

Thesis for the Degree of Doctor of Philosophy

**Nordic Microalgae-Assisted Valorization of  
Anaerobic Digestion Liquid and Gas  
Effluents**

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UNIVERSITY  
OF BORÅS

## **Nordic Microalgae-Assisted Valorization of Anaerobic Digestion Liquid and Gas Effluents**

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Cover photo: A simplified illustration of microalgae transforming wastewater into clean water. Designed using Google Gemini.



## Abstract

Anaerobic digestion (AD) is widely applied for the treatment of organic waste, yet dewatering of the AD digestate produces a liquid effluent rich in ammonium and organic pollutants that can pose environmental risks if discharged untreated or add extra load to wastewater treatment systems. In addition, upgrading biogas produced through AD generates a CO<sub>2</sub> stream, which requires appropriate handling to minimize emissions. This study explores the use of microalgae as a sustainable strategy to address both challenges by converting nutrient-rich effluents into valuable biomass while simultaneously enabling CO<sub>2</sub> biofixation.

The project began with a screening phase in which native Nordic microalgal strains, *Chlorella vulgaris*, *Coelastrella* sp., *Chlorococcum* sp., *Scotiellopsis reticulata*, and *Desmodesmus* sp., were assessed for their growth performance and nutrient removal efficiency in AD effluents from chicken manure, pulp and paper sludge, and food waste. This stage identified strains capable of efficiently assimilating volatile fatty acids and ammonium nitrogen. Based on these results, *Coelastrella* sp., *Chlorella vulgaris*, and *Chlorococcum* sp. were selected for further evaluation of total ammonia nitrogen tolerance in AD effluent of municipal sewage sludge under different dilution conditions. Subsequent experiments investigated the effects of light availability and CO<sub>2</sub> sparging on the growth, nitrogen removal and biomass composition of *Chlorella vulgaris* and *Chlorococcum* sp. While CO<sub>2</sub> supplementation enhanced biomass accumulation and fatty acid production, higher nitrogen removal was achieved under conditions without CO<sub>2</sub> sparging. The final stage, cultivations was scaled up in 4.5 L reactors to assess *Chlorococcum* sp. performance under different CO<sub>2</sub> concentration (0.04, 3, 6, and 9%) and bioreactor configurations. Aeration with 6% CO<sub>2</sub> yielded the highest biomass production and was selected for the comparison of the effect of reactor design (bubble column bioreactors, airlift bioreactors, and bubble column bioreactors containing carriers). Bubble column bioreactors showed superior nutrient removal efficiency, whereas airlift bioreactors supported faster cell growth.

Overall, this thesis demonstrates that native Nordic microalgae offer strong potential for the treatment of AD effluents while simultaneously utilizing CO<sub>2</sub> through biofixation. The findings provide a foundation for integrating microalgal cultivation into waste management systems, mitigating environmental impact while generating valuable biomass with potential for applications such as biofertilizers, biochar, biodiesel and bioplastics production.

**Keywords:** anaerobic digestion effluent, Nordic microalgae, nitrogen removal, light:dark cycle, CO<sub>2</sub> concentration, reactor configuration



## List of publications included in the thesis

The thesis is based on the following papers:

- I. **Mohammadkhani, G.**, Mahboubi, A., Plöhn, M., Funk, C. & Ylittervo, P. (2024) The potential of Nordic microalgae in nutrient removal from anaerobic digestion effluents. *Physiologia Plantarum*, 176(1), e14153. doi: <https://doi.org/10.1111/ppl.14153>
- II. **G. Mohammadkhani**, A. Mahboubi, M. Plöhn, C. Funk, P. Ylittervo, Total ammonia removal from anaerobic digestion effluents of municipal sewage sludge using Nordic microalgae, *Algal Research*, 84 (2024) 103802. doi: <https://doi.org/10.1016/j.algal.2024.103802>
- III. **G. Mohammadkhani**, A. Mahboubi, M. Plöhn, C. Funk, P. Ylittervo, Effect of light and CO<sub>2</sub> on nutrient removal and biochemical composition of Nordic microalgae cultivated in anaerobic digestion effluent, (2025) (Submitted)
- IV. **G. Mohammadkhani**, A. Mahboubi, C. Funk, P. Ylittervo, Cultivation of Nordic *Chlorococcum* sp. in anaerobic digestion effluent: Effects of CO<sub>2</sub> concentration and reactor configuration, (2025) (Submitted)

### **Statement of contribution**

My contribution to the above-mentioned publications are as follows:

**Paper I:** Responsible for designing and conducting all experiments, data processing and analysis, and full manuscript preparation and revision.

**Paper II:** Responsible for the design of the experiment, conducting the experimental work, majority of processing and analysis of the data \*, and writing the original manuscript draft and revision.

**Paper III:** Responsible for the design of the experiment, conducting the experimental work, majority of processing and analysis of the data \*, and writing the original manuscript draft and revision.

**Paper IV:** Responsible for designing and conducting all experiments, data processing and analysis, and full manuscript preparation and revision.

\* FTIR analysis and data processing was conducted by Martin Plöhn at Umeå University.

## List of publications not included in the thesis

- I. **Ghasem. Mohammadkhani**, Päivi Ylittervo, Carlo Esposito, Francesco Secundo, Ikumi Umetani, Lu Feng, Saumita Chakravarty, Vaishali Rani, Amir Mahboubi, Christiane Funk, Microalgae within the Circular Bioeconomy, (2025), (Review paper: Submitted)
- II. N. Perrin, **G. Mohammadkhani**, F. Homayouni Moghadam, C. Delattre, A. Zamani, Biocompatible fibers from fungal and shrimp chitosans for suture application, *Current Research in Biotechnology*, 4 (2022) 530-536
- III. E. R. Kanishka B Wijayarathna, **G. Mohammadkhani**, F. H. Moghadam, L. Berglund, J. A. Ferreira, K. H. Adolfsson, M. Hakkarainen, A. Zamani, Tunable Fungal Monofilaments from Food Waste for Textile Applications. *Global Challenges* (2024), 8, 2300098. <https://doi.org/10.1002/gch2.202300098>
- IV. E.R.K.B. Wijayarathna, **G. Mohammadkhani**, A.M. Soufiani, K.H. Adolfsson, J.A. Ferreira, M. Hakkarainen, L. Berglund, I. Heinmaa, A. Root, A. Zamani, Fungal textile alternatives from bread waste with leather-like properties, *Resources, Conservation and Recycling*, 179 (2022) 106041
- V. S.E. Svensson, M. Abdollahi, F.H. Moghadam, N.K. Kalita, M. Hakkarainen, E.R.K.B. Wijayarathna, **G. Mohammadkhani**, J.A. Ferreira, A. Zamani, Valorization of Bread Waste to Fungal-Based Products for Medical Textile and Food Applications, *ACS Sustainable Resource Management*, 1 (2024) 385-394

## **The journey**

My research journey has been anything but linear, and that is exactly what has made it unique. What follows is a condensed reflection on the experiences that shaped my academic life.

I began my studies in 2007 in my hometown, Shiraz, Iran, where I pursued a bachelor degree in Materials Engineering at Shiraz University. By the end of my bachelor studies, Biomaterials emerged as a compelling direction. Building on the diverse materials knowledge I had acquired, I was eager to explore this field in a new setting. This interest led me to the University of Tehran for an M.Sc. in Biomedical Engineering-Biomaterials, where I also met my wife, Sara, who was my classmate. The master research focused on designing and fabricating a biosensor for detecting digoxin, a heart-failure medication, using localized surface plasmon resonance, a thesis I completed in 2014.

After finishing my degree, I began preparing for the TOEFL exam with the intention of pursuing a Ph.D. abroad, following the path many of my classmates had taken to North America. I had only a limited window to secure admission before mandatory military service. Unfortunately, I was unable to complete the exam and secure a position in time, so I returned to Shiraz and entered military service. Although my service as a master graduate in Iran was not physically demanding, it offered little opportunity for productive work, and the distance from Sara made this period even more difficult. After completing my service, I returned to Tehran and began working as a biomedical engineer, first as an orthopedic arthroplasty specialist and later as an atrial fibrillation solutions product specialist for the Medtronic distributor company, where I provided clinical support and training in cath labs for some years. During this period, I also resumed preparing for the TOEFL exam with renewed determination. While the industry experience was valuable, over time the work became repetitive, and I realized that I did not want to spend my entire career doing the same work year after year. I wanted a path that offered continuous intellectual challenge and growth.

Having previously considered North America as my main destination for doctoral studies, I initially focused my Ph.D. applications on Canada. I eventually came super close to securing a Ph.D. position in Canada in a field related to biomaterials, but Sara and I felt that even Canada was still too far from home. Around this period, my friends Arman and Naeimeh suggested an alternative path: applying to the master program in Resource Recovery in Borås, Sweden, which also offered tuition-fee scholarships. Naeimeh, who was already studying there, encouraged and helped me throughout the process. I applied, was awarded the scholarship, and made the difficult decision to pursue a second master degree.

This decision marked the beginning of a new chapter. While Sweden is one of the best places to study resource recovery, the field was completely new to me, and I had a steep learning curve ahead. However, I settled into work and uncovered a new enthusiasm for biotechnology. As part of this journey, my master thesis, on wet spinning of fungal and shellfish chitosan for medical applications, was later selected as the best master thesis of the international master program in resource recovery, confirming my aptitude for a completely new field. After graduating, I continued as a research assistant with Professor Akram Zamani, my master thesis supervisor, further expanding my understanding of fungal fiber production. When a Ph.D. position in microalgae became available in our department, I felt it was the perfect next step. Although I had no prior experience with microalgal cultivation, I have always been motivated by new challenges. It was indeed a challenge, as it was the first project on microalgae at the University of Borås. This means we had to build the algal laboratory from scratch; an opportunity I was excited to help shape. The university-funded nature of the project also offered academic freedom to explore diverse research directions under the guidance of my supervisors.

I began my Ph.D. on 22<sup>nd</sup> of November 2021 and entered the fascinating world of microalgal science. As a newcomer to both microalgae cultivation and anaerobic digestion, I relied heavily on the support and guidance of my supervisors, colleagues, and collaborators, who helped me navigate this unfamiliar field. Today, I see myself as an enthusiastic four-year-old algal researcher, still curious, still learning, and eager to contribute further to this remarkable area of science. This journey is only just beginning.

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## List of abbreviations

AD	Anaerobic digestion
VFA	Volatile fatty acid
AD-VFA	Anaerobic digestion-volatile fatty acid effluents
TAN	Total ammonia nitrogen
FA	Free ammonia
CKM	Chicken manure
PPS	Pulp and paper sludge
FW	Food waste
BC	Bubble column bioreactor
ALR	Airlift bioreactor
BP	Biomass productivity
$R_c$	Carbon dioxide fixation rate
RE	Removal efficiency
FAME	Fatty acid methyl esters
SDGs	Sustainable Development Goals

## **1. Introduction**

The rapid pace of climate change and depletion of natural resources threatens global sustainability [1]. Industrialization, population growth, and short-term economic priorities often accelerate waste generation, particularly organic waste and wastewater, which exceed the capacity of existing management systems [2]. In many cases, short-term economic goals dominate long-term environmental objectives [3], leading to the improper disposal of these materials that contributes to biodiversity loss [4] and intensifies greenhouse gas emissions [5]. This challenge is aggravated by the dominant linear “take-make-dispose” model, which drives resource overuse and environmental degradation [6]. In response, strategies focused on reducing, reusing, and recycling have become essential to minimize resource input and waste output, while promoting the recovery of valuable nutrients and materials [7]. To address these systemic inefficiencies, microalgae represent a promising biotechnological platform, providing efficient pathways for nutrient recovery and sustainable biomass production. By converting waste into valuable metabolites, microalgal systems not only support biomass valorization but also mitigating climate impact through carbon dioxide biofixation and lessen dependence on fossil resources [8].

### **1.1 Organic waste generation and treatment**

Organic waste refers to biodegradable materials derived from plant or animal sources, including the organic fraction of municipal solid waste, materials from agriculture, (e.g., crop residues and manure), waste from the food industry, (e.g., processing and restaurant waste), and primary and biological sludge from wastewater treatment [9, 10]. Organic waste represents a significant global challenge, as the increasing demands of a growing population results in the daily production of tons of agricultural and food waste. For instance, the European Union generates over 58 million tonnes of food waste annually, equivalent to 130 kg per person [11]. The management of these organic waste streams requires effective treatment technologies to minimize environmental impacts and maximize resource recovery. To treat organic waste, several conventional treatment methods are being used, including composting, incineration, landfilling, and anaerobic digestion (AD). Although landfilling is simple, it is the most greenhouse gas intensive option, with emissions ranging from 400 kg CO<sub>2e</sub> to 781 kg CO<sub>2e</sub> per tonne of organic waste [12, 13]. Incineration with high energy recovery efficiency produces negligible methane emissions, since the process involves combustion. It results in relatively lower emissions than landfilling, ranging from -124 kg CO<sub>2e</sub> to 1.85 kg CO<sub>2e</sub> per tonne of

organic waste. The negative emissions are due to the energy recovery that offsets fossil fuel use [14, 15]. Composting produces a valuable bio-fertilizer and reduces landfill, but its emissions vary widely, from -106 kg CO<sub>2</sub>e to 926 kg CO<sub>2</sub>e per tonne of organic waste [12, 14, 16]. Among these approaches, AD has emerged as a promising method due to its dual benefits of waste treatment and generating renewable energy through biogas production. However, it demands major infrastructure investment, along with precise control of waste characteristics and reactor conditions. AD shows a net reduction in emissions, ranging from -36 kg CO<sub>2</sub>e to -25 kg CO<sub>2</sub>e per tonne of organic waste [12, 15, 17]. The negative emissions are due to the production of renewable natural gas and the avoidance of methane emissions from landfilling [12, 17]. Therefore, AD is recognized as an environmentally favorable method for organic waste valorization, which will be discussed in the following section.

## **1.2 Anaerobic digestion**

AD is a biological process in which microorganisms decompose organic material in the absence of oxygen, generating biogas and digestate [18]. Since 1980, this well-established process has been widely applied to treat diverse organic wastes such as food waste, agricultural residues and sewage sludge [19]. AD relies on a symbiotic microbial community that sequentially converts organic matter into biogas through four distinct steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis [20]. The primary objective of AD is to stabilize organic matter, reduce odors, pathogen concentrations, and the mass of solid organic material.

Beyond waste stabilization, AD serves as a tool for climate mitigation by providing a controlled environment to capture and utilize methane that would otherwise be released into the atmosphere during the natural, unmanaged decomposition of organic matter [21]. Furthermore, it also aims to produce renewable energy in the form of biogas, which can be used for heating, electricity, and as a vehicle fuel if upgraded [22, 23]. Biogas composition can vary depending on the feedstock, but it typically contains 35-75% methane, 25-65% carbon dioxide, 1-5% hydrogen along with minor quantities of water vapor, ammonia, and hydrogen sulfide [24]. Biogas upgrading is essential for utilizing biogas as a renewable energy source, either as a vehicle fuel or for injection into the natural gas grid. The aim of biogas upgrading is to increase the methane content by removing carbon dioxide and hydrogen sulfide [25].

Apart from biogas production, AD produces digestate, dividing into solid and liquid fraction. European countries produce 222 to 258 million tonnes of digestate annually [26]. In particular, Sweden total digestate production (wet weight) from biogas facilities reached 3.7 million tonnes in 2024 [27]. Projections for future development suggest that Sweden biogas output could

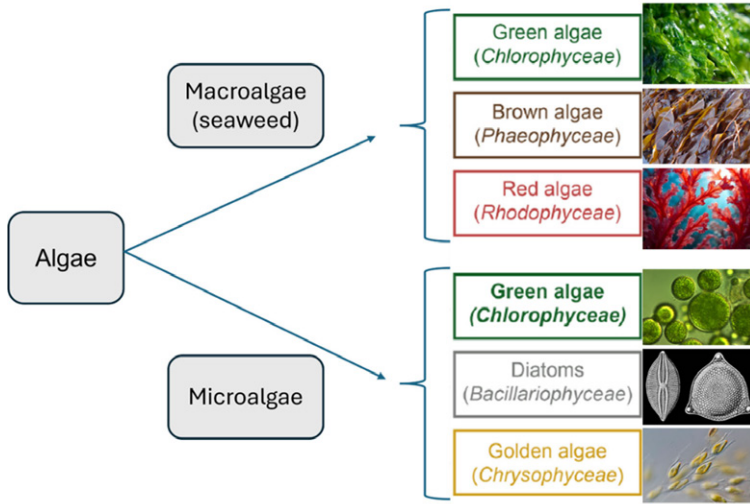
increase from approximately 2 TWh today to 7 TWh by 2030, leading to a significant rise in digestate production, exceeding 10 million tonnes per year [28]. Digestate is typically composed of 80-90% liquid fraction and 10-20% solid fraction, depending on feedstock and separation technologies [29]. Solid digestate serves as a valuable fertilizer due to its richness in nitrogen, phosphorus, potassium, and organic matter, all of which enhance soil fertility and lessen dependence on synthetic fertilizers [30]. The liquid part of digestate is also rich in nutrients, making it useful for farming. Still, whether it can be applied directly depends on the source of the waste [31]. If effluents are discharged untreated, they can cause eutrophication and spread waterborne diseases, making proper wastewater treatment necessary for protecting ecosystems and supporting sustainable water use [32, 33]. Furthermore, additional treatment is required for this effluent to comply with discharge regulations, particularly to lower nitrogen levels [34]. In Nordic countries, large wastewater treatment plants ( $\geq 10000$  population equivalents) typically operate with total nitrogen concentrations of about 10-15 mg L<sup>-1</sup> when meeting EU removal requirements [35]. However, Swedish plants use a much stricter limit of 6 mg L<sup>-1</sup> to further protect the sensitive aquatic environments of the Baltic Sea [36].

Wastewater treatment plants use several post-treatment methods to remove ammonium, which are typically grouped into physical, chemical, and biological processes. Although physical methods like ammonia stripping, ion exchange, and adsorption can effectively remove contaminants, they are expensive, require high energy, and generate secondary pollutants such as sludge and ammonia gas emissions [37, 38]. Chemical approaches, including breakpoint chlorination and struvite precipitation, are quick and simple, yet they are demanding in terms of labor and involve high expenses for chemicals such as chlorine and magnesium salts [39, 40]. Biological options such as nitrification-denitrification and anammox are regarded as environmentally friendly [41], but nitrification demands substantial aeration, denitrification depends on added carbon sources, and anammox is vulnerable to shifts in environmental conditions [42].

Another biological option is microalgae cultivation. An effective strategy for nutrient removal from AD effluent should reduce energy and chemical use while allowing nutrient recovery for other applications. Microalgae contribute to wastewater treatment by absorbing nitrogen and phosphorus [43] and removing pollutants such as heavy metals and chemical oxygen demand [44]. In addition, they produce biomass that can be utilized in various valuable ways, which will be discussed in Section 1.3.1.

### 1.3 What is microalgae

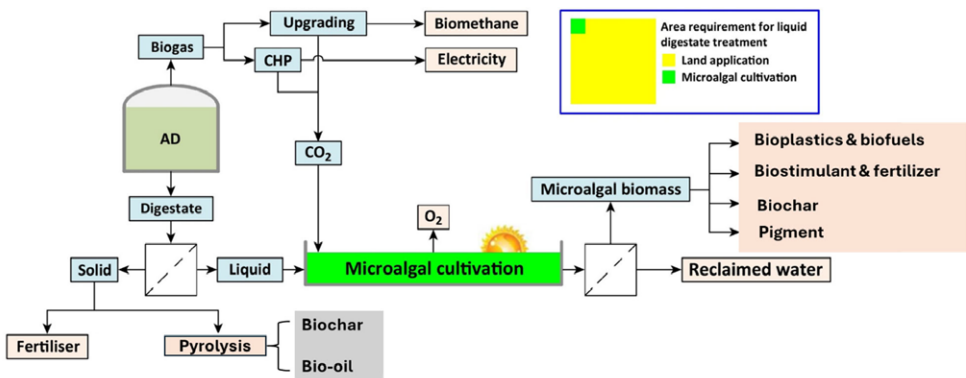
Microalgae are eukaryotic, photosynthetically active, and ubiquitous organisms (unicellular or multicellular) that thrive in water [45]. Currently, there are 182,629 species and infraspecific names exist in the database Algaebase [46]. Microalgae contribute up to 60% of the oxygen released into the atmosphere during photosynthesis [47]. Like terrestrial plants, they use sunlight and CO<sub>2</sub> in photosynthesis, but more efficient (10 times greater) with additional advantages such as consuming pollutants, having minimal resource requirements, and not competing with agriculture for precious resources [48]. However, unlike plants, they lack roots, stems, or leaves; instead, each individual cell is photosynthetic and capable of absorbing nutrients directly. This cellular simplicity allows microalgae to thrive in diverse environments, tolerating wide ranges of temperature, salinity, pH, and light intensity [49]. While microalgae are microscopic organisms that typically range from 0.5 to 30 µm in size [50], macroalgae (seaweeds) can be up to 70 m long [48]. Algae can be classified due to their pigment content, including green algae (*Chlorophyceae*), brown algae (*Phaeophyceae*), red algae (*Rhodophyceae*), diatoms (*Bacillariophyceae*) and golden algae (*Chrysophyceae*) (Figure 1). Cyanobacteria (blue green algae) are prokaryotic photosynthetic organisms. Therefore, many authors exclude cyanobacteria from algal groups as modern taxonomy recognizes cyanobacteria as bacteria [51]. In this thesis, the term microalgae specifically refers to green Nordic microalgae, with the species identified in the experimental work section. Nordic microalgal strains are originally from the Nordic region. These algae have a unique adaptation to the sub-arctic climate, which is defined by very cold, long, and dark winters (with an average temperature of -4 °C) and short, cool, and bright summers (with an average temperature of 16 °C). A collection of green microalgae strains from this region has already been established and documented. These strains exhibit high tolerance to low temperatures, limited light conditions, pharmaceuticals, heavy metals, and other compounds typically found in municipal and industrial wastewater [52].



**Figure 1.** Algae classification according to their pigments. (Adapted from [53, 54], with Permission from Taylor & Francis).

### 1.3.1 Waste-to-resource conversion with microalgae

Due to their rapid growth, efficient CO<sub>2</sub> sequestration, and ability to thrive in non-arable land, microalgae have attracted considerable attention for diverse biotechnological applications. In particular, microalgae offer efficient nutrient recovery and wastewater remediation [55]. When it comes to valorization of AD liquid and gas effluents, microalgae are not only employed for bioremediation (e.g., removal of heavy metals and pharmaceutical residues) but also contribute to managing two major waste streams from AD treatment, including nitrogen and phosphorus recovery from AD effluents, and CO<sub>2</sub> assimilation via biomass accumulation (Figure 2).



**Figure 2.** Integrated system for anaerobic digestion and microalgal cultivation. (Adapted from [56], with Permission from Elsevier)

Moreover, microalgae convert 9-10% solar energy into biomass (about 280 ton per ha per year) [50], which can be utilized for various valuable applications depending on the cultivation source and biomass composition. For instance, if the cultivation medium is AD effluent, safety and regulatory measures consider the resulting biomass unsuitable for food, feed, pharmaceutical, or cosmetic applications. Under such conditions, however, microalgal biomass can still be valorized into biofertilizers, biostimulants [57, 58] biochar [59], bioplastics [60], and biofuels [47].

Biofertilizers enhance the consumption of essential nutrients (N, P, K) and micronutrients while introducing bioactive compounds [61, 62]. Application typically occurs via wet cell suspensions or dry biomass. In wet applications, studies have shown ammonium nitrogen increases of up to 4.37 times in melon cultivation [63]. Moreover, dry biomass applications in lettuce and Hawthorn orchards have demonstrated growth increases of up to 121% and yield improvements of 29.6%, respectively, while simultaneously promoting soil carbon and nitrogen sequestration [64]. Regarding biochar, microalgal biomass composition, mainly their high lipid and protein levels, dictates the final quality and yield of the biochar [65], setting it apart from traditional wood-based (lignocellulosic) sources. Furthermore, alkaline pH and high nitrogen and ash content of algal biochar make it particularly effective as a slow-release nitrogen fertilizer for soil applications, unlike synthetic fertilizers that tend to wash away quickly. When it comes to biofuel, due to the environmental and economic concerns associated with first and second generation biofuels (derived from food crops and lignocellulosic biomass, respectively), research has shifted toward third generation like fuels derived from microalgae. For instance, biodiesel as a primary product of this feedstock can be synthesized from cellular lipids [66] through extraction and transesterification with energy contents ranging from 39 to 41 kJ/g [67]. In addition, production of bioplastic from microalgae offer significant benefits over land-based feedstocks, including superior productivity, year-round cultivation, and no competition with food production for land or water [68]. Starch-rich species like *Chlorella vulgaris* have shown potential for food packaging, and the absence of lignin in species such as *Nannochloropsis oceanica* eliminates energy-intensive pretreatment steps [69]. Furthermore, autotrophic microalgae can assimilate inorganic carbon that provides an economic advantage over heterotrophic bacteria by reducing carbon source expenses, which can account for up to 50% of the total production costs for polyhydroxyalkanoate plastics [70].

It is important to note that despite the potential of microalgae to reduce dependency on non-renewable resources, several obstacles still limit their competitiveness against fossil-based and

synthetic alternatives. High production costs, low culture stability, inhibition, energy-intensive harvesting processes, expensive pretreatments of the algal biomass caused by the rigid cell wall, and uncertain regulations remain significant challenges that must be addressed to fully realize their potential in sustainable bioeconomic development.

### **1.3.2 Factors affecting microalgal growth**

Microalgal growth is influenced by a wide range of biotic and abiotic factors. Abiotic factors include light, nutrients, pH, carbon dioxide [71], and reactor designs [72] while biotic factors includes initial cell density and pathogenic organisms [73]. The following sections highlight some of the key factors affecting microalgal cultivation.

#### ***1.3.2.1 Light***

Light is the essential energy source for microalgae, which are photosynthetic organisms. The conversion of energy begins in the thylakoid membranes of the chloroplasts, the sites where the light-dependent reactions of photosynthesis take place, similar to higher plants. These lipid-based membranes contain pigment-protein complexes that initiate the electron transport chain [74]. Microalgal pigments (chlorophylls and carotenoids) capture light energy, driving reactions that produce oxygen and generate high-energy molecules ATP and NADPH. More specifically, chlorophyll-a drives photosynthesis by absorbing blue and red light, while carotenoids act as accessory pigments that either broaden the usable light spectrum or protect the cells by converting excess energy into heat [75]. Ultimately, the captured light energy is converted into chemical energy, which is subsequently used during the light-independent reaction, known as the Calvin-Benson cycle. During the Calvin-Benson cycle, CO<sub>2</sub> is fixed into sugars and other carbohydrate compounds that form the basis of biomass [76].

Light quantity and quality strongly influence microalgal growth and play a key role in regulating metabolic processes [77]. The microalgal growth rate increases linearly at low light levels until a saturation threshold is reached. Above this light saturation threshold, excessive light inhibits growth and can cause irreversible damage to the photosynthetic machinery [78]. That is because the optimum light conditions varies among different microalgal species.

Microalgae shows significant metabolic flexibility, growing through three primary pathways depending on how they obtain energy and carbon. Autotrophic microalgae use light and inorganic carbon, mainly CO<sub>2</sub>, to produce energy through photosynthesis. Heterotrophic microalgae rely on organic carbon sources such as glucose, acetate, or glycerol and can grow in darkness. Mixotrophic microalgae combine both strategies, using CO<sub>2</sub> photosynthetically

while also consuming organic carbon, resulting in even higher growth rates and biomass productivities than heterotrophic cultivation [79, 80]. This metabolic versatility is most evident during light:dark transitions. In the absence of light, microalgae cease photosynthesis and shift toward a heterotrophic mode of metabolism. Lacking the light-driven production of ATP and NADPH, cells shift their energy generation from chloroplasts to mitochondrial respiration to sustain basic cellular functions. During this transition, cells catabolize internal carbon reserves, such as starch and lipids, or utilize available external organic substrates. During this phase, pigment synthesis diminishes, chlorophyll gradually degrades, and growth rates decline significantly unless supported by an external organic carbon source for continued biomass production [81].

### ***1.3.2.2 Nutrients***

There are different micro and macronutrients required for microalgae to grow. Regarding micronutrients, key elements include Mg, S, Na, Cl, Ca, Fe, Mo, Mn, Zn, Cu, B, and Co. These micronutrients are commonly available in wastewater and seawater [71]. The most important macronutrients are nitrogen, phosphorus and carbon.

Nitrogen is an essential nutrient for microalgae, required for the synthesis of proteins, nucleic acids, and pigments such as chlorophyll [82], and it typically represents about 1% to 14% of the dry biomass [71]. Microalgae can utilize different inorganic nitrogen sources, including  $\text{NO}_3^-$ ,  $\text{NO}_2^-$  and  $\text{NH}_4^+$ , which are subsequently converted into organic compounds (e.g., proteins and amino acids). Of these sources,  $\text{NH}_4^+$  is the most preferred [83] because it can be directly incorporated into amino acids through the GS-GOGAT cycle, a pathway that uses the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT). However,  $\text{NO}_3^-$  must first be reduced to  $\text{NO}_2^-$  by nitrate reductase, and then  $\text{NO}_2^-$  must be converted to  $\text{NH}_4^+$  by nitrite reductase before it can enter the GS-GOGAT pathway [84]. Although  $\text{NH}_4^+$  is the most preferred nitrogen form, high concentrations of ammonium nitrogen can inhibit microalgal growth or even lead to cell death [85-87].

In aqueous environments, there is an equilibrium between  $\text{NH}_4^+$  and  $\text{NH}_3$  (free ammonia (FA)). The equilibrium constant is a function of pH and temperature, which means  $\text{NH}_4^+$  and  $\text{NH}_3$  can be present simultaneously in cultivation systems. The sum of ammonium nitrogen and ammonia nitrogen is referred to as total ammonia nitrogen (TAN). Several studies have shown that FA is the main inhibitor of microalgal growth [83, 86, 88]. However, as noted previously, other findings suggest that ammonium ions themselves can also be inhibitory [85-87]. Therefore, it is crucial to evaluate the tolerance of different microalgal strains to varying concentrations of

ammonium and TAN in order to effectively treat ammonia- and ammonium-rich effluents like AD effluent.

In addition to nitrogen, phosphorus is crucial for microalgal growth, representing 0.05 to 3.3% of the dry biomass [71]. It plays key roles in biological processes and is a fundamental component of molecules such as ATP, DNA, RNA, and membrane phospholipids. Phosphorus is found in different forms such as orthophosphate, polyphosphate and pyrophosphate. Microalgae actively absorb phosphorus mainly as orthophosphate ( $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ ), which is incorporated into organic molecules through phosphorylation, an energy-dependent process. Under phosphorus limitation, growth is restricted but lipid accumulation often increases [89]. Conversely, when phosphorus is abundant, microalgae can perform luxury uptake, storing excess phosphate in polyphosphate bodies [90].

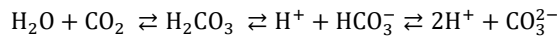
Another important growth limiting nutrient is carbon, which accounts for around 50% of the microalgal biomass [71]. Carbon reserves are vital for microalgae to support energy-intensive processes like cell division and DNA replication [91]. Carbohydrates typically account for 20 to 50% of cell weight [92, 93], while carbon is also stored as lipids, where polar lipids forming membranes and neutral lipids serving as energy reserves. Oleaginous microalgae can accumulate 20 to 40% of their biomass as lipids [94].

#### ***1.3.2.3 Carbon dioxide***

Around 1.8 to 2 kg  $\text{CO}_2$  is needed to produce 1 kg microalgal biomass [95]. Since ambient air contains only about 0.04%  $\text{CO}_2$  and  $\text{CO}_2$  diffusion is much more limited in liquid compared to air, additional carbon supplementation is necessary to sustain optimal microalgal growth. Carbon can be supplied either in the form of bicarbonate or by sparging of  $\text{CO}_2$ -rich air in the culture.  $\text{CO}_2$  concentration directly affects microalgal growth and biomass. However, excessive  $\text{CO}_2$  can lower pH, hindering photosynthesis and growth, underscoring the need for optimal  $\text{CO}_2$  levels [96], which vary by strain since each microalgal species has distinct tolerance and performance thresholds. Therefore, it is interesting to evaluate the effect of  $\text{CO}_2$  levels on microalgal growth. One opportunity for supplying  $\text{CO}_2$  is to capture it from various industrial sources, including flue gas from power plants [97],  $\text{CO}_2$  emissions from cement kilns [98] and  $\text{CO}_2$  stream from biogas plants [99]. Redirecting this stream to microalgal cultivation offers a two-fold benefit: it reduces greenhouse gas emissions while simultaneously producing valuable algal biomass.

#### **1.3.2.4 pH**

The pH of the culture medium is influenced by factors including its composition, buffering capacity, dissolved CO<sub>2</sub> levels, temperature, and cellular metabolic activity [71]. While the optimal range for growth of most microalgae is pH 7 to 9, there are species that can grow at very low or very high pH [100]. When the culture medium is too acidic (lower than the optimal pH), microalgae experience interference with nutrient absorption and disruption of essential cellular functions. Conversely, excessive alkalinity (higher than optimal pH) impairs the cells ability to utilize carbon dioxide and severely limits the rate of cell division [101]. Microalgae thrive in the aquatic environment, where CO<sub>2</sub> reacts with water to form carbonic acid (H<sub>2</sub>CO<sub>3</sub>), which then dissociates into bicarbonate (HCO<sub>3</sub><sup>-</sup>) and carbonate (CO<sub>3</sub><sup>2-</sup>) through the following equilibrium reactions [102].



Therefore, there will be three forms of dissolved inorganic carbon CO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup> with different proportions. The pH of the aqueous solution governs these reactions. When pH rises, equilibrium shifts toward HCO<sub>3</sub><sup>-</sup> and CO<sub>3</sub><sup>2-</sup>. HCO<sub>3</sub><sup>-</sup> is dominant when pH is between 6.3 to 10. When CO<sub>2</sub> is assimilated, it releases hydroxide ions (OH<sup>-</sup>), leading to an increase in medium pH. Consequently, the equilibrium shifts towards CO<sub>3</sub><sup>2-</sup> which is not preferred CO<sub>2</sub> source for microalgae [103].

Moreover, the pH can be affected by the nutritional composition of the media. For instance, consumption of NO<sub>3</sub><sup>-</sup> releases OH<sup>-</sup> ions and increases the pH, while ammonium assimilation releases H<sup>+</sup> and reduces the pH [104]. On the other hand, a high pH shifts the ammonium-ammonia equilibrium towards ammonia, which is volatile and can be stripped from the system [105]. As a result, the nitrogen source will be reduced for microalgae. For large-scale cultivation, the pH can be regulated by CO<sub>2</sub> aeration instead of using chemical buffers due to high cost of buffer utilization.

#### **1.3.2.5 Bioreactor designs**

To improve CO<sub>2</sub> delivery and utilisation efficiency, several methods have been reported. The availability of CO<sub>2</sub> for the cells can be restricted by short residence times of CO<sub>2</sub> bubbles and insufficient mixing. Gas transfer is a crucial aspect of photobioreactor design. When CO<sub>2</sub> is introduced into the system as bubbles, the efficiency of the entire process depends on the dynamics of those bubbles. Their behavior such as how they grow, break apart, join together, and rise directly affects the uniform distribution of gas throughout the liquid and the overall

transfer efficiency of the CO<sub>2</sub> to the microalgae, which eventually influence microalgal biomass productivity [106]. The mass transfer of CO<sub>2</sub> is affected by parameters like sparger design and characteristics, reactor geometry, and operational factors that can increase CO<sub>2</sub> retention time in the liquid phase and enhance mass transfer coefficients [107]. Therefore, bioreactor design is crucial for overcoming gas exchange limitations.

The Airlift bioreactor (ALR), with its centric-tube column, generates a regular cyclic flow pattern that promotes effective mixing and uniform light distribution. This mechanism significantly enhances light conversion efficiency and ultimately leads to higher microalgal growth rates [108, 109]. In contrast, the bubble column bioreactor (BC) is characterized by a more random fluid motion that creates low-frequency circular loops [108]. Low-frequency loop refers to a large-scale, slow, and random liquid recirculation patterns created by rising bubbles. This chaotic movement results in mixing that is generally less efficient. Consequently, the BC design is expected to result in suboptimal availability of both nutrients and light, ultimately leading to lower overall photosynthetic productivity [109]. However, adding carriers into BC may improve their performance by offering surfaces for microalgae attachment, subsequently enhancing nutrient uptake and biomass yield [110]. Furthermore, carriers improve the transfer of gas and liquid, increasing CO<sub>2</sub> utilization and fixation rates, which further boosts microalgal growth [110]. They can also extend the CO<sub>2</sub> residence time in the liquid phase, contributing to better CO<sub>2</sub> removal efficiency [111]. Despite these advantages, the presence of carriers may limit the light penetration due to shading effects. Serrano-Blanco, et al. [112] showed that this trade-off is beneficial. When cultivating *Tetrademus obliquus* in primary municipal wastewater using a suspended-solid photobioreactor with a 12.5% fill ratio of Kaldnes K1 carriers for 9 days, they achieved a cell productivity 2.8 times higher than the control. Although only 3% of cells were attached to the carriers, it facilitated the development of a diverse microalgae-bacteria biofilm and supported high nutrient removal efficiencies compared to the control condition. Importantly, the potential for shading did not negatively impact kinetic parameters, as specific growth rates remained comparable to the control [112].

In addition to above-mentioned designs, closed photobioreactor systems also include horizontal tubular, flat-panel, and plastic-bag photobioreactors. Horizontal tubular photobioreactors are designed with a large surface area per unit volume to maximize sunlight exposure, but they often suffer from oxygen accumulation and the need for high-velocity mechanical pumping to prevent cell settling. In contrast, as mentioned previously, the vertical ALR manages these issues more effectively by using internal draft tube to create organized flow that minimize

oxygen buildup and shear stress while achieving higher cell densities. However, ALR complex flow patterns make them challenging to use for scaling up. Moreover, while vertical flat panels offer superior photosynthetic efficiency and irradiated surface areas, they are limited by difficulties in temperature control and high hydrodynamic stress. Plastic-bag systems represent a low-cost, scalable option, but they are frequently hindered by photo-inhibition and poor mixing [113].

Hence, it would be interesting to investigate the effect of reactor designs on microalgal growth and nutrient removal efficiency.

#### **1.4 Aim of the thesis**

AD is a widely adopted method for stabilizing organic waste, but its operation generates two problematic resource streams, including highly polluting liquid effluents, rich in ammonium and organic contaminants, as well as CO<sub>2</sub> emissions produced when biogas is upgraded. Therefore, the primary aim of this thesis was to investigate the efficacy of using microalgae as a sustainable solution to this challenge, focusing on the efficient recovery of nutrients and fixation of CO<sub>2</sub> in liquid and gaseous waste streams, which simultaneously generates valuable microalgal biomass. This thesis has been conducted in several stages:

In **Paper 1**, five different Nordic microalgal strains were examined for their ability to grow and nutrient removal from industrial AD effluents obtained from chicken manure, pulp and paper sludge and food waste.

In **Paper 2**, three Nordic microalgal strains were chosen, based on the findings of paper I, to investigate total ammonia nitrogen tolerance when they were cultivated in AD effluent of municipal sewage sludge using different dilution conditions.

In **Paper 3**, two Nordic microalgal strains were selected, based on the findings of paper II, to evaluate the effect of continuous light and CO<sub>2</sub> availability on microalgal growth and biomass composition of microalgal biomass.

**Paper 4** aimed to scale up cultivations to 4.5 L reactors to investigate the impact of different CO<sub>2</sub> concentrations and bioreactor configurations on microalgal growth, NH<sub>4</sub><sup>+</sup> and P removal efficiency and biochemical composition of microalgal biomass.

## 2. Experimental work

### 2.1 Waste streams collection and pretreatment

Anaerobic digestion-volatile fatty acid effluents (AD-VFA) from pulp and paper sludge (PPS) [114], chicken manure (CKM) [115] and food waste (FW) [116] were kindly provided by fellow PhD students. The substrates were collected from Östrand pulp mill (Östrand, Sweden), the egg-laying farm Sjömarkens Hönsgård AB (Borås, Sweden) and solid waste treatment company Renova AB (Gothenburg, Sweden), respectively. The effluents originated from AD processes that were terminated prior to methanogenesis to maximize volatile fatty acid (VFA) accumulation, followed by separation using an immersed membrane bioreactor. The VFA-rich effluents were used as culture media in **Paper 1**. AD effluent from municipal sewage sludge was used as the culture medium in **Papers 2-4** and collected from Borås Energy and Environment (Borås, Sweden). In **Paper 2**, the effluent was diluted 2 (X2), 3 (X3), 5 (X5), 7 (X7), and 10 (X10) times; X5 and X7 was used in **Paper 3**; and X5 was used in **Paper 4**.

All effluents used as culture media were filtered through 10  $\mu\text{m}$  pore-size filter paper and subsequently autoclaved. In **Paper 1**, the pH was adjusted to 7.1, whereas in **Papers 2-4** the effluents were used without pH adjustment. The control medium was BG11 at 7.1 [117].

### 2.2 Microalgae cultivation

Five indigenous Nordic microalgal strains used in this thesis were kindly provided by the University of Umeå (Table 1). These strains were isolated in Sweden from either fresh water or municipal wastewater [52]. Precultures were prepared by growing microalgal strains in BG11 under sterile conditions in Erlenmeyer flasks (250 or 500 mL; 30% working volume) on an open-air shaker at 120 rpm, 25 °C, with continuous light at 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for five days. These precultures were then used to inoculate the respective experimental media to an optical density of 0.1 (OD<sub>680</sub> in **Paper 1** and OD<sub>750</sub> in **Papers 2-4**). The final working volumes and light intensities were: 35 mL and 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (**Paper 1**); 100 mL and 80  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (**Paper 2**); 650 mL and 50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (**Paper 3**); 3.5 L and 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (**Paper 4**). Cultivations in **Paper 1** were performed in triplicate, whereas those in **Papers 2-4** were conducted in duplicate.

**Table 1.** Nordic microalgae used in this thesis.

Family	Strain name and ID	Isolation source	Usage in Papers
Scenedesmaceae	<i>Scotiellopsis reticulata</i> (UFA-2)	Dåva (MWW) <sup>a</sup>	1
Scenedesmaceae	<i>Desmodesmus</i> sp. (2-6)	Skåne - Lake Ringsjön (FW) <sup>b</sup>	1
Scenedesmaceae	<i>Coelastrella</i> sp. (3-4)	Umeå (MWW) <sup>a</sup>	1 & 2
Chlorellaceae	<i>Chlorella vulgaris</i> (13-1)	Umeå (MWW) <sup>a</sup>	1 – 3
Chlorococccaceae	<i>Chlorococcum</i> sp. (MC-1)	Bäckhammar (FW) <sup>b</sup>	1 – 4

<sup>a</sup> Municipal wastewater<sup>b</sup> Fresh water

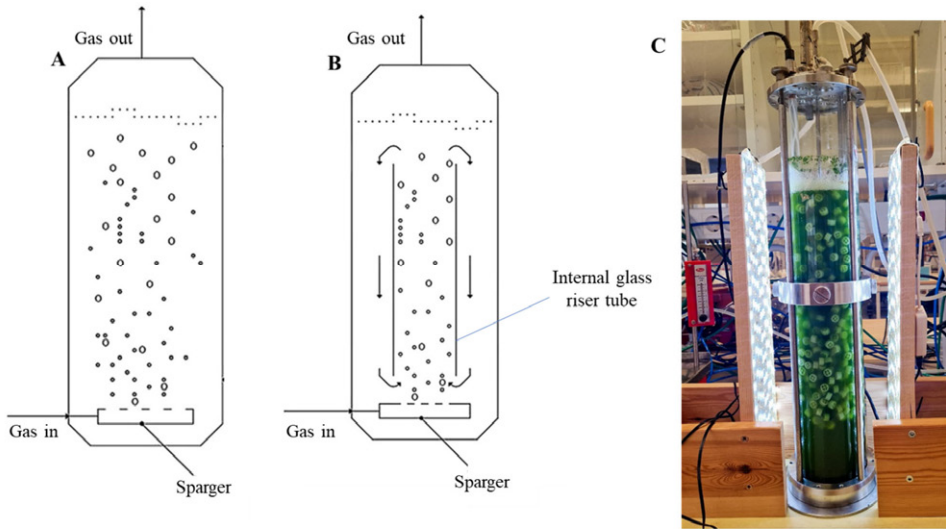
Microalgal cultures were continuously aerated with either air or air enriched with CO<sub>2</sub> in **Paper 3** (300 mL min<sup>-1</sup>) and **Paper 4** (500 mL min<sup>-1</sup>). The gas mixtures were filtered through 0.3 µm Whatman HEPA-VENT filters prior to entering the cultivation media. The cultivation regimes evaluated in **Paper 3** is presented in Table 2.

**Table 2.** Microalgal cultivation regimes (photoperiod and CO<sub>2</sub> sparging). Adapted from **Paper 3**.

Cultivation name	Light (L)-Dark (D) regime	Aeration
24L-CO <sub>2</sub>	24 h Light:0 h Dark	Air mixed with 3% CO <sub>2</sub>
24L-Air	24 h Light:0 h Dark	Air
12L-CO <sub>2</sub> /12D-Air	12 h Light:12 h Dark	Air mixed with 3% CO <sub>2</sub> <sup>a</sup> Air <sup>b</sup>
12L-Air/12D-Air	12 h Light:12 h Dark	Air

<sup>a</sup> Air-CO<sub>2</sub> mixture was provided during the light period<sup>b</sup> Air was provided during the dark period

In **Paper 4**, the study was performed in two stages: first, the effects of different CO<sub>2</sub> concentrations (0.04, 3, 6 and 9% v/v) on microalgal growth was evaluated in BC. Second, reactor configurations were compared using the optimal CO<sub>2</sub> concentration (6%). Three setups were tested: a standard BC, an ALR with a centered glass draft tube (40 cm height, 6 cm width), and a BC with carriers (Figure 3).



**Figure 3.** Schematic representation of the bubble column bioreactor (A) and airlift bioreactor (B), and bubble column bioreactor with carriers (C). Pictures A and B were adapted from [118], with Permission from Elsevier.

### 2.3 Culture growth monitoring

Culture growth was monitored daily or every other day by collecting 1 mL samples from each culture. Number of cells ( $\text{cells mL}^{-1}$ ) were determined with a CellDrop FL (Denovix, USA) [119] and pH values were recorded as well. Optical densities were measured at 530, 680, and 750 nm (Thermo Scientific™ GENESYS 140 Visible Spectrophotometer, Sweden). Optical densities were measured directly when values were  $\leq 1$  and diluted when higher. The wavelengths 530, 680, and 750 nm were selected based on their specific absorption properties in microalgae. Absorption at 680 nm corresponds with the peak of chlorophyll-a [75], reflecting photosynthetic pigment content and cellular activity, and thus serving as a reliable indicator of microalgal growth. In contrast, absorption at 750 nm lies outside the chlorophyll absorption range and is commonly used as a turbidity reference, correlating more directly with total biomass (cell number or dry weight) since pigment interference is minimized [120]. Comparing OD680 and OD750 over time provides complementary information: a proportional increase in both values indicates true algal biomass accumulation, whereas a disproportionate rise in OD750 without a corresponding increase at OD680 suggests contamination or non-chlorophyll particles contributing to turbidity. The 530 nm wavelength is associated with pigments such as carotenoids [75] present in the microalgal biomass.

At the end of the cultivation period, biomass was harvested by centrifugation (5 min at  $3000 \times g$ ) and washed with Milli-Q water. Dry weight was determined gravimetrically [121],

with biomass oven-dried at 105 °C for 24 h in **Paper 1** and freeze-dried in **Papers 2-4**. In **Paper 4**, daily 10 mL aliquots were additionally collected for biomass determination alongside routine sampling.

## 2.4 Cultivation media characterization

The concentration of NH<sub>4</sub>-N and total P was quantified using Nanocolor® test kits (Ammonium 100 ortho- and total-Phosphate 15; MACHEREY-NAGEL, Düren, Germany). All analyses were carried out with a Nanocolor 500D photometer (MACHEREY-NAGEL GmbH & Co. KG, Germany), which was used to determine the concentrations according to the manufacturer protocols. The concentration of ammonia nitrogen expressed as NH<sub>3</sub>-N was calculated using Eq. 1, which relates NH<sub>4</sub>-N to NH<sub>3</sub>-N through the temperature-dependent dissociation constant pKa, which is equal to 0.09018 +  $\left(\frac{2729.92}{T(K)}\right)$ , where T(K) is the temperature in Kelvin.

$$\text{NH}_3 - \text{N} = \frac{\text{NH}_4\text{-N} * 10^{-\text{pKa}}}{10^{-\text{pH}}} \quad (\text{Eq. 1})$$

TAN was defined as the sum of ammonium and ammonia nitrogen (TAN = NH<sub>4</sub>-N + NH<sub>3</sub>-N). Free ammonia (FA), expressed as NH<sub>3</sub>, was then obtained from TAN using the equilibrium relationship shown in Eq. 2 [86].

$$\text{FA} = \frac{\text{TAN}}{1 + \left(\frac{10^{-\text{pH}}}{10^{-\text{pKa}}}\right)} \times \frac{17}{14} \quad (\text{Eq. 2})$$

VFA concentrations were quantified using a gas chromatograph (Clarus 550, Perkin-Elmer, Norwalk, CT, USA) equipped with an Elite-WAX ETR capillary column (30 m × 0.32 mm × 1.00 μm) and a flame ionization detector (FID). The injector and detector temperatures were set to 250 °C and 300 °C, respectively, while the oven temperature was maintained at 200 °C. Nitrogen served as the carrier gas at a flow rate of 2 mL min<sup>-1</sup> and a pressure of 20 psi. For sample preparation, 200 μL of an acid mixture, comprising 25% (v/v) formic acid and 25% (v/v) ortho-phosphoric acid in a 1:3 ratio, was added to 1.0 mL of sample, followed by centrifugation at 10,000 × g for 5 min. The resulting supernatant was filtered through a 0.22 μm syringe filter (ABLUO, GVS, USA) to remove particulates. A final mixture containing 250 μL of the filtrate, 250 μL of butanol (1 g L<sup>-1</sup>) as the internal standard, and 500 μL of Milli-Q water was prepared for GC analysis [122].

## 2.5 Kinetic parameters

Biomass productivity (BP, g<sub>dw</sub> L<sup>-1</sup> d<sup>-1</sup>) was calculated from the change in biomass concentration over the cultivation period using Eq. 3.

$$P = \frac{X_1 - X_0}{t_1 - t_0} \text{ (Eq. 3),}$$

where  $X_0$  and  $X_1$  represent the biomass concentrations at the start and end of the experiment.

In this thesis,  $t_0$  is zero and  $t_1$  is 15 (**Paper 3**) or 12 (**Paper 2**).

The carbon dioxide fixation rate ( $R_C$ ,  $\text{g}_{\text{CO}_2} \text{ L}^{-1} \text{ d}^{-1}$ ) was calculated from the carbon fraction of the biomass ( $C$ ), obtained by elemental analyzer, together with biomass productivity (BP) [123], as shown in Eq. (4):

$$R_C = C_C \times \text{BP} \times \left( \frac{\text{MCO}_2}{\text{MC}} \right) \text{ (Eq. 4)}$$

where  $\text{MCO}_2$  and  $\text{MC}$  are the molar masses of  $\text{CO}_2$  and elemental carbon (44 and 12  $\text{g mol}^{-1}$ ), respectively [123, 124].

TAN removal efficiency (RE, %) was determined according to Eq. 5.

$$RE (\%) = \frac{C_1 - C_2}{C_1} \times 100 \text{ (Eq. 5),}$$

where  $C_1$  is the initial TAN concentration ( $\text{mg L}^{-1}$ ) and  $C_2$  is the final TAN concentration at the end of cultivation.

## 2.6 Biomass characterization

### 2.6.1 Protein content and C:N ratio in algal biomass

Protein content in the microalgal biomass was determined by first measuring the total nitrogen content and then converting it to protein using a conversion factor of 4.78 [125], as applied in **Papers 3 and 4**. Total nitrogen was quantified using an Element FlashSmart CNHS (Thermo Fisher Scientific, USA), where nitrogen was detected via a thermal conductivity detector. For each measurement, 3 to 4 mg of freeze-dried biomass was weighed into a tin capsule and loaded into the autosampler. The combustion reactor operated at 950 °C, while the GC oven was maintained at 65 °C. Helium was used as the carrier gas at 140  $\text{mL min}^{-1}$ , and pure oxygen served as the combustion gas at 250  $\text{mL min}^{-1}$ . In addition to protein determination, biomass C:N ratios were calculated from the elemental analyzer data.

### 2.6.2 Carbohydrate content

Total carbohydrate content in the microalgal biomass was quantified following the detailed protocol described by Chen, et al. [126], as applied in **Papers 3 and 4**. Sample absorbance, measured at 490 nm, was compared against a glucose calibration curve prepared from known glucose standards.

### 2.6.3 Fatty acid profiles

Fatty acid compositions was determined using a direct transesterification procedure following Cavonius, et al. [127], as described in **Papers 3 and 4**. The resulting fatty acid methyl esters (FAME) were analyzed by gas chromatography using a GC-FID system (Perkin Elmer, Clarus 590) equipped with a DB-23 capillary column (Agilent, USA). Nitrogen served as the carrier gas at a flow rate of  $0.64 \text{ mL min}^{-1}$ , and the instrument operated under a constant pressure of  $94.45 \text{ kPa}$ . The oven program began at  $50 \text{ }^\circ\text{C}$  for 2 min, followed by a temperature increase of  $12 \text{ }^\circ\text{C min}^{-1}$  to  $175 \text{ }^\circ\text{C}$ . The temperature was then raised at  $2 \text{ }^\circ\text{C min}^{-1}$  until reaching  $230 \text{ }^\circ\text{C}$ , where it was held for 11 min.

## 3. Results and discussion

### 3.1 Microalgae growth and ammonium removal in diverse waste streams

Results from **Paper 1** is presented and discussed in this section. Five Nordic microalgal strains (Table 3) [52] were grown on AD-VFA effluents derived from pulp and paper sludge (PPS), chicken manure (CKM), or food waste (FW) to assess their growth performance as well as ammonium nitrogen and VFA removal efficiency. The pH values of the received effluents were 8.0, 8.2 and 5.5, respectively. All media were subsequently adjusted to pH 7.1 prior to inoculation.

**Table 3.** Amount of volatile fatty acids and ammonium in anaerobic digestion effluent of pulp and paper sludge (PPS), chicken manure (CKM) and food waste (FW), as received.

Parameter Measured <sup>a</sup>	Medium		
	PPS	CKM	FW
Acetic acid ( $\text{g L}^{-1}$ )	$0.74 \pm 0.02$	$0.24 \pm 0.00$	$1.09 \pm 0.26$
Propionic acid ( $\text{g L}^{-1}$ )	$0.11 \pm 0.00$	$0.02 \pm 0.00$	$0.16 \pm 0.04$
Isobutyric acid ( $\text{g L}^{-1}$ )	$0.02 \pm 0.00$	0.00	$0.02 \pm 0.01$
Butiric acid ( $\text{g L}^{-1}$ )	0.00	0.00	$0.98 \pm 0.21$
Iso Valeric acid ( $\text{g L}^{-1}$ )	$0.08 \pm 0.00$	$0.03 \pm 0.00$	$0.07 \pm 0.02$
Valeric acid ( $\text{g L}^{-1}$ )	0.00	0.00	$0.13 \pm 0.03$
Caproic acid ( $\text{g L}^{-1}$ )	0.00	0.00	$0.70 \pm 0.08$
Total VFAs ( $\text{g L}^{-1}$ )	$0.94 \pm 0.02$	$0.30 \pm 0.00$	$3.14 \pm 0.65$
$\text{NH}_4\text{-N}$ ( $\text{mg L}^{-1}$ )	$217.28 \pm 7.87$	$417.85 \pm 7.87$	$156.00 \pm 15.75$

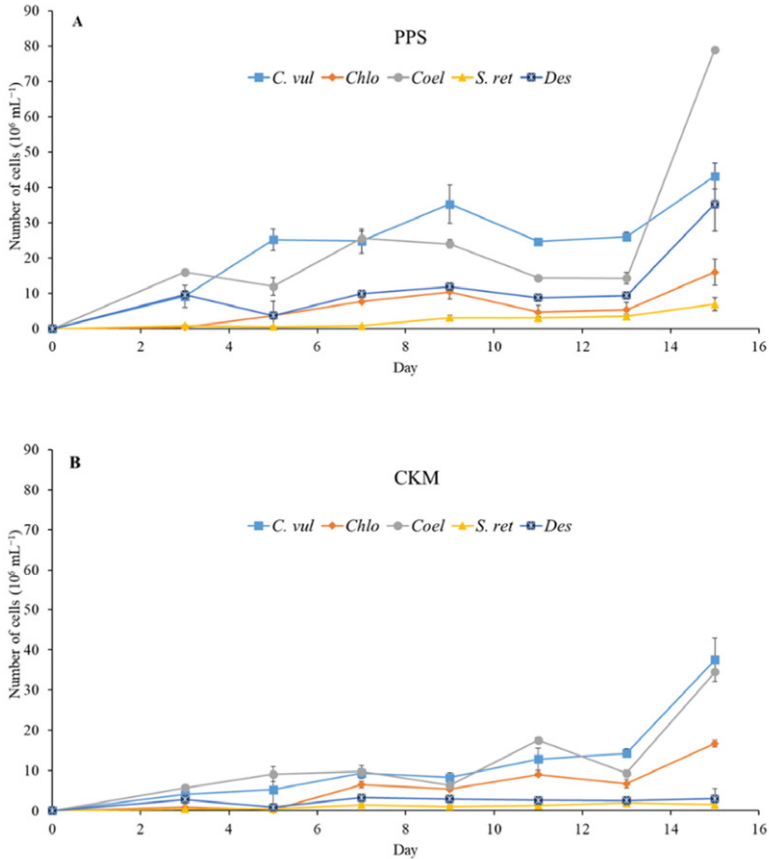
<sup>a</sup> Concentration of VFAs and  $\text{NH}_4\text{-N}$  represent the initial content of the pure media before the addition of the algal pre-culture. The corresponding table in **Paper 1** shows the initial contents after adding pre-culture, and as *Scotiellopsis reticulata* (*S. ret*) required larger inoculum volume to standardize the starting optical density, resulting in lower initial VFA values contributed by pre-culture compared to the other strains in that table.

None of the strains were able to grow in AD-VFA effluents from FW. Although all media were initially adjusted to pH 7.1, the pH of FW dropped rapidly to below 6.0 shortly after inoculation. This acidic shift likely inhibited microalgal growth, as most strains prefer neutral to slightly alkaline conditions. Moreover, at lower pH levels, VFAs shift from their ionic form (dissociated form) into an uncharged (undissociated form), which can be inhibitory for microalgal growth [128]. In undissociated state, the acids can easily penetrate the cell membranes of microalgae, leading to cell death. While the PPS medium also contained a significant amount of acetic acid, its higher pH stability kept the acid in a dissociated, non-toxic state. In contrast, the FW medium contained a much higher total VFA load ( $3.14 \text{ g L}^{-1}$ ) and a more diverse mixture of long-chain acids, such as butyric ( $0.98 \text{ g L}^{-1}$ ) and caproic acids ( $0.70 \text{ g L}^{-1}$ ). The combination of high acid variety and an unstable, dropping pH created an unfavorable environment that prevented any biomass production in the FW medium.

The growth performance of five Nordic microalgal strains cultivated in AD-VFA effluents from PPS and CKM is presented in Figure 4. In PPS medium, *Chlorella vulgaris*, *Coelastrella* sp., and *Desmodesmus* sp. exhibited rapid growth and reached higher cell densities. *Coelastrella* sp. achieved the highest density of  $79.00 \times 10^6 \text{ cells mL}^{-1}$  after 15 days, followed by *Chlorella vulgaris* with  $43.20 \times 10^6 \text{ cells mL}^{-1}$ . In contrast, *Chlorococcum* sp. and *Scotiellopsis reticulata* showed slower growth, reaching only  $16.00 \times 10^6$  and  $6.94 \times 10^6 \text{ cells mL}^{-1}$ , respectively. The lower performance of these strains may be attributed to their larger cell sizes, which are inversely correlated with maximal population density [52, 129].

In CKM medium, a similar trend was observed. *Chlorella vulgaris* and *Coelastrella* sp. again showed the highest cell densities, reaching  $37.5 \times 10^6$  and  $34.5 \times 10^6 \text{ cells mL}^{-1}$ , respectively. However, *Scotiellopsis reticulata* ( $1.51 \times 10^6 \text{ cells mL}^{-1}$ ) and *Desmodesmus* sp. ( $3.02 \times 10^6 \text{ cells mL}^{-1}$ ) exhibited the lowest growth. *Chlorococcum* sp. reached an intermediate density of  $16.7 \times 10^6 \text{ cells mL}^{-1}$ , which was higher than *S. reticulata* and *Desmodesmus* sp., but still substantially lower than the best-performing strains. Notably, *Chlorella vulgaris*, *Coelastrella* sp., and *Desmodesmus* sp. exhibited shorter lag phases in both media, suggesting better adaptability to the effluent conditions. In contrast, *Chlorococcum* sp. and *Scotiellopsis reticulata* displayed longer lag phases, which may be explained by several factors. First, both strains have relatively larger cell sizes, which are often associated with slower division rates and reduced population growth potential. Second, their metabolic requirements may be less flexible, requiring more time to acclimate to the complex composition of AD effluents. Finally, *Scotiellopsis reticulata* required larger inoculum volumes to reach the target optical density,

which diluted the available nutrients and may have delayed the onset of exponential growth. Overall, microalgal strains reached higher cell densities in PPS compared to CKM.



**Figure 4.** Growth curves of the five native Nordic microalgae strains *Chlorella vulgaris* (*C. vul*), *Chlorococcum* sp. (*Chlo*), *Coelastrella* sp. (*Coel*), *Scotiellopsis reticulata* (*S. ret*) and *Desmodesmus* sp. (*Des*) cultivated on anaerobic digestion effluent derived from PPS (A) and CKM (B). Reproduced from **Paper 1**.

Biomass production data (Table 4) further supports the cell density trends. *Coelastrella* sp. produced the highest dry biomass in both PPS and CKM, with  $1.84 \pm 0.08 \text{ g L}^{-1}$  and  $1.99 \pm 0.04 \text{ g L}^{-1}$ , respectively, confirming its strong potential for cultivation in anaerobic digestates. *Chlorella vulgaris* also performed well, yielding  $1.82 \pm 0.12 \text{ g L}^{-1}$  in PPS and  $1.42 \pm 0.17 \text{ g L}^{-1}$  in CKM. *Desmodesmus* sp. produced  $1.31 \pm 0.21 \text{ g L}^{-1}$  in PPS, aligning with its moderate cell density, but only  $0.25 \pm 0.06 \text{ g L}^{-1}$  in CKM.

The higher biomass yields observed in PPS compared to CKM may be caused by the elevated initial acetic acid concentration in PPS, which has previously been linked to enhanced microalgal growth and biomass accumulation [130]. Moreover, the higher initial ammonium nitrogen concentration in CKM can slow the growth of microalgal strains. Overall, *Coelastrella* sp. and *Chlorella vulgaris* demonstrated superior growth and biomass productivity in both media.

**Table 4.** Biomass dry weight and produced biomass per consumed ammonium, and ammonium removal of the Nordic algal strain in PPS or CKM after 15 days. Adapted from **Paper 1**.

Media	Strain	Biomass (g L <sup>-1</sup> )	Biomass/consumed NH <sub>4</sub> <sup>+</sup> -N	NH <sub>4</sub> <sup>+</sup> -N removal %
PPS	<i>C. vulgaris</i>	1.82 ± 0.12	9.33	100.00
	<i>Chlorococcum</i> sp.	1.18 ± 0.12	6.05	100.00
	<i>Coelastrella</i> sp.	1.84 ± 0.08	9.44	100.00
	<i>S. reticulata</i>	1.05 ± 0.09	6.56	100.00
	<i>Desmodesmus</i> sp	1.31 ± 0.21	6.72	100.00
CKM	<i>C. vulgaris</i>	1.42 ± 0.17	9.79	38.69 ± 1.85
	<i>Chlorococcum</i> sp.	0.84 ± 0.15	5.25	42.67 ± 0.57
	<i>Coelastrella</i> sp.	1.99 ± 0.04	11.37	46.73 ± 4.62
	<i>S. reticulata</i>	0.31 ± 0.07	1.94	18.20 ± 0.55
	<i>Desmodesmus</i> sp	0.25 ± 0.06	2.08	32.01 ± 0.43

Despite their slower growth and extended lag phases, *Chlorococcum sp.* and *Scotiellopsis reticulata* were still able to completely remove the available ammonium in PPS medium (Table 4). This indicates that while their biomass productivity was lower, their nutrient uptake capacity remained effective. The other three strains also removed 100% of the ammonium nitrogen in PPS. This performance highlights the competitiveness of Nordic strains; for instance, it is reported that [131] ammonium assimilation ceased after six days in cultures with an initial ammonium concentration of 220 mg L<sup>-1</sup>. Although the reported inhibitory and toxic thresholds for *Chlorophytes* are about 332 mg L<sup>-1</sup> and 548 mg L<sup>-1</sup>, respectively [132], *C. pyrenoidosa* has been shown to tolerate up to 350 mg L<sup>-1</sup> in artificial anaerobic effluent [83]. In our study, the initial ammonium concentrations at the start of cultivation were 195 mg L<sup>-1</sup> (PPS) and 375 mg L<sup>-1</sup> (CKM) for all strains, except *S. reticulata* started at 160 mg L<sup>-1</sup> (PPS) and 330 mg L<sup>-1</sup> (CKM). These values are lower than the "as-received" concentrations in Table 3 because they account for the dilution occurring when the algal pre-culture was added.

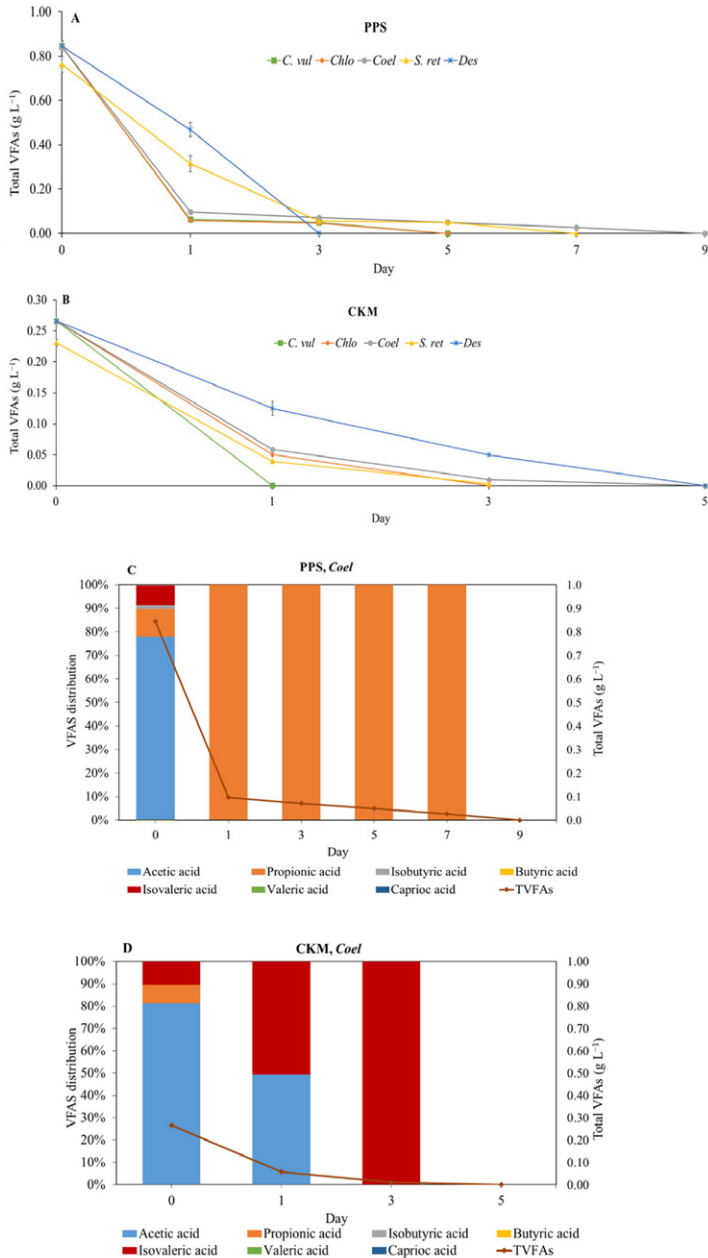
None of the strains were able to fully assimilate ammonium in CKM within 15 days. *Coelastrella sp.* achieved the highest removal (46%), whereas *Scotiellopsis reticulata* removed only 18% (Table 4). The reduced efficiency in CKM compared to PPS is likely due to its high nitrogen load, which can inhibit algal growth. Biomass yield per unit of consumed ammonium further illustrates strain performance. *Coelastrella sp.* achieved the highest ratios in both PPS (9.44 g biomass g<sup>-1</sup> ammonium) and CKM (11.37 g biomass g<sup>-1</sup> ammonium). *Chlorella vulgaris* was also highly efficient in both media. The remaining strains showed comparable biomass yields per consumed ammonium in PPS, but *Scotiellopsis reticulata* and *Desmodesmus sp.* had the lowest values in CKM.

### 3.1.1 Volatile fatty acids consumption by Nordic microalgae

VFAs serve as carbon sources for microalgae, and their relative composition strongly influences growth and biomass production [133]. In PPS medium, all VFAs were consumed within 3 to 9 days, whereas in CKM they were depleted more rapidly, within 1 to 5 days, due to lower initial concentrations of VFAs (Table 3). Among the strains, *Coelastrella sp.* was the most effective, combining high biomass production with efficient ammonium removal. It assimilated all acetic acid within the first day, while complete consumption of propionic acid required 9 days (Figure 5C). This pattern reflects strain-specific preferences for organic acids, linked to differences in metabolic pathways [130, 134]. Acetic acid is readily converted to acetyl-CoA, a precursor for fatty acid biosynthesis via the tricarboxylic acid or TCA cycle [135]. Its transport into cells requires less energy than butyric acid, contributing to a prioritization of acetic acid consumption

over butyric acid [79, 136]. Propionic and butyric acid metabolism follow more complex pathways: butyric acid is degraded to acetyl-CoA via  $\beta$ -oxidation, while propionic acid enters the tricarboxylic acid cycle through oxaloacetate [130, 137]. Propionic acid is assimilated more slowly than butyric acid and cannot serve as a sole carbon source [136, 137]. In CKM, *Coelastrella* sp. consumed acetic acid completely within 24 hours, while propionic acid was assimilated even faster due to its much lower initial concentration (0.02 g L<sup>-1</sup> vs. 0.22 g L<sup>-1</sup> for acetic acid) (Figure 5D).

Considering biomass production and nutrient removal (NH<sub>4</sub>-N and VFAs), *Coelastrella* sp., *Chlorella vulgaris* consistently performed best in both PPS and CKM media. *Chlorococcum* sp. outperformed *Scotiellopsis reticulata* and *Desmodesmus* sp., particularly in CKM, which represents a medium with elevated ammonium concentrations. Therefore, these three strains were selected for subsequent experimental work, focusing on microalgal cultivation in AD effluent of municipal sewage sludge under different nitrogen levels.



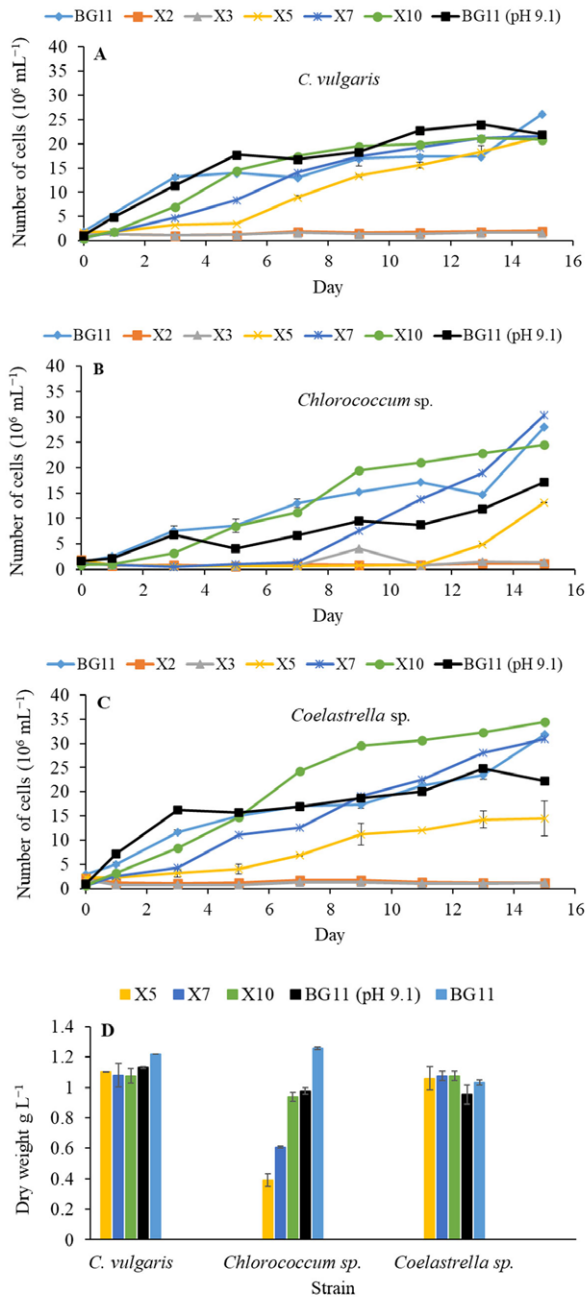
**Figure 5.** Total VFAs consumption by the Nordic microalgae *Chlorella vulgaris* (*C. vul*), *Chlorococcum* sp. (*Chlo*), *Coelastrella* sp. (*Coel*), *Scotiellopsis reticulata* (*S. ret*) and *Desmodesmus* sp. (*Des*) in PPS (A) and CKM (B). Changes in the VFAs concentrations and VFAs distribution during the cultivation of *Coelastrella* sp. in PPS (C) and CKM (D). Reproduced from **Paper 1**.

### 3.2 The effect of nitrogen content

The results and discussion in this section reflect the outcomes detailed in **Paper 2**. *Chlorella vulgaris*, *Chlorococcum* sp. and *Coelastrella* sp. were cultivated in AD effluent obtained from municipal sewage sludge. The effluent contained an initial ammonium nitrogen concentration of  $1540 \pm 20 \text{ mg L}^{-1}$ , and was diluted 2, 3, 5, 7 and 10 times with Milli-Q water, designated as X2, X3, X5, X7 and X10, respectively. The five diluted effluents were autoclaved, after which the pH increased from 8.2 to approximately 9.2, and they were subsequently used as cultivation media without further pH adjustment. BG11 served as the control medium, with its pH set to 7.1. In addition, a BG11 variant with pH adjusted to 9.1 was prepared and tested to compare microalgal performance under conditions similar to the effluents elevated pH.

#### 3.2.1 Growth performance of microalgae

All three microalgal strains were able to grow in effluent dilutions X5, X7 and X10, while no growth was observed in X2 and X3 due to ammonium toxicity (Figure 6A, B and C). Growth dynamics varied across strains and media: *C. vulgaris* exhibited lag phase in X5 but ultimately performed consistently across X5, X7, and X10. *Chlorococcum* sp. showed rapid growth in X10 but experienced extended lag phases in X5 and X7, which correlated with its lower biomass yields in those media. In contrast, *Coelastrella* sp. started growing without delay in all tested dilutions and consistently outperformed the other strains in X7 and X10. Biomass measurements (Figure 6D) confirmed these trends as *C. vulgaris* and *Coelastrella* sp. produced significantly higher dry weights than *Chlorococcum* sp. in X5, while *Chlorococcum* sp. achieved its highest biomass in BG11 control. The reduced biomass of *Chlorococcum* sp. in X5 and X7 is likely linked to its prolonged lag phase and larger cell size, which may limit population density [129]. Notably, the biomass yields of *C. vulgaris* in X5, X7 and X10 were comparable to those reported in previous studies for cultivation of the same strain in municipal and piggery wastewater, showing the effectiveness of this Nordic strain under varying nitrogen conditions [52, 138].



**Figure 6.** Growth curves as number of cells  $\text{mL}^{-1}$  of *C. vulgaris* (A), *Chlorococcum* sp. (B) and *Coelastrrella* sp. (C), and biomass dry weight (D) of the same strains cultivated on anaerobic digestion effluents at different dilution conditions as well as BG11 control (pH 7.1) and BG11 (pH 9.1). Reproduced from **Paper 2**.

### 3.2.2 Total ammonia nitrogen (TAN) removal and free ammonia (FA) effect

The TAN removal efficiencies (Table 5) demonstrate that all three microalgal strains, *Chlorella vulgaris*, *Chlorococcum* sp. and *Coelastrrella* sp., effectively assimilated ammonium from diluted AD effluent. *C. vulgaris* consistently removed over 90% of TAN across all media. *Coelastrrella* sp. achieved its highest removal (95.5%) in X10, and *Chlorococcum* sp. reached complete removal (100%) in X10, although its lower biomass yield indicates that high nitrogen removal does not necessarily translate to high biomass yield [139].

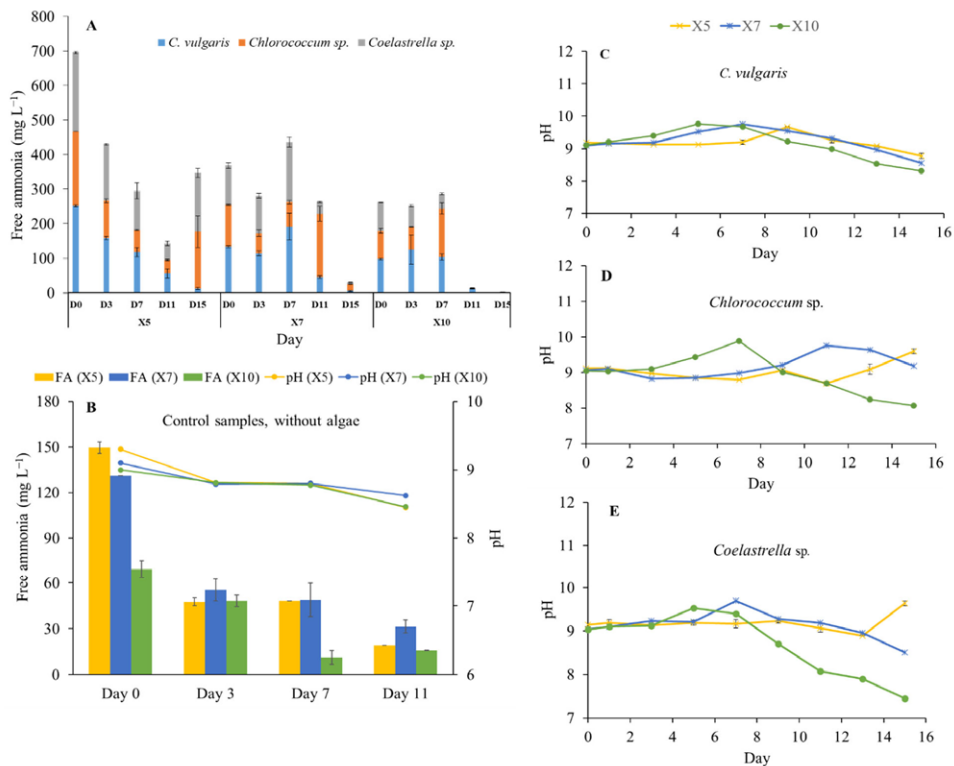
**Table 5.** Initial total ammonia nitrogen concentration and total ammonia nitrogen removal efficiency of the Nordic algal strain in anaerobic digestion effluent with different concentrations after 15 days. Adapted from **Paper 2.**

Medium	Strain	Initial TAN (mg/L)	RE%
	<i>C. vulgaris</i>	444.82 ± 5.29	90.70 ± 1.20
X5	<i>Chlorococcum</i> sp.	417.51 ± 0.00	53.40 ± 10.10
	<i>Coelastrrella</i> sp.	426.62 ± 5.07	53.90 ± 2.50
	<i>C. vulgaris</i>	266.19 ± 7.24	90.50 ± 1.20
X7	<i>Chlorococcum</i> sp.	254.03 ± 0.25	86.00 ± 0.20
	<i>Coelastrrella</i> sp.	243.40 ± 15.29	87.80 ± .070
	<i>C. vulgaris</i>	191.23 ± 0.18	90.90 ± 0.40
X10	<i>Chlorococcum</i> sp.	178.61 ± 10.12	100.00 ± 0.00
	<i>Coelastrrella</i> sp.	179.22 ± 6.98	95.50 ± 2.20

Figure 7 shows the FA removal by different strains (Figure 7A), pH values and FA removal in control samples without algae due to ammonia stripping (Figure 7B) and pH values during the cultivation period for different strains (Figure 7C, D and E). In control samples, FA declined steeply between day 0 and day 3, particularly in X5, while pH also decreased. This sharper early FA reduction compared to algal cultures reflects passive NH<sub>3</sub> volatilization under initially alkaline conditions (pH around 9.1). As pH dropped further, the equilibrium shifted toward NH<sub>4</sub><sup>+</sup>, limiting new FA formation and slowing the FA reduction. Thus, controls show strong early stripping but weaker long-term removal. In algal cultures (Figure 7A), FA reduction was slower in the first three days compared to controls, especially for *C. vulgaris* in X5. However, by day 7 onward, generally, FA levels in algal cultures dropped more substantially and continued to decline through day 15. This sustained reduction indicates active biological uptake, which remains effective even after pH shifts reduce the volatilization. However, FA levels did not decline consistently; they fluctuated in response to pH changes. For example, in X5 with *Coelastrella* sp., FA increased between day 11 and day 15 as pH rose from 9.07 to 9.64, shifting the equilibrium toward NH<sub>3</sub>. Similarly, in X7 with *Chlorococcum* sp., FA rose from day 7 to day 11 when pH increased from 9.00 to 9.75. These cases highlight that FA removal is not solely determined by assimilation but is strongly influenced by pH fluctuation, which governs the NH<sub>4</sub><sup>+</sup>-NH<sub>3</sub> balance.

The pH profiles highlight strain-specific strategies. *C. vulgaris* (Figure 7C) maintained relatively stable pH across 15 days, limiting NH<sub>3</sub> formation while steadily reducing TAN. *Chlorococcum* sp. (Figure 7D) showed the highest pH at day 7 in X10, coinciding with a temporary increase in FA. After day 7, pH declined and FA levels dropped again, confirming that both equilibrium shifts and biological uptake shaped the trend. *Coelastrella* sp. (Figure 7E) exhibited the strongest pH decline, especially after day 8 in X10, shifting the balance toward NH<sub>4</sub><sup>+</sup> and reducing FA toxicity.

Together, these results show that microalgae reduce nitrogen through two complementary mechanisms. First, direct assimilation of NH<sub>4</sub><sup>+</sup> into biomass, sustaining FA reduction beyond the early phase. Second, indirect control of FA levels via pH modulation, which influences the NH<sub>4</sub><sup>+</sup>-NH<sub>3</sub> equilibrium.



**Figure 7.** Free ammonia (FA) removal by different strains (A), pH values and FA removal in control samples without algae (B) and pH values during the cultivation period for different strains (C, D and E; **Paper 2**).

In summary, although *Chlorococcum sp.* exhibited lag phases of 7 days in X5 and 11 days in X7, its TAN removal efficiency after 15 days was comparable to that of *Coelastrella sp.*, which did not experience any lag in these media. In X10, *Chlorococcum sp.* achieved complete TAN removal (100%), followed by *Coelastrella sp.* with 95%. Moreover, *C. vulgaris* consistently showed the highest TAN removal in X5 and X7 (both 90%). In addition, *C. vulgaris* produced the highest biomass dry weight across all media, reaching 1.1 g/L in X5. Taken together, these findings highlight *C. vulgaris* and *Chlorococcum sp.* as the most promising candidates for efficient TAN removal and sustainable biomass production. Therefore, these two strains were selected for the next experimental work, which was evaluation of the effect of light:dark cycles.

In addition, up to this stage of the thesis, *C. vulgaris* and *Coelastrella sp.* showed very similar trends in both growth and nutrient removal, but *C. vulgaris* is a widely studied and well-established reference strain, allowing easier comparison with existing literature. In

contrast, *Chlorococcum* sp. was retained as the second candidate because it is less frequently studied, offering additional novelty to the work.

Regarding the selection of effluent dilutions, while all strains grew successfully at dilution factors of 5, 7, and 10 times, both X7 and X10 produced comparable results in nutrient removal and biomass production. Therefore, X5 and X7 were chosen for cultivating *C. vulgaris* and *Chlorococcum* sp. in the next phase because they were the more sustainable options, requiring less water for dilution compared to X10 and still supporting microalgal growth.

### **3.3 The effect of light:dark cycle and CO<sub>2</sub> aeration**

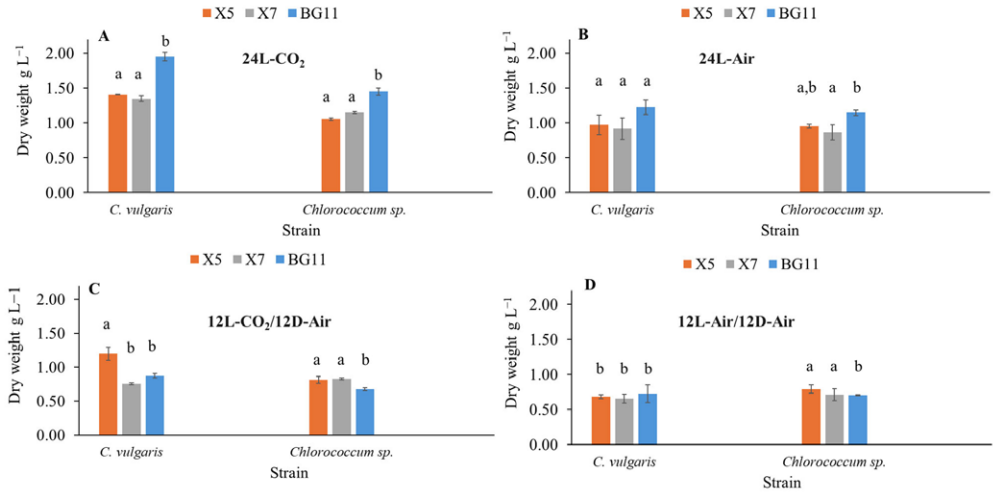
The results and discussion presented in this section correspond to the findings reported in **Paper 3**. *C. vulgaris* and *Chlorococcum* sp. were cultivated in X5 and X7 diluted AD effluent under different cultivation regimes, as presented in Table 2. Although a new batch of municipal sewage sludge AD effluent was collected for this experiment, its characteristics were highly comparable to those used in **Paper 2**. The starting pH ranged from 8.1 to 8.3, and the initial ammonium concentration was  $1580 \pm 28 \text{ mg L}^{-1}$ , which is very close to the  $1540 \pm 20 \text{ mg L}^{-1}$  measured in **Paper 2**. After autoclaving, the pH of the diluted effluents increased to 9.1 - 9.3, and the media were used directly for cultivation without further pH adjustment.

#### **3.3.1 Biomass production and carbon dioxide fixation rate**

The dilution factor of the AD effluent did not significantly influence the biomass dry weight of either strain after 15 days ( $p > 0.05$ ; Figure 8), except for *C. vulgaris* grown under the 12L-CO<sub>2</sub>/12D-Air regime (Figure 8C). Under this condition, *C. vulgaris* produced 60% more biomass in X5 compared to X7. When cultivated under continuous CO<sub>2</sub> aeration (24L-CO<sub>2</sub>), *C. vulgaris* achieved the highest biomass yields in both X5 and X7, reaching 1.41 and 1.05 g L<sup>-1</sup>, respectively. Harwati, et al. [140] reported that *Chlorococcum* sp. grown in BG11 under 24L-CO<sub>2</sub> and 24L-Air with a light intensity of  $54 \mu\text{mol m}^{-2} \text{ s}^{-1}$  produced 1.03 and 0.53 g L<sup>-1</sup> biomass in 10 days. These values are lower than those obtained in the present study, where *Chlorococcum* sp. cultivated in BG11 under 24L-CO<sub>2</sub> and 24L-Air reached 1.45 and 1.15 g L<sup>-1</sup>, respectively, after 15 days.

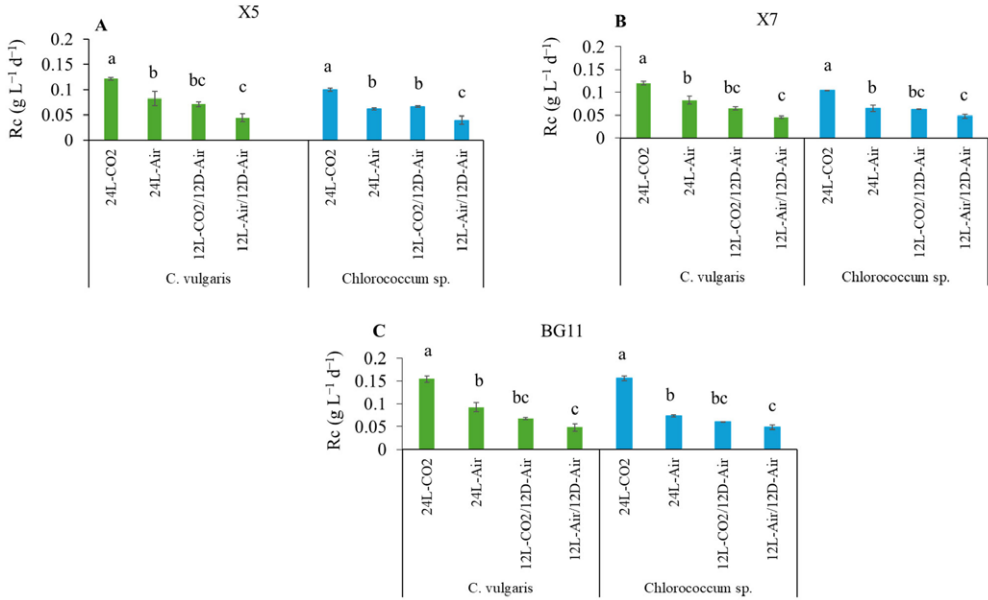
Comparing all cultivation conditions, both strains produced the lowest amount of biomass dry weight when grown under 12L-Air/12D-Air (Figure 8D), likely due to limited availability of CO<sub>2</sub>, which restricted photosynthetic efficiency [141]. Photosynthesis requires light, whereas during the dark phase microalgae rely on stored energy reserves, resulting in a temporary halt in biomass accumulation. Although microalgae may heterotrophically utilize organic carbon

present in AD effluent during the dark period, continuous photoautotrophic growth with an unrestricted CO<sub>2</sub> supply is generally more efficient than heterotrophic metabolism [142].



**Figure 8.** Biomass dry weight of *C. vulgaris* and *Chlorococcum sp.* cultivated for 15 days in BG11 or anaerobic digestion effluents (X5 and X7) under different cultivation regimes. Mean values that do not share a common letter differ significantly (p value < 0.05). Letter groupings are assigned independently for each strain within each cultivation regime.

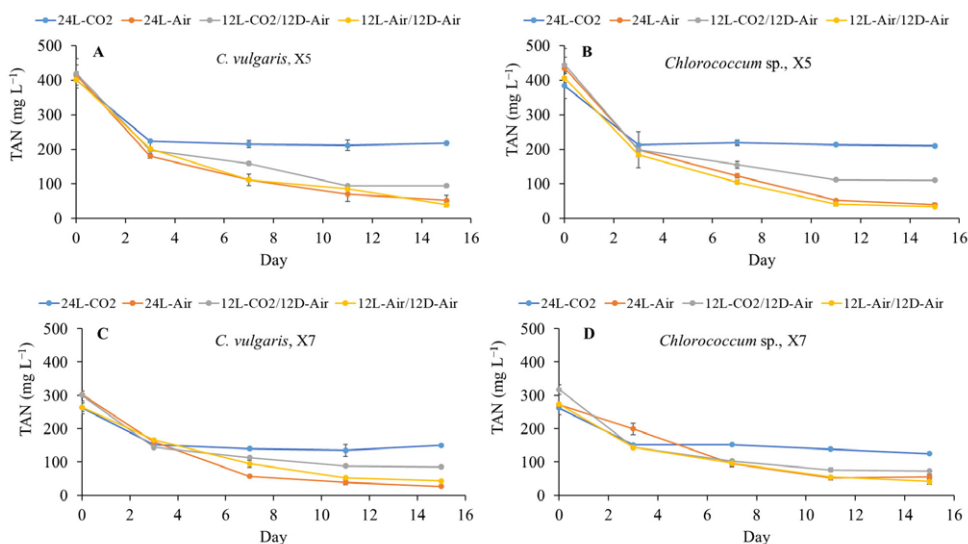
The carbon dioxide fixation rates of both strains,  $R_c$  (gCO<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup>), calculated using Eq. (4), were compared in Figure 9. The highest carbon dioxide fixation rate was attained under 24L-CO<sub>2</sub> regime. While in X5 and X7 the highest  $R_c$  was achieved by *C. vulgaris* (0.121 and 0.119 gCO<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup>, respectively), in BG11 *Chlorococcum sp.* and *C. vulgaris* reached the highest  $R_c$  of (0.156 and 0.154 gCO<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup>, respectively). Tang, et al. [143] reported a maximum  $R_c$  of 0.288 g L<sup>-1</sup> d<sup>-1</sup> for *S. obliquus* SJTU-3 cultivated in BG11 under continuous light (180 μmol m<sup>-2</sup> s<sup>-1</sup>) and air supplemented with 10% CO<sub>2</sub>. This value exceeded the  $R_c$  measured in this study, likely because the higher light intensity used by Tang, et al. [143] promoted greater biomass accumulation and, consequently, higher CO<sub>2</sub> fixation [144].



**Figure 9.** Carbon dioxide fixation rates,  $R_c$  ( $\text{g CO}_2 \text{ L}^{-1} \text{ d}^{-1}$ ), of *C. vulgaris* and *Chlorococcum sp.* cultivated in anaerobic digestion effluents; X5 (A) or X7 (B), or in BG11 medium (C) after 15 days under different cultivation regimes. Mean values that do not share a common letter differ significantly ( $p < 0.05$ ). Letter groupings are assigned separately for each strain within each growth medium. Adapted from **Paper 3**.

### 3.3.2 Total ammonia nitrogen removal

The maximum TAN removal efficiencies of both strains, regardless of the dilution factor of AD effluent, were achieved in cultivation regimes without CO<sub>2</sub> supplementation (12L-Air/12D-Air and 24L-Air) (Figure 10). In contrast, the lowest efficient TAN removal occurred under 24L-CO<sub>2</sub> regime, followed by 12L-CO<sub>2</sub>/12D-Air. When CO<sub>2</sub> was supplied (24L-CO<sub>2</sub> and 12L-CO<sub>2</sub>/12D-Air) the culture pH remained between 7 and 7.5, whereas in the absence of CO<sub>2</sub> sparging (24L-Air and 12L-Air/12D-Air) the pH increased to 8-9. Since the NH<sub>4</sub><sup>+</sup>-NH<sub>3</sub> equilibrium constant is approximately 9.25, lower pH values favor the presence of NH<sub>4</sub><sup>+</sup>, while NH<sub>3</sub> becomes dominant only when pH exceeds 9.25 [87, 145]. Therefore, under CO<sub>2</sub>-enriched conditions, the lower pH limited the conversion of NH<sub>4</sub><sup>+</sup> to NH<sub>3</sub>, reducing ammonia stripping. Although CO<sub>2</sub> supplementation enhanced microalgal growth and resulted in higher biomass yields in 24L-CO<sub>2</sub> and 12L-CO<sub>2</sub>/12D-Air compared to the air-only regimes, the reduced NH<sub>3</sub> formation hindered TAN removal. In the absence of CO<sub>2</sub>, pH values of about 8.5 promoted higher NH<sub>3</sub> formation, and more stripping, which then led to higher TAN reduction than optimal culture growth. Moreover, high CO<sub>2</sub> availability may have decreased ammonium uptake by the microalgae due to metabolic prioritization of carbon assimilation.



**Figure 10.** Total ammonia nitrogen (TAN) removal by *C. vulgaris* and *Chlorococcum* sp. grown in anaerobic digestion effluent; X5 (A and B) or X7 (C and D) in different cultivation regimes. Reproduced from **Paper 3**.

### 3.3.3 Biomass composition of microalgal biomass

The highest FAME accumulation was achieved by *C. vulgaris* in X7 under 24L-CO<sub>2</sub> regime (30%), exceeding *Chlorococcum* sp. (26%) under the same conditions (Table 6). This corresponds with the highest biomass C/N ratio recorded for *C. vulgaris* in X7 under 24L-CO<sub>2</sub> (15.31). Greater nitrogen limitation promoted increased fatty acid production, consistent with previous findings [146, 147]. Continuous light and CO<sub>2</sub> supply (24L-CO<sub>2</sub>) resulted in the highest FAME synthesis across both strains and all media (p value < 0.05). High CO<sub>2</sub> concentrations have been shown to elevate intracellular metabolic intermediates such as acetyl-CoA (AcCoA), a key precursor for fatty acid biosynthesis. An increase in AcCoA is directly associated with enhanced lipid production [148]. In 24L-CO<sub>2</sub> cultivation regime, continuous CO<sub>2</sub> aeration maintained the pH at approximately 7.5. This observation aligns with previous findings showing *C. vulgaris* achieved its highest lipid content at pH 7.5 under 24L-CO<sub>2</sub> when cultivated across a range of initial ammonium concentrations (50, 120, 240, 360 and 500 mg L<sup>-1</sup>) and pH levels (6.5, 7.5, 8.5 and 9.5) [86].

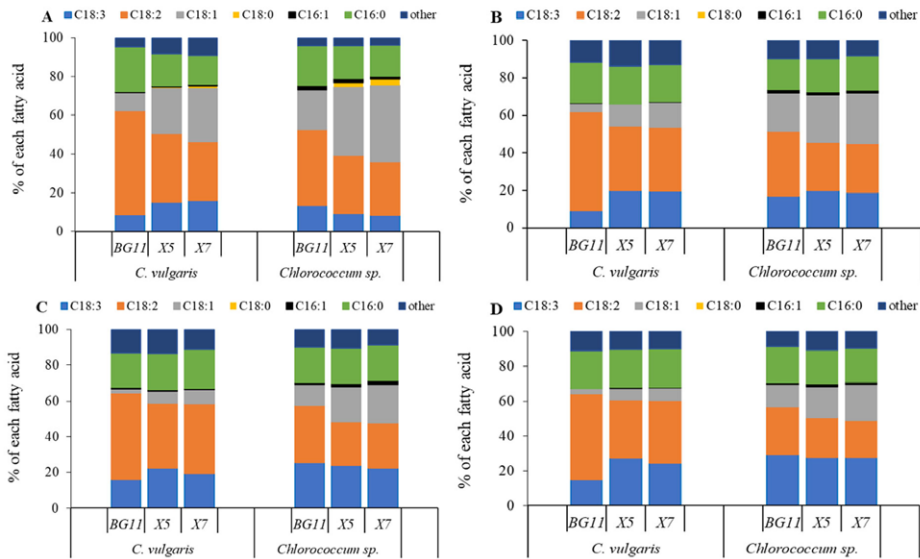
In general, cultivation regimes that promote lipid accumulation often involve nutrient stress or higher biomass C/N ratio, leading to reduced protein production [149, 150]. In this study, *Chlorococcum* sp. showed higher protein content than *C. vulgaris* under 24L conditions (24L-CO<sub>2</sub> and 24L-Air) in both X5 and X7 media. This observation is consistent with the lower biomass C/N ratios measured for *Chlorococcum* sp. compared to *C. vulgaris* under the same 24L regimes.

**Table 6.** Total FAME, protein and C/N ratio of *C. vulgaris* and *Chlorococcum* sp. grown in AD effluent; X5 or 7 X7 after 15 days using different cultivation regimes. Adapted from **Paper 3**.

Parameter	Strain	Media	Cultivation regime			
			24L-CO <sub>2</sub>	24L-Air	12L-CO <sub>2</sub> /12D-Air	12L-Air/12D-Air
Total FAME (%)	<i>C. vulgaris</i>	X5	26.61 ± 0.82	17.80 ± 3.59	8.45 ± 1.00	7.97 ± 0.82
		X7	30.42 ± 0.01	21.23 ± 2.23	10.35 ± 0.87	10.19 ± 0.01
	<i>Chlorococcum</i> sp.	X5	20.39 ± 0.18	12.06 ± 1.06	10.56 ± 0.29	8.69 ± 0.18
		X7	24.44 ± 0.01	13.81 ± 1.94	11.13 ± 0.20	10.04 ± 0.00
Protein (%)	<i>C. vulgaris</i>	X5	19.04 ± 0.10	26.6 ± 1.72	35.44 ± 1.45	36.32 ± 1.82
		X7	17.75 ± 0.64	24.40 ± 0.84	32.52 ± 0.64	31.26 ± 1.89
	<i>Chlorococcum</i> sp.	X5	29.8 ± 0.57	28.98 ± 0.84	35.68 ± 0.57	32.14 ± 0.84
		X7	30.04 ± 2.19	26.29 ± 0.67	34.75 ± 0.20	29.99 ± 0.30
C/N ratio	<i>C. vulgaris</i>	X5	14.01 ± 0.05	9.69 ± 0.86	6.80 ± 0.23	6.53 ± 0.2
		X7	15.31 ± 0.53	10.75 ± 0.53	7.59 ± 0.08	7.42 ± 0.21
	<i>Chlorococcum</i> sp.	X5	8.64 ± 0.00	8.60 ± 0.30	6.85 ± 0.09	7.22 ± 0.04
		X7	8.51 ± 0.53	9.27 ± 0.41	7.03 ± 0.07	8.11 ± 0.04

The fatty acid profiles of both microalgal strains are shown in Figure 11. For *C. vulgaris*, C18:2 (linoleic acid) was the predominant fatty acid across all cultivation regimes and media. C18:2 is an important polyunsaturated fatty acid (PUFA: containing multiple carbon-carbon double bonds) and an essential omega-6 component of the human diet [151]. Conversely, *Chlorococcum* sp. cultivated in X5 and X7 under 24L-CO<sub>2</sub> regime primarily produced C18:1 (oleic acid), a monounsaturated fatty acid (MUFA: containing only a single double bond), which is an excellent biodiesel component, valued for enhancing both cold-flow properties and oxidative stability [140]. Moreover, the cetane number, which shows the combustion quality of diesel fuel, improves with higher concentrations of monounsaturated fatty acids such as C18:1 [152]. This strain preferentially assigns carbon flux towards C18:1 MUFA synthesis rather than C18:2 PUFA, reflecting a different metabolic strategy distinct from that of *C. vulgaris*.

According to the European biodiesel quality standard EN 14214 (2004), the content of C18:3 (linolenic acid) must not exceed 12%, and the proportion of polyunsaturated fatty acids with four or more double bonds must remain below 1% [153, 154]. *Chlorococcum* sp. cultivated in AD effluent media X5 and X7 under 24L-CO<sub>2</sub> regime met these requirements, with C18:3 levels of 9.19% and 8.42%, respectively. *C. vulgaris* grown in X5 and X7 exceeded this threshold (15.18% and 16.03%) and therefore did not comply with the C18:3 specification. In addition, 24L-CO<sub>2</sub> was the only condition that enabled the production of C18:0 (stearic acid), a saturated fatty acid (SFA: containing no double bonds). Stearic acid contains long-chain hydrocarbons that release high energy during combustion, contributing to a higher calorific value [155].



**Figure 11.** Fatty acid profiles of *C. vulgaris* and *Chlorococcum* sp. grown in anaerobic digestion effluents; X5 and X7 under different cultivation regimes: A (24L-CO<sub>2</sub>), B (24L-Air), C (12L-CO<sub>2</sub>/12D-Air), D (12L-Air/12D-Air). Reproduced from **Paper 3**.

For the next stage of the experimental work (Section 3.4 and 3.5, corresponding to **Paper 4**), *Chlorococcum* sp. was selected as the target strain because it is considerably less studied than *C. vulgaris*, making it a promising candidate for further investigation. Regarding the growth medium, the same AD effluent derived from municipal sewage sludge was used as the cultivation medium. The effluent used in next sections was taken from the same batch collected for **Paper 3**.

### 3.4 The effect of different CO<sub>2</sub> concentrations

*Chlorococcum* sp. was cultivated in municipal sewage sludge AD effluent with an initial ammonium concentration of approximately 240 mg L<sup>-1</sup> and a pH of 9.1. The study presented in **Paper 4** was carried out in two stages. In the first stage, the influence of different CO<sub>2</sub> concentrations (0.04, 3, 6 and 9% v/v) on microalgal growth was assessed in BC bioreactors. The 0.04% CO<sub>2</sub> condition represents ambient air without any additional CO<sub>2</sub> supplementation

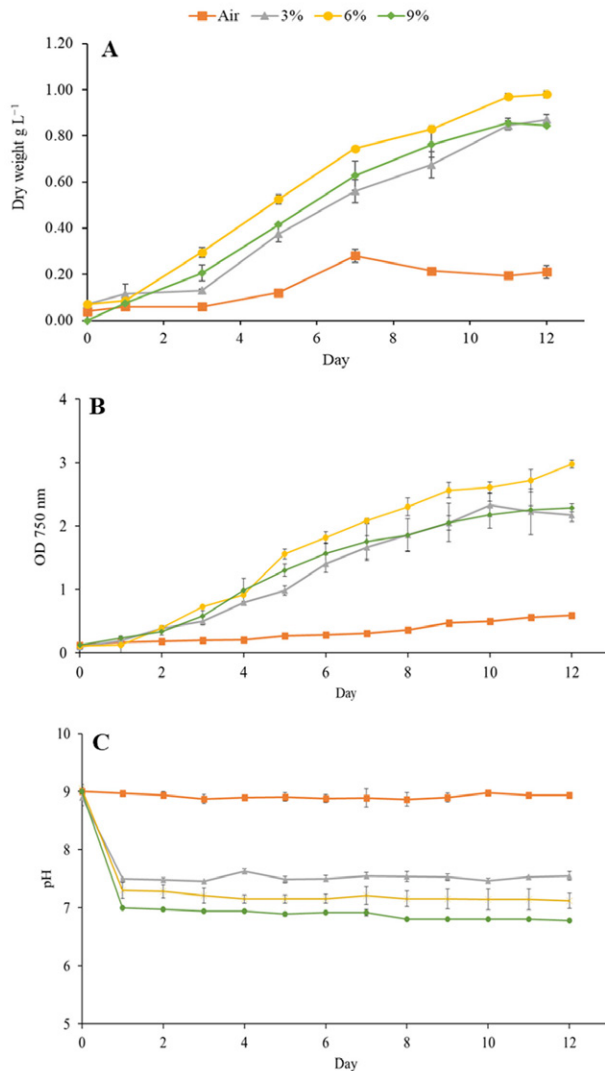
#### 3.4.1 The effect on microalgal growth & nutrient removal

The optimal CO<sub>2</sub> concentration for the growth of *Chlorococcum* sp. was 6% (Figure 12 A and B), which resulted in the highest biomass yield of 0.98 ± 0.01 g L<sup>-1</sup>. Under this condition, optical density values after 12 days were also significantly higher than those obtained at 9% CO<sub>2</sub> concentration (p value of 0.026 < 0.05), consistent with findings previously reported by [140].

Supplying 6% CO<sub>2</sub> supply led to a 4.6 fold increase in the final biomass concentration compared to cultivation under 0.04% CO<sub>2</sub>. The weak growth observed under 0.04% CO<sub>2</sub> can be explained by limited photosynthetic activity relative to CO<sub>2</sub>-enriched conditions [156]. Additionally, the high pH of the medium (approximately 9, Figure 12 C) was likely outside the optimal pH range for *Chlorococcum* sp., further constraining growth as the optimal pH range of *Chlorococcum* sp. lies between neutral to moderately alkaline, approximately pH 7.3 to 8.5 [157, 158].

The balance between dissolved CO<sub>2</sub>, bicarbonate (HCO<sub>3</sub><sup>-</sup>), and carbonate (CO<sub>3</sub><sup>2-</sup>) shifts with pH: CO<sub>2</sub> dominates below pH 6.3, HCO<sub>3</sub><sup>-</sup> between pH 6.3 and 10.3, and CO<sub>3</sub><sup>2-</sup> above pH 10.3. At pH 9 the equilibrium is dominated by bicarbonate, while the concentration of dissolved CO<sub>2</sub> is negligible. Although the carbon is not yet locked as carbonate at this stage (carbonate only becomes significant above pH 10), the complete absence of free dissolved CO<sub>2</sub> and the highly alkaline conditions likely created a suboptimal environment for growth, which slow photosynthesis and enhances respiration rate [159].

Notably, supplying 9% CO<sub>2</sub> did not enhance biomass production compared to 3% or 6% CO<sub>2</sub>. This outcome may be linked to the lower pH observed under 9% CO<sub>2</sub> (6.76 to 6.99), in contrast to the higher pH range recorded at 3% CO<sub>2</sub> (7.46 to 7.63)



**Figure 12.** Biomass dry weight (A), optical densities (B), and pH values (C) of *Chlorococcum* sp. cultures grown in anaerobic digestion effluents under different CO<sub>2</sub> concentrations. Reproduced from **Paper 4**.

When it comes to ammonium nitrogen removal, aeration with 0.04% CO<sub>2</sub> led to significantly greater NH<sub>4</sub><sup>+</sup>-N removal (58.04%) compared to the CO<sub>2</sub>-enriched treatments, aligning with findings reported in **Paper 3**. This condition also achieved the highest phosphorus removal efficiency, reaching 40.06% (Table 7). Cultivation under 3% CO<sub>2</sub> resulted in significantly higher NH<sub>4</sub><sup>+</sup>-N removal efficiency compared with 6% and 9% CO<sub>2</sub>, whereas no significant difference was observed between the 6% and 9% conditions.

Regarding air aeration conditions, a duplicate control reactor containing only the growth medium (without microalgae) were also tested to realize the ammonia stripping under this condition with the highest pH values. In the control, the initial  $\text{NH}_4^+\text{-N}$  concentration of  $242.50 \pm 17.68 \text{ mg L}^{-1}$  declined to  $152.50 \pm 3.54 \text{ mg L}^{-1}$ , corresponding to a removal efficiency of  $36.99\% \pm 3.13\%$ . The pH in control remained between 8.8 and 9.0. At an average pH of 8.9, the  $\text{NH}_3/\text{NH}_4^+$  equilibrium shifts to roughly 30.9%  $\text{NH}_3$  and 69.1%  $\text{NH}_4^+$ , indicating that a portion of the observed ammonium reduction likely resulted from conversion to gaseous  $\text{NH}_3$  and subsequent stripping during aeration.

**Table 7.** Nutrient removal efficiency, biomass productivity and  $\text{CO}_2$  fixation rate by *Chlorococcum* sp. cultures grown in anaerobic digestion effluents using different  $\text{CO}_2$  concentrations. Mean values that do not share a common letter within the same column differ significantly ( $p$  value  $< 0.05$ ). Reproduced from **Paper 4**.

$\text{CO}_2$ (%)	Removal efficiency (%)		Productivity	$\text{CO}_2$ fixation rate
	$\text{NH}_4\text{-N}$	P	$\text{mg biomass L}^{-1} \text{ d}^{-1}$	$\text{mg CO}_2 \text{ L}^{-1} \text{ d}^{-1}$
0.04	$58.04 \pm 4.94^a$	$40.06 \pm 2.49^a$	$14.58 \pm 2.94^b$	$27.05 \pm 5.44^a$
3	$40.64 \pm 3.48^b$	$25.48 \pm 4.93^b$	$66.66 \pm 1.17^a$	$146.42 \pm 2.24^{ab}$
6	$34.56 \pm 7.09^c$	$22.01 \pm 0.43^b$	$76.25 \pm 0.58^a$	$162.55 \pm 2.28^b$
9	$27.62 \pm 2.17^c$	$16.81 \pm 3.21^b$	$66.66 \pm 0.00^a$	$140.35 \pm 5.34^c$

As shown in Table 7, the highest  $\text{CO}_2$  fixation rate,  $162 \text{ mg L}^{-1} \text{ d}^{-1}$ , occurred at 6%  $\text{CO}_2$ , while air aeration yielded the lowest rate ( $27 \text{ mg L}^{-1} \text{ d}^{-1}$ ). The maximum fixation rate achieved at 6%  $\text{CO}_2$  surpassed values previously reported in **Paper 3** for *C. vulgaris* ( $121 \text{ mg L}^{-1} \text{ d}^{-1}$ ) and for *Chlorococcum* sp. grown under 3%  $\text{CO}_2$  ( $99 \text{ mg L}^{-1} \text{ d}^{-1}$ ).

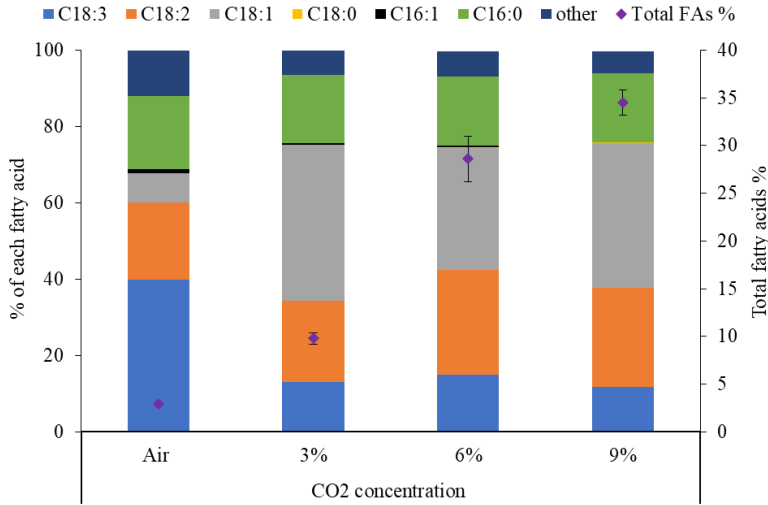
### 3.4.2 The effect on Fatty acid production

The highest FAMES yield was obtained under 9%  $\text{CO}_2$  aeration (34.48%), followed by 6, 3 and 0.04% (Figure 13). This shows that increasing  $\text{CO}_2$  availability promote fatty acid accumulation, aligning with the findings of Harwati, et al. [140], who reported increased lipid content in *Chlorococcum* sp. at  $\text{CO}_2$  aeration levels of 1, 3 and 6% compared to 0.04%. Furthermore, Sun, et al. [160] examined *Chlorella sorokiniana* under  $\text{CO}_2$  concentrations ranging from 1% to 10% and found that 10%  $\text{CO}_2$  aeration significantly boosted the lipid content relative to lower tested concentrations (0.04, 1, 2 and 5%).

The  $\text{CO}_2$  level affected not only the total fatty acid content but also the fatty acid profile. At 0.04%  $\text{CO}_2$ , the fatty acid profile was dominated by PUFAs, particularly C18:3 (39.86%) and C18:2 (20.18%), while MUFA C18:1 was low (7.61%) and C16:0 (palmitic acid) accounted for 19.01%. Under  $\text{CO}_2$  supplementation (3, 6 and 9%), C18:3 sharply declined to 13.12%, 14.94%, and 11.67%, respectively, accompanied by a marked rise in C18:1, reaching 40.83% at 3%  $\text{CO}_2$ .

This shift from PUFAs to MUFAs was consistent with the previous results by Li, et al. [161], who observed *C. vulgaris* produced increased levels of C18:1 under 10% and 15% CO<sub>2</sub>, and elevated C18:3 content under ambient CO<sub>2</sub> (0.04% CO<sub>2</sub>). The C16:1 (palmitoleic acid) fraction, already low at 0.04% CO<sub>2</sub>, declined to nearly undetectable levels under higher CO<sub>2</sub> levels.

This pattern indicates a metabolic shift from synthesizing building materials (membrane lipids) to accumulating storage lipids as carbon availability increases. Under carbon-limited conditions (0.04% CO<sub>2</sub>), the cells must allocate every available carbon atom toward essential building materials, such as photosynthetic membranes (thylakoids), resulting in a fatty acid profile dominated by the PUFA C18:3, a major component of these membranes [162]. However, when carbon is abundant (3, 6 and 9% CO<sub>2</sub>), the metabolic priority shifts toward energy storage, leading to enhanced synthesis of triacylglycerols (TAGs), with C18:1 becoming the predominant fatty acid incorporated into these storage TAGs lipids [163]. This metabolic shift can be explained by the C18 fatty acid biosynthetic pathway. PUFA synthesis requires two key enzymes: a delta-12 desaturase that converts C18:1 to C18:2, followed by a delta-15 desaturase that converts C18:2 to C18:3 [162]. The data shown in Table 3 of **Paper 4** provide evidence of the regulation of this pathway. Under high CO<sub>2</sub>, the sharp decline in the end product C18:3 (from 39% to 11%) alongside the substantial accumulation of its precursor C18:1 (from 7.6% to 40%) may suggest that the high CO<sub>2</sub> concentration turned off these desaturase enzymes. This inhibition creates a metabolic bottleneck, limiting membrane lipid C18:3 formation and leading to the buildup of C18:1, which is then directed toward storage lipid synthesis.



**Figure 13.** Fatty acid composition and total fatty acid produced by *Chlorococcum* sp. cultivated in anaerobic digestion effluents under different CO<sub>2</sub> concentrations.

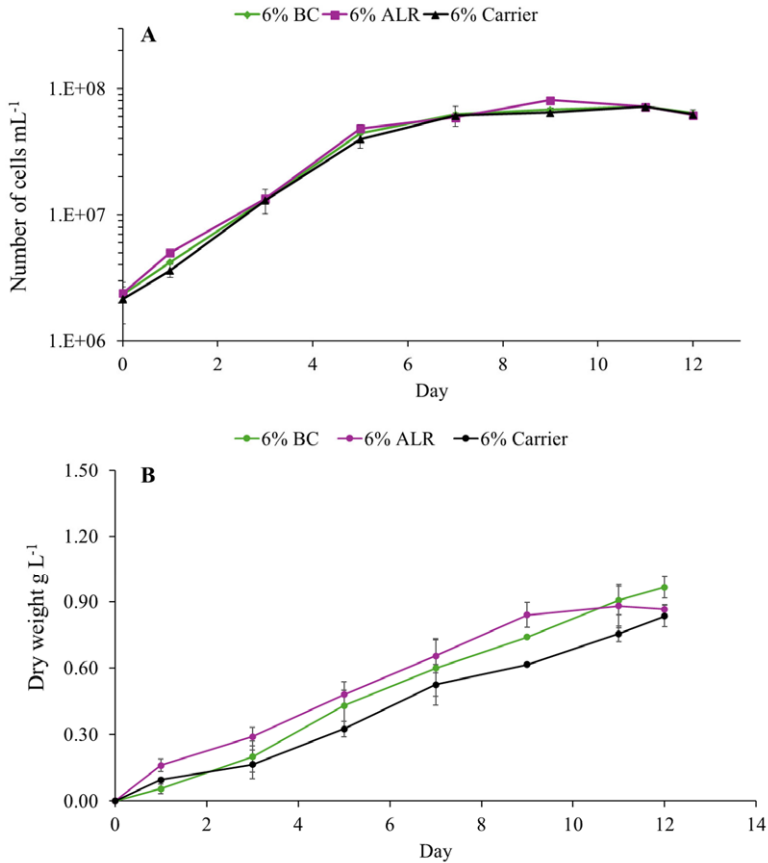
Finally, 6% CO<sub>2</sub> concentrations were selected for the second stage of this experimental work in **Paper 4** as this CO<sub>2</sub> concentration led to highest biomass production, highest CO<sub>2</sub> fixation rate among all conditions, comparable NH<sub>4</sub>-N and P removal compared to 3% as well as comparable total fatty acid production to 9% CO<sub>2</sub>.

### 3.5 The effect of reactor configurations

The optimal CO<sub>2</sub> concentration (6%) was applied to compare different reactor configurations. Three systems were evaluated: a standard BC, an ALR reactor, and a BC containing carriers (Figure 3).

Figure 14 shows the cell numbers and biomass concentration of *Chlorococcum* sp. cultivated in different reactor configurations over 12 days. The highest cell density ( $81 \times 10^6$  cells mL<sup>-1</sup>) was achieved with microalgae cultivated in ALR on day 9, followed by BC ( $72.5 \times 10^6$  cells mL<sup>-1</sup>) and BC with carriers ( $72 \times 10^6$  cells mL<sup>-1</sup>) both on day 11. Despite the higher cell count in the ALR, the cultures in BC produced more biomass ( $0.96 \pm 0.05$  g L<sup>-1</sup>) than ALR ( $0.86 \pm 0.02$  g L<sup>-1</sup>). This is likely because the ALR entered the stationary phase earlier due to its superior mixing and circulation, which prevent sedimentation and enhance light exposure [164]. Although microalgae cultivated in ALR showed more biomass production than the others until day 9, it declined after entering the stationary phase. Oncel, et al. [165] found that *Spirulina platensis* grew faster in an ALR (0.45 day<sup>-1</sup>) than in a BC (0.33 day<sup>-1</sup>). Likewise, *Nannochloropsis* sp. achieved higher cell concentrations in an ALR due to improved flow

patterns and light distribution [109]. However, Uyar, et al. [166] observed that *Chlorella sorokiniana* grew best in a BC, where a microporous sparger generated smaller bubbles and substantially increased volumetric mass transfer compared to ALR and stirred-tank systems.



**Figure 14.** Number of cells (A) and biomass concentration (B) of *Chlorococcum* sp. cultures grown in anaerobic digestion effluents using different reactor configurations. Adapted from **Paper 4**.

The highest removal rates for  $\text{NH}_4^+\text{-N}$  and phosphorus was observed in cultures grown in BC reactor, reaching 37.61% and 25.87%, respectively (Table 8). Microalgae cultivated in ALR and BC with carriers showed similar  $\text{NH}_4\text{-N}$  and phosphorus removal, with no significant differences between them. The biomass productivity was between 65.00 to 77.08  $\text{mg L}^{-1} \text{d}^{-1}$ , and the  $\text{CO}_2$  fixation rate ranged from 132.45 to 155.49  $\text{mg CO}_2 \text{L}^{-1} \text{d}^{-1}$ . Although these parameters were comparable across all reactor configurations, the BC with carriers exhibited a

slightly higher CO<sub>2</sub> fixation rate (155.49 mg CO<sub>2</sub> L<sup>-1</sup> d<sup>-1</sup>). This may be due to the carriers slowing the escape of CO<sub>2</sub> bubbles, thereby increasing their residence time in the liquid phase. However, this increase in CO<sub>2</sub> fixation rate was not statistically significant and the presence of carriers did not lead to any specific improvements in growth or nutrient removal, which may be attributed to light shading effects caused by the carriers themselves.

**Table 8.** Nutrients removal efficiency, biomass productivity, and CO<sub>2</sub> fixation rates under different reactor designs. Mean values that do not share a common letter within the same column differ significantly (p value < 0.05). Reproduced from **Paper 4**.

CO <sub>2</sub> (%)	Removal efficiency (%)		Productivity mg biomass L <sup>-1</sup> d <sup>-1</sup>	CO <sub>2</sub> fixation rate mg CO <sub>2</sub> L <sup>-1</sup> d <sup>-1</sup>
	NH <sub>4</sub> -N	P		
Reactors				
BC	37.61 ± 2.27 <sup>a</sup>	25.87 ± 0.48 <sup>a</sup>	65.00 ± 3.54 <sup>a</sup>	132.45 ± 5.18 <sup>a</sup>
ALR	23.22 ± 1.10 <sup>b</sup>	15.69 ± 2.18 <sup>b</sup>	67.50 ± 4.71 <sup>a</sup>	135.58 ± 12.40 <sup>a</sup>
Carrier	25.97 ± 2.09 <sup>b</sup>	16.79 ± 2.94 <sup>b</sup>	77.08 ± 5.30 <sup>a</sup>	155.49 ± 11.89 <sup>a</sup>

The individual fatty acids, total FAMES, protein and carbohydrate content were similar across all systems, showing that reactor design did not significantly affect these parameters (Data not shown here; see Table 5 in **Paper 4**). The overall consistency of these key biochemical components highlights the metabolic robustness of *Chlorococcum* sp. This stability is advantageous for industrial applications, as it suggests that reactor choice can be based on cost and productivity considerations without compromising biomass quality.

#### 4. Conclusion and Future work

This doctoral thesis investigated the potential of native Nordic microalgae as a biological solution for the treatment and valorization of AD liquid and gas effluents.

##### Conclusion

The initial screening established that native Nordic strains, particularly *Coelastrella* sp., *C. vulgaris* and *Chlorococcum* sp., were able to thrive in AD effluents like PPS and CKM. They were able to assimilate both inorganic nutrients and organic carbon in the form of volatile fatty acids, with a marked preference for acetic acid. While *Coelastrella* sp. and *C. vulgaris* were initially identified as the most consistent performers across both media, *Chlorococcum* sp. was specifically noted for its robustness in CKM, outperforming other native strains like *Scotiellopsis reticulata* and *Desmodesmus* sp. in ammonium-rich environments.

The research further defined the upper limits of nitrogen tolerance in AD effluent of municipal sewage sludge. It was concluded that ammonium nitrogen concentrations higher than 400 mg L<sup>-1</sup> are toxic to the tested strains. In more concentrated media (X5 and X7), *Chlorococcum* sp. demonstrated high resilience by achieving nitrogen removal efficiencies comparable to *Coelastrella* sp., despite experiencing significant lag phases of 7 and 11 days. In X10 media, *Chlorococcum* sp. reached complete (100%) ammonia nitrogen removal. Consequently, *C. vulgaris* and *Chlorococcum* sp. were selected for subsequent stages; *C. vulgaris* was retained as a well-established reference strain, while *Chlorococcum* sp. was chosen for its scientific novelty and robust physiological adaptation.

The investigation into light:dark cycles and carbon dioxide sparging clarified a critical process trade-off. While carbon dioxide supplementation is the primary driver for biomass accumulation and fatty acid synthesis, it was found to hinder nitrogen removal efficiency. In regimes without carbon dioxide sparging, the biological elevation of pH facilitates ammonia stripping. This suggests that for maximum remediation, aeration strategies must balance the need for inorganic carbon with the pH-induced nitrogen loss.

The final stage proved that reactor configuration dictates growth kinetics but does not compromise the biochemical quality of the biomass. The ALR achieved the highest cell density due to enhanced mixing and light distribution, whereas the BC supported higher total biomass dry weight and superior ammonium-nitrogen removal. The presence of carriers did not lead to any specific improvements in growth or nutrient removal, which may be attributed to light shading effects caused by the carriers themselves.

Finally, this research aligns with and supports multiple United Nations Sustainable Development Goals (SDGs). It supports SDG 13 (Climate Action), SDG 14 (Life Below Water), and SDG 15 (Life on Land) by preventing eutrophication while mitigating greenhouse gas emissions. Moreover, this research contributes to SDG 6 (Clean Water and Sanitation). The study also supports SDG 11 (Sustainable Cities and Communities) through enhancing the resilience of urban environments by integrating microalgal systems into municipal infrastructure.

### **Future Work**

The following research directions are proposed to optimize system performance, ensure year-round operational stability in Nordic climates, and validate the economic and environmental viability of the process.

1. Future research should transition from sterilized media to non-sterile conditions to evaluate the robustness of these Nordic strains against native microbial competitors found in AD effluent.
2. Investigating algal-bacterial co-cultivations to enhance system stability and performance.
3. To improve the economics of nutrient recovery, future studies should focus on higher density algal cultivations designed for faster uptake of nitrogen and phosphorus.
4. Optimize gas-liquid mass transfer and carbon dioxide residence time to maximize CO<sub>2</sub> removal using different cultivation designs, for example algal immobilization using filamentous fungi biomass.
5. Future analysis should verify the quality of algal biomass for biochar, biopolymers (such as PHA or PHB), biofuels and biofertilizers production.
6. Future research should focus on the performance of these Nordic strains at lower temperatures to ensure year-round wastewater treatment capability in Nordic climates, where extreme seasonal fluctuations directly influence enzymatic activity, biomass productivity, and the solubility of gases like carbon dioxide.
7. Transition from laboratory experiments to pilot-scale applications to validate its feasibility under industrial conditions. A critical challenge for industrial implementation is the continuous treatment of large wastewater volumes without long-term storage. Future research must, therefore, determine optimal large-scale bioreactor designs

capable of maintaining year-round performance despite extreme seasonal variations in light and temperature in Nordic countries.

8. A comprehensive techno-economic and life cycle assessment is essential to determine the commercial and environmental viability of integrating these microalgal systems into existing municipal biogas and sewage infrastructures. This analysis should account for the energy requirements of energy-intensive harvesting and dewatering steps, pilot-scale pumping, the costs of carbon capture technology, and the potential revenue from recovered biomass.

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Ghasem Mohammadkhani

January 2026, Borås

## Declaration about the use of generative AI

Generative AI tools (Microsoft Copilot and Gemini 3) have been used to improve the language and enhance the overall readability of this thesis.

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