assessing the scale-up potential

The scaling up process of microalgal systems is notoriously challenging with many factors to consider including, but not limited to, mixing, shear stress, mass transfer, and light path, transparency and translucency (Qiang, et al., 1998). All these factors become increasingly complex to optimize as a system is scaled up, due to physical, technological, environmental, and labor limitations. Of the many factors to consider, light constitutes the most complex and arguably one of the major limiting factors for scale up. The type of photobioreactor (tubular, flat panels, raceway ponds), the opacity of the culture medium, the degree and type of mixing, and the material of the photobioreactor all influence the light penetrance, time of light exposure per cell, and the light path and flux (Jacob-Lopes, et al., 2020). During the present study, the second phase of experiments aimed to scale up the system tested in phase one from 600 mL working volume to 4 L (approx. 6X increase). In both experiments, standard glass laboratory bottles (DWK Life Science DURAN™) were used as make-shift lab-scale photobioreactors with the aim of emulating a simple industrial tubular reactor. For the optimization experiment, 1 L bottles with diameter of 101 mm and 230 mm height were used, while 5 L bottles with 182 mm diameter and 335 mm height were used for the second phase. The increased working volume and wider photobioreactor resulted in a much darker media and decreased light penetrance as well as a lower rate of mixing as the aeration remained the same between

experiments. Despite the expected limitations, growth rate between experiments remained similar, and surprisingly higher in the scale-up experiment (0,27 d^{-1} and 0,34 d^{-1} for the optimization and scale-up phases respectively). Additionally, biomass productivity increased from 1590,9 $mg.L^{-1}.d^{-1}$ during the first experiment to 1632,06 $mg.L^{-1}.d^{-1}$ in scale-up. While biomass growth seemed unaffected, the maximum phycocyanin concentration was significantly ($p < 0.05$) lower at the end of the scale-up experiment than that of the optimization phase (132,93 and 252,76 mg/g DW respectively). The difference in phycocyanin production, however, may be overcome through further optimization of extraction processes, more sterile cultivation, and improved light systems.

5. Future Work

The present study was significantly limited by time and budget as well as a lack of knowledge or experience dealing with algal cultivation systems. Given more time, further optimization would greatly improve the accuracy and optimization of abiotic factors such as light intensity, temperature, salinity, nutrient availability, and aeration. Investigating growth on additional variations of media to wastewater, comparing more varied photoperiods against continuous illumination, and altering the light intensity are essential steps to further optimize this process. Particular attention would need to be given to improve the aeration system of the photobioreactor set-up and supplement the inlet air with CO_2 to improve biomass productivity. During the second phase of cultivation, flocculation became apparent at a higher cell density and cells appeared to sediment toward the end of the cultivation period. This could be due to rapidly increasing pH which promotes flocculation. Monitoring the bacterial population present throughout cultivation using DNA sequencing would be beneficial in understanding nutrient removal as well as purity of phycocyanin and provide insight into the relationship between cyanobacteria and contaminating organisms during cultivation. Concerning wastewater treatment, monitoring the removal of COD, TOC, and other sources of nitrogen and phosphorous throughout cultivation would allow for a more thorough assessment of the treatment capability of *A. platensis* on the given stream of wastewater. Finally, this study requires further investigation into the feasibility of this process and the economic viability of algal wastewater treatment coupled with the valorization of biomass for this particular case.

6. Conclusion

This study successfully outlines the potential of implementing a biorefinery concept coupling wastewater treatment with the production of a high-value compound, algal pigments. The removal rate and efficiency of NH_4^+ and PO_4^{3-} were similar to those seen in recent wastewater treatment studies and resulted in a near complete reduction of total nitrogen in the growth media at the end of cultivation. Concerning pigment production, this study achieved high concentrations of phycocyanin during both experiments with values consistent with existing literature in the field. Higher nutrient concentrations, present in the 75% FWW group, and shorter light exposure time (14h:10h photoperiod) seemed to favor phycocyanin production. However, more accurate extraction and purification steps would be needed to confirm the accuracy of this comparison. Finally, the scale-up potential of this process seems promising as biomass growth, productivity and final concentrations remained similar between the growth experiments. There is great opportunity here in Skåne for further research in both alternative wastewater treatment systems and algal biotechnology, with advancing systems that allow for indoor cultivation regardless of environmental climate, allowing for local production of high-value compounds.

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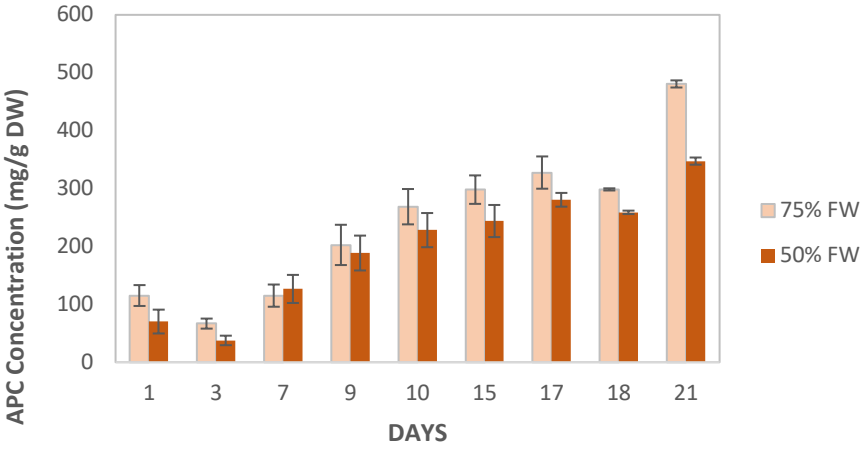
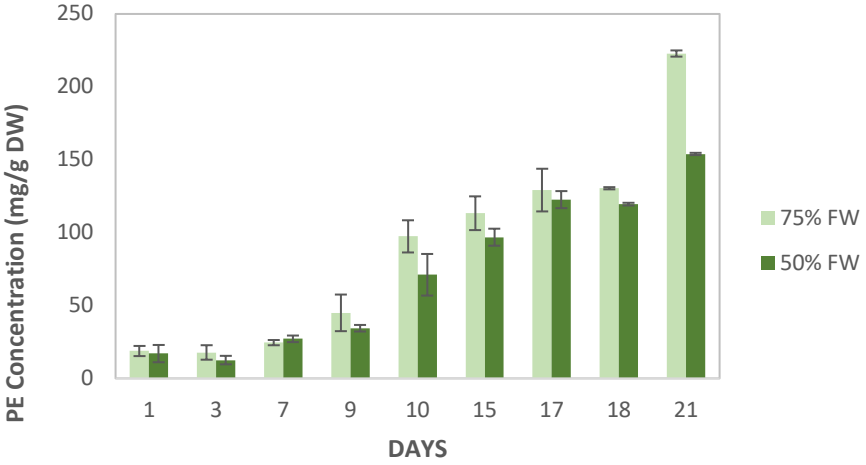
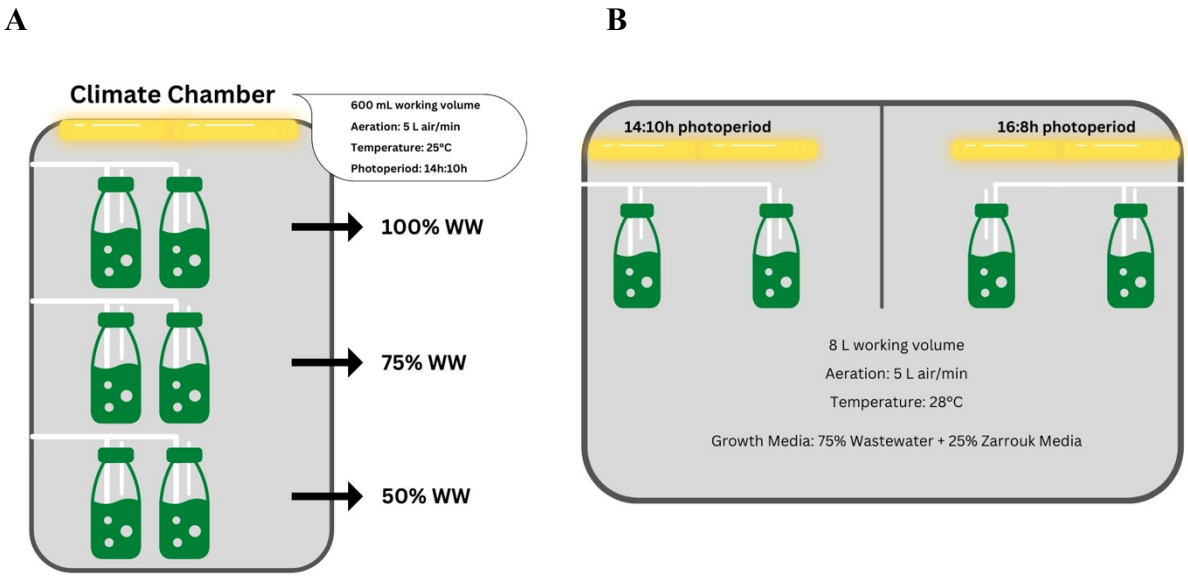
Appendix



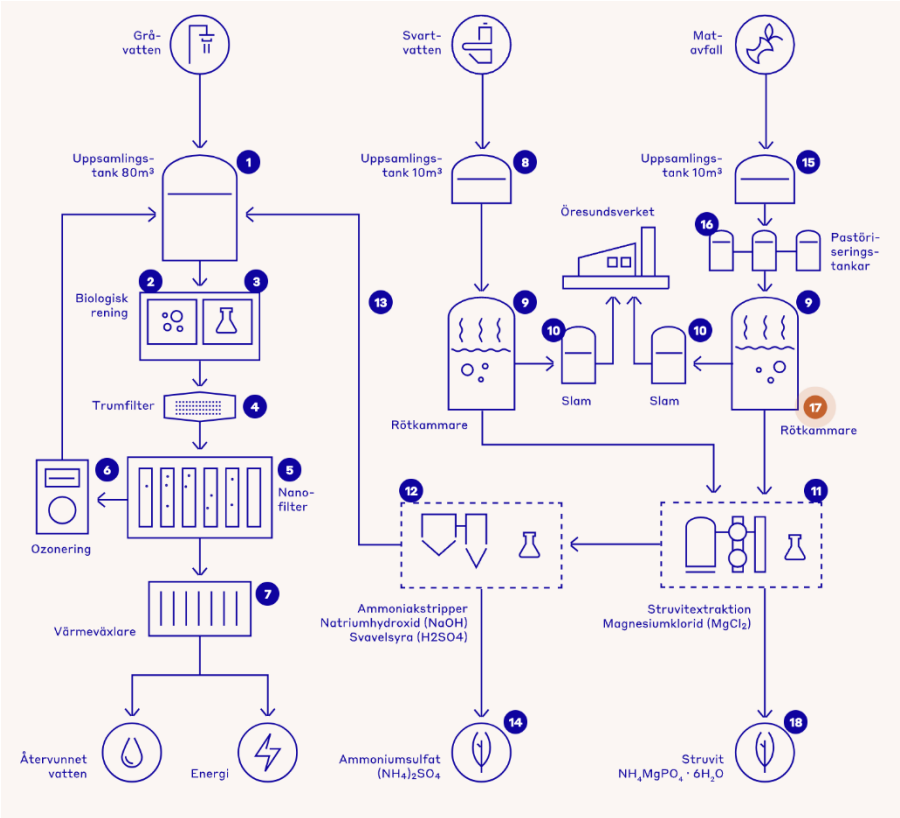
Supplementary figure 1: Allophycocyanin content during the optimization experiment of A. platensis grown on different compositions of WW (75% FWW and 50% FWW).



Supplementary figure 2: Phycoerythrin content during the optimization experiment of A. platensis grown on different compositions of WW (75% FWW and 50% FWW).



Supplementary figure 3: Graphic layout and parameter specifications for (A) optimisation experiment 1 and (B) scale-up experiment 2.



Supplementary figure 4: Schematic of the layout at RecoLab treatment facility, indicating the separation of waste streams (gray, black and food WW), and the flow of processing that each stream undergoes. Treatment 17, highlighted on the diagram, indicates the point at which samples were taken for use in this study.