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Microalgal Biotechnology: Prospects and Applications

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1. Introduction

There is a current worldwide interest in finding new and safe antioxidants from natural sources such as plant material to prevent oxidative deterioration of food and to minimize oxidative damage to living cells [1]. Microalgae are photosynthetic microorganisms that are able to rapidly generate biomass from solar energy, CO$_2$ and nutrients in bodies of water. This biomass consists of important primary metabolites such as sugars, oils and lipids, for which process pathways exist for the production of high-value products including human and animal feed supplements, transport fuels, industrial chemicals and pharmaceuticals. Algal biomass and algae-derived compounds have a very wide range of potential applications, from animal feed and aquaculture to human nutrition and health products. Some algae are considered as rich sources of natural antioxidants. Although macroalgae have received much attention as potential natural antioxidants [2]. Furthermore, the qualities of the microalgal cells can be controlled, so that they contain no herbicides and pesticides, or any other toxic substances, by using clean nutrient media for growing the microalgae. The value of microalgae as a source of natural antioxidants is further enhanced by the relative ease of purification of target compounds. Reports on the antioxidant activity of microalgae are limited. Because cyanobacteria are largely unexplored, they represent a rich opportunity for discovery; the expected rate of rediscovery is far lower than for other better-studied groups of organisms Li et al. 2007 [3]. In this chapter, we focus on many desirable chemicals are the products of secondary metabolism triggered under conditions not conducive to fast growth. For those chemicals to be produced by microalgae, one needs to develop new strains (faster growth, higher substrate tolerance, etc.) by classical selection or genetic manipulation so microalgal biomass can be produced consistently. Highlight the role of dietary antioxidants and their potential benefits in health and disease directly or indirectly by the plant nutrition and animal feed to produce healthy organic food. Investigate the different biological activities of algae and the relations with its biochemical
composition, pigments and different constituents which may vary with salt stressed culture conditions and describe the antioxidant characteristics of algae.

2. What are microalgae?

Microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that produce carbohydrates, proteins and lipids as a result of photosynthesis. They can grow rapidly and live in harsh conditions due to their unicellular or simple multicellular structure. Examples of prokaryotic microorganisms are Cyanobacteria (Cyanophyceae) and eukaryotic microalgae are for example green algae (Chlorophyta) and diatoms (Bacillariophyta). Microalgae are present in all existing earth ecosystems, not just aquatic but also terrestrial, representing a big variety of species living in a wide range of environmental conditions. It is estimated that more than 50,000 species exist, but only a limited number, of around 30,000, have been studied and analyzed [4]. Sunlight, water, nutrients and arable land are the major requirements for growing algae. Microalgae have the ability to fix CO$_2$ using solar energy with efficiency 10 times greater than that of the terrestrial plants with numerous additional technological advantages. Algae are more efficient at utilizing sunlight than terrestrial plants, consume harmful pollutants, have minimal resource requirements and do not compete with food or agriculture for precious resources [5].

3. Algal metabolites

Metabolites are the intermediates and products of metabolism. The term metabolite is usually restricted to small molecules. A primary metabolite is directly involved in the normal growth, development, and reproduction. A secondary metabolite is not directly involved in those processes, but usually has important ecological function. The induction of secondary metabolism is linked to particular environmental conditions or developmental stages. Secondary metabolites are those chemical compounds in organisms that are not directly involved in the normal growth, development or reproduction of organisms. The exploration of these organisms for pharmaceutical purposes has revealed important chemical prototypes for the discovery of new agents, stimulating the use of sophisticated physical techniques and new syntheses of compounds with biomedical application. In this regard, both secondary and primary metabolisms have been studied as a prelude to future rational economic exploitation (Figure 1). The secondary metabolism is of restricted distribution, while the primary metabolism furnishes intermediates for the synthesis of essential macromolecules [6].

4. What are phytochemicals?

“Phyto” is the Greek word for plant. The term "phytochemicals" refers to a wide variety of compounds produced by plants. Phytochemicals are chemical compounds formed during the plants normal metabolic processes. There are many “families” of phytochemicals and they help the human body in a variety of ways. Phytochemicals may protect human from a host of diseases. These chemicals are often referred to as “secondary metabolities” of which
there are several classes including alkaloids, flavonoids, coumarins, glycosides, gums, polysaccharides, phenols, tannins, terpenes and terpenoids. Phytochemicals are naturally occurring, nonnutritive chemicals. They appear to work alone and in combination, and perhaps in conjunction, with vitamins [8].

5. Microalgal bioactive compounds

Microalgae are significant resource for bioactive metabolites, particularly cytotoxic agents with applications in cancer chemotherapy. From the marine microalgae such as from the blooms of Phaeocystis sp., antibiotic substances were listed. Phaeocystis pouchetii is reported to produce chemicals such as acrylic acid, which constitutes about 7.0% of the dry weight. The antibiotic substances thus produced are transferred throughout the food chain and found in the digestive tract of Antarctic penguins. Production of β carotene and vitamins by the halotolerant alga Dunaliella sp. is documented. These compounds have much importance for the Mariculture activities [9]. Cyanobacteria have been identified as one of the most promising group of organisms from which novel and biochemically active natural products are isolated. Cyanobacteria such as Spirulina, Anabaena, Nostoc and Oscillatoria produce a great variety of secondary metabolites. Cyanobacteria produce a wide variety of bioactive compounds, which include 40% lipopeptides, 5.6% amino acids, 4.2% fatty acids, 4.2% macrolides and 9% amides. Cyanobacterial lipopeptides include different compounds like cytotoxic (41%), antitumor (13%), antiviral (4%), antibiotics (12%) and the remaining 18% activities include antimalarial, antimycotics, multi-drug resistance reversers, antifeedant, herbicides and immunosuppressive agents [7]; besides the immune effect, blue green algae improves metabolism. Cyanobacteria are also known to produce antitumor, antiviral,
antifungal compounds and have a cholesterol-lowering effect in animals and humans [10]. Many of the pharmaceutically interesting compounds in cyanobacteria are peptides, including cyanobacterial toxins and important candidates for anti-cancer drugs. Peptide synthetases are common in cyanobacteria and responsible for the production of cyanobacterial hepatotoxins and other peptides. Polyketide synthetases are also involved in the biosynthesis of certain cyanobacterial bioactive compounds (e.g. microcystins). A number of extracts were found to be remarkably active in protecting human lymphoblastoid T-cells from the cytopathic effects of HIV infection. Active agents consisting of sulfolipids with different fatty acid esters were isolated from *Lyngbya lagerheimii* and *Phormidium tenue*. Cyanovirin is a protein isolated from an aqueous cellular extract of *Nostoc clippusporum* prevents the in vitro replication and cytopathicity of primate retroviruses. Cryptophycin 1, an active compound isolated from *Nostoc* strain, exerts antiproliferative and antimitotic activities by binding to the ends of the microtubules, thus blocking the cell cycle at the metaphase of mitosis. Research has been focused on its potent antitumor activity and a synthetic analogue, cryptophycin-52, is at present in Phase II clinical trials. Sulfated polysaccharide, calcium spirulan. A novel water soluble extracts of cyanobacteria have found to be an antiviral agent. This compound appears to be selectively inhibiting the penetration of enveloped viruses into host cells, thereby preventing the replication. The effect was described for many different viruses like herpes simplex, measles, and even HIV-1. Among eukaryotic microalgae, a glycoprotein prepared from *Chlorella vulgaris* culture supernatant exhibited protective activity against tumor metastasis and chemotherapy-induced immunosuppression in mice [11]. Hereafter, a brief discuss of the commercial application of the most explored compounds from algae and the biosynthetic pathways of fatty acids, steroids and carotenoids.

5.1. Fatty Acids (FA)

Microalgae include essential fatty acids (EFAs) such as linoleic, arachidonic, linolenic, 3-linolenic acids etc. that must be in diet for healthy growth. These acids cannot be synthesized fast enough by body to meet needs [12]. Fatty acids are structural components of many lipids, and the types and amounts of fatty acids vary considerably among algae. In recent years, fatty acids compositions in large scale production of microalgae including marine algae have created considerable interest among researchers. This is mainly because of the health benefit of mono and polyunsaturated fatty acids (MUFA and PUFA) that can be found in plants including microalgae. Moreover, polyunsaturated fatty acids (PUFAs) play key roles in cellular and tissue metabolism, including the regulation of membrane fluidity, electron and oxygen transport, as well as thermal adaptation [13]. The biosynthesis of EPA occurs through a series of reactions that can be divided into two distinct steps. First is the de novo synthesis of oleic acid (18:1 ω9) from acetate, followed by conversion to linoleic acid (18:2 ω-6) and α-linolenic acid (18:3 ω-3). The subsequent stepwise desaturation and elongation steps form an ω-3 PUFA (Fig. 2). Inside the cell, EPA is normally esterified (by cyclooxygenase and lipoxygenase activities) to form complex lipid molecules and plays an important role in higher animals and humans as the precursor of a group of eicosanoids,
hormone-like substances such as prostaglandins, thromboxanes and leukotrienes that are crucial in regulating developmental and regulatory physiology (Figure 2) [14]. Consumption of n-3 PUFAs from both seafood and plant sources may reduce coronary heart disease (CHD) risk as reported by Mozaffarian et al. [15] in a cohort study of 45,722 men. Thus, many health supplement stores now sell preparation of microalgae such as *Spirulina* and *Chlorella* packed in capsule or caplets, or even in food and beverages known to have therapeutic values in treating hypercholesterolemia, hyperlipidaemia and atherosclerosis [16]. The fatty acid contents of microalgae are influenced by the environmental and cultural condition selected for its growth [17]. Some of the environmental conditions include heterotrophic, photoautotrophic and nitrogen deprivation or stimulation. Although some microalgae species are cultivated as sources of these fatty acids, transgenic algae engineered to produce EPA, like transgenic oilseed crops, could provide an alternative sustainable source of oil for human consumption [18]. However, the possibility for deploying transgenic organisms nutritionally enhanced with EPA is currently limited by continued consumer antipathy to transgenic food products. One alternative would be to use EPA from transgenic algae as a high potential food source in aquaculture. In this way, the significant health benefits of these fatty acids could be delivered into the human diet, without the requirement of direct ingestion of genetically modified food.

**Figure 2.** A simplified biosynthesis scheme of eicosapentaenoic acid and eicosanoid (prostaglandins, thromboxanes, leukotrienes) modified from Sayanova and Napier [19].

### 5.2. Sterols

Sterols are one of the most important chemical constituents of microalgae [20]. Sterols are the main component of eukaryote organisms and different classes of organisms have divergent sterols patterns. It is because of this that sterols act as a fingerprint for organic
matter input into an aquatic environment. Furthermore, sterols have a relatively high resistance to degradation when settled in anoxic sediments and persist in the environment for a longer period of time. Of all the sterol compounds, cholesterol is the most abundant and ubiquitous one in the environment, which is due to it having a variety of sources [21]. Most biologically produced sterols are planar 3β-hydroxy tetracyclic structures commonly containing a methyl- or ethyl-substituted C7-C11 hydrocarbon side chain, and exhibiting a range of methyl-substitution (C4, C14) patterns on the polycyclic nucleus with varying degrees and positions of unsaturation (C5, C7, C8). The rigid structure of the sterols (Figure 3), caused by the fused ring system, provides the cell membrane integrity and stability thus, holds the membrane together. In general, there is not a specific sterol that can be uniquely linked to one algal source. Many of the sterols previously discussed are also found in other groups of algae [22].

Figure 3. Some sterols found in marine and freshwater microalgae (modified from Ponomarenko et al. [34])

5.3. Pigmentation in aquacultures

Astaxanthin (Figure 4) is a red pigment common to several aquatic organisms including microalgae, seagrasses, shrimp, lobsters and fish such as salmon and trout. Crustaceans are unable to synthesize carotenoids de novo and require astaxanthin (or appropriate
precursors) in their diet in order to acquire the adequate color for seafood market acceptance [23]. Several natural sources—such as the algae *Dunaliella salina* and *Spirulina maxima*—or synthetic β-carotene, canthaxanthin and astaxanthin have been used for this purpose. Astaxanthin is, in fact, one of the most expensive components of salmon farming, accounting for about 15% of total production costs [48]. Among the several natural sources of astaxanthin applied in aquaculture, the green unicellular freshwater alga *Haematococcus pluvialis* has been explored by biotechnology companies [24].

Figure 4. Chemical structure of β-carotene and of the xanthophylls astaxanthin and lutein, main carotenoids from microalgae with commercial interest

β-Carotene is one of the important members of the family of carotenoids; a group of natural fat-soluble stereoisometric pigments. β-Carotene shows pro-vitamin A activity and as such it plays an important role in the human body [25]. β-Carotene can be also used as a coloring agent. Therefore, β-carotene has several applications in food, pharmaceuticals and cosmetics. The great demand of β-carotene has been met by industry, mainly by synthetic production. Increasing demand for natural carotenoids has resulted in growing interest in extracting β-carotene from different natural sources. *Dunaliella salina* is the main source for the natural β-carotene in the market. The estimated market size for natural β-carotene is 10-100 tonnes/year and its price is >750 €/Kg [26]. In addition, β-carotene (like other carotenoids) is a strong antioxidant, scavenging potentially harmful oxy radicals, which are commonly associated with the induction of certain cancers (Leach et al., 1998) and there is an inverse relation between the consumption of certain carotenoids and the risk of cancer [25]. The demonstrated antioxidant activity of carotenoids is the basis of the protective action of these compounds against oxidative stress in many organisms and situations. Effects of carotenoids on human health are, in general, associated with their antioxidant properties. Notwithstanding, not all of the biological activities ascribed to carotenoids must be necessarily linked to their ability to prevent accumulation of free radicals and reactive oxygen species. The halophilic green biflagellate microalga *Dunaliella salina* has since long been recognized as an efficient biological source of this carotenoid. Many epidemiological studies suggest that humans fed on a diet high in β-carotene from *Dunaliella*, which maintains higher than average levels of serum carotenoids, have a lower incidence of several types of cancer and degenerative diseases [27]. The xanthophyll astaxanthin has many applications in nutraceuticals, cosmetics, and food and feed industries. Recently, a variety of
additional potential applications of this carotenoid, mainly related to human health and nutrition properties, have been claimed [28]. Lutein is among the most important carotenoids in foods and human serum and, together with zeaxanthin, is the essential component of the pigment present in the macula lutea (or yellow spot) in the eye retina and in the eye lens. Lutein is used as food dyes and especially as feed additives in aquaculture and poultry farming. During the last few years, additional applications for lutein have received considerable interest, especially those related to human health. Mainly on the basis of epidemiological studies, lutein is currently considered as effective agent for the prevention of a variety of human diseases. The microalga *Muriellopsis* sp. and other chlorophycean species are able to accumulate lutein as a part of their biomass. An established commercial system for the production of lutein from microalgae does not exist yet, although the basis for outdoor production of lutein-rich cells of strains of *Muriellopsis* and *Scenedesmus* at a pilot scale has already been set up [29].

5.4. Mycosporine-like amino acids

A remarkable group of marine natural products are the mycosporine-like amino acids (MAAs). An outstanding characteristic of these compounds is their high UV absorption with molar absorptivities ($\epsilon$) of around 40 000 l mol$^{-1}$ cm$^{-1}$ (e.g. Takano et al. [30]). MAAs are water-soluble, low molecular-weight (generally <400) compounds composed of either an aminocyclohexenone or an aminocyclohexenimine ring, carrying nitrogen or amino alcohol substituents [31]. They are found in a wide variety of marine, freshwater and to a smaller degree in terrestrial organisms. There is limited evidence that MAAs are derived from early steps of the shikimate pathway. However, the biochemical pathway of MAA synthesis is still largely unknown, as well as its genetic base. The most primitive organisms capable of MAA synthesis are cyanobacteria [32].

6. Biological activity of microalgae

Many of the microalgal metabolites have chemical structure and possess interesting biological activity. Microalgae are a unique source of therapeutic substances, particularly from cyanobacteria. Among cyanobacteria *Spirulina* sp. has undergone numerous and rigorous toxicological studies that have highlighted its potential therapeutic applications in the area of immunomodulation, anticancer, antiviral, and cholesterol reduction effects.

6.1. Antioxidant activity

Hydrogen peroxide is a product of microalgae and plants through of photosynthesis, photorespiration, respiration and other metabolic processes, as result from the enzymatic activity of glycolate oxidase, urate oxidase and amino acid oxidase. However, major pathway for production of H$_2$O$_2$ is conversion from superoxide (O$_2^-\$) produced through the transfer of an electron from ferredoxin of photosystem I (PSI) to O$_2$ (Mehler reaction) by the action of Superoxide Dismutase (SOD). However, it is suspected that those antioxidants are responsible for some side effects such as liver damage and carcinogenesis. Antioxidants can
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involve with the oxidation process by scavenging free radicals, chelating catalytic metals and by acting as oxygen scavengers [33]. Recently many researchers are interested in finding any natural antioxidants having safety and effectiveness, which can be substituted for current and commercial synthetic antioxidants, BHA and BHT. Microalgae have become good candidates for sources of natural antioxidants, as revealed by a number of recent studies [34-35]. Algae contain several enzymatic and nonenzymatic antioxidant defense systems to maintain the concentration of ROS (O


peroxide and H


O


2


) to protect cells from damage [36]. The main cellular components susceptible to damage by these ROS are lipids (peroxidation of poly-unsaturated fatty acids in membranes), proteins (denaturation), carbohydrates and nucleic acids. The essential for ROS detoxification during normal metabolism and particularly during stress, are antioxidant defenses system [37]. The primary scavenging enzymatic defenses system include SOD, catalase (CAT) and glutathione peroxidase, (GPX) and peroxiredoxin (PrxR) [38]. These enzymic detoxification system involving the action of SOD and reductase, either quench toxic compounds or regenerate antioxidants with the help of reducing power provided by photosynthesis [39]. However, at low levels, H


2


 resulted in induction of defense genes such as glutathione S-transferase and glutathione peroxidase. The hydrophilic antioxidants AA and GSH effectively scavenge oxygen radicals. Carotenoids and TOH remove ROS directly from the pigment bed [40]. Also, Foyer and Noctor [41] reported that the changes in ROS, fluctuations in the antioxidants concentrations in photosynthetic cells might have important consequences not only for defense metabolism but also for the regulation of genes associated with adaptive responses. Several bioactive metabolites produced by cyanobacteria and algae have been discovered by screening programs, employing target organisms quite unrelated to those for which the metabolites evolved [42]. Shanab et al. [43] studied the antioxidant activity of aqueous extracts of nine microalgal species namely, *Nostoc muscorum*, *Anabaena flos aquae*, *Anabaena oryzae*, *Nostoc humifusum*, *Oscillatoria* sp., *Spirulina platensis*, *Phormidium fragile*, *Wollea saccata* and *Chlorella vulgaris*. Antioxidant activity of the algal extracts was performed using 2,2 diphenyl-1-picrylhydrazyl (DPPH) test and 2,2'-azino-bis(ethylbenzthiazoline-6-sulfonic acid (ABTS) radical action assay which revealed higher antioxidant activity than DPPH method. Concerning DPPH, the antioxidant activity of nine tested algal species ranged between 30.1 and 72.4% comparing with the standard antioxidant BHT (80.2%). Using ABTS method, which was more sensitive than the DPPH method (Figure 5), the antioxidant activity ranged between 31.2 and 75.9% (Standard BHT showed 85.6%); *Spirulina platensis*, *Oscillatoria* sp., *Anabaena flos-aqua* and *Nostoc muscorum* recorded the highest (75.9, 75.6, 73.6 and 72.8%, respectively) antioxidant activity which is could be attributed to the extracellular and intracellular secondary metabolites content (Total phenolic content, terpenoids and alkaloids) of these microalgae (Tables 1,2). The extracellular phytochemicals metabolites (%) released in the algal cultures show large variability. *Anabaena oryza*, *Phormidium fragile* and *Wollea saccata* (Table 1) recorded the highest extracellular total phenolic compounds (0.0085, 0.0078 and 0.0074%, respectively). The highest terpenoids contents were recorded by *Phormidium fragile*, *Spirulina platensis* and *Wollea saccata* (0.0055, 0.0050 and 0.0049%, respectively). The maximum values of the extracellular alkaloids were recorded by *Anabaena oryza*, *Phormidium fragile*, *Anabaena oryza*, *Spirulina platensis* and *Phormidium fragile* (0.075, 0.068 and 0.068%, respectively). While,
the highest percentages of these metabolities complaining with the released extracellularly metabolities, *Spirulina platensis*, *Nostoc muscorum* and *Oscillatoria* sp. recorded the greatest intracellular total phenolic compounds (0.71, 0.6 and 0.55% respectively), while *Wollea saccata* showed the least content (0.1%) as shown in Table (2).

Concerning terpenoids, *Anabaena flos aquae*, *Spirulina platensis* and *Wollea saccata*, recorded the highest contents (0.15, 0.14 and 0.14% respectively). Alkaloids determination in algal cultures showed that *S. platensis*, *Oscillatoria* sp. and *Chlorella vulgaris* showed the highest contents (3.02, 2.6 and 2.45% respectively). Phycobiliprotein pigments (Table 3) were determined in water extracts of the tested algal species. Normally, phycobilin pigments in cyanobacteria comprised phycocyanin, allophycocyanin and phycoerytherin (the blue, gray and red colors respectively). *Phormidium fragile* recorded the higher C-phycocyanin (CPC) content (0.13 mg/ml) while *Anabaena oryzae* and *A. flos aquae* recorded the least and absence of phycocyanin (0.0089 and 0.0 mg/l respectively).

![Figure 5. Antioxidant activity of algal water extracts at 100 g/ml against DPPH (a) and ABTS (b) radicals](image)

<table>
<thead>
<tr>
<th>Table 1. Secondary metabolites (as mg/100 g) in algal filtrates (extracellular) [43]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metabolites</td>
</tr>
<tr>
<td>T. phenolic</td>
</tr>
<tr>
<td>Terpenoids</td>
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<tr>
<td>Alkaloids</td>
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<th>Table 2. Secondary metabolites (as %) in algal cells (intracellular) [43]</th>
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<td>Metabolites</td>
</tr>
<tr>
<td>T. phenolic</td>
</tr>
<tr>
<td>Terpenoids</td>
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<tr>
<td>Alkaloids</td>
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<tr>
<td>LSD 0.05</td>
</tr>
</tbody>
</table>
Table 3. Phycobilins pigments (mg/ml) in different aqueous filtrate of some microalgae [43]

<table>
<thead>
<tr>
<th>Algal species</th>
<th>CPC</th>
<th>APC</th>
<th>CPE</th>
<th>Total phycobilins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nostoc muscorum</td>
<td>0.089±0.010</td>
<td>0.140±0.020</td>
<td>0.0±0.0</td>
<td>0.229±0.020</td>
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<tr>
<td>Anabaena flos aquae</td>
<td>0.000±0.000</td>
<td>0.017±0.000</td>
<td>0.0±0.0</td>
<td>0.017±0.000</td>
</tr>
<tr>
<td>Chlorella vulgaris</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Oscillatoria sp</td>
<td>0.021±0.000</td>
<td>0.001±0.000</td>
<td>0.0±0.0</td>
<td>0.022±0.000</td>
</tr>
<tr>
<td>Spirulina platensis</td>
<td>0.030±0.000</td>
<td>0.113±0.000</td>
<td>0.0±0.0</td>
<td>0.143±0.000</td>
</tr>
<tr>
<td>Anabaena oryzae</td>
<td>0.009±0.000</td>
<td>0.002±0.000</td>
<td>0.0±0.0</td>
<td>0.011±0.000</td>
</tr>
<tr>
<td>Wollea sacca</td>
<td>0.002±0.000</td>
<td>0.033±0.000</td>
<td>0.0±0.0</td>
<td>0.035±0.000</td>
</tr>
<tr>
<td>Nostoc humifusum</td>
<td>0.012±0.000</td>
<td>0.019±0.000</td>
<td>0.0±0.0</td>
<td>0.031±0.000</td>
</tr>
<tr>
<td>Phormedium fragile</td>
<td>0.130±0.010</td>
<td>0.050±0.000</td>
<td>0.0±0.0</td>
<td>0.160±0.030</td>
</tr>
<tr>
<td>LSD 0.05</td>
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<td>0.0057</td>
<td></td>
<td>0.003</td>
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</table>

*Nostoc muscorum* contained the greatest allophycocyanin (APC) pigment (0.14 mg/l), while, *Oscillatoria* sp have the least content (0.001 mg/l). Concerning total phycobilin pigments (phycocyanin and allophycocyanin), *Nostoc muscorum*, *Phormedium fragile* recorded the highest contents (0.229 and 0.16 mg/l) followed by that of S. platensis (0.143 mg/ml), while *Anabaena oryzae* showed the least total phycobilin pigment content (0.011 mg/l). *Chlorella vulgaris* is a green alga which have other pigments (Chlorophyll and carotenoids) than the phycobilins (absent).

Aqueous extracts of the tested algal species showed wide range of colours (green, blue, violet, pink, light-blue) in spite of the fact that eight of the tested algae were of cyanobacteria and only one species was a green alga, their water extracts showed highly variable colors (Figure 6) which may be attributed in part to their phycobiliprotein constituents (ratios of phycocyanin to allophycocyanin and approximately absence of phycoerytherin pigments), and in part to the produced major polar secondary metabolites. All these substances may not only caused the alteration of the pH values of the algal aqueous extracts, but also the induced biological activities which may be attributed to the synergistic effects of these compounds. The aqueous extract of the tested algal species (8 cyanobacteria and one green alga) have variable colors (Figure 6) ranging from green, violet, blue, light blue and pink color, which can be used as an additive coloring agents to different food products (natural, non-toxic) instead of the synthetic coloring substances which may be carcinogenic [43].

![Figure 6. Color of aqueous extracts of the different microalgae](image-url)
Phycobiliprotein pigments were known by its antioxidant activity [44], increasing of these pigments production as a result of doubling nitrate concentration in the growth culture media, led to a progressive increase in the antioxidant activity recorded by both DPPH and ABTS assays in the two cyanobacteria under investigation. Keeping in mind that, synergetic effect occurred between the polar secondary metabolites especially the phenolic compounds and the polysaccharides in antioxidant activity. Increasing nitrate concentrations in the culture media of both cyanobacteria species (N. muscorum and Oscillatoria sp) led to a marked enhancement in phycobiliprotein production (Table 4) which was translated in an obvious increase in antioxidant activity (by DPPH and ABTS) in both species under study while decreasing the nitrate content, phycobilin pigments production were consequently decreased in both species and its complete absence was recorded on nitrogen starvation especially in case of Oscillatoria sp., and no allophycocyanin pigments were produced as found by Shanab et al.[43]. They also investigated that increasing nitrate conc. and the consequent increase in phycobilin pigments production, have the major role in enhancing the antioxidant activity may be attributed. The decrease in nitrate conc. was followed by an obvious decrease in phycobilin pigment and even an absence of one of its constituents on nitrate starvation. The antioxidant activity in both species (by both assays) was apparently not affected comparing with the control (1.5 g/l nitrate). Under stress conditions, it was known that, deviation in metabolic pathways may occur. In presence of nitrate, nitrogenous compounds, including the phycobilin pigments were increasingly produced leading, together with other antioxidant active secondary metabolites (as phenolics), to a marked increase in biological activity.

**Table 4.** Antioxidant activity of the nitrogen stressed promising algal species using DPPH and ABTS radicals [43]

The decrease in nitrate content induced a stress condition and not only a decrease in nitrogen skeleton compounds as phycobilin pigment production, but an increase in the carbon skeleton compounds (as phenolics) as a result of metabolic alterations under these stress conditions. So on decreasing nitrate content, the antioxidant activity remain at a level comparable or even higher than the control due to the synergistic effect of the phycobilin pigment and the phenolic compounds produced in excess under stress nitrate condition which have high redox potentials. On nitrogen starvation the recorded antioxidant activity
Comparable to those in presence of high nitrate content (6-9 g/l) was largely due to the high production of the carbon skeleton compounds (phenolic compounds) which show potent antioxidant activity [45]. Shalaby et al. [46] stated that cultivation of *Spirulina platensis* under salt stress conditions (0.02 M as control), 0.04 and 0.08 M NaCl led to a remarkable alteration of algal metabolism as well as an enhancement or induction of biologically active compounds. Biochemical analysis of salt stressed algal revealed that lipid content was slightly increased together with certain saturated and unsaturated fatty acids especially the polyunsaturated ones (γ-linolenic acid, omega 3 fatty acid).

6.2. Anticancer activity

Today cancer is the largest single cause of death in men and women, and chemoprevention has been a promising anticancer approach aimed at reducing the morbidity and mortality of cancer by delaying the process of carcinogenesis. A variety of compounds from nature sources have been shown to be beneficial for the inhibition of cancer, such as flavonoids, phenolic acids, carotenoids, etc.; the mechanisms which suppress tumorgenesis often involve inhibition of tumor cell mediated protease activity, attenuation of tumor angiogenesis, promotion of cell cycle arrest, induction of apoptosis and immunostimulation, etc. In addition, Chinery et al. [47] also reported their use with the chemotherapy agents 5-fluorouracil and antioxidants could cause complete remissions in colorectal cancer, where only partial remission is possible with chemotherapy agents only; therefore, antioxidants have been proposed to have potential for the prevention and treatment of diseases associated with active oxygen species, especially in cancer diseases. Moreover, experimental and epidemiological evidence suggests that anti-inflammatory drugs may also decrease the incidence of mammary cancer, tumor burden, and tumor volume [48]. The medicinal value of cyanobacteria was appreciated as early as 1500 BC, when strains of Nostoc were used to treat gout, fistula and several forms of cancer. Cyanobacteria are a rich source of potentially useful natural products. Over 40 different Nostocales species, the majority of which are *Anabaena* and Nostoc spp. Produce over 120 natural products (Secondary metabolities) having activities such as anti-HIV anticancer, antifungal, antiinflammatory and antimicrobial. Cyanovirin (CV-N, cyanovirin-N), a 101 amino acid protein extracted from *Nostoc ellipsosporum* was found to have potent activity against all human immunodeficiency viruses such as HIV-1, M and Tropic strains of HIV-1, HIV-2, SIV (Simian), and FIV (Feline) [7]. The cosmopolitan distribution of cyanobacteria indicates that they can cope with a wide spectrum of global environmental stress, such as heat, cold, desiccation, salinity, nitrogen starvation, photooxidation, anaerobiosis and osmotic stress. They have developed a number of mechanisms by which cyanobacteria defend themselves against environmental stressors. Important among them are the production of photoprotective compounds such as mycosporine-like amino acids (MAAs) and Scytonemin enzymes such as superoxide dismutase, catalase and peroxidases repair of DNA damage and synthesis of shock proteins [49]. Shanab et al. [43] investigated anticancer efficiency of the algal water extracts against Ehrlich Ascites Carcinoma cell (EACC) and Human hepatocellular cancer cell line (HepG2). Anticancer efficiency of the algal water extracts was investigated against Ehrlich
Ascites Carcinoma cell (EACC) and Human hepatocellular cancer cell line (HepG2). The anticancer efficiency of the algal aqueous extracts illustrated in Figure (7) and using EACC and HepG2 cell lines, recorded that the anticancer activity ranged between 15.68 to 87.25% in case of EACC cell line and from 9.5 to 89.4% using HepG2 cell line Nostoc muscorum aqueous extracts recorded the highest anticancer activity in both cell lines (87.25% in case of EACC and 89.4% in case of HepG2), followed by Oscillatoria sp. (67.40 and 77.8% in EACC and HepG2 respectively). In case of N. muscorum, the anticancer activity against EACC cell line ranged between 83.0 and 90.4% at all nitrate concentrations (increase and decrease) compared to the control (85.9%). Comparable anticancer activity was recorded at both the highest nitrate conc and starvation (90.4 and 89.9% respectively). The anticancer activity against HepG2 cell line recorded more or less comparable activities were recorded at most nitrate conc compared to the control (85.6, 86.9, 88.7 and 88.6% at 3, 6, 9 and 1.5 g/l). At nitrate starvation the highest anticancer activity against HepG2 cell line was recorded (92.3%). In case of Oscillatoria sp., the anticancer activity against EACC and HepG2 recorded an increase in activity on both increasing and decreasing nitrate conc compared with the control. Higher activity was recorded against both cell lines at higher nitrate conc (82.6 and 75.9% in case of EACC and HepG2 respectively) and at nitrate starvation (82.9 and 82.0% respectively) compared to the control (68.3 and 70.4% against EACC and HepG2 respectively). Water extracts of the tested promising algal species demonstrated higher anticancer efficiencies against both EACC and HepG2 cell lines (87.25 and 89.4% respectively) in case of N. muscorum and 67.40 and 77.8% in case of Oscillatoria sp.). Under stress nitrogen conditions, these two cyanobacteria species recorded higher anticancer activities on excess limitation or starvation of nitrate comparing with its normal content in growth media.

Figure 7. (a, b). Anticancer activity of algal water extracts at 100 µg/ml against EACC (a) and HepG2 (b) cell lines [43]
The recorded maximum activity in both species against both cell lines at the highest nitrate content (9 g/L) may be attributed mainly to the higher content of the phycobiliprotein pigments produced under excess nitrate contents. Nitrate limitation and starvation, in spite of the caused decrease in phycobilin pigment production due to metabolic alteration expected under stress conditions, the carbon skeleton compounds as phenolic may replace phycobilin shortage in inducing similar anticancer activity of or even higher efficiency caused by great phycobilin contents at higher nitrate supplementation [43]. These results demonstrated that the compounds responsible for anticarcinogenic activity was highly polar as the phycobilins, phenolic compounds and polysaccharides which induced apoptosis of the cancer cells as reported by Aboul-Enein et al [50], which go parallel with these results coincides with the results obtained by Wang et al [51] who reported that the aqueous extract of red algae mainly contain c-phycocyanin, exhibited higher antiproliferation inducing apoptosis body formation. The authors explained that phycocyanin interact with membrane associated B-tubulin and glyceraldehydes-3-phosphate dehydrogenase (GAPDH), caused polymerization of microtubules and actins filaments leading to arrested the cell cycle at G0/G1 phase. As these aqueous extracts exhibited antioxidant and anticancer activities, its effect as coloring agent is amplified by these biological efficiencies which are very important for human health. Also, it can be used for the manufacture of pharmaceutical drugs (antioxidant and anticancer).

Table (5) recorded the anticancer efficiency of nitrate stressed N. muscorum and Oscillatoria sp. against EACC and HepG2 cell lines. The promising cyanobacterial species N. muscorum and Oscillatoria sp. induced both the highest antioxidant (by DPPH and ABTS methods) and anticancer activities (using EACC and HepG2 cell lines) which may be attributed to their large content in total phycobiliprotein pigments together with the higher secondary metabolites content (phenolic compounds, terpenoids, alkaloids [43].

6.3. Antimicrobial activity

The antimicrobial activity of microalgae has been attributed to compounds belonging to several chemical classes –including indoles, terpenes, acetogenins, phenols, fatty acids and volatile halogenated hydrocarbons [52] for instance, the antimicrobial activity of supercritical extracts obtained from the microalga Chaetoceros muelleri were related to its lipid composition [53]. However, the antimicrobial activity detected in several pressurized extracts from Dunaliella salina may be explained not only by several fatty acids, but also by such compounds as - and -ionone, -cyclocitral, neophytadiene and phytol. Efforts to identify the compounds directly responsible for those antimicrobial features –e.g. chlorellin [54] have been on the run, but are still relatively incipient owing the some new classes of compounds found. Microalgal cell-free extracts are already being tested as additives for food and feed formulation, in attempts to replace antimicrobial compounds of synthetic origin currently in use – including subtherapeutical doses of antibiotics employed as prophilatic measure in animal breeding [55].

Recall, in this regard, the growing resistance of some bacterial strains arising from the widespread and essentially unrestricted use of antibiotics in cattle handling, and by domestic
consumers use via self-prescription [56]. However, a key factor for their eventual economic feasibility is the possibility of operating large photobioreactors under aseptic conditions, which are able to produce biomass and metabolites to sufficiently high levels [57].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Oscillatoria sp.</th>
<th>EACC</th>
<th>HepG2</th>
<th>EACC</th>
<th>Nostoc musorum</th>
<th>EACC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1.5 g/L NaNO₃)</td>
<td>70.4±1.87</td>
<td>68.30±0.58</td>
<td>88.60±2.96</td>
<td>85.90±3.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 g/L</td>
<td>72.10±2.60</td>
<td>69.70±1.64</td>
<td>85.60±2.30</td>
<td>84.60±4.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>70.60±0.80</td>
<td>72.60±2.34</td>
<td>86.90±1.80</td>
<td>85.60±2.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>75.90±2.61</td>
<td>82.60±4.65</td>
<td>88.70±0.95</td>
<td>90.40±4.82</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.75</td>
<td>72.60±3.05</td>
<td>68.30±0.72</td>
<td>86.70±1.00</td>
<td>83.00±1.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.37</td>
<td>74.80±2.50</td>
<td>78.50±5.96</td>
<td>86.00±2.70</td>
<td>84.00±0.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>82.00±4.85</td>
<td>82.90±3.40</td>
<td>92.30±1.65</td>
<td>89.90±1.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>0.57</td>
<td>0.907</td>
<td>1.65</td>
<td>0.905</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5. Anticancer activity of the nitrate stressed promising algal species using EACC and HepG2 cell lines [43]

6.3.1. Antiviral activity

A number of infectious diseases caused by viruses have emerged (and re-emerged) in recent years. Although several antiviral drugs have been specifically developed, drug-resistant mutations are constantly occurring – so new antiviral active principles are necessary, especially those from sources that do not constitute (or are exposed to) viral pools. This is why microalgae have received a strong attention as potential suppliers of antiviral agents [58]; Viral growth is generally divided into three stages, and antiviral action may take place at a single or more stages: Stage I, which consists on adsorption and invasion of cells; Stage II, or eclipse phase, during which the cell is forced to synthesize multiple copies of said virus; and Stage III, or maturity and release of virus particles. For instance, the anti-HSV activity of the antiviral compound acyclovir® is expressed at stage II, but the anti-HSV factor from *Dunaliella* sp. inactivates the viral function at stage I. Sulphated exopolysaccharides from marine microalgae have been claimed to interfere with Stage I of some enveloped viruses they offer competitive advantages because of their broad antiviral spectrum against e.g. HSV and HIV-1 [59]. Apparently, their inhibitory effect arises from interaction with the positive charges on the virus or on the cell surface – which prevents penetration of the former into the host cells; they may also selectively inhibit reverse transcriptase in the case of HIV, thus hampering production of new viral particles after infection yet the exact step during viral replication when they act remains to be elucidated. Antiviral highly sulfated polysaccharides from several species of red microalgae consist mainly of xylose, glucose and galactose [60]; they are unusually stable when exposed to extreme pH and temperature [61]. Despite their successful antiviral performance, the metabolic pathways leading to sulfated polysaccharides are still poorly known. Their secretion by unicellular red algae was originally characterized via radiolabeling – which showed biosynthesis of the carbon chain, and sulfation of the resulting polysaccharide to
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occur in the Golgi apparatus [62]; these findings were confirmed in Porphyridium sp. [63] and other red microalgae [64]. More recently, [65] used 14C pulse-chase experiments and ultrastructural microscopy to conclude that brefeldin A—a membrane-traffic inhibitor of the Golgi apparatus, decreases the contents of the bound and the soluble forms of polysaccharides, while inhibiting cell-wall binding of polysaccharides to a greater extent than its soluble counterpart (in both actively growing and resting cells). Discovery of small molecules that can specifically disrupt a particular protein-protein interface remains a challenge—but is of a particular interest in virology, since the antiviral drugs currently available target only viral proteins.

6.3.2. Antibacterial activity

Most efforts were devoted to the study of antibiotic resistance in bacteria for several reasons: (i) bacterial infections are responsible for most community-acquired and nosocomial infections; (ii) the large and expanding number of antibacterial classes offers a more diverse range of resistance mechanisms; and (iii) the ability to move bacterial resistance determinants into standard, well-characterized bacterial strains facilitates more detailed studies of the underlying molecular mechanisms [66]. Pratt et al. [67] isolated the first antibacterial compound from a microalga, Chlorella; a mixture of fatty acids, viz. chlorellin, was found to be responsible for that inhibitory activity against both Gram+ and Gram- bacteria. Research aimed at identifying antibacterial active principles produced by microalgae has meanwhile boomed [68]. This realisation arose e.g. from the risk associated with several multidrug-resistant Staphylococcus aureus (MRSA) strains, which have been causing an increased concern in healthcare institutions worldwide—since they are not susceptible to most conventional antibiotics. Hence, discovery of novel antibacterial compounds following distinct biochemical mechanisms of action is urged. Antibiotics are typically less effective against Gram- bacteria because of their complex, multilayered cell wall structure—which makes it more difficult for the active compound to penetrate them [69]; this justifies why the antibacterial activity of the supernatant (and methanolic extracts) is more potent against Gram+ than Gram- bacteria [68,70]. The exact mechanism of action of fatty acids remains unknown: they may act upon multiple cellular targets, even though cell membranes are the most probable ones—as membrane damage will likely lead to cell leakage and reduction of nutrient uptake, besides inhibiting cellular respiration; conversely, Desbois [71] claimed a peroxidative process. Furthermore, compounds synthesized by Scenedesmus costatum, and partially purified from its organic extract, exhibited activity against aquaculture bacteria because of their fatty acids longer than 10 carbon atoms in chain length—which apparently induce lysis of bacterial protoplasts. The ability of fatty acids at large to interfere with bacterial growth and survival has been known for quite some time, but recent structure-function relationship studies suggest that said ability depends on both their chain length and degree of unsaturation. Such compounds as cholesterol can antagonize antimicrobial features [53] so both composition and concentration of free lipids should be taken into account [72]. Among microalgal-derived oxylipins, the antibacterial activities of polyunsaturated aldehydes deserve a special mention. Such compounds are synthesized by diatoms, e.g. S. costatum and Thalassiosira rotula. One illustrative example is
decadienal—probably derived from (the polyunsaturated) arachidonic acid (C20:4 n-3), which exhibits a strong activity against such important human pathogens as MRSA and *Haemophilus influenza*—with MIC values of 7.8 and 1.9 µg/mL, respectively, and well as against *E. coli* and *Pseudomonas aeruginosa*, and *S. aureus* and *Staphylococcus epidermidis* (Gram- and Gram+ bacteria, respectively). Furthermore, it impairs growth of diverse marine bacteria, such as (the Gram-) *Aeromonas hydrophila*, *L. anguillarum*, *Alteromonas haloplanktii*, *Photobacterium phosphoreum* and *Psychrobacter immobilis*, and the (Gram+) *Planococcus citreus* and *Micrococcus luteus* [73].

6.3.3. Antifungal activity

Algae are one of the chief biological agents that have been studied for the control of fungi plant pathogens [74]. Various strains of cyanobacteria are known to produce intracellular and extracellular metabolites with diverse biological activities such as antibacterial, antifungal and antiviral activity [75]. These biologically active compounds include antibiotics and toxins [76]. Hussien *et al.* [77] screened the effect of culture filtrates of nine algal strains (*Anabaena flos-aquae*, *Anabaena oryzae*, *Chlorella vulgaris*, *Nostoc muscorum*, *Nostoc humifusum*, *Oscillatoria* sp., *Phormedium fragile*, *Spirulina platensis* and *Wollea saccata*) at concentrations of 10, 20, 30 and 40% on mycelium growth of the plant pathogenic fungi *Cercospora beticola* causing leaf spot disease in sugar beet comparing with different concentrations of the synthetic fungicide Topsin M70 (100, 200, 300 and 400 ppm). They found that generally, all the algal culture filtrates reduced the fungal mycelium growth but the highest fungal mycelium growth inhibition percentages were achieved by *Spirulina platensis*, *Oscillatoria* sp. and *Nostoc muscorum* (Figure 8) at the concentrations of 30% (100, 100 and 82%, respectively) and at 40% (100, 100 and 100%, respectively). While, the chemical synthetic fungicide Topsin M70 completely inhibited the fungal mycelium growth at the concentrations of 200, 300 and 400 ppm. Fungal spores production (number of spores) was completely inhibited by the previous three algal culture filtrates at 300 and 400 ppm particularly at the concentration of 40%. Same results were obtained by Topsin M70. The antifungal activity of the algal culture filtrates has been attributed to the presence of bioactive compounds i.e. total phenolic compounds, total saponins and alkaloids in the algal culture filtrates.

6.4. Nematicidal activity

Root-knot nematodes, *Meloidogyne* spp., are among the most damaging nematodes in agriculture, causing an estimated US$ 100 billion loss/year worldwide [78]. The application of chemical nematicides has been found as an effective measure for controlling nematodes but they have toxic residual effect on the environment particularly on non-target organisms and human health. In addition, the use of chemical nematicides is prohibited in organic farming. Nematicidal potential of cyanobacteria has remained unexplored except for a few reports, which suggest that endospores of *Microcoleus* and *Oscillatoria* spp. killed nematodes [79].
Figure 8. The efficacy of (A) *Nostoc muscorum*, (B) *Oscillatoria* *sp* and (C) *Spirulina platensis* culture filtrates (40 mL.L$^{-1}$) in suppressing the fungal mycelia growth diameter of *Cercospora beticola* [77].

Culture filtrates of Microcoleus vaginatus inhibited hatching of *Meloidogyne incognita* eggs and killed second stage juveniles. Microalgal metabolites have attracted attention, because they are a resource for toxins, and potential new drugs [80]. Shawky et al. [81] studied the nematicidal effect of nine culture filtrates of algal strains (*Nostoc muscorum, Anabaena flos*...
aquae, Anabaena oryzae, Chlorella vulgaris, Wollea saccata, Phormedium fragile, Oscillatoria sp., Nostoc humifusum and Spirulina platensis), Azolla pinnata aqueous extract filtrate (1:2w/v) and compost watery extract filtrate (1:5w/v) in controlling the population of the root knot nematode, Meloidogyne incognita in cucumber under both laboratory and greenhouse conditions. Laboratory experiment revealed that high juvenile mortality percentage occurred during all the exposure periods of all treatments, the best results were after 72 hr exposure. Only five cyanobacterial strains, namely, Spirulina platensis, Oscillatoria sp., Anabaena oryzae, Nostoc muscorum and Phormedium fragile, followed by compost watery extract, significantly increased juveniles mortality over 70% at the highest concentration of 1:10 (84.3, 80.4, 78.9, 75.4, 72.5 and 70.1%, respectively). Azolla pinnata aqueous extract filtrate achieved 69.8% at the same concentration while, Anabaena flos aquae and Chlorella vulgaris recorded the lowest effect on mortality percentage (52.1 and 40.1%, respectively) at the concentration of 1:10. In the greenhouse experiment, the combination of mixing five algal culture filtrates of S. platensis, Oscillatoria sp., A. oryzae, N. muscorum and P. fragile with A. pinnata aqueous extract filtrate and compost extract achieved the highest reduction in the number of the 2nd stage juveniles in soil, the numbers of galls, developmental stages, females, egg masses, egg numbers/egg mass in roots of cucumber plants comparing with the individual treatment and the non treated control. In addition, all combinations significantly improved fresh weight of roots and shoots and increased the yield of cucumber plants.

6.5. Molluscicidal activity

The snail intermediate hosts of schistosomiasis are the sites of intense multiplication of this parasite, thus their control strategies are considered a priority of the reduction of schistosomiasis transmission [82]. Although chemical molluscicides are to certain extent quite successful in curbing the disease concerned. However, in view of their side effects, interest in environmentally friendly approaches and use of biological control agents have been revived [83]. Mostafa and Gawish [84] stated that the algal culture filtrate of Spirulina platensis was proved for its molluscicidal activity against Biomphalaria alexandrina snails the intermediate host of Schistosoma mansoni in Egypt that accommodates their survival and fecundity and also for its effect on hatchability of snail’s eggs and viability of the free living larval stages of the parasite (miracidia and cercariae). The culture filtrate proved to have a lethal effect on snails with 90 LC 0.23% of the filtrate. B. alexandrina snails stopped egg laying after one week of continuous exposure to the sublethal concentrations 0.19 and 0.17%, while those exposed to 0.07% laid few ones. It could be due to the phytochemical constituents of the culture filtrate i.e. total phenolic compounds, alkaloids and total saponins. This was confirmed by histological examination that showed a severe damage in the hermaphrodite gland cells of snails (Figure 9) exposed to these concentrations. B. alexandrina eggs of 3 and 7 days old failed to hatch post exposure to 0.1% of algal filtrate, while 22% and 10% only hatched after exposure to 0.05% of this filtrate. Free cell culture filtrate shows also marked miracidicidal and cercaricidal activities as 2% of this filtrate killed most of these organisms within 15 minutes of exposure. It is concluded that the byproducts of the blue-green alga Spirulina platensis has a lethal effect against adult B. alexandrina snails, reduced or stopped their oviposition, hence minimize the snail populations
available for the parasite transmission. Therefore it may be a potential source of effective compounds for control of *Schistosoma mansoni*.

Figure 9. Section in the hermaphrodite gland of *Biomphalaria alexandrina* snail: control (A), exposed to 0.07% (B) and to 0.17% (C) concentrations of *Spirulina platensis* culture filtrate. G=Spermatogonia, T=Spermatid, Z=Spermatozoa, I=Oogonia, O=Oocyte V= vacules D=degeneration [84]

7. Other applications and products from microalgae

Microalgae have found commercial applications as natural sources of valuable macromolecules, including carotenoids, long-chain polyunsaturated fatty acids, and phycocolloids. As photoautotrophs, their simple growth requirements make them attractive for bioprocesses aimed at producing high added-value compounds that are in large demand by the pharmaceutical market. The productivity and biochemical composition of microalgae depend strongly on the mode of cultivation, medium composition, and nutrient profile. Consequently, numerous efforts aimed at elucidating the practical impacts of the aforementioned parameters have been developed [56]. Thus, there is a growing interest in the area of research on the positive effect of algae on human health and other benefits.

7.1. Food

The first use of microalgae by humans dates back 2000 years to the Chinese, who used *Nostoc* to survive during famine. Microalgae for human nutrition are nowadays marketed in different forms such as tablets, capsules and liquids. They can also be incorporated into pastas, snack foods, candy bars or gums, and beverages [85-86]. Owing to their diverse chemical properties, they can act as a nutritional supplement or represent a source of natural food colorants. The commercial applications are dominated by four strains: *Arthrospira*, *Chlorella*, *Dunaliella salina* and *Aphanizomenon flos-aquae*. *Arthrospira* is used in human nutrition because of its high protein content and its excellent nutritive value [87-88].
addition, this microalga has various possible health-promoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal *Lactobacillus*, and suppression of elevated serum glucose level [85-86]. A significant amount of *Arthrospira* production is realized in China and India. *Chlorella* can also be used as a food additive owing to the taste- and flavour-adjusting actions of its coloring agent [85]. *D. salina* is exploited for its β-carotene content that can reach 14% of dry weight [89]. For human consumption, Cognis Nutrition and Health, the world’s largest producer of this strain, offers *Dunaliella* powder as an ingredient of dietary supplements and functional foods. The last major commercial strain application is *A. flos-aquae*. According to many research studies, used alone or in combination with other nutraceuticals and natural food products, *A. flos-aquae* promotes good overall health [85,90]. The consumption of *Arthrospira (Spirulina)* by the Kanembu was also reported by Delisle et al. [91] in a survey of household food consumption and nutritional adequacy in Wadi zones of Chad. Table (6) presents a comparison of the general compositions of human food sources with that of different microalgae.

<table>
<thead>
<tr>
<th>Commodity</th>
<th>Protein</th>
<th>Carbohydrate</th>
<th>Lipid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bakers’ yeast</td>
<td>39</td>
<td>38</td>
<td>1</td>
</tr>
<tr>
<td>Meat</td>
<td>43</td>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>Milk</td>
<td>26</td>
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</tr>
<tr>
<td>Rice</td>
<td>8</td>
<td>77</td>
<td>2</td>
</tr>
<tr>
<td>Soybean</td>
<td>37</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td><em>Anabaena cylindrica</em></td>
<td>43-56</td>
<td>25-30</td>
<td>4-7</td>
</tr>
<tr>
<td><em>Chlamydomonas reinhardtii</em></td>
<td>48</td>
<td>17</td>
<td>21</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>51-58</td>
<td>12-17</td>
<td>14-22</td>
</tr>
<tr>
<td><em>Dunaliella salina</em></td>
<td>57</td>
<td>32</td>
<td>6</td>
</tr>
<tr>
<td><em>Porphyridium cruentum</em></td>
<td>28-39</td>
<td>40-57</td>
<td>9-14</td>
</tr>
<tr>
<td><em>Scenedesmus obliquus</em></td>
<td>50-56</td>
<td>10-17</td>
<td>12-14</td>
</tr>
<tr>
<td><em>Spirulina maxima</em></td>
<td>60-71</td>
<td>13-16</td>
<td>6-7</td>
</tr>
<tr>
<td><em>Synechococcus sp.</em></td>
<td>63</td>
<td>15</td>
<td>11</td>
</tr>
</tbody>
</table>

**Table 6.** General composition (%) of dry matter of different human food sources and algae [92].

### 7.2. Feed

Microalgae can be incorporated into the feed for a wide variety of animals ranging from fish (aquaculture) to pets and farm animals. In fact, 30% of the current world algal production is sold for animal feed applications [92] and over 50% of the current world production of *Arthrospira* is used as feed supplement [85]. In 1999, the production of microalgae for aquaculture reached 1000 t (62% for molluscs, 21% for shrimps, and 16% for fish) for a global world aquaculture production of 43×10⁶ t of plants and animals [93]. The importance of algae in this domain is not surprising as they are the natural food source of these animals. The main applications of microalgae for aquaculture are associated with nutrition, being used fresh (as sole component or as food additive to basic nutrients) for coloring the flesh of salmonids and for inducing other biological activities. The most frequently used species are *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis,*
Many nutritional and toxicological evaluations have proved the suitability of algal biomass as feed supplement [92]. *Arthrospira* is largely used in this domain and concerns many types of animal: cats, dogs, aquarium fish, ornamental birds, horses, cows and breeding bulls. Algae positively affect the physiology (by providing a large profile of natural vitamins, minerals, and essential fatty acids; improved immune response and fertility; and better weight control) and their external appearance (resulting in healthy skin and a lustrous coat) of animals. In poultry rations, algae up to a level of 5-10% can be used safely as partial replacement for conventional proteins. Prolonged feeding of algae at higher concentrations produces adverse effects. The yellow color of broiler skin and shanks as well as of egg yolk is the most important characteristic that can be influenced by feeding algae [92].

### 7.3. Agricultural purposes

Humans have practiced agriculture for more than 10,000 years, but only in the past 50 years or so have farmers become heavily dependent on synthetic chemical fertilizers and pesticides. It contributes to numerous forms of environmental degradation, including air and water pollution, soil depletion and diminishing biodiversity. Synthetic chemical pesticides and fertilizers are polluting soil, water, and air, harming both the environment and human health. Soil is eroding much faster than it can be replenished—taking with it the land’s fertility and nutrients that nourish both plants and those who eat them. Chemical fertilizers can gradually increase the acidity of the soil until it begins to impede plant growth. Chemically fertilized plots also show less biologic activity in the soil food web (the microscopic organisms that make up the soil ecosystem) than do plots fertilized organically with manure or other biologic sources of fertility [95]. The best way, however, is to use as much as possible microbial products, functional bio-fertilizers and bio-controllers and reduce the amount of the use of chemical fertilizers or pesticides. Heterocystous cyanobacteria and several nonheterocystous cyanobacteria are known for their ability to fix atmospheric nitrogen. The fertility of many tropical rice field soils has been mainly attributed to the activity of nitrogen-fixing cyanobacteria. An estimation showed that more than 18 kg N ha⁻¹ year⁻¹ was added to the soils by cyanobacteria. Inoculation of cyanobacteria to increase the fertility of soils has been successfully attempted. Recently, nitrogen-fixing cyanobacteria have been reported to dominate desert crusts worldwide. This is believed to contribute significantly to the fertility of desert soils and may eventually facilitate vegetation of deserts [96]. Algae as biofertilizers are a promising alternative to avoid soil pollution caused by agrochemicals. Also, they recover the nutrients content to soil as they secrete exo-polysaccharides that improve soil structure and bio-active substances that enhance the plant growth. Algae are known to be one of the most promising sources as bio-control agents of any residues, thereby having positive impact on human health [97]. Microorganisms play an important role in various chemical transformations of soils and thus, influence the availability of major nutrients like nitrogen, phosphorus, potassium and sulphur to the plants. Cyanobacteria and phosphate-solubilizing bacteria were used as biofertilizers to increase crop production [98]. The cyanobacterial ability to mobilize insoluble forms of inorganic phosphates is evident from the finding of kleiner and Harper
who reported more extractable phosphates in soils with cyanobacterial cover than in nearby soils without cover. Cyanobacteria can fix about 25 kg N/ha/season. Apart from nitrogen fixation, inoculation with cyanobacteria is also reported to reduce considerably the total sulphides and ferrous iron content of the soil. Blue-green algae constitute an important group of microorganism capable of nitrogen fixation. Most of the species possess nitrogen fixation ability to the order Nostocales and Stigonematales. Over 100 species of blue-green algae are known to fix atmospheric nitrogen. These have been found to be very effective on the rice and banana plantation. In field condition, overall increase in the gram yield of rice is amounted to about 586 kg/ha. In case of crops other than rice, algalization increased nearly 34 per cent yield. India is one of the countries where agro-chemical conditions appear to be favourable where blue-green algae technology has been put forward. In some parts of the country, production of BGA inoculants has been commercialised. Producing inoculum in artificially controlled conditions is well defined, but relatively expensive. On the other hand open-air soil culture is simpler, less expensive and easily adaptable by the farmers. Field scale production of algae biofertilizer is also possible. 20-25 kg dry algae can be obtained on 40 m field. Adopting this method, 15 t/ha of wet BGA can be obtained by the farmers. Farmers can also produce algae for countryard of the house [96]. Blue-green algal extracts comprise a great number of bioactive compounds that influence plant growth and development. They mostly contain growth phyto-regulators like gibberellins, auxin, cytokinin, ethylene and abscisic acid [100]. This group of microorganisms have been reported to benefit plants by producing growth promoting regulators resemble gibberellin and auxin, vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol and polymers especially exopolysaccharides that were reported to enhance growth and productivity of plants like Daucus carota [101], Santalum album [102], Oryzae sativa [103], Lilium alexandriae hort [104] and Beta vulgaris L.[100]. Non- nitrogen fixing cyanobacteria can enrich phosphorus and potassium contents in soils, laying indirect major role in plant growth promotion [105]. Cyanobacteria also enhance the soil biological activity in terms of increasing the total bacterial, total cyanobacterial counts, CO$_2$ evolution, dehydrogenase and nitrogenase activities. Many researches suggested that up to 50% of the recommended dose of the mineral nitrogen fertilizers could be saved by using some species of nitrogen fixing cyanobacteria. The obtained results emphasized the prospects and potentials of using cyanobacteria biofertilizers as renewable natural nitrogen resources for many crops. They are none polluting, inexpensive, utilize renewable resources (inorganic nutrients and atmospheric CO$_2$) in addition to their ability in using free available solar energy, atmospheric nitrogen and water [106-108].

7.4. Mitigation of CO$_2$: Why algae for CO$_2$ sequestration?

Many options that have been proposed and that are in use for capturing CO$_2$ can be seen as economically, socially and environmentally short-sighted. A common approach is taking measures to offset any immediate effects, often by simple relocation of the emissions. Injection of flue gases into oceanic or geological sinks is examples of such “end-of-pipe” solutions [109]. Algae cultivation can yield a broad range of useful end products, apart from biofuels. The sequestration of CO$_2$ into algal biomass can become profitable also through the
production of high value products such as pigments and high-grade lipids, which are extractable from several species of algae. Brennan and Owende [110] also mention high value products such as animal feed supplements being extractable from the microalgae species Chlorella, Scenedesmus and Spirulina. The urgent need for substantive net reductions in CO₂ emissions to the atmosphere can be addressed via biological CO₂ mitigation, coupled with transition to more extensive uses of biofuel, nuclear and renewable energy sources. Microalgae have attracted a great deal of attention for CO₂ fixation and biofuel production because they can convert CO₂ (and supplementary nutrients) into biomass via photosynthesis at much higher rates than conventional biofuel crops can. This biomass may then be transformed into methane or hydrogen, using processes mediated by anaerobic bacteria; an integrated process for hydrothermal production of methane via microalgae has been discussed recently [111-112]. Of particular interest is the production of oils by microalgae because of the ease of their synthesis (a lack of a nitrogen source usually suffices to trigger this form of secondary metabolism). Lipid extraction and re-esterification are accomplished with short-chain alcohols and other by-products of secondary metabolism (i.e. polyunsaturated fatty acids, bcarotenes or polymers [111]. Upon extraction, such oils can be hydrolyzed and then re-esterified with methyl- or ethyl alcohol moieties to obtain biodiesel. Microalga-mediated CO₂ fixation and biofuel production can be rendered more sustainable by coupling microalgal biomass production with existing power generation and wastewater treatment infrastructures (Figure 10). Microalgae can utilize low-quality water, such as agricultural runoff or municipal, industrial or agricultural wastewaters, as a source of water for the growth medium as well as a source of nitrogen, phosphorus and minor nutrients [113].

7.5. Wastewater treatments

Wastewater nitrogen and phosphorous as microalgae nutrients aquaculture systems involving microalgae production and wastewater treatment (e.g. of amino acids, enzyme, or food industries wastewaters) seems to be quite promising for microalgae growth combined with biological cleaning. This allows nutrition of microalgae by using organic compounds (nitrogen and phosphorous) available in some manufactures wastewater, not containing heavy metals and radioisotopes. Additionally, microalgae can mitigate the effects of sewage effluent and industrial sources of nitrogenous waste such as those originating from water treatment or fish aquaculture and at the same time contributing to biodiversity. Moreover, removing nitrogen and carbon from water, microalgae can help reduce the eutrophication in the aquatic environment. Aslan and Kapdan [114] used C. vulgaris for nitrogen and phosphorus removal from wastewater with an average removal efficiency of 72% for nitrogen and 28% for phosphorus (from 3 to 8 mg/L NH₄+ and 1.5–3.5 mg/L PO₄³⁻). Other widely used microalgae cultures for nutrient removal are Chlorella [115] and Spirulina species [116]. Nutrient removal capacities of Nannochloris [117], Botryococcus braunii [118] and cyanobacterium Phormidium bohneri have also been investigated [119-120]. Environmental applications production of biodiesel and other bio-products from microalgae can be more environmentally sustainable, cost-effective and profitable, if combined with processes such as wastewater and flue gas treatments. In fact various studies demonstrated
the use of microalgae for production of valuable products combined with environmental applications [113,121].

7.6. Biofuel production

Microalgae can potentially be employed for the production of biofuels in an economically effective and environmentally sustainable manner. The production of these biofuels can be coupled with fuel gas CO$_2$ mitigation, wastewater treatment and the production of high-value chemicals. The efficiency is low but there is much room for improvement. The use of microalgae is seen as, at least, a partial solution to climate change and energy problem [122]. Many microalgae are exceedingly rich in oil which can be converted to biodiesel using existing technology. More than 50% of their biomass as lipids, sometimes even up to 80%, and oil levels of 20-50% are quite common [123].

Figure 10. Integration of microalgal bioreactors into existing wastewater and power generation infrastructures. The overall process uses microalgae to capture industrially produced waste CO$_2$ in photobioreactors, coupled with treatment of nutrients in wastewater. CO$_2$ is converted into algal biomass by photosynthesis in the presence of light. After processing (biological, physical or thermochemical), the biomass generated can be used for production of biodiesel, methane or other fuels and co-products (e.g. animal feeds and polymers).

Lipids production and biodiesel extraction from algae depend on algal species and extraction solvent system [124]. There is a unique opportunity to both treat wastewater and provide nutrients to algae using nutrient-rich effluent streams. By cultivating microalgae, which consume polluting nutrients in municipal wastewater, and abstracting and processing this resource, then the goals of sustainable fuel production and wastewater treatment can be combined [174,125]. The efforts span over many areas of “algae to fuels”
technologies including production system development, algae harvest, algae strain development and genetic modification, algae products development, etc. Screening and genetic modification of algae strains will play an increasingly important role. Genetic engineering has the potential to improve the overall algal biomass yield and lipid yield. Discovery of new strains and genetically modified strains capable of secreting hydrocarbons to extracellular spaces will open some new opportunities; however, challenges with recovering the secreted liquids or volatiles remain. There is a need to develop high throughput screening and analysis methods. Current harvest and dewatering are still too energy intensive. New techniques and strategies must be devised to lower the costs. Direct conversions such as in situ transesterification and hydrothermal liquefaction offer the possibility to process wet algae. Fractionation of algal biomass, before or after oil extraction, deserves a closer look because it may play an important role in offsetting the costs. New techniques to disrupt algae cellular structures to improve oil extraction efficiency are needed [126].

7.7. Heavy metals and phycoremediation

Metals are directly or indirectly involved in all phases of microbial growth. Many metals such as sodium, potassium, iron, copper, magnesium, calcium, manganese, zinc, nickel and cobalt are vital for biological functions, while others such as aluminum, cadmium, silver, gold, mercury and lead are not known to have necessary biological functions. All these elements can interact with microbial cells and be accumulated as a result of different mechanisms [127]. Some of these mechanisms have biotechnological importance and can be applied for the bioremediation of metals from industrial effluents. The capability of some microbial species to adsorb some heavy metals on their surface [128-129] or accumulate them within their structure is a chief route for the removal of heavy metals from contaminated environment [130-132]. Another fashion for the detoxification of heavy metals by microorganisms is the chelation of these metals inside or outside their cells after converting them into other forms to reduce their toxicity. In 2007, Lefebvre et al. [133] working with some cyanobacterial strains (Limnothrix planctonica, Synechococcus leopoldiensis and Phormidium limnetica) demonstrated their ability to convert Hg$^{2+}$ into elemental mercury Hg$^{0}$ and meta-cinnabar ($\beta$-HgS) under pH controlled and aerated conditions. The transformation of mercury into $\beta$-HgS was attributed to the interaction with metal binding sulfhydryl protein as an intermediate step in metal sulfide synthesis. Moreover, some of the freshwater algae Limnothrix planctonica and Selenastrum minutum were recorded for their ability to bio-transform Hg$^{2+}$ into a form with the analytical properties of $\beta$-HgS under aerobic conditions due to the presence of some protein and non-protein thiol chelators [134]. Furthermore, Lengke et al. [135] investigated the gold bioaccumulation by cyanobacterium Plectonema boryanum from gold (III)-chloride solutions. They confirmed that the reduction mechanism of gold (III) to metallic gold by this organism involves the formation of an intermediate gold (I)-sulfide due to a chelation process via some thiol compounds. Recently Essa and Mostafa [136] studied the efficiency of three cyanobacterial isolates (Spirulina platensis, Nostoc muscorum, and Anabaena oryzae) individually or as a mixed culture to
precipitate some heavy metals (Hg$^{2+}$, Cd$^{2+}$, Cu$^{2+}$ and Pb$^{2+}$) out of their solutions through using the culture biogas produced during their aerobic growth in a batch bioreactor. Variable capabilities of metal bioprecipitation were recorded by the three algal isolates. FT-IR studies showed the existence of –OH groups in the metal precipitate produced by the algal isolates while –NH groups were identified only in the metal precipitates produced by N. muscorum, and A. oryzae. This study highlighted a novel approach for heavy metals bioremediation through the transformation of these metals into nitrogen complexes and/or hydroxide complexes via using the culture biogas produced by some cyanobacterial species.

8. Microalgal production

Microalgae for human nutrition are nowadays marketed in different forms such as tablets, capsules and liquids. They can also be incorporated into pastas, snack foods, candy bars or gums, and beverages. In addition, this microalga has various possible healthpromoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal Lactobacillus, and suppression of elevated serum glucose level [86-87]. Owing to their diverse chemical properties, they can act as a nutritional supplement or represent a source of natural food colorants. The commercial applications are dominated by four strains: Arthrospira, Chlorella, D. salina and Aphanizomenon flos-aquae. Arthrospira is used in human nutrition because of its high protein content and its excellent nutritive value [87,88,137]. A significant amount of Arthrospira production is realized in China and India. The world’s largest producer Hainan Simai Enterprising Ltd. is located in the Hainan province of China. This company has an annual production of 200 t of algal powder, which accounts for 25% of the total national output and almost 10% of the world output. The largest plant in the world is owned by Earthrise Farms and stretches over an area of 440,000 m$^2$ (located at Calipatria, CA, USA; Figure 11). Their production process is presented in Figure 12. Their Arthrospira-based products (tablets and powder) are distributed in over 20 countries around the world. Many other companies sell a wide variety of nutraceuticals made from this microalga. For example, the Myanmar Spirulina Factory (Yangon, Myanmar) sells tablets, chips, pasta and liquid extract, and Cyanotech Corp. (a plant in Kona, Hawaii, USA) produces products ranging from pure powder to packaged bottles under the name Spirulina pacifica. Cyanotech Corp. has developed an orginal process for drying the biomass in order to avoid the oxidation of carotenes and fatty acids that occurs with the use of standard dryers. The patented process employs a closed drying system that is kept at low oxygen concentrations by flushing with nitrogen and carbon dioxide. The process relies on a very cold ocean water crown from a depth of 600 m just offshore to provide dehumidification and actually dries microalgal products in less than 6 s (Figure 13). Chlorella is produced by more than 70 companies; Taiwan Chlorella Manufacturing and Co. (Taipei, Taiwan) is the largest producer with 400 t of dried biomass produced per year. Significant production is also achieved in Klötze, Germany (130 – 150 t dry biomass per year) with a tubular photobioreactor. This reactor consists of compact and vertically arranged horizontal running glass tubes with a total length of 500,000 m and a total volume of 700 m$^3$ (Figure 14). The world annual sales of
Chlorella are in excess of US$ 38 billion [85]. The most important substance in Chlorella is β-1,3-glucan, which is an active immunostimulant, a free-radical scavenger and a reducer of blood lipids (9, Ryll et al., Abstr. Europ. Workshop Microalgal Biotechnol., Germany, p. 56, 2003). However, various other health-promoting effects have been clarified (efficacy on gastric ulcers, wounds, and constipation; preventive action against atherosclerosis and hypercholesterolemia; and antitumor action) [85,122,138].

Figure 11. Earthrise Farms Arthrospira production plant (Calipatria, CA, USA)

Figure 12. Earthrise Farms microalgal production process
9. Conclusion

Meeting the increasing water demands with limited resources advocates Egypt to find innovative and sustainable approaches for management. It is essential to maximize the benefits of the available resources and to minimize the wastes and losses, not only in water resources but also in all economical and social resources, and in an integrated framework believing that everything is related to everything. So would it not be possible to kill several birds with one stone, using algae for absorbing CO$_2$ at the same time as providing nutrient recovery from food industrial effluents and domestic wastewater and producing renewable energy (fuels), as well as other pharmaceutical products, food, feed and fertilizer from the biomass? In recent years, microalgal culture technology is a business oriented line owing to their different practical applications. Innovative processes and products have been
introduced in microalgal biotechnology to produce vitamins, proteins, cosmetics, health foods and animal feed. For most of these applications, the market is still developing and the biotechnological use of microalgae will extend into new areas. With the development of algal cultures and screening techniques, microalgal biotechnology can meet the challenging demands of food, feed, pharmaceutical industries, fuels and biofertilizers. The general needs of the human society are continuously increasing. We need every new compound which may be useful for the human society. More food, new drugs, and other goods are highly necessary for the benefit of humankind. The only question is the existence of sufficient natural and technical resources to fulfill these demands. Fortunately, in the area of the research of bioactive microbial products it seems that the ever expanding scientific and technical possibilities are increasing together with the continuously widening needs of the human.

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10. References


