Impact of different sidewater streams on the growth of microalgae - a case study on Spirulina growth and microorganisms

Linnea Dahl

Independent project • 15 Hp
Swedish University of Agricultural Sciences, SLU
Landscape Architecture, Horticulture and Crop Production Sciences /Biosystem and technology
Horticultural Management Programme - Gardening and Horticultural Production
2025

Impact of different sidewater streams on the growth of microalgae - a case study on Spirulina growth and microorganisms

Påverkan av olika sidovattenflöden på tillväxten av mikroalger – en fallstudie om Spirulina-tillväxt och mikroorganismer

Linnea Dahl

Supervisor:	**Samar Khalil, Sveriges LantbruksUniversitet, Department of biosystem and technology**
Examiner:	Anna-Karin Rosberg, Sveriges Lantbruksuniversitet, Department of biosystem and technology

Credits:	15 credits
Level:	G2E
Course title:	Självständigt arbete I Trädgårdsvetenskap
Course code:	EX0844
Programme/education:	Trädgårdsingengör - odling
Course coordinating dept:	Department of Biosystems and Technology
Year of publication:	2025
Keywords:	Fish Water, Food Wastewater, Microbial content, Nutrient Recycling, Wastewater treatment, Water recycling

Swedish University of Agricultural Sciences

Faculty of: Landscape Architecture, Horticulture and Crop Production Sciences

Department: Biosystems and technology

Abstract

The growing demand for alternative water and nutrient sources in horticulture has sparked interest in reusing industrial sidewater streams with rich nutrient content. However, effective purification and utilization methods are essential to prevent contamination and ensure safe and efficient reuse of these water streams. Microalgae have long been explored as a tool for wastewater purification and nutrient recovery. If successfully implemented, biofertilizers derived from these processes could provide an alternative to conventional fertilizers, improving soil health and reducing reliance on synthetic inputs.. This study investigates the potential of microalgae (Spirulina, *Arthrospira platensis*) to grow in different wastewater streams—blackwater, Food Wastewater (FWW), and aquaculture water—to assess biomass production and microbial content. The goal is to determine whether microalgae can produce valuable biomass for potential use as a biofertilizer. The experiment was conducted in small-scale cultures, measuring biomass growth through optical density and analysing microbial quality before and after cultivation of microalgae. Results indicate that FWW supported the highest Spirulina growth, followed by blackwater, while aquaculture water and a control treatment with plant fertilizer showed significantly lower biomass accumulation. Microbial enumeration after microalgae cultivation showed increased bacterial and fungal presence across all treatments, highlighting important considerations for safety and processing in biofertilizer applications. However, this also suggests the potential for synergistic and symbiotic interactions between microalgae and microbial life. Further research is essential to optimize these relationships, improving wastewater treatment efficiency and enhancing beneficial microbial dynamics. The study encountered challenges in system design particularly due to significant water loss, which impacted result reliability by artificially inflating concentrations and loss of replicates. Future investigations with optimized conditions and larger-scale trials are necessary to assess the feasibility of microalgae-based wastewater treatment and nutrient recycling.

Keywords: Fish Water, Food Wastewater, Microbial content, Nutrient Recycling, Wastewater treatment, Water recycling

Table of contents

Abbreviations

Abbreviation:	**Description**:
FWW	Food WasteWater
HRAP	High-Rate Algal Ponds
COD	Chemical Oxygen Demand
BOD	Biological Oxygen Demand
DO	Dissolved Oxygen
ROS	Reactive Oxygen Species
CFU	Colony Forming Unit
MA	Malt Agar
TSA	Tryptic Soy Agar
TNTC	To Numerous To Count
PAR	Photosynthetically Active Radiation

1. Introduction

1.1 Challenges in Horticulture and the Need for Sustainable Alternatives

As global food production must increase to meet the demands of a growing population, horticulture faces challenges in securing sustainable water and nutrient sources. Synthetic fertilizers have played a crucial role in modern horticulture, but their excessive use has led to severe environmental consequences. Over-fertilization can result in soil degradation, nutrient runoff, and water eutrophication, threatening ecosystems and biodiversity (Pooja et al., 2022). Additionally, industrial fertilizers contribute to greenhouse gas emissions and may lead to the accumulation of heavy metals in soils, crops, and the food chain (Gonçalves et al., 2023). With finite global reserves, e.g. of phosphorus a critical plant nutrient, there is an urgent need to explore alternative, more sustainable nutrient sources (Hultberg et al., 2013).

One potential solution is utilizing wastewater streams as alternative nutrient sources. These side streams often contain valuable organic matter and essential nutrients that could be recovered for agricultural use. However, they also pose risks, including contamination from pathogens, heavy metals, and organic pollutants (Abdelfattah et al., 2023). Improperly managed wastewater can contribute to disease outbreaks, while nutrient runoff from hydroponic systems and industrial waste can lead to further environmental degradation (Hultberg et al., 2013; Al-Jabri et al., 2021). To ensure safe and efficient reuse, effective purification methods are essential.

An emerging approach to both wastewater purification and nutrient recovery is microalgae cultivation. Microalgae can efficiently absorb contaminants and excess nutrients, improving water quality while generating biomass with potential applications as biofertilizer (Pooja et al., 2022). By integrating microalgae-based treatment systems, horticulture could reduce reliance on synthetic fertilizers, minimize pollution, and contribute to a more circular and sustainable practice.

1.2 Aim of Study

The aim of this bachelor’s thesis is to evaluate the ability of microalgae to grow in different wastewater streams and estimate their biomass production potential for future use as fertilizer. Additionally, the study examines their impact on microbial quality in reused water streams. This information is essential for assessing the feasibility of using microalgae for nutrient and water recycling, as well as their potential as a sustainable biofertilizer.

The study questions are:

1. In which wastewater stream used in the study is most suitable for Spirulina biomass growth?
2. How does the cultivation of spirulina effect the numbers of bacteria and fungi?

The hypothesis is that the streams with highest amounts of nutrient will support the largest production of Spirulina biomass along with a higher number of microorganisms.

This study does not include a growing trial to test the effectiveness of microalgae as fertilizer, nor does it identify the specific microorganisms present in the microbial enumeration

2. Background

2.1 Microalgae: A Versatile and Sustainable Resource

Microalgae include both prokaryotic cyanobacteria and single-celled eukaryotic microorganisms, with some cyanobacteria capable of nitrogen fixation (Gonçalves et al., 2023). Cyanobacteria are believed to be among the first organisms on Earth, playing a crucial role in Earth's oxygenation and geochemical shift two billion years ago (Faulds, 2023). In her master's thesis, Faulds (2023) describes cyanobacteria as “unmatched” in their ability to fix nitrogen and carbon. They exhibit diverse metabolic strategies—autotrophic, heterotrophic, and mixotrophic—allowing them to thrive in various environmental conditions (Abdelfattah et al., 2023).

Microalgae have a higher photosynthetic efficiency than terrestrial plants, contributing to approximately 50% of the oxygen in the atmosphere while rapidly converting sunlight and nutrients into biomass (Abdelfattah et al., 2023). Their ability to produce biomass efficiently allows for yields up to five times higher per unit area than terrestrial energy crops, all without relying on arable land (Faulds, 2023; Slinksienė et al., 2022). They are also considered a low-maintenance crop, requiring minimal space and expertise for cultivation (Pooja et al., 2022). By optimizing factors such as pH, nutrient levels, and light availability, their productivity can be further enhanced, (Abdelfattah et al., 2023). Östlund (2024) emphasizes that an external input of carbon is essential for the optimal growth of microalgae, as it supplies the necessary carbon for photosynthesis and biomass production. Faulds (2023) discusses the limitations in microalgae cultivation due to light availability. One significant challenge is self-shading, which occurs when densely packed algae cells absorb most of the available light near the surface, preventing sufficient light from reaching cells deeper in the culture. This uneven light distribution can hinder the growth of the algae. To address this issue, photobioreactors are often used, employing artificial lighting and design strategies to ensure more uniform light exposure throughout the culture, thereby mitigating the effects of self-shading. (Faulds, 2023).

Spirulina is a genus of cyanobacteria, with *Arthrospira platensis* being the species used in this study. *A. platensis* thrives in warm, freshwater environments with high alkalinity, preferring temperatures between 30–35°C and a pH range of 9–11 (Faulds, 2023). Its adaptability to nutrient-rich conditions makes it a promising candidate for wastewater treatment and biomass production.

2.2 Wastewater as a Resource: The Role of Microalgae in Water Treatment

Traditional wastewater treatment involves multiple steps, including the removal of solid particles and microbial breakdown of organic matter. These processes generate large amounts of biohazardous sludge, which is costly to manage and requires extensive infrastructure (Abdelfattah et al., 2023). Microalgae present a promising alternative, as they can thrive in wastewater while simultaneously removing pollutants and producing valuable biomass (Pooja et al., 2022). By utilizing the remaining carbon, nitrogen, and phosphorus in wastewater, microalgae can help reduce nutrient loads while also breaking down toxins (Pooja et al., 2022). Through photosynthesis, they generate oxygen, which supports bacterial degradation of organic matter, creating a self-sustaining purification cycle (Abdelfattah et al., 2023).

Co-culturing microalgae with bacteria, activated sludge, fungi, or nanoparticles has been shown to significantly enhance wastewater treatment by improving nutrient removal, organic matter degradation, and biomass production (Abdelfattah et al., 2023). Each combination offers unique advantages, making them promising alternatives to traditional wastewater treatment methods.

In addition to traditional microalgae-based systems, High-Rate Algal Ponds (HRAPs) utilize algae to absorb nutrients and convert carbon dioxide to oxygen gas, which is then used by heterotrophic bacteria to oxidize organic contaminants and release CO_2 for the algae (Álvarez-González et al., 2022). Previous research has demonstrated that HRAPs can effectively remove up to 69% of Chemical Oxygen Demand (COD), 95% of ammonium nitrogen, 83% of total inorganic nitrogen, and 81% of phosphate, significantly improving wastewater quality while supporting nutrient recovery (Álvarez-González et al., 2022).

Microalgae's high adaptability to different conditions allows flexibility in both wastewater characteristics and system design where the most suitable microalgae strains can get selected for the type of wastewater and system design (Abdelfattah et al., 2023). Their high surface-to-volume ratio enhances biosorption, where pollutants passively adhere to their cell surfaces; bioaccumulation, where contaminants are actively absorbed and stored; and biodegradation, where harmful substances are broken down metabolically (Abdelfattah et al., 2023). These processes enable them to efficiently capture and transform pollutants, reducing Biological Oxygen Demand (BOD)—the oxygen needed by microorganisms to break down organic matter—and Chemical Oxygen Demand (COD)—the total oxygen required to oxidize both organic and inorganic pollutants—by 45–65% (Abdelfattah et al., 2023).Selecting the right microalgae strains and optimizing carbon source concentrations are crucial for maximizing biodegradation efficiency (Abdelfattah et al., 2023).

Studies have demonstrated that *Arthrospira platensis* (previously known as *Spirulina platensis*), can remove heavy metals cadmium and chromium from wastewater through biosorption (Abdelfattah et al., 2023).

In addition to nutrient and pollutant removal, microalgae contribute to pathogen reduction through multiple mechanisms, including nutrient competition, changes in pH and dissolved oxygen (DO) levels, attachment and sedimentation, and the production of antimicrobial compounds (Abdelfattah et al., 2023). However, according to the literature, further research is needed to optimize these processes for large-scale applications.

Microalgae offer additional environmental benefits. By sequestering CO_2, they contribute to carbon reduction efforts, and it is believed to be a helpful tool against global warming (Abdelfattah et al. 2023).

Al-Jabri et al. (2021) study the use of microalgae to recycle waste nitrogen in fertilizer production instead of letting it end up in a landfill. Although the results of the study showed a lower biomass production compared to the control group grown with urea there is still a promise for the technique when used in combination of utilization of other types of waste streams (for example waste heat and/or flue gas) could make the whole recycling business both more effective and cheaper. The authors did not intend for the microalgae in that study to be used as fertilizer but instead as fish feed in aquaculture.

2.3 Microalgae-Based Fertilizers: A Step Towards Sustainable Horticulture

Bio-fertilizers pose fewer risks of salinization or eutrophication compared to industrial fertilizers and contain lower levels of toxins while being easier to handle (Pooja et al., 2022). Gonçalves et al. (2023) expand on this argument by highlighting that bio-fertilizers not only supply essential nutrients but also improve soil health by promoting beneficial microbial activity and increasing the amount of organic matter, while also limiting the spread of certain pathogens.

Microalgae biomass has a wide range of applications, with its use as a bio-fertilizer (Pooja et al., 2022; Gonçalves et al., 2023) becoming increasingly popular. It functions as a slow-release fertilizer, limiting nutrient leakage by ensuring nutrients are made available in alignment with plant uptake (Álvarez-

González et al., 2022; Slinksienė et al., 2022). Studies comparing industrial fertilizers with microalgae bio-fertilizers grown from wastewater, as well as combinations of the two, found that combining microalgae with inorganic fertilizers could provide a more balanced nutrient supply. However, the study also highlighted that nitrogen availability in microalgae-based fertilizers was a limiting factor. The slow-release nature of the microalgae fertilizer was too slow for optimal chlorophyll content in plants when used alone (Abdelfattah et al., 2023).

A study performed by Slinksienė et al. (2022) using leachate from landfills as a nitrogen source for *Chlorella sp*. showed a potential for a higher biomass yield compared to the control, a universal nutrient medium. Adding an organic carbon source, such as technical glycerol (a byproduct of biodiesel production), further increased biomass production. The dried microalgae biomass was then used as an additive to granular nitrogen fertilizer which seemed promising.

2.4 The Circular Economy Potential of Microalgae in horticulture

There is potential to create a circular economy aspect in horticulture, where wastewater and nutrients from different sidestreams is seen as a resource rather than a waste product. Microalgae can be used to treat wastewater while simultaneously producing valuable biomass that can be sold as bio-fertilizer, thus reducing overall production costs and minimizing the environmental footprint (Álvarez-González et al., 2022; Gonçalves et al., 2023). Álvarez-González et al. (2022) also suggest that microalgae-based wastewater treatment could be as cost-effective, if not more so, than conventional treatment methods. By optimizing microalgae-based wastewater treatment, industries could significantly reduce water pollution while benefiting from valuable biomass production (Abdelfattah et al., 2023).

2.5 Challenges and Considerations for Large-Scale Implementation

Despite the many advantages of microalgae-based systems, some challenges remain. Gonçalves et al. (2023) point out that microalgae can accumulate heavy metals and other contaminants from wastewater, posing risks for both crops and consumers. Additionally, the nutrient composition of microalgal biomass may not always be stable or optimized as plant fertilizer. However, Slinksienė et al. (2022) found no heavy metal contamination in their study of microalgae biomass.

Studying the microbial quality of wastewater used in microalgae cultivation is vital for ensuring public health and environmental safety. It helps assess the risk of harmful pathogens or the production of toxic byproducts like phycotoxins. Microbial quality also ensures compliance with safety regulations for treated water and biofertilizer products. Furthermore, understanding microbial dynamics can optimize the cultivation process, improving nutrient removal efficiency and biomass production. It also helps assess the potential environmental impact of wastewater reuse, ensuring that harmful microorganisms aren't introduced into ecosystems. Microbial quality is important to the safety, efficiency, and sustainability of microalgae-based wastewater treatment.

Another potential issue is the absorption of antibiotics and other pollutants (Abdelfattah et al., 2023). Some pollutants may generate reactive oxygen species (ROS) during bioaccumulation, which can damage cellular components, disrupt metabolism, and cause cell death (Abdelfattah et al., 2023).

Microalgae growth may also be limited by solid particles or coloured wastewater, which can hinder the availability of photosynthetically active radiation (PAR) needed for growth (Abdelfattah et al., 2023).

The economic feasibility of large-scale microalgae production is another area of debate. Gonçalves et al. (2023) notes that production costs and energy demands can be high depending on cultivation and processing technique. However, Abdelfattah et al. (2023) argue that microalgae-based wastewater treatment could be cost- and energy-efficient compared to traditional methods. One strategy to lower costs is the use of wet (fresh) microalgae biomass rather than using energy-intensive drying processes (Slinksienė et al., 2022).

While these findings are promising, the literature is requesting further research to refine and optimize microalgae-based systems for large-scale implementation.

3. Material and Methods

3.1 Experiment setup

The growing trial was conducted in 100 ml glass flasks using five replicates for each of the four different treatments: blackwater (toilet water), Food Wastewater (FWW), aquaculture water, and commercial plant fertilizer as a control. Each flask contained a total of 50 ml, consisting of 45 ml of the respective water and 5 ml of Spirulina culture, following the ratio of 10% microalgae in relation to the total volume applied by Carrez (2022). Spirulina was cultivated in each wastewater type for one week, and biomass production was measured daily.

To provide aeration and mixing, a small piece of tubing was inserted into each flask and connected to a small aquarium air pump, generating bubbles to oxygenate the water. (Figure 1). A loose ball of cotton was placed in the neck of each flask to keep the tubing in place while ensuring the system remained ventilated but not completely open.

The flasks were placed in a greenhouse chamber maintained at a constant temperature of 20°C using natural light conditions of approximately 10 hours per day.

The blackwater and FWW were sourced from Recolab in Helsingborg, Sweden, and filtered through a 0.45 μm Supor® PES Membrane, to reduce the presence of larger particles that risked blocking the light. Previous studies on FWW and Blackwater from the same source in Helsingborg showed that: The FWW contained 278.82–547.18 mg/L total nitrogen, 21.21–52.79 mg/L total phosphorus, and 138.08–479.92 mg/L ammonium ($N–NH_4$) (Faulds, 2023). The blackwater contained 950–1500 mg/L total nitrogen, 100–130 mg/L total phosphorus, and 950–1250 mg/L $N–NH_4$ (Carraz, 2022).

The aquaculture water was obtained from a previous tilapia (*Oreochromis niloticus*) aquaculture system at SLU Alnarp, it contained 48mg/l nitrogen and 4.8mg/L phosphorus (personal communication, Samar Khalill, SLU- Department of biosystem and technology).

The control consisted of tap water supplemented with a commercial fertilizer (Emmaljunga, Växtnäring), with a ratio of NPK 5-1-4, at a 200:1 dilution, following the manufacturer's instructions. The resulting concentrations are 2.48 mg/L of nitrogen (N) and 0.5 mg/L of phosphorus (P), calculated by multiplying the nutrient percentages by the dilution factor and converting to mg/L.

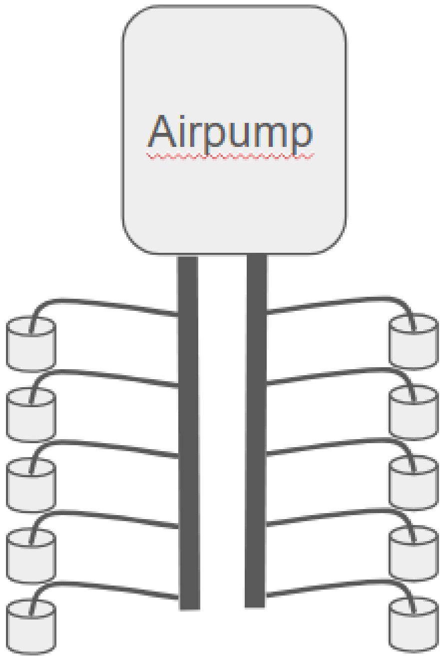

Figure 1. Schematic illustration of the air pump system.

3.2 Optical Density

Spirulina biomass was measured every 24 hours using a spectrophotometer to compare its growth across different wastewater treatments. A 150 µL sample was collected from each flask, and absorbance (optical density) was measured at 680 nm (λ = 680 nm) (Östlund, 2024) in absorbance mode using a Multiskan SkyHigh spectrophotometer (Thermo Fisher Scientific) reflecting the presence of chlorophyll-a and indicate Spirulina biomass.

3.3 Microbial enumeration

Viable count method based on Colony Forming Unit (CFU) was performed for enumeration of microbial content before and after the cultivation of microalgae.

A serial dilution method (10^0 to 10^{-5}) was used to enumerate microorganisms, with 10% Malt Agar (MA) for general fungal biota and 10% Tryptic Soy Agar (TSA) for general bacterial biota. A 0.5 mL sample from each flask (replicate) was sequentially transferred into test tubes containing 4.5 mL of 0.85% NaCl solution to achieve the desired dilutions. The dilutions 10^0-10^2 were used on MA and 10^3-10^5 on TSA. An amount of 100 µL of each dilution was applied using drop test and two replicate plates per medium. The plates were then incubated in 37 degrees Celsius for 24 hours for TSA plates and 36 hours for MA plates.

3.4 Statistics

The statistical analysis was made in the program Minitab 19. Tests performed on the Spirulina biomass and microbial count (log-transformed) was one-way ANOVA and Tukey Pairwise Comparisons with a significance level of $p<0.05$.

4. Results

4.1 Optical density

The initial values, day 0, vary slightly, notably the FWW, even though the same concentration of Spirulina was added. This indicates how many other particles in the water also absorb the wavelength 680nm. (Figure 2).

On the last day, day 6, a loss of water caused the numbers of replicates to vary. Specifically, blackwater had 2 replicates, aquaculture had 5, Food Wastewater had 3, and the control had 4. This variation in replication is important to note when comparing the results across treatments. Further discussed in section 4.3.

The Food Wastewater (FWW) treatment showed significantly (significance level: $\alpha=0.05$, $p=0.001$) the highest absorbance values of all treatments after cultivation. The absorbance values notably increased after day 4, a sample peaking at 0.964 on day 5 and maintaining the highest average of 0.596 by the last day (day 6). (Figure 2).

The Blackwater treatment also demonstrated high growth, though it was less consistent than the FWW. A peak sample absorbance of 0.583 was observed on day 2, followed by a clear dip the next day, with a recovery once again toward the end of the cultivation period. By the last day, the treatment had reached an average absorbance of 0.3175. (Figure 2).

In contrast, the Aquaculture treatment showed much lower absorbance values throughout the experiment. The highest sample absorbance recorded was 0.203 on day 2, after which the growth stagnated and even slightly declined. By the final day, the average absorbance was only 0.0618. (Figure 2).

The Control treatment displayed consistently low absorbance values, a single sample peaking at 0.127 on the fifth day. The highest average was observed on the last day with absorbance at 0.111. (Figure 2).

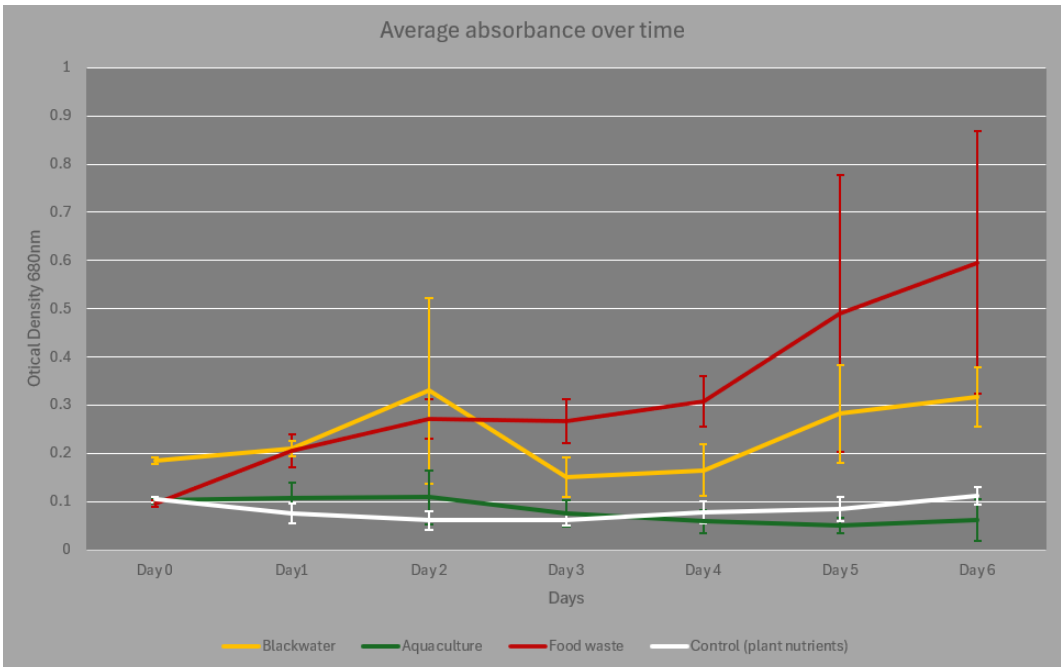

Figure 2: Line graph displaying the average absorbance of Spirulina biomass reflecting the presence of chlorophyll-a at 680 nm in the different waste water side- streams throughout the cultivation period of one week. The significance differences between the values at the last day of measurements (day 6) with p value 0.001 were evaluated using Minitab 19 software.

4.2 Microbial enumeration

The number of post-treatment replicates varied across treatments due to water loss during the trial period. Specifically, blackwater had 1 replicate, aquaculture had 5, Food Wastewater had 3, and the control had 4. Losing one more replicate from the blackwater compared to the OD measurement since not enough water was left to conduct both tests. This variation in replication is important to note when comparing the results across treatments. Further discussed in section 4.3.

4.2.1 Bacterial content

The bacterial growth, measured before (Pre) and after (Post) cultivation, increased across all treatments (see Figure 3). The Food wastewater followed by the blackwater treatments showed the highest values. The control had no detected bacteria colonies pre-treatment. The aquaculture treatment had the lowest bacterial presence after cultivation.

The control treatment showed the significantly (α=0,05, p< 0, 0001) largest increase in bacterial growth after cultivation compared to the others, although they all experienced an increase.

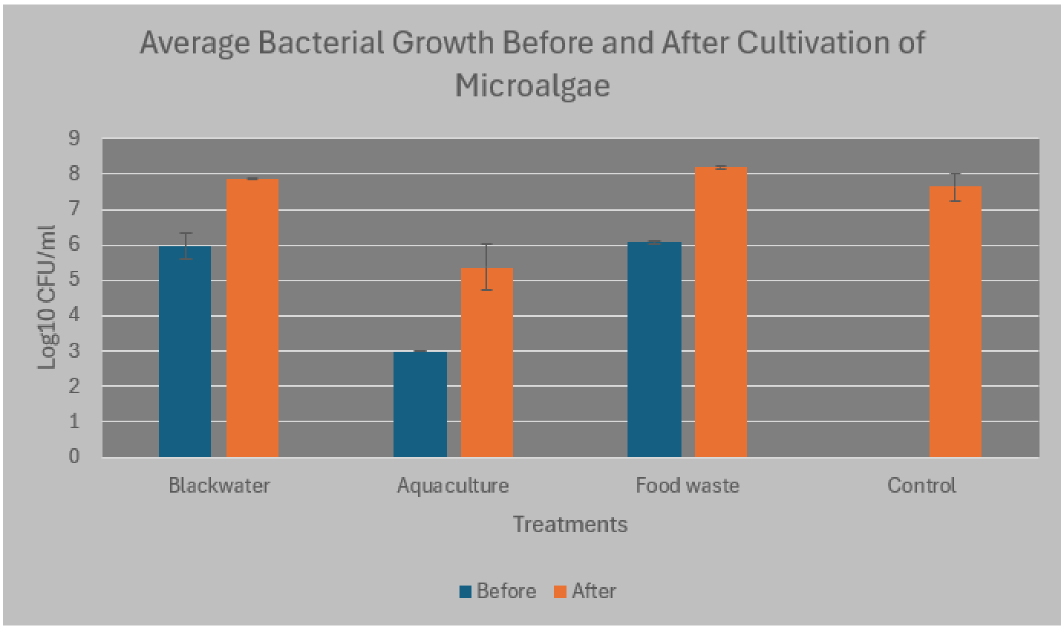

Figure 3: Average bacterial growth on Tryptic Soy Agar (TSA) before and after the cultivation of microalgae, as influenced by different wastewater side-streams. The bar chart presents the log-transformed (log10) colony-forming units (CFUs) of bacterial growth, with five replicates per treatment. Post-treatment samples had varying numbers of replicates (1, 5, 3, and 4, respectively).

4.2.2 Fungal content

Fungal growth increased across all treatments after cultivation (See figure 4)., but the extent of this increase varied. The control treatment had no detectable fungal presence before cultivation with Spirulina but developed extremely high fungal growth afterward and was thereby considered Too Numerous To Count (TNTC) within the frame of the used dilutions. The Food Wastewater was also TNTC post-cultivation in the used dilutions.

The aquaculture treatment started with a low fungal count, second only to the control, but also experienced a substantial rise, with one replicate reaching too many colonies to count (TNTC).

The blackwater treatment exhibited fungal growth both before and after cultivation, showing a smaller increase relative to other treatments.

Because of the TNTC-values there was no statistical analysis made.

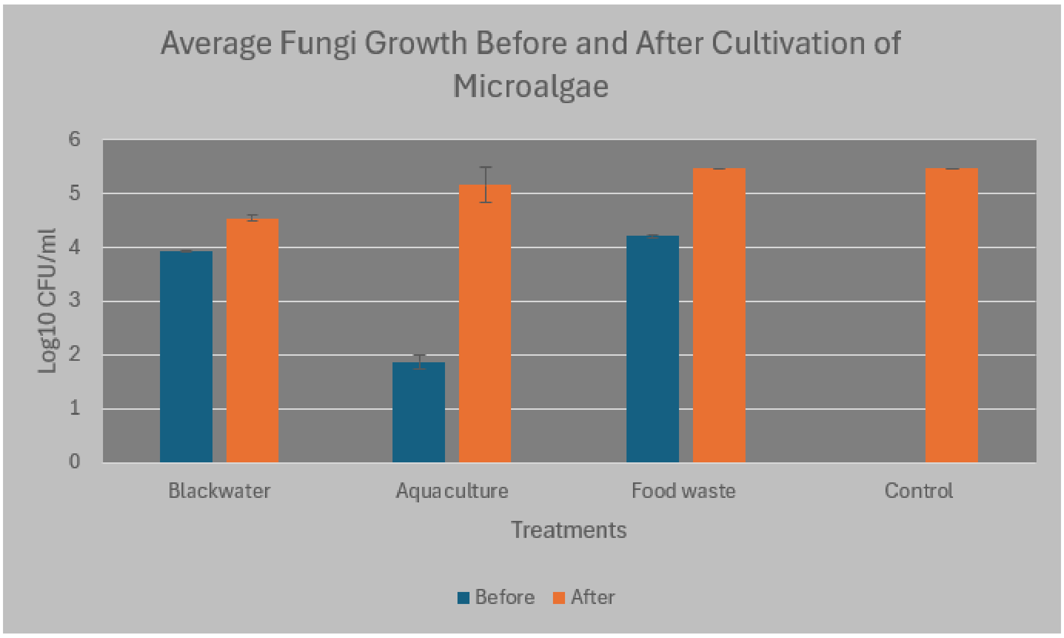

Figure 4: Average fungal growth on Malt Extract Agar (MEA) before and after the cultivation of microalgae, as influenced by different wastewater side-streams. The bar chart presents the log-transformed (log10) colony-forming units (CFUs) of fungal growth, with five replicates per treatment. Post-treatment samples had varying numbers of replicants (1, 5, 3, and 4, respectively). Food Waste and Control treatments exceeded the measurable limit (TNTC) in the used dilution, so their true values are not accurately represented in the graph.

4.3 Complications

The trial faced complications that likely impacted the results and their reliability. A significant water loss occurred throughout the growing trial. By the final day (day 6), some flasks had completely dried out, preventing the collection of samples for absorbance tests or Microbial enumeration. This is believed to be due to the small initial volumes, the periodic sampling of water during the trial (although small, it represented a portion of the total volume), and excessive evaporation. The cotton balls in the necks of the flasks were insufficient to prevent evaporation, especially given the small water volumes. Additionally, the air supply created considerable bubbling, which likely exacerbated evaporation. Another clue to evaporation being a major factor is further supported by the varying degrees of water loss seen across treatments. The blackwater and Food Wastewater (FWW) replicates experienced the most losses, likely because these liquids, being darker in colour compared to the almost colourless aquaculture and control waters, absorbed more light and heated up more readily which would lead to higher degrees of evaporation. This complicates results since the remaining liquid would be more concentrated regardless of treatment success.

5. Discussion

5.1 Optical density

Preferably another type of control should have been implemented to reduce the error occurring from other particles absorbing the same wavelength that does not indicate Spirulina biomass. Replicates without added spirulina culture could help eliminate this issue and is a point for improvement for further studies.

The Food Wastewater (FWW) treatment demonstrated the highest absorbance values, indicating the most substantial Spirulina biomass growth. Previous studies have shown (Faulds, 2023) that FWW provides a highly nutrient-rich environment with total nitrogen content of 278.82 -547.18.mg/L and phosphorus content of 21.21 - 52.79 mg/L. These findings provide some insight into the potential nutrient composition of the FWW used in this experiment.

The blackwater treatment supported notable Spirulina growth, though to a lesser extent than FWW. This difference may be due to variations in nutrient composition or the presence of inhibitory factors in blackwater. Although this study did not measure nutrient concentrations or other limiting factors, but previous research by Carraz (2022) on blackwater from the same source (Reco lab- Helsingborg, Sweden) reported total nitrogen content of 950–1500 mg/L and total phosphorus content of 100–130 mg/L, which should have provided more nutrients for better growth than the reported FWW concentrations.

One potential factor for this discrepancy is ammonia, which can become toxic to microalgae at high concentrations (Faulds, 2023). Carraz (2022) reported $N–NH_4$ of 950 – 1250 mg/L in his blackwater while Faulds reported only 138.08 - 479.92 in the FWW. Additionally, blackwater may include organic matter that fosters bacterial competition, pathogens, microbial contaminants, or trace pharmaceuticals, all of which could negatively impact Spirulina growth. This was not investigated in the current study and of great need for further research.

In contrast, the aquaculture wastewater treatment exhibited significantly lower growth compared to blackwater and FWW, suggesting that its nutrient content was insufficient to sustain substantial Spirulina biomass production. This could be due to lower nitrogen (48 mg /L) or phosphorus (4.8 mg/L) levels measured from experiments at SLU compared to the other wastewater sources.

The control treatment, which contained liquid plant fertilizer, showed only minimal Spirulina growth. This suggests that the nutrients in the fertilizer were either not in an optimal form for Spirulina uptake or were present in insufficient concentrations. This aligns well with the fact the NPK fertilizer had concentrations; 2.48 mg/L of nitrogen (N) and 0.5 mg/L of phosphorus (P). Substantially lower than those in the wastewater streams.

These findings highlight the potential of wastewater-derived nutrients originated from FWW and blackwater as a viable alternative to conventional fertilizers, offering a sustainable approach to both Spirulina biomass production and wastewater treatment. The obtained results indicated also the need of high levels of nitrogen and phosphorus content to obtain a good growth of microalgae. However, further research is needed to analyse nutrient composition and other environmental factors influencing Spirulina growth in different wastewater sources.

Overall, the results suggest that utilizing nutrient-dense waste streams, particularly FWW and blackwater, holds promise for enhancing microalgal growth, potentially improving water and nutrient recycling and biofertilizer production.

Although, challenges in system design and the water loos in some of the treatments especially blackwater are factors affecting the obtained results. By the end of the measurements period, a loss of some replicants occurred in some of the treatments, limiting the possibility of comparison between the treatments. Blackwater treatment being the most affected one, having only two replicates. The significant water loss would also lead to increased concentrations, which could result in a higher concentration of Spirulina relative to the remaining water volume. Therefore, it remains unclear whether the observed results reflect an actual increase in Spirulina biomass or simply a higher concentration due to the reduced volume of water.

5.2 Microbial enumeration

5.2.1 Bacterial content

The highest bacterial counts were observed in the Food Wastewater (FWW) and blackwater treatments after cultivation, indicating that these environments supported bacterial growth. This may be due to their high nutrient content, which can promote bacterial, while Spirulina may have contributed by supplying oxygen and organic carbon compounds through photosynthesis. Such interactions between microalgae and bacteria are well-documented, as co-culturing microalgae with bacteria can enhance nutrient removal, organic matter degradation, and biomass production (Abdelfattah et al., 2023) Microbial communities in wastewater treatment systems often function through symbiotic relationships between microalgae and bacteria. In High-Rate Algal Ponds (HRAPs), algae absorb nutrients and release oxygen, which bacteria use to oxidize organic matter, releasing CO_2 that in turn supports algal growth. Similar interactions may have played a role in the observed bacterial growth in the FWW and blackwater treatments, though further investigation is needed to confirm these dynamics (Álvarez-González et al., 2022).

In contrast, the aquaculture treatment exhibited the lowest bacterial presence after cultivation. This suggests that fewer nutrients were available to support bacterial growth or that other environmental conditions in this medium were less favourable for microbial growth. Similarly, the control treatment, which contained only commercial fertilizers, had lower bacterial counts post-cultivation compared to FWW and blackwater. However, without identifying the bacterial species present, it remains unclear whether these microbial communities were beneficial, neutral, or negative to Spirulina growth. Future studies should include bacterial identification to better understand the nature of these interactions.

Microbial colonies were detected when samples were plated on Tryptic Soy Agar (TSA). However, some of these colonies could potentially be Spirulina itself rather than bacteria. Additional tests on selective media with Spirulina and bacteria would help distinguish between the two.

Another important limitation of this study is the variation in post-treatment replicates across treatments. This discrepancy resulted from water loss during the trial period, which reduced the available sample volume in some bottles. Since treatments with more replicates provide a more reliable average, those with fewer replicates may not fully capture the variability in bacterial growth. Future studies should aim to maintain a balanced number of replicates to improve comparability.

Additionally, water loss could have artificially increased bacterial concentrations, making it difficult to determine whether observed bacterial growth was because of Spirulina cultivation, the wastewater sources, or simply the reduced sample volume. As discussed in relation to absorbance measurements, evaporation can lead to misleadingly high microbial densities. Future research should take this into account and implement measures to minimize water loss for more reliable results. Although a study conducted by Qiu et al. (2022) suggests that bacterial content decreases with a reduction in available water volumes, this finding contradicts the interpretation in the current study, where an increase in bacterial concentrations was observed simultaneously with a decrease in water volume. This might be an indication of the symbiosis between microalgae and bacteria to support the later despite the reduction of water volume or another indication of the unreliability of the results.

5.2.2 Fungal content

In interpreting the results, it is important to acknowledge the limitations posed by the TNTC values observed in certain treatments, along with the loss of replicates. As a result, statistical analysis was not performed, which limit the robustness of the conclusions drawn. The results indicated high fungal growth in all treatments, the TNTC values highlighting the need for higher dilutions to obtain readable results. Therefore, future studies should focus on maintaining a stable number of replicates and using higher dilutions to improve the reliability of the data.

However, the data suggests that the conditions in all treatments were conducive to fungal growth, with some media—particularly the control and Food Wastewater—supporting extensive fungal development.

The complete absence of fungi in the control before cultivation and its rapid increase post-cultivation indicates that the medium was highly favourable for fungal development. The source of the fungi establishment probably coming from another source than the control water itself, e.g. the spirulina culture, the air supply tubing or other material that was not sterile before application.

The role of specific nutrients or microbial interactions in driving these differences remains unclear and would require further investigation.

5.3 Overall Findings and Implications

The findings in the current study suggest that different waste streams provide varying levels of suitability for Spirulina cultivation. Food Wastewater (FWW) appears to be the most promising medium in terms of biomass yield, which could make it a viable candidate for biomass-based fertilizer production. The observed bacterial growth in this treatment may not necessarily be a drawback, as bacteria can play a symbiotic role by aiding in nutrient breakdown and enhancing microalgal productivity. However, further analysis is needed to determine the nature of these bacterial communities and their potential impact on the final biomass product. Additionally, the need for post-harvest processing should be considered to ensure safe application as a biofertilizer.

The blackwater treatment also showed moderate growth, indicating its potential for nutrient recycling. However, further research is needed to evaluate its safety, along with that of other wastewater side-streams.

The aquaculture and control treatments resulted in lower biomass production, suggesting they may be less effective for large-scale Spirulina cultivation.

A pattern emerged showing that higher Spirulina biomass coincided with increased bacterial and fungal growth. The FWW treatment, which produced the most Spirulina, also had the highest bacterial count and one of the highest fungal counts (TNTC), suggesting that nutrient-rich wastewater supports both Spirulina and microbial growth. Similarly, blackwater, which promoted moderate Spirulina growth, also saw microbial expansion, although the increase in bacterial and fungal populations was less pronounced compared to FWW, indicating a more balanced microbial environment. In contrast, the aquaculture treatment, which showed relatively low Spirulina growth, still experienced substantial bacterial and fungal growth, with one replicate reaching too many fungal colonies to count (TNTC). This suggests that while the medium was not optimal for Spirulina, it contained organic matter or conditions that promoted microbial expansion. Or perhaps that the microbial expansion prohibited optimal Spirulina growth.

The control treatment showed minimal Spirulina biomass but notable bacterial and fungal growth. The absence of fungi before cultivation and the rapid increase in fungal colonies post-cultivation indicates that the medium was favourable for fungal development, even without wastewater nutrients. Bacterial numbers also increased remarkably.

Overall, these results highlight that while certain wastewater sources can effectively support Spirulina growth, they also encourage bacterial and fungal expansion. This creates a potential trade-off: wastewater sources that enhance Spirulina production may also promote microbial growth, which could have implications for applications as fertilizer. The extent to which microbial growth impacts Spirulina cultivation—whether through competition, symbiosis, or contamination—remains uncertain and would require further investigation.

5.4 Improvements for future studies

Future studies should implement a more optimal system design. Recommended is to work with substantially larger water volumes than 50 ml used here to avoid the issue of evaporation and compromised concentrations. Additionally, system design can be further optimized by integrating heating and artificial lighting to enhance microalgae growth.

An optimal balance must be established to maintain temperatures within the range of 30–35°C (Faulds, 2023), ensuring that elevated temperatures do not exacerbate issues related to evaporation. To marginalize the effects of water volume fluctuations, higher temperatures would require increased water volumes.

In larger water volumes, artificial lighting may become essential to minimize the risk of self-shading, were dense cell concentrations limit light penetration. Faulds (2023) highlights how photobioreactors address this challenge through strategic lighting solutions. Implementing similar approaches, such as interlighting within the culture, could help with light distribution and improve overall productivity. A speculative suggestion for a potential low-tech solution in small-scale cultivation could involve using LED string lights or similar light sources wrapped around the growing containers to improve light distribution.

A carbon source, such as carbon dioxide, could also be beneficial for stimulating optimized growth. Östlund (2024) considers it essential for microalgae growth in his cultivation trials. Due to technical limitations in the current study, no carbon source was supplied, and this should be seen as an area for improvement in future research.

Regarding the OD measurements, steps should be taken to ensure that the readings specifically reflect Spirulina biomass and are not influenced by other particles that could interfere with the results. One suggestion to address this is to include additional control treatments without Spirulina, which would allow for measurement of how much each water source absorbs at the 680 nm wavelength on its own.

Further studies should investigate microbial composition in more detail and consider microbial community interactions with Spirulina to determine whether these bacteria contribute positively (e.g., nutrient cycling) or negatively (e.g., competition or contamination) to the cultivation. Additionally tests should be performed to conclude if Spirulina itself (or other microalgae) can grow on the selective media used for the tests.

5.5 Supplementary Notes

By the second day, the blackwater no longer had an unpleasant odour, while the Food Wastewater (FWW) still emitted a noticeable smell. However, FWW eventually lost its Odor, though the exact timing is unclear. The difference in odour reduction between blackwater and FWW could be due to variations in microbial activity. Blackwater likely experienced more efficient microbial breakdown of organic matter, leading to faster odour neutralization, while the more complex organic compounds in FWW may have been harder to decompose, slowing the process. Environmental factors, such as pH and oxygen levels (neither measured), could have also influenced the rate of odour reduction. The data show increased microbial growth across treatments, suggesting that microbial communities played a key role in breaking down odorous compounds. Additionally, Spirulina's photosynthetic activity may have contributed to changes in water chemistry, such as oxygenation and pH shifts, further affecting odour breakdown.

It was also observed that the cotton balls in the flask necks had absorbed moisture, likely due to splashing air bubbles or condensation from trapped evaporation, although no condensation was visible on the glass.

By the fourth day, precipitates had formed in several treatments, though it was unclear whether they were Spirulina or other substances. The precipitation may have resulted from an increase in pH, which is known to occur with microalgae growth (Carraz, 2022), although pH levels were not measured during the experiment.

6. Conclusion

The results of this study suggest that Food Wastewater (FWW) and blackwater may have potential for supporting Spirulina biomass production, while aquaculture wastewater and the control treatment showed significantly lower growth. Both FWW and blackwater also supported large microbial content. However, the microbial quality needs further investigation. Without measurements of nutrient levels or other environmental factors, the cause of the observed differences cannot be definitively determined. The results are inconclusive due to significant water evaporation, which led to the loss of replicates and a reduction in water volume. This would have artificially inflated absorbance and microbial quality values and compromised the reliability of the samples. This could result in misleading representations of actual Spirulina biomass and microbial growth.

The system design in this study was not optimal. Future work should handle larger volumes to mitigate the evaporation and concentration issues. Additionally, a carbon source, along with potential heating and artificial lighting is recommended to maximise microalgae growth.

References

Abdelfattah, A., Ali, S.S., Ramadan, H., El-Aswar, E.I., Eltawab, R., Ho, S-H., Elsamahy, T., Li, S., El-Sheekh, M.M., Schagerl, M., Kornaros, M. & Sun, J. (2023) 'Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects', *Environmental Science and Ecotechnology*, 13, p.100205. Available at: https://doi.org/10.1016/j.ese.2022.100205 [Accessed 9 Feb. 2025].

Al-Jabri, H., Das, P., Thaher, M., Khan, S., and AbdulQuadir, M. (2021) 'Potential utilization of waste nitrogen fertilizer from a fertilizer industry using marine microalgae', *Science of The Total Environment*, 755, 142532. Available at: https://doi.org/10.1016/j.scitotenv.2020.142532 [Accessed 5 Feb. 2025].

Álvarez-González, A., Uggetti, E., Serrano, L., Gorchs, G., Ferrer, I. and Díez-Montero, R. (2022) 'Can microalgae grown in wastewater reduce the use of inorganic fertilizers?', *Journal of Environmental Management*, 323, 116224. Available at: https://doi.org/10.1016/j.jenvman.2022.116224 [Accessed 31 Jan. 2025].

Faulds, H. (2023) *'Production of natural pigments from A. platensis grown on wastewater from local treatment facility*', Master's thesis, Lund University. [Accessed 18 Feb. 2025].

Carraz, B. (2022) *'Growing algae from nutrients in wastewater*', Technical internship report, Lund University & ENSIL-ENSCI. (unpublished)

Hultberg, M., Carlsson, A.S. and Gustafsson, S. (2013) *'Mikroalger i växthuset – ett redskap för minskat näringsläckage*', LTJ-fakultetens faktablad, 2013:6. Sweden University of Agricultural Sciences (SLU), Alnarp.

Gonçalves, J., Freitas, J., Fernandes, I. and Silva, P. (2023) 'Microalgae as biofertilizers: A sustainable way to improve soil fertility and plant growth', *Sustainability*, 15(16), 12413. Available at: https://doi.org/10.3390/su151612413 [Accessed 29 Jan. 2025].

Pooja, K., Priyanka, V., Sekhar Rao, B.C., and Raghavender, V. (2022) 'Cost-effective treatment of sewage wastewater using microalgae Chlorella vulgaris and its application as bio-fertilizer', *Energy Nexus*, 7, 100122. Available at: https://doi.org/10.1016/j.nexus.2022.100122 [Accessed 27 Jan. 2025].

Qiu, Y., Zhou, Y., Chang, Y., Liang, X., Zhang, H., Lin, X., Qing, K., Zhou, X., & Luo, Z. (2022). The Effects of Ventilation, Humidity, and Temperature on Bacterial Growth and Bacterial Genera Distribution. *International journal of environmental research and public health*, 19(22), 15345. https://doi.org/10.3390/ijerph192215345

Slinksienė, R., Sendzikiene, E., Mikolaitiene, A., Makareviciene, V., Paleckiene, R., and Ragauskaite, D. (2022) 'Use of microalgae biomass for production of granular nitrogen biofertilizers', *Green Chemistry Letters and Reviews*, 15(2), pp. 416-426. Available at: https://doi.org/10.1080/17518253.2022.2071593 [Accessed 7 Feb. 2025].

Östlund, A. (2024) *'Integrating microalgae in wastewater treatment - Nutrient recovery and biomass production using Chlorella vulgaris in anaerobically digested black water treatment*', Master's thesis, Lund University. [Accessed 18 Feb. 2025].

Publishing and archiving

Approved students' theses at SLU can be published online. As a student you own the copyright to your work and in such cases, you need to approve the publication. In connection with your approval of publication, SLU will process your personal data (name) to make the work searchable on the internet. You can revoke your consent at any time by contacting the library.

Even if you choose not to publish the work or if you revoke your approval, the thesis will be archived digitally according to archive legislation.

You will find links to SLU's publication agreement and SLU's processing of personal data and your rights on this page:

- https://libanswers.slu.se/en/faq/228318
- ☒ YES, I, Linnea Dahl, have read and agree to the agreement for publication and the personal data processing that takes place in connection with this