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Microalgae

Current and Potential Applications

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Microalgae - Current and Potential Applications

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Meet the editor



Sevcay Aydin is a professor in the Biology Department, Istanbul University, Türkiye. She earned her bachelor's degree from the Biology Department, Ege University, Türkiye, and her Ph.D. from the Environmental Biotechnology Program, Istanbul Technical University. Dr. Aydin holds a professorship in Environmental Engineering, with research interests spanning environmental biotechnology, renewable energy production, bioremediation, and environmental engineering. With forty-nine scientific articles in high-impact journals, Dr. Aydin imparts her knowledge by teaching biotechnological approaches to water treatment, environmental technologies, and bioenergy production. She focuses on optimizing bioenergy potential from various wastes and creating cost-effective strategies for polluted soil bioremediation. Specifically, she explores microalgae applications to enhance biogas production and address environmental challenges and biodiversity loss.

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Microalgae as a Promising Candidate for Mitigating Climate Change
and Biodiversity Loss

by Şuheda Reisoglu and Sevcen Aydın

Preface

Microalgae, as microscopic photosynthetic organisms, have emerged as pivotal subjects of scientific inquiry due to their unparalleled biodiversity and versatile biochemical capabilities. Within their cellular structures, these microorganisms harbor complex metabolic pathways and biochemical reactions that enable the synthesis of diverse and valuable compounds. Beyond their fundamental roles in oxygen production and carbon sequestration, microalgae present an extensive array of applications. In *Microalgae – Current and Potential Applications*, readers are invited to reveal the intricate realm of microalgae, unravelling their biodiversity, applications, and potential contributions to a sustainable future. Eight chapters of this book navigate through various facets, from the cellular structure of microalgae to their pivotal roles in bio-fertilizer synthesis, phycoremediation, and the production of bioactive compounds. The book offers insights into contemporary research frontiers, addressing biodiesel production, the prolific synthesis of docosahexaenoic acid (DHA), and the crucial role microalgae play in mitigating climate change and biodiversity loss. With each chapter, readers explore the complexities of microalgae technology, learning about cutting-edge developments, challenges, and transformative potential inherent in these microscopic organisms. This collective exploration aspires to contribute not only to the understanding of microalgae but also to the broader perspective on sustainable biotechnological practices and the global impact of microalgae technology.

Chapter 1 is the introductory chapter.

Chapter 2 delves into the critical task of identifying microalgae biodiversity. The chapter specifically centers on microalgae, sequentially delving into their cellular structure and plastid evolution. The chapter navigates through sample collection protocols and identification methods, crucial components in understanding the intricate diversity of microalgae within ecosystems. This comprehensive exploration contributes to the preservation of biodiversity and the untapped potentials of microalgae and their byproducts.

Chapter 3 addresses the practical implementation and contemporary outlook regarding the extraction of microalgae biomass from crops in wastewater, which is a crucial raw material for the synthesis of biofertilizers, biostimulants, and feed additives, fostering resource reuse, transformation, recovery, and conservation. The overarching goal of this approach is to advance bioeconomic and circular economy processes. The chapter provides insights into organic waste origins, waste management strategies, challenges in aquaculture systems, and the role of microalgae in assisting aquaculture. Additionally, it explores sustainable agricultural practices facilitated by microalgae, contributing to a holistic understanding of the potential applications and benefits in the context of resource efficiency and environmental sustainability.

Chapter 4 discusses the prospective application of microalgae biodiversity in the intricate field of phycoremediation. The chapter encompasses a comprehensive

exploration of the mechanisms underlying the tolerance and resistance of microalgae to heavy metals. It scrutinizes the feasibility of employing consortia of microorganisms as opposed to individual species, particularly in addressing the challenges posed by complex polluted matrices. Furthermore, the chapter delves into the potential for metabolic engineering of microalgae, aiming to enhance their inherent functionalities. The chapter also investigates the multifaceted usage of microalgae, not only in liquid environments but also within solid devices designed for phycoremediation. This exploration seeks to unveil the myriad possibilities and applications that arise from understanding and manipulating microalgae in the context of environmental remediation strategies.

Chapter 5 conducts a thorough exploration of the realm of microalgae-derived lead compounds and their diverse bioactivities. This investigation serves as a foundational cornerstone for the intriguing concept of a multi-product cell factory. The examination encompasses a broad overview of contemporary downstream processing (DSP) methods currently operational within industrial-scale microalgal biorefineries, shedding light on the pivotal techniques that drive efficiency and productivity in this domain. This chapter transitions into an in-depth examination of the cutting-edge developments in research and development pertaining to multi-products derived from specific microalgal species. Notably, the focus extends to well-known varieties such as *Spirulina*, *Chlorella* spp., and *Haematococcus pluvialis*. These microalgae are showcased as exemplars in the ongoing efforts to unlock and harness their diverse bioeconomic potential. By delving into the specifics of these species and their multi-product outputs, the chapter underscores the significance of such endeavors in shaping the future trajectory of sustainable biotechnological practices within the realm of microalgae.

Chapter 6 is a comprehensive synthesis of the strides made in operating the biodiesel production process, particularly in the realm of transesterification. Focusing on the in situ, direct, and supercritical oil transesterification processes of microalgae, with a specific focus on the genera *Chlorella*, *Scenedesmus*, *Spirulina*, and *Nannochloropsis*, the chapter presents a view of the advancements achieved under various operating conditions.

Chapter 7 delves into the remarkable potential of microalgae as a prolific source of DHA, an omega-3 polyunsaturated fatty acid crucial for human health. Microalgae, known for their rapid growth and cost-effectiveness, accumulate high lipid levels, and this chapter explores their role as efficient DHA producers. Addressing the growing demand for this essential nutrient, the chapter addresses the optimization of microalgae cultivation conditions, diverse engineering techniques for enhanced DHA yields, and the concise perspective of dietary consumption.

Chapter 8 discusses microalgae as candidate tools for mitigating climate change and biodiversity loss in a holistic approach. The chapter starts with the integration of biodiesel and biogas production from microalgae biomass, maximizing energy yield. It then goes on to explore the potential of microalgae in heavy metal bioremediation, highlighting studies that integrate wastewater treatment and biodiesel production. The chapter emphasizes the environmental risks posed by contaminated water from various industries and discusses conventional chemical and biological treatment

methods. It highlights the potential of microalgae in biological wastewater treatment, showcasing their efficiency in nutrient removal and their valuable biomass. The chapter indicates the significance of microalgae-derived biofertilizers in fostering sustainable agriculture. It addresses challenges like soil degradation and the environmental impact of chemical fertilizers. The chapter explores the role of microalgae-based products in addressing the interconnected challenges of climate change and biodiversity loss, in terms of various contexts, such as soil bioremediation, promoting soil fertility, and enhancing microbial diversity. Finally, the chapter discusses the global market and economic aspects of microalgae technology, emphasizing their biodiversity and ability to synthesize valuable compounds.

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Introductory Chapter: Microalgae – A Versatile and Valuable Resource for Sustainable Development

Sevcan Aydin

1. Introduction to Microalgae

Microalgae, microscopic organisms that have silently thrived for over a billion years, represent an evolutionary tapestry of unparalleled complexity [1]. They have evolved to adapt to various environmental conditions, such as light, temperature, salinity, pH, nutrient availability, and stress factors, resulting in a remarkable diversity of morphological, physiological, biochemical, and genetic traits. Their adaptive journey has taken them from primordial oceans to diverse ecosystems, ranging from arctic tundras to the sun-drenched expanses of deserts [2]. Their astounding genetic diversity, estimated to encompass around 72,500 species, forms the foundation of their versatility and resilience [3]. These organisms, hidden from the naked eye, emerge as architects of Earth's ecosystems. Their contribution to the oxygenation of Earth's atmosphere and their pivotal role in the carbon cycle underscores their significance in global biogeochemical cycles [4]. The intricate ballet of pigments and proteins involved in microalgal photosynthesis not only sustains their existence but also has profound implications for atmospheric composition and climate regulation [5].

Microalgae have long been recognized as important primary producers and ecological agents in aquatic ecosystems, contributing to the global carbon cycle, oxygen production, nutrient cycling, and food webs [6]. However, in recent decades, microalgae have also emerged as a promising source of valuable products and services for various human applications, such as food, feed, fertilizer, biofuel, bioremediation, biotechnology, and biopharmaceuticals [7]. Microalgae have several advantages over other biomass sources, such as high productivity, fast growth, wide distribution, low land and water requirements, and the ability to utilize waste streams and CO₂ as inputs [8]. Moreover, microalgae can produce a wide range of compounds with commercial interest, such as lipids, proteins, carbohydrates, pigments, antioxidants, vitamins, minerals, polysaccharides, and bioactive molecules [9].

The interest and demand for microalgae and their products have increased significantly in the last few years due to the growing awareness and concern about the global challenges of energy security, food security, environmental protection, and human health [10]. Microalgae offer a sustainable and renewable alternative to fossil fuels, animal feed, chemical fertilizers, synthetic drugs, and other conventional resources,

which are becoming scarce, expensive, and harmful to the planet and its inhabitants [11]. Microalgae also have the potential to mitigate the impacts of climate change and biodiversity loss, by sequestering carbon, reducing greenhouse gas emissions, producing bioenergy, and restoring and conserving ecosystems [12]. Nonetheless, despite the considerable potential and prospects offered by microalgae, numerous obstacles and impediments must be addressed in order to realize the widespread and economically efficient production and utilization of both microalgae and their derivatives. These include the optimization and integration of the cultivation, harvesting, and processing technologies, the improvement and modification of the microalgal strains and products, the development and standardization of the quality and safety criteria and regulations, the assessment and minimization of the environmental and social impacts, and the enhancement and diversification of the market and consumer acceptance [13].

Microalgae, with their intricate molecular machinery, hold the potential to redefine our relationship with the environment, providing sustainable solutions to global challenges. The purpose of this book is to provide a comprehensive and updated overview of the current and potential applications of microalgae in various fields, highlighting the challenges, opportunities, and future prospects. The book is intended for researchers, students, professionals, and policymakers interested in the field of microalgae and its applications. The book is divided into seven chapters, each covering a different topic related to the applications of microalgae. Chapter 1 introduces the diversity and classification of microalgae and the methods and strategies for their identification and characterization. Chapter 2 reviews the main challenges of traditional aquaculture and microalgae for sustainable agricultural practices as biofertilizers in agriculture and aquaculture. Chapter 3 explores the use of microalgae as bioremediation agents. Chapter 4 describes the production and extraction of various high-value compounds from microalgae, such as lipids, proteins, polysaccharides, and bioactive molecules, and their quality, functionality, and applications. Chapter 5 provides an overview of the progress made in optimizing the in situ, direct, and supercritical oil transesterification processes for microalgae belonging to the genera *Chlorella*, *Scenedesmus*, *Spirulina*, and *Nannochloropsis* under different operating conditions. Chapter 6 focuses on the production of docosahexaenoic acid (DHA), an omega-3 fatty acid with numerous health benefits, from microalgae. And finally, chapter 7 evaluates the potential of microalgae for mitigating the two major global threats of climate change and biodiversity loss by analyzing the role of microalgae in carbon sequestration, greenhouse gas emission reduction, bioenergy production, and ecosystem restoration and conservation.

We hope that this book will inspire and stimulate the readers to explore the versatile and valuable resource of microalgae and to contribute to the sustainable development of humanity and the planet.

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Unlocking the Richness of Microalgae Biodiversity for Potential Applications

Amal Badr and Walid Fouad

Abstract

Identifying microalgae biodiversity is essential to unleashing the diverse potential applications for microalgae. The aim of the chapter is to define the various tools and methodologies possible for revealing microalgae diversity. Identifying microalgae from natural environments, hotspots, and extreme environments enables us to isolate naturally tolerant species that may thrive and exhibit unique characteristics. Identifying microalgae will enable researchers to uncover new applications for these versatile organisms, including biofuel production, wastewater treatment, and pharmaceutical development. Hence, it is crucial to define the different possible identification approaches, beginning with the sample collection protocols and extending to the identification methods employed to determine the diversity of microalgae in an ecosystem.

Keywords: microalgae, biodiversity, identification tools, algal richness, culture enrichment

1. Introduction

Algae are a highly diverse group of photosynthetic organisms that can thrive in various aquatic and soil environments. They are categorized based on their size, with macroalgae being visible to the naked eye and microalgae being microscopic. Macroalgae, also known as seaweed, height can vary from a few cm and can reach up to 53 m in height if present in ideal conditions [1], such as the Ochrophyta genus *Sargassum* and *Macrocystis* [2, 3]. Microscopic algae can range from 0.2–200 μm and are further classified into picoalgae (algae size between 2 and 5 μm), and microalgae [4, 5]. Algae can be prokaryotic or eukaryotic, with eukaryotic algae belonging to various phyla such as Rhodophyta (red algae), Chlorophyta (green algae), Glaucophyta, Euglenophyta, Dinoflagellates, Cryptophyta, Heterokonts, Ochrophyta (brown algae), Eustigmatophyta, Bacillariophyta (diatoms), and Haptophyta. Despite algae being tremendously rich with essential by-products that are useful for many industrial applications, only about 30,000 species of algae have been identified and studied due to limitations in research on microalgal biodiversity [6, 7]. The lack of taxonomists and researchers in this field has resulted in inadequate records of microalgae diversity and the loss of their potential uses [8].

Why is it important to study biodiversity? A remarkable feature of our planet Earth is its ability to sustain a great variety of life. However, one of the most prevalent occurring environmental changes is the loss of this biological variation. Scientists have determined that the leading cause of species extinction is due to the drastic anthropogenic changes we currently face [9, 10]. Research indicated that the rate at which different resources are deteriorating is increasing, and water quality is decreasing exponentially with the declining species diversity [11]. The decrease in diversity would eventually lead to the homogenization of an ecosystem and, as a result, would lead to a decrease in the functionality of this ecosystem [12, 13]. The absence of heterogeneity in an ecosystem would further result in the loss of energy fluxes and matter that are essential to all ecological processes, which also includes the loss of control over the abundance, biomass, and distribution of organisms in an ecosystem [10, 14, 15]. For instance, an example of a food chain would include microalgae and picoalgae as the primary producer in an aquatic ecosystem. The absence or abundance of algae can lead to the interruption of this food chain due to the low evenness, which means that the community has a few abundant species that are dominating the community and could consequently result in the loss of the following consumers in the food chain [16]. The functionality of an environment is considered preserved when evenness is high, and the community contains various species that equally exist, with no one species gaining the upper hand and dominating an ecosystem [17–19].

The United Nations (UN) has expressed concern about the loss of habitats and species, which threatens the functionality of our ecosystem and planet Earth. In response, the UN declared 2010 as the International Year of Biodiversity and developed the Strategic Plan for Biodiversity 2011–2020 (CBD, 2010), which aims to improve the science base and biodiversity-related technologies by 2020. To achieve this goal, biodiversity analysis and studies must be intensified to preserve and protect the remaining biodiversity [20]. This chapter will focus on microalgae and will discuss the different methods used to collect environmental samples and identify microalgae diversity present in their natural environments, which will ultimately facilitate the conservation of biodiversity and preserve the known and unknown potentials of microalgae and their by-products.

2. Microalgae cellular structure importance to microalgae identification

Microalgae are microorganisms that belong to the kingdom Protista. Microalgae have different morphological characteristics that, when observed under the microscope, aid in taxonomic-level identification and classification. However, this is not the case when it comes to picoalgae such as *Synechococcus* and *Chrysochromulina*, which are extremely small-sized and are difficult to be identified on a morphological level [21, 22]. Hence, they are mostly classified through ribosomal RNA (rRNA) or other identical tools that do not depend on morphological characteristics.

2.1 Microalgae basic cellular structure

Microalgae cellular organization that can be used to distinguish microalgae includes unicellular (non-motile and motile), colonial (coenobitic and non-coenobitic), and filamentous (simple, branched, and heterotrichous). A feature that can clearly help in the identification of microalgae is their ability to locomotion [23, 24]. The motility of microalgae is of two types: flagellar motility and gliding motility [25].

Euglena and *Pedinomonas* are examples of microalgae that have flagella. Microalgae that do not have flagella still have the ability to glide, such as *Synechococcus* and *Oscillatoria* [26, 27]. Chlorophyta is the only phylum of microalgae that grows in the form of coenobitic colonies (such as *Volvox*) [28]. While non-coenobitic microalgae retain a gelatinous matrix or are found to be attached by a gelatinous stalk [29, 30]. Eukaryotic microalgae cells have organelles that are membrane-bound and unlike bacteria [31]. The prokaryotic cyanobacteria, also known as blue-green algae, are considered photosynthetic bacteria. Their deoxyribonucleic acid (DNA) lies freely in the cytoplasm, yet they, like microalgae, can carry oxygenic photosynthesis (conversion of CO₂ and water to sugars using the energy from light) even though they lack a true chloroplast. In cyanobacteria, photosynthetic electron transport occurs in the thylakoid membranes present in the cytoplasm [32, 33]. Through evolution, eukaryotic microalgae have gained their cellular complexity from cyanobacteria endosymbiosis.

2.2 Plastid evolution and microalgae pigments

The plastid in microalgae originated as a result of endosymbiosis with cyanobacterium (as the primary chloroplast ancestors) [34, 35]. Further evolution of the plastid in microalgae occurred through a series of endosymbiotic events in which the primary endosymbiosis gave rise to the plastid of red algae, green algae, and plants chloroplast [35–37]. The secondary symbiosis resulted in more diversification and complexity of the plastid of microalgae species. Euglenoids, chlorarachniophytes, and a small group of dinoflagellates have up-taken the green algae chloroplast through the secondary endosymbiosis resulting in the three-membrane plastid [37]. The secondary endosymbiosis of glaucophytes and red algae further resulted in the presence of four-membrane-bound plastids that can be identified in Chlorarachniophytes, Haptophytes, and Heterokonts.

Certain plastid genes have been noted to track the origins of the plastid to cyanobacteria. RuBisCO genes, responsible for the production of ribulose-1,5-bisphosphate carboxylase/oxygenase, a critical enzyme in carbon fixation, have created confusion about the endosymbiotic events and the existence of microalgae from cyanobacteria [38]. Interestingly, some algae groups contained similar non-cyanobacteria enzymes. However, this has been elucidated by confirming that several gene duplications and subsequent gene divergence resulted in the paralogous sequences of the gene that have been transferred through horizontal gene transfer, giving rise to the Rubisco phylogeny [39]. By comparing the genome of cyanobacteria to that of a eukaryotic microalgae plastid, it was found that the number of genes significantly decreased. This is because several plastid genes have been moved to the nucleus, and some genes have been lost due to their redundant function [34]. In addition, there are various heterotrophic microalgae that are non-photosynthesis, including certain lineages of Euglenophytes (*E. gracilis* and *E. longa*), diatoms (*Nitzschia* sp. strain NIES-3581), and green algae *Polytomella* spp. and *Helicosporidium* sp. Although these microalgae lack genes related to photosynthesis in their plastids, the plastids still play a crucial role in their survival by participating in various biological pathways other than photosynthesis [40–42].

The type of pigmentation present within the plastids also reflects the plastid lineage and phylogeny of the microalgae. The three major pigment classes present in microalgae are chlorophylls, carotenoids, and phycobiliproteins. The pigments absorb light at different wavelengths making their productivity depend on the light regimen they are exposed to. Microalgae, such as Chlorophyta (green algae), contain chlorophyll *a* and

b pigments that produce absorption peaks at wavelengths between 300 and 700 nm, which is the wavelength of the visible spectrum [43]. Chlorophyll *a* absorbs light in the red-violet region and is present in all the microalgae phyla, while chlorophyll *b* absorbs light in the red-blue region, and both reflect green light [44–46]. Hence, the green appearance of the green microalgae. Cyanobacteria, on the other hand, lack chlorophyll *b* and favor low light intensities. Even though cyanobacteria lack chlorophyll *b*, however, the capture of photons is compensated by the presence of Phycobilisomes (PBS) light-harvesting antennae that contain chromophores, also known as phycobilins that are capable of absorbing orange, green, and blue-green light at wavelength 620 nm, 545 nm, and 495 nm, respectively [47, 48]. Carotenoids are terpenoid pigments that play an important role in photosynthesis as they harvest light within 400 and 550 nm and transfer it to chlorophyll *a* [49]. They also play an important role in protecting the microalgae cell and its photosynthetic machinery from reactive oxygen species (ROS) [50]. Unicellular and multicellular microalgae can have one or more chloroplasts present in a cell. Chloroplast division in microalgae occurs once per cell cycle [51], while in a plant, each cell may have up to 20 chloroplasts in each mesophyll cell of a plant [52]. Microalgae cells under the algae order Chlorococcales and Volvocales follow a cell cycle known as the multiple fission cycle (Cn). Under optimum conditions (in terms of light and temperature), they can divide into 8–16 identical daughter cells in the cell division cycle of about 6–12 h [53, 54].

3. Acquisition of environmental samples for studying algal diversity

Microalgae are typically found in environmental sources such as rivers, lakes, oceans, and soil. To identify the diversity of microalgae in a particular ecosystem, selecting the appropriate sample site is crucial. When choosing a sampling site, certain conditions must be taken into account. The Environmental Protection Authority (EPA) recommends developing a monitoring plan that considers various factors to ensure consistency in sampling. These measures are necessary to maintain sample integrity, as any changes to the conditions may alter the sample and lead to misleading information. Mismanagement of water and soil resources can result from inaccurate analysis of such samples [55, 56].

3.1 Sample collection methods

3.1.1 Manual sampling (grab/hand samples)

Water or soil samples can be collected manually by hand in a collection bottle. Using this method limits sampling to surface sampling. This method is cost-effective and does not eliminate microalgae depending on their size; however, this method limits the collection to microalgae with high buoyancy due to the presence of gas vacuoles that are mainly present in cyanobacteria [57–59].

3.1.2 Plankton nets

Nets are made up of nylon or silk and have a cylinder or valve attached to the bottom for sample collection. The nets come in different mesh sizes that can range from 10 to 1000 microns [60]. Plankton nets are used to quantitatively and qualitatively identify microalgae present in water sources. The net can be used to collect samples

horizontally from a water surface, by adding weights to the net a vertical sample can be collected from the depth of the water source. The mesh size also plays an important role in the identification of microalgae from environmental samples. The size of the mesh selected can lead to the elimination of certain microalgae from the analysis of the environmental sample. Furthermore, the identified microalgae only mirror the filtering capability of the mesh [55]. Small-sized mesh samples are easily clogged, while large-sized mesh leads to the loss of smaller-sized microalgae.

3.1.3 Van Dorn sample bottle

The Van Dorn samplers enable depth sampling of water sources. The sampler consists of a hollow tube that is made up of PVC (Polyvinyl chloride) or acrylic that is connected to a rope. The tube is fitted with a seal at both ends that seals to lock the water collected in. The sampler is deposited in the water source to the desired depth, and the seals are released to lock the water in. The sample volume collected is known and the sample is drained through a drain valve [61]. The Schindler-Patalas trap is a similar tool that combines the Van Dorn and a phytoplankton net. The trap box is fitted with a mesh net at the bottom of the wall of the trap through which the collected sample is filtered after collection.

3.1.4 Van Veen grab sampler

The Van Veen grab sampler can be used to collect samples from soil or sediments. The sampler consists of claws that can grab sediments. Soil samples need to be either soaked in autoclaved distilled water or an enrichment media to extract and enhance the soil microalgae into a liquid media to enable and ease the microalgae identification.

3.2 Sample processing

Sample preservation may be necessary to maintain the integrity of the morphological features of the microalgae collected. Common fixatives used in preserving and fixing microalgae and protists are formaldehyde and Lugol's iodine solution. Fixative stains at a low concentration can aid in morphological identification. However, the addition of a higher concentration may lead to the loss of flagellum and sometimes cell loss [62] and may hinder the molecular screening process [63].

To isolate a single cell from liquid media, one can use a micropipette or a glass capillary tube. The latter can be made by manually stretching a Pasteur pipette under a flame to create a capillary tube. This technique is useful for isolating single microalgae cells from a small sample drop under an inverted microscope. However, it may require expertise in both the isolation technique and microalgae identification. Another method of microalgae isolation is the streak plating of environmental samples on solid agar, usually a concentration of agar between 0.8 and 2%. This can be directly from an environmental sample or following a serial dilution of the sample. The plating of environmental samples can limit the identification of non-motile microalgae. However, several other methodologies may be involved in the identification of microalgae. Serial dilution is one of the most common methods that includes the preparation of serial dilution of a collected water sample or soaked soil sample with autoclaved distilled water. The dilution factor is maintained at each step [64]. The dilution set created can be cultured on an enrichment liquid or solid media. This method enables isolating

single microalgae cells from the environment [65, 66]. The enrichment of a dilution on a liquid enrichment media can enable the identification of motile and non-motile microalgae. Fluorescence-activated cell sorting (FACS) and flow cytometry (FCM) cell sorting are two high-throughput automated isolation and screening techniques. The technique allows isolation and identification of microorganisms based on particle volume, fluorescence, and the cell's ability to scatter light [67–69].

3.3 Enrichment of sample by nutrient media

An easy and inexpensive approach that is commonly used for the identification of an environmental sample is the use of culture media to enrich species in an environmental sample, followed by using microscopy to identify and isolate the species observed in the sample or a more advanced identification tool. Even though sample enrichment is considered a “golden tool” to analyze the diversity of an unknown sample, it is similar to the direct identification method, which depends on the resolution of the microscope and the expertise of the workforce [70]. Currently, the enrichment of environmental samples is usually followed up with identification on a molecular level to confirm the phylogenetic classification of the microalgae isolated [66, 71–77]. Microalgae can either survive as free organisms or co-exist in the presence of other organisms, such as lichens, in which the microalgae are said to live in symbiosis with other fungi and bacteria [78]. Therefore, enriching an environmental sample can help identify species that are not easily observable but are present only in large populations. Hence, optimization of nutrient composition, pH, temperature, and light intensity to enrich microalgae species and mimic the naturalistic conditions is necessary to ensure the identification of maximum diversity enrichment. Commonly used media like Bold's Basal Media (BBM) and Blue-green media (BG-11) can be used to prevent favoritism toward specific genera or phyla. However, selective enrichment media may also be required to nourish specific microalgae phyla that require extra elements, such as diatoms that need silica [79] found in F/2 nutrient media [80, 81] (Table 1). This section focuses on the different culturing requirements of microalgae since it is important to consider their effects on microalgae identification, growth, and by-product production. Some culturing parameters that may require optimization for improvement of microalgae identification and isolation include the following:

Synthetic media	Main phyla enriched	Illumination	Carbon source	Species enriched	Ref.
BBM	Chlorophyta	25 $\mu\text{mol photons m}^{-2} \text{ day}^{-1}$ (continuous) Fluorescent lamps-5 Days	5% CO_2 aeration	• <i>Desmodesmus sp.</i> • <i>Chlamydomonas sp.</i>	[82]
		2000 lx (12:12 h) Fluorescent lamps-10 days	2% CO_2 aeration	• <i>Chlorella</i> • <i>Parachlorella</i> • <i>Desmodesmus sp.</i> • <i>Micractinium sp.</i>	[83]
		200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (continuous)—14 days	0.5 g L^{-1} Sodium carbonate (agar media)	• <i>Chlorella singularis</i>	[84]

Synthetic media	Main phyla enriched	Illumination	Carbon source	Species enriched	Ref.
BG-11	Cyanophyta and Chlorophyta	150 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (continuous)—Fluorescent lamps –14 Ddays	98% air: 2% CO_2 aeration	<i>Chlamydomodium fusiforme</i> (culture maintenance and initiation)	[85]
		$75 \pm 7 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (continuous)—Fluorescent lamps – 3 months (initiation culture)	Shaking 130 rpm for atmospheric CO_2 dissolution (~410 ppm) and sodium carbonate in BG-11 (0.19 mM)	<i>Anabaena sp. strain UTEX 2576</i> (culture maintenance BG-11 missing nitrogen to maintain heterocyst)	[86]
		125 $\mu\text{E m}^{-2} \text{ s}^{-1}$ (12:12 h) Fluorescent lamps	Sodium carbonate in BG-11 (0.19 mM)	• <i>Ankistrodesmus</i> • <i>Botryococcus braunii</i> • <i>Botryococcus sudeticus</i> • <i>Chlorella vulgaris</i> • <i>Nannochloris oculata</i> • <i>Scenedesmus dimorphus</i> • <i>Anabaena variabilis</i> • <i>Synechocystis sp.</i>	[87]
Walne's medium	Diatoms	1300–2500 lx	Not mentioned	• <i>Skeletonema sp.</i>	[88]
		30 $\mu\text{Em}^{-2} \text{ s}^{-1}$ (12:12 h) Fluorescent lamps-10 Days	Not mentioned	• <i>Amphora sp.</i>	[89]
Guillard's F/2 medium	Chlorophyta and Diatoms	120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (continuous)—Fluorescent lamps	Continuous aeration	• <i>Dunaliella tertiolecta</i> • <i>Chlorella sp.</i> • <i>Tetraselmis sp.</i> • <i>Nitzschia sp.</i> • <i>Thalassiosira rotula</i> • <i>Bacillariophyta sp.</i> • <i>Phaeodactylum tricornutum</i> • <i>Cylindrotheca closterium</i> • <i>Cymbella sp.</i> • <i>Navicula sp.</i>	[90]
		250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (12:12 h)—Fluorescent lamps – 14 days	Continuous aeration	• <i>Navicula incerta</i>	[91]
		80 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (continuous)—Fluorescent lamps	Not mentioned	• <i>P. tricornutum</i>	[91]
		3000 lx (18 h light:6 h dark) – Fluorescent lamps	Not mentioned	• <i>Pennate Diatoms</i> • <i>Chlorella sp.</i>	[92]
		90 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and 200 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ —Fluorescent lamps – 8 days	Continuous aeration and Sodium bicarbonate in media	• <i>C. vulgaris</i> • <i>Scenedesmus sp.</i> • <i>Coelastrum sp.</i>	[93]

Synthetic media	Main phyla enriched	Illumination	Carbon source	Species enriched	Ref.
Jaworski's medium	Chlorophyta	10.8 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (continuous)—Fluorescent lamps	Continuous aeration and Sodium bicarbonate in media	• <i>Chlorella sp.</i> • <i>Scenedesmus sp.</i> • <i>Monoraphidium sp.</i> • <i>Carteria sp.</i>	[94]
		200–250 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ (18 h light:6 h dark)—LED light—20 days	Continuous aeration and Sodium bicarbonate in media and Glycerol	• <i>C. vulgaris</i> • <i>B. braunii</i> • <i>Scenedesmus sp.</i>	[95]

Table 1.
Synthetic nutrient media commonly used for microalgae enrichment.

3.3.1 Light

Microalgae are photosynthetic microorganisms that absorb light energy and convert it to chemical energy in the photosynthetic apparatus, the photosystem. The photosystem complex contains pigments such as chlorophyll or carotenoid that absorb light energy. Light energy is measured in irradiance; photons of light that are used photosynthetically are known as photosynthetically active radiation (PAR). PAR is usually reported in $\mu\text{mol m}^{-2} \text{s}^{-1}$ or in $\mu\text{E m}^{-2} \text{s}^{-1}$. Light intensity has been vastly studied to increase algae production and growth rates [67–69]. Artificial light is widely used in photobioreactors, with light-emitting diodes (LEDs) being a common choice. Research has focused on the impact of light color on growth, carbohydrate, protein, and pigment content. For instance, red light has been found to increase biomass, while blue light increases the cell concentration of *Chlorella kessleri* [96]. In a study by Jin et al., monochromatic red light (660 nm) resulted in decreased biomass and cellular components, leading to reduced carbohydrate, carotenoid, and protein levels in *Dunaliella salina*. Conversely, monochromatic blue light (445 nm) did not significantly increase biomass production but led to the accumulation of lipids, proteins, and carbohydrates. Combining red and blue light resulted in higher dried biomass and cell content compared to white light [97, 98]. White light increased the cellular productivity and protein content of *Isochrysis zhanjiangensis*, while red light increased the specific growth rate. Greenlight improved the pigment content of *Isochrysis sp.* but reduced biomass, and blue light promoted carbohydrate content [99]. In another study, *Chlamydomonas reinhardtii* exposed to yellow light resulted in the highest biomass concentration, while blue and red light separately resulted in the lowest biomass [100]. The photosynthetic process and growth of microalgae are significantly affected by the duration, wavelength, and intensity of light exposure [101, 102]. Hence, the choice of light color is essential for both microalgae cultivation and the production of specific biochemical by-products, which at the end might favor one species over another, impacting population diversity.

3.3.2 Nutrients

The growth of microalgae and the synthesis of proteins, lipids, and carbohydrates require macronutrients, mainly nitrogen (N), phosphorus (P), and potassium (K). Nitrogen is considered a crucial element as it forms a significant portion of structural

molecules in microalgae, including nucleic acid, amines, and is also needed for cell wall components and pigment production [103–105]. Microalgae can obtain various forms of nitrogen from their surroundings, inorganic nitrogen, such as nitrates, nitrites, ammonium, and the organic nitrogen in the form of urea. Ammonium nitrate is taken up more easily and at a higher rate by microalgae. Nitrates, on the other hand, are converted into ammonium inside the microalgae chloroplast, making ammonium a better option in terms of the energy needed for the nitrate-to-ammonium conversion [106, 107]. However, microalgae exposure to high ammonium concentration can suppress nitrate uptake, possibly due to a negative feedback loop, which may be attributed to the high production of glutamine in the cell upon exposure to ammonium [108]. Hence nitrates are currently being commonly used in synthetic media. Phosphorus is another essential macronutrient that is required by microalgae to produce nucleic acid, phospholipids, and ATP, as well as for cell division and growth [109]. Insufficient phosphorus can result in decreased lipid content [110] and a decline in chlorophyll *a* levels, as phosphorus is necessary for its production [111]. Even though they are required in minute quantities, vitamins significantly influence the cellular biochemistry of microalgae. Microalgae exist in a mutualistic symbiotic relationship with bacteria, where they obtain vitamin B (cobalamin, thiamine, and biotin) produced by bacteria in exchange for carbon fixation [112]. As microalgae are unable to produce these vitamins *de novo*, they must be supplemented in synthetic media [113, 114].

3.3.3 Carbon sources

Microalgae require carbon sources for cell growth and the production of necessary primary metabolites such as carbohydrates, lipids, and proteins. Microalgae also can utilize different carbon sources. They can be cultured under autotrophic conditions using inorganic carbon such as carbon dioxide (CO₂) or bicarbonate and light to produce energy through the process of photosynthesis. Yeh et al. (2010) tested the impact of using sodium bicarbonate (NaHCO₃) as a carbon source on *Chlorella vulgaris*, the higher the concentration of NaHCO₃ (1 g/L), the higher the biomass production along with the optimum light intensity [115]. Microalgae can also be heterotrophs under lightless conditions using organic carbon sources such as glucose or glycerol [116–118], while microalgae in the presence of light in mixotrophic cultures can use either organic or inorganic carbon sources. *Phaeodactylum tricornutum* showed an increase in biomass and lipid production when grown in a media with glycerol as a carbon source and 24-h illumination regimen [119]. Similarly, *Chlorella sorokiniana* [120] and diatom *Thalassiosira pseudonana* [121] showed better biomass and lipid production when grown on glycerol compared to glucose. Glucose assimilation was considered higher in heterotrophic culture resulting in nine times higher biomass of *Auxenochlorella protothecoides* when compared to autotrophic mode indicating that the uptake of glucose by *A. protothecoides* may be inhibited by light [122]. This was also observed when tested on *Chlorella* and *Scenedesmus* cultures [123]. Yet, *Pavlova lutheri* cultured on sucrose showed better biomass and lipid production under mixotrophic conditions when compared to heterotrophic conditions and other carbon sources such as glucose and glycerol [124].

Although the use of an organic carbon source with a light source allows microalgae to function under mixotrophic conditions as an alternative to autotrophic and heterotrophic conditions, the presence of organic carbon reduces the microalgae CO₂ capture efficiency for photosynthesis [125, 126]. Additionally, the presence of inorganic carbon sources or dissolved CO₂ is known to decrease pH due to the dissociation

of the inorganic carbon and the release of H^+ ions, leading to media acidification and reduced microalgae activity [127]. Some algae can utilize both inorganic carbon sources and CO_2 capture, while others only depend on the inorganic carbon sources if present, and this process is pH-dependent. Since the bicarbonate form of inorganic carbon is mainly predominant at pH 9, while at lower pH, both the aqueous form of CO_2 and the bicarbonate form are equally present [128]. Therefore, managing the pH of microalgae culture is crucial as it plays a vital role in making essential elements available for microalgae growth and regulating microalgae biochemical pathways.

3.3.4 pH

pH is one of the important parameters that needs to be maintained to conserve the physiology of microalgae. However, in a natural habitat, pH is quite variable and not necessarily maintained. Therefore, microalgae collected from the environment may have developed tolerance to the pH of the extreme ends. Hence, the need to optimize pH conditions for the enrichment of a complete diversity or specific microalgae depending on the research aim. In a culture media, the presence of the inorganic carbon source bicarbonate is converted to CO_2 by the enzyme carbonic anhydrase to release hydroxyl ions that result in an increase in pH. This increase in pH results in the inhibition of cell growth due to the reduction of nutrient accessibility; an increase in pH will eventually result in a decrease in cell growth [129]. Freshwater microalgae have been reported to tolerate high pH (>10) and extremes of pH in general, while marine algae are limited to growth within the pH range of 8.1–8.3 [129, 130]. An increase of pH (from pH 8.5 to pH >11) in the culture media also leads to the flocculation of algal cells, a desired feature in the industry, and is used to harvest algae, but is troublesome when studying diversity [131].

3.3.5 Temperature

Another parameter that affects microalgae growth is temperature. The optimal temperature for microalgae to carry out cell functions and photosynthesis is generally between 15 and 30°C. Within the optimum temperature, ideally, an increase in temperature has a positive effect on the photosynthetic process and cell division. However, an unfavorable increase above the optimal temperature range can sharply decrease the growth rate of microalgae. This is because enzymatic functions are drastically affected by the high temperatures, hence affecting important enzymes of the electron transport chain, Calvin cycle, and membrane stability, eventually influencing the growth rate of the microalgae [132]. However, this is not always the case in cultures since microalgae are highly adaptive with the potential to adapt to extreme environments such as arid deserts [132–135]. Furthermore, microalgae can be pre-adapted and acclimatized to certain temperatures depending on their habitat when cultured in the laboratory settings [136].

4. Microalgae identification methods

4.1 Direct morphological identification

The direct identification of microalgae from environmental samples starts by collecting a sample either from water or soil as representative of the habitat. This is

followed by direct observation through morphological characteristics of the different microalgae species under the microscope. There are several identification guidelines and keys available to aid in the identification of microalgae [31, 137–140]. This method depends mainly on the presence of a research-grade microscope.

Scanning and transmission electron microscopes (SEM and TEM) are not options that are readily available in all research labs. However, they are used to study fine ultrastructure details on the species level of microalgae. Moreover, viewing microalgae under SEM and TEM requires specific cell preparation to preserve the ultrastructure of the microalgae. The preparations involve fixation, dehydration, and sputtering of the sample with gold particles [141–143]. Automated microalgae identification is a modern form of morphological level identification that is currently made available for the taxonomic identification of microalgae from an environmental sample. An automated system depends on machine learning and a training set that consists of a set of captured images of microalgae that a taxonomist has previously identified. This training set is used to train the software to classify the different microalgae genera [144–149]. Yet, identifying microalgae based on their physical characteristics is challenging as their phenotypic traits are heavily influenced by the environment and their response to stress [150, 151]. To overcome this issue, molecular-based identification has emerged as an essential alternative approach that enables the identification of microalgae irrespective of any changes in their physical or morphological traits.

4.2 Molecular-level identification

Molecular-level identification is the process of identifying microalgae on a genetic level. This process involves various steps and can be categorized into two main approaches: non-polymerase chain reaction (PCR)-based or PCR-based methods. These methods can be applied directly to environmental samples, enriched samples, or isolated species. Additionally, molecular level identification can serve as a confirmation tool for morphological level identification (**Figure 1**). This section will discuss the technologies utilized in both approaches for identifying microalgae diversity.

4.2.1 Non-PCR-based identification

4.2.1.1 Fluorescence in situ hybridization (FISH)

FISH is a technique that depends on the hybridization of a fluorescently labeled DNA sequence to its complementary sequence in a cell, followed by identification using epifluorescence microscopy. FISH has been used and optimized to identify harmful microalgae species mainly. This method has successfully identified dinoflagellates *Gambierdiscus* spp. [152], *Biecheleria baltica* [153] and the toxic *Alexandrium* sp. [154–157], Chromophyta *Heterosigma akashiwo* [158].

4.2.1.2 Restriction fragment polymorphism (RFLP)

The identification of different organisms has been facilitated by RFLP, a technique that analyzes specific DNA sequence variations. This involves digesting DNA with restriction endonucleases and observing the digestion pattern through gel electrophoresis. Consequently, RFLP has been widely employed in gene mapping and DNA fingerprinting [159]. However, its use in microalgae identification has been limited [160]. PCR-RFLP or Terminal-RFLP (T-RFLP) are alternative methods to RFLP

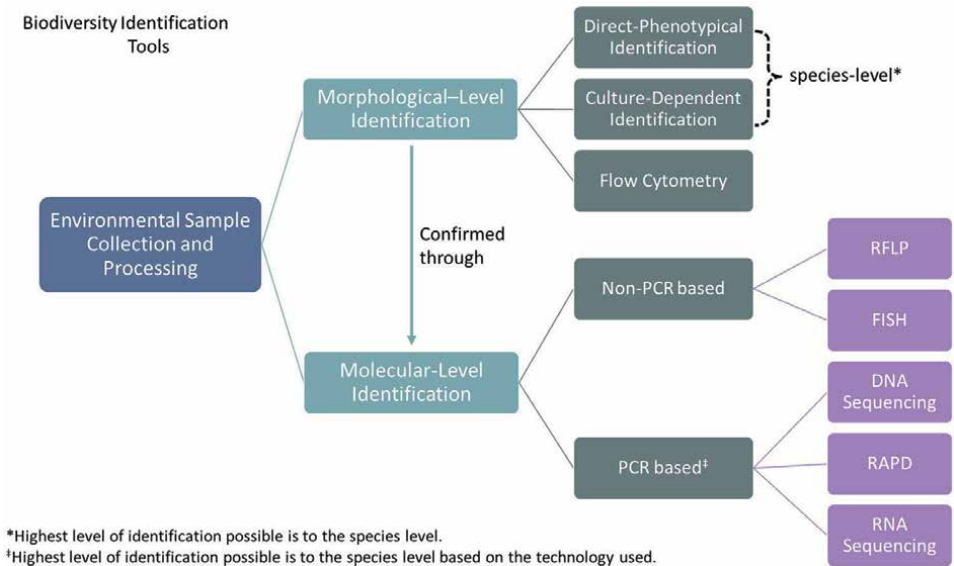


Figure 1.
Various methods that can be utilized to identify microalgae in environmental samples.

analysis, and they depend on the combination of PCR for DNA amplification of DNA barcodes and restriction fragment analysis, as discussed next.

4.2.2 PCR-based identification

Polymerase chain reaction (PCR)-based molecular level identification depends on amplifying short conserved nucleic acid sequences known as DNA barcodes. Different DNA barcodes can also be used to identify different phyla of microalgae. For instance, the 16S ribosomal DNA (rDNA) sequence is mainly used for cyanobacteria identification region [161], while the 18S rDNA is used for eukaryotic microalgae by using primers designed to amplify the variable regions or the internal transcribed spacers (ITSs) in addition to the identification of eukaryotic microalgae through plastid genes [162] (**Figure 2**). The level of identification, whether to the genus or species level, depends on the reference database used for alignment. This method has

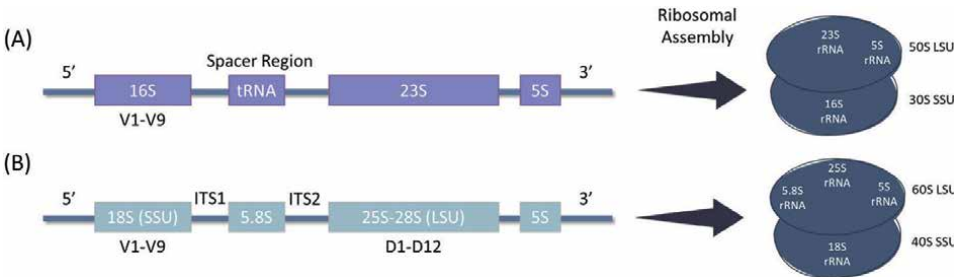


Figure 2.
Schematic diagram representing the ribosomal gene cluster (rDNA) of the (A) 16S and (B) 18S rDNA that are commonly used for the identification of cyanobacteria and eukaryotic microalgae, respectively. The hypervariable regions are denoted with V1-V9 on the 16S and 18S rDNA.

Microalgae phyla	Ref.	DNA barcode*	Genus	Tools
Chlorophyta	[163]	18S rDNA V4 region and <i>rbcL</i>	• <i>Scenedesmus</i> sp. • <i>Tetradesmus dimorphus</i> • <i>Stichococcus</i> sp. • <i>Parachlorella kessleri</i> • <i>Chloroidium saccharophilum</i> • <i>Monoraphidium</i> sp. • <i>Chlorococcum</i> sp.	Sanger sequencing
	[162]	16S rDNA (plastid)	• <i>Chlorella vulgaris</i>	Sanger sequencing
	[164, 165]	<i>rbcL</i>	• <i>Chlorella</i> strains	Sanger sequencing
	[166]	<i>rbcL</i> , ITS, 16S rDNA (plastid) and <i>tufA</i>	• <i>Scenedesmus</i> strains	Bidirectional sequencing
	[167]	16S V5-V6 rDNA and 16S-23S ITS region	• <i>Chlorogloeopsis</i> sp. • <i>Leptolyngbya boryana</i> • <i>Thermoleptolyngbya albertanoae</i> • <i>Synechococcus bigranulatus</i> • <i>Oculatella</i> sp. • <i>Desertifilum</i> sp. • <i>Leptolyngbya geysericola</i> • <i>Geitlerinema splendidum</i> • <i>Cyanobacterium aponinum</i>	454 pyrosequencing and Illumina MiSeq™ platform
Cyanophyta	[163]	16S rDNA-ITS region	• <i>Synechocystis</i> sp. • <i>Aphanocapsa</i> sp. • <i>Chroococcidiopsis thermalis</i> • <i>Nostoc</i> sp. • <i>Cylindrospermopsis raciborskii</i> • <i>Pseudanabaena</i> sp.	Sanger sequencing
	[168]	16S rDNA-ITS region	• <i>Aphanizomenon</i> sp. • <i>Olichospermum</i> sp. • <i>Limnoraphis</i> sp. • <i>Microcystis</i> sp.	Sanger sequencing
	[169]	16S-23S ITS region	• <i>Scytonema hyalinum</i>	Illumina MiSeq™ platform
	[170]	16S rDNA	• <i>C. stanieri</i> • <i>Prochloron</i> sp. • <i>Synechocystis</i> sp. PCC6803	Sanger sequencing
	[171]	16S rDNA	• <i>Nostoc</i> strains	Sanger sequencing
	[172]	16S rDNA	• <i>Phormidium retzii</i>	RAPD and Sanger sequencing

Microalgae phyla	Ref.	DNA barcode*	Genus	Tools
Bacillariophyta	[173]	COI, <i>rbcL</i> , SSU, and LSU	• <i>Asteroplanus karianus</i> • <i>Cerataulina pelagica</i> • <i>Chaetoceros muellerii</i> • <i>Cyclotella</i> sp. • <i>Entomoneis</i> sp. • <i>Licmophora paradoxa</i> • <i>Melosira varians</i> • <i>Navicula bottnica</i> • <i>Phaeodactylum tricornutum</i> • <i>Skeletonema costatum</i> • <i>Thalassiosira gravida</i>	Bidirectional sequencing
	[174]	COI, 23S rRNA gene, SSU and LSU	• <i>Sellaphora</i> • <i>Navicula</i> • <i>Nitzschia</i> • <i>Fragilariopsis</i> • <i>Tragilariopsis</i> • <i>Pseudonitzschia</i>	Sanger sequencing
	[175]	18S V4 rDNA	• <i>Leptocylindrus</i> • <i>Chaetoceros</i> • <i>Thalassiosira</i> • <i>Skeletonema</i> • <i>Pseudonitzschia</i>	454 pyrosequencing
	[176, 177]	COI, <i>rbcL</i> , SSU, and LSU	• <i>Frustulia strains</i>	Sanger sequencing
	[178]	COI	• <i>Alexandrium</i> • <i>Scrippsiella</i> • <i>Karlodinium</i> • <i>Karenia</i>	Sanger sequencing
Dinophyta	[179]	16S rDNA and 18S rDNA V3-V4 region	• <i>Karlodinium veneficum</i> • <i>Gyrodinium</i> sp. • <i>Gymnodinium</i> sp. • <i>Gonyaulax</i> sp. • <i>Symbiodinium</i> sp. • <i>Amphidiniella</i> sp. • <i>Ceratium</i> sp.	Illumina MiSeq™ platform
	[180]	COI, calmodulin, rad24, and actin	• <i>Amphidinium carterae</i> • <i>Alexandrium tamarense</i> • <i>Heterocapsa triquetra</i> • <i>Karenia brevis</i> • <i>Karlodinium micrum</i> • <i>Lingulodinium polyedrum</i> • <i>Symbiodinium</i>	Sanger sequencing
	[181]	COI and <i>cob</i>	• <i>A. tamarense</i>, • <i>Pfiesteria piscicida</i> • <i>Karlodinium veneficum</i> • <i>Prorocentrum minimum</i> • <i>Akashiwo sanguinea</i> • <i>P. micans</i> • <i>Prorocentrum nanum</i> • <i>D. acuminata</i> • <i>Ceratocorys horrida</i>	Sanger sequencing

Microalgae phyla	Ref.	DNA barcode*	Genus	Tools
Haptophyta	[182]	18S rDNA V4 region	• <i>Phaeocystis</i> • <i>Chrysochromulina</i> • <i>Prymnesium</i> • <i>Braarudosphaera</i> • <i>Gephyrocapsa/Emiliana</i>	Ion torrent (IT) sequencing
	[183]	LSU	• <i>Chrysochromulina</i> • <i>Umbilicosphaera</i> • <i>Calcidiscus</i> • <i>Coronosphaera</i> • <i>Scyphosphaera</i> • <i>Helicosphaera</i> • <i>Coccolithus</i>	High-Throughput Sequencing
	[184]	SSU rDNA V4 region	• <i>Haptolina fragaria</i> • <i>Pleurochrysis pseudoroscoffensis</i> • <i>Chrysochromulina</i> • <i>Prymnesium polylepis</i> • <i>Phaeocystis</i> • <i>Emiliana</i> • <i>Isochrysis galbana</i> • <i>Imantonia rotunda</i> • <i>Diacronema emmorea</i>	454 pyrosequencing
Euglenophyta	[185]	COI and 18S rDNA	• 23 euglenida species	Sanger sequencing
	[186]	16S V3-V4 (for cyanobacteria) and 18S V4 rDNA (eukaryotic microalgae)	• <i>Colacium vesiculosum</i> • <i>Discoplastis spathirhyncha</i> • <i>Euglena sp.</i> • <i>Flexiglena variabilis</i> • <i>Lepocinclis sp.</i> • <i>Monomorphina pyrum</i> • <i>Phacus sp.</i> • <i>Trachelomonas sp.</i>	Illumina MiSeq™ platform

*DNA barcode abbreviations: ribulose-1,5-bisphosphate carboxylase/oxygenase (*rbcL*), large subunit ribosomal DNA (LSU rDNA), small subunit ribosomal DNA (SSU rDNA), cytochrome c oxidase I (COI), encoding elongation factor (*tufA* gene), internal transcribed spacer region (ITS), cytochrome oxidase B (*cob*), calcium-modulated protein (*calmodulin*), damage checkpoint rad24 protein (*rad24*).

Table 2.
Common DNA barcodes utilized for the identification of microalgae.

gained popularity in taxonomical identification and phylogenetic analysis as it eliminates the need for culturing microalgae to confirm their presence, allowing the ease of direct identification from environmental samples. The identification and analysis of DNA barcodes have been facilitated by the development of various biotechnology tools (Table 2). The following are some tools that can be used to identify microalgae through DNA barcodes.

PCR-Restriction Fragment Polymorphism (PCR-RFLP) and Random Amplified Polymorphic DNA (RAPD) are two PCR-based techniques that have been utilized to

identify different microalgae species. PCR-RFLP is a method that involves amplifying a DNA barcode before digesting the amplified region with restriction nucleases. This technique has been successfully used for the identification of microalgae such as *Stihococcus* sp., *Nitzschia* sp., *C. vulgaris*, and *Navicula accomoda* through the PCR of 18S rDNA and its digestion [187]. PCR-RFLP has also been used to identify the dinoflagellate *Gambierdiscus* sp. isolated from the Caribbean Sea and Mexican coast was also identified through the amplification of the LSU rRNA D1-D2 sequences of the 18S rDNA [188]. Random Amplified Polymorphic DNA (RAPD) is another PCR-based technique that is useful for identifying genetic variation in different species [189]. RAPD has been used in the identification of different *Chlorella* strains [190], *Microcystis* species [191], *Dunaliella* species [192, 193], and diatom *Chaetoceros* species [194]. However, with the emergence of sequencing technology has provided a more reliable and accurate identification method over RAPD.

Sanger sequencing, also known as chain termination sequencing or first-generation sequencing, has been widely used as a confirmatory tool for identifying microalgae post-enrichment and isolation from environmental samples. The effectiveness of this method is dependent on the utilized DNA barcode, with a multiple primer approach often employed for identifying microalgae diversity across various phyla (Table 2). For instance, Ballesteros et al. used 16S rDNA and 16S–23S rDNA regions for cyanobacteria identification and 18S rDNA and ribulose-bisphosphate carboxylase (*rbcL*) gene for chlorophyta identification [163]. Other DNA barcodes used in identifying eukaryotic microalgae include the mitochondrial gene cytochrome c oxidase subunit 1 (*cox1* or *COI*), ITS (ITS1–5.8S–ITS2), and elongation factor EF-Tu (*tufA*) gene [195].

Metagenomics involves the direct study of genomes in their natural habitat without isolation, and this is made possible due to the development of next-generation sequencing. Samples can be either directly used for DNA extraction, such as soil samples or water samples can be filtered with the appropriate filter paper followed by DNA extraction, and samples can be sequenced using Illumina MiSeq™ and HiSeq™ platform [186, 196–198]. Next-generation sequencing provides the best tool to identify the highest microalgal diversity from environmental samples; however, similar to any DNA-based tool, it depends on the sequences available in the database and is not usually combined with single species isolation.

4.3 Biochemical marker for microalgae identification

Several approaches have been employed to identify microalgae based on their pigment, also known as chemotaxonomic markers. Additionally, carbohydrates, lipids, and fatty acids may be used as chemotaxonomic markers. Microalgal photosynthetic pigments constitute a diverse range and are a highly valuable product with a vast array of commercial applications, including the food, cosmetics, and health industries [50, 199–201]. Despite the variety of chemotaxonomic markers, microalgae are known for their adaptability and plasticity as their cell composition and phenotypic characteristics are influenced by environmental changes such as temperature and nutrient composition [202, 203]. However, these changes can make it challenging to detect microalgae using such markers. Therefore, additional tests are necessary to account for the impact of various environmental conditions on microalgae cells, including limited nutrients, elevated CO₂, and prolonged darkness, which affect cell composition [204, 205]. To address this issue, some research has focused on identifying specific macromolecules for different species [206, 207]. Serive et al. [208]

determined that photosynthetic pigments cannot be used as the sole method of identification since the expression of these pigments depends upon various physiological and environmental factors, such as light and nutrients, and hence must be confirmed using other taxonomic identification tools [208].

Despite the fact that chemotaxonomic markers are not reliable in identifying microalgae, quantifying their biochemical composition after identification from their environment is an essential next step. To achieve this, Fourier-transformed infra-red (FTIR) spectroscopy is a powerful tool for the rapid identification of microalgal lipids, carbohydrates, and proteins through biomass characterization *via* the identification of functional groups [209, 210] (**Table 3**). However, FTIR is limited for use on environmental samples containing unidentified species, as it can only identify species whose chemical composition is already present in its library [204]. For the identification of fatty acid from microalgae, gas chromatography-flame ionization detection (GC-FID), gas chromatography-mass spectroscopy (GC-MS), and nuclear

Identification tool	Microalgae biochemical product	Method of identification	Refs.
FTIR	Lipids, Carbohydrates, Proteins	The vibrational energy of functional groups such as CH ₂ , OH, and C=O induced by the absorption of infrared radiation.	[211–215]
GC-FID	Fatty acid methyl esters (FAMES)	GC connected to flame ionization detectors is based on the burning of esterified fatty acids using a hydrogen flame after separation using gas chromatography.	[216, 217]
GC-MS	Fatty acid methyl esters (FAMES)	GC connected to a mass spectrometer detects FAMES by measuring ions based on their mass-to-charge ratio.	[218, 219]
HPLC	Fatty acid methyl esters (FAMES) and pigments	HPLC separates and quantifies components of liquid samples based on the retention time of the sample in the stationary phase of the column.	[220–224]
HPLC-DAD	Pigments	Combines HPLC and spectrometry, and it identifies compounds based on their absorbance in the ultraviolet-visible spectrum.	[208, 225, 226]
NMR	Fatty acids, amino acids, pigments and carbohydrates	Identification of carbon-hydrogen frameworks within organic molecules based on their absorption of electromagnetic radio waves	[227–230]
UV-vis spectrometry	Pigments	Depends on the absorption of pigments within the near-UV (180–390 nm) or visible (390–780 nm) radiation spectrum.	[220, 231, 232]

Abbreviations: Fourier-transform infrared spectroscopy (FTIR); Gas chromatography-Flame Ionization Detection (GC-FID); Gas chromatography-mass spectroscopy (GC-MS); High-performance liquid chromatography (HPLC); Nuclear magnetic resonance (NMR); HPLC-diode-array detector (HPLC-DAD); Ultraviolet-visible (UV-vis) spectrophotometry.

Table 3.
Biochemical identification tools.

magnetic resonance (NMR) have been commonly used (**Table 3**). NMR has also been utilized to identify carbohydrates and pigments from microalgae. Other tools, such as high-performance liquid chromatography (HPLC) and ultraviolet-visible (UV-vis) spectrophotometry, have been employed for pigment analysis, ultimately allowing for the classification of microalgae up to the phyla level based on their pigments [233].

4.4 Conclusion

Identification of microalgae is important for determining their value. Microalgae are rich in nutrients such as proteins, vitamins, minerals, essential fatty acids, and different pigments, making them a valuable source for human food, animal feed, and various industrial applications. In addition, microalgae are fast-growing and can be easily cultivated up to large scales, which make them a cost-effective solution for environmental clean-up. Different species of microalgae are known to be effective in bioremediation. Hence, researchers are continuously investigating different targeted strategies using such species for cleaning up polluted environments. This can lead to a more sustainable and eco-friendly approach to environmental remediation. By identifying the specific species of microalgae, it is possible to determine the potential health and industrial benefits. Therefore, accurate identification of microalgae is crucial for assessing their nutritional value and potential applications in different industries; additionally, biochemical characterization of microalgae can help in the discovery of new bioactive compounds with potential in various applications.

Identification and isolation of microalgae from their natural habitats are crucial for their utilization and extraction of diverse by-products. Various factors come into play when isolating and maintaining microalgae, such as sample collection, post-collection processing, and identification methods. Direct morphological identification, which involves identifying different phenotypical features of microalgae under the microscope, is a golden method that requires highly qualified taxonomists. To further ease the identification of microalgae from their environmental samples, enrichment of microalgae using different nutrients and optimizing their growth enables the ease of identifying low-density microalgae through enriching them. Molecular level identification is another means of identification that can be used to confirm the morphological level identification of microalgae or can be used to directly identify microalgae without culturing or isolating it. Utilizing several identification methods provides a holistic approach to identifying microalgae from environmental samples. Thus, the need for continuous optimization of the different identification methods and to aid the continuous monitoring of the microalgae diversity in our environment.

Conflict of interest

The authors declare no conflict of interest.

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Current Status of the Microalgae Application in Agriculture and Aquaculture

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Abstract

Microalgae are organisms with great potential for the use of goods and services in areas of social and commercial interest. The application of bioproducts of microalgal origin for the purpose of feed in aquaculture and agriculture directly influences the generation or mitigation of environmental impacts. Its use in the treatment of wastewater allows the reduction of nutrients such as nitrogen, phosphorus, and CO₂, providing a system that acts in the reuse and use of water resources, generating the return of cleaner water to bodies of water and acting in the reduction of the gases greenhouse effect. Microalgae biomass is presented as an alternative to generating a wide variety of value-added products that, in recent years, given its biotechnological potential, have been gaining ground in agribusiness. This document aims to show the application and current perspectives for obtaining biomass of microalgae from crops in wastewater that can be used as raw material for the production of biofertilizers, biostimulants, feed additives that encourage reuse, transformation, recovery, and savings of resources to promote bioeconomic and circular economy processes. Generate strategies to develop practices in the agricultural sector with high productivity, quality, and efficiency, which in turn can be sustainable, friendly, and provide economic advantages, part of the need to use bioresources and implement circular bioeconomy concepts.

Keywords: biofertilizers, circular economy, sustainability, organic waste, nutrient recovery

1. Introduction

With the arrival of the Green Revolution, in the middle of the twentieth-century, the development of techniques in the production of agricultural businesses intensified [1]. Basically, the transformations were perceived in using modern machinery and in developing chemical inputs and fertilizers. In other words, the agrarian transformation has positively impacted the increase in food production, agricultural land, irrigation, and soils, as well as helped to rapidly increase the economic development of several countries [2]. At the same time, this practice has led to serious nutritional

imbalances, modifying, for example, the relationship between nitrogen (N), phosphor (P), and potassium (K), salinization, groundwater pollution, and reduction of soil fertility as time passes. In addition, these products are responsible for considerable greenhouse gas emissions in addition to requiring high-energy consumption. Therefore, it is imperative to seek more sustainable alternatives to overcome these limitations [3].

Biofertilizers fit into this context as a viable, healthy, and pollution-free option, currently being considered the best substitute for chemical fertilizers. Among the various types of biofertilizers, formulations based on microorganisms, including cyanobacteria and microalgae, have been growing substantially due to their excellent ability to increase soil fertility, improve nutrient quality and increase the productivity of different crops [4]. In parallel, the use of microalgae in the biostimulants market makes it possible to reduce the number of fertilizers needed for crops and thus indirectly increase agricultural sustainability. In this case, the application of these products at a rate of 1 L/ha can reduce the need for chemical fertilizers by more than 5% in addition to promoting increments of up to 10% in the growth and rooting of several plants [5].

Briefly, microalgae play a vital role in the sustainability of our planet, mainly because it reduces the environmental impact of transforming CO₂ into O₂. Microalgae are rapid-growth microorganisms, that reach high cell productivity in a short time and use mainly sunlight as an energy source. They have been studied as a source for the production of a wide range of products, such as animal feed, food, pharmaceuticals, and biofuels [6]. However, microalgae have even greater potential to boost the global bioeconomy, expanding its current production capacity and exploring new applications. These emerging applications include the production of agro-industrial products such as biofertilizers, biostimulants, biopesticides, and aquaculture. In the latter, progress has been made with new formulations based on microalgae and vegetable oils, significantly reducing the dependence on marine ingredients [7], and in turn, showing positive effects for the growth of salmonids and marine species that vary depending on the microalgal species used [8, 9].

In addition, they make the cultivation of food more sustainable when collaborating with the treatment of wastewater and its efficient reuse in aquaculture farms, soil irrigation, and plantations [5].

By exploiting their full potential, we can reap even more benefits from these microscopic organisms, promoting a more sustainable and ecologically balanced future as well as promoting economic success. This occurs mainly because microalgae are able to produce different inputs from the recovery of nutrients and significant biological material derived from different organic residues. In fact, the use of biofertilizers from waste recycling is currently considered the basis of sustainable agriculture, since it adopts modern recycling techniques, reduces the environmental impact, and reduces the carbon footprint, in addition to avoiding the high costs associated with landfills [10].

Although the use of these biological alternatives is still relatively low compared to the use of synthetic products, representing no more than 5% of the world market, the growing practice of organic aquaculture and agriculture, the need to improve water and soil organic matter, and a regulatory framework favorable are the main factors driving this market [11]. Furthermore, with the advent of integrated pest and other disease management programs that promote the use of biological inputs, the aquaculture and agriculture sector is expected to see an increase in the use of microalgae-based processes and products in the future really close.

2. Sources of organic waste

In recent years, organic waste has been studied with great frequency due to its serious disposal problems and low efficiency of use. Among these residues are some more complex ones, such as agricultural and forest residues, and animal, industrial, and municipal residues, due to their great source of pollution, pathogens, odors, and increase of greenhouse gases [12].

It is estimated that rice, wheat, and corn straw residues (the most produced in the agricultural sector) reach more than 700, 350, and 200 million tons (Mt) per year, respectively. In addition, the global production of wood waste can exceed 4 Gt per year, of which more than 20% is caused by loss of production [13]. Most animal waste is generated from the fishing, poultry, and livestock industries, for example, due to their high production and disposal of inedible or unsuitable portions for human consumption. The methane gas (CH₄) emitted by cattle (due to the enteric fermentation that occurs in the animals' digestive process) is also one of those responsible for the greenhouse effect, which, intensified, generates global warming [14].

As for industrial waste, sewage sludge contains various hazardous materials, pathogens, heavy metals, and difficult-to-treat microplastics. Other large residual volumes come from the most diverse sectors, such as the pharmaceutical industry, chemical industry, and fermentation products. The characteristics of industrial waste are high chemical oxygen demand (COD), biological oxygen demand (BOD), and strong smell resulting from the presence of organic fillers and volatile compounds [15].

Over the years, the growing generation of municipal waste and established disposal practices, combined with the still high cost of storage, have resulted in increasing volumes of accumulated waste and, historically, in serious environmental and public health problems. Irregular disposal has caused contamination of soils, watercourses, and groundwater, and also caused many diseases in the population. The high number of nutrients and organic materials produced by, for example, cleaning products and human waste are said to generate large residual loads in municipal treatment plants, yielding around 0.10 tons/capita/year [16]. In addition, the composition of urban waste is very heterogeneous, in which gravimetric analyses reveal a significant frequency of the fraction composed of organic materials (food remains, pruning, and other putrescible), representing on average more than 50% of the total waste collected.

2.1 Integrated organic waste management

The management of organic waste prioritizes the reduction, reuse, recycling, and environmentally appropriate final disposal of waste. Another essential concept is that it is the responsibility of all of society to participate in the management of waste and the life cycle of products, as opposed to the linear model of "production-consumption-disposal" [17]. Thus, all generators, individually and collectively, in addition to those who act directly or indirectly at any stage of the product's life cycle, are responsible for waste management, obviously considering the specificity of each one in the production chain. This topic has been discussed in the corporate environment for some years, especially after the environmental pillar gained even more prominence on the world stage because of the ESG (Environmental, Social, and Governance). Therefore, it is not possible to ignore reality, since the global trend has given more value to sustainable practices [18].

Composting and biodigestion (with or without energy conversion) are the most recommended technologies worldwide for recycling organic waste [19]. However, the World Economic Forum has pointed to the “circular economy” as a model that makes it possible to reintroduce waste into the production chain in order to reduce pressure on natural resources. This new paradigm leads to a change in the very concept of waste, which is now considered a resource, since, in large part, it can be reused. This enormous potential is already recognized by several countries, whose organic waste management is intrinsically linked to the local economy, promoting income, employment, and mitigating environmental impacts [20].

For a reuse system to be implemented, the treatment system must be complemented with operations that transform the substances into a material that can be used in the production process, as a raw material [21]. This guarantees the quality of the treated waste and its suitability for its intended use.

One way of treating industrial waste is the installation of an effluent treatment plant, where the waste can pass through the physical, chemical, or biological phase, as shown in **Figure 1**. In the first, the pollutants are removed, and the solid and liquid phases are separated. Already the biological treatment is carried out using bacteria and other microorganisms. These consume polluting organic matter through the respiratory process [22].

Briefly, conventional physical methods include screens, sedimentation, flotation, decantation, and filtration to remove suspended sediments and floating solids. The conventional chemical and physical-chemical treatment methods include coagulation-flocculation, adsorption, chemical oxidation, chemical precipitation, ion exchange, and electrochemical. After this phase, the processes become more intensive and are based on the conversion of organic materials by microbial decomposition. Here, many types of settings are presented. However, the activated sludge treatment process stands out for its low operating cost. In fact, this method consists of physically, mechanically, chemically, and biologically stimulating the aerobic biota of raw effluents [4].

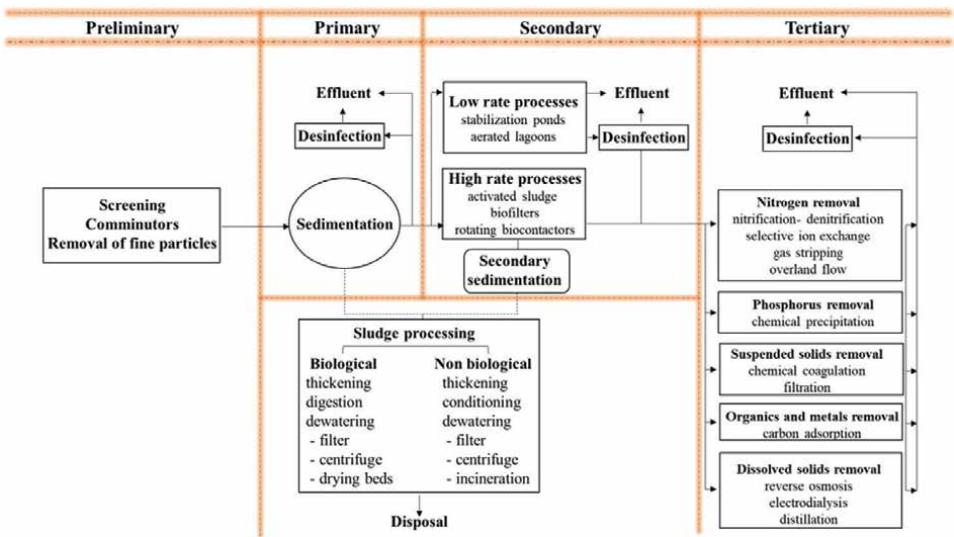


Figure 1.
Conventional wastewater treatment processes.

Although there are necessary technologies to reduce the disposal of most of the waste generated, the high costs, poor sustainability, and lack of greater integration in the management of this waste have been pointed out by specialists as the reasons why it is still a global challenge. Thus, waste treatment based on the stimulation and enhancement of nutrients with the parallel use of its products, betting on a circular economy, has been gaining prominence.

3. Nutrients recovery of organic wastes

In production systems, not all raw materials are converted into products, as chemical reactions produce many different substances than those initially intended. For example, in agriculture, it is very common to apply substances from the petrochemical industry. Chemical fertilizers are commonly used to provide crops with the necessary nutrients, such as nitrogen and phosphorus, for their growth and the production of food. However, when these nutrients are not fully utilized, they can be lost from agricultural fields. Leaching is an unwanted process, in which soil nutrients are transported to depths beyond those occupied by plant roots, which can bring economic and environmental damage to the farmer [23].

Eutrophication is a process observed in different water bodies and characterized by an increase in nutrients and excess substances used and/or lost in nature. This process leads to hypoxia and anoxia, reduced water quality, altered food web structure, habitat degradation, loss of biodiversity, and production of harmful algae [24]. Chemical fertilization, in addition to contaminating soil and water, releases nitrogen-based gaseous compounds into the air, such as ammonia and nitrogen oxides. Thus, the amount of oxygen decreases, which causes the death of various species [23].

The high cost of products and the environmental problems generated by chemical fertilizers and inputs have stimulated approaches that emphasize integrated management strategies in production systems. Bioremediation processes have been proposed as an environmentally correct alternative for the remediation of residues from water bodies and agricultural environments [25]. Within this context, microalgae have been of great interest to many scientific researchers, as they assimilate considerable amounts of residual nutrients (COD, N, and P) with the ability to convert them into many products of commercial interest, resulting in economic gain and reduction of harmful substances [5].

Basically, as shown in **Figure 2**, the nutrients contained in the residues are assimilated in the microalgae cells for their growth, consuming mainly inorganic CO₂ as a carbon source. This CO₂ is first absorbed by water forming carbonic acid, dissociating into bicarbonate and hydrogen ions [22]. Microalgae produce biomass and release O₂ which is then assimilated by bacteria to oxidize organic compounds into inorganic ones. Subsequently, through nitrification, the microalgae consume the oxygen released in their metabolic activity, reducing cultivation costs, while the phosphate macronutrients are integrated into the energy supply by phosphorylation. NADP⁺ and adenosine diphosphate (ADP) are incorporated into NADPH and adenosine triphosphate (ATP), regulating, respectively, the metabolic pathways of microalgae [26].

Thus, both microalgae and bacteria can assimilate considerable amounts of nutrients from waste and incorporate them into their biomass. These microorganisms are capable of surviving in extreme environmental conditions and due to their distinct

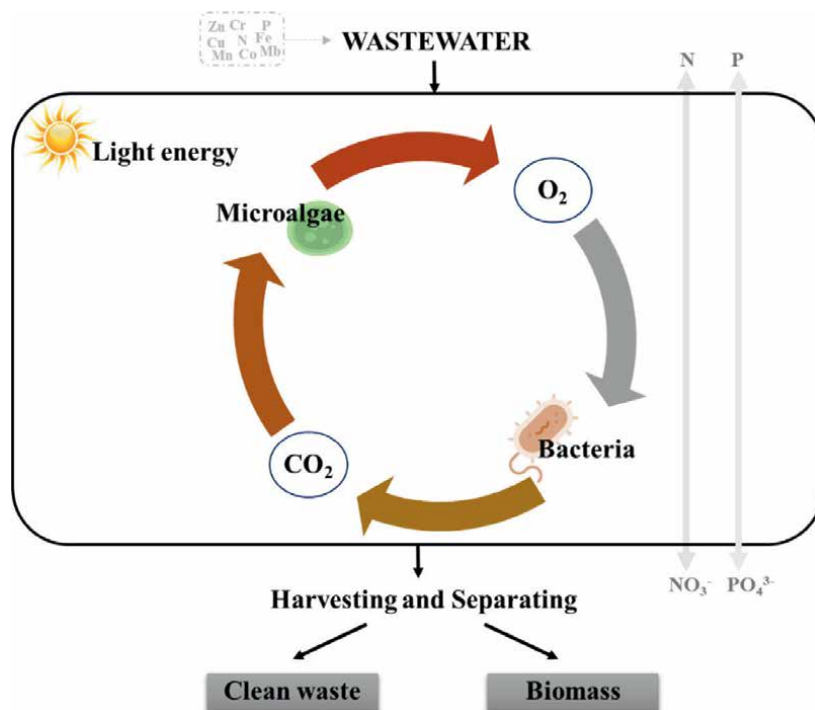


Figure 2.
Simplified representation of the microalgae biological phenomena during the treatment of wastewater.

structural, physiological, and morphological characteristics are reflected in a high degree of processes involving their use. The biotechnological potential of microalgae is mainly due to their ability to synthesize a wide range of products such as food, pharmaceuticals, biofuels, bioenergy, animal feed, and agricultural products [6]. In addition, the cultivation of microalgae in wastewater is considered an effective tool, as it is natural and inexpensive, for the assimilation of nutrients and other contaminating compounds from the environment, and can therefore be used as an alternative for the tertiary treatment of wastewater [25].

The production of microalgae involves the stages of cultivation, biomass recovery, and obtaining products. The recovery stage is a production bottleneck, representing a high cost for the process. In addition, some separation systems compromise the cellular integrity of microalgae. Among the possible biomass concentration methods are sedimentation, centrifugation, flotation, flocculation, conventional filtration, and membrane separation methods, such as microfiltration, ultrafiltration, and even direct osmosis [22]. However, despite the abundance of studies on biomass harvesting, there is still no common consensus on the most appropriate and economically viable methodology for obtaining microalgae bioproducts [27].

In this context, despite the steps and types of systems used, microalgae-based processes that aim to reduce pollutants associated with biomass production as inputs of interest to agribusiness are booming markets. Today, many companies are focusing on microalgae raw materials as an agricultural commodity and replacing traditional aquaculture production methods. Thus, its applicability in these two fields of study will be discussed below.

4. Overview of aquaculture systems

Aquaculture production has become increasingly important and is growing rapidly in terms of providing a dietary source of protein for human consumption. With the increasing world population, the demand for aquaculture is high and it is estimated that the industry is expected to grow at a rate of more than 7% within 5 years, contributing more than 50% of the total weight of the aquatic animal harvest for consumption human [28, 29].

Among the most common culture units that farmers recognize in aquaculture are ponds, conduits, and aquaculture recirculation systems (RAS). Although these methods differ in terms of labor requirement, energy consumption, dependence on natural resources, simplicity, and costs, they have in common the large flow of circulating water and its indirect disposal to external sources [30]. In this context, many challenges are encountered in traditional aquaculture which is best shown in **Figure 3**.

4.1 Main challenges of traditional aquaculture

Water quality is associated with the productivity and health of aquatic animals, as there are ideal development conditions that vary according to the cultivated species.

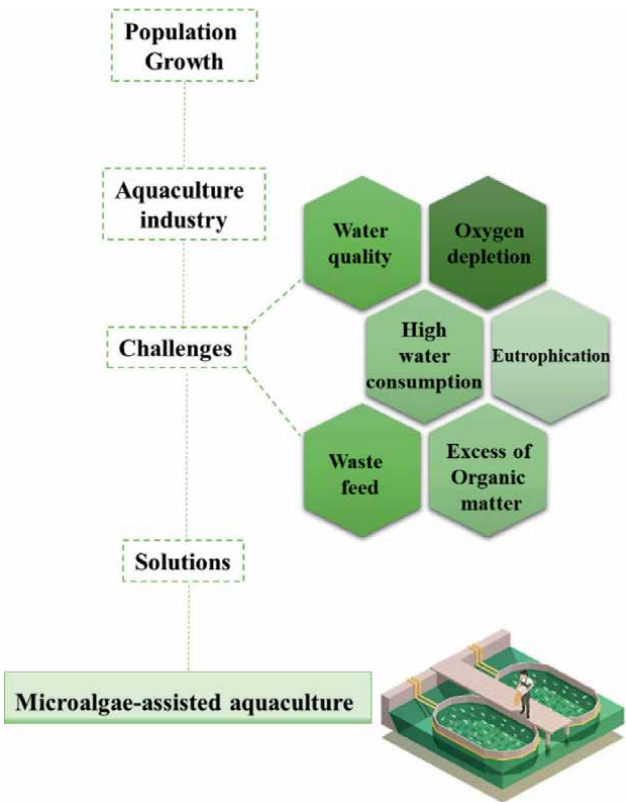


Figure 3.
Challenges and solutions for aquaculture systems.

Among the main factors to be monitored, the chemical demand for dissolved oxygen and excreted metabolic waste are the main problems encountered in the ecosystem [31]. Ocean and river acidification, associated with elevated carbon dioxide content, harms calcifying organisms such as mollusks and crustaceans [24]. In fact, the accumulation of particulate organic matter that accelerates microbial activity and the consumption of dissolved oxygen in culture waters increases morbidity in aquaculture. On the other hand, oxygen depletion accelerates the production of various toxins, which not only threaten the survival of aquatic species but also disturb the ecological balance of nature [32].

Aquaculture wastewater contains organic matter in the form of dissolved nutrients or suspended solids. Many studies reveal that in addition to organic matter from feed waste, excrement from aquatic animals can be considered the main factor contributing to eutrophication in aquaculture [33]. Nutrient enrichment in waterways due to excess feed used on aquaculture farms, to increase growth rates, exceeds average levels of nitrogen, organic matter, and phosphorus. Thus, eutrophication leads to harmful algal blooms, increased frequency of anoxic events, and fish death. These conditions lead to health implications, economic losses, and even ecological disasters if discarded without treatment [24].

Water scarcity and depletion of natural water resources are significant challenges facing the world today and particularly represent a major problem in the aquaculture industry. In the state of the art, the safety of aquatic organisms is closely linked to good quality water resources in order to guarantee the efficient supply of nutrients for the survival of diverse species [31]. This requires sustainable innovative practices as a possible way to solve the problems faced in the field of traditional aquaculture.

4.2 Microalgae-assisted aquaculture

Arguably worldwide, the most important application (in volume) of microalgae is in the production of fish, crustaceans, mollusks, seaweed, and others. Since, in natural environments, microalgae are the basis of a large part of the reproductive chains, larviculture, and growth, their high production becomes necessary, given the high nutritional value of its biomass [34].

In aquaculture, high loads of water used for production are usually discharged into the environment during water changes, as well as during harvesting [30]. Nutrients dissolved in aquaculture wastewater are mainly from unused feed and aquatic animal secretions. Therefore, in terms of nutrient supply, aquaculture wastewater is a promising alternative medium for microalgae cultivation.

Previous studies have shown that some species of microalgae grow well in aquaculture wastewater to sufficiently remove most of the contained nutrients. Ansari et al. [35] reported the use of different species of microalgae (*Chlorella sorokiniana*, *Scenedesmus obliquus*, and *Ankistrodesmus falcatus*) to treat aquaculture wastewater, in which they achieved removal efficiencies between 75.5 and 98.5% of nitrate (NO_3) and phosphorus in 15 days of cultivation. Likewise, high nitrate, carbon dissolved, and phosphorus removal efficiencies were found for *Parachlorella kessleri* at the inoculation concentration of only 0.1 g/L [36]. These same authors report NO_3 removal efficiency (85.7–97.1%) and total phosphorus removal efficiency between 90.2–98.9% for *Chlorella vulgaris*, *Scenedesmus quadricauda*, *Chlorococcum* sp., and *Scenedesmus obliquus*. In addition to removal efficiencies, the use of microalgae biomass as a supplement for aquatic animals has grown substantially, as at the same time that it increases their growth, it decreases feed consumption, thus consequently

lowering production costs. This type of diet increases the nutritional value and disease resistance of most fish and crustaceans [33].

Briefly, the concept of microalgae-assisted aquaculture involves different stages: (i) Microalgae are inoculated in ponds or aquatic systems. Here, they digest part of the organic waste secreted by the animals, improving the self-purification of the aquaculture system; (ii) The effluent water from the systems is used to cultivate microalgae and thus absorb the nutrients contained therein in separate tanks using various methods; (iii) The microalgae are then harvested in a cost-effective and environmentally friendly way; (iv) Freshly harvested biomass will be used as value-added feed and food, thus reducing the cost of rearing and production in aquaculture, and (v) treated effluent is recycled for use again in aquaculture systems [37].

Among the various microalgae cultivation systems developed, the Raceway system and the Rotating Algal Biofilm (RAB) are the two main systems used in microalgae-assisted aquaculture. The first involves pond systems in a circulation channel from suspended cell culture, reaching average productivity between 1 and 10 g/L for raceway tanks and photobioreactors, respectively [38]. Despite the low operational and investment costs due to its simplicity, the low cell concentration makes this type of system unfeasible, since the demand for time increases energy consumption and the harvesting and dehydration processes become more expensive (Ahmad et al., 2021). Thus, the microalgae cultivation system by biofilm becomes promising because it involves the growth and production of microalgae directly adhered to the surface of a support, in which its biomass is simply obtained by scraping. Thus, the cost of the harvesting process is practically non-existent [37].

Although the integration of microalgae in aquaculture systems alongside the treatment of effluents results in several technical, sustainable, and economic advantages, there are still several challenges that must be faced mainly in the use of microalgae biomass as an aquatic feeding medium [31]. It is mentioned that the cellulose-rich cell walls of microalgae make it difficult for aquatic animals to digest and therefore their structures must first be broken down for nutrients to be efficiently absorbed in their organism. The antinutrient effects and the bioaccumulation of heavy metals in microalgae from the treatment of highly polluting waste can also negatively affect the environment in which they live [39]. Thus, new technologies are being studied to eliminate these disadvantages and allow better assimilation of microalgae biomass as value-added products in aquaculture in the coming years. The main advantages and disadvantages of microalgae-assisted aquaculture are presented below (**Table 1**).

5. Microalgae for sustainable agricultural practices

The use of microalgae in modern agriculture has aroused great interest due to the wide range of benefits they can provide, both in increasing yields and crop health, while preserving the environmental balance [40]. Although several products can be used to improve crop productivity, microalgae biomass has high levels of nutrients that provide beneficial effects to plants. It is important to point out that different biological compounds can improve agricultural productivity through different modes of action and applicability, as shown in **Figure 4**. For example, microalgae contain plant hormones that function as plant growth promoters and at the same time protect against pests and pathogens. The concomitant assimilation of wastewater treatment, as previously discussed, also brings many benefits, mainly by keeping soils and water

Advantages	Disadvantages
Wastewater remediation	Energy consumption
Water replacement	Deficiency in digestibility
Recycling and water quality	Anti-nutritional factors
High-value food	Bioaccumulation of heavy metals
Healthier diets	Harvest and dehydration expenses
Reduced feed costs	
Prevention of oxygen depletion	
Prevention of toxic microorganisms	

Table 1.
Main advantages and disadvantages of microalgae-assisted aquaculture.

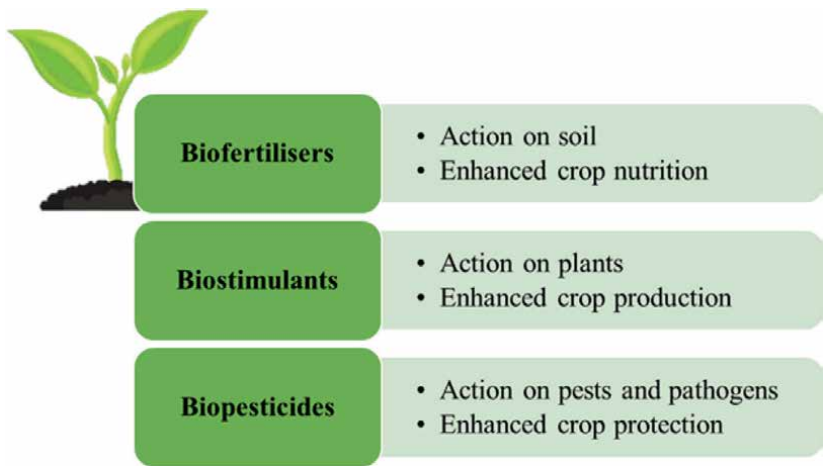


Figure 4.
Main applications of microalgae-based products in agriculture.

bodies free of contamination and consequently promoting the circular bioeconomy, by using its biomass as an organic matter [41].

Biofertilizers are biologically based compounds that promote agricultural processing, improving the availability of nutrients in the environment [42]. In addition to beneficial microorganisms, it is possible to find in biofertilizers various nutrients and plant hormones that, once applied to the soil, colonize the rhizosphere and even the interior of plants. Biostimulants are capable of improving the photosynthetic metabolism of plants due to respiratory control, ion absorption, and nucleic acid synthesis. Biopesticides act as biological controllers of pathogenic fungi and bacteria due to their antimicrobial, antioxidant, antiviral, and antifungal functions [40].

Although still under study and development, microalgae-based biofertilizers have proven to be a viable alternative to traditional, costly, and unsustainable agricultural techniques. Among the main benefits that biofertilizers offer to plants and the surrounding environment are: (i) an increase in organic matter in the soil; (ii) improvement in soil porosity; (iii) increased supply of oxygen in the rhizosphere; (iv) increase in soil water retention; (v) release of plant growth promoting substances;

(vi) improvement in salinity and control of soil acidity; (vii) prevention of weed growth, and (viii) increased availability of phosphates for plants [38]. These and other benefits are further presented below.

5.1 Fertility and soil quality

Cropland (i.e. plantation and rangeland), which occupies over 40% of Earth's land, is a major target of soil-based carbon sequestration. Scientists have estimated that soils – particularly agricultural soils – can sequester over an additional billion tons of carbon each year [43]. However, the conversion of natural ecosystems to agricultural land disrupts soil structure, releasing much of this stored carbon and contributing significantly to climate change.

The soils are increasingly impoverished due to extensive agricultural practices carried out in recent years. The excessive use of chemical defenders, nitrogen fertilizers, and the frequent use of heavy machinery alter the soil structure, resulting in disturbances, erosion, nutrient depletion, and difficulties in water maintenance [44].

From the point of view of health and quality, microalgae-based biofertilizers can increase the stock of organic carbon in soil through the excretion of carbon (exopolysaccharides) that are previously absorbed by the metabolism of microalgae through photosynthesis [45]. Reports have evaluated the potential of various types of biofertilizers, and the results have shown that compared to the control, microalgae-based biofertilizers are an advance for and renewal of organic carbon stocks in soils and negative biological emission of gases [43].

Considering the biological nitrogen fixation capacity, it is possible to conclude that microalgae can replace chemical nitrogen fertilizers, benefiting the economy in agricultural production [41]. In fact, microalgae have an advanced organism that fixes nitrogen gas by a cell called heterocyst, which creates a micro anaerobic environment where the enzyme nitrogenase can work smoothly. Even though it is the main constituent of our atmosphere (more than 75%), gaseous nitrogen (N_2) is not assimilable by most organisms, therefore, microalgae do not compete with plants for nitrogen demand, which ends up helping soil availability [46].

In the process of biological fixation, fixing microorganisms convert nitrogen into ammonia. Once fixed, one of the main mechanisms for transferring nitrogen from microalgae to plants is through the mineralization of the biomass after its death [47]. As the amount of ammonium salt is low in the soil, this fixation becomes extremely important for the maintenance of the environmental nitrogen cycle.

Other elements such as phosphorus, calcium, magnesium, zinc, iron, and potassium can also be found in microalgae biomass. These elements are typically involved in redox reactions, playing an important role in plant metabolism and growth [41].

5.2 Promotion of plant growth, disease, and pest control

As the environment changes and the crop passes through different stages of development, the ability to stimulate specific and natural responses in the plantation can make a significant contribution to achieving the goals of growth and integrated plantation management. Biostimulants, which have a very definite result, can provide the farmer with a balanced, protected, and proactively prepared plantation for all stages of development [41].

Biostimulants or phytohormones produced from microalgae contain complex bioactive molecules that have varied functionalities depending on their growth

conditions [48]. Among the main intracellular hormones of microalgae, cytokinins are widely studied molecules, however, there are other substances with the ability to promote plant growth and development, such as auxin, polyamines, and jasmonic acid. These are excreted from microalgae for in vitro growth and regeneration of valuable plants and this interaction occurs through the interaction between microalgae biomass and plant roots [15].

Although with development, practices characterized by successive planting have made the agriculture industry increasingly susceptible to disease and pests due to the plentiful availability of food, breeding sites, and chemical pesticide residues that are not easily decomposed, and thus remain in the soil as waste toxic [49]. Given this situation, microalgae can be identified as articulators of defense mechanisms in plants, such as an increase in RNA, and also in enzymatic activities described as antifungal, insecticides, nematicides, and herbicides [44].

Finally, microalgae biomass has many benefits when compared to traditional synthetic agricultural media. Many studies have already been reported in the literature and mainly involve the use of microalgae-based biofertilizers. Its main effects can be better observed in **Table 2**.

Strain	Target Crop/Soil	Benefits	Reference
<i>Anabaena</i> sp.	Rice	Improvement of iron and copper deficiency	[44]
<i>Anabaena variabilis</i>	Rice	Improves phosphate bioabsorption	[44]
<i>Anabaena torulosa</i>	Wheat	Increase in nitrogen availability in the soil	[50]
<i>Chlorella</i> sp.	Corn	Increased leaf length and weight	[51]
<i>Chlorella</i> sp.	Maize	Increased soil organic carbon	[52]
<i>Chlorella vulgaris</i>	Okra	Increased germination and yield Faster maturity	[53]
<i>Dunaliella salina</i>	Tomato	Increase in carotenoids Better enzymatic activities of plants	[54]
<i>Dunaliella salina</i>	Wheat	Increase in tolerance to salt stress	[55]
<i>Nannochloropsis oculata</i>	Tomato	Increase of N, P in the leaves Increase in sugars/carotenoids in fruits	[56]
<i>Nannochloropsis oculata</i>	Tomato	Increase in ammonium, phosphorus, and potassium in soil Increase in sugar and carotenoids contents in fruit	[56]
<i>Nostoc</i> sp.	Rice	Increase in grain yields comparable to those obtained with chemical fertilizers	[57]
<i>Oscillatoria augustissima</i>	Pea	Reduces the use of chemical fertilizers by 50% Improved nutritional value of pea seeds	[58]
<i>Scenedesmus quadricauda</i>	Pea	Increased protein, chlorophyll, and carotenoid content Better enzymatic activities in the leaves	[59]
<i>Scenedesmus</i> sp.	Lettuce	Inhibition of fungal growth	[60]
<i>Spirulina platensis</i>	Corn	Increased grain yield	[61]

Table 2.
Benefits of using microalgae-based products in agriculture.

6. Conclusions

The need to adopt sustainable practices in agribusiness is increasingly evident worldwide. Many producers still fear that ecological methods do not meet their production needs or are unaware of their benefits. The current consensus is that we need to urgently transition from a petrol-based market to a sustainable circulating bioeconomy.

Reducing the effects of chemical products, including traditional fertilizers, was already a goal set by the UN (United Nations) over 20 years ago. However, the main target imposed has not yet been reached. More than ever, reducing the use of chemical inputs is a more than immediate need to preserve the integrity of the environment and public health.

Microalgae have long been considered a rich source of value-added products and an attractive business opportunity. The agriculture and aquaculture sectors have been important, as microalgae biomass has shown remarkable results in improving aquafeed, soil quality, and crop development, growth, and yield.

To date, microalgae systems and processes have been well explored. In contrast, microalgae bioproducts, although known to have positive effects, their commercial implementation is limited by high-energy demand and production costs. In this sense, the treatment of organic waste using microalgae is a valuable strategy, as in addition to obtaining environmental benefits (such as cleaning watercourses, reduction of degrading compounds, and greenhouse gas emissions), there is a parallel advantage in the economy through the recovery of nutrients and conversion of its biomass for use in several areas of application.

Finally, the challenges related to microalgae processes associated with organic waste still need extensive field research, further evaluation, and, above all, increasing awareness of its benefits in the areas of social interest and the global economy. Thus, bearing in mind that biologically based products can improve the quality of life on Earth, we can be sure that the commercialization and production of microalgae-based products will be an imminent success in the future.

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The Potential of Microalgae in Phycoremediation

Lucia Barra and Silvestro Greco

Abstract

Heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs) can have negative impacts on the marine and freshwater biota. Utilizing microbes, particularly microalgae, which are able to extract metals and hydrocarbons from environmental matrices, the impact of these contaminants in freshwaters, seas, and wastewaters can be reduced. In fact, the contaminants can be passively absorbed and actively accumulated within the organelles of microalgae, reducing their negative impact. River streams, untreated municipal, agricultural, and industrial wastewaters, represent one of the most important issues for the anthropogenic pollution. Microalgae and bacteria can grow in polluted waters containing different metals as cadmium (Cd), lead (Pb), zinc (Zn), chromium (Cr), copper (Cu), and mercury (Hg). They are able to accumulate metal elements within biomass in a dose-dependent manner and are employed in bioremediation thanks to their growth ability in polluted sites. In the following chapter, we analyze the potential of employing microalgae in phycoremediation, their mechanisms of tolerance and resistance to heavy metals, the possibility to use consortia of microorganisms instead of single specie to cope with complex polluted matrices, the possibility to metabolic engineer microalgae to gain their function, and the employment of microalgae in both liquid and solid devices.

Keywords: microalgae, genetic diversity, heavy metals, hydrocarbons, bioremediation

1. Introduction

1.1 Origin of pollution

Anthropogenic pollution is affecting marine environments worldwide, posing serious issues to the sustainability of current economic activities. Mining, intensive agriculture, metallurgical, tanning, and canning industries have led to significant contamination of terrestrial and marine ecosystems with toxic metals and organic pollutants such as petroleum hydrocarbons and other emerging contaminants.

The release of pollutants, such as polycyclic aromatic hydrocarbons (PAHs), heavy metals, pesticides, and plastic waste, has resulted in climate change, habitats' destruction, and loss of biodiversity. Anthropogenic impacts have disrupted ecosystems, altered natural processes, and posed significant threats to human health and the well-being of the ecosystems. Among different classes of pollutants, we will address, in

this chapter, two particular classes, whose impacts are crucial for the sustainability of our planet and future generations, e.g., heavy metals (HMs) and polycyclic aromatic hydrocarbons (PAHs). Heavy metals, including lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), and chromium (Cr), are primarily derived from industrial activities, such as mining, metal smelting, and manufacturing processes. These metals can enter in the ecosystems through atmospheric deposition, leaching from mining sites, and industrial wastes [1].

Additionally, agricultural practices, such as the use of fertilizers and pesticides, can contribute to heavy metal contamination in soil and water systems. Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds that are formed during the incomplete combustion of organic materials, such as fossil fuels, wood, and tobacco. Major sources of PAHs include vehicle emissions, industrial combustion processes, and residential heating using solid fuels. Activities, such as oil spills, industrial discharges, and improper waste disposal, also contribute to PAH contamination in aquatic environments. Both HMs and PAHs are persistent pollutants that can bioaccumulate in organisms through a process called biomagnification that affects species tropic chain at different levels till arriving to human beings [2]. Effective management strategies, including pollution prevention, proper waste management, and the implementation of strict regulations, are necessary to mitigate the sources and impacts of HMs and PAHs on the environment.

Bioremediation, i.e., the use of living organisms to remove or neutralize pollutants from the environment, has emerged as a promising and sustainable approach to cope with different pollutants. Bringing novel pollution-tolerant microbes in culture and documenting their ability to degrade or uptake selected pollutants in the laboratory are likely to facilitate bioremediation research by providing novel species and information on their tolerance/resistance thresholds. Among the diverse microorganisms used for bioremediation, bacteria and microalgae have gained significant attention due to their unique capabilities to mitigate pollution while offering additional benefits. Several microbe-based approaches to promote hydrocarbons' degradation and metals' removal are currently under investigation.

In this chapter, we explore the mechanisms by which microorganisms and microalgae, in particular, contribute to bioremediation process.

1.2 Why microorganisms can be useful?

The use of microorganisms in the bioremediation of heavy metals (HMs) offers several advantages over traditional remediation methods. First, microorganisms possess the ability to react with heavy metals and transform them into less toxic forms through various metabolic processes. This natural ability of microorganisms can avoid the recurrence of costly chemical treatments. Second, microorganisms can thrive in different environments, including contaminated sites with extreme pH, temperature, light, and salinity conditions. Their adaptability makes them able to survive in challenging environments where chemical-physical remediation methods may be less effective or unfeasible.

Furthermore, microorganisms have the capability to bioaccumulate heavy metals within their biomass. This property makes them suitable for the removal and recovery of heavy metals from contaminated waters or soils. The metal-laden microorganisms can be harvested and further processed, allowing for the potential extraction and recycling of valuable metals. In addition, the use of microorganisms in heavy

metals remediation is generally considered environmentally friendly and sustainable. Microbial processes occur naturally and do not produce harmful byproducts or contribute to secondary pollution. The use of microorganisms in remediation can also be combined with other eco-friendly techniques, such as phytoremediation, where plants and microorganisms co-work to remove contaminants.

Lastly, microorganisms can be engineered or selected for specific heavy metal tolerance or remediation capabilities, enhancing their efficiency in targeted remediation processes. Genetic engineering and microbial consortia approaches can be employed to optimize the remediation potential of microorganisms and tailor them to specific contamination scenarios. Overall, the use of microorganisms in heavy metals remediation offers a cost-effective, adaptable, sustainable, and environment-friendly approach that holds significant promise for addressing heavy metals pollution and restoring contaminated environments [3]. Below, we present the peculiar processes of photosynthetic eukaryotes, such as microalgae, which are small photosynthetic organisms either prokaryotic (cyanobacteria) or eukaryotic and which can live in all types of water and use carbon dioxide (CO₂) and direct light as their only sources of energy and carbon to make organic chemicals [4]. Microalgae are able to cope with the presence of HMs and PAHs in the environments, making them promising microorganisms to be investigated for future applications in the phycoremediation field:

1. **Photosynthetic activity:** microalgae harness the power of photosynthesis, a process that utilizes light energy to convert carbon dioxide into organic matter, thereby reducing greenhouse gas emissions. This photosynthetic activity also plays a pivotal role in bioremediation. Microalgae absorb pollutants, such as heavy metals and organic contaminants, from their surroundings. The energy acquired through photosynthesis allows them to metabolize and break down these pollutants into less toxic forms, effectively removing them from the environment.
2. **Nutrient uptake and eutrophication control:** excessive nutrient runoff, particularly nitrogen and phosphorus, leads to eutrophication, a phenomenon causing harmful algal blooms (HABs) and oxygen depletion in aquatic ecosystems. Microalgae can help combat eutrophication by efficiently assimilating and incorporating these nutrients into their biomass. By doing so, they compete with harmful algal species for resources, limiting their growth and mitigating the negative impacts of eutrophication.
3. **Biosorption and bioaccumulation:** microalgae possess the ability to accumulate and sequester various pollutants through biosorption and bioaccumulation processes. Biosorption involves the passive binding of contaminants onto the surface of microalgal cells or extracellular polymeric substances (EPS) produced by microalgae. Through this mechanism, microalgae can remove heavy metals, organic pollutants, and even radioactive substances from the environment.

Bioaccumulation, on the other hand, involves the active uptake and concentration of contaminants within the cellular structures of microalgae. These contaminants can be transformed, stored, or further metabolized within the cells. Microalgae, with high bioaccumulation potential, can be employed in bioremediation strategies to reduce the concentration of pollutants in contaminated areas.

4. **Enzymatic degradation:** microalgae possess a diverse range of enzymes that play a crucial role in the degradation and detoxification of pollutants. For example, certain microalgae produce extracellular enzymes like lipases, proteases, and peroxidases that can break down complex organic compounds, including petroleum hydrocarbons. These enzymatic activities enhance the bioremediation efficiency of microalgae by facilitating the degradation of recalcitrant pollutants into less harmful byproducts.
5. **pH adjustment and oxygen production:** microalgae can influence the pH of their surroundings through their metabolic activities. They can remove excess carbon dioxide from the environment during photosynthesis, leading to a rise in pH. This ability to regulate pH is particularly useful in bioremediation processes, as certain pollutants are more soluble and easily removable under specific pH conditions. Additionally, microalgae are oxygenic organisms, releasing oxygen as a byproduct of photosynthesis. The oxygen released by microalgae enhances the aerobic conditions necessary for the activity of other microorganisms involved in the bioremediation process.

1.3 Chemical versus biological remediation processes

Classical chemical remediation processes are employed to mitigate environmental contamination caused by various pollutants. These processes involve the use of chemical agents or reactions to transform or remove contaminants from soil, water, or air. Heavy metals have been removed using a variety of standard techniques, including adsorption, chemical precipitation (carbonate, hydroxide, and sulfide precipitation), chemical oxidation, chemical reduction, solvent extraction, reverse osmosis ion exchange, and electrodialysis [5]. One widely used chemical remediation technique is chemical oxidation, which involves the introduction of oxidizing agents, such as hydrogen peroxide, ozone, or persulfate, into contaminated sites. These agents react with the pollutants, breaking them down into less harmful substances. Another method is the chemical reduction, where reducing agents like zerovalent iron or sodium dithionite are utilized to convert toxic contaminants into less harmful or nontoxic forms. Additionally, chemical precipitation is used to remove heavy metals from wastewater by adding chemicals that cause the metals to form insoluble precipitates, which can then be easily separated. Chemical remediation processes offer efficient and targeted approaches for treating contaminated environments, but their implementation requires careful consideration of the specific contaminants, site conditions, and potential impacts on surrounding ecosystems. However, at concentrations between 1 and 100 mg/L, these traditional methods are ineffective for entirely removing the heavy metals. Other traditional methods have also been described, including the utilization of inorganic adsorbents such as ores, clay, metallurgical wastes, industrial waste materials, and alum, as well as organic adsorbents like plants and animal-derived waste products. However, they are neither a cheap, energy-intensive, nor an ineffective procedure, and they do not totally remove the heavy metals from the effluent [6, 7]. For the above-mentioned reasons, the phycoremediation research field is rising in the last 10 years. In **Table 1** are shown the classic methods for chemical remediation.

Methods: Physical	Advantages	Disadvantages
Adsorption	Economical, absorbent, low sludge volume, flexibility in operation, applicability in wide pollutants, and fast kinetics	Adsorption chemicals are required, and adsorbent-dependent performance
Ion exchange	Potentials for metal recovery and maximized degree of regeneration and can be metal selective	Has high operation cost and sensitivity toward the particles
Membrane filtration	High efficiency, space saving, low solid waste generation, and low chemical consumption	Requires high power, restoration of the membrane is required, and high initial capital cost
Photocatalysis	Removes heavy metals and organic pollutants	Time-consuming process
Reverse osmosis	Pure effluent generation and removes most of the heavy metals	Expensive method required
<i>Chemical methods</i>		
Chemical precipitation	No metal selectivity and simple technique	Ineffective in higher concentration, large sludge generated, high maintenance, and sludge disposal cost
Coagulation and flocculation	Cost-effective techniques, aids dewatering, bacterial inactivation capacity, and good sludge settling features	High utilization of chemical and solvent, high sludge disposal required, and cannot stand alone to remediate the heavy metals
Electrochemical treatment	Moderate metal selectivity, a low chemical required, and pure metal can be achieved	Requires high electricity supply, requirement of floc filtration, and high capital cost
Evaporation	Pure effluents generated that can be reused	Energy-intensive process, sludge disposal required, and a costly process

Table 1.
Remediation of heavy metals using various conventional methods (Monteiro et al. [8]; Cheng et al. [5]).

2. Phycoremediation

Phycoremediation, among the various phytoremediation strategies, specifically focuses on the use of macro- and microalgae for environmental remediation purposes. In this chapter, we will focus on microalgae's capabilities to remediate environments from heavy metals pollution. Phycoremediation harnesses the natural capabilities of microalgae to remove or transform pollutants from air, water, and soil. Microalgae have a remarkable ability to uptake and accumulate contaminants, thanks to their high surface area-to-volume ratio and efficient uptake mechanisms. They can accumulate and sequester pollutants within their biomass, thus reducing their presence in the surrounding environment. Moreover, certain species of microalgae have the capability to metabolize and degrade various organic contaminants, such as hydrocarbons, pesticides, and pharmaceuticals, through their unique enzymatic machinery. This capacity makes them valuable agents for the biodegradation and detoxification of organic pollutants in aquatic and terrestrial environments. Phycoremediation is a sustainable and eco-friendly approach that operates through natural biological processes, minimizing

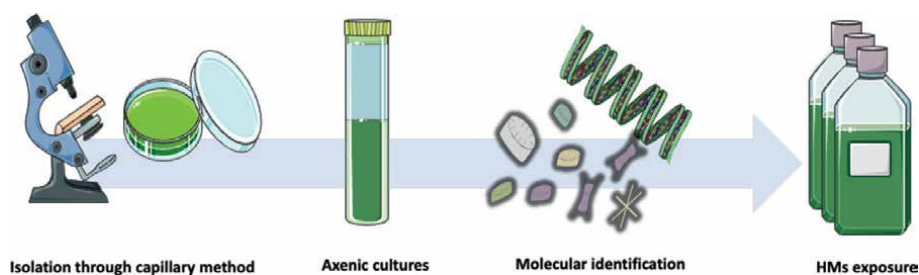


Figure 1.
Flowchart of operation working for microalgae use in the bioremediation of heavy metals, HMs.

the need for extensive infrastructure or chemical interventions. Furthermore, the biomass generated during phycoremediation processes can be harnessed for various value-added applications. Microalgal biomass can serve as a potential source of biofuels, feedstock for livestock and aquaculture, or as a raw material for the production of bioactive compounds. By harnessing the power of these microscopic organisms, phycoremediation offers a greener and more holistic solution to environmental cleanup, while also providing opportunities for resource utilization and sustainable development. In **Figure 1** is explained the flowchart of microalgae phycoremediation.

2.1 Benefits of using microalgae, a realm of biodiversity

Microalgae are highly adaptable organisms, which are capable of thriving in diverse environmental conditions, including saline, brackish, or wastewater-impacted environments. Their rapid growth rates and high biomass production further contribute to their efficacy as phycoremediation agents. They encompass a vast realm of biodiversity, comprising numerous genera and species with diverse characteristics and functionalities.

Different microalgae have been utilized to remediate wastewater all around the world for the past 30 years [9]. Microalgae *Scenedesmus incrassatulus* were used in an experiment by Pena-Castro and associates [10] to extract copper (II), cadmium (II), and chromium (VI). *S. incrassatulus* eliminated 25–78% of the heavy metals, according to the findings. Similar to Omar [11], who investigated the removal of Zn using *Scenedesmus quadricauda* and *Scenedesmus obliquus*, demonstrated that *S. obliquus* had a better removal efficiency than *S. quadricauda*. Additionally, Soeprbowati and Hariyati [12] investigated the capacity of the microalgae *Chlorella sp.*, *Spirulina sp.*, *Chaetoceros sp.*, and *Porphyridium sp.* to remove heavy metals including Cd, Cu, and Pb. *Chlorella sp.* provided the maximum removal effectiveness of Pb (90%), Cd (62%), and Cu (83%) after 14 days of cultivation. However, *Spirulina sp.* and *Chaetoceros sp.* reacted with the lowest level of effectiveness, while *Porphyridium sp.* demonstrated good decrease of Cd (70%) and Cu (96%). These findings revealed that different microalgae species reduce heavy metals content to varying degrees, and that these species are also influenced by the types of heavy metals present in the culture medium or wastewater. By using adsorption and desorption techniques, dead microalgal cells (passive adsorbed biomass, PAB) and microalgae-based products can remove heavy metals. Daneshvar and collaborators [13] investigated the use of microalgal char in the removal of Cr (VI) from water. According to the study, microalgal biochar completely eliminated Cr (VI) from water. Cheng [14] also studied the biosorption and kinetics of Cd (II) removal using living and dead cells of *Chlorella vulgaris*.

The biosorption capacity of *Scenedesmus* sp. biomass for the removal of Cr (VI) from the solution was also studied by Pradhan and associates [15]. The findings showed that 89% of Cr (VI) was deposited in *Scenedesmus* sp. biomass. According to an analysis using Fourier transformation-infrared spectroscopy (FT-IR), microalgae cells contain a variety of functional groups, including amides, aldehydes, carboxylic acids, halides, and phosphates, all of which are positively charged. This allows microalgae to adsorb and aid the biosorption process. Using the Pb (II)-tolerant microalga *S. obliquus*, Danouche and colleagues [16] investigated the mechanism of Pb (II) reduction. In all the studies cited, the percentages of metals' uptake capability were higher than 80%. The most part of experiments has been conducted with species belonging to Chlorophyceae and Diatoms genera.

Another source of HMs can be represented by the acid mine drainage; acid mine drainage can be directly discharged into water systems, which damages aquatic habitat and has detrimental effects on the ecosystem. However, by eliminating the hazardous heavy metals that are present in acid mine drainage, microalgae can remediate it [9]. The microalgae *Chaetoceros muelleri*, *Tetraselmis chuii*, *Pavlova lutheri*, and *Nannochloropsis gaditana* were utilized by Richards and Mullins [17] to extract heavy metals from municipal leachate and hypersaline effluent. The findings showed that 95% of the heavy metals in both wastewaters were removed by microalgae. Furthermore, a significant amount of lipids was generated by *Nannochloropsis gaditana* and *Chaetoceros muelleri*. Using a biorefinery strategy, the integration of microalgae-based heavy metal wastewater treatment with microalgal lipid production may offer a new way to keep the circular bio-economy alive. In order to investigate this integrated strategy on a laboratory scale, Yang and colleagues [18] heterotrophically cultured *Chlorella minutissima* UTEX 2341 in the presence of heavy metals as Cd, Cu, Mn, and Zn in order to produce lipids. The findings showed that *Chlorella minutissima* UTEX 2341 eliminated heavy metals by intracellular accumulation and extracellular immobilization. The presence of Cd and Cu in the media also increased the accumulation of lipid. The removal of Ni and Cu using *C. vulgaris* and *Desmodesmus* sp. was also studied by Rugnini and associates [19]. The findings showed that both microalgae were able to survive for up to 12 days when Cu and Ni were present, and *Desmodesmus* sp. was able to remove more than 90% of the Cu from the water samples. The capacity of *Nannochloropsis oculata* for the uptake of Cu (0,05, 0,1, 0,15, 0,2, and 0,25mmol/L) from the acid mine drainage was assessed by Martinez-macias and colleagues [20]. They also investigated that *N. oculata* species' lipid and biomass production eliminated 92% of the Cu content, according to the findings. Among them, the most conspicuous part was removed by microalgal metabolism, while a small percentage has to be ascribed to adsorption mechanism.

Additionally, it generated biomass rich in saturated and monounsaturated fatty acids (SFAs), which was used as a fuel for the manufacture of biodiesel.

There is a significant volume of copper-rich water produced by the copper industry. The removal of Cu and Mo from wastewater by *C. vulgaris* had the highest removal efficiency of the two microalgae that Urrutia and associates [21] examined. *Chlorella kessleri*, a microalga, was also employed by Sultana and associates [22] as a biosorbent agent to remove heavy metal ions from synthetic wastewater.

The above-mentioned studies give an idea on microalgal biodiversity that extends far beyond the examples provided here. The microalgal biodiversity, in fact, is huge, with almost 64 classes (phyla) and more than 70,000 species that could represent the basin to draw for investigation of the microalgal diversity, with bioremediation aims [23]. The most recent and diversified taxon group, from an evolutionary and biogeochemical

point of view, is the diatoms' group that has been estimated to have approximately 10^5 species [24]. Most of the diatoms' biodiversity, for example, is hidden due to cryptic and pseudocryptic nature of species belonging to the group. For this reason, in recent years, taxonomy has been using molecular tools, in addition to morphological identification to better decipher the richness of the microalgal realm [25].

There are thousands of genera and species of microalgae, each with unique characteristics and potential applications. The study and exploration of microalgae biodiversity hold immense value in unlocking their full potential for various industries and environmental applications. **Table 2** illustrates examples of microalgae successfully employed in phycoremediation.

Microalgal species	Experimental condition	Heavy metal removal efficiency	Reference
<i>Chlorella sp.</i>	IT: 14 days	Pb: 90%, Cd: 62%, Cu: 83%	Soeprbowati and Hariyati [12]
<i>Chaetoceros sp.</i>	IT: 14 days	Pb: 81%	Soeprbowati and Hariyati [12]
<i>Porphyridium sp</i>	IT: 14 days	Cd: 70%, Cu: 96%	Soeprbowati and Hariyati [12]
<i>Spirulina sp.</i>	IT: 14 days	Cd: 73%	Soeprbowati and Hariyati [12]
<i>Chlorella vulgaris</i> , <i>Arthrospira platensis</i>	pH: 5–5.5, T: 20 °C	Ni (II), Zn (II), Cd (II), Cu (II)	Piccini <i>et al.</i> [27]
<i>Pseudochlorococcum typicum</i>	T: 20.1°C, LI: 30 mE m ² s ⁻¹ , D:N cycle: 16 h:8 h	Hg ²⁺ : 97%, Pb ²⁺ : 70%, Cd ²⁺ : 86%	Shanab <i>et al.</i> [28]
Live and dead cells of <i>Chlorella vulgaris</i>	T: 25°C, CT: 5–15 min	Live cells: Cd removal by live cells (95.2%) and dead cells (96.8%)	Cheng [14]
<i>Scenedesmus quadricauda</i> biochar	CT: 4 h, pH: 2, T: 5–35°C	Cr (VI): 100%	Daneshvar <i>et al.</i> [13]
<i>Chlorella sorokiniana</i> , <i>Scenedesmus acuminatus</i>	IT: 7 days, T: 25.2°C, LI: 100 µmol m ² s ⁻¹ , D:N cycle: 16 h:8 h	Cu (na)	Hamed [29]
<i>Desmodesmus sp.</i> MAS1 and <i>Heterochlorella sp.</i> MAS3	pH: 3.5, IT: 7 days, T: 23.1°C, LI: 60 µmol m ² s ⁻¹	Cd: >58%	Abinandan <i>et al.</i> [30]
<i>Scenedesmus sp.</i> 10 mg l	pH: 1.0, T: 23°C	Cr (VI): 92.89%	Pradhan <i>et al.</i> [15]
<i>Chlamydomonas reinhardtii</i>	pH: 9.5, IT: 180 h	As: 38.6%	Saavedra <i>et al.</i> [31]
<i>Chlorella vulgaris</i>	pH: 5.5, IT: 180 h	As: 32.4%	Saavedra <i>et al.</i> [31]
<i>Scenedesmus almeriensis</i>	pH: 9.5, IT: 180 h	As: 41.7%	Saavedra <i>et al.</i> [31]
<i>Chlorella vulgaris</i> , <i>Scenedesmus quadricauda</i>	pH: 2, T: 25°C, IT: 240 h, pH: 6 for Cr (III) and 1 for Cr (VI), T: 25°C, IT: 120 h	Cr (VI): 43%; Cr(III): 98.3%	Sibi [32], Khoubestani <i>et al.</i> [33]

Microalgal species	Experimental condition	Heavy metal removal efficiency	Reference
<i>Chlorella sp.</i> and <i>Chaetoceros sp.</i>	pH: 6, T: 25°C, IT: 180 h	<i>Chlorella sp.</i> and <i>Chaetoceros sp.</i> removed Pb 78 and 60%, respectively	Molazadeh <i>et al.</i> [34]
<i>Chlorella pyrenoidosa</i>	T 26–31°C	Ammonium nitrogen 100%, total nitrogen 57.14%, total phosphorus 75.51%, copper 73.39%, lead 72.80%, and cadmium 48.42%	Rahmani <i>et al.</i> [35]
<i>Nannochloropsis oculata</i>	LI: 2000 lux, D:N cycle 18 h:6 h, T: 25°C	Oil 66.5%, COD 54%	Ammar <i>et al.</i> [36]
<i>Nannochloropsis oculata</i>	D:N cycle 14 h:10 h, T:21°C, pH 7.5–9	Ammonium nitrogen 100%, COD 70%, iron 100%	Parsy <i>et al.</i> [37]
<i>Nannochloropsis oculata</i>	LI: 57 µmol m ⁻² s ⁻¹ , D:N cycle 12 h:12 h, T: 21°C, pH: 7	PAHs 94%, NAP 96%, APT 95%, FLU 91%, PHE 83%, BbF 95%, DA 90%, BaP 95%, iron 96.80%	Marques <i>et al.</i> [38]
<i>Galdieria sulphuraria</i>	T: 42°C, D:N cycle 24 h, LI: 4000 lux	Total nitrogen 100%	Rahman <i>et al.</i> [39]

Table 2.
Modified from Grosswami *et al.* [26], different microalgae reported to remove heavy metals, hydrocarbons, nitrogen N, phosphorus P, and chemical oxygen demand, COD; T, temperature; IT, incubation time; LI, light intensity; DN, day-night cycle.

2.2 How do microalgae work to cope with heavy metals in the environment?

Two main processes have been detected in the study of microalgae responses to metals' tolerance and resistance, they can be resumed in passive and active mechanisms, e.g., passive biosorption and active intake of metals through specific transporters together with antioxidant responses and redox cellular state changes. In the following sections, we will sum up the processes one by one. Ion exchange, chelation, complexation, hydroxide condensation, covalent binding, redox interaction, biomineralization, and precipitation of insoluble metal complexes are some of the molecular mechanisms by which HMs are biosorbed onto cell walls and EPS. These mechanisms involve electrostatic, van der Waals, or hydrophobic interactions between positively charged HM cations and negatively charged groups on the cell surface [40].

1. Passive biosorption is a key mechanism by which microalgae can remove heavy metals from the environment. Microalgae have a high surface area-to-volume ratio, which facilitates the binding and accumulation of heavy metals onto their cell walls, extracellular polymeric substances (EPS), and intracellular compartments. The cell wall of microalgae contains various functional groups, such as carboxyl, amino, hydroxyl, sulfhydryl, sulfate, phosphate, carbonyl, amide, imidazole, thioether, and phenol groups, which can interact with heavy metal ions through electrostatic attraction, ion exchange, complexation, and coordination reactions. The constitutive macromolecules of the cell wall, which contain a variety of negatively charged functional groups, act as the interface between the intracellular compartment and the external environment, creating a background moiety that can bind to ions from the surrounding medium in the absence of

steric or conformational barriers [41]. The biosorption capacity of microalgae depends on factors, such as the metal concentration, pH, temperature, biomass concentration, and the presence of other ions in the environment. Certain species of microalgae, such as *Chlorella*, *Spirogyra*, and *Scenedesmus*, have demonstrated efficient biosorption capabilities for a range of heavy metals, including copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn). Biosorption offers a cost-effective and eco-friendly approach for the removal and recovery of heavy metals from contaminated water bodies. Death microalgal biomass, PAB, can be employed for the passive biosorption process.

2. Molecular processes and antioxidant responses: when exposed to heavy metals, microalgae activate a series of molecular processes and antioxidant responses to mitigate the toxic effects. Microalgae respond to HM-generated oxidative stress by managing the cell redox status and overexpressing heat shock proteins (HSPs), just like other cell types do [42]. MicroRNAs (miRNAs) are essential parts of the gene regulatory system that controls cellular HMs. By causing the complexation of excess HMs, oxidative stress defense, and signal transduction for biological control purposes, their contribution to posttranscriptional cleavage and translational inhibition of target mRNAs or methylation of target DNAs regulates responses intended to maintain cellular homeostasis [43]. Additionally, it appears that a number of genes involved in HM absorption, transport, and detoxification are activated by metal-responsive transcription factors (TFs), creating a systemic defense against HM toxicity in the cell. Microalgae consider alternative strategies, such as sexual reproduction, expression of metal-modifying enzymes, and phenotypic plasticity as a means of surviving HM toxicity [44]. Heavy metals can induce the cells to produce reactive oxygen species (ROS), which can cause oxidative stress and damage to proteins, lipids, and DNA. More specifically, they act by impairing the photosynthetic apparatus, preventing enzyme activity, and/or stopping cellular divisions [45], or by obstructing the normal function of the thylakoid membrane and chlorophyll biosynthesis, acidifying the cytoplasm, or harming the cell membrane [46]. Microalgae employ various antioxidant defense mechanisms to counteract the harmful effects of ROS and maintain cellular homeostasis. Some of these molecular processes include:

- Activation of antioxidant enzymes: microalgae upregulate the activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and ascorbate peroxidase (APX). These enzymes scavenge ROS, convert them into less harmful molecules, and maintain the redox balance within the cells.
- Accumulation of antioxidants: microalgae increase the synthesis and accumulation of antioxidant compounds such as glutathione (GSH), ascorbic acid (vitamin C), tocopherols (vitamin E), and carotenoids. These antioxidants directly neutralize ROS and protect cellular structures from oxidative damages. Increased expression of peptides that include metal-binding amino acids, such as proline, histidine, and glutamate, is another strategy used by microalgae. In order to counteract the otherwise harmful effects of HMs in the cytoplasm, these organometallic complexes are normally transported and partitioned them into vacuoles [47]. When HMs are released from their organic carrier in the acidic environment of vacuoles, they are most likely stabilized and chelated by sulfides or organic acids in vacuoles [48].

- Induction of stress-responsive genes: exposure to heavy metals triggers the activation of stress-responsive genes, including those involved in metal detoxification, chelation, and transport. For instance, metallothionein and phytochelatin genes are upregulated, leading to the synthesis of metal-binding proteins that sequester heavy metals and prevent their toxic effects.
- Activation of repair mechanisms: Microalgae activate DNA repair mechanisms, including DNA damage sensing and repair enzymes, to mitigate the genotoxic effects of heavy metals. These repair mechanisms help maintain genomic stability and prevent mutations.

Overall, the biosorption capacity of microalgae enables them to sequester heavy metals from the environment, while their molecular processes and antioxidant responses protect them from the toxic effects of heavy metal-induced oxidative stress. Understanding these mechanisms is crucial for harnessing the potential of microalgae in bioremediation strategies and developing effective strategies for environmental cleanup and sustainable resource utilization, also through the use of genetic engineered microalgae. For the active intake of metals, microalgae employ various mechanisms through membrane transporters to fulfill their nutritional requirements. The net metal flow is therefore lowered, and the expulsion of trace metal complexes may potentially impact the chemical speciation of the HMs. Several metal efflux pumps do regulate the algal membrane permeability by actively moving HMs into and out of the cell [49]. The membrane transporters facilitate the uptake of essential metals, such as iron (Fe), copper (Cu), manganese (Mn), zinc (Zn), and others. It can happen that for some of these membrane transporters, toxic metals, such as cadmium (Cd), chromium (Cr), and lead (Pb), are internalized because the transporters cannot discriminate between toxic and essential cations. The mechanism can be broadly categorized into two types: primary active transport and secondary active transport:

1. Primary active transport: primary active transport involves the direct expenditure of energy (typically adenosine triphosphate (ATP)) to drive the uptake of metals against their concentration gradient. This process is mediated by ATP-binding cassette (ABC) transporters, which are energy-dependent membrane proteins. These transporters utilize the energy derived from ATP hydrolysis to actively pump metal ions across the cell membrane.
2. Secondary active transport: secondary active transport relies on the preexisting electrochemical gradient of ions generated by primary active transporters or ion pumps. The energy stored in the electrochemical gradient is then utilized to drive the uptake of metals. Two common types of secondary active transporters involved in metal uptake are symporters and antiporters:
 - Symporters: Symporters cotransport metal ions along with other ions across the membrane in the same direction. The movement of one ion down its electrochemical gradient provides the energy required to transport the other ion against its concentration gradient. For example, the sodium-dependent phosphate symporter (NaPi) mediates the cotransport of sodium and phosphate ions, with the sodium gradient providing the energy for phosphate uptake.

- **Antiporters:** Antiporters facilitate the exchange of one ion for another across the membrane. They couple the movement of one ion against its concentration gradient with the movement of another ion down its gradient. For instance, the proton-coupled divalent metal transporter (DMT) exchanges protons for divalent metal ions, allowing the uptake of metals using the proton gradient as the driving force.

These membrane transporters play a crucial role in maintaining metal homeostasis within microalgae cells, in the most part, but not always discriminating between essential and toxic metals. The expression and activity of these transporters are regulated by the availability of metals and the specific needs of the microalgae. It's important to note that the specific membrane transporters and their mechanisms can vary among different microalgae species and even within different growth culture stages of the same species. The understanding of these transport mechanisms is essential for exploring the bioavailability and uptake of metals in microalgae and harnessing their potential in bioremediation, nutrient supplementation, and other applications. In **Figure 2** are shown the cellular mechanisms which are implied in microalgae responses to HMs.

2.2.1 Mechanisms against cadmium, copper, and zinc metals and species for metals remediation

Microalgae employ specific mechanisms to cope especially with a high concentration of cadmium (Cd), and essential metals such as zinc (Zn) and copper (Cu), when

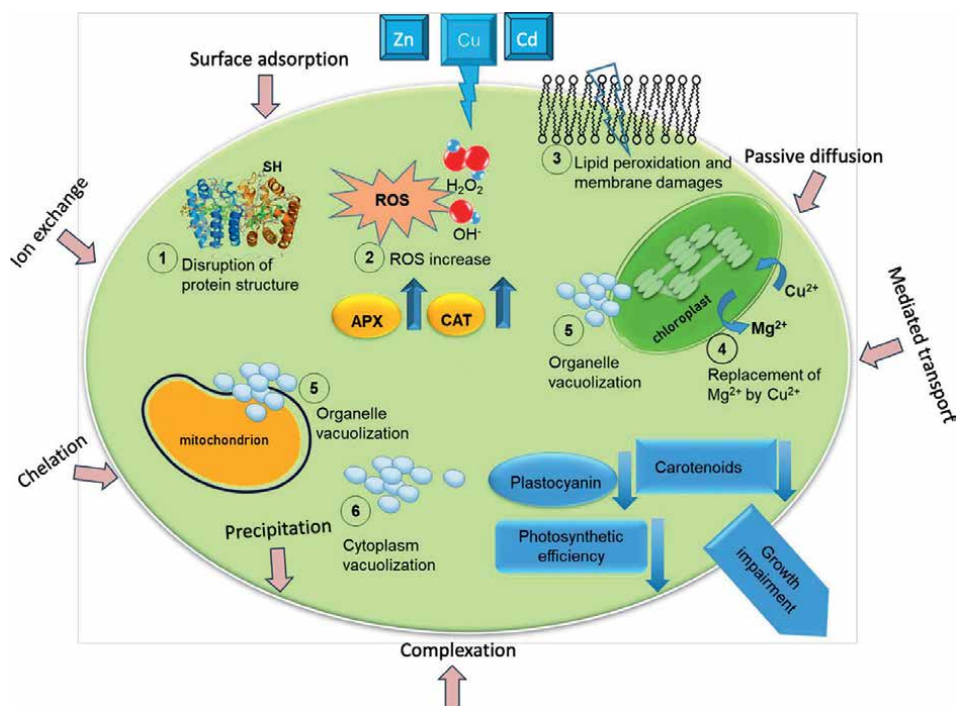


Figure 2. Modified from Cavalletti et al. [50], the figure illustrates the effects of high concentrations of cadmium (Cd), copper (Cu), and zinc (Zn).

their concentrations exceed the normal cellular functions, and mitigate their toxic effects; obviously, the mechanisms acted against these metals can also work with other elements. Cd is a heavy metal that can cause severe damage to cellular components and disrupt biological processes in cells. Here are some specific mechanisms used by microalgae to cope with Cd toxicity:

1. **Metal binding and sequestration:** microalgae possess cell wall components, such as polysaccharides and proteins, which can bind and sequester cadmium ions. These functional groups present on the cell surface can form complexes with cadmium through coordination reactions, reducing its bioavailability and preventing its entry into the intracellular compartments. This sequestration mechanism helps protect the vital cellular machinery from cadmium-induced damage [51].
2. **Phytochelatin synthesis:** phytochelatin (PCs) are small cysteine-rich peptides that are synthesized by microalgae in response to cadmium exposure. PCs have a high affinity for heavy metals, including cadmium, and form stable complexes with them. The synthesis of PCs is catalyzed by the enzyme phytochelatin synthase (PCS), which is induced upon cadmium stress. PCs function as metal chelators, binding to cadmium ions and sequestering them in vacuoles or other intracellular compartments, reducing their toxicity.
3. **Antioxidant defense system:** cadmium exposure leads to the generation of reactive oxygen species (ROS) within microalgal cells, causing oxidative stress. Microalgae activate their antioxidant defense system to scavenge ROS and maintain redox homeostasis. This includes the upregulation of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and ascorbate peroxidase (APX), which detoxify ROS and protect cellular components from oxidative damage.
4. **Enhanced metal efflux:** microalgae can enhance the efflux of cadmium ions from their cells, reducing the intracellular cadmium concentration. This efflux is facilitated by membrane transporters such as ATP-binding cassette (ABC) transporters, metal ion transporters, or plasma membrane H⁺ -ATPases. These transporters actively pump cadmium ions out of the cells, reducing the cellular cadmium burden and minimizing its toxic effects.
5. **Metallothionein synthesis:** microalgae synthesize metallothioneins (MTs) in response to Cd exposure. MTs are small, cysteine-rich proteins that have a high affinity for metal ions, including Cd. These proteins bind to copper ions and sequester them, reducing their availability and toxicity. The induction of MT synthesis is mediated by metal-responsive transcription factors, enabling microalgae to regulate Cd homeostasis [45].

The next examples are specific mechanisms used by microalgae to cope with copper and zinc toxicity:

6. **Enhanced copper efflux:** microalgae enhance the efflux of excess copper ions from their cells to minimize copper toxicity. This is achieved through the activity of membrane transporters, such as ATP-binding cassette (ABC) transporters, copper-transporting P-type ATPases, or copper-effluxing P-type ATPases. These

transporters actively pump copper ions out of the cells, reducing the intracellular copper concentration and preventing copper-induced damage [50].

7. Metal chelation: microalgae produce various chelating agents, such as organic acids, amino acids, and peptides, which can bind to copper ions and form complexes. These chelators, including citric acid, oxalic acid, and phytochelatins, reduce the bioavailability and toxicity of copper by sequestering it in nontoxic forms. Copper chelation helps protect cellular components from copper-induced oxidative stress and damage.
8. Zinc efflux: microalgae enhance the efflux of excess zinc ions from their cells to prevent zinc accumulation and toxicity. This is achieved through the activity of membrane transporters, such as ATP-binding cassette (ABC) transporters and zinc-effluxing P-type ATPases. These transporters actively pump zinc ions out of the cells, reducing the intracellular zinc concentration and protecting cellular components from zinc-induced damage.
9. Zinc precipitation and complexation: microalgae can precipitate excess zinc ions by forming insoluble complexes or precipitates. They produce molecules such as phosphate and sulfate, which can bind to zinc ions and form insoluble zinc phosphates or zinc sulfates. This precipitation mechanism reduces the bioavailability of zinc and limits its potential toxicity to microalgae.

Several microalgae species have demonstrated potential for heavy metals remediation, also described in Section 2.1. Some of the commonly studied species include:

- *Chlorella vulgaris*: this species has shown a high capacity for cadmium (Cd), copper (Cu) and zinc (Zn) biosorption and accumulation, making it suitable for metals remediation applications. For instance, transcriptomic analysis of *Chlorella vulgaris* revealed that intracellular carotenoids and proline contents as well as the activity of antioxidant enzymes like catalase (CAT), peroxidase (POX), polyphenol oxidase (PPO), and superoxide dismutase (SOD) had significantly increased after exposure to Cd cations. In response to metal stress, photosystem II (PSII) and CO₂ assimilation appear to be blocked in microalgae; a corresponding reduction in growth rate and cell density has been seen for different *Chlorella vulgaris* strains during exposure to high doses of Cd [52].
- *Scenedesmus obliquus*: *Scenedesmus obliquus* has been extensively studied for its ability to tolerate and remove cadmium from contaminated aqueous solutions, showing that heat-inactivated cells removed a maximum of Cd. The highest extent of metal removal, analyzed at various pH values, was at pH 7.0. [53].
- *Chlamydomonas reinhardtii*: This model microalga has been utilized to investigate the molecular mechanisms involved in cadmium (Cd) and lead (Pb) tolerance and detoxification thanks to extracellular polymeric substances (EPS), intracellular starch granules, lipid droplets, and glutathione that were significantly increased under Cd and Pb treatments [54].
- *Arthrospira platensis*: this species, also known as Spirulina, has shown a high potential of copper (Cu) bioaccumulate thanks to its capability to produce biomass and can be used in remediation efforts [55].

- *Euglena gracilis*: *Euglena gracilis* has been studied for its ability to tolerate and remove cadmium (Cd), lead (Pb), and mercury (Hg) from contaminated waters and wastewater and its capability to hyperaccumulate these metals [56].
- *Dunaliella salina*: *Dunaliella salina* is a halophilic microalga that can tolerate elevated levels of zinc together with pigments and antioxidant molecules' accumulation and has potential for zinc bioremediation applications [57].
- *Microcystis aeruginosa*: This cyanobacterial species has demonstrated the ability to tolerate and remove cadmium and zinc at high concentration from water bodies without being affected in its growth [58].

These microalgae species possess inherent mechanisms to cope with cadmium (Cd), copper (Cu), and zinc (Zn) stress, making them suitable candidates for these elements' remediation. Their ability to sequester, chelate, and detoxify cadmium (Cd), copper (Cu), and zinc (Zn) highlights their potential in mitigating metals pollution and improving environmental quality.

2.2.2 The use of microorganism consortia in bioremediation

Microorganism consortia play a crucial role in bioremediation as they can exhibit enhanced pollutant degradation capabilities compared to individual microorganisms. Microalgae and cyanobacteria could be considered suitable for metals and hydrocarbons depletion from aquatic environments, since they are able to passively adsorb them onto their cellular wall polymers and, in living cells, they can accumulate HMs and PAHs through membrane transporters from the cytosol to organelles. Microalgae and cyanobacteria are suitable candidates for HMs and PAHs bioremediation because of their autotrophy and their ability to achieve fast growth leading to a high biomass productivity.

In fact, it has been demonstrated that some of the genes involved in heavy metals decontamination are activated in decontamination catabolism PAHs. Among microalgae, chlorophytes show the highest bioaccumulation and heavy metals removal (Cd, Cu, and Zn) capability from liquid medium making them suitable candidates for bioremediation [59].

Numerous studies have shown that when cyanobacterial biomass increases, metal concentrations, ammonium ions, and mineral phosphorus levels in the water decline. With the use of micro- and macroalgae and cyanobacteria, the efficacy of purification can reach 90–97% [60]. It has been demonstrated that cyanophytes contribute to the breakdown of hydrocarbons and have a significant role in the removal of oil-based contaminants from water [61]. Cyanobacterial biomass is regarded as one of the most successful bio-accumulators due to its widespread distribution, affordable cultivation methods, and high absorption capacity. Cyanobacteria are categorized as complex biological objects that function as bio-sorbents. This ability depends on the kind of microbe and the growth conditions; assemblages of microalgae and cyanobacteria might be advantageous in the restoration of polluted environments. It is influenced by the composition of their cell walls as a structure that immobilizes the metals. The consortia consist of multiple species or strains of microorganisms that work synergistically to degrade and remove pollutants from contaminated environments. There are some key aspects of using microorganism consortia for bioremediation as functional diversity that allows

them to collectively target a wide range of pollutants, including organic compounds, heavy metals, and hydrocarbons. The consortium members can complement each other's metabolic pathways, ensuring more efficient degradation and removal of various pollutants, giving synergistic interactions, where the metabolic activities of one species or strain can support or enhance the activities of the others. For example, certain species may produce growth factors, enzymes, or metabolites that stimulate the growth or activity of other consortium members [62]. This synergy promotes the overall degradation efficiency and adaptability of the consortium to diverse environmental conditions. Microorganism consortia can utilize complementary nutrient sources available in the contaminated environment, ensuring efficient utilization of available resources and preventing resource competition among the consortium members. Some species may specialize in the degradation of specific compounds or metabolize different carbon sources, thereby enhancing the overall pollutant removal efficiency. Microorganism consortia provide redundancy, meaning that if one member fails to thrive or loses activity due to unfavorable conditions or pollutant toxicity, other members can compensate for the loss; this redundancy improves the stability and resilience of the consortium, ensuring pollutant degradation even under changing environmental conditions.

Moreover, microorganism consortia can exhibit complex biodegradation pathways involving multiple enzymatic reactions and intermediate metabolites. This complexity allows for the breakdown of complex or recalcitrant pollutants that cannot be efficiently degraded by individual microorganisms. The consortium members work together to sequentially degrade the pollutants into simpler and less toxic compounds. Microorganism consortia have a higher environmental adaptability than single strains to different pH levels, temperature ranges, or salinity conditions. Examples of microorganisms' consortia used in bioremediation include those employed in the degradation of petroleum hydrocarbons, chlorinated solvents, pesticides, and various industrial pollutants; there are several examples in the literature of microbial consortia based on bacterial species belonging to *Veillonellaceae*, *Pseudomonadaceae*, and *Clostridiaceae* which are able to remediate metalloid polluted environments [63] or *Bacillus* species can decontaminate hydrocarbon polluted sites [64]. In the last decade, attention has been placed on the capability of bacterial-microalgal consortia for the bioremediation of pesticides. For the past 50 years, nutrient removal from wastewater, agro-industrial effluent, and heavy metals pollution has been accomplished using bacterial-microalgal consortia [65]. The rate at which pesticides are accumulated or converted by bacterial-microalgal consortia has been measured in numerous pilot-scale investigations. These microbes are frequently cultivated after being extracted from an area with high pesticide contamination levels, where they are combined with other microbes to biodegrade or bioenhance organic pollutants [66]. A consortium of proteobacteria including *Ralstonia*, *Pseudomonas*, *Burkholderia*, *Sphingomonas*, and *Acinetobacter* was employed in conjunction with microalgae including *Chlorella* sp., *Selenastrum* sp., *Phormidium* sp., and *Scenedesmus* sp. to digest a variety of organic contaminants [67]. Polyaromatic hydrocarbons (PAHs) are primarily broken down by the cytochrome P450 monooxygenase (CYP) enzyme family, with wild-type enzymes from *Bacillus* and *Pseudomonas* species being modified to increase degradation activity [68]. The use of genetically altered bacteria and/or microalgae can provide fitness increases through upregulation of enzymatic activity, ameliorating the rate-limiting steps in metabolic pathways [69].

2.2.3 The use of microalgae free cell compared to immobilized cells

In bioremediation applications, microalgae can be utilized in two different forms: free cells or immobilized cells. Each approach offers distinct advantages and considerations, depending on the specific bioremediation requirements. Let us explore the use of microalgae free cells and immobilized cell devices in bioremediation:

1. **Microalgae free cells:** microalgae free cells refer to individual cells that are suspended in a liquid medium. They are widely used in bioremediation due to their ease of handling and scalability. Here are some key points regarding the use of microalgae free cells:
 - **Versatility:** microalgae free cells can be easily adapted to various bioremediation scenarios, including wastewater treatment, the removal of heavy metals, and the degradation of organic pollutants. They can be introduced directly into the contaminated medium, allowing for efficient contact and interaction with the pollutants.
 - **Nutrient availability:** microalgae free cells require a suitable nutrient supply to support their growth and metabolic activities. Adequate concentrations of carbon, nitrogen, phosphorus, and other essential elements need to be maintained to ensure optimal bioremediation performance.
 - **Harvesting and reusability:** harvesting microalgae free cells can be relatively straightforward by employing sedimentation, self-flocculation, centrifugation, or filtration techniques. Additionally, free cells can be harvested and reused in subsequent bioremediation processes, enhancing cost-effectiveness.
2. **Immobilized cell devices:** Immobilized cells refer to microalgae cells that are attached or confined to a solid support matrix or carrier material. This immobilization technique offers several advantages for bioremediation applications:
 - **Enhanced stability and longevity:** immobilization provides physical support and protection to microalgae cells, increasing their stability and longevity during bioremediation processes. The immobilization matrix acts as a barrier against shear forces and environmental fluctuations, ensuring the sustained activity of the microalgae.
 - **Improved retention:** immobilized cell devices allow for the retention of microalgae cells in a specific location or within a reactor system, providing better control over their distribution and interaction with the target contaminants. This can enhance the efficiency of pollutants' removal and reduce the risk of cells' washout.
 - **Reusability and removability:** immobilized cells can be reused in multiple cycles of bioremediation, reducing the need for continuous addition of fresh cells and can be simply removed. This can be cost-effective and environmentally friendly.
 - **Scalability:** immobilized cell devices are easily scalable, allowing for the design and construction of larger-scale bioremediation systems. The immobilized cells

can be incorporated into fixed-bed reactors, fluidized-bed reactors, or other immobilization setups, enabling efficient pollutant treatment in large volumes.

The choice between microalgae free cells and immobilized cell devices depends on factors, such as the nature of the contaminants, the scale of the remediation project, and the specific requirements of the bioremediation process. Both approaches have been successfully employed in various bioremediation applications, highlighting their potential to address environmental pollution and promote sustainable remediation practices. **Table 3** shows examples of biopolymer matrices already employed with immobilized microalgae.

Matrix type	Phase shape	Application	Species	
			Motile	Not motile
Luffa sponge	solid	Cadmium removal		<i>Chlorella sorokiniana</i>
Luffa sponge	solid	Chromium III removal		<i>Chlorella sorokiniana</i>
Plain printing paper	45 g m ⁻² , newsprint “Kölner Stadt-anzeiger” DuMont Schauberg	Microalgae growth for feeding in PBR	<i>Isochrysis</i> spp. <i>Tetraselmis suecica</i>	<i>Phaeodactylum tricornutum</i> <i>Nannochloropsis</i> spp.
Polyvinylidene fluoride (PVDF)		N and P in wastewater removal		<i>Chlorella vulgaris</i>
Alginate	beads	Value-added products		<i>Botryococcus</i> Spp., <i>Chlorella vulgaris</i>
Several polymers not specified		N and P in wastewater removal		<i>Chlorella vulgaris</i>
Several polymers not specified		N and P in wastewater removal		<i>Scenedesmus</i> spp.
Several polymers not specified		N and P in wastewater removal	<i>Dunaliella</i> spp.	
Blank alginate Na and Ca	beads	Nickel removal		<i>Chlorella vulgaris</i>
Kappa-carrageenan	beads 5 mm diameter	Zinc chromium and cadmium		<i>Scenedesmus acutus</i>
Polyurethane	foam	Zinc chromium and cadmium		<i>Chlorella vulgaris</i>
Balginate Ca and bario	beads	CdCl ₂ and CuSO ₄ x 17 days		<i>Nannochloropsis gaditana</i>
Aginate Ca	beads	CdCl ₂ and CuSO ₄ at 0–10–60 min and 24 h (total Cu removal) N and P wastewater treatment and metals in wastewater	<i>Tetraselmis chui</i>	

Matrix type	Phase shape	Application	Species	
			Motile	Not motile
Polysulfone and epoxy resin	beads	Cu(II), Fe(II), Ni(II), and Zn(II)		<i>P. laminosum + microcystis</i>
Alginate Ca	beads	Chromium		<i>Anacystis nidulans</i>
Zeolite 13X-Algal-Alginate Beads (ZABs)	beads	CuSO ₄		<i>Chlorella vulgaris</i>
Blank-Alginate Beads (BABs)	beads	CuSO ₄		<i>Chlorella vulgaris</i>
Chlorella-Alginate Beads (CABs)	beads	CuSO ₄		<i>Chlorella vulgaris</i>
Alginate beads	beads	N and P wastewater		<i>Chlorella sorokiniana</i>
Silica gel	biofilm	Thorium		<i>Jania rubens/Saccharomyces cerevisiae</i>

Table 3.
Illustrated examples of different microalgae immobilized in polymeric matrices.

2.3 Gain the response: The genetic engineering of microalgae for ameliorating the bioremediation fitness

Genetic engineering of microalgae holds significant promise for improving bioremediation capabilities. By manipulating the genetic makeup of microalgae, scientists can enhance their ability to degrade pollutants, tolerate higher concentrations of toxic substances, and improve overall remediation efficiency. The molecular toolkits created for microalgal bioengineering include numerous promoters, vectors, reporter genes, and regulatory elements as well as the genome technologies that allow gene editing as the technology based on zinc-finger nucleases (ZFNs), transcription activator-like endonucleases (TALENs), clustered regulatory interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) systems, and RNA interference (RNAi) [70]. Any desired DNA sequence can be targeted using these molecular techniques to produce knockout, knockdown, or insertion-deletion (indel) mutants. The new horizons of genetic modification, made possible by recent technological developments in microalgal molecular cloning, include the modification of the simultaneous expression of numerous genes and the production of marker and transgene-free knockout mutants [71]. Below, there are some key aspects of genetic engineering in microalgae for bioremediation:

1. Gene overexpression: different mechanisms can be fine-tuned in response to heavy metals tolerance and resistance, such as the overexpression of genes implied in metal transportation, chelation, biotransformation, oxidative stress response regulation, and cell surface bioengineering [72]. This allows for increased production of enzymes or proteins responsible for pollutant breakdown, thereby enhancing the microalgae's remediation potential. CrMTP4, a metal-tolerant protein

(MTP) from the family of metal transporters known as cation diffusion facilitators, was overexpressed in *C. reinhardtii*; this resulted in a notable improvement of both bioaccumulation efficiency and resistance to the toxicity of Cd [73].

2. Metabolic pathway engineering: microalgae can be genetically modified to optimize metabolic pathways relevant to bioremediation. A chromate reductase (ChrR) has been identified in *C. vulgaris* that converts Cr (VI) to Cr (III) by the oxidation of glutathione (GSH), providing a high level of resistance to Cr toxicity [74]. Arsenic biotransformation is done in a variety of microalgae strains via a number of different mechanisms, the most prevalent of which is the conversion of As (V) to As (III). Tolerance to the As element can be increased by interfering with the arsenic reduction enzymatic pathway. According to mercury resistance mediated by microalgae, *Selenastrum minutum*, *Chlorella fusca*, and *Galdiera sulphuraria* strains are able to biotransform Hg²⁺ to less harmful elemental Hg when mercuric reductase is expressed [75]. Genes encoding enzymes like cytochromes or dehalogenases can be overexpressed to increase the breakdown of organic pollutants. Phosphate transporters and/or ABCC transporters, and their overexpression, were explicitly blamed for the increased tolerance and bioaccumulation of As and Cr [76]. Other HM volatilization mechanisms in microalgae have been reported as photoreduction pathway for the biotransformation of Cr in *C. vulgaris* [77], intracellular and extracellular metal nanoparticle biosynthesis, and reductive interactions with functional groups of biomolecules inside and outside the cell. Modifying genes involved in nutrient uptake, detoxification pathways, or stress response mechanisms can improve the effectiveness of pollutant breakdown and metabolic activity by adding or changing genes implicated in pollutant degradation pathways.
3. Heavy metal tolerance: genetic engineering, in microalgae, can involve the introduction of genes encoding metal-binding proteins or transporters that can sequester or actively transport heavy metals, reducing their toxicity within their cells. MTs are a class of polypeptides that are either genetically encoded (classes I and II) or produced (class III) by an enzyme and are present in all living things [78]. They play a significant role in metal homeostasis and trafficking. They have a higher (15–35%) percentage of cysteine residues and a lower (15–20%) percentage of histidine residues. Also, phytochelatins (PCs) are involved in metals chelation, helping microalgae in coping with a high concentration of HMs. Glutamate-cysteine ligase catalyzes the first step in the biosynthesis of PCs, which is the creation of γ -glutamylcysteine from cysteine and glutamic acid. Following the ligation of γ -glutamylcysteine with glycine by GSH synthase, GSH is produced and functions as the primary redox buffer in eukaryotic cells, shielding them from oxidative and metal stressors. The strategy linked to phytochelatins and metallothioneins is widely used in cyanophytes as *Synechococcus sp.* and seven other blue-green microalgae strains that have been found to contain MT genes [79]. The ability of MTs and their host cells to bind and remediate HM has been hypothesized to depend on the length of the oligopeptide chain and the distribution of cysteine residues. Some strains of microalgae with a better tolerance for HMs do appear to produce MTs with longer chains and more cysteine residues. Through the heterologous expression of enzymes for the synthesis of long, cysteine-rich MTs from hypertolerant species in transgenic strains, it is possible way to engineer microalgae with increased HM removal capability [80].

4. Stress response enhancement: microalgae can be genetically engineered to enhance their stress response mechanisms, enabling them to better cope with pollutants and adverse environmental conditions. This may involve the introduction of stress-related genes or transcription factors that regulate the expression of stress-responsive genes, improving the microalgae's ability to withstand and remediate contaminated environments when HMs induce the generation of superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (HO), and singlet oxygen ($^1O_2$)—collectively known as ROS [81]. In microalgae cells under HMs stress, various antioxidant enzymes' encoding genes were increased, according to transcriptomic studies of *D. salina* and *C. reinhardtii* exposed to Cd and Pb, respectively [82]. In addition to upregulating antioxidant enzymes, *Auxenochlorella protothecoides*, *C. vulgaris*, and *C. reinhardtii* cells overexpressed thioredoxin (Trx), heat shock proteins (HSPs), and carotenoids in response to toxic amounts of metals [83]. Similar studies conducted under HM stress in *Amphora coffeaeformis*, *Navicula salinicola*, and *D. salina* revealed a significant upregulation of genes associated with antioxidant defense [84]. Superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), guaiacol peroxidase (GPX), and glutathione-S-transferase (GST) are well-known enzymatic antioxidants that actively convert superoxide radicals to hydrogen peroxide, till the final oxygen acceptor, while proline, ascorbic acid, and GSH are nonenzymatic antioxidants that may directly cope with ROS via complexation [85]. Heat shock proteins (HSPs) function as molecular chaperones to protect and repair proteins during HM stress, and are part of no-direct mechanisms used by cells [86]. HSPs play important roles in the transport, folding, unfolding, assembly, and disassembly of proteins as well as the destruction of misfolded or aggregated proteins in microalgae and other organisms. In the green microalga *Tetraselmis suecica*, the function of HSPs, such as HSP20, HSP70, and HSP100, in preventing protein denaturation has been demonstrated [87].
5. Bioaccumulation and biomonitoring: genetic engineering can be utilized to enhance microalgae's ability to accumulate and bioconcentrate pollutants, enabling more efficient remediation. By introducing or modifying genes associated with pollutant uptake, transport, or storage, microalgae can accumulate higher concentrations of pollutants, facilitating their removal from the environment. Due to the need for thiols and thiol-containing molecules like GSH and PCs and their capacity to chelate metal cations, sulfur metabolism is essential for the mitigation of microalgal HM. When *C. reinhardtii* was exposed to Hg^{2+} , it was found that the genes involved in sulfur metabolism—biotin biosynthesis genes—and S-transporter genes were upregulated [88]. Numerous HM transporters in microalgae and plants still need to be molecularly identified and described in terms of localization, transport characteristics, and specificity. Therefore, research in this area is essential for learning more about the routes used by HM trafficking and accumulation in microalgae. Additionally, genetic engineering can enable microalgae to serve as biomonitoring tools by introducing genes that produce detectable biomarkers in response to specific pollutants, aiding in pollution detection and monitoring efforts.

To conclude, it is impossible to disregard the importance of genetic engineering in creating and comprehending the mechanisms by which microalgae detoxify such contaminants. In order to strengthen the widespread usage of genetically modified

microalgae and to close the knowledge gap in this area, more concentrated research is being acquired to address the growing environmental threat that waters pose to sustainable development. While genetic engineering offers tremendous potential for improving microalgae bioremediation capabilities, it is essential to consider the potential risks and ethical implications associated with releasing genetically modified organisms into the environment. Careful regulation, risk assessment, and monitoring are crucial to ensure the safe and responsible use of genetically engineered microalgae for bioremediation purposes.

3. Patents on use of microalgae in bioremediation

Patents related to microalgae bioremediation can cover a wide range of aspects, including:

1. Novel microalgae strains: patents may be filed to protect newly discovered or genetically engineered microalgae strains specifically designed for bioremediation applications. These strains may exhibit enhanced pollutant uptake, degradation capabilities, or stress tolerance.
2. Bioremediation methods and systems: patents can be obtained for innovative methods and systems that utilize microalgae for bioremediation purposes. These patents may cover the design and configuration of bioreactors, cultivation techniques, harvesting and biomass processing methods, and other aspects related to the efficient and effective use of microalgae in pollutant removal.
3. Genetic engineering techniques: patents may be filed to protect novel genetic engineering techniques or tools used for modifying microalgae genomes. These techniques can enable the development of microalgae strains with enhanced bioremediation capabilities, such as improved pollutant degradation pathways or increased metal uptake and tolerance.
4. Bioproducts and value-added applications: in some cases, patents may focus on the extraction and utilization of value-added products produced during microalgae bioremediation.

For example, patents may cover the extraction of biofuels, bioplastics, or bioactive compounds from microalgae biomass generated during the bioremediation process. These patents protect the intellectual property associated with the specific methods, compositions, and applications of these bioproducts. Nowadays, we still do not have patents on “Genetically Engineered Microalgae Strain for Enhanced Heavy Metal Remediation”, while we can find some examples of patents on “Integrated Microalgae Bioremediation System for Wastewater Treatment” (patent number RU2758690C; US11577111B2) as bioderived compositions for use in environmental remediation (patent number US20150223470A1). These methods have been developed for integrated purification of complex multicomponent wastewater, containing perfluoro- and polyfluoroalkyl substance (PFAS). We can also find biotreatment methods for efficiently purifying biogas slurry with microalgae (patent number CN103663715A), algal system for improving water quality based on *Spirogyra grevilleana* (patent

number WO2014201298A1). “Highly Efficient Microalgae Device for Bioremediation Applications” includes methods of wastewater treatment with microalgae culture supplemented with organic carbon or consortia made of bacteria-microalgae for N content decreasing (patent number WO2018053071A1; CN108623099A). Several examples of using microalgae grown in bioreactor for water decontamination can be found in the literature, e.g., USA patented the systems US20110151547A1.

4. Future perspectives of using microalgae in bioremediation

The future of using microalgae in bioremediation holds great promises and potentials. As research in this field continues to advance, several exciting future perspectives emerge:

- **Enhanced genetic engineering and tailored microalgae strains:** genetic engineering techniques will develop even better, allowing for precise manipulation of microalgae genomes and the development of microalgae strains with enhanced pollutant degradation capabilities, higher stress tolerance, and increased biomass production. The targeted genetic modifications will optimize metabolic pathways, improve pollutant uptake, and facilitate the production of valuable compounds during the bioremediation process. Customized microalgae strains will exhibit a higher pollutant uptake, degradation, and tolerance, making them highly effective in cleaning up diverse pollution scenarios.
- **Integration of advanced technologies:** the integration of advanced technologies, such as omics approaches (genomics, transcriptomics, proteomics, and metabolomics), will provide a deeper understanding of microalgae’s molecular responses to pollutants. This knowledge will enable the identification of key genes, enzymes, and metabolic pathways involved in bioremediation processes. Additionally, advanced monitoring techniques, remote sensing, and modeling tools will allow for real-time assessment and optimization of bioremediation strategies using microalgae.
- **Algal bioreactors and bioprocessing:** algal bioreactors will be developed and optimized for large-scale production of microalgae biomass dedicated to bioremediation applications. These systems will incorporate innovative designs, efficient nutrient recycling, and controlled environmental conditions to maximize microalgae growth and pollutant removal.
- **Synergistic bioremediation approaches:** future research will focus on developing synergistic approaches that combine microalgae bioremediation with other remediation strategies, such as phytoremediation, bacterial consortia, or physical remediation methods. These integrated approaches will leverage the strengths of different remediation techniques to achieve enhanced and comprehensive pollutant removal, making them more effective and sustainable.
- **Field applications and commercialization:** as research progresses and the efficacy of microalgae-based bioremediation becomes more established, the field applications and commercialization of microalgae-based bioremediation systems will likely increase. Industries, environmental agencies, and stakeholders will recognize the potential of microalgae as a cost-effective and eco-friendly solution

for tackling pollution. The development of scalable, efficient, and economically viable microalgae bioremediation systems will drive their widespread adoption in different environmental settings.

5. Conclusions

Microalgae have demonstrated remarkable potential as bioremediation agents, owing to their diverse mechanisms and unique capabilities. Through their photosynthetic activity, microalgae can metabolize and break down HMs and PHA, effectively removing them from the environment. Their ability to efficiently uptake and assimilate nutrients helps mitigate eutrophication and control harmful algal blooms. Additionally, microalgae exhibit biosorption and bioaccumulation abilities, enabling them to sequester heavy metals, organic pollutants, and even radioactive substances. Their enzymatic degradation capabilities further contribute to the breakdown and detoxification of complex contaminants. Moreover, microalgae play a crucial role in pH adjustment and oxygen production, creating favorable conditions for other microorganisms involved in bioremediation processes, such as bacteria or fungi. Harnessing these mechanisms provides a sustainable and eco-friendly approach to address various environmental challenges. The concept of coupling environmental remediation with the generation of sustainable resources adds an economic dimension to phycoremediation, making it an attractive option for environmental restoration and resource recovery. Further research and optimization of microalgae-based bioremediation strategies hold great promise for mitigating pollution and restoring the health of our ecosystems.

Authors' contribution

Both authors substantially contributed equally to the conception of the work and to the interpretation of data for the work; they equally participated in drafting and revising it.

Conflict of interest

The authors declare no conflict of interest.

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Microalgae as Multiproduct Cell Factories

Kolos Makay and Claudia Grewe

Abstract

Microalgae are a highly diverse group of microorganisms that are currently produced at industrial scale at comparably high specific costs for various applications (e.g., food supplements and cosmetics). Although a multitude of high-value, market-relevant products, such as fucoxanthin and eicosapentaenoic acid (EPA), are present in the biomass, currently single-value chain products are produced and marketed, limiting profitability of microalgal biotechnology, as well as potential application. The chapter provides an overview on microalgae-based lead compounds and their bioactivities providing the basis for the multiproduct cell factory concept. Furthermore, a general overview of current downstream processing (DSP) methods is given that are currently used for microalgal biorefineries at industrial scale. The latest advancements in the research and development of multi-products are showcased, highlighting its role in facilitating the microalgal bioeconomy.

Keywords: high-value compounds, multi-product, DSP, algae-based bioeconomy, cell factories

1. Introduction

Microalgae are predominantly photosynthetic and mainly single-celled microorganisms. Their metabolic complexity enables them to produce a diverse range of algae-specific compounds; thus, microalgae have emerged as promising multiproduct cell factories. Moreover, the composition, as well as the proportion of their biochemical composition, is highly variable and controllable by adjusting cultivation conditions. Therefore, microalgae are subject of increasing interest of various industrial sectors seeking solutions to prevail future global sustainability challenges. Among these, microalgae can contribute to food, feed, and high-value chemicals supply [1–3]. However, the establishment of a microalgae-based bioeconomy faces substantial challenges, first and foremost economic hurdles in both upstream (USP) and downstream processing (DSP) [4], as well as market entry barriers and consumer acceptance.

DSP plays a crucial role in the production process, involving several unit operations such as solid-liquid separation, milling, extraction, and chromatographic separation of the desired compound. In its complexity, it contributes substantially to the production costs and represents a major economic bottleneck for new microalgae-based products [5]. This limitation reasons that commercial exploitation of microalgae is currently restricted to a few single-value chained products (e.g., astaxanthin from *H. pluvialis* [6]

and phycocyanin (PC) from *A. platensis* [7]) [8–10]. However, using microalgae as multiple product cell factories to produce several high- and medium-value components and utilizing the residual biomass for the production of low-value bulk commodities (e.g., food and feed additives, fertilizers) seems to be a promising way to generate sufficient value to render the overall bioprocessing economically viable. To achieve this goal, straightforward, optimized, and most importantly scalable unit operations have to be combined for DSP allowing microalgal biomass to be fractionated into their most valuable components, including n-3 and n-6 very-long-chain polyunsaturated fatty acids (VLC PUFAs), carotenoids, (phycobili) proteins and functional polysaccharides (e.g., β -glucans). While mechanically based solid liquid separations such as centrifugation for cell harvest and bead milling for disintegration do not represent big contributors to energy requirements and specific costs, extraction and adsorption-based chromatographic techniques are costly in terms of specific energy input and operational expenditures (OPEX). If cheaper solutions, for example, aqueous two-phase extractions (ATPE) and liquid-liquid chromatography for fractionation can be established and their robustness and efficiency can be shown; as a consequence, an increased number of microalgae-producing companies and products will enter the market, which, in turn, will enhance product recognition and acceptance [5, 9].

2. Current status of microalgal products

Microalgae present a unique diversity in the biosphere in terms of their phylogenetic characteristics, which results in an array of metabolic pathways. This metabolic flexibility leads to the synthesis of a wide range of chemical constituents with diverse biological activities (e.g., anti-inflammatory, anti-aging, and anti-proliferative), making microalgae a potential candidate for sustainable multiproduct cell factories [11, 12]. Driven by this unique metabolomic versatility, microalgae have recently gained significant interest for bioprospecting from industries such as Nestlé [13] and L'Oréal [14] owing to their potential as sustainable sources of bioactive and functional compounds.

Attempts to develop microalgae-based products, including food, cosmetics, and pharmaceuticals, are not new [11]. Harder and Von Witsch (1942) and Kathrein (1964) suggested using microalgae as a sustainable source of lipids and carotenoids, and substantial research was conducted on using microalgae as a source of single-cell protein in the 1970s [15]. However, owing to unresolved economic hurdles, to date, only a few species of microalgae have been produced commercially and are currently exploited as single-value chain products by the industry (see **Table 1**) [4].

For instance, the dried biomasses of *Spirulina* and *Chlorella* spp. have been utilized as dietary supplements for essential amino acids and vitamins in various forms (e.g., tablets, capsules, and powders) or have been incorporated into foods (e.g., smoothies and pasta) to enhance the nutritional value of these products [24–26]. *H. pluvialis* and *D. salina*, the leading natural sources of commercial astaxanthin and β -carotene, respectively. These carotenoids are high-value antioxidants that can accumulate in the cells in high quantities (astaxanthin up to 5% and β -carotene up to 10% DW, respectively); thus, their biomass and processed extracts are commonly used as natural colorants and increase the nutritional value of feed and food applications [27–29]. *E. gracilis* is a widely commercialized microalgal species in East Asia since it produces paramylon in large quantities (> 50% of DW). Due to its immunomodulatory

Microalgae	Single-value component and its content [w/w% dry biomass]	Primary product form	Biomass producers (examples)
<i>Arthrospira platensis</i> (<i>Spirulina</i>)	phycocyanin ($\leq 20\%$) [16]	Extract	Cyanotech Corp.
	Spirularin®	extract	n.s.
<i>Haematococcus pluvialis</i>	astaxanthin ($\leq 5\%$)	Biomass/extract	Algotech LTD, Algalif Iceland ehf
<i>Dunaliella salina</i>	β -carotene ^{e31} ($\leq 10\%$) [17]	Biomass/extract	BASF SE, NBT Ltd.
	glycerol ($\leq 10\%$) [17]	Extract	n.s.
	Pepha®CTIVE	Extract	n.s.
<i>Chlorella vulgaris</i>	protein ($\leq 60\%$) [8]	Biomass	Roquette Frères, Algaspring B.V.
<i>Tetraselmis</i> spp.	EPA, C20:5 ($\leq 2\%$)	biomass/extract	Reed Mariculture Inc.
<i>Euglena gracilis</i>	paramylon ($> 50\%$) [18]	Biomass	Euglena Co., Ltd.
<i>Porphyridium cruentum</i>	ARA, C20:4 ($\leq 2.2\%$) [19]	Extract	Necton S.A.
	phycoerythrin ($\leq 8\%$) [20]	Extract	
	SILIDINE®OH & SILIDINE®	Extract	n.s.
<i>Cyanidium caldarium</i>	TEGO® Stemlastin	Extract	n.s.
<i>Phaeodactylum tricornutum</i>	EPA ($\leq 4.5\%$) [21]	Extract	Simris Group AB
	fucoxanthin ($\leq 1.9\%$) [21]	Biomass/extract	
	MEGASSANE®	Extract	n.s.
<i>Skeletonema costatum</i>	Grevilline™PF	Extract	n.s.
<i>Nannochloropsis</i> spp.	EPA ($\leq 5\%$) [22]	Biomass/extract	Xiaozao Tech
	Pepha®TIGHT	Extract	n.s.
<i>Isochrysis galbana</i>	DHA (≤ 1.8) [23]	Biomass/extract	Reed Mariculture Inc.
	fucoxanthin (≤ 1.45) [23]	Biomass/extract	
	Lipout™	extract	n.s.
<i>Schizochytrium</i> (protist)	DHA ($\leq 20\%$)	Biomass/extract	Xiaozao Tech

n.s.: Not specified.

Table 1.
List of commercially produced microalgae and their extracts.

functions, the β -1,3- glucan is used for the preparation of high value-added nutritional supplements and medicinal products [30, 31].

Some microalgal species, such as *Nannochloropsis* spp., *Tetraselmis* spp., and *I. galbana*, and the protist *Schizochytrium* are known to contain considerable amounts of n-3 and n-6 VLC PUFAS such as EPA and DHA (**Table 1**), which are products of both high market relevance and commercial value. Microalgal species with high PUFA content are currently utilized as feed in aquaculture and animal farming as important sources of these fatty acids [32–35]. Biomass is used as rotifer feed or is incorporated into formulations for larval and juvenile feed, which is essential for breeding crustaceans and fish [36, 37].

Other single-value chained products from microalgae are lipophilic astaxanthin extract (5, ..., 10%) from *H. pluvialis* [6], algal oil from *P. tricornutum* [38]), the hydrophilic extracts Pepha®TIGHT from *Nannochloropsis oculata* [39], and SILIDINE® from *Porphyridium cruentum* (**Table 1**). These extracts have found industrial applications owing to their anti-oxidative, anti-inflammatory, anti-aging, and skin regeneration properties in nutraceuticals and cosmeceutical industries [40, 41]. However, beside containing the main valuable and effect causing component(s) of interest alone, also a myriad of other extracted cellular products are present in the raw extracts. Further fractionation of these extracts to obtain a high level of purity for one valuable compound is currently limited to niche products with high market value but very low market volumes (e.g., analytical grade phycoerythrin (PE) from *P. purpureum* [42]), where the special application justifies the effort and price of the required purification steps [5]. Hence, the generation of economically feasible microalgal products can either be accomplished due to the hyperaccumulation of a specific high-value product or in the co-accumulation of multiple high and low-value products.

3. High-value lead compounds from microalgae

Lead compounds are biomolecules with sufficient potential (measured by potency, bioavailability, therapeutic efficacy, etc.) to progress into a full product development program in the cosmeceutical, nutraceutical, and pharmaceutical industries [43]. However, these lead compounds are only commercially exploitable from microalgae if there is no alternative source, the respective microalga contains the lead compound in higher contents, or the compound possesses specific activities compared to other sources (e.g., galactolipid (GL) esterified EPA compared to EPA from triacylglycerol (TAG)). Such microalgal metabolites that have been recognized for their potential as next-generation lead compounds are special lipids such as carotenoids (e.g., fucoxanthin and astaxanthin), n-3 and n-6 VLC PUFAs, phycobiliproteins, phenols, and polysaccharides. Thus, this section provides a general overview of these high-value lead compounds that are currently commercialized with high market prices and considerable volume [44–47].

3.1 Lipids

Lipid molecules are a broad class of structurally diverse compounds that are insoluble in water but soluble in organic solvents such as chloroform [48]. Among these, considerable interest of the microalgal-produced lipids are glycolipids, phospholipids, carotenoids, triglycerides, and sterols [49] (**Figure 1**). Due to their special structure and functionalities, these are used for diverse applications, for example, in nutraceuticals, aquaculture, and cosmeceutical formulations [50].

3.1.1 Very-long-chain polyunsaturated fatty acids

In addition to being building blocks of cellular membranes, n-3 and n-6 VLC PUFAs have been shown to play a significant role as mediator molecules [51], in pre- and postnatal brain development [52], regulation of cytokine storms [53], antidepressant mechanisms [54], and prevention of cardiovascular diseases [55]. As the *de novo* synthesis of n-3 and n-6 VLC-PUFAs is restricted to microorganisms, among

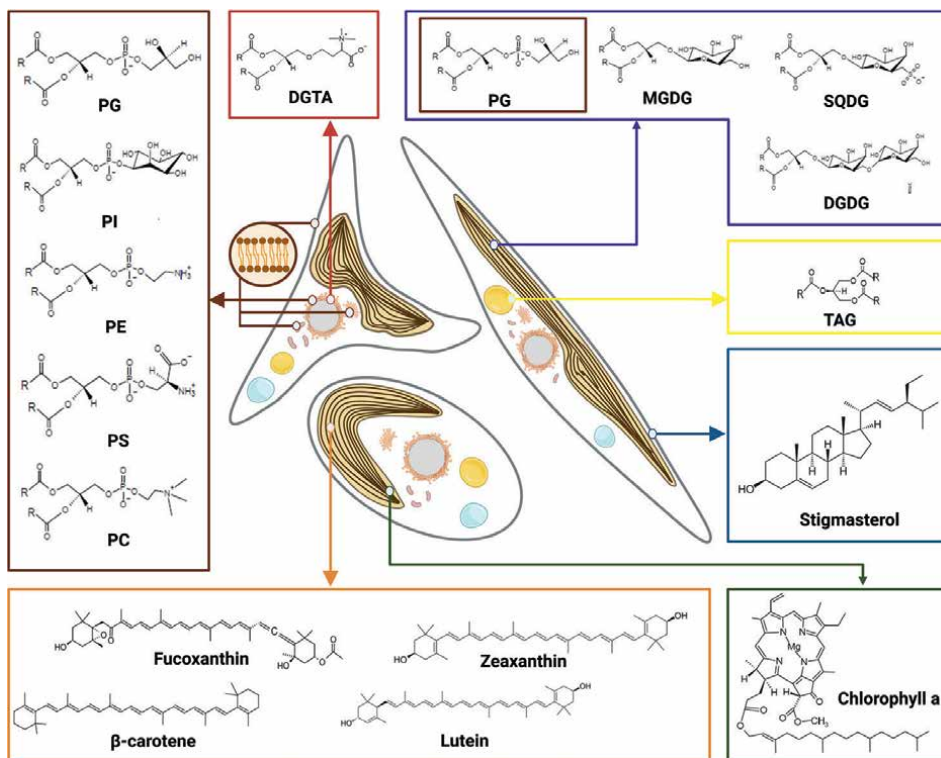


Figure 1.
 Schematic figure of the three different isoforms (triradiate, ovoid, and fusiform, from left to the right) of the oleaginous microalgae *P. tricornutum* representing the localization of the major lipid constituents of the microalgal lipidome such as glycolipids (purple), phospholipids (brown), special ether bond-based betaine lipids (red), chlorophylls (green), carotenoids (orange), and neutral lipid such as triacylglycerols (yellow) (R represents fatty acid acyl chains with different length (12–22 carbon atoms, for example, EPA. Created with BioRender.com).

which marine microalgae, such as the EPA containing oleaginous marine algae *P. tricornutum*, are considered rich and sustainable sources of these PUFAs (arachidonic acid (ARA, C20:4, n-6), EPA and docosahexaenoic acid (DHA, C22:6n-3)) [1, 3]. Principally, FAs are present in the glycerolipidome of cells, which refers to the complete set of glycerolipids in which two fatty acid acyl chains (R_1 and R_2) are esterified at the sn-1 and sn-2 positions of the glycerol backbone, whereas the third hydroxyl group of the glycerol molecule is esterified with headgroups of different polarities or a third fatty acid acyl chain (R_3) [56, 57].

Microalgae synthesize several types of glycerolipids, which are based on the chemical structure of the headgroup, subclassified into glycolipids, phospholipids, special ether bond-based betaine lipids, and the neutral lipid fractions, such as triacylglycerols. **Figure 1** shows the lipidome and its distribution in the commercially exploited diatom *P. tricornutum*. Among these lipid classes, n-3 and n-6 VLC PUFAs in microalgae are predominantly reported to be esterified into medium-polar thylakoid localized lipid classes [58, 59]. Incorporating these PUFAs into membranes enables algae to maintain optimal membrane fluidity. This characteristic is particularly vital in cold environments as it ensures the efficient light-driven electron transfer through the thylakoid membranes, thereby sustaining the functionality of the photosynthetic apparatus [56]. Interestingly, n-3 and n-6 VLC PUFAs, which are esterified to the GL

fractions, have demonstrated enhanced bioavailability and bioactive properties (e.g., antitumor-, antiproliferative-, and anti-inflammatory activities). These bioactive effects surpass those observed with the triacylglycerol-esterified form derived from fish oil, however, the underlying mechanism behind this phenomenon is still not fully understood [60–62].

3.1.2 Carotenoids

Microalgae have long been recognized for their ability to synthesize and accumulate carotenoids, which have been shown to exhibit several remarkable health-promoting properties. They are well-known for their neutralizing activity against harmful free radicals in the body, thus acting as protective agents against oxidative damage. In addition, carotenoids exhibit further biological activities, such as anti-inflammatory, immune-enhancing, and anticancer properties, making them high-value compounds [63, 64]. In microalgae, these pigments accumulate to higher contents than in alternative sources, such as heterotrophic microorganisms or plants, making them attractive sources for extraction. Astaxanthin (6.000, ..., 7.150 US\$ kg⁻¹ [65]) β -carotene (300, ..., 3.000 US\$ kg⁻¹ [66]), and fucoxanthin (40.000, ..., 80.000 US\$ kg⁻¹ [67]) are the most valuable commercially sold carotenoids [68–70] at scale.

Astaxanthin is a red secondary ketocarotenoid produced predominantly by green microalgae species. *H. pluvialis* accumulates astaxanthin contents of up to 5% being nature's richest source of this dye. The molecule is known for its superior antioxidant activity, making it highly effective in neutralizing free radicals and suppressing inflammatory processes [6].

β -Carotene, in turn, is a common orange-colored hydrocarbon (**Figure 1**) and acts as provitamin A, thus contributes to healthy vision and supports immune functions [69]. β -Carotene is found in all microalgae species as primary carotenoid. However, in *D. salina* under unfavorable cultivation conditions, it can accumulate up to 10%, which made *D. salina* an attractive choice for production purposes [71].

Fucoxanthin has a unique chemical structure characterized by an allenic bond and an epoxy group (**Figure 1**). This structural characteristic contributes to its additional health benefits such as anti-obesity and antidiabetic properties. Fucoxanthin serves as primary carotenoid and is mainly distributed in the chloroplast of diatoms. In *P. tricornutum* fucoxanthin content can reach up to 1.9% [21, 72]. This 5, ..., 60-fold higher fucoxanthin content compared to brown seaweeds [73] makes *P. tricornutum* a suitable alternative production source.

Although frequently being investigated and purified from Chlorophyceae, lutein and zeaxanthin from microalgae can product wise currently not compete with the industrial scale extraction from *Tagetes erecta* petals, which moreover lack chlorophyll [74]. This may change in the course of successfully applied multiproduct biorefinery approaches, where a separation will also yield these purified carotenoids.

3.1.3 Phytosterols

Microalgae are also sources of phytosterol compounds (e.g., *Pavlova lutheri*, 0.8, ..., 2.6%, *P. tricornutum* 0.1, ..., 0.3% [75]). Phytosterols are plant and algae-derived cholesterol analogs, which are vital for the structural integrity and proper functioning of cell membranes and serve as precursors for the synthesis of hormones, vitamins, and bile acids [47]. Two microalgal phytosterols, β -sitosterol and stigmasterol, have been extensively studied for their ability to reduce low-density lipoprotein (LDL)

cholesterol levels, exhibit anti-inflammatory and neuroprotective activities, and have been shown to suppress tumor growth [76]. Therefore, they are potential candidates for the development of novel pharmaceuticals targeting inflammatory diseases such as arthritis and oxidative stress-related disorders. The utilization of microalgal sterols in the formulation of drug delivery systems, including liposomes and nanoparticles, is gaining momentum since these microalgal-based sterol systems improve drug solubility, stability, and bioavailability and enhance therapeutic efficacy. Moreover, they have been shown to be promising in reducing side effects [77, 78].

3.2 Phenols

Microalgae-derived phenols are a diverse group of secondary metabolites that make up a small percentage of microalgal biomass (0.1, ..., 0.9%). For example, in the case of *N. oculata*, the phenolic content can range between 0.1, ..., 0.34%, while in *I. galbana* 0.2, ..., 0.8%) have been reported [79]. These phenolic compounds have been found to possess various beneficial properties. Microalgal phenolic compound enriched extracts were shown to exhibit potent antioxidant- and anti-inflammatory properties and considerable antibacterial- and antifungal activity against pathogenic strains [80]. Furthermore, phenol-rich fractions have also demonstrated cytotoxicity and induced apoptosis in various malignant cancer cell lines. Therefore, these substances are promising chemopreventive agents and are currently being investigated to examine their potential in cancer treatment [81, 82]. In addition, several phenolic compounds derived from microalgae have been shown to exert neuroprotective effects as well. They can cross the blood-brain barrier and exert nervous system-localized neurotrophic activities, making them potential candidates for the treatment and prevention of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases [83–85].

3.3 Proteins and peptides

Microalgae-derived protein hydrolysates, containing typically oligopeptides (10, ..., 20 amino acids), often possess radical scavenging activities and exhibit inflammatory pathway modulatory functions, thus have potential applications in targeting oxidative stress and inflammation-related conditions (e.g., arthritis and inflammatory bowel diseases) [86]. Furthermore, certain microalgae-derived oligopeptides also exhibit antihypertensive effects by inhibiting enzymes involved in blood pressure regulation such as angiotensin-converting enzyme [87], while the inhibition of the growth and proliferation of cancer cells was also proven [88].

PC and PE are two types of phycobiliproteins (PBP) found in certain microalgae [89] (e.g., Rhodophyta, *P. purpureum* (8%) [20]) and Cyanobacteria (*A. platensis*, ($\leq 20\%$) [16]). PC is a blue PBP that has been approved safe for consumption by regulatory agencies and is used primarily as a natural food colorant [90]. Furthermore, it exhibits anti-oxidative and immunomodulatory properties, and research suggests its potential in various therapeutic applications, including as a neuroprotective and anticancer agent [16, 91, 92]. PE is a red-colored PBP primarily found in red microalgae (Rhodophyta), which exhibits fluorescence properties and has been utilized as a fluorescent probe in various scientific applications, including flow cytometry and microscopy. Like PC, PE has also shown potential therapeutic activities (e.g., anti-oxidative and anti-cancer); however, its industrial applications are primarily focused on the use of natural fluorescent dyes [90, 93, 94].

3.4 Polysaccharides

Polysaccharides are classified into two main groups: high-molecular-weight (HMW) polysaccharides and low-molecular-weight (LMW) polysaccharides or also referred sometimes as oligosaccharides as the boundary is not precisely defined [95]. Among the HMW polysaccharides, the extracellular ones are of major interest because of their remarkable versatility and wide-ranging applications [96]. For instance, certain species of *C. vulgaris* produce polysaccharides that exhibit immunomodulatory and anti-oxidative activities [97], and thus have potential in immune health and functional food products. Additionally, some microalgae, such as *Porphyridium* spp. and *Tetraselmis* spp., produces sulfated polysaccharides that have been shown to possess antimicrobial- and anticoagulant activities [19, 98]. These sulfated polysaccharides, along with chrysolaminarin (β -glucan) from *P. tricornutum* (contains up to 32%) [99]), also showed immunomodulatory, anti-oxidative, and antitumor activities, thus holding potential for biomedical research [72, 100, 101]. Additionally, these HMW polysaccharides possess exceptional water-binding capacity and viscosity [100]. A recently exploited sulfated polysaccharide is sacran, derived from the freshwater cyanobacterium *Aphanothece sacrum*. The chain length exceeds 30 μm and the extremely high molecular weight of 10 to 22 MDa accounts for its elasticity and water-binding capacity. The manifold beneficial characteristics make the biopolymers excellent matrices, emulsifiers, stabilizers, and thickening agents in formulations for the food, pharmaceutical, and cosmetic industries [102].

4. Downstream processing

Exploiting the full potential of microalgae requires an effective DSP strategy to extract, purify, and concentrate the desired products from the complex biomass or their culture broth. However, the DSP of microalgae presents unique challenges, owing to the intrinsic, highly variable characteristics of these microorganisms [103]. In addition to physical characteristics (e.g., cell size, cell wall composition, thickness, and rigidity), which primarily affect harvesting and cell disruption processes. The diverse range of biochemical characteristics of microalgae plays also a significant role in tailoring the overall DSP. Therefore, these differences in the localization (e.g., intra- or extracellular polysaccharides) and content of the compounds of interest necessitate the development of specialized processes for different genera [104]. Thus, the following section aims to discuss the main steps involved in DSP applied for microalgae.

4.1 Harvesting

In general, harvesting techniques aim to concentrate the microalgal suspension for the next DSP unit operation as the diluted algal suspension (0.03, ..., 0.5% DM) cannot be used directly from the effluent of the photobioreactor for efficient cell disruption or extraction. However, the small size of the single-celled microalgae (usually below 10 μm), the low density difference between cell and medium, and the low solid volume fraction connected to a large volume of the algal suspension result in a long processing time. Therefore, harvesting is a substantial contributor to the high production expenses. Numerous harvesting methods, such as centrifugation, filtration, and flotation, have been examined to optimize the productivity and economic viability of this DSP unit operation [105–107].

Disk stack centrifuges (separators) are currently predominantly employed for concentrating microalgal suspensions, while hydrocyclones present an additional option to remove extraneous solid matter when harvesting from open ponds. Depending on the physicochemical properties of the algal suspension, a concentration factor of 50 to 150 for closed photobioreactors and up to 400 for open ponds can be achieved, resulting in slurries with dry matter contents of up to 25%. The feed capacity for the separator can reach up to $90 \text{ m}^3 \text{ h}^{-1}$. However, to achieve the required separation efficiency, which is equivalent to minimal discharge of centrifuged cells with the clear run of the separator, the physiochemical properties of the suspension have to be considered. Here, the particle size (e.g., *H. pluvialis* 15, ..., 50 μm (cyst state) vs. *Nannochloropsis* 2, ..., 4 μm), the viscosity of the algae suspension (e.g., *Porphyridium sordidum* of 4.2 mPas at 5.0 g L^{-1} [108] vs. *Spirulina* of 1.25 mPas at 3.40 g L^{-1} and 25°C [109]), as well as the solid volume fraction, influence the feed flow rate at a given harvest efficiency. Both achievable harvest cell biomass concentration and the daily harvest volume is dependent upon the photobioreactor design. Particularly for raceway ponds pre-concentration steps, such as pH-induced flocculation and precipitation, have been investigated. These methods do not possess industrial relevance as the multivalent cations (Al^{2+} , Al^{3+}) or the base (20 mM NaOH) cannot be efficiently removed from the clear phase, preventing re-usage of the medium. Therefore, flotation or vibration sieves are applied, wherever it is feasible with respect to cell and suspension characteristics [106], due to the low volume fractions of the cells in the microalgal suspension (1, ..., 2% (v/v) corresponding to 0.3, ..., 0.5 g L^{-1} dry matter). In the pre-concentration step, the suspension is concentrated to, and this pre-thickened slurry is further processed to obtain a final slurry of a dry biomass content of 10 to 25%. Due to acceleration of up to 20,000 g the energy consumption of disk stack centrifuges is high, but the biomass-specific energy consumption is, depending on the type of algae, between 0.4 and 0.8 kWh kg^{-1} acceptable and moreover cost effective [105, 107, 110].

Among the filtration techniques of small and compressible algal cells, dead-end filtration suffers from the high cake resistance and resulting low filtrate flow rates. Under extreme conditions (e.g., 3 M NaCl), depth filtration using silane-treated glass wool can be applied [111]. With the development of suitable polymer and ceramic membrane materials, crossflow filtration has become an option for harvesting microalgal biomass. In this technique, a tangential flow is applied across the membrane surface, which prevents a rapid decline in flux, setting it apart from dead-end filtration. Thus, with crossflow filtration (CF), a concentration factor of approximately 300 in a single step has been reported for instance for *C. minutissima* [112]. The energy demand of crossflow filtration techniques, ranging from 0.64 to 3 kWh kg^{-1} [112–114], is comparable to that of centrifugal harvesting techniques. However, it is important to consider that CF relies on specific membrane suppliers, necessitating filter replacements with uncertain standing times, as well as process interruptions due to backflushing cycles. Consequently, disk stack centrifuges are currently the preferred and most cost-effective harvesting method for microalgae, especially for small and single-cell morphologies.

4.2 Cell disruption

To facilitate the release of intracellular molecules and enhance the recovery of special membrane-bound high-value components (e.g., carotenoids and GL- esterified n-3 and n-6 VLC PUFAs), cell disruption is a pre-requisite [115]. As key performance

indicator, the disintegration degree is defined and targeted at values of greater than 90%, preferably above 95%. The thickness and structure of the cell wall predestinate the required power demand and exposure time of the cell disintegration method applied for this purpose [104]. For instance, a study conducted by Tan et al. demonstrated that an effective disruption of the cell wall of *A. platensis* can be reached by one freeze-thaw cycle. Its cell membrane, consisting of four 15 nm peptidoglycan layers [116], was easily disrupted using a freezing/thawing cycle of 2/24 hours being sufficient to maximize the yields of PC. This approach is used at scale for industrial PC production if slurry is used as raw material for extraction.

In contrast, the cell wall of certain microalgae, such as *Nannochloropsis* spp., presents a unique composition consisting of algaenan, cellulose, and hemicellulose layers. This structure results in a cell wall thickness of approximately 110 nm [117], which provides exceptional rigidity for the cells. Consequently, a significantly higher energy input is required to disrupt these cells. Among the mechanical disintegration techniques, stirred bead milling (SBM) and high-pressure homogenization (HPH) are the preferred methods for industrial-scale applications [118]. While industrial-scale bead mills with rotating drums do not provide sufficient specific energy input, the SBM is established as an efficient and favorable disintegration method owing to its high throughput, easy scalability, and high disruption efficiency. In SBM, cells are damaged and ground into smaller particles by applying a force through collisions between the cells and beads, pressure differences, and shearing between the spheres. The effectiveness of microalgal cell lysis was found to be primarily dependent on the sphere diameter (0.35, ..., 1.9 mm) to cell diameter ratio (60, ..., 230), the material of the beads (e.g., glass, zirconium, steel), the filling degree (0.8, ..., 0.85), the circumferential speed (8, ..., 14 m s⁻¹), the geometry of the stirring elements, as well as the dry matter content (up to 25%) [119]) and the temperature of the microalgal slurry [119–122].

HPH is also a mechanical technique used to rupture the cells by suitable conduction of flow of the biomass suspension through a homogenizing valve, converting high hydrostatic into hydrodynamic energy. The efficiency of cellular disintegration relies predominantly on the pressure applied (up to 2500 bar), the number of cycles, the geometry of the valve, and the solid content (up to 25%) [123]) of the slurry. Although this technique has shown effective cellular disintegration, an excessive amount of heat is generated that may influence the product composition. As the valve is not cooled directly, the process operation requires additional intermediate cooling, while the SBMs typically employ a cooling double jacket [123–125].

The importance of species-specific tailoring for achieving optimal cellular disintegration degrees (>90%) was demonstrated by several studies. Montalescot et al. revealed that specific processing parameters in an SBM were required for *N. oculata* and *P. cruentum* [120]. The optimal conditions proved to be 0.6 mm zirconia beads with a filling ratio between 80 and 85% and an agitator speed ranging from 8 to 10 m s⁻¹, for *N. oculata* whereas for *P. cruentum*, satisfactory disintegration degrees were achieved at 45% filling ratio with 1.3 mm glass beads. However, it should be noted that these optimized conditions were reported to have an exceptional high biomass-specific energy consumption due to the 7 to 200 times lower dry matter content, compared to other studies. In parallel, studies have focused on systematically optimization of SBM operating conditions, resulting in a considerable reduction in biomass-specific energy demands. For instance, it was shown by Postma et al. that the minimum specific energy consumption was obtained for *C. vulgaris*, *Neochloris oleoabundans* and *T. suecica* with 1.42 kWh kg⁻¹, 1.78 kWh kg⁻¹, and 0.47 kWh

kg⁻¹ respectively, while still achieving >95% cellular disintegration degree [121]. Furthermore, several studies have optimized HPH process parameters and found that it is also possible to achieve cellular disintegration degrees higher than 95% for microalgae species possessing a rigid cell wall, such as *N. gaditana*, with a specific energy input of less than 0.5 kWh kg⁻¹ [126]. Based on energy considerations, there is currently no universally preferred method for cell disruption. Further DSP influencing phenomena such as stable emulsion formation hampering extractability need to be considered in each individual case.

4.3 Extraction

In microalgal biorefinery, one of the crucial steps is to effectively extract the valuable components of interest. This extraction process commonly uses both dry and wet biomass [127, 128]. Currently, water-based extraction techniques are the most frequently applied extraction methods for the generation microalgae-based extracts. They offer several advantages that contribute to their widespread use such as safety, environmental friendliness, and mild extraction conditions (e.g., ambient pressure) [129]. For the generation of hydrophilic extracts (containing e.g., carbohydrates and polyphenols), hot water extraction by maceration is frequently used (e.g., for Peph@TIGHT from *N. oculata*). In the process, the harvested biomass slurry is diluted and under continuous stirring at elevated temperatures (up to 100°C) for several hours extracted. Following extraction, the raw extract is centrifuged for the removal of cells and debris and subsequently ultrafiltrated to remove macromolecules, which may precipitate or have allergic potential. Finally, the clarified extracts are formulated into products (e.g., topically applied cosmetics) and preserved according to their application [39].

For the extraction of the lipophilic compounds, many methodologies have been investigated; however, solvents, such as chloroform, are considered highly toxic. In agreement with the 2009/32/EC directive, which regulates the use of extraction solvents for foods [130], only a small number of solvents can be used without any restrictions (e.g., ethanol, ethyl acetate). Currently, ethanolic maceration is a widely used technique for the extraction of several lipophilic compounds (e.g., fucoxanthin, n-3, and n-6 VLC-PUFAs). Many studies have evaluated the feasibility of ethanol-based maceration for lipid extraction using multiple species of marine microalgae (e.g., *P. tricornutum*). Thus, ethanolic extraction of lipophilic molecules seems to be a feasible extraction method; however, to efficiently extract valuable compounds of interest with ethanol, large biomass-to-solvent ratios (usually 1:10 w/v) are required. Furthermore, to increase the extraction yield, elevated temperatures and prolonged extraction times are used. Due to the flammability and explosiveness of ethanol, additional safety precautions, such as explosion protection measures, must be implemented. Additionally, the large volume of extracts produced using ethanolic extraction needs to be evaporated before transport or further processing for solvent recycling.

Therefore, as an alternative to conventional organic solvent extraction, supercritical CO₂ (ScCO₂)-based extraction has gained attention and is currently in use as green alternative for the extraction of lipophilic molecules. Specialized processing companies are producing algal extracts such as astaxanthin oleoresins from *H. pluvialis* [131], as well as fucoxanthin and EPA containing oleoresins from *P. tricornutum* [132, 133]. However, only a few companies have experience with ScCO₂ at industrial scale, and as this process requires long processing hours and a high technical demand this

technique is the most expensive method available. Furthermore, because of the low solubility of, for example, carotenoids in ScCO_2 , high extraction pressures (up to 1000 bar) are required for efficient recovery of the highly apolar astaxanthin fatty acid esters from *H. pluvialis*. Therefore, in industrial processes the use of ethanol as an entrainer recovers carotenoids, in this case astaxanthin, at reduced pressures [6] and process costs.

4.4 Multiproduct refinery

While the number of bioactive compounds of potential commercial interest from microalgae is continuously increasing, the launch of new microalgal-based products remains challenging. Microalgae typically contain low amounts of target compounds in their biomass. This low content makes the DSP even more crucial for extracting and concentrating as many valuable components as possible. Therefore, instead of commercializing single-value chain products, such as algal biomass and raw extracts (Table 1), it is important to establish a biochemical class-based production platform for multiple products to fully capitalize the components produced by microalgae [8, 134]. A possible multiproduct purification scheme is presented in Figure 2, generating nine valuable fractions, an additional residual, and insoluble biomass fraction.

Prior to extraction, the harvested algal biomass is either processed directly in the form of slurry (frozen and thawed). Alternatively, the biomass is disrupted and dried, or solely dried. Then, the concept of cascade extraction is implemented to microalgal bioprocessing. The biomass is consecutively extracted using solvents of different polarities (e.g., water and ethyl acetate) to obtain two fractions: one enriched in hydrophilic (e.g., proteins and carbohydrates) and one in lipophilic compounds (e.g., n-3 VLC-PUFAs and carotenoids). The generated extracts are then separated using

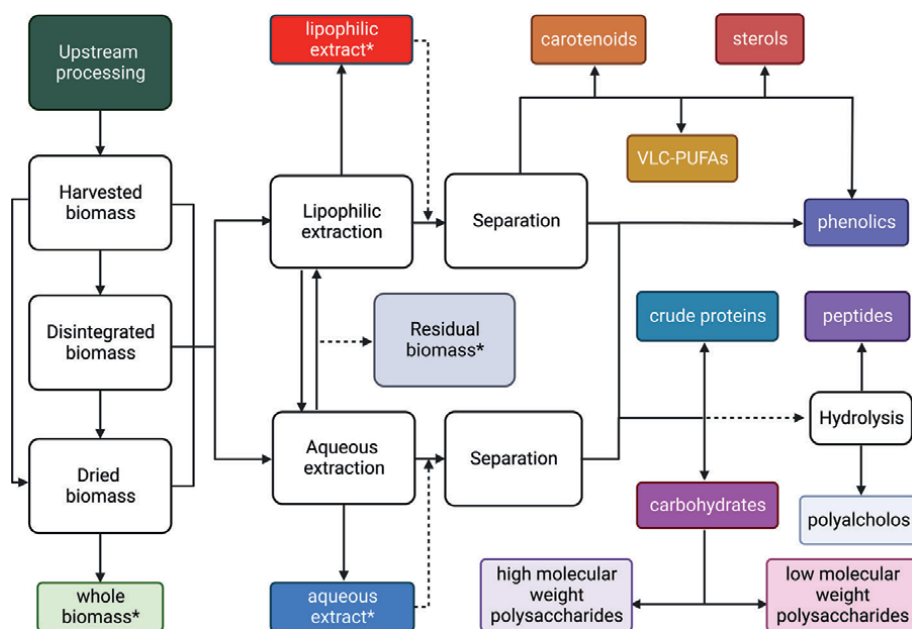


Figure 2. Multiproduct generation platform from microalgal biomass. * represents the single-value chained products and residual biomass that is currently discarded. Created with BioRender.com.

different fractionation techniques based on the nature of the molecules found in the extract (e.g., based on the molecular size; HMW sulfated polysaccharides from LMW polysaccharides). The residual biomass from the cascade extraction may undergo additional hydrolysis, resulting in polyalcohols (e.g., inositol, glycerol, sorbitol), bioactive peptide-rich fractions, or leave the bioprocessing chain being sold as low-value bulk commodities (e.g., fertilizer). Therefore, this approach can result in a spectrum of high- and low-value products, which balances the production costs of both bulk and specialty microalgae-based products. Subsequently, the total revenue from a given algal biomass can increase, and the production of sustainable algae-based bulk commodities in an economically viable way becomes possible. However, it is important to note that the focus should not be solely on optimizing individual unit operations, but rather on enhancing the efficiency of the overall process. Improving a single-unit operation based on a benchmark may lead to a more intricate and expensive overall process. Therefore, increasing the efficiency of utilization by implementing a zero-waste multiproduct biorefinery has become a prominent area of research.

In a study conducted by Gallego et al., a sequential accelerated solvent extraction-based platform was developed to recover PE, sulfated polysaccharides, and carotenoids (β -carotene and zeaxanthin) from *P. cruentum* [135]. Similarly, Derwenskus et al. applied this pressurized liquid extraction (PLE) technique for the co-production of EPA and fucoxanthin from the diatom *P. tricornutum* (**Figure 1**) [21]. They obtained EPA- and fucoxanthin-rich fractions through a single extraction step, applying ethanol, which contained approximately 90% of the initial fucoxanthin and EPA contents. The obtained fraction was further purified by precipitation and filtration, resulting in product purities exceeding 90%. Furthermore, the use of subcritical n-hexane at 100°C led to direct precipitation of the carotenoid at a temperature drop to ambient conditions. Similarly, Zhang et al. investigated the concept of an integrated biorefinery process to produce three valuable compounds, fucoxanthin, EPA, and chrysolaminarin, *via* cascade extraction [136]. In their study, freeze-dried *P. tricornutum* was processed using microwave-assisted ethanolic extraction. The obtained extract and residual biomass were subsequently subjected to n-hexane extraction *via* maceration to obtain fucoxanthin- and EPA-rich fractions. After n-hexane extraction, the residual biomass was further processed using sulfuric-acid-catalyzed hot water extraction to obtain chrysolaminarin. Although the compounds of interest were isolated, their yields were relatively low (34 and 23%, respectively) [136].

While these studies represent progress in multiproduct generation, they also underscore the importance of scalable DSP unit operations because the scalability of microwave-assisted extractions and PLE processes from the laboratory to the industrial scale remains a general cost issue and currently exceeds the technical state of the art at scale. Here, solvent extraction is applied at ambient or low overpressure, which results in lower extraction yields compared to PLE. To ease scale-up classical maceration and mixing techniques using various solvents have been studied. For instance, Chaiklahan et al. evaluated different scenarios of sequential product recovery, such as PC, FAs, and polysaccharides from *A. platensis*, using conventional maceration techniques to extract dried biomass [137]. PC was the main product of the extraction process, and approximately 67% of the original PC content from the raw materials could be recovered. Crude fatty acid and polysaccharide-rich extracts were also obtained, which have shown to increase the overall revenue. Similarly, Li et al. applied cascade extraction under atmospheric conditions using cold water, 95% ethanol, and hot water to recover PE, lipids, and polysaccharides from *P. purpureum* [138].

The cascade extraction process achieved considerable recovery rates for the target compounds (63.3, 74.3, and 75.2%, respectively).

However, when high-purity fractions have to be generated, it is necessary to separate the compound of interest from other molecules, which primarily reduce purity and value of the lead component. For this purpose, different separation techniques, depending on the nature of the molecule, can be used such as solid-liquid phase chromatographic techniques (e.g., adsorption or molecular sieve-based). Several studies have demonstrated the successful use of silica gel for purifying microalgal oil, including the separation of specific compound-rich fractions, such as γ -linolenic acid from *Spirulina* [139], EPA and ARA from *Porphyridium* spp. [140], and carotenoids, such as fucoxanthin [141]. These purifications are currently restricted to the laboratory scale [119]. Despite these efforts, microalgae extracts are rarely purified to single components owing to the high cost of purification with respect to the “ceutical” application and a lacking market demand for highly pure compounds. Thus, simple, robust, and scalable isolation methods will aid broader availability of valuable microalgae-based fractions at reasonable market prices. For this purpose, liquid-liquid separation techniques, such as centrifugal partition chromatography (CPC), high-speed countercurrent chromatography (HSCCC), and aqueous two-phase systems (ATPS), have been increasingly investigated in natural product recovery.

ATPSs are formed when two nonmiscible aqueous solutions are mixed (e.g., two polymers, polymer-salt, ethanol-salt, or ionic liquids (IL)-salt or polymer) [142]. ATPSs have been investigated in the study of Suarez Ruiz et al. using different systems, including PEG400-Cholinium dihydrogen phosphate, PEG400-citrate, and Iolilite 221PG-citrate, for the development of a single-step fractionation method for microalgal carbohydrates yielding a high recovery for glucose [143]. In a different study, 98% of pigments were recovered from the polymer-rich phase, separated from a protein-rich cholinium dihydrogen phosphate phase using a disintegrated *N. oleoabundans* suspension at a PBR-typical concentration (2.5 g L^{-1}). In the IL phase, a recovery of 82 and 93% for proteins and soluble sugars, respectively, was obtained. In addition, 79% of the insoluble polysaccharides and 71% of the lipid molecules were recovered from the interface [144] underlining the suitability of the multistep fractionation approach. Sarkar et al. further confirmed the efficacy of the ATPS approach [145]. Using an aqueous ammonium sulfate solution (40%) combined with ethanol at a ratio of 0.25:1 (v/v), it was possible to separate proteins, carbohydrates, and chlorophylls simultaneously in a single step from the biomass of *C. thermophila*. The recovery yields were 71, 40, and 22%, respectively [145]. Thus, the use of ATPS for extracting multiple products from microalgae has recently gained significant interest. Although the scalability of ATPE was demonstrated up to 1000 L systems (e.g., Human insulin-like growth factor [142]), its industrial application in microalgae processing has not been examined, yet. Technically scalability of ATPE is comparably simple as it combines a stirred tank with a centrifuge. Thus, studies should aim to investigate the still existing gap to explore the possibility of using this technique in the microalgal biorefinery processing [142–144].

CPC and HSCCC are separation techniques that use two immiscible liquid phases, composed mainly of organic solvents. One phase is held stationary in sequentially joint separation chambers (CPC) or in a multilayer coil (HSCCC) by centrifugal force, whereas the other phase is forced to flow through the stationary phase. Dissolved components are separated on the basis of their distribution coefficient between the two phases. Unlike other chromatographic techniques, a solid support is not required; therefore, irreversible sample adsorption is prevented. In addition to a recovery rate

of over 95% and a purity rate of over 99% [146], CPC and HSCCC also provide high injection capacities, making this technique an efficient DSP unit operation in the field of natural product isolation [147, 148]. Recently, the applicability of these techniques in algal biorefineries has been investigated. For instance, in a study by Bárcenas-Pérez (2021), HSCCC was employed to isolate fucoxanthin from an extract of *P. tricornutum* using a multiple-sequential-injection HSCCC method. With ten consecutive sample injections, 38 mg of fucoxanthin with 97% purity and 98% recovery was obtained. The process throughput was 0.19 g extract h⁻¹, while the fucoxanthin throughput was 3 mg h⁻¹ employing a 135 mL rotor [149]. In another study, Bárcenas-Pérez et al. al (2021) focused on the separation of DHA ethyl ester and docosapentaenoic acid (DPA, C22:5, n-3) ethyl ester from algal oil using HSCCC. Similarly, consecutive sample injections of 1 g of algal oil were performed, resulting in the isolation of 1.8 mg of DHA ethyl ester with 99% purity and 99% recovery. In addition, 2.16 mg of DPA n-6 ethyl ester with 97% purity and 92% recovery was obtained. The process throughput for algal oil was 1.15 g h⁻¹, and the throughput of the two target compounds was about 0.11 g h⁻¹ [150]. Additionally, a novel two-step HSCCC method was developed, resulting in the isolation of 980 mg EPA ethyl ester-rich fraction with a purity of 71%. This fraction was further purified, yielding 700 mg of EPA ethyl ester with a purity of 99% and a recovery of 98% with an EPA ethyl ester throughput of 0.05 g h⁻¹ [151]. Furthermore, Fábryová et al. developed a new isolation procedure for the purification of lutein from *C. vulgaris*. They utilized a multi-injection HSCCC method, processing a total of 250 mg of extract per hour, which resulted in a total recovery of 60 mg lutein within a processing time of 8 h at a purity of 92%. Fábryová et al. also demonstrated that HSCCC separation can be used for the pre-fractionation of astaxanthin fatty acid esters from *H. pluvialis* [152, 153].

The abovementioned studies collectively emphasize the potential of liquid-liquid chromatographic techniques as versatile tools for the recovery and purification of valuable natural compounds derived from microalgae since these techniques offer efficient separation, high purity, and high recovery rates. Therefore, these separation techniques may represent a useful and moreover scalable tool for the efficient production of purified microalgae-based compounds such as fucoxanthin and VLC PUFAs. With respect to scalability CPC rotors of a volume of 50 L are currently available [154], which represents a scale-up factor of 200 to 500 for CPC methods developed. Processing capacities of crude extracts lie, assuming optimized methods, in industrial relevant dimensions of several kg of extract per day.

Summarizing the currently applied and possible DSP unit operations, **Table 2** presents an overview as a showcase of the state of the art and the potential multi-product biorefinery for *P. tricornutum*. At industrial level, the applied DSP process currently involves four-unit operations, including solid liquid separation, cellular disintegration, drying, and SC-CO₂ extraction. At the end of this production process,

Number of DSP unit operations	Gained fractions	Valuable fractions	Discarded fractions	References
4	2	1	1	Industrial process
5	3	2	1	[21]
5	10	10	0	Potential

Table 2.
Current and possible DSP unit operations for *P. tricornutum*.

one fucoxanthin and EPA-enriched fraction are commercialized, while the residual biomass is not used.

As shown by Derwenskus et al., by implementing one additional DSP unit operation, the number of valuable fractions also increases. In this study, an EPA and fucoxanthin-rich fraction was obtained by PLE subsequent to harvesting and disintegration [21]. Further crystallization and filtration of precipitating fucoxanthin yielded two purified fractions. However, if after harvesting and cellular disintegration, adequate bi-phasic extraction techniques are utilized (e.g., ethyl acetate, water) the process would already result in the hydrophilic and lipophilic enriched extracts (see **Figure 3**). Subjecting these extracts to liquid-liquid separation techniques, such as CPC and ATPPE, up to 10 different products could be generated. For *P. tricornutum*, from the lipophilic fraction fucoxanthin, VLC PUFAs, polyphenols, and sterols could be purified, while from hydrophilic extracts the more polar polyphenols, proteins, and chrysolaminarin could be obtained. These hydrophilic compounds then could be further fractionated by ATPS into proteins and carbohydrates and separated by filtration based on their different molecular size. The residual cell debris from the extraction could consequently undergo additional hydrolysis to generate polyalcohols, peptides, and amino acids. The residual solids would leave the value chain and potentially utilized as fertilizers. This concept then would result in a close to zero-waste biomass utilization of *P. tricornutum* without a significantly higher number of different unit operations.

In summary, previous studies have presented advancements and a step forward in the extraction and purification of multiple high-value compounds. However, the financial implications of the multiproduct biorefinery concept, resulting from the implementation of additional DSP unit operations at the industrial scale, still need to be explored. Additionally, the choice of process units, design characteristics, and process conditions for each step of the algal biorefinery chain affects the performance of the subsequent processing steps. Thus, owing to the lack of information on the

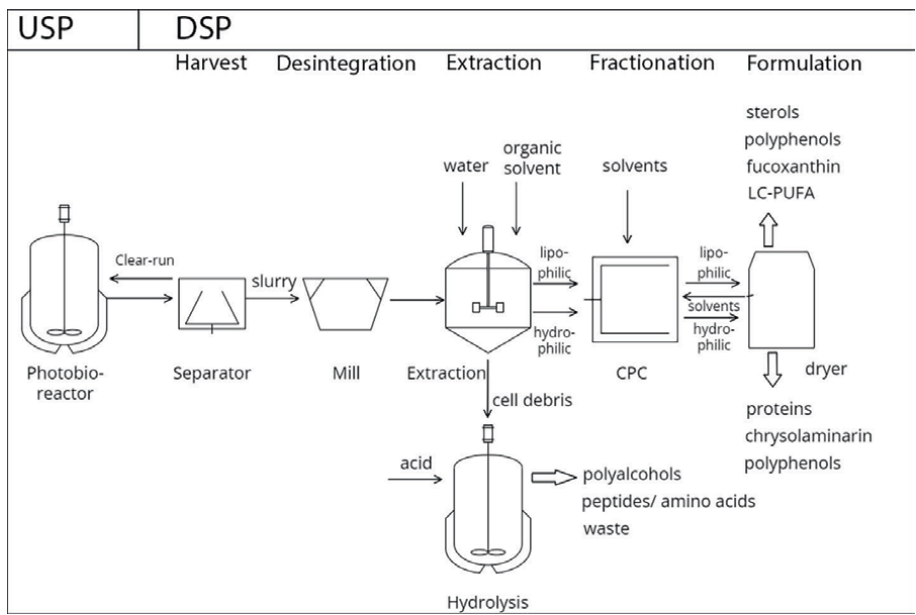


Figure 3. Indicative flow diagram containing unit operations and products of the multiproduct DSP concept for *P. tricornutum*.

total specific energy demands, processing times and yields of the overall process, further investigations on the subject are required.

5. Conclusion

In summary, this chapter highlights the large potential of microalgae as multiproduct cell factories. Despite the economic and legislative hurdles faced in establishing a microalgae-based bioeconomy, the unique metabolic versatility of microalgae makes them promising candidates as sustainable bioresources. The current status of microalgal products is presented and it is evident that the commercial exploitation of microalgae is currently limited to a few single-value chained products. However, identifying market-relevant products derived from algae of high commercial interest, such as fucoxanthin and EPA, and the utilization of the currently discarded residual biomass seems to be a promising way to enhance the credibility of a microalgae-based bioeconomy.

It is also evident that there have been notable achievements in the research and development of multiproduct biorefinery approaches; however, these are still limited to laboratory-scale experiments. Additionally, research activities have been focusing on the microalgae-specific optimization of single DSP unit operations, thus neglecting the overall performance of the whole multiproduct DSP platform. Furthermore, there is a need to develop suitable purification methods for multiple products with a high throughput, yield, and recovery. For this approach, liquid-liquid separation techniques, such as CPC and ATPE, appear to be promising. However, further economic feasibility investigation (e.g., product-specific energy input, solvent reusability, yield) for the generation of multiple products with consecutive liquid-liquid chromatographic techniques has to be carried out in order to provide further insights into microalgae-based bioprocessing.

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Third-Generation Biodiesel: Different Production Processes

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Abstract

The production of biodiesel from microalgae faces several problems to be solved, among them is the necessity of increasing their lipid content, optimizing the harvesting, and improving the conversion of lipids to bioenergy, therefore reducing the energy cost of the production process prior to its commercial launch. Research focused on optimizing the biodiesel production process known as transesterification has various objectives such as eliminating the biomass drying stage, unifying the extraction and transesterification stages, improving the reaction yield using supercritical conditions, providing heating with microwave and ultrasonic radiation, reusing enzymatic and heterogeneous catalysts, among others. This chapter aims to summarize the advances that have been achieved with the various operating conditions for the *in situ*, direct, and supercritical oil transesterification process of microalgae from the genera *Chlorella*, *Scenedesmus*, *Spirulina*, and *Nannochloropsis*.

Keywords: transesterification, microalgae, reaction conditions, yield, biodiesel

1. Introduction

Research and development on microalgae as a feedstock for third-generation biofuel have focused on increasing their production, this is due to their ability to synthesize and accumulate lipids [1]. The main interest of these studies is their doubling rate, which varies from 3.5 to 24 h, resulting in a high growth rate and high biomass yield in short periods [1–3].

The overall success of biodiesel production from microalgae begins with the selection of the right strain, which must exhibit optimal biomass production performance, as well as a high accumulation of triglycerides, and must adapt to extreme environments [4].

Obtaining lipids from microalgae begins with their growth, followed by harvesting, filtration, drying, and, finally, oil extraction [5]. Microalgal biomass production cost is estimated at \$5.8 USD/kg, with an energy requirement of 33 MJ/kg dry biomass for lipid recovery [6].

Biodiesel has similar physicochemical properties to diesel, making it suitable for use in compression-ignition internal combustion engines; it is also considered to have a net contribution of carbon to the environment, high flash point, complete combustion with low sulfur emissions, and high cetane number [7]. Emissions from its combustion depend on the percentage used and the oil used for its combustion [8]. In addition, its calorific value is lower than that of diesel, being 39.45 MJ/kg versus 44.24 G/kg for diesel [9]. The biodiesel obtained from castor oil has an energy of 26.03 MJ/kg [10].

To optimize the production of biodiesel by means of transesterification, researchers have used different reaction conditions, as explained below.

2. Microalgae

Microalgae are unicellular photosynthetic eukaryotic microorganisms that inhabit freshwater and brackish water aquatic environments [11]. They produce fatty acids as precursors for the synthesis of several types of lipids, which vary between species: polar and neutral lipids, waxes, sterols, phospholipids, glycolipids, carotenoids, terpenes, carotenoids, tocopherols, and quinones, for the synthesis of proteins and cell growth [12], of which only neutral lipids are suitable for biodiesel production.

Chemical stimuli such as nutrient deficiency (nitrogen, phosphorus, sulfur, and silicon), salinity, and pH, as well as physical stimuli such as temperature and light intensity, influence the composition of lipids and fatty acids contained in microalgae [13, 14]. The C:N ratio is the main factor responsible for the synthesis and accumulation of intracellular lipids [15]; both elements are important in the photosynthetic metabolic pathway of microalgae [16]. Several strains of microalgae have been studied in different culture media, with the intention of increasing biomass productivity or lipid productivity; however, these studies have not focused on the utilization of sunlight and contaminated natural waters.

The fatty acids present in microalgae include linear molecules with a length of 12 to 22 carbon atoms in even numbers, both saturated and unsaturated. Unsaturated fatty acids vary in the number and position of double bonds in the main carbon chain, monounsaturated fatty acid contains only one double bond, while polyunsaturated fatty acids contain two or more double bonds [17]. The lipid contents of various species of microalgae have been reported to range from 11 to 63% on a dry weight basis and are listed in **Table 1**.

Among the fatty acids present in vegetable oils are the saturated palmitic (C16:0) and stearic (C18:0) fatty acids, the saturated fatty acids oleic (C18:1) and linoleic (C18:2), as well as the polyunsaturated fatty acids linolenic (C18:3), eicosapentaenoic (C20:5) and docohexanoic (C22:6) [19–21]. Microalgae with a high percentage of fatty acids, mainly those containing acids from C16 to C18, are suitable for the production of biodiesel [22].

Scenedesmus dimorphus contains fatty acids mainly from C16 to C18 and can also be obtained from C20 to C22 depending on the culture conditions. In BG-11 medium, it can accumulate from C14 to C20, the ones found in higher percentage were palmitic acid at 24.23%, linolenic acid at 44.01%, hexacosanoic acid (C26:0) at 19.12%, while when cultured in lactic acid-rich wastewater the lipid profile deviates to the accumulation of palmitic acid with 25.01% and oleic acid with 53.24% [23].

Microalgae	% Lipids	Reference
<i>Chlamydomonas reinhardtii</i>	21	[18]
<i>Chlorella vulgaris</i>	36–40	[11]
<i>Scenedesmus</i> sp.	40	[12]
<i>Chlorella</i> sp.	28–32	[13]
<i>Scenedesmus dimorphus</i>	31	[14]
	10.23	[15]
<i>Chlorella minutissima</i>	31	[16]
<i>Scenedesmus obliquus</i>	12–14	[18]
<i>Nannochloropsis salina</i>	50–55	[17]

Table 1.
Percentage weight basis.

In another research where it is cultivated in municipal wastewater, the lipids obtained range from C15 to C18, predominantly palmitic acid with 21.6%, oleic acid with 23.3%, and linolenic acid with 24.01%, when the biomass is subjected to salt stress with NaCl at 5% P/V, the lipid profile changes, increasing the percentage of palmitic acid to 40.5% and stearic acid from 2.5 to 11.2%, while linolenic acid decreases to 4.16% [24]. In other words, the lipids of *Scenedesmus dimorphus* include palmitic, oleic, and linolenic acid in different percentages depending on the growth metabolism.

A comparative study of biomass and lipid content of *Chlorella vulgaris* reported higher biomass productivity with the use of mixotrophic growth (53%, 3 g/L) in comparison to autotrophic (20%, 0.8 g/L) and heterotrophic (16%, 0.2 g/L) growth, highlighting that microalgae can use both organic and inorganic carbon, nevertheless, in both cases, light is required for growth [25]. In another experiment, it was reported that *Chlorella* strains are capable of bio-sequestration of heavy metals such as Hg, Cd, Pb, Au, Ag, Cu, Cr (VI), and Ni [26]. The lipid profile of *Chlorella vulgaris* contains saturated and unsaturated fatty acids, specifically palmitic acid, linoleic acid, oleic acid, and stearic and linolenic acids. Generally, the acids present range from C16 to C18, with a higher percentage of palmitic acid [27–30].

Chlorella minutissima presented 57.1% palmitic acid (C16:0), 5.92% stearic acid (C18:0), 26.3% oleic acid (C18:1) when grown in Guillard f/2 medium under 150 Lux illumination [31]. Meanwhile, *Scenedesmus* sp stores 25.35% of palmitic acid and 74.67% of oleic acid [32]. Finally, *Spirulina platensis* has been shown to store palmitic acid and oleic acid [33].

Different species of green microalgae and their growth metabolisms vary in lipid contents; however, it is observed that they tend to accumulate palmitic and oleic acid as main sources.

3. Conversion of biomass to bioenergy

Biodiesel is a chemically long-chain fatty acid monoalkyl ester, one of the processes to obtain it is transesterification, which consists of combining

vegetable oil with alcohol, obtaining biodiesel and the value-added residue glycerol [27, 32, 34, 35].

Among the different alternatives for the production of biodiesel from microalgae as raw material, there is the conventional transesterification of oils, transesterification under supercritical operating conditions, direct transesterification of wet biomass, and *in situ* transesterification of dried microalgae, in addition to the effect of different types of catalysis.

Cao *et al.* investigated the possibility of avoiding the steps of dehydration of the biomass and extraction of its lipids by proposing a method of direct transesterification of wet biomass, observing a yield inversely proportional to the humidity, the yield of the reaction remains stable for humidities lower than 30%, the advantage of this alternative is the reduction of the energy requirements for the production of biodiesel [21]. Direct transesterification is a method that does not require dehydration of the biomass and extraction of lipids, in **Table 2** it is observed that direct transesterification has been studied in supercritical conditions, in these conditions a catalyst is not required for the reaction, the reason for which the product does not require purification.

Sivaramakrishnan *et al.* investigated the direct transesterification of the microalgae *Scenedesmus* sp. using dimethyl carbonate (DMC) and lipase (Lipozyme-Novozyme CAL-B from *Candida antarctica*, Novozymes Denmark) immobilized on Celite (commercial brand of diatoms), with 1% water for 36 h at 50°C, stirring at 180 rpm obtaining a yield of 92%, before the synthesis, a process of cell disruption is carried out with ultrasound, the use of lipase in this process showed a yield of 90% up to the fifth cycle, addition, this process generates glycerol carbonate as a by-product [12]. the most common methylating agent is methanol, as a by-product of the transesterification reaction, glycerin is obtained, which is oversupplied in the market, consequently, its price has decreased, the use of different methylating agents such as dimethyl carbonate, and methyl acetate produced as a by-product glycerol dicarbonate and triacetin [44].

Another alternative method considers the use of supercritical methanol coupled to oil extraction with CO₂ under supercritical conditions, for the case of biodiesel production from *Chlorella* sp. the advantage of this process is the utilization of the extraction residues in the production of drugs because carbohydrates, trace elements, and minerals are not damaged in the process and can be easily separated from CO₂ by decompression [37]. Supercritical transesterification of microalgae oils offers better yields than when using dry or wet biomass, on the other hand, the use of cosolvents improves the process.

Patil *et al.* compared the yield of a simultaneous microwave-assisted extraction and transesterification reaction under supercritical conditions of the microalgae *Nannochloropsis* obtaining yields of 80.13 and 84.15% respectively, they conclude that microwave radiation is more effective in cell lysis, due to that the reaction time is shorter, the process in supercritical conditions consumes 600 KJ while the assisted transesterification consumes only 254.5 KJ, that is, this alternative is an easy, fast and efficient process with a lower energy requirement [36].

Biodiesel is influenced by the physicochemical characteristics of the feedstock from which it is produced. Because of this relationship, microalgae oil should contain long-chain fatty acids with a low degree of unsaturation, preferably palmitoleic (16:1), oleic (18:1), and myristic (14:1) acids, thus allowing the reduction of toxic

Microalgae	Operating conditions	Performance	Reference
Transesterification under supercritical conditions			
<i>Nannochloropsis</i>	Algae moisture/methanol (W/V) 1:8, 250°C, 25 min.	84.16%	[36]
<i>Nannochloropsis sp</i>	Wet algae/ethanol (W/V) 1:9, 25 min, 265°C, 80 Bar	30.9%	[17]
<i>Chlorella sp</i>	20 gr algae mesh 40, 2 L/min CO ₂ , 60°C, 20 MPa	63.7%	[37]
<i>Schizochytrium limacinum</i>	Biomass 1.6% moisture: methanol 1:6, 210°C, 7.6 MPa, 60 min.	84%	[38]
<i>Chlorella prothothecoides</i>	Microalgae oil: methanol 1:19, 320°C, 152 bar, 31 min	90.8%	[39]
	Microalgae oil: ethanol 1:33, 340°C, 170 bar, 35 min	87.8%	
<i>Chlorella sp</i>	Biomass 75% moisture: methanol 1:10 P/V, 265°C, 50 min	42.62%	[40]
<i>Nannochloropsis oculata</i>	Biomass 75% moisture: methanol 1:10 P/V, 265°C, 50 min	21.67%	
<i>Spirulina platensis</i>	Biomass: methanol 1gr:8 ml, cosolvent hexane 4 ml: gr biomass 40% moisture, 244.8 °C, 6.61 MPa	99.32%	[41]
<i>Chlorella</i>	Microalgae oil: methanol 1:30, 270°C, 8.1 MPa, 30 min	97.1%	[42]
<i>Spirulina platensis</i>	Microalgae oil: methanol 1:40, 0.003 g CO ₂ /gr methanol, 300°C, 20 MPa, 30 min	72%	[43]
	Microalgae oil: ethanol 1:40, 0.001 g CO ₂ /gr methanol, 300°C, 20 MPa, 30 min	68%	
<i>Schizochytrium limacinum</i>	Biomass: methanol 1:10 P/V, 260°C, 20 MPa, 20 min	61.4%	[44]
	Biomass: methanol 1:10 P/V, 270°C, 20 MPa, 40 min	>90%	
	Biomass: dimethyl carbonate 1:10 P/V, 270°C, 20 MPa, 30 min	>50%	
	Biomass: methyl acetate 1:10 P/V, 270°C, 20 MPa, 30 min	>40%	
<i>Schizochytrium limacinum</i>	Biomass: etanol 1:10 P/V, 320°C, 20 MPa, 50 min	>95%	[45]
	Biomass: etanol 1:10 P/V, 380°C, 20 MPa, 60 min	>60%	
Direct transesterification			
<i>Chlorella vulgaris</i>	200 mg biomass with 5.3% moisture:3 ml methanol, H ₂ SO ₄ (mol:80.8 mol), 60°C, 120 min, 200 rpm	94.6%	[23]
	200 mg biomass with 5.3% moisture:3 ml methanol, KOH (mol:19.9 mol), 60°C, 120 min, 200 rpm	70.5%	

Microalgae	Operating conditions	Performance	Reference
<i>Chlorella vulgaris</i>	Biomass at 86–91% moisture: methanol 1:66.9, lipase <i>Burkholderia</i> 2028 U/g, 45°C, hexane: methanol 1:65, 500 rpm, 48 h	58.21%	[46]
<i>Chlorella vulgaris</i>	Biomass moisture 40.6%, Biomass: methanol 1:9, biomass: IL (Phosphonium carboxylate) 1:8 W, 102.4°C, 4.6 h	98.6 ± 1.82%	[47]
In situ transesterification			
<i>Chlorella</i> sp.	Biomass: methanol 100 mg:12 ml, H ₂ SO ₄ 3%, 65°C, 2 h	45.26%	[19]
	Biomass: methanol 100 mg:12 ml, H ₂ SO ₄ 4%, 65°C, 2 h	53.19%	
	Biomass: methanol 100 mg:12 ml, H ₂ SO ₄ 3.5%, 65°C, 2 h	60.71%	
	Biomass: methanol 100 mg:12 ml, H ₃ PO ₃ 3%, 65°C, 2 h	39.12%	
	Biomass: methanol 100 mg:12 ml, H ₂ SO ₄ 3% (E1), NaOH 1%W (E2), 65°C, 2 h	43.92%	
<i>Spirulina platensis</i>	Biomass: methanol 1gr:5 ml, NaOH 0.5 gr/gr, 60°C, 3 h	30%	[20]
	Biomass: methanol 1 g:5 ml, H ₂ SO ₄ 1 gr/gr, 60°C, 3 h	79.5%	
	Biomass: methanol 1gr:5 ml, HCl, 1 gr/gr, 60°C, 3 h	58.6%	
	Biomass: methanol 1 g:5 ml, HNO ₃ 1 g/gr, 60°C, 3 h	40.3%	
	Biomass: methanol 1 g:5 ml, H ₃ PO ₄ , 1 gr/gr, 60°C, 3 h	3.6%	
<i>Scenedesmus</i> sp.	0.5 gr algae, 5 ml DMC ¹ /gr microalgae, 1% W/W water/microalgae, 50°C, 36 h, 180 rpm	92%	[12]
<i>Chlorella pyrenoidosa</i>	4 ml methanol, 8 ml n-hexane, H ₂ SO ₄ 0.5 M, 120°C, 180 min	92.5%	[21]
<i>Nannochloropsis gaditana</i> (11.1% saponifiable lipids LS and 75% moisture)	Methanol/LS 171.1 mg/gr, 5% V/V acetyl chloride, 100°C, 105 min, 2.5 atm, 104 ml/gr hexane	100%	[22]
<i>Nannochloropsis</i> sp	1gr biomass, lipid: methanol 1:200, KOH 2%W, methanol: hexane 1:0, 60°C, 2 h	49.9%	[48]
	1gr biomass, lipid: methanol 1:200, KOH 2%W, methanol: hexane 1:1, 60°C, 2 h	56.1%	
	1gr biomass, lipid: methanol 1:200, KOH 2%W, methanol: hexan 1:1.5, 60°C, 2 h	48.43%	
	1gr biomass, lipid: methanol 1:300, KOH 2%W, methanol: hexan 1:1.5, 60°C, 2 h	76.77%	
	1gr biomass, lipid: methanol 1:400, KOH 2%W, methanol: hexan 1:1.5, 60°C, 2 h	78.87%	
	1gr biomass, lipid: methanol 1:400, KOH 2%W, methanol: hexane 1:1.5, 60°C, 4 h	90.95%	

Microalgae	Operating conditions	Performance	Reference
<i>Spirulina sp</i>	Biomass: methanol 50gr:100 ml, KOH 2%W, biomass: cosolvent 1:200 chloroform: methanol, 180 W ultrasound, 15 min	1.6% W Biomass 95.2% FAME content	[49]
<i>Chlorella sp</i>	Biomass: methanol 1gr:120 ml, 10%W catalyst ² , 80°C, 3 h	33%	[50]
	Biomass: methanol 1gr:120 ml, 20%W catalyst ² , 80°C, 3 h	47%	
<i>Chlorella vulgaris</i> , <i>Scenedesmus bijurjos</i> , <i>Euglena sp</i> , <i>Oscillatoria quadripunctulata</i> , <i>Microcystisaeruginosa</i> and <i>Chlamydomonas debaryana</i>	Biomass: methanol 1gr:12 ml, 4%W Fe ₂ O ₃ -Al ₂ O ₃ , 6 h	95.6%	[51]
<i>Chlorella pyrenoidosa</i>	1 gr biomass, methanol: CPME ³ : HCl 10:1.5:1, 80°C, 380 rpm, 150 min	92%	[52]
	1 gr biomass, methanol: 2-MeTHF ⁴ : HCl 10:1.5:1, 80°C, 380 rpm, 150 min	91%	
	1 gr biomass, methanol: chloroform: HCl 10:1.5:1, 80°C, 380 rpm, 150 min	76%	
Microwave-assisted transesterification			
<i>Nannochloropsis</i>	Dry biomass/methanol (W/V) 1:12, KOH 2%W, 6 min, 60–64°C	80.1%	[36]
<i>Chlorella pyrenoidosa</i>	Wet biomass (77%): methanol+ H ₂ SO ₄ 3% V, (2gr:6 ml), 70°C, 30 min.	50%	[24]
	Wet biomass (77%): ethanol+ H ₂ SO ₄ 3% V, (2gr:6 ml), 70°C, 30 min.	80.6%	
	Wet biomass (77%): isopropanol+ H ₂ SO ₄ 3% V, (2gr:6 ml), 70°C, 30 min.	85.7%	
Transesterification of oils with homogeneous catalyst			
<i>Chlorella vulgaris</i>	Methanol: oil 6:1, NaOH 1%, 55°C, 15 min.	>98%	[25]
<i>Tetraselmis sp</i>	Methanol: oil 6:1, 0.5 g NaOH, 65°C, 300 rpm, 3 h	Not specified	[26]
<i>Chlorella vulgaris</i>	Methanol: oil 10:1, 1.5% H ₂ SO ₄ V/V, 60°C, 40 min (E1)	84.01%	[28]
	Methanol: oil 10:1, KOH 1.5%, 60°C, 15 min microwave 700 W (E2)		
	Methanol: oil 10:1, 1.5% H ₂ SO ₄ V/V, 60°C, 40 min (E1)	83.23%	
	Methanol: oil 10:1, KOH 1.5%, 60°C, 3 h (E2)		
<i>Chlorella vulgaris</i>	38 mg oil:3 ml methanol, H ₂ SO ₄ (mol:50.7 mol), 60°C, 120 min, 200 rpm	96.6%	[23]
	38 mg oil:3 ml methanol, KOH (mol:2.5 mol), 60°C, 120 min, 200 rpm	90.4%	
<i>Chlorella sp</i>	Methanol: oil 30:1, H ₂ SO ₄ 10%P, 4 h	87.5%	[53]
	Ethanol: oil 30:1, H ₂ SO ₄ 10%P, 4 h	75.4%	

Microalgae	Operating conditions	Performance	Reference
Transesterification of oils with heterogeneous catalyst			
<i>Nannochloropsis oculata</i>	Methanol: oil 6:1, 80% CaO/Al ₂ O ₃ 2% W/W, 50°C, 4 h, 1100 rpm	23	[29]
	Methanol: oil 30:1, 80% CaO/Al ₂ O ₃ 2% W/W, 50°C, 4 h, 1100 rpm	97.5%	
<i>Tetraselmis</i> sp.	Isocetane: oil 5 ml:10 ml, 1gr immobilized <i>Candida rugosa</i> , 60 ml methanol, 40°C, 180 rpm, 10 h	Not specified	[26]
<i>Nannochloropsis oculata</i>	Methanol: oil 15:1, 3.5% W/W ZnO: Mn ⁺² encapsulated in PEG ⁵ , 60°C, 4 h	87.5%	[30]
<i>Scenedesmus</i> sp.	Methanol: oil 11:1, goat bone nanocatalyst 2% P, 60°C, 1500 rpm, 3 h	92%	[31]
<i>Chlorella</i> sp., <i>Scenedesmus</i> sp. <i>Synechocystis</i> sp., <i>Spirulina</i> sp.	Methanol: oil 20:1, 5% P sulfonated peanut shell biochar, 65°C, 4 h	92%	[33]
<i>Chlorella vulgaris</i>	Methanol: oil in hexane (5 mg: ml) 0.15 ml:0.5 ml, 8.6 mg ROL/MNP-AP-GA ⁶ , 45°C, 24 h	69.8%	[54]

¹Dimethylcarbonate.²LiOH-pumice.³Cyclopentyl methyl ether.⁴2-Methyltetrahydrofuran.⁵Polyethylene glycol.⁶*Rhizopus oryzae* lipase (ROL)-Superparamagnetic nanoparticles with 3-aminopropyl triethylenesilane-gutavaldehyde (MNP-AP-GA).**Table 2.***Third-generation biodiesel production processes.*

emissions, improving the cetane number and oxidative stability, without affecting lubricity, viscosity, and cloud point. Common methyl esters in biodiesel are methyl palmitate, methyl stearate, methyl oleate, methyl linoleate, methyl linolenate, and methyl laureate [55].

4. Conclusions

The production of third-generation biodiesel has focused on the variation of operating conditions to increase yield. Direct transesterification has the advantage of converting wet biomass directly avoiding the drying and extraction stages, as well as transesterification under supercritical conditions, another advantage of the latter is that it does not require the use of a catalyst, consequently, the biodiesel does not need purification. Both processes have yields inversely proportional to the amount of moisture in the biomass, the use of a cosolvent increases the reaction yield.

Microwave and ultrasound-assisted transesterification have yields greater than 75% in shorter reaction times, which is attributed to the higher mass transfer. The transesterification of oils with homogeneous and heterogeneous catalysis presents yields higher than 87%, the advantage of heterogeneous catalysts is their reusability, easy recovery, and elimination of the biodiesel purification stage.

Among the variables that affect the reaction yield is that a high temperature favors mass transfer, and an excess of alcohol favors the formation of products, however, it must be optimized to avoid yield losses due to the dissolution of biodiesel in alcohol, likewise an excess of catalyst can reduce the reaction yield.

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Conflict of interest

The authors declare no conflict of interest.

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Driving into the Factory of Docosahexaenoic Acid (DHA), Microalgae

Nahid Hosseinzadeh Gharajeh and Mohammad Amin Hejazi

Abstract

Microalgae, with their rapid growth and cost-effective cultivation, have emerged as a potent source of bioactive compounds, including lipids. Docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid, is an important fraction of microalgal lipids, which holds a crucial place in human nutrition and health. This chapter underscores microalgae's potential as a prolific factory for DHA production. Limited availability of conventional sources has stimulated interest in sustainable alternatives, with microalgae proving to be an effective solution. Microalgae can synthesize DHA *de novo*, eliminating the need for resource-intensive intermediaries. Optimization of cultivation conditions, including light intensity and nutrient availability, has boosted DHA production. Genetic engineering techniques enhance yields by overexpressing key biosynthetic genes, while innovative cultivation strategies such as mixotrophic and phototrophic modes increase biomass accumulation and DHA content. Biorefinery approaches utilize residual biomass for value-added product production, enhancing overall sustainability. By harnessing microalgae's inherent capabilities through cultivation optimization, genetic manipulation, and innovative processing, a reliable and sustainable DHA source is established, promoting enhanced human health and nutrition to meet the growing demand for this essential nutrient.

Keywords: microalgae, docosahexaenoic acid, cultivation optimization, genetic engineering, biorefinery approaches

1. Introduction

The essential fatty acids has garnered substantial attention due to their pivotal role in maintaining optimal health and well-being. Among these, docosahexaenoic acid (DHA), an omega-3 very-long-chain (VLC) fatty acid, has emerged as a compound of paramount importance with diverse physiological benefits [1–3]. As our knowledge of DHA advantages expands, the discrepancy between the recommended DHA dietary intake and its actual consumption gains more attention [4]. With the growing global population and occurrences of chronic diseases, the demand for DHA is set to increase [5]. This raises the need to explore innovative and sustainable sources for DHA production, especially considering the declining availability of traditional marine sources and the environmental concerns associated with overfishing [6]. In this

context, microalgae have emerged as an intriguing and promising solution to bridge the gap between DHA demand and supply. Microalgae are unicellular photosynthetic organisms abundant in various aquatic ecosystems that possess a remarkable ability to synthesize and accumulate lipids rich in DHA [7]. This unique characteristic has attracted the attention of researchers and industries prompting a comprehensive exploration of their potential as a reliable and sustainable source of DHA.

The journey into DHA production from microalgae is a multidisciplinary endeavor. It involves the seamless integration of biology, biotechnology, engineering, and environmental science. Scientists delve into the intricate mechanisms within microalgae cells, deciphering the molecular basis and metabolic pathways responsible for DHA synthesis [8, 9]. This knowledge serves as the blueprint for enhancing DHA yields through genetic engineering and optimization of growth conditions. Moreover, the cultivation of microalgae occurs within controlled environments. These cultivation systems mimic the natural conditions necessary for optimal growth, ensuring that microalgae flourish and efficiently convert sunlight and nutrients into DHA. Combining advanced technology and ecological considerations transforms microalgae into miniature biorefineries, producing DHA while minimizing resource consumption and waste generation.

2. Potential of microalgae for sustainable lipid production

Lipids are significant chemical compounds produced by microalgae (up to 70%) [10, 11]. The accumulation of high levels of lipids with various and unique fatty acid profile in microalgae nominates them as a viable and sustainable source for valuable lipid production [12]. The world is currently facing the significant challenge of the population growth and paucity of food and eco-friendly fuel sources. Interest in the production of lipid or valuable fatty acids (FAs) from oleaginous microalgae as a potential stock is rooted in their higher lipid content and fast growth rate [13]. Microalgae have several other advantages including high efficiency in photosynthesis, carbon dioxide sequestration, simple growth requirements, efficient land utilization, ability to grow in wastewater, and the year-round harvest. Compared with the land food sources, microalgae do not require arable land or potable water for growth; this avoids the competition for limited resources [14–16]. Microalgae produce 10–400 times more energy per acre in comparison to land plants [16]. The lipids and fatty acids from microalgae are considered as energy storage boxes, involved in metabolic processes and cell membrane-related functions. Unlike traditional sources of lipids, such as vegetable oils and animal fats, microalgae produce lipids rich in highly unsaturated fatty acids (HUFAs). They are essential and valuable fatty acids not commonly synthesized in our body and needed to be obtained by diet.

3. Heterogeneity of algal fatty acids

Tremendous species diversity in microalgae provides a rich reservoir with different characteristics of lipid composition and yield. There is a recent trend toward the evaluation of different microalgae and their lipidomic profiling. As befits their variable morphology and physiology, microalgae contain miscellaneous and unique acyl lipid composition [17]. Further, adaptation to adverse ecosystems and diverse habitats has reflected in exceptional variety of lipid patterns and unique fatty acid profile in microalgae [15, 17]. They synthesize a group of essential fatty acids that are not synthesized by other plants, animals, and humans [18].

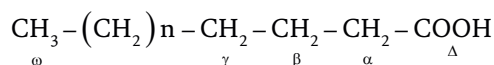
Unlike traditional sources of lipids, such as vegetable oils and animal fats, microalgae produce lipids rich in highly unsaturated fatty acids (HUFAs). Fatty acids of HUFA as part of complex lipids might be involved in cellular metabolism and physiological responses such as energy maintenance and transport, cell membrane structure and thus cell activity, cell signaling, modification of transcription, mechanical protection and thermal as well as electrical insulation [19, 20].

HUFAs include polyunsaturated fatty acids (PUFAs) and omega-3 long-chain and very-long-chain PUFA (LC and VLC-PUFA) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) [16, 18, 21]. Numerous health benefits are attributed to these fatty acids which makes them highly desirable in various industrial sectors of food additives and in cosmeceutical, nutraceuticals, pharmaceutical industries [22].

4. Architecture of microalgal lipid and fatty acids

Microalgal lipids are heterogeneous molecules with various structures ranging from short fatty acids (FAs) to more complex forms of phospholipids (PLs), triacylglycerols (TAGs), and sterols and their esters [20]. Complex lipids differ structurally according to the FA chain length along with the presence, location, and orientation of double bonds in their FAs; the branching of the hydrocarbon chain; the addition of polar groups; and glycosylation. The structural difference among FAs and lipids provides their functional differences upon cell and microalgal responses [19, 23, 24].

FAs are a series of hydrocarbon chains with a methyl (CH₃) group on one end (methyl, ω or n terminal) and carboxylic acid (COOH) the opposite end of the molecule (acid or α terminal). The ω3 FAs contain multiple double bonds with one bond at the third carbon relative to the methyl end [25]. The systematic nomenclature for fatty acids may also imply the carboxyl group (Δ) [20]. In brief, when designated as ω-? or n-?, the location of the first double bond in reference to the methyl end is attended and when named as Δ-? the position relative to functional carboxylic acid end is considered. Further, the carbon immediately after the carboxyl end is named α, and the subsequent carbons are β and γ, respectively. The formulation below outlines the structure of fatty acids.



The most abundant microalgal fatty acids are even-numbered, in straight-chain form, while odd-numbered and branched-chain FAs exist in respectively in ruminant tissues and bacteria [26]. The carbon number of fatty acids ranges from 2 to 30. Based on the chain length, fatty acids are classified as SCFA = short-chain fatty acids (C4–C13), MCFA = medium-chain fatty acids (C14–C17), LCFA = long-chain fatty acids (C18–C20), and VLCFA = Very-long-chain fatty acids (> C20) [27].

The lipid content in the microalgae is diverse; some produce SCFA and MCFA, while others synthesize VLCFAs. The enriched FA profile of SCFA, MCFA, and MUFA and lipid content of microalgae make them a possible source of biofuel [28]. In contrast, there are other microalgae that generally accumulate a large quantity of VLC-PUFAs as their major components that are of much current nutritional interest [17].

The unsaturation degree varies from 1 to ≥2 double bond(s), categorically referred to as monounsaturated fatty acids (MUFAs) and PUFAs, respectively. Apart from the position of the double bonds, *cis* or *trans* orientation of these bonds makes the

lipid structure even more complex. These variation factors may pack FAs in lipid membranes by modifying its biological, biochemical, and biophysical properties. The majority of the naturally occurring unsaturated FA in microalgae are of *cis* type, *trans* configurations rarely occur [19, 29].

5. Current sources of DHA

The HUFAs were traditionally sourced from marine fish resources [30]. In fact microalgae are the original producers of n3 HUFAs and DHA that enters the food chain *via* consumption by the subsequent organism, fish [16, 18, 21]. Biocontamination by heavy metals, toxic and carcinogen pollutants, and bio-destruction by depletion of particular fish species and overfishing due to rising population have raised concerns about finding sustainable sources replacing fish that has the additional limitation of peculiar taste and odor [6]. This has drawn the attention toward evaluating the primary sources of microalgae [31].

The accumulation of a diverse array of FAs in the microalgae brings them forwards as the main HUFA including DHA source. This would revolutionize biotechnology in the fields of nutrition, aquaculture, nutraceutical, and pharmaceutical industry [32] benefiting human health, global marketing, and the environment [33]. Since microalgal DHA + EPA is comparable with fish, replacing the fish oil with microalgal oil can be viable and sustainable [34]. This will thereby minimize the pressure of ocean fisheries and preserve the biodiversity [35]. **Table 1** shows the fatty acid profile of some microalgal species.

Currently, the major genera of omega 3 HUFA commercial microalgae belong to Chromista, of which the families Crypthecodiniaceae and Thraustochytriaceae are known as DHA producers [46, 47]. *Crypthecodinium cohnii* as the source of VLC-PUFA as well as DHA holds the primary place in commercialization [48–50]. The DHASCOT oil from *Crypthecodinium cohnii* (with 45% w/w DHA content) is marketed as the infant product.

6. DHA as essential bioactive fatty acids

A significant portion of human food, including plants and animals, contains n6 lacking n3 PUFAs [46]. Further, land plants mainly include shorter chain (16–18 carbon) n3 PUFAs such as α -linolenic acid (ALA). The n6 PUFA, linoleic acid (LA), can be converted to fatty acids with longer chains, and conversion of n3 ALA leads to synthesis of EPA and DHA. However, in human body, the conversion is not efficient and its rate is trivial (1 to 10%). Thus, these n3 HUFAs are very crucial part of the human diet and need to be supplied through regular daily intake of 0.2–0.3 g day⁻¹ [51].

The n3 fatty acids render an array of profound benefits in human health including normal growth, development of specific and nonspecific immunity, optimal fetal, ocular, cardiovascular, neurological and cognitive development, and functioning [1]. DHA and EPA are linked to alleviate and reduce the risk of asthma, arthritis, depression, psoriasis, autoimmune disorders, coronary cardiac disease, hypertension, inflammation, premenstrual tension, various atopic disorders, diabetes, skin disease, hypertension, hyperlipidemia, atherosclerosis, thrombosis, Alzheimer, and cancer [52–54]. Moreover, DHA and EPA function as natural antimicrobials, anti-inflammatories and antioxidants [54–61]. They constitute membrane phospholipids in certain tissues and

Species	ΣSFA	ΣMUFA	ΣPUFA	Σn3 PUFA	Σn6 PUFA	n3: n6	UFA:SFA	DHA	EPA	TFA	References
<i>Aurantiochytrium BL10</i>	35	1	65				2	40	5	73	[36]
<i>Cryptocodinium cohnii</i>	47.3	0.81	51.9				1.12	51.12	—	15.2	[37]
<i>Tisodrysis lutea</i>	28.9	19.8	50.8				2.4	13	—	21	[38]
<i>Monodus subterraneus</i>	29	29	41.4				2.4	—	32	17	[39]
<i>D. salina</i>	34.8	32.2	33.1				1.9	15.4	—	27.1	[39]
<i>D. tertiolecta</i>	37.8	1.2	60.9				1.6	0.01	0.1		[40]
<i>Volvox carteri</i>	34.1	54.7	8				1.8	—	—		[41]
<i>Chlamydomonas reinhardtii</i>	27	15	58	29	29	1	2.7	—	—		[42]
<i>Spirulina platensis</i>	34.3	39.3	25.1	7.9	17.2	0.5	1.9	3.51	1.9	8.03	[43]
<i>Isochrysis galbana</i>	43.3	30	24.4	21.7	2.8	7.9	1.3	18.8	3.2	17.2	[44]
<i>Chlorella vulgaris</i>	22.2	35.4	38.9	29.2	9.7	3	3.4	20.9	2.9	13.3	[44]
<i>D. sp. ABRINW-II</i>	12	10	78	54	24	2.3	7.3	23.8	1.2	20	[45]

Table 1.
 Comparison of fatty acid profile in different microalgal species. TFA is represented as percent of biomass dry weight and all others are as percent of TFA.

provide the viscosity of cell membranes [62–65]. DHA, the prominent fraction of the membrane phospholipids in neural and retinal systems, is more imperative for cell signaling, brain function, and vision [66]. Fetal neurodevelopment and infant cognitive development and visual acuity are especially attributed to DHA intake during early life [67, 68]. Lower intelligence quotients are determined in formula-fed than breastfed infants due to the rich DHA content of human milk [69]. Compared to EPA, DHA is reported to more effectively act on controlling the blood pressure and regulating the heart rate [70]. DHA is also implicated to be more efficient on treating neural system disorders (multiple sclerosis, Alzheimer's, and Parkinson) [71] and preventing the onset of cancerous growths than EPA [72]. In the human body, dietary DHA might be retro-converted to EPA, while the production of DHA from EPA is not observed [73]. The detailed essentiality and health claims of DHA have been comprehensively discussed in some studies by Oscarsson and Hurt-Camejo [74], Horrocks and Yeo [2, 73], Sijsma and De Swaaf [75], Li, Pora [3, 76].

7. Bioengineering of microalgal DHA production

Large-scale production of lipid from microalgae entails delicate bioengineering approaches [77] in either genetic or non-genetic tailoring form [78]. To optimize lipid production, several factors might be considered, such as the cultivation conditions, the microalgal strain and its biological properties, and the harvesting and extraction methods [79].

Cultivation and growth parameters including temperature, light, and nutrients significantly impact lipid content and composition [80]. Cultivation conditions might be complex as different strains may respond differently to the same conditions due to different molecular adjustment mechanisms to various stimuli [55]. This highlights the importance of comprehensive experimental analyses, unique to each strain, prior to embarking optimization programs. Accordingly, optimal condition favoring HUFA production is a fatty acid-, species-, or strain-specific property [55, 81]. Selection of a suitable microalgal strain is critical for achieving high lipid yields. Screening of a hyperproducer strain, optimization of physical conditions, breeding, and biochemical or metabolic engineering are the approaches to enhance lipid or valuable FA yield [81, 82]. The co-cultivation of microalgae species has been explored as a strategy for simultaneous production of EPA and DHA. Thurn, Stock [83] studied the photoautotrophic co-cultivation of *Tisochrysis lutea* and *Microchloropsis salina*, which resulted in the production of appropriate balance of EPA and DHA in the studied microalgae. Overall, metabolic engineering of microalgae holds great potential for enhancing DHA production.

While the role of organic carbon sources in the biosynthetic pathways of PUFAs remains unclear, Villanova, Fortunato [84] demonstrated an elevated EPA and DHA content in *Phaeodactylum tricornutum* when cultivated under mixotrophic conditions using the carbon source of glycerol. DHA synthesis was also examined in *Tisochrysis lutea* CCMP1324 cultures using autotrophic (with glucose and acetate as the carbon source), mixotrophic, and heterotrophic approaches (with glycerol). The mixotrophic conditions demonstrated the highest DHA production and productivity [85]. Mixotrophic growth also led to an increase in TFA content in the dry weight and TFA production in the medium. Specific extraction methods are also essential for maximizing DHA yields. Traditional methods, such as centrifugation and solvent extraction, can be time-consuming and energy-intensive. Newer technologies, such as organic solvents-water-organic solvents, flocculation, and membrane filtration, offer

more efficient and sustainable alternatives [86, 87]. In a study on *Cryptocodinium cohnii*, cultivation was carried out in fed-batch conditions using cost-effective industrial by-products such as raw glycerol and corn steep liquor [88]. The fermentation resulted in an appropriate lipid content and productivity. The subsequent saponification extracting reaction followed by fractionation process at different temperatures yielded a PUFA rich fraction.

8. Biochemical engineering for DHA production

In order to develop an optimized microalgal platform, administering a varied range of environmental factors to an individual isolate may contribute to outreach the desired fatty acid content [89, 90]. Biochemical engineering is the strategy of enhancing the microalgal lipid/FA production *via* controlling the culture and nutritional conditions. During biochemical engineering, the metabolic fluxes generated in photobiosynthesis are channeled into the biosynthesis of lipids or the desired FAs [46, 91, 92].

Several studies have been conducted to identify the main factors and the conditions that might enhance DHA production in numerous microorganisms including *Schizochytrium* and *Aurantiochytrium* [93–97]. Once the microalga *Cryptocodinium cohnii* was cultured in the salinity of 1.5 M NaCl, total DHA content was augmented up to 56.9% of TFA [16]. These were achieved *via* optimizing the concentration of fructose and monosodium glutamate (MSG) as well as agitation speed. Zhu et al. highlighted the importance of optimizing the medium and culture conditions for the growth and DHA production of *Schizochytrium* [98]. They emphasized that the data on the cultivation factors of *Schizochytrium* affecting its biomass and DHA production is limited but essential for industrial-scale DHA production. To improve the DHA production and prevent lipid peroxidation, Sun et al. developed a cooperative adaptive-evolution method in *Schizochytrium sp.* [99] to develop strains with high DHA production capacity and strong antioxidant defenses. This approach resulted in strains with improved DHA production and stability.

Of different environmental parameters, temperature is a crucial factor for DHA production [78]. Bindea, Rusu [100] highlighted the importance of finding the optimum temperature that balances increased DHA production and cell growth. They found that low temperature favors DHA production but it limits the cell growth. Therefore, the temperature should be optimized in a way that maximizes DHA as well as biomass production.

The highest DHA yield in *Schizochytrium sp.* was obtained in GM media containing glycerol. Glycerol in addition to proteose peptone as respectively carbon and nitrogen carbon sources, in combination with ethanol, improved DHA production [101]. Chalima et al. [97] optimized DHA production of the cells in terms of cultivation factors of nutrients and temperature. Based on the results, microalgal growth was remarkably improved with the application of ammonium as nitrogen source.

Non-genetic tailoring offer some advantages including cost-effectiveness and the use of available resources. However, the inherited shortcoming of this method is that it is time-consuming procedure. Another drawback is that the abiotic stress inevitably accompanies reduced biomass yield [78]. The physiological stress required for high lipid content coincides with microalgal growth cessation [79, 102, 103]. Achieving the appropriate balance between the optimal biomass and lipid volumetric productivity of microalgae relies on various biotic and abiotic factors [104]. Therefore, various

stresses should be meticulously programmed to reach the concurrent production of biomass and DHA.

Hosseinzadeh et al. [45] introduced an isolate *Dunaliella* sp. ABRIINW-I1 from Urmia lake as DHA hyperproductive microalga. Their data revealed that the combination of salinity, light, and temperature significantly increased the production of biomass, TFA, and DHA. The maximum growth and DHA level were achieved at 0.5 M NaCl, 25°C, and 200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. The highest DHA production in *Dunaliella* sp. ABRIINW-I1 was in the same range as commercial *Thraustochytrids* [45].

9. Metabolic engineering for DHA production

Apart from the biochemical engineering, another promising approach for higher production of targeted substances in the microalgae is metabolic engineering. It deals with genetic, transcriptomic, and regulatory processes within cells. In parallel, omics technology including genomics, transcriptomics, proteomics, metabolomics, and lipidomics explores the mechanisms underneath the cell responses toward stressors [53, 105]. Developing improved strains accumulating large quantities of the desired FAs requires providing a deep insight into microalgal biology, including its genetic, transcriptomic, and metabolic information.

Wang et al. used metabolic engineering techniques to improve the synthesis of DHA and fatty acids of odd chains in a strain of *Schizochytrium* sp. [106]. They achieved the significant improvements in the contents and titers of DHA and odd-chain fatty acids, leading to a decrease in the total fatty acid production cost. One approach to optimize DHA production is through the reconstruction and analysis of genome-scale metabolic models (GSMMs). Ye et al. reconstructed a GSMM of *Schizochytrium limacinum* SR21 and used it to predict the requirements for DHA production [107]. They identified six amino acids, malate, and citrate as potential supplements to enhance DHA production. Additionally, they used a Minimization of Metabolic Adjustment algorithm to identify 30 genes as potential targets for DHA over-production. Utilization of response surface methodology (RSM) is an effective approach used for the enhancement of DHA production. Nazir et al. applied RSM for cultivation conditions of *Aurantiochytrium* SW1 to optimize its biomass, TFA, and DHA production [108].

Manipulating the metabolic pathways through genetic modification is one of the strategies in metabolic engineering. Efforts have been undertaken to augment the EPA and DHA production by altering the metabolic pathways in microalgae [109]. For example, once the endogenous type 2 diacylglycerol acyltransferase (DGAT2B) was overexpressed in the *Phaeodactylum tricornutum*, higher lipid yields were resulted with enhanced levels of DHA in triacylglycerols (TAG) [110]. In addition to genetic manipulation, other approaches have been explored to optimize DHA production in microalgae. For instance, the addition of stimulators such as 2-phenylacetic acid (PAA) improves the growth, DHA, and the relevant transcriptome in *Aurantiochytrium* sp. [111]. This method is considered practical and efficient without any need to comprehensive understanding of the molecular targets.

The authors of this chapter have analyzed the expression of lipid synthesis genes in the native microalga *Dunaliella* under the stress conditions. Based on their results, under high TFA production, in the first 24 hours, all the glycerolipid and FA synthesis genes were up-regulated yielding in improved TAG synthesis. The fatty

acid elongation genes, during the time span of 4 to 24 h, were up-regulated in all the conditions. The over-expression of the VLCFA elongase as well as $\Delta 5$, $\Delta 7$, $\Delta 8$, $\Delta 9$, $\Delta 12$, $\Delta 15$, $\omega 3$, $\omega 6$, and long-chain FA desaturase genes in high TFA samples suggested the increased elongation and desaturation toward VLC-PUFA and highly unsaturated fatty acids synthesis. Regarding DHA production pathway, the main genes involved in the recently discovered alternative pathway of polyketide synthase (PKS) were enriched (data not published).

The $\Delta 6$ -desaturase, $\Delta 4$ -desaturase, and $\Delta 5$ -elongase were determined as rate-limiting enzymes in enhancement of DHA production in microalgae. The expression of $\Delta 5$ -desaturase in *Phaeodactylum tricornutum* UTEX646 leads to 8 times increase of DHA production compared to the native strain [112].

10. Perspective

At present, with elevated fried- and fast-food consumption, the balance between omega-3 and omega-6 fatty acids has been disrupted and this has been linked to increased prevalence of chronic diseases [113, 114]. While dietary recommendations for EPA and DHA range from 250 to 1000 mg/day for adults, the actual consumption is around 163 mg/day [115]. Further, a significant portion of the population consumes less than 100 mg/day [116]. For a global population of around seven billion in 2019, meeting a daily dosage of 500 mg per person would reveal a DHA deficit of 0.4–1.0 million tons/year. A detailed overview of the widening discrepancy between DHA availability and the quantities required to meet optimal dietary intake has been discussed by Tocher et al. [4]. Addressing the challenge of filling the gap between DHA supply and demand has become an immediate and future imperative. As the global population grows and ages, there is mounting requirements for EPA/DHA to combat escalating chronic diseases.

Direct incorporation of microalgal-derived EPA/DHA into human foods and supplements circumvents the reliance on fish farming with the relevant limitations. Moreover, microalgae offer the advantage of remediating water pollution during intensified aquaculture through wastewater recycling, establishing ecological equilibrium and circular economy where microalgae support fish growth while mitigating pollutants [117].

In strain selection, only a few members out of the broad microalgal community has been qualified in terms of food-grade lipids and fatty acids [118]. Certain species have become favorite experimental organisms and most of the studies have been performed on them, leaving the local isolates unexplored. The studies should include a broad range of microalgal strains other than the model microalgae. By persistent isolation, screening and investigating the indigenous isolates from the immediate environment, a wide array of microalgal origins with exploitable potential will be available [16].

Modern techniques such as riboswitches, RNAi technology for posttranscriptional downregulation of specific genes, and coupling nuclear promoters for gene regulation may have a great contribution to the field of genetic engineering [119]. Genetically modified microalgae generated by genetic engineering technique would aid in exploring the underlying biology and achieving valuable biomolecules including DHA. The transgenes associated with the overproduction of with specific post-translation modifications should be activated within microalgal chloroplasts which are free of unwanted glycosylation [120].

11. Conclusion

In the realm of enhancing the global health, microalgae exhibit the potential to become a primary source of DHA, surpassing traditional methods. The remarkable ability of microalgae to synthesize DHA, coupled with advancements in cultivation techniques, metabolic engineering, and downstream processing, offers a sustainable and innovative solution to address the increasing DHA demand. The pivotal role of DHA in human health underscores the urgency to bridge the gap between DHA supply and the recommended dietary intake. With the global population expanding and chronic diseases on the rise, the demand for DHA is growing. Microalgae offer a versatile platform to fulfill this demand, not only by producing DHA but also by promoting sustainable practices through nutrient recycling and wastewater bioremediation. Furthermore, the versatility of microalgae as biorefineries presents a multifaceted advantage. Microalgae yield not only DHA but also a spectrum of other valuable compounds, including proteins, carotenoids, and biofuels.

However, challenges remain, including scaling up production, cost-effectiveness, and integrating microalgal-based DHA into mainstream markets. Rigorous research is essential to optimize growth conditions, genetic modifications, and cultivation strategies to achieve consistent and economically viable DHA production.

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Microalgae as a Promising Candidate for Mitigating Climate Change and Biodiversity Loss

Şuheda Reisoglu and Sevcan Aydin

Abstract

The modern world is confronted with the pressing challenges of reducing carbon dioxide (CO₂) emissions as the leading promoter of climate change while also addressing the energy and food crisis. Microalgae, being photosynthetic organisms, possess the unique ability to utilize solar energy for the conversion of CO₂ into organic matter. Their ability to remove CO₂ from the atmosphere proves advantageous in mitigating climate change. Algae have demonstrated effectiveness in wastewater treatment (WWT) by removing pollutants. In agriculture, microalgae offer a green alternative to chemical fertilizers, benefiting crop growth. The ease and cost-effectiveness of sampling and culturing microalgae contribute to their popularity. This chapter examines the sustainable applications of microalgae, including WWT, bioproduct recovery, bioremediation, energy production, agriculture, and biodiversity conservation. The examination of the growing global microalgae market, particularly in relation to biotechnological applications, was also reviewed as an indicator of the immense potential offered by microalgae.

Keywords: climate change, biodiversity loss, microalgae, sustainability, ecosystem services

1. Introduction

Climate change is characterized by persistent modifications in Earth's climate patterns, primarily attributed to anthropogenic factors such as the combustion of fossil fuels, deforestation, and industrial activities. The consequential rise in global temperatures and atmospheric carbon dioxide (CO₂) levels has been unequivocally linked to far-reaching alterations in hydrological cycles, including precipitation and evaporation dynamics. Furthermore, climate change has been observed to engender an escalation in the magnitude and frequency of extreme weather phenomena [1]. These disturbances hold profound implications for biodiversity, exerting a significant negative impact on ecosystems worldwide. Consequently, climate change has emerged as a significant driver of biodiversity loss, exerting substantial impacts on ecosystems at a global scale [2]. One of the primary consequences of climate change is the loss of habitats, predominantly attributed to alterations in temperature and precipitation patterns. The changing climate disrupts ecosystems, transforming previously suitable

habitats into unsuitable environments for numerous species. This loss of habitat contributes significantly to the ongoing decline in biodiversity.

Biodiversity encompasses the diversity of genes, species, and ecosystems found within a given area or across the planet. It includes the variation in genetic makeup within species, which provides the basis for adaptation and evolutionary processes. Species diversity, on the other hand, refers to the number and abundance of different species in a specific area, reflecting the richness and composition of biological communities. The impact of climate change on biodiversity is of great scientific concern, given the critical role biodiversity plays in supporting ecosystem stability, resilience, and functionality. In a study by Thomas et al. [3], a concerning outlook is presented, suggesting that climate change has the potential to drive the extinction of over a million terrestrial species within the next 50 years. This stark projection highlights the urgent need to address climate change as a primary driver of biodiversity loss. Implementing effective mitigation measures and adaptation strategies becomes imperative to safeguard biodiversity and ensure the long-term viability of ecosystems.

Microalgae, as photosynthetic unicellular microorganisms, play a crucial role in enhancing the productivity, biodiversity, and functioning of aquatic ecosystems. Particularly, phytoplankton, a subgroup of microalgae adapted to living in suspension in water, account for a substantial portion of the global net primary production in marine and freshwater environments, contributing approximately 49% [4]. The taxonomic diversity of microalgae is extensive, encompassing species that exhibit significant evolutionary divergence. Despite their small size, ranging from 0.2 to 200 μm , microalgae have a remarkable capacity to contribute to climate change mitigation through their ability to fix CO_2 [5]. This process of carbon fixation by microalgae holds great potential for sequestering CO_2 from the atmosphere, offering possibilities for sustainable utilization such as bioenergy production and the development of value-added products [6]. The significance of microalgae extends beyond their CO_2 -removing potential in combating climate change, as they possess remarkable capabilities to address various environmental challenges. Their capacity for biomonitoring enables effective assessment of water pollution levels, facilitating remediation efforts through bioremediation processes. Microalgae in wastewater treatment has garnered significant attention as a promising strategy with several notable benefits. This approach is characterized by its low energy demand, making it an energy-efficient option for treating wastewater. Microalgae exhibit remarkable adaptability and can thrive under various environmental conditions, allowing for effective wastewater treatment. Additionally, microalgae offer the potential to recover and transform nutrients present in wastewater into highly valuable bioactive compounds. This integrated approach not only addresses wastewater management but also presents an opportunity for resource recovery and the production of valuable products. The utilization of microalgae in wastewater treatment holds great promise in advancing sustainable practices and contributing to the circular economy [7]. Furthermore, microalgae contribute to the production of eco-friendly energy sources, offering a renewable and sustainable alternative to fossil fuels. Industries in the food and pharmaceutical sectors capitalize on the diverse biochemical composition of microalgae, utilizing them to produce various value-added products. Moreover, microalgae play a crucial role in promoting sustainable agriculture through various mechanisms. They enhance the growth of beneficial bacteria in the soil, contributing to a healthier soil microbiome. Microalgae help improve soil organic carbon content, which enhances soil fertility and nutrient availability for plants. They also have the ability to fix atmospheric nitrogen, making it accessible to plants and reducing the

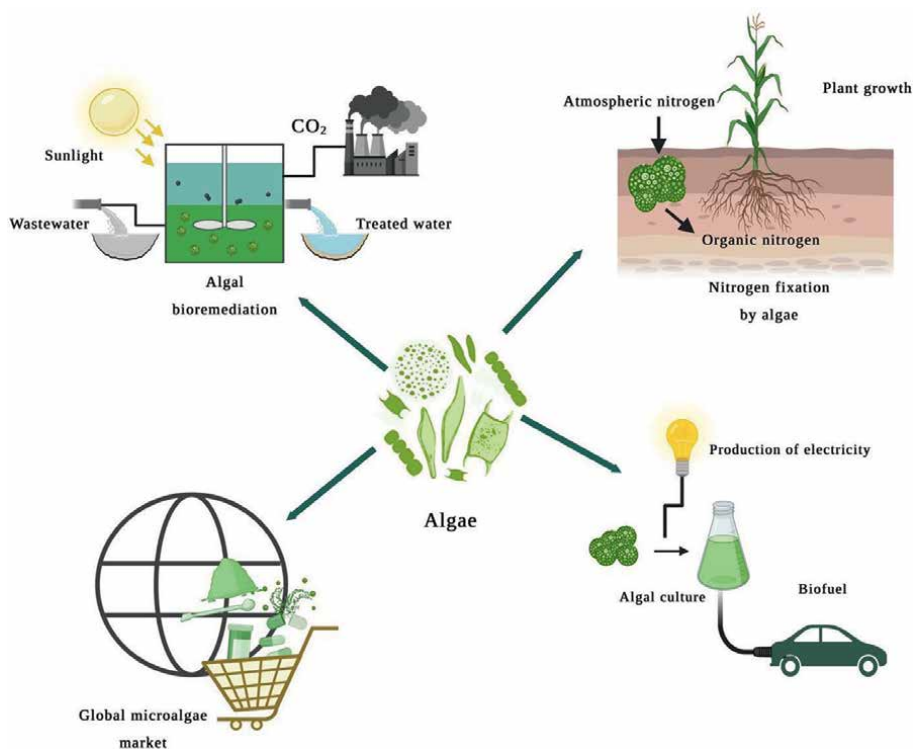


Figure 1.
Algal applications for sustainability.

need for synthetic nitrogen fertilizers. Furthermore, they produce growth hormones that stimulate plant growth and development. Overall, the utilization of microalgae in agriculture holds significant potential for enhancing productivity, sustainability, and resilience in food production systems. Microalgae possess multifaceted attributes that are significant in addressing climate crises, including biodiversity loss, while promoting sustainable practices across different sectors [8].

This chapter aims to highlight the importance of microalgae in addressing climate change and biodiversity loss issues by uncovering the vast potential in promoting environmental sustainability. Through this comprehensive review, their potential in sectors like bioremediation and bioenergy, wastewater treatment, green agriculture for sustainable Earth, and value-added products in mitigating the biodiversity crisis will be highlighted. The chapter also examines the increasing global microalgae market. **Figure 1** illustrates various applications of algae for achieving environmental sustainability against climate change and biodiversity loss.

2. Roles of microalgae-based systems for bioremediation and biofuel production

Energy production is currently confronted with a significant crisis due to a growing global population and the escalating demand for energy. The widespread use of fossil fuels has resulted in elevated levels of CO₂ and other greenhouse gases in the atmosphere. Consequently, there is an urgent requirement for renewable and

environmentally friendly fuel to satisfy the world's energy needs. Algae can play a crucial role in meeting this demand by serving as a primary source of biofuel, owing to their advantageous qualities such as safety, non-competitiveness, and rapid growth [9]. Algal cells are known to contain a diverse array of valuable components, including fatty acids, carbohydrates, lipids, and proteins. Microalgae exhibit a remarkable capacity to accumulate lipids, with their oil content typically ranging from 20% to 50%. However, certain species have demonstrated an exceptional ability to accumulate lipids, reaching levels as high as 80%. These oil-rich algal species are considered highly promising to produce biofuels, as they offer a renewable alternative to conventional fossil fuels such as diesel, kerosene, and gasoline.

The utilization of algal biofuels brings forth several noteworthy benefits. Firstly, algal biofuels are environmentally advantageous due to their carbon neutrality. Their combustion releases an amount of CO₂ equivalent to the CO₂ they initially absorbed during growth, thus minimizing the net contribution of greenhouse gases to the atmosphere. This characteristic makes algal biofuels an attractive option for mitigating climate change and reducing overall carbon emissions. Moreover, algal biofuels offer considerable potential for economic sustainability. As nonfood feedstocks, microalgae cultivation for biofuel production avoids competition with food crops, thus alleviating concerns regarding food security. Additionally, the high oil content of certain algal species enables efficient extraction and conversion processes, resulting in improved energy output and cost-effectiveness [10]. Extensive research has been conducted on algal species known for their high oil content, particularly in relation to biofuel production. Among these species, several have gained recognition for their remarkable oil accumulation capabilities. One such species is *Dunaliella tertiolecta*, which boasts an impressive oil content ranging from 60% to 67%. Another noteworthy species is *Botryococcus braunii*, known for its substantial oil content of approximately 43.06%. *Chlorococcus spp.* has been identified as possessing an oil content of about 38%. *Scenedesmus obliquus* is also worthy of mention, as it demonstrates oil content within the range of 35% to 55%. In addition, *Arthrospira platensis*, a species widely recognized for its valuable properties, exhibits an oil content of approximately 35.31% [11, 12].

The production of biodiesel from microalgae involves a two-step process. The first step involves the extraction of lipids from microalgal cells, while the second step entails the transesterification of the extracted lipids using alcohol and a catalyst [13]. In addition to biodiesel production, microalgae have demonstrated their potential in the generation of other biofuels. Through photobiological processes, microalgae can be employed for the production of bio-hydrogen. Anaerobic digestion of microalgae can yield bio-methane, and fermentation processes can be utilized to produce bioethanol (**Figure 2**). These multifaceted applications of microalgae indicate their versatility as a sustainable resource for the production of different types of biofuels. With the strategic utilization of microalgae's inherent capabilities, the development of environmentally friendly and renewable energy solutions becomes feasible [8].

Rahman et al. [14] conducted a study where a two-step transesterification process was employed to produce biodiesel from the microalgae species *Spirulina maxima*. The resulting biodiesel was subjected to analysis based on ASTM standards, and it was observed that the produced biodiesel met the requirements specified for biodiesel quality. Another study by Harun et al. [15] focused on assessing the potential of microalgae, specifically *Chlorococcus spp.*, as a viable substrate for bioethanol production using yeast, namely *Saccharomyces bayanus*. The researchers aimed to extract lipids from *Chlorococcus spp.* and subsequently ferment them to produce bioethanol.

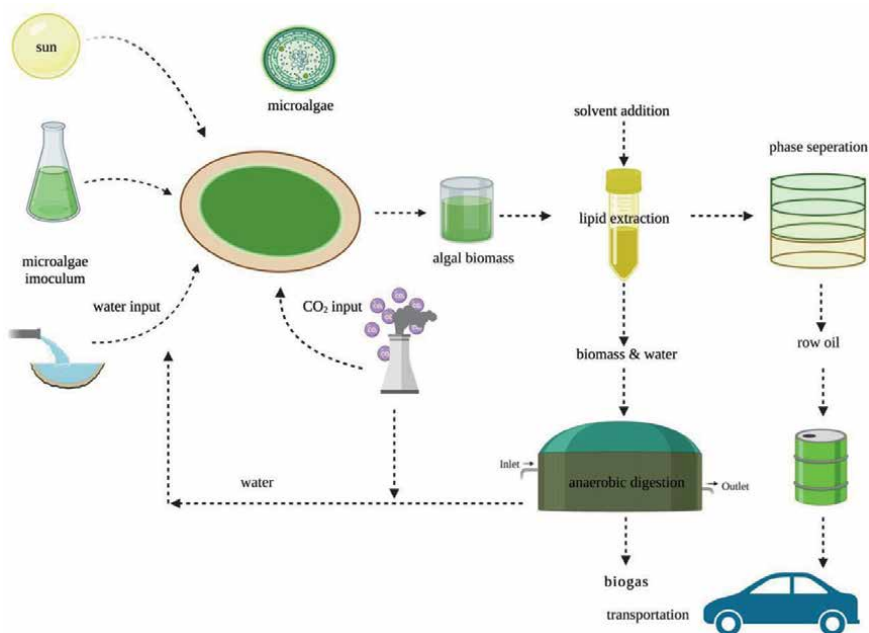


Figure 2.
 Biofuel production from microalgae.

Lipids were extracted from *Chlorococcus spp.*, and the resulting lipid-extracted microalgae debris was used as a feedstock for bioethanol production. The researchers observed a maximum ethanol concentration of 3.83 g L^{-1} , which was obtained from a concentration of 10 g L^{-1} of the lipid-extracted microalgae debris. This corresponds to a productivity level of 38% w/w. These findings suggest that microalgae, specifically *Chlorococcus spp.*, have the potential to serve as a valuable feedstock for bioethanol production.

Anaerobic digestion (AD) serves as a pivotal step in the generation of biogas and is a naturally occurring biological process with a long history. Organic components undergo biodegradation into biogas through the cooperative actions of diverse bacterial and archaeal species in oxygen-free conditions during AD [16]. Utilizing microalgae in AD has proven to be an efficient, practical, and cost-effective method for biogas production. While lignocellulosic wastes have traditionally been widely used in AD, their slow biodegradability can be attributed to the complex structure and nature of the biomass. Microalgae, in contrast, contain no lignin, leading to higher biodegradability compared to lignocellulosic biomass. Consequently, estimated methane yields from various common microalgae biomass are significantly higher than those from different wastes such as corn residues, wheat and rice straws, and sugarcane. In contrast to contemporary microalgae-based biodiesel production, which primarily utilizes high lipid-containing species, anaerobic digestion (AD) allows for the utilization of any microalgal species or residual biomass, regardless of their lipid content. This flexibility enables the inclusion of a wider range of microalgae in the AD process, either individually or through co-digestion. It has been observed that the generation of methane from microalgae through AD is more energy-intensive compared to microalgae-based biodiesel production. However, the advantage of AD lies in its ability to extract energy from microalgae biomass, regardless of its lipid content.

Furthermore, the integration of biodiesel and biogas production from the same biomass can be achieved by utilizing lipid-extracted residues. These residues, obtained from biodiesel facilities, contain significant amounts of proteins and carbohydrates that can be biodegraded into biogas during the AD process. This integrated concept offers the potential for maximizing the energy yield and resource utilization from microalgae biomass [17].

Industrial and mining activities have led to the release of hazardous pollutants, with heavy metals being a significant concern that poses both environmental and health risks. The remediation of wastewater contaminated with heavy metals typically relies on conventional methods such as chemical precipitation and ion exchange [18]. However, these methods are often expensive and inefficient, particularly when it comes to meeting regulatory limits for heavy metal concentrations. In contrast, the utilization of microalgae for the treatment of heavy metal-loaded wastewater offers several advantages. Microalgae-based bioremediation exhibits potential applications across a wide range of environmental conditions, including varying pH levels, temperatures, the presence of other ions, and microalgal tolerance to different types of wastewater and heavy metals. This versatility makes microalgae a promising option for the bioremediation of heavy metal-contaminated wastewater. In addition to the capacity of being a natural feedstock particularly to produce biodiesel, microalgal biomass holds promise for the development of added-value bioproducts, including pigments, carbohydrates, and proteins [19].

Several studies have focused on the dual purpose of microalgal cultivation, aiming to simultaneously achieve wastewater treatment and biodiesel production. This approach capitalizes on the ability of microalgae to remove heavy metals from wastewater while also harnessing their lipid content for the production of biodiesel. By integrating these two objectives, researchers aim to create a sustainable and economically viable process that addresses both environmental concerns and the growing demand for renewable energy sources.

In a study conducted by Arora et al. [20], a synergistic approach combining bioremediation of arsenic and efficient biodiesel production was investigated. The selected microalgal strains, particularly *Chlorella minutissima* and *Scenedesmus spp.*, demonstrated effective removal of arsenic species (III, V) from synthetic soft water, regardless of their speciation. Also, these microalgal species exhibited a significant increment in lipid production by accumulating large lipid droplets. The biodiesel obtained from the microalgae showed favorable characteristics and comparability to plant oil methyl esters, indicating its suitability for its usage as a fuel. The results suggest that this approach offers dual benefits, including the effective removal of carcinogenic metal arsenic from potable water sources and a high yield of lipid production.

3. Roles of microalgae-based wastewater treatment and bioproducts recovery

Water is a fundamental and indispensable resource utilized as a primary raw material in a wide range of industries, encompassing pharmaceuticals, agriculture, health, beverage, agrochemicals, and oil and gas sectors, as well as for domestic usage. The release of contaminated water directly from these applications poses significant environmental risks and has become a growing concern due to the presence of diverse contaminants. Wastewater contains a variety of compounds that exist at concentrations that can be toxic to organisms. Additionally, important quantities of inorganic

and organic nutrients are discharged into the surroundings, leading to elevated levels of chemical oxygen demand (COD) and biological oxygen demand (BOD). The excessive introduction of phosphorus (P) and nitrogen (N) into aquatic environments results in eutrophication, which engenders environmental issues like the generation of solid wastes and the emission of unpleasant substances into the atmosphere [21]. Furthermore, the proliferation of undesirable microorganisms facilitated by eutrophication poses a threat to aquatic organisms and compromises the potability of drinking water, thereby contributing to prevalent health concerns in areas adjacent to the discharge zone. Direct exposure to these toxic substances through inhalation, ingestion, or physical contact, even at minimal concentrations, may lead to substantial risks to human health and heighten the probability of cancer development [22].

In situations where water becomes contaminated and purification becomes imperative, it is crucial to select an appropriate treatment strategy to reach the purification aims. Conventional approaches commonly employed include various physical and chemical applications. Among these, chemical treatment stands out as one of the most effective wastewater treatment (WWT) approaches, as it aims to modify water characteristics by reducing turbidity, adjusting pH, and removing dissolved substances, thereby enhancing water quality. Although prominent techniques in chemical treatment such as chlorination, chloramination, UV light, and ozonation exhibit higher efficacy when utilized in secondary WWT where the objective is to decompose organic matter through microbial processes, their significant drawbacks are associated with high costs, limitations in dewatering, and extensive maintenance requirements [23]. In contrast, biological treatments depend on the metabolic processes of microbial to enzymatically degrade and transform wastewater pollutants into biomass and relevant gases, including CO₂, methane (CH₄), nitrogen (N₂), and sulfur dioxide (SO₂). This enzymatic degradation leads to a reduction in the levels of BOD in addition to a decrease in COD levels within the wastewater effluents, ultimately resulting in an enhancement of their quality. Biological wastewater treatment processes encompass the utilization of diverse microorganisms, such as bacteria, fungi, yeast, and microalgae, to facilitate the process of biodegradation. The primary objective of biological treatments is to establish a system effectively that is able to facilitate the treatment of discharged wastewater based on its inherent potential for decomposition [24].

The utilization of microalgae in biological WWT has emerged as a highly promising technology for the advanced treatment and recovery of nutrients from wastewater. This approach has garnered increasing attention in recent years since it presents a reliable solution for addressing liquid or solid waste generated by traditional methods while simultaneously transforming these wastes into valuable products with added benefits. Microalgae, which are oxygen-evolving photosynthetic microorganisms similar to plants, are widely present in various water environments, including fresh and marine water, as well as different types of wastewater. Extensive research has demonstrated the capacity of microalgae to effectively harness nutrients and thrive in wastewaters. This ability stems from their substantial requirement for nitrogen and phosphorous, alongside solar energy and either CO₂ or organic substances as carbon sources. These essential elements are crucial for microalgae to synthesize vital components such as proteins, nucleic acids, and phospholipids while concurrently decreasing the concentration of these substances within the water [25].

The original purpose of employing microalgae in WWT was to address excessive nutrient levels in secondary effluent and mitigate the risk of eutrophication in natural water bodies. However, it is worth noting that the biomass production of microalgae

during wastewater treatment holds considerable additional value. The harvested algal biomass can be utilized as a valuable feedstock in biorefinery processes or other applications, offering potential economic and environmental benefits [26]. Also, the symbiotic relationship between microalgae and bacteria is characterized by the utilization of resources and the exchange of byproducts. Microalgae engage in photosynthesis, utilizing CO_2 to produce O_2 . Heterotrophic bacteria benefit from the generated oxygen, which they utilize to assimilate and degrade organic carbon, nitrogen, and phosphorus compounds. In turn, the aerobic metabolism of bacteria releases CO_2 , inorganic nitrogen, and phosphorus, which serve as nutrients for microalgae, facilitating their further photosynthetic activity. Microalgae-bacteria consortia not only effectively eliminate contaminants and nutrients but also generate valuable biomass that can be utilized in various applications. An illustrative instance involves the utilization of microalgae cultivated using cattle dairy wastewater, which has proven beneficial for fertilizing pasture. This application has resulted in elevated levels of essential minerals such as phosphorus, calcium, magnesium, and manganese, along with an increased content of dry matter [27].

Recent studies have revealed that the presence of algae can efficaciously help in the removal of nutrients from wastewater. For instance, Cai et al. [28] reported that the utilization of algae granules in synthetic wastewater exhibited a high level of efficiency in eliminating phosphorus, as well as recovering and reusing it from the resulting phosphorus-rich algae biomass. Moreover, aside from their effectiveness in capturing CO_2 and removing nutrients from wastewater, microalgae have demonstrated potential as a viable source for energy generation. Furthermore, microalgae offer a promising approach for the removal and inactivation of pathogenic microorganisms due to the various mechanisms such as pH elevation, the production of microalgal toxins, and nutrient competition they employ. The process of photosynthesis, essential for microalgal growth, leads to an increase in pH and the enrichment of oxygen concentration in wastewater. As a result, the alterations in pH and dissolved oxygen levels can efficiently hinder the growth of pathogens in wastewater. In a study conducted by Mezzari et al. [29], it was found that the suppression of antibiotic-resistant *Salmonella typhimurium* was observed within a 48-hour period due to the favorable pH and dissolved oxygen levels created by the photosynthetic activity of microalgae. Another potential of the microalgae-derived treatment in wastewater was investigated by Aydin et al. [30], and they focused on the bioaugmentation of *Haematococcus pluvialis*, a freshwater species of Chlorophyta known for its exceptional potential to produce color pigment, astaxanthin, in an aerobic membrane bioreactor (MBR). The objective of this study was to investigate the capability of *H. pluvialis* in the removal of antibiotics in wastewater, alleviating biofouling and evaluating its influence on the structure of the microbial community. The experiment involved the setup of three reactors: two control MBRs, one with antibiotics and one without, and an MBR augmented with *H. pluvialis*. The results demonstrated that the augmented reactor exhibited a 33% delay in membrane biofouling and a 6% increase in COD removal compared to the control reactors. Furthermore, the bioaugmented reactor exhibited the highest removal of antibiotics, ranging from 69.75% (C2) to 89.73% (HP). Additionally, the study revealed that the presence of *H. pluvialis* had a significant impact on the indigenous microbial communities and the biofilm within the MBR. The biodegradation network was altered, leading to a modification in the relative abundance of microbial taxa. Specifically, there was an increase in the relative abundance of *Proteobacteria*, while the presence of *Firmicutes* and *Bacteroidetes* was noticeably reduced.

Microalgae have demonstrated significant potential in the production of various bioproducts, offering a diverse range of applications including biofuels, chemicals, food, feed, and pigments. The advantages of utilizing microalgae lie in their high productivity, the ability to cultivate them on marginal land using fresh or saltwater, which minimizes competition with food crops, and the potential to integrate biomass growth with the treatment of waste streams [31]. However, the extraction of these bioproducts can pose difficulties due to factors such as their solubility, small molecular weight, or low concentrations. In this regard, membrane filtration provides a flexible solution, enabling the separation of bioproducts from the growth media. Additionally, membrane filtration can assist in deliberate cell disruption, facilitating the release of intracellular products. Moreover, this technique can be utilized to harvest gases, such as oxygen, during the process. A study was conducted to explore the potential of thin stillage digestate as a growth medium for microalgae, with the objectives of investigating its effectiveness in removing nitrogen, phosphorus, and organic carbon and evaluating the potential of the resulting biomass for the production of biofuels and bioproducts. Thin stillage digestate was subjected to a pre-treatment process involving struvite recovery, which is a value-added product commonly used as a fertilizer. The treated digestate was then utilized as a growth medium for cultivating *Chlorella sorokiniana* microalgae. The resulting microalgal biomass was analyzed and found to contain significant amounts of potential bioproducts, including protein, starch, and lipid. These bioproducts have the potential to be integrated into existing corn ethanol plants, reducing the need for corn feedstock while increasing the protein content of dried distiller's grain and improving corn oil yield. Additionally, the study investigated the microbial community present in the photobioreactor and discovered the potential for ammonium nitrification, which could be utilized to mitigate the inhibitory effects of high ammonium concentrations on microalgae growth. Overall, this biorefinery concept holds promise for the development of an eco-friendly and economically sustainable bioethanol industry [32].

Another valuable material produced by microalgae as a bioproduct is color pigments. The market demand for natural pigments derived from microalgae has significantly increased due to their remarkable coloring properties. These natural pigments are sought after as renewable and sustainable color enhancers for both food and feed applications. Additionally, they offer certain health benefits, making them highly desirable in the market. The increasing demand for natural pigments poses challenges due to the significant water and nutrient requirements in large-scale microalgae production, leading to high costs and hindering commercialization. To address these challenges, researchers have recently investigated the usage of wastewater for microalgae cultivation as a potential solution for natural pigment production. Furthermore, the potential application of anaerobic digestion at the end of the process to recover bioenergy from the residual biomass holds promise from both environmental and economic perspectives. In this regard, Jiang et al. [33] conducted a study to investigate the capacity of a diatom species, *Phaeodactylum tricornutum*, in the removal of nutrients present in swine wastewater while simultaneously producing the fucoxanthin pigment, which has the potential to exhibit anticancer, anti-inflammatory, and anti-obesity properties. *P. tricornutum* exhibited the highest capability for nutrient removal from swine wastewater, particularly in terms of total phosphorus, nitrogen, and ammonia nitrogen. The study also confirmed the presence of high levels of fucoxanthin, consistent with previous reports. These findings highlight the significant potential of *P. tricornutum* for swine wastewater treatment coupled with the production of fucoxanthin.

In the realm of the bioeconomy, the adoption of innovative processes plays a crucial role in the production of bioproducts while simultaneously reducing the consumption of virgin resources. Microalgae-based WWT emerges as a significant contribution to this practice. This approach has attracted attention due to its minimal energy requirements, the adaptable growth characteristics of microalgae in various environmental conditions, and the potential to transform wastewater nutrients into bioactive compounds of significant value. These factors highlight the promising potential of microalgae-based wastewater treatment as a sustainable and resource-efficient solution in the bioeconomy.

4. Roles of microalgae-derived biofertilizer in the sustainability of green agriculture

In recent years, the rapid growth of the global population, along with the impacts of climate change, has placed significant strain on natural sources and placed a considerable burden on environmental conservation and agricultural sustainability. One of the critical areas of concern is ensuring an adequate food supply for the expanding population. The huge demand for potential food has placed substantial pressure on soil resources, leading to soil degradation. This degradation entails the loss of biodiversity and ecosystem services, and it is further exacerbated by the impacts of climate change, including altered precipitation patterns, increased frequency of extreme weather events, and shifts in soil temperature regimes. Soil degradation encompasses a range of adverse changes, such as reduced fertility, diminished organic matter content, and impaired water-holding capacity [34]. To address this challenge, the harnessing of fertilizers in agriculture plays a crucial role in ensuring an adequate food supply for the increasing global population. Fertilizers help bridge the nutrient gap in the soil, replenishing the necessary elements that are depleted through intensive farming practices. By supplementing the soil with nutrients, fertilizers contribute to increased crop yields and improved agricultural productivity. This, in turn, helps meet the rising food demands and ensures food security for the growing population [35].

Chemical fertilizers, produced through industrial means and offered in diverse commercial variations, frequently include inorganic micronutrients. Their primary purpose is to enhance crop yields and improve soil fertility. Nevertheless, the continuous application of fertilizers that are in inorganic form has been associated with negative outcomes. These include a decline in soil organic matter, increased soil acidity, degradation of soil physical and structural properties, contamination of groundwater and surface water, as well as potential carcinogenic effects for humans. The prolonged utilization of inorganic fertilizers has detrimental effects on the ecosystem, poses challenges to food security, and restricts the potential for land reuse in agricultural production [35]. Furthermore, the utilization of chemical fertilizer contributes to the release of various GHGs, with CO₂ being the primary contributor. These emissions pose significant global concerns, not only impacting human health but also disrupting ecosystems. Considering these concerns, organic farming has gained considerable momentum as a rapidly growing agricultural technique on a global scale. This approach places a strong emphasis on utilizing biofertilizers to improve soil fertility while simultaneously avoiding the utilization of agrochemicals, genetically modified organisms, and synthetic substances commonly found as additives in food. This shift toward bio-based fertilizers is motivated by multiple factors, including the increasing

demand for organic food in markets. To meet these market demands and produce organic food, farmers are increasingly adopting and implementing the use of bio-based fertilizers. This approach not only enhances soil fertility but also aligns with the principles of sustainable agriculture and environmental conservation [36].

Biofertilizers are intricately formulated products that leverage the symbiotic relationship between microorganisms and plants, or incorporate natural compounds from bacteria, fungi, and other organisms. They play a pivotal role in augmenting both the chemical and biological properties of the soil, thereby stimulating robust plant development and restoring soil fertility [37]. Extensive research has consistently highlighted the minimal impact of biofertilizers on human health and their ability to circumvent environmental pollution concerns. Notably, among the diverse array of biofertilizers, formulations rooted in photosynthetic organisms, particularly prokaryotic cyanobacteria and microalgae, have garnered significant recognition. These innovative formulations showcase remarkable aptitude in bolstering the bioavailability of nutrients, enriching soil fertility, and amplifying crop yields. A notable advantage lies in the biomass of photosynthetic organisms, which boasts a rich composition of proteins, carbohydrates, and vitamins, rendering them ideal for the development of eco-friendly fertilizers [38].

Microalgae represent a unique and diverse group of microorganisms, comprising both cyanobacteria (prokaryotic organisms) and eukaryotic organisms. They offer a multitude of advantages for addressing the complex agricultural landscape. These microscopic organisms have the potential to improve soil fertility, enhance plant growth, and reduce reliance on chemical fertilizers and pesticides. Using microalgal biomass as biofertilizer plays a beneficial role in soil nutrient cycling, facilitating the availability of essential nutrients for plant uptake (**Figure 3**). Upon direct contact of microalgal biomass with the soil, specific factors such as moisture, pH, and light percentages activate viable microalgae cells and sustain their metabolic activity. This process facilitates the capture of atmospheric N_2 and supplies vital macronutrients

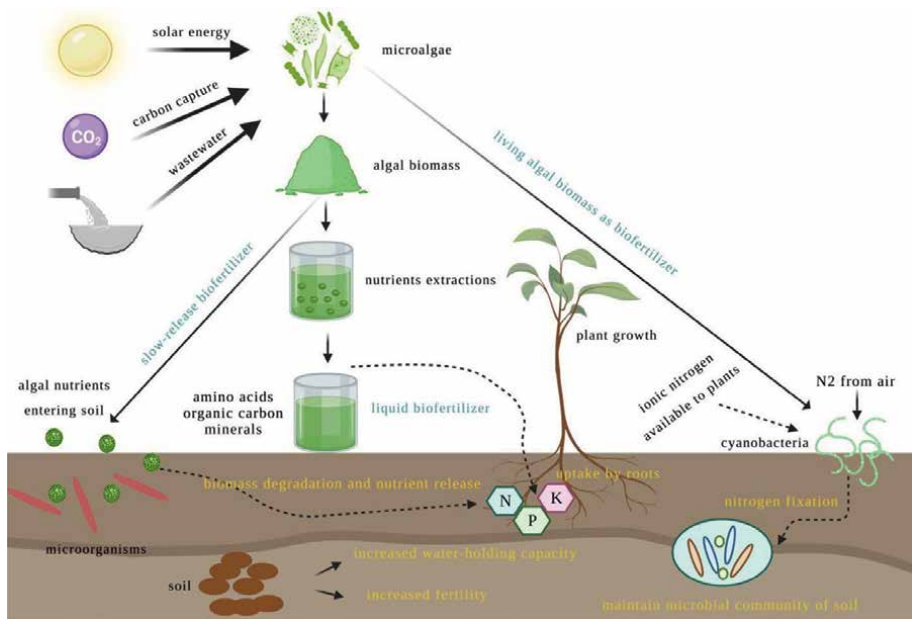


Figure 3.
Microalgae-derived biofertilizers on soil and crop improvement.

and micronutrients that are essential for plant development. Microalgae also produce bioactive compounds such as phytohormones that positively influence plant growth and protect against phytopathogens and pests. Another noteworthy benefit is their ability to capture CO₂ through photosynthesis, making them valuable for carbon sequestration. Furthermore, certain species of microalgae produce exopolysaccharides (EPS) that improve soil structure, enhancing water retention and aeration [39].

Additionally, microalgae can establish an endosymbiotic relationship with plant roots and other soil microorganisms. Extensive research on the symbiotic relationship between microalgae and plants was demonstrated in a study using a hydroponic system with *Chlorella vulgaris* and *Mentha spicata*. It was observed that the co-cultivation of microalgae and plants, coupled with aeration, had the most significant influence on the increment in plant weight. This effect was primarily attributed to the improvement of novel shoot and leaf [40].

Moreover, measurements of photosynthetic pigments indicated no growth-related stress on the plant. Microalgae also form endosymbiotic relationships with bacteria, creating a mutually beneficial association that enables their survival and proliferation in various ecosystems. This symbiotic consortium presents opportunities for applications in WWT, reclamation of nutrient, production of feedstock, generation of biofuel, and biofertilizer development. Several studies have corroborated the efficacy of microalgae and other microorganisms, even in challenging environments such as deserts, in enhancing soil fertility. These beneficial microorganisms contribute to improved soil properties, including increased water retention; enhanced stability; pollutant removal; and the provision of a favorable substrate for plant growth [41].

A straightforward method for utilizing microalgae as a fertilizer involves directly applying the microalgal extract to the soil. However, to make this practice feasible and applicable, it is essential to optimize the energy requirements associated with it. Another alternative is the foliar spray method, where cellular extracts of microalgae are applied to the foliage of plants. This technique has also shown promising results, yielding profitable outcomes. Dineshkumar et al. [42] successfully implemented *Spirulina platensis* and *Chlorella vulgaris* as biofertilizers using the soil drench treatment technique, resulting in a significant enhancement of rice yield by 20.9%. Additionally, microalgal biofertilizers exhibited favorable effects on various parameters related to vegetative growth, including plant height, leaf area, fresh weight, and dry weight, thus demonstrating their positive impact on crop performance. While further research is necessary to establish the widespread application of the foliar spray method, this technique is increasingly preferred due to its efficient nutrient delivery to plants, despite the influence of various critical factors such as temperature, light intensity, nutrient concentration, humidity, and application rate. Garcia-Gonzalez and Sommerfeld [43] have reported on the utilization of *Acutodesmus dimorphus* microalgal extract usage as a foliar spray application, which has shown promising results in enhancing the growth rate of tomato plants. Following the treatment with *A. dimorphus* extract, notable improvements were observed, including increased plant height and a greater number of flowers and branches per plant.

Mahmoud et al. [44] conducted a study to investigate the impact of two different microalgal strains, *Chlorella vulgaris* and *Anabaena cylindrica*, in the development of *Spinacia oleracea* (spinach plant) and their potential in reducing heavy metal accumulation in the plant. Microalgae suspensions, prepared from both strains at 1% and 2% of dry weight, were applied either to the soil or as a foliar spray on plant seeds sown in soils contaminated with heavy metals. The results demonstrated that the application of microalgae significantly stimulated the growth of the spinach plant, with growth

enhancement ranging from 21% to 29% compared to the control group. The most effective application was obtained with *C. vulgaris* at a concentration of 2% and when applied to the soil. Furthermore, microalgae supplementation increased the uptake of macronutrients, such as nitrogen (N), phosphorus (P), and potassium (K), by the plant. Interestingly, the application of *A. cylindrica* at a concentration of 1% and as a foliar spray showed better performance in terms of both fresh matter and dry matter yield of the plant, as well as superior macronutrient uptake compared to *C. vulgaris*. Also, regardless of the application method or microalgae strain, the presence of microalgae resulted in a reduction in the accumulation of heavy metals, specifically cadmium, lead, and copper, in the plant. This highlights the ability of these microorganisms to accumulate and prevent the uptake of these contaminants from the soil into the plant. Furthermore, Renuka et al. [45] have elucidated the prospective utilization of microalgae cultivated in wastewater as biofertilizer for enhancing the development rate of wheat plant. The study reports that microalgal strains such as *Scenedesmus*, *Chlorococcum*, and *Phormidium* cultivated in wastewater have shown a significant improvement in wheat growth, although not to the same extent as commercial microalgal cultures.

Microalgae offer a valuable source of organic carbon, addressing the issue of declining soil quality and soil fertility in croplands [46]. Via photosynthesis, microalgae generate extracellular polymeric substances (EPS), thereby enhancing the availability of organic carbon present in the soil. A pot experiment investigating the impact of microalgae biofertilizer reported a significant increase in soil organic carbon, which can be attributed to the photosynthetic activities of the microalgal species involved [47]. Furthermore, the assimilation of CO₂ by microalgae can significantly increase soil organic carbon content. Research findings indicate that the utilization of biofertilizers results in a substantial rise of approximately 71.2% in soil organic carbon through the process of carbon sequestration [48]. Additionally, certain microalgae strains have the capacity to fix nitrogen, apart from their role in CO₂ fixation. In cyanobacteria, for instance, while vegetative cells are responsible for CO₂ fixation, specialized heterocystous cells perform nitrogen fixation, converting atmospheric nitrogen into ammonia [49].

One notable benefit of the application of microalgal biofertilizers is the enhancement of physical properties such as soil structure and water retention. Carillo et al. [50] observed that the utilization of microalgal biofertilizer led to augmented agglomeration of soil particles and increased water-holding capacity. Studies concerning cyanobacteria have highlighted the positive impact of biofertilizer application on both soil structure maintenance and fertility enhancement and subsequent improvements in crop yields [39].

Moreover, microalgal biofertilizers are able to contribute to the proliferation of bacterial community in soil. This, in turn, can lead to an overall enhancement of the microbial community in soil, thereby indirectly influencing plant growth. Marks et al. [51] demonstrated that the application of biofertilizers containing *Chlorella sorokiniana*, cultivated in WWT, resulted in a 25% increase in bacterial abundance in the soil. This finding underscores the potential of microalgal biofertilizer in promoting beneficial interactions between microorganisms and plants in the soil ecosystem. Studies indicate that the implementation of microalgae as biofertilizer or biostimulants in soil not only enhances plant development and crop productivity but also contributes to the proliferation of bacterial biomass, symbiotic interactions among microorganisms, and the enrichment of soil structure. Microalgae, capable of treating degraded soil, hold significant potential as biofertilizers, offering a viable alternative to chemical fertilizers in the realm of sustainable agriculture.

5. Roles of microalgae-based product to prevent the global biodiversity crisis

Humanity is currently confronted with two pressing and closely interconnected environmental issues: climate change and biodiversity loss. As we step into a critical decade, the global efforts to address these challenges are becoming increasingly pivotal, with the development of ambitious strategies on both the international biodiversity and climate change fronts. Scientific evidence consistently highlights climate change as a major contributing factor to the current biodiversity loss and the primary factor of climate change is the emissions of greenhouse gases (GHG).

The emission of GHGs primarily stems from the combustion of fossil fuels, including coal, oil, and natural gas, in the automotive and industrial sectors. These activities contribute to carbon emissions during both the extraction and consumption processes. Also, the growing demand for meat and milk products has resulted in a substantial increase in livestock population and the conversion of extensive land areas into pastures and farmlands for livestock rearing. Ruminant animals, including cow, buffalo, and sheep, generate substantial quantities of methane through enteric fermentation during microbial digestion, thereby adding to the accumulation of GHGs in the atmosphere [52]. The production of 1 kg of meat requires 7 kg of grain and between 5000 and 20,000 L of water, whereas the production of 1 kg of wheat necessitates between 500 and 4000 L of water. Methane is also generated through the anaerobic fermentation of livestock manure. The importance of the need for transforming our animal farming industry by utilizing readily available plant-based ingredients, which can match the nutrient content of any sort of meat at a fraction of the cost, is seriously emphasized [53].

The main source of N_2O emissions into the atmosphere (60%) originates from microbial activity on nitrogen-rich organic matter present in both uncultivated soils, besides wastewater. Human activities, particularly in agriculture, contribute to the remaining nitrous oxide emissions. The implementation of nitrogenous fertilizers on crops has a widespread practice aimed at increasing yields. However, it is common for most farmers to apply excessive amounts of these fertilizers. This practice results in N_2O emissions from the soil, primarily through microbial processes such as nitrification and denitrification. Synthetic and organic fertilizers both contribute to increased N_2 availability in the soil, which promotes microbial activity and subsequently leads to the release of N_2O . However, organic fertilizer releases N_2 into the soil at a slower pace compared to synthetic fertilizer. This gradual release enables plants to effectively absorb a larger proportion of the N_2 as it becomes available. Synthetic fertilizer, on the other hand, rapidly releases N_2 that cannot be immediately utilized by plants, thereby providing excess nitrogen to microbes for conversion into N_2O . Currently, atmospheric CO_2 concentration is higher than at any time in the past 2 million years, while methane and nitrous oxide levels are higher than in the last 800,000 years [54].

Shivanna [55] indicates that if climate change mitigation measures are not fully and precisely implemented, significant contractions in climatic ranges are likely to occur, resulting in a considerable decline in global biodiversity and ecosystem services by the end of the twenty-first century. In other words, taking immediate and stringent action to reduce GHG emissions holds the potential to mitigate the risk of widespread biodiversity loss among common and widely distributed species. Through the implementation of these measures, the concurrent decline in ecosystem services that are facilitated by these species can be effectively averted. Efforts to mitigate climate change focus on reducing GHG emissions through various means,

such as transitioning to renewable energy sources, improving energy efficiency, implementing sustainable agricultural practices, and adopting cleaner technologies. Implementing nature-based solutions capitalizes on the intricate interplay between the climate system, oceans, land, and the encompassing natural environment. It is essential to manage this approach in a manner that preserves and sustains the multitude of contributions that nature provides to human societies and well-being, without jeopardizing their integrity [56]. Visions of nature-based strategies are emerging through the application of biotechnological transformations, encompassing the entire industrial chain. This worldwide trend embraces strategies that seek to diminish reliance on fossil fuel resources. In addition to designing safe foods, these strategies aim to significantly mitigate the impacts of the climate crisis on ecosystems and biodiversity loss. To achieve these goals, a substantial increase in the utilization of biological biomass is anticipated. Notably, the utilization of microalgal biomass to produce sustainable and environmentally friendly products stands out as one of the cleanest nature-based approaches that exhibit almost no GHG emissions [57]. Microalgal biomass has attracted significant interest in recent years due to its diverse range of potential applications. These applications include bioenergy production, wastewater treatment, industrial CO₂ removal, and the production of biochemical compounds that offer benefits for human and animal health.

By harnessing microalgal biomass, it becomes possible to address multiple challenges such as mitigating climate change and reducing global biodiversity loss. One of the key advantages of microalgal biomass is its potential for bioenergy production. Microalgae can be cultivated to produce biofuels, which serve as a renewable and sustainable alternative to fossil fuels. This not only helps reduce GHG emissions but also decreases reliance on finite and environmentally damaging energy sources. WWT is another area where microalgal biomass shows promise. Microalgae have the ability to efficiently remove pollutants and nutrients from wastewater, thereby improving water quality and reducing the environmental impact of wastewater discharge. This contributes to the preservation of biodiversity in aquatic ecosystems. Additionally, microalgae have the capacity to capture and utilize CO₂, making them a valuable tool for industrial carbon capture and utilization. By incorporating microalgae into industrial processes, CO₂ emissions can be mitigated, helping to combat climate change and its detrimental effects on biodiversity. Furthermore, the production of biochemical compounds from microalgal biomass offers numerous health benefits for both humans and animals. These compounds can be used in pharmaceuticals, nutraceuticals, and other applications, promoting well-being and potentially reducing the reliance on synthetic alternatives that may have negative environmental impacts [58].

Another challenge is that climate change poses an escalating threat to soil degradation, which consequently results in the loss of biodiversity and crucial ecosystem services. Soils play a vital role in providing various ecosystem services, including sustainable plant production, water quality control, regulation of biological pests and diseases, nutrient and contaminant filtration, carbon storage, GHG regulation, waste detoxification, and recycling, as well as flood and climate change mitigation, among others. When soil degradation occurs, it impairs the capacity of the soil to support a diverse array of microorganisms, leading to a decline in soil biodiversity, complexity, and functionality. The loss of soil biodiversity and compromised ecosystem services can have implications for human health, as the soil plays a crucial role in supporting healthy plant growth, nutrient uptake, and the purification of water resources [59]. The utilization of microalgae for the bioremediation of degraded soils has been the subject of investigation, yielding promising outcomes. Contaminants, including

heavy metals and pesticides, have a substantial impact on soil quality and the overall health of ecosystems. However, microalgae present an environmentally friendly and cost-effective solution for mitigating soil pollution. These microorganisms possess the ability to degrade pollutants and convert them into less harmful forms [60]. They enhance soil fertility by promoting overall microbial activity and facilitating beneficial microbial interactions. Microalgae support the growth of beneficial microbes in the soil, thereby influencing nutrient cycling and promoting nutrient availability for plants [61].

Moreover, numerous scientific studies have provided evidence for the probiotic-producing capabilities of various microalgae species. Microalgae have the ability to influence the microbial diversity in their environment through the secretion of various bioactive compounds including amino acids and extracellular polymeric substances (EPS), which function as a form of probiotic. For instance, Aydin et al. [30] investigated the application of *H. pluvialis* microalgae in an aerobic membrane system. The study observed a notable increase in the population of microbial communities affecting reactor performance within the reactor. This increase was attributed to the presence of bioactive molecules and EPS secreted by the microalgae, which demonstrated probiotic properties by promoting the growth and development of microorganisms beneficial in reactor performance. The findings suggest that microalgae-produced EPS and bioactive compounds hold the potential to enhance the abundance and activity of beneficial microbial biodiversity.

In summary, climate change is a pressing issue that affects all sectors of society and poses a threat to biodiversity, which represents the planet's vitality. To preserve and restore biodiversity, it is crucial to limit deteriorative activities caused by mankind and address the global climate crisis. Mitigating climate change requires reducing GHG emissions, which are major drivers of climate change. The unsustainable use of fossil fuels for transportation, heating, and other industrial purposes, coupled with the increasing global population, has rapidly increased GHG emissions and triggered global warming and climate change. In addition, the harness of chemicals in agriculture and livestock farming to fulfill the rising food demand of the growing global population contributes significantly to greenhouse gas GHG emissions. To safeguard the present biodiversity and secure the future of life on our planet by mitigating the impacts of climate change, it is crucial to shift away from current energy sources toward renewable energy, enhance energy efficiency, adopt cleaner technologies, and establish sustainable agricultural practices. In this pursuit, prioritizing nature-based approaches is of utmost importance, as they are highly sustainable and result in minimal GHG emissions.

In recent years, biotechnological applications have emerged as a clean and environmentally friendly option by harnessing the beneficial microorganisms naturally present in the environment. Microalgae have shown great potential in combating climate change and preventing biodiversity loss through the production of renewable biofuels such as biodiesel and biogas. They have a renewable and GHG emission-free nature, making them an attractive option for energy production. Furthermore, utilizing these microorganisms in WWT, promoting sustainable agriculture through the usage of biofertilizers and biostimulants that enhance plant growth and soil biodiversity, and employing their bioproducts in various sectors such as health, cosmetics, and animal husbandry in the fight against climate change and biodiversity loss offer promising multifaceted applications. These approaches present adaptable and sustainable applications, offering a promising pathway to tackle climate change and mitigate global biodiversity loss.

6. Global market and economic analysis of microalgae technology for sustainability

Microalgae have garnered significant attention in the biomanufacturing field due to their extensive biodiversity and their capacity to synthesize a wide range of compounds. Beyond their role in carbon sequestration and oxygen production, microalgae offer a vast repertoire of metabolic pathways and biochemical reactions, allowing them to produce diverse and valuable compounds. The enormous biodiversity of microalgae encompasses numerous species with distinct genetic backgrounds, physiological characteristics, and biochemical capabilities. Microalgae can be engineered or selected for specific traits and optimized to produce various compounds. The ability of microalgae to perform complex biosynthetic pathways and their capacity for high-yield compound production make them valuable candidates for sustainable biomanufacturing. Their photosynthetic nature, coupled with the utilization of solar energy and CO₂, further enhances their appeal as an environmentally friendly cell factory [62].

Microalgae exhibit a greater proportion of valuable cellular compositions in comparison to agriculturally cultivated biomass including edible food products and lignocellulosic biomass. Studies demonstrate the suitability of lipids produced from microalgae for conversion into alternative biofuels. Furthermore, microalgae play a significant role in the circular bioeconomy, as they contribute to transforming CO₂ and water into oxygen through photosynthesis, thereby promoting the sustainability of the Earth [63]. In addition to their ecological benefits, microalgae biotechnology has emerged as a contributor by generating beneficial biomass for various applications. This refers to the utilization of microalgae to produce high-value compounds, such as proteins, lipids, polysaccharides, antioxidants, and pigments, which find applications in industries such as food, feed, cosmetics, pharmaceuticals, and specialty chemicals [64].

Numerous countries have recognized the significance of cultivating microalgae and have established a presence in the market for algae-based products over several years. Microalgae possess the unique capability to utilize solar energy, water, CO₂, and inorganic nutrients to undergo photosynthesis, producing O₂ while retaining biomass that is rich in proteins, vitamins, antioxidants, and other essential nutrients pivotal for human consumption. Among the commercially cultivated microalgal species worldwide, *Spirulina* and *Chlorella* are prominent examples [65]. Over the past 15 years, China has emerged as one of the leading global producers regarding the collection and storage of microalgal biomass. Among various microalgal species, *Spirulina* has experienced the most significant commercial success in China, primarily to produce aquaculture feeds and human foods. According to Li et al. [66], the biomass collection and storage of *Spirulina* in China is substantial. It is estimated that over sixty *Spirulina* cultivating plants, occupying a cultivation land area of 7,500,000 m², generate approximately 9600 tons of dry microalgal biomass annually. The retail value of these *Spirulina* products exceeds USD 650 million per year. This level of productivity indicates an average biomass yield of around 13 hectares per year; roughly 70 kg of microalgal biomass are marketed and sold to consumers.

The industry in Japan exhibits a strong emphasis on the biofuel application of microalgae and collaborates closely with the European Union (EU) in this regard. The Japanese market for microalgal biofuel is valued at approximately USD 1170 million, reflecting its significant size. Ongoing investments are being made in research and development programs, specifically targeting algal science, with a particular

emphasis on biofuel production technologies. Furthermore, there is a notable drive to expand into various other microalgal markets, including foods, chemicals, and supplements. This diversification of applications indicates a broader utilization of microalgae beyond biofuel production [67]. In Europe, on the other hand, the industry is currently in the early stages of development compared to countries in the Eastern hemisphere. EU has recognized the utilization of microalgae to ensure the sustainability of the economy while effectively balancing different economically growth activities and concurrently preserving natural resources. This approach is driven by the aim to meet the demands of a rapidly growing population while ensuring the long-term viability of ecosystems and minimizing environmental impacts. The EU is actively exploring the potential of microalgae as a valuable resource for sustainable economic development [68].

EU is actively pursuing the development of a circular economy to address global issues including loss of biodiversity, climate crisis, environmental pollution, and waste management. This approach aims to establish systems that can combat these issues while protecting diversity. Unlike the linear economy model, which relies on resource exploitation and generates significant waste with minimal environmental considerations, the circular economy promotes sustainable practices that optimize resource utilization and minimize waste generation. The EU recognizes the importance of transitioning away from linear economy businesses and is dedicated to researching and implementing strategies that leverage microalgae's potential. By this way, the EU is taking progressive steps toward achieving a more sustainable and environmentally conscious utilization of resources [69]. Recently, Europe has been at the forefront of the global *Spirulina* market, and one contributing factor to Europe's dominant position is the openness of consumers in the region toward experimenting with new products. Europe has witnessed the highest volume of product launches containing *Spirulina*, accounting for 70% of global introductions in this market. In comparison, while Asia-Pacific accounts for 14% of products, North America represents 10%, the Middle East, as well as Africa, holds 5%, and Latin America 1%. This data indicates that microalgae is relatively less recognized in the Asia-Pacific region but possesses significant potential for expansion within this industry. By introducing, educating, and raising awareness about the benefits of microalgae, it is possible to increase public awareness and exposure to these products. This, in turn, can improve consumers' understanding of microalgae and their potential when it comes to selecting products [70].

The present utilization of microalgae encompasses various applications, including food, feed, health-related products, and cosmetics. Microalgae biomass prices for these applications range from 5 to 500 €/kg, and the market size reaches up to 100 kt/year. Furthermore, microalgae demonstrate significant potential in sectors like biofuels, biofertilizers, wastewater treatment, and chemicals, where the biomass price is notably lower, below 5 €/kg. Despite the lower biomass price, these sectors offer immense market opportunities [64]. The most widely consumed microalgae species worldwide are *Spirulina*, *Chlorella*, and *Haematococcus*. *Spirulina*, with its remarkable capacity to flourish in alkaline environments, is highly suitable for cultivation in open systems. Consequently, numerous companies have undertaken *Spirulina* production endeavors. The global annual production of dry biomass from *Spirulina* is estimated at around 15,000 tons, with China being responsible for a significant contribution of 10,000 tons. Alongside the protein-rich biomass, there is also substantial production of phycocyanin extract, with an estimated worldwide output of 200 tons per year [71].

Microalgae are abundant sources of compounds with important applications in the health and cosmetic sectors. These organisms produce a variety of bioactive carbohydrates, antioxidants, pigments, and fatty acids, among other constituents. Carotenoids derived from microalgae play a significant role in health protection. Beta-carotene obtained from microalgal species *Dunaliella salina*, for instance, has shown remarkable efficacy in laboratory studies, particularly in inhibiting angiogenesis. This property makes it valuable in preventing the formation of abnormal blood vessels, which is associated with conditions such as cancer [72]. Astaxanthin, derived from *H. pluvialis*, has been indicated to exhibit blood pressure-lowering effects. It has been identified as an antihypertensive agent, and it displays robust antioxidant activity and surpasses other carotenoids in terms of its antioxidant capacity. Also, carotenoids, including astaxanthin, have demonstrated anticancer properties, particularly in the prevention of various types of cancers such as breast, prostate and intestinal cancer, besides leukemia [73].

Among the diverse applications of microalgae, skin care has gained significant attention and utilization. Specifically, microalgal genera *Arthrospira* and *Chlorella* have been extensively studied and widely employed in the skin-care industry. Microalgal biomass and extracts are incorporated into various skin-care products, including, skin moisturizers, antiaging formulations, refreshing treatments, anti-irritant peelers, and hair-care products, in addition to sun-protection. Also, cyanobacteria contain specific compounds that possess photo-protection, moisture, and anti-inflammatory, antioxidant, and regenerative properties. These properties make them valuable in various health and wellness treatments, which aim to promote overall well-being and rejuvenation [74].

Microalgae offer versatile applications in various fields, including the production of biomaterials. These biomaterials aim to decrease the dependence on conventional fossil-fuel-derived materials while offering enhanced properties for diverse applications. A notable area of interest is the development of bioplastics derived from microalgae, which offer environmental friendliness and biodegradability compared to current fossil-derived plastics. Microalgae possess the capability to produce a range of ingredients for the synthesis of bioplastics, such as polyester polymers named polyhydroxybutyrate (PHB). The improvement of PHB production efficiency by utilizing microalgal biomass derived from high-rate algal ponds holds the potential to decrease operational costs, thereby establishing microalgae as a sustainable and promising feedstock in the production of bioplastic in the foreseeable future. Nevertheless, it is crucial to acknowledge that microalgae-derived bioplastic production is currently in the comprehensive research phase and has not yet achieved commercialization. Further studies and advancements are necessary to optimize the production processes, scale up the technology, and address economic viability before widespread commercial implementation can be realized [75].

7. Conclusion

This chapter provides a comprehensive overview of the industrial applications of microalgae and their potential role in mitigating climate change and biodiversity loss. Although microalgae-derived approaches are still in the early stages of exploration, they hold significant promise as a future pathway for sustainable and green manufacturing. Microalgae exhibit the unique ability to harness solar energy and convert CO₂ directly into organic carbon, enabling the production of a diverse array of high value

bioproducts. Recent advancements in the field have positioned microalgae as promising sources for biofuels, biofertilizers, and the synthesis of complex macromolecules like proteins and nucleic acids that are valuable for human consumption and health. These bioactive molecules secreted by microalgae can have the ability to influence microbial diversity by enhancing the development of certain bacterial species. Furthermore, the advancement and widespread implementation of microalgae-based approaches present a viable means to mitigate the primary causal factor of climate change, GHG emissions. By effectively reducing GHG emissions at a global scale, the adverse impacts of the climate crisis on biodiversity loss and other ecological concerns can be alleviated. Furthermore, the growing global microalgae market signifies the promising potential of industrially developed and widely adopted microalgae-derived methods and bioproducts. These innovative solutions provide sustainable alternatives that can cater to a broader consumer base, contributing to the transition toward a more environmentally conscious and resilient future. However, the success and feasibility of microalgae-derived approaches rely on key factors such as efficient biomass accumulation and high yields of the target products. Continued research and development in this field are essential for unlocking the full potential of microalgae as a sustainable and environmentally friendly resource to mitigate global climate, food, and energy crisis.

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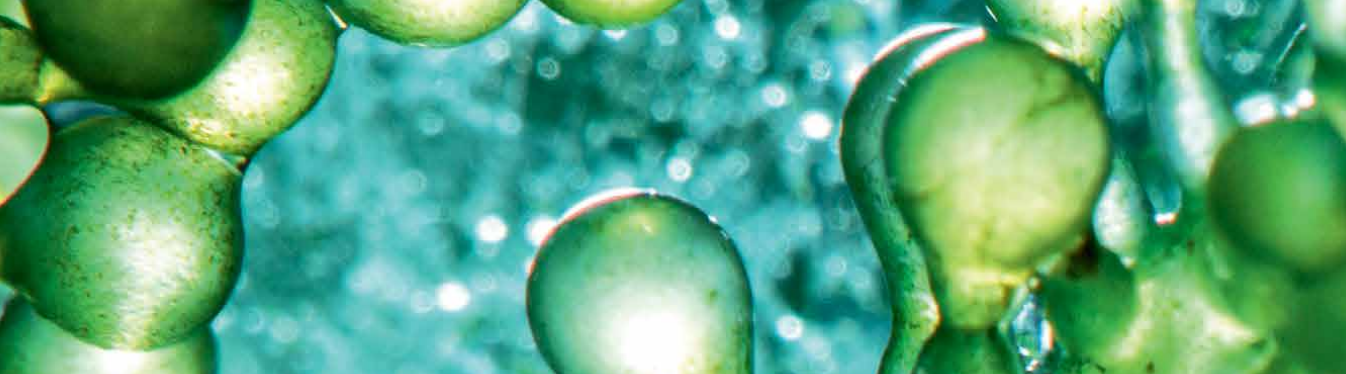
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This book offers a profound exploration of microalgae, unraveling the potential applications of these photosynthetic powerhouses. With eight chapters, this book discusses the diverse applications of microalgae and microalgae-derived compounds and addresses such topics as cutting-edge developments in biodiesel production, the synthesis of docosahexaenoic acid (DHA), the pivotal role of microalgae in mitigating climate change, and more. This book is essential for those intrigued by sustainable biotechnological practices and the global impact of microalgae technology.

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