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The Cultivation, Harvesting, and Multiple Roles of Bioactive Compounds in Microalgae in the Field of Biotechnology

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Abstract

Algal technologies have advanced significantly over the past few decades. Biopharmaceuticals, nutraceuticals, animal feed, and renewable energy are among the applications of microalgae that have recently attracted significant attention worldwide. In addition to their use in modern agriculture, the fascinating group of organisms can also be used in human health, as food additives, as they facilitate nutrient availability. In recent studies, algal biomass was demonstrated to be beneficial in sequestering CO_2. A variety of wastewater and industrial effluents have been demonstrated to produce algal biomass. However, because of its high productivity and potential for accumulating significant amounts of lipid, microalgae initially caught the eye of scientists as a renewable source of biofuels. By modifying cultivation conditions, harvesting, and extracting value-added products, an algal biofuel economy is being developed. All value-added products can be extracted from algal biomass more efficiently and environmentally friendly by utilizing a complete biorefinery approach. This review highlights potential research and development areas for microalgae biotechnology.

Keywords: Microalgae, Food additive, Biofuel, Wastewater treatment, CO_2 sequestration

Introduction

Microalgae are a type of unicellular, microscopic, polyphyletic, autotrophic, photosynthetic organisms that grow from carbon dioxide (CO_2) (Nicoară *et al*., 2023; Voiţă-Mekereş *et al*., 2023). In addition to eukaryotes, prokaryotes like blue-green algae are also often classified as algae (Mutanda *et al.*, 2011). Due to their unicellular structure and simple multicellular structure, microalgae can survive harsh environmental conditions and proliferate rapidly (Mata *et al.*, 2010). Approximately 3 billion years ago, the first photosynthetic organisms appeared in the ocean, replacing CO_2 with oxygen as the main component of the atmosphere. In addition to constituting atmospheric oxygen, photosynthesis increases dissolved oxygen levels in seawater (Popa-Nedelcu *et al*., 2020; Galea-Holhoș *et al*., 2023). As well, microalgae feed zooplankton, which in turn nourishes fish, who, in turn, feed humans. In addition to their bioactive components, they also contain substances that can be exploited for commercial purposes. Besides aquatic ecosystems, microalgae exist in all terrestrial ecosystems as well, and they come in a variety of species living in a variety of conditions (Mata *et al.*, 2010). In the literature, only about 35,000 of the estimated 200,000-800,000 species have been described, illustrating their amazing diversity (Odjadjare *et al.*, 2017). To detect and classify new species of microalgae, molecular methods like denaturing gel electrophoresis and next-generation sequencing have emerged. Traditionally, microalgae are classified on pigmentation, life cycle, and cell structure.

Increasing demand for alternative energy sources, the depletion of fossil fuel reserves, and the global energy crisis have led to the discovery of a wide variety of bioactive compounds in microalgae. In modern agriculture, this fascinating group of microalgae is also crucial for increasing nutrient availability, maintaining soil organic carbon, and enhancing crop production through soil microbial activity. Furthermore, they are developed for cosmetics and nutraceutical applications, as well as in the treatment of wastewater and the mitigation of atmospheric CO_2.

Transesterification is a method for converting triacylglycerol (TAG) in microalgal lipids to biodiesel through the reaction of TAG with methanol. In this process, fatty acid methyl esters (biodiesel) and glycerol are produced in the presence of an acid or alkali catalyst. Bioethanol and biogas can be produced from microalgal biomass by fermentation or anaerobic digestion. Microalgae starch can also be biodegraded into bio-hydrogen via biodegradation. Biomass from microalgae is an inexpensive source of protein that can be added to animal and fish feeds. Astaxanthin, carotenoids, polyunsaturated fatty acids, and others are bioactive compounds produced by certain microalgae

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species. Many microalgae in the environment are unknown and uncharacterized, which implies that a large number of potentially beneficial microalgae may remain undiscovered. Microalgae have huge potential biotechnological applications, as this review highlights areas that need further research and innovation.

Definition and Characteristics of Algae

Macroalgae and microalgae are two types of algae. Using sunlight, water, and atmospheric CO_2, microalgae are autotrophic, unicellular organisms. A source of energy is lipids, which are formed by converting atmospheric CO_2 into lipids. There are microalgae in a variety of sizes ranging from nanometers to millimeters (Saharan *et al.*, 2013). Microalgae have no roots, stems, or leaves, and about 100,000 species have been identified. Approximately 35000 species have been characterized so far. The algae were divided into filamentous and phytoplankton populations and further into diatoms, green, blue-green, and golden algae.

Several microalgae, such as *Chlorella* and *Scenedesmus*, are capable of surviving in the most extreme conditions (e.g., high temperature and high CO_2). Marchetti *et al.* (2013) report that microalgal biochemical composition varies according to species and environmental conditions. Light, pH, temperature, nutrients, as well as micro and macro metabolites of microalgae are primarily affected by environmental conditions, including light, pH, temperature, and nutrient levels. Microalgal biomass consists of high proportions of proteins (6-71%), lipids (7-23%), and carbohydrates (5-64%). These ratios depend on the growth conditions and algal species. In the production of biofuels, lipids are the most valuable microalgal product. Under favorable and optimal conditions, different species of microalgae can synthesize up to 50-70% oil/lipid per dry weight, and for microalgae with 30% and 70% oil content (by weight), yields range from 58,700 Loil/ha to 136,900 Loil/ha. In contrast, crops produce the following oil yields: corn yields 172 loads per hectare, soybeans 446 loads per hectare, coconuts 2689 loads per hectare, and soybeans 5 950 loads per hectare. Compared with microalgae, soybean and palm oil contain less than 5% of the total biomass in oil.

Cultivation of Microalgae

There are various types of algal cultivation systems, but most consist of either open ponds or raceways and closed bioreactors.

Open Pond Culture Systems

Reactors with open ponds (OPRs) are exposed to the environment. Raceway ponds and High Rate Algal Ponds (HRAPs) are commonly used for wastewater treatment (Passos & Ferrer, 2014; Zhao *et al.*, 2014). There are relatively low constructions, installation, and maintenance costs associated with reactors with open ponds (Richardson *et al.*, 2012). In addition to contamination with other algae and predators, vaporization, and uncontrollable growth parameters are some of the drawbacks of OPR (Koller *et al.*, 2012). There is a relatively low biomass concentration.

Closed Photobioreactors

Photobioreactors are closed systems that can be used for microalgae cultivation. Tubes, flat tanks, bubble columns, and serpentines are frequently used. In PBRs, algae growth is controlled, which leads to high algal biomass productivity. Optimizing and controlling the cultural environment conditions can also minimize contamination by other algae species. While PBRs have some advantages, they also have some disadvantages: higher operating and maintenance costs. Biomass concentrations range between 20 and 100 grams of dry matter of algae per square meter per day.

Culture Using Deep Seawater

Fisheries, aquaculture, agriculture, medicine, cosmetics, and energy production have benefitted from deep seawater (DSW) since the 1980s. Ocean water consists of 95% DSW. A thermocline 200 meters below the ocean surface is another name for deep ocean water. Under the thermocline, temperatures drop rapidly with depth. In addition to their low temperature, they maintain a high nutrient level, are aged, stable, and possess antiseptic properties. Microalgae strains cannot compete for nutrients in DSW because pathogenic bacteria and viruses cannot flourish there (Tan *et al.*, 2015).

In recent decades, deep-sea water (DSW) has also drawn substantial attention due to its readily accessible quantity and potential for recycling energy. Various elements and nutrients in DSW may stimulate microalgae to produce specific components/metabolites. This culture method resulted in a higher biomass (2.44 g/L) and higher oil production (176.6 mg/L/day) compared to a 50% DSW in BG-11 medium culture used by Tan *et al.* (2015) to determine the growth and oil-rich microalgae *Chlorella sorokiniana* CY1. Additionally, other studies have shown that DSW can be used to increase biomass yield from a variety of marine microalgae species with small changes in composition or nutrient additions.

Cultivation Strategy

In biotechnological processes, there are various cultivation strategies, which can be employed: batch (continuous), fed-batch, semi-continuous, and continuous.

Because of its advantages, the continuous model is most suitable for laboratory-scale and research applications.

The advantages of continuous systems are that they do not require post-processing preparation of reactors (an essential step in batch fermentation), they can be automated, and they produce fresh and active algae cells continuously over an extended period. In addition, steady-state results in the constant production of biomass and products in consistent quality and quantity. There is limited literature on the design and construction of efficient and robust full-scale cultivation systems, and these systems are expensive and difficult to operate and install (Koller *et al.*, 2012).

Factors Determining Microalgae Cultivation

Growing microalgae depends on nutrient concentrations, quality of nutrients, carbon dioxide concentrations, pH (4–11), light exposure (1,00–10,000 lux), biological factors, culture density, water quality, toxic compounds, salinity (12–40 g/L), turbulence, heavy metals, biological factors, and synthetic organisms, in addition to conditions during bioreactor operation (Mata *et al.*, 2010).

Different cultivation factors have been examined by various studies and their effects on algal growth include:

- The effects of a reduction in pH caused by CO_2, which inhibited algal growth, were examined by Moheimani (Moheimani, 2005). For *Pleurochrysis carterae*, the pH range for maximum productivity in a plate photobioreactor is pH 7.7–8.0, while the pH range for maximum productivity in an outdoor raceway pond is pH 9.1–9.6. For the outdoor raceway pond, the author determined that a depth of 16–21 cm is the optimal depth.
- It was reported by Richmond (2003) that the bubble residence time is not enough to absorb CO_2 in shallow suspensions at neutral pH, resulting in great CO_2 losses to the atmosphere.
- Two uncatalyzed reactions can accelerate the absorption of CO_2 into alkaline waters, as explained by Weissman and Goebel (1987). One is the hydration of CO_2 followed by an acid-base reaction, and the other is a direct reaction with the hydroxyl ion to form bicarbonate. According to this author, the former reaction occurs faster at pH values below 8, while the second occurs at pH values above 10. It can be important to consider both 8 and 10.
- In *Nannochloropsis oculata* cultures with increased CO_2 concentration, (Chiu *et al.*, 2009) found an increase in biomass production and lipid accumulation.
- According to Brazilian researchers (De Morais & Costa, 2007), *Scenedesmus obliquus* and *Chlorella kessleri* are capable of fixing CO_2 in thermal power plants using biochemical processes.
- As a carbon source, carbon dioxide (and bicarbonate) is used to study photosynthetic algae species. Studying the proximate chemical compositions of algae under nitrogen and salt stress is the main purpose of this study. It has been concluded that when species are exposed to environmental stress, their basic cellular composition differs.
- All five *Chlorella* strains investigated by (Illman *et al.*, 2000) showed an increase in lipids with nitrogen reduction. A 63% increase in lipid content by dry weight was observed in C. *emersonii*, 56% in C. *minutissima*, and 40% in C. *vulgaris*.
- Macedo and Algre (2001) found that decreased nitrogen concentrations and temperature decreased were more effective in reducing *Spirulina* lipid content by approximately 3 times.

Nutrients

Growing microalgae requires inorganic carbon, inorganic nitrogen (ammonium or nitrate), and phosphorus (some species can use organic carbon from wastewater) (Pittman *et al.*, 2011). Inorganic nitrogen sources such as urea are also suitable and cost-effective alternatives. CO_2 or organic forms of carbon can be added to algae cultures, such as glycerol and acetates. As a carbon source, CO_2 from the environment must be used for large-scale cultivation of microalgae, which is not only low-cost but also CO_2 emission-reducing. Nitrogen is another important element required for microalgae. Microalgae also require nitrogen, and ammonium is one of the main sources of nitrogen for fast-growing microalgae, but intermittent feeds of nitrate can promote growth in the absence of nitrate in the medium. When microalgae are stressed by insufficient nitrogen, their growth rate and productivity are lower, but they produce more lipids (reserve compounds).

For the growth of microalgae biomass, phosphorus is the third most important nutrient. The application of phosphates should be excessive since some phosphorus compounds cannot be biodegraded (e.g. phosphorus combined with metal ions). However, for effective cultivation, trace species like metals (Mg, Ca, Mn, Zn, Cu, Mb), vitamins, and other trace elements must be added (especially in laboratory-scale cultivation).

Light Provision

Light provision is another important factor. In microalgal bioreactors, measurement of the intensity and utilization efficiency of light as well as dark-to-light ratios and high-intensity light periods are crucial.

In comparison with an unaerated column, Jones and Horrison 2014 reported better growth in a bioreactor. Because of aeration, the microalgal culture mixes better, which prevents sedimentation, maintains homogeneity, and enables a better exchange of nutrients between cells.

Temperature

Algae cultivation in closed and open outdoor systems is primarily limited by temperature, after light. Generally, Mesophilic species grow best at 20 to 25 °C, whereas thermophilic strains (*Chaetoceros, Anacystis nidulans*) can grow at up to 40 °C, while psychrophilic strains (*Asterionella formosa*) can grow as low as 17 °C.

In the laboratory, temperature effects are well documented for many microalgae species, but the magnitude of temperature effects on biomass production outdoors is not well understood. Even though many microalgae can tolerate temperatures as low as 15°C below their optimal, exceeding the optimum temperature by just 2–4°C may result in total culture loss. During hot days, closed culture systems may overheat to 55 °C, causing overheating problems. Using an evaporative water cooling system, the temperature can be lowered to around 20–26 °C economically (Moheimani, 2005).

Salinity

A microalgae's growth and cell composition can be affected by salinity, whether in an open or closed system. As a result of high evaporation, every alga has its optimum salinity range. Changes in salinity typically have three effects on phytoplankton both osmotically and chemically (Moheimani, 2005): ion (salt) stress; cellular ionic ratio changes; and change in membrane permeability. To control salinity, freshwater or salt can be added as needed.

Microalgae Harvesting and Biomass Concentration

A bioreactor effluent must be separated from the microalgae biomass after cultivation. As a result of the harvesting process, 2–7% of algae in slurry material are harvested (Saharan *et al.*, 2013). The extraction of large quantities of water and the process of large volumes of algal biomass can be achieved by several physical, chemical, or biological methods.

In addition to sedimentation and centrifugation, filtration and ultrafiltration methods are also commonly used to harvest, sometimes in combination with flocculation. To increase sedimentation, centrifugal recovery, and filtration efficiency, flocculation aggregates microalgal cells. Cell density, microalgae species, and culture conditions determine which method is best.

Proper harvesting procedures should be chosen according to the desired quality of the product. Gravitational sedimentation, flocculation-enhanced sedimentation, or settling ponds can all be used for low-value products. Food and aquaculture applications, however, require continuous centrifugation if the product has a high value. Furthermore, not every method of harvesting microalgae is suitable for all species, for example, filtering is only suitable for large microalgae (*Scenedesmus platensis*), while centrifugation is suitable for any microalgae species. Additionally, cleaning and sterilization of centrifugation devices are easy. The biomass should also be capable of adjusting its density or moisture to the subsequent process (Pahl *et al.*, 2013). A combination of harvesting methods is necessary to obtain the optimal biomass parameters, such as pre concentration (mechanical dewatering and centrifugation) and post concentration (screw centrifugation or thermal drying). It is also important to consider the economic factors when choosing the right method or methods of harvesting biomass. Researchers have reviewed the methods for harvesting, thickening, and dewatering microalgae biomass at scales of both research (pilot and laboratory) and industrial scales (Pahl *et al.*, 2013).

Applications of Microalgae

In addition to carotenoids and polyunsaturated fatty acids, microalgae are also a source of phycocolloids and polyphenols. The simple growth requirements of photoautotrophs make them ideal for bioprocesses that aim to produce drugs with high added value. Culture method, medium composition, and nutrient composition all play a significant role in microalgal productivity and biochemical composition.

Therefore, many efforts have been made to clarify the practical significance of the above parameters. Due to these reasons, algae are being studied for their beneficial effects on human health.

Commercial Application of Microalgae

Microalgae and Human Food

A variety of types of compounds can be produced by microalgae, depending on the conditions of growth. In addition to proteins, carbohydrates, lipids, carotenoids, vitamins, and mineral salts, these compounds are also found in foods (Hamed, 2016). There has been extensive research on their composition over the last few years, comparing several species like *Chlorella vulgaris*, *Botryococcus braunii*, *Arthrospira platensis*, *Nannochloropsis* sp., *Phaeodactylum tricornutum*, *Dunaliella salina*, *Haematococcus pluvialis* and *Scenedesmus almeriensis* (Jones & Harrison, 2014).

It is possible to detect possible applications of microalgae-based on the characteristics of their cellular content, such as long-chain fatty acids (most notably omega-3 and eicosapentaenoic acid), carotenoids, and proteins, through the characterization of their cellular content (Tibbetts *et al.*, 2015). Food industry applications for microalgae include a wide range, and species such as *Chlorella vulgaris* and *Arthrospira platensis* are currently considered safe by the FDA (Food and Drug Administration) (Caporgno & Mathys, 2018). Because of their high protein content (between 42% and 58% w/w on a dry basis), phytobiliproteins (coloring substances with excellent antioxidant properties) are used in food and beverages (Stanic *et al.*, 2018). The color and vitaminic properties of carotenoids are also appreciated in the food field, particularly beta-carotene. The intestinal tract converts beta-carotene directly into retinol (vitamin A), as it is a precursor of provitamin A. D. *Salina* is already used as a food coloring additive (E 160 IV), containing more than 80% beta-carotene extract. Microalgae also produce lipids, which are used in the food industry along with coloring substances. To prevent cardiovascular disease, the World Health Organization recommends taking 200–500 mg of long-chain fatty acids, including omega-3, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). For lactating women and other adults, omega-3 foods and drinks are enriched with the lipid content extracted from microalgae, specifically *Ulkenia* sp., and *Schizochytrium* sp.

Nutraceuticals also use microalgae as food supplements in the form of powders and tablets. Because they contain vitamins, amino acids, and polyunsaturated fatty acids, *Chlorella vulgaris*, and *Arthrospira platensis* are among the most widely used microalgae in the nutraceutical industry. Besides *Dunaliella salina*, *Haematococcus pluvialis* is also used in nutraceuticals as an antioxidant and vitamin supplement (Bishop & Zubeck, 2012).

Microalgae and Cosmetics

Among the most important microalgae in the cosmetics industry are C. *Vulgaris*, which synthesizes collagen with anti-aging properties, and *Dunaliella salina* and *Haematococcus pluvialis*, which contain carotenoids for coloration and UV protection. Linoleic acid can be produced from microalgae, which produce excellent lipids, as an active ingredient for softening the skin (Guillerme *et al.*, 2017).

A relatively recent trend has been the application of microalgae for cosmetics; these microorganisms produce metabolites that regulate cellular regeneration and protect them against external aggression. In this context, when these compounds to the skin they could instigate the same effect. C-phycocyanin and astaxanthin are bioactive extracts of microalgae potentially usable in cosmetics. The bioactive compounds from microalgae may protect against skin cancer, maintain skin health, maintain dehydration, reduce photooxidation and hyperpigmentation, as well as some dermatological conditions (Ariede *et al.*, 2017; Hu *et al.*, 2019).

Microalgae and Food Colorant

The microalgal pigment has commercial uses like cosmetic ingredients and natural food coloring. Food colorants are used in the production of margarine to provide the yellow color. However, different microalgae besides beta-carotene have carotene, phycobiliproteins, phycocyanin, phycoerythrin, lutein, and astaxanthin. Phytocyanin is an antioxidant and anti-inflammatory pigment found in red algae, which is used in food and cosmetic products. Beta carotene is used to improve the color of egg yolks and fish flesh, to improve the health and fertility of grain-fed cattle, and to enhance the health and fertility of grain-fed cattle. The benefits of beta-carotene in preventing heart disease have been proven in several studies. This natural carotenoid is fat-soluble. Beta-carotene is much more effective against cancer and heart disease due to its fat-soluble nature. The National Cancer Institute recently announced beta-carotene as an anticarcinogenic. Beta-carotene has become much more valuable with these new findings, which may increase its demand. However, food coloring derived from microalgae is limited, because the color is not photostable and bleaches after cooking. It is nevertheless likely that microalgae-derived food coloring will find a huge market despite these limitations. Beta-carotene from *Dunaliella salina* is utilized as an orange coloring and as a vitamin C supplement.

Industrial Application

Microalgae and Biofuel

There are many advantages to using microalgae for biodiesel production, compared to other feed stocks (Rosenberg *et al.*, 2008; Rodolfi *et al.*, 2009). Microalgae have many practical advantages, such as ease of cultivation, low maintenance, and unsuitable water for human consumption.

Triacylglycerol (TAG) and hydrocarbons are the major components of microalgae lipids (Hama & Kondo, 2013). As the metabolic rate of microalgae slows, TAGs are the primary storage components of energy reserves. Using microalgal lipid as a substrate, TAGs can be converted to biodiesel through transesterification progress, where methanol reacts with methyl alcohol in the presence of a catalyzer to form fatty acid methyl esters (biodiesel) and glycerol **(Figure 1)**.

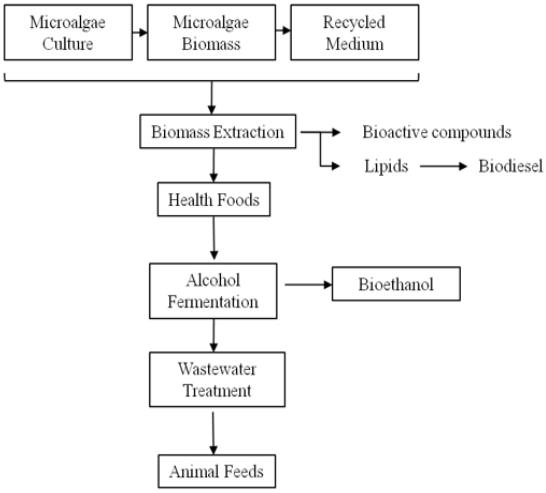

Figure 1. Transesterification reaction between triglyceride and alcohol in biodiesel production.

There are several types of fatty acids produced by different microalgae species, and the lipids they produce must be characterized. Oil production can be enhanced by causing many microalgae species to accumulate significant amounts of lipids. Most species can synthesize up to 70%, however, some species can reach 90% dry weight under certain conditions. The lipid composition of some microalgal species differs from that of others while certain fatty acids are better suited to transesterification into biodiesel than others.

Generally, microalgal oils contain polyunsaturated fatty acids with four or more double bonds, which are vulnerable to oxidation during storage, therefore making them less suitable as biodiesels (Zheng *et al.*, 2012). In biodiesel, the most common fatty acids are those with a carbon chain length of 14 to 22 carbon atoms. These fatty acids are therefore desired for the production of high-quality biodiesel (Zheng *et al.*, 2012). A low polyunsaturated fatty acid composition is considered the best biodiesel formula to decrease issues with oxidative stability and cold flow.

Several factors affect the fatty acid composition, including nutrition, the environment, cultivation conditions, and growth phases. C18:1 was accumulated in all treated species as a result of nitrogen deficiency, as well as C20:5 to some extent in *Botryococcus braunii*. Various algae species were found to have different fatty acid compositions, as reported by other authors (Gouveia & Oliveira, 2009).

According to **Table 1**, which compares biodiesel production efficiency, land use, and oil yield per hectare of microalgae and other vegetable oil crops, the microalgae oil yield is generally higher than that of other vegetable oil crops (Mata *et al.*, 2010).

Microalgae are superior in oil yield and biodiesel production when compared with seed plants in terms of biomass productivity. From a land-use perspective, microalgae and palm oil biodiesel are more advantageous due to their higher biomass productivity and oil yield.

Table 1. Comparison of microalgae with other biodiesel feedstocks (Vollmann *et al.*, 2007; Nielsen 2008).

Plant source	Seed oil content (% oil by wt in biomass)	Oil yield (L oil/ha year)	Land use (m^2 year/kg biodiesel)	Biodiesel productivity(kg biodiesel/ha year)
Corn/Maize (*Zea mays* L.)	44	172	66	152
Hemp (*Cannabis sativa* L.)	33	363	31	321
Soybean (*Glycine max* L.)	18	636	18	562
Jatropha (*Jatropha curcas* L.)	28	741	15	656
Camelina (*Camelina sativa* L.)	42	915	12	809
Canola/Rapeseed (*Brassica napus* L.)	41	974	12	862
Sunflower (*Helianthus annuus* L.)	40	1070	11	946
Castor (*Ricinus communis*)	48	1307	9	1156
Palm oil (*Elaeis guineensis*)	36	5366	2	4747
Microalage (low oil content)	30	58,700	0.2	51,927
Microalgae(medium oil content)	50	97,800	0.1	86,515
Microalgae (high oil content)	70	136,900	0.1	121,104

Microalgae Used as Biofertilizer

In addition to living in hot and cold deserts, soil crusts, rock crevices, and ocean depths, algae also live in a wide range of other environments. These habitats are dominated by unicellular, colonial microalgae, as well as lichens, which significantly contribute to carbon sequestration through carbon sequestration. Microalgae intervene with a positive effect on soil quality for sustainable agriculture. Through their ability to enhance soil microbial activity and facilitate better microbial interactions, algae increase soil fertility (Yan *et al.*, 2013). A variety of crops have benefited from cyanobacterial inoculation in the soil in several ways, including optimized seed germination, growth, grain yield, and nutritional content. Several studies have also demonstrated that green microalgae are suitable for use as biofertilizers and can improve seed germination, plant growth, nutrition, and crop yield.

Although cyanobacteria have a well-established ability to fix nitrogen, recent research has shown that they can also be useful in a variety of other ways, such as mineralizing nutrients, producing growth hormones, biocontrol, reclaiming wastelands, and improving plant defense mechanisms.

In the last decade, algae biotechnology has developed significantly; several algae have had their genomes sequenced, and some are still in development. In the context of their widespread applications, including biofuels and biofertilizers, genetically modified algae have been studied extensively. Cyanobacteria such as *Nostoc* and *Anabaena* strains are the most commonly used organisms as biofertilizers because of their ability to fix atmospheric nitrogen. Researchers are improving the N-fixing mechanism in heterocysts to enhance their N-fixation capacity (Yan *et al.*, 2013).

Microalgae are Used as Animal Feed

A significant contribution to global food production comes from livestock maintenance. During the Industrial Revolution, human populations moved to urban areas and food styles changed, resulting in a continuous increase in meat consumption. Vegetarians constitute only a small percentage (21.8%) of the global population, and the meat market continues to grow. A new study has projected that global meat production will increase by 1.5% in 2018, while meat exports will increase by 2.6%. Bovine meat is expected to grow at the fastest rate by 2%, followed by pig meat by 1.6 % and ovine meat by 0.8 % (Dineshbabu *et al.*, 2019). According to Becker *et al.* in 2003, 30% of microalgae biomass produced for feed or preparations for feed were sold as animal feed. Since then, human food supplement companies have become more aware of the potential of microalgae for food, and more are developing algae-based food additives Becker (2004). Despite this, microalgae are still essential in the preparation of animal feed.

Several microalgae have been tested for nutrient profiles and have been found to provide significant benefits as a feed supplement. Several important microalgal species can be utilized as supplements in animal feeds, such as *Arthrospira* sp., *Schizochytrium* sp., *Porphyridium* sp., *Chlorella* sp., *Arthrospira* sp., *Isochrysis* sp., *Pavlova* sp., and *Schizochytrium* sp., *Nannochloropsis* sp., *Chlorella* sp., (Madeira *et al.*, 2017).

Microalgae are Used as Aquaculture Feed

It is a powerful part of the mariculture industry to mass cultivate microalgae, which can be fed to crustaceans (shrimp), mollusks (clams and oysters), and fish. Microalgae feeds are especially valuable for species that have very particular dietary requirements, such as shrimp and clams. Microalgae are commonly used to supply amino acids, fats, or other unidentified growth factors or as sources of carotenoids. To provide essential amino acids, fatty acids, or other growth factors, or to provide carotenoids for coloring, microalgae are commonly used. Microalgae are typically produced in aquaculture facilities, frequently by fertilizing incoming seawater and fed directly to animals as a diluted, living culture.

For aquaculture applications of microalgae, two ways can be naturally used: (i) Natural phytoplankton populations, either as they naturally occur or as they are cultivated with nutrients added. Natural phytoplankton has the major disadvantage of not being controlled, being consumed by predators, and being contaminated by a variety of species better adapted to the existing environment. (ii) As a source of high-quality feed with known nutritional properties, or as a bacteria-free culture that will not introduce unwanted pathogens into animal cultures, an algae monoculture may be beneficial. Large-scale monocultures have the major difficulty of being susceptible to viruses, bacteria, and fungi, as well as predatory bacteria, rotifers, crustaceans, and even microplantonic larvae of benthic organisms.

Many aquaculture operations maintain cultures in duplicate due to the unpredictable nature of microalgae cultures collapsing due to predation.

Microalgae Use in Pharmaceuticals

Primary and secondary metabolites produced by algal organisms are novel and biologically active. The pharmaceutical industry may be interested in these metabolites because they are biologically active and novel. Because algae occur in aquatic natural communities, where producers and competitors interact with each other in an inhibitory manner, bioactive compounds are expected to exist in algae. There are numerous bioactive compounds found in microalgae that can be harnessed for commercial use. Their pharmaceutical and nutritional value has made them important sources of proteins and value-added compounds. In culture, microalgae can produce bioactive compounds that are difficult to synthesize chemically, making them a valuable natural source of bioactive molecules. In vitro, both cell extracts and growth media extracts of numerous unicellular algae have proven to be antibacterial against Gram-positive and Gram-negative bacteria (e.g. *Chlamydomonas pyrenoidosa*, *Chlorella vulgaris*). Furthermore, extracts of green algae, diatoms, and dinoflagellates have been reported to have a wide range of antifungal activity in vitro. Microalgae produce potential pharmaceuticals such as *Ochromonas* sp., *Prymnesium parvum*, and several blue-green algae. In addition to antialgal, antibacterial, antifungal, and antiviral properties, bacteria produce metabolites with diverse biological properties. Antimicrobial agent production is influenced by temperature, pH, incubation period, medium constituents, light intensity, and medium components.

Environmental Applications of Microalgae

A variety of studies have demonstrated the ability to produce valuable products from microalgae as well as biological indicators of pollution and reduce environmental impact if combined with processes such as wastewater and flue gas treatment.

Microalgal Remediation of Wastewater and Simultaneous Biomass Production

In aquaculture systems that involve microalgae farming combined with wastewater treatment (e.g. amino acids, enzymes, or wastewater from food industries) nitrogen and phosphorus from wastewater can be used as microalgae nutrients. As a result of this process, microalgae can be nourished by organic compounds (nitrogen and phosphorous) found in the wastewater of some manufacturing plants, and which do not contain heavy metals or radioactive isotopes. The microalgae also contribute to biodiversity while reducing the effects of sewage effluent and industrial sources of nitrogenous waste. Moreover, microalgae are capable of removing nitrogen and carbon from water, thus reducing eutrophication in aquatic environments. Based on the average removal efficiency for nitrogen and phosphorus (from 3 to 8 mg/L NH4+ and 1.5–3.5 mg/L PO43−) of *Chlorella vulgaris* from wastewater, Aslan and Kapdan (2006) found a 72% removal efficiency for nitrogen and 28% for phosphorus.

Various microalgae species, such as *Chlorella* (Worku & Sahu 2014) and *Spirulina*, are also widely used to remove nutrients. Different studies have also reported that *Nannochloris*, *Botryococcus brauinii*, and the cyanobacterium *Phormidium bohneri* are capable of removing nutrients from the environment (Milano *et al.*, 2016).

Flue gas CO_2 Emissions as Microalgae Nutrient

As a result of fossil fuel combustion, greenhouse gases like carbon dioxide (CO_2) are released into the atmosphere and contribute substantially to climate change and global warming. Approximately 1.6% per year is expected to be added to the global energy-reacted CO_2 emissions by 2035. In 2000, 110 billion metric tons of wastes were generated in several sectors, such as industrial, power generation, and transportation. The amount of CO_2 emitted by the year 2035 is expected to exceed 140 billion metric tons (Milano *et al.*, 2016). Plants and microalgae use photosynthesis to fix $CO_2$to form complex sugars. As a result, using flue gas from industrial processes (e.g. power plants) to provide CO_2 for microalgae growth has a great potential to decrease CO_2 emissions while offering a very promising alternative to current GHG emissions mitigation strategies.

As demonstrated by Zeiler *et al.* (1995) a green algae *Monoruphidium minutum* is capable of producing substantial biomass using simulated flue gases containing high levels of carbon dioxide, sulfur, and nitrogen oxides. It has been shown that Chlorophyta is more efficient than terrestrial plants at fixing CO_2 and capturing solar energy.

Chlorococcum littorale, a marine alga that shows exceptional tolerance to high CO_2 concentrations, is one of the microalgae that can be used to fix CO_2 from flue gas. It has also been shown that *Chlorella* strains from hot springs can fix CO_2 from industrial flue gas containing up to 40% CO2 at high temperatures of 42 °C. Also exhibited good tolerance to high CO_2 concentrations were the microalgae S. *Obliquus* and C. *Kessleri* isolated from Presidente Médici's waste treatment ponds.

Bioremoval of Heavy Metals by Microalgae

The following heavy metals are among the most common: silver (Ag), chromium (Cr), arsenic (As), iron (Fe), cadmium (Cd), mercury (Hg), copper (Cu), lead (Pb), zinc (Zn). A heavy metal is a metal or metalloid with a density greater than 5 grams per cubic centimeter. However, most prokaryotic and eukaryotic organisms are toxic when they are exposed to elevated levels of these metal ions.

By US Environmental Protection Agency regulations, As, Cd, Cr, Hg, and Pb have maximum contamination limits (MCL) of 0.01, 0.005, 0.1, 0.002, and 0.015 mg/L, respectively (Leong& Chang, 2020). It is hight toxic, carcinogenic, mutagenic, and teratogenic to expose yourself to low concentrations of heavy metals and their compounds. By directly contacting, inhaling, or ingesting these heavy metals, severe health risks are posed to human beings, including mutations and genetic damage to the central nervous system and increased cancer risk.

To detect the potential toxic effects of heavy metals on microalgae, they are often used as biological sensors. The tolerance of certain cyanobacterial species to heavy metal stress allows them to naturally grow in contaminated water. Examples include *Anabaena*, *Oscillatoria*, *Phormidium*, and *Spirogyra*.

It is also possible for microalgae to form complexes with pollutants in wastewater in addition to heavy metals. Total suspended solids (TSS) and total dissolved solids (TDS) are both reduced as a result of flocculation. In addition to immobilizing heavy metals, gene regulation, exclusion, and chelation, microalgae species also reduce heavy metals via redox reactions using antioxidants or reducing enzymes.

Despite the presence of heavy metals in cells, microalgae can form complexes of proteins and heavy metals without changing their activity. As a result, the heavy metalions are separated within vacuoles which help to regulate heavy metalion concentration in cytoplasm (Priatni *et al.*, 2018). The interaction between heavy metals and phytochellatins (PCs) minimizes heavy metal stress by activating the synthesis of these thiol-rich peptides and proteins.

Microalgae Fine Chemicals and Bioactive Compounds

A variety of high-value chemical compounds can be extracted from microalgae species, including pigments, antioxidants, carotenoids, b-carotenes, polysaccharides, triglycerides, fatty acids, phycobiliproteins, peptides, vitamins, lipids, proteins **(Tables 2 and 3)** and biomass. These compounds can be used as food colorants, additives for animal feed and aquaculture, and cosmetics, as well as being nutritional and therapeutic (Miguel *et al.*, 2021; Martínez *et al.*, 2022). Some bioactive compounds in microalgae can be used as pharmaceuticals since some of them act on enzymes in the body. Ravindran *et al.* (2016) reported that bacteria and peptides from microalgae have antimicrobial properties and various neurological properties. Despite their high lipid content, microalgae residue still retains a large amount of nitrogen and phosphorus, which makes them an excellent fertilizer. They also contain a lot of essential fatty acids, and some marine algae such as *Nannochloropsis salina*, *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Arthrospira* (*spirulina*), and *Platensis* (cyanobacterium) contain. Docohexaenoic acid (DHA), linoleic acid, arachidonic acid, and eicosapentaenoic acid (EPA) are omega-3 polyunsaturated fatty acids (PUFA) found in microalgae (oil) used as an alternative to fish oil. Various health benefits are associated with these omega-3 long-chain polyunsaturated fatty acids, which are found in both plant and animal sources (Biller & Ross, 2014).

Table 2. Biomass composition of microalgae expressed on a dry matter basis (Biller & Ross, 2014).

Strain	**Protein (%)**	**Lipid (%)**	**Carbohydrates (%)**
Botryococcus braunii	40	33	2
Dunaliella bioculata	49	8	4
Chlamydomonas rheinhardii	48	21	17
Chlorella pyrenoidosa	57	2	26
Chlorella vulgaris	41-58	10-22	12-17
Dunaliella salina	57	6	32
Dunaliella tertiolecta	29	11	14
Euglena gracilis	39-61	14-20	14-18
Porphyridium cruentum	28-39	9-14	40-57
Prymnesium parvum	28-45	22-39	25-33
Scenedesmus dimorphus	8-18	16-40	21-52
Scenedesmus obliquus	50-56	12-14	10-17
Scenedesmus quadricauda	47	1.9	-
Spriogyra sp.	6-20	11-21	33-64
Spirulina maxima	60-71	6-7	13-16
Spirulina platensis	42-63	4-11	8-14
Synechoccus sp.	63	11	15
Tetraselmis maculata	52	3	15
Pseudochoricystis ellipsoidea	10.2	38	34
Chlorogloeopsis fritschii	41.8	8.2	37.8
Chlorella emersonii	9.03	29.3	37.9
Chlorella zofingiensis	11.2	56.7	11.5
Chlorella FC2 IITG	10.4	37.3	24.5

Table 3. Some high-value bioproducts are extracted from microalgae (Ariede *et al.*, 2017).

Product Group	**Applications**	**Examples (producer)**
Phycobiliproteins carotenoids	Pigments, cosmetics, pro-vitamins, pigmentation	Phycocyanin (*Sprrulina platensis*) Beta-carotene (*Dunaliella salina*) astaxanthin and leutein (*Haematococcus pluvialis*)
Polyunsaturated fatty acids (PUFAs)	Food additive, nutraceutics	Eicosapentaenoic acid (EPA)(*Chlorella minutissima*) docosahexaenoic acid(DHA) (*Schizochytrium* sp.,) Arachidonic acid (AA)(*Parietochlorisincise*)
Vitamins	Nutrition	Biotin (*Euglena gracilis*) α-tocopherol (Vitamin E) (*Prototheca moriformis*, *Chlorella* spp.,)

Conclusion

Microalgae inhabit a wide variety of ecosystems, but only a small percentage has been exploited for human purposes. Microalgae have been primarily studied for biodiesel production over the years, but, likely, many more species have yet to be identified and characterized. To make microalgal biomass economically feasible, further research and innovation are needed to combine the phytoremediation of wastewater with biomass production. Pharmaceutical, nutritional, and cosmetic industries can benefit from microalgae products, but their potential has largely been untapped. To make microalgae exploitation economically feasible, sustainable, and competitive, creative and innovative upstream and downstream processing methods are required. There is a reasonable chance that microalgae with novel properties and uses will be discovered by bioprospecting in habitats that have not yet been explored.

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References

Ariede, M. B., Candido, T. M., Jacome, A. L. M., Velasco, M. V. R., de Carvalho, J. C. M., & Baby, A. R. (2017). Cosmetic attributes of algae-A review. *Algal Research*, *25*, 483-487.

Aslan, S., & Kapdan, I. K. (2006). Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. *Ecological Engineering*, *28*(1), 64-70.

Becker, W. (2004). Microalgae in human and animal nutrition, in Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Wiley Online Library Hoboken NJ, USA.

Biller, P., & Ross, A. B. (2014). Pyrolysis GC–MS as a novel analysis technique to determine the biochemical composition of microalgae. *Algal Research*, *6*, 91-97.

Bishop, W. M., & Zubeck, H. M. (2012). Evaluation of microalgae for use as nutraceuticals and nutritional supplements. *Journal of Nutrition & Food Sciences*, *2*(5), 1-6.

Caporgno, M. P., & Mathys, A. (2018). Trends in microalgae incorporation into innovative food products with potential health benefits. *Frontiers in Nutrition*, *5*, 58.

Chiu, S. Y., Kao, C. Y., Tsai, M. T., Ong, S. C., Chen, C. H., & Lin, C. S. (2009). Lipid accumulation and CO2 utilization of Nannochloropsis oculata in response to CO2 aeration. *Bioresource Technology*, *100*(2), 833-838.

De Morais, M. G., & Costa, J. A. V. (2007). Carbon dioxide fixation by Chlorella kessleri, C. vulgaris, Scenedesmus obliquus and Spirulina sp. cultivated in flasks and vertical tubular photobioreactors. *Biotechnology Letters*, *29*, 1349-1352.

Dineshbabu, G., Goswami, G., Kumar, R., Sinha, A., & Das, D. (2019). Microalgae–nutritious, sustainable aqua-and animal feed source. *Journal of Functional Foods*, *62*, 103545.

Galea-Holhoș, L. B., Delcea, C., Siserman, C. V., & Ciocan, V. (2023). Age estimation of human remains using the dental system: A review. *Annals of Dental Specialty*, *11*(3), 15.

Gouveia, L., & Oliveira, A. C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology*, *36*(2), 269-274.

Guillerme, J. B., Couteau, C., & Coiffard, L. (2017). Applications for marine resources in cosmetics. *Cosmetics*, *4*(3), 35.

Hama, S., & Kondo, A. (2013). Enzymatic biodiesel production: an overview of potential feedstocks and process development. *Bioresource Technology*, *135*, 386-395.

Hamed, I. (2016). The evolution and versatility of microalgal biotechnology: A review. *Comprehensive Reviews in Food Science and Food Safety*, *15*(6), 1104-1123.

Hu, F., Liu, W., Yan, L., Kong, F., & Wei, K. (2019). Optimization and characterization of poly (lactic-co-glycolic acid) nanoparticles loaded with astaxanthin and evaluation of anti-photodamage effect in vitro. *Royal Society Open Science*, *6*(10), 191184.

Illman, A. M., Scragg, A. H., & Shales, S. W. (2000). Increase in Chlorella strains calorific values when grown in low nitrogen medium. *Enzyme and Microbial Technology*, *27*(8), 631-635.

Jones, S. M., & Harrison, S. T. (2014). Aeration energy requirements for lipid production by Scenedesmus sp. in airlift bioreactors. *Algal Research*, *5*, 249-257. doi:10.1016/j.algal.2014.03.003

Koller, M., Salerno, A., Tuffner, P., Koinigg, M., Böchzelt, H., Schober, S., Pieber, S., Schnitzer, H., Mittelbach, M., &Braunegg, G. (2012). Characteristics and potential of micro algal cultivation strategies: a review. *Journal of Cleaner Production*, *37*, 377-388.

Leong, Y. K., & Chang, J. S. (2020). Bioremediation of heavy metals using microalgae: Recent advances and mechanisms. *Bioresource Technology*, *303*, 122886.

Macedo, R. V. T., & Alegre, R. M. (2001). Influence of the nitrogen level in the cultivation of spirulina maxima in two levels of temperatures: part II: production of lipids. *Food Science and Technology*, *21*(2), 183-186. doi:10.1590/S0101-20612001000200011

Madeira, M. S., Cardoso, C., Lopes, P. A., Coelho, D., Afonso, C., Bandarra, N. M., & Prates, J. A. (2017). Microalgae as feed ingredients for livestock production and meat quality: A review. *Livestock Science*, *205*, 111-121.

Marchetti, J., Bougaran, G., Jauffrais, T., Lefebvre, S., Rouxel, C., Saint-Jean, B., Lukomska, E., Robert, R., & Cadoret, J. P. (2013). Effects of blue light on the biochemical composition and photosynthetic activity of Isochrysis sp.(T-iso). *Journal of Applied Phycology*, *25*, 109-119.

Martínez-Ruiz, M., Martínez-González, C. A., Kim, D. H., Santiesteban-Romero, B., Reyes-Pardo, H., Villaseñor-Zepeda, K. R., Meléndez-Sánchez, E. R., Ramírez-Gamboa, D., Díaz-Zamorano, A. L., Sosa-Hernández, J. E., et al. (2022). Microalgae bioactive compounds to topical applications products—a review. *Molecules*, *27*(11), 3512.

Mata, T. M., Martins, A. A., & Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: a review. *Renewable and Sustainable Energy Reviews*, *14*(1), 217-232.

Miguel, S. P., Ribeiro, M. P., Otero, A., & Coutinho, P. (2021). Application of microalgae and microalgal bioactive compounds in skin regeneration. *Algal Research*, *58*, 102395.

Milano, J., Ong, H. C., Masjuki, H. H., Chong, W. T., Lam, M. K., Loh, P. K., & Vellayan, V. (2016). Microalgae biofuels as an alternative to fossil fuel for power generation. *Renewable and Sustainable Energy Reviews*, *58*, 180-197.

Moheimani, N. R. (2005). *The culture of coccolithophorid algae for carbon dioxide bioremediation* (Doctoral dissertation, Murdoch University).

Mutanda, T., Ramesh, D., Karthikeyan, S., Kumari, S., Anandraj, A., & Bux, F. (2011). Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production. *Bioresource Technology*, *102*(1), 57-70. doi:10.1016/j.biortech.2010.06.077

Nicoară, N. D., Marian, P., Petriș, A. O., Delcea, C., & Manole, F. (2023). A Review of the Role of Cognitive-Behavioral Therapy on Anxiety Disorders of Children and Adolescents. *Pharmacophore*, *14*(4), 35-39.

Nielsen, D. C. (2008). Oilseed productivity under varying water availability, in: *Proceedings of the 2008 Central Plains Irrigation Conference*, Greeley, Colorado, February 19-20. Colorado State University. Libraries.

Odjadjare, E. C., Mutanda, T., & Olaniran, A. O. (2017). Potential biotechnological application of microalgae: a critical review. *Critical Reviews in Biotechnology*, *37*(1), 37-52.

Pahl, S. L., Lee, A. K., Kalaitzidis, T., Ashman, P. J., Sathe, S., & Lewis, D. M. (2013). Harvesting, thickening and dewatering microalgae biomass. *Algae for Biofuels and Energy*, 165-185.

Passos, F., & Ferrer, I. (2014). Microalgae conversion to biogas: thermal pretreatment contribution on net energy production. *Environmental Science & Technology*, *48*(12), 7171-7178.

Pittman, J. K., Dean, A. P., & Osundeko, O. (2011). The potential of sustainable algal biofuel production using wastewater resources. *Bioresource Technology*, *102*(1), 17-25.

Popa-Nedelcu, R., Delcea, C., Siserman, C., & Carmen Domnariu, D. C. (2020). The relationship between personality disorders and domestic violence in forensic context. *Romanian Journal of Legal Medicine, 28*(2), 166-171.

Priatni, S., Ratnaningrum, D., Warya, S., & Audina, E. (2018). Phycobiliproteins production and heavy metals reduction ability of Porphyridium sp. In *IOP Conference Series: Earth and Environmental Science* (Vol. 160, p. 012006). IOP Publishing.

Ravindran, B., Gupta, S.K., Cho, W.M., Kim, J.K., Lee, S.R., Jeong, K.H., Lee, D. J., & Choi, H.C. (2016). Microalgae potential and multiple roles—current progress and future prospects—an overview. *Sustainability*, *8*(12), 1215.

Richardson, J. W., Johnson, M. D., & Outlaw, J. L. (2012). Economic comparison of open pond raceways to photo bio-reactors for profitable production of algae for transportation fuels in the Southwest. *Algal Research*, *1*(1), 93-100.

Richmond, A. (2003). Biological principles of mass cultivation. *Handbook of microalgal culture: Biotechnology and applied phycology*, 125-177.

Rodolfi, L., Chini Zittelli, G., Bassi, N., Padovani, G., Biondi, N., Bonini, G., & Tredici, M. R. (2009). Microalgae for oil: Strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor.*Biotechnology and Bioengineering*,*102*(1), 100-112.

Rosenberg, J. N., Oyler, G. A., Wilkinson, L., & Betenbaugh, M. J. (2008). A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. *Current Opinion in Biotechnology*, *19*(5), 430-436.

Saharan, B. S., Sharma, D., Sahu, R., Sahin, O., & Warren, A. (2013). Towards algal biofuel production: a concept of green bio energy development. *Innovative Romanian Food Biotechnology*, *12*, 1.

Stanic-Vucinic, D., Minic, S., Nikolic, M. R., & Velickovic, T. C. (2018). Spirulina phycobiliproteins as food components and complements. *Microalgal Biotechnology*, 129-149.

Tan, C. H., Show, P. L., Chang, J. S., Ling, T. C., & Lan, J. C. W. (2015). Novel approaches of producing bioenergies from microalgae: A recent review. *Biotechnology Advances*, *33*(6), 1219-1227.

Tibbetts, S. M., Milley, J. E., & Lall, S. P. (2015). Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. *Journal of Applied Phycology*, *27*, 1109-1119.

Voiţă-Mekereş, F., Delcea, C., Buhaş, C. L., & Ciocan, V. (2023). Novichok Toxicology: A Review Study. *Archives of Pharmacy Practice*, *14*(3), 62-66.

Vollmann, J., Moritz, T., Kargl, C., Baumgartner, S., & Wagentristl, H. (2007). Agronomic evaluation of camelina genotypes selected for seed quality characteristics. *Industrial Crops and Products*, *26*(3), 270-277.

Weissman, J. C., & Goebel, R. P. (1987). *Design and analysis of microalgal open pond systems for the purpose of producing fuels: a subcontract report* (No. SERI/STR-231-2840). Solar Energy Research Inst.(SERI), Golden, CO (United States).

Worku, A., & Sahu, O. (2014). Reduction of heavy metal and hardness from ground water by algae. *Journal of Applied & Environmental Microbiology*, *2*(3), 86-89.

Yan-Gui, S., Xin-Rong, L., Ying-Wu, C., Zhi-Shan, Z., & Yan, L. (2013). Carbon fixation of cyanobacterial–algal crusts after desert fixation and its implication to soil organic carbon accumulation in desert. *Land Degradation & Development*, *24*(4), 342-349.

Zhao, B., Ma, J., Zhao, Q., Laurens, L., Jarvis, E., Chen, S., & Frear, C. (2014). Efficient anaerobic digestion of whole microalgae and lipid-extracted microalgae residues for methane energy production. *Bioresource Technology*, *161*, 423-430.

Zheng, H., Gao, Z., Yin, F., Ji, X., & Huang, H. (2012). Lipid production of Chlorella vulgaris from lipid-extracted microalgal biomass residues through two-step enzymatic hydrolysis. *Bioresource Technology*, *117*, 1-6.