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A REVIEW ON POTENTIAL OF MICROALGAE FOR DIFFERENT BIO-PRODUCTS AND THEIR APPLICATIONS

¹Aniketa Kumari, ² Dr. Shalini Sharma.

¹Research Scholer, ²Associate Professor,

¹Biotechnology Department, Aniketa kumari.

¹Shoolini Institute of Life sciences and Business management , Solan (H.P.), India 173212.

Abstract: Microalgae are autotrophic microorganisms that live in marine, freshwater, and soil habitats and generate organic compounds from photosynthesis. Because of its high metabolic flexibility and adaptability to varied growing environments, integrated approaches for its cultivation are currently being developed with the goal of isolating various biologically active compounds in order to boost the profitability of algae production. The number of studies on their potential use as a source of biologically useful compounds is gradually increasing. Their extraordinary potential for CO₂-fixation additionally demonstrates their critical importance in sustaining Earth's ecosystems. Furthermore, they play an important role in the removal of pollutants from different environments. In addition to ecological benefits, many micro algal species have great nutritional value and produce valuable bio-products like Pigments, lipids, bioactive chemicals, specific polysaccharides, bio-hydrogen, and even bio polyesters with plastic-like characteristics have the potential for market success. The review focuses at the main parameters (temperature, pH, component composition) that influence the biomass growth process and the production of biologically active substances in microalgae. An examination of various methods for isolating and producing the main biologically active substances of microalgae (proteins, lipids, polysaccharides, pigments, and vitamins) are presented and new technologies and approaches aiming for using microalgae as promising ingredients in value-added products.

Index Terms – Micro algae, Biomass, Bio-product, Pollutants, Plastic-like.

1. INTRODUCTION

- a. **General aspects:** - Algae are among the oldest organism existing on our planet, the earth and can justifiably be regarded as the bio-ancestral forerunners of life. The estimated age of algae is about 540 million years. Algae are a collective term applied to a group of primitive, autotrophic and thaloid plant with wide diversity. Most algae, like higher plants, utilize sunlight to produce their own food and nutrients. They are ubiquitous, ranging from microscopic unicellular 1µm to multicellular kelps inhabiting the oceans, fresh water bodies, soils, rocks and play a fundamental role in ecosystem.
- b. **Habitat:** - Freshwater algae are some of the most diverse and widespread organisms on the planet. They live in a wide variety of ecological environments, including lakes, rivers, peat swamps, inland salt lakes, snow, ice, damp soil, wetlands, desert soils, wastewater treatment plant and are symbionts in and on many fungi and animals. Algal ecosystem plays an important role in management of aquatic ecosystem, productivity, their biodiversity interaction with organisms and water quality.
- c. **Evolution and origin:** - There are about 1, 40,000 algal species estimated to be found on the earth, many of which are highly nutritious and can be eaten and there are also small number of algae which are highly toxic when consumed. Recent estimates showed that human being utilizes only 221 species of algae, 145 species as food and 101 species for phycocolloid production.

Algae provide food and feed for people and livestock as well as serve as thickening agents in ice-cream and shampoos and used as drug in curing many diseases. In earlier periods, coastal people used algae as food during famine to ward off starvation. One interesting legend on discovery of algae as food is, it all began with when a lady by the name **Nei Tebanikarawa** had a vision that the algae in the neighborhood pond were edible. It made her to try it out one day. After that the people in the village began collecting and using it as food (Maata et al, 2004). However, the current growth in human population and limited food sources has encouraged scientists to look for new food sources. Amongst the non-conventional food sources, algae as alternate source of food have enthralled attention of most of the world. This is because of the richness of algae in proteins, carbohydrates, vitamins, minerals and fatty acids (Hopp, 1979).

The members from Chlorophyta, Phaeophyta, Rhodophyta and Cyanophyta are now consumed in all countries that possess marine coasts as well as in countries where algae are abundant in fresh water bodies such as lakes, streams, ponds and rivers. Algae are used as food in variety of ways. They are served raw in salads and pickled or fermented into relish. They enrich soups, stews, and sauces and are also used as condiments. Algae are even roasted, employed as tea and served as a dessert, sweetmeat, jelly, or a cooked vegetable. The caloric value of edible algae ranges from 4405 - 5410 (Cyanophyta), 4700 - 4940 (Chlorophyta), 4160 - 5160 (Phaeophyta) and 3290 - 5400 (Rhodophyta) calories per gram respectively (Cummins and Wuycheck, 1971). The

digestibility of seaweeds ranges from 39 to 73 % with net energy availability of 48 to 67 % (Kishi et al, 1982). The problem of deficiency of proteins in food is another reason that fascinated attention of scientists towards algae as a food throughout the world, as algal biomass has been considered a potential protein source for human consumption (Venkataraman and Baker, 1985). Algae as a source of proteins are more beneficial since the cost of algal proteins is much less as compared to those of animal or other vegetable proteins (Golueke et al, 1960). Microalgae similar to macro algae **are also rich in proteins, carbohydrates, lipids, amino acids, trace elements and vitamins** (Waaland, 1981), some microalgae like Spirulina, Chlorella, Nostoc, Anabaena etc. are rich source of proteins and trace elements and therefore have been used as a safe source of food for centuries in some cultures, but it is only really in the last fifteen years, or so, that they have started to become increasingly popular in developed countries like the UK and the USA.

Spirulina, a blue-green alga, is traditionally harvested from natural lakes by Aztec Indians and several North American tribes as a protein source. Until the late 1960s, spirulina was considered undesirable and toxic in ponds. This species' later was rediscovered as a valuable food source. The first experimental wide-scale harvesting of Spirulina was carried out in Mexico to feed the poor population of the country. Spirulina is now mostly used to extract phycocyanin, a blue photosynthetic pigment. Commercial applications include natural food colouring and cosmetic ingredients.

Another microalga used as proteinaceous food is the green alga **Chlorella**. The Carnegie Institute launched a significant research programme in the 1950s to investigate the potential for mass culture of Chlorella to feed the world's hungry. Chlorella, like Spirulina, is now mostly available in health food stores and as fish feed. Several by-products of Chlorella are employed as fruit and vegetable preserves (Hills and Nakamura, 1978).

Herbal remedies are one of the hottest trends in the field of biology today (Blumenthal, 1999) in which the activities of the plants are used for treating diseases like asthma, cholera, diarrhea, dysentery, dermatological infections, intestinal disorders, recurrent fevers, ulcers, uremia and cancer. Similarly, the fungi as a source of antibiotics have received much attention. Nevertheless, a large group of lower plants, algae has been very little studied. Amongst algae, that have been reported to inhibit growth of microorganisms are mostly planktonic and benthic marine forms by workers like (Ramamurthy, 1970, Sachithanathan and Sivapalan, 1975, Hornesy and Hide, 1974).

Microalgae constitute promising bio-catalysts to be implemented in the increasing field of **"White Biotechnology"**. This is considered for the production of feed, food, chemicals, and various "energy carriers" (Milledge, 2012).

1.1 The broad range of micro algal products:

a. High-prized products

In addition to the consumption of micro algal biomass rich in proteins and minerals for food and feed, these organisms establish advantageous chemical substances such as pigments, enzymes, and sugars, lipids with valuable fatty acids, sterols, and vitamins. Other scarce bioactive compounds, in particular such as displaying immune responses, anticancer, anti-inflammatory and antibiotic activity, are also reported (Olaizola, 2000). As a result, algae might function as a chemical platform for cosmetic applications (e.g., colouring pigments and particularly anti-aging skin supplements: extracts from *Chlorella vulgaris* have been reported to support collagen repair mechanisms), pharmaceutical and therapeutic applications (Bumbak, 2011), food technology and the production of "green energy carriers" such as biogas and biodiesel (Y chisti, 2007, 2008) bio-hydrogen, and bioethanol. Besides from micro algal lipids and pigments, the unique carbohydrates produced by these organisms are rapidly gaining interest due to their potential therapeutic applications. For e.g. β -1, 3-glucan, a natural soluble fibre act as immune-stimulator, antioxidant and reducer of blood cholesterol extracted from Chlorella strains.

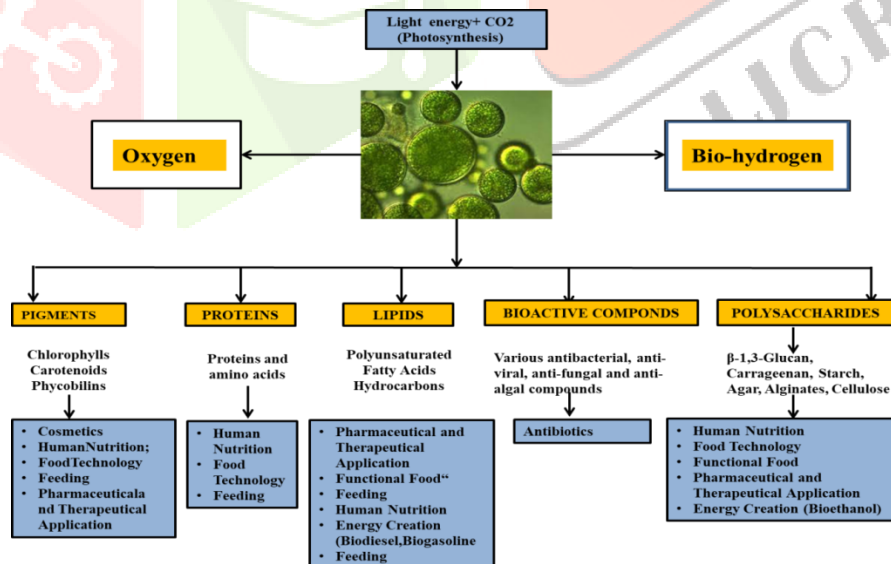


Fig.1.1 product synthesised by microalgae and their application

In addition to the therapeutic use, carbohydrate can be used in the production of food and beverages, mainly as fat substitute for texturizing. β -1,3-glucan can be added to unique food products such as functional beverages, functional bread, ready-to-eat soups, functional snack foods, and a range of sauces, creamers, bakery products, and other food products (Spolaore et al, 2006).

b. Cultivation conditions for product formation: - Micro algal production has just recently transitioned from "wild" cultivating and harvesting practices to controlled cultivation methods in technologically advanced systems like racing ponds or photo bioreactors (Pulz, 2001). Microalgae frequently have the ability to rapidly adapt to highly variable process conditions, such as salinity, temperature, pH, and illumination; illumination features take light intensity into account, dark-light cycles and spectral range into account. Depending on the environmental factors that the organisms are exposed to, different organisms will produce different amounts of biomass, lipids, pigments, bio polyesters, and carbohydrates (Santos et al, 2013). Fig 1.1 provides a

schematic overview of potential micro algal products like lipids, pigments, biomass, carbohydrates, bio polyester, molecular oxygen or bio-hydrogen. In addition, the different areas of application of these products, such as agriculture, aquaculture, generation of green energy carriers, and pharmaceutical and nutritional purposes are indicated.

Table1.1. A generalized set of conditions for culturing micro-algae

Parameters	Range	Optimum conditions
Temperature (°C)	16-27	18-24
Salinity (g.l ⁻¹)	12-40	20-24
Light intensity (lux)	1,000-10,000 (depends on volume and density)	2,500-5,000
Photoperiod (light: dark, hours)	-	16:8(minimum) 24:0 (maximum)
p ^H	7-9	8.2-8.7

2. VARIOUS MICRO ALGAL BIO PRODUCTS AND THEIR APPLICATIONS

2.1 Algae as a source for nutrition

Micro algal biomass is of interest for human nutrition due to its high protein content and progressively applied as dietary or “health food”. It is a precious source of protein and polyunsaturated fatty acids (PUFAs) for aquaculture as well as for feeding cattle, pigs, and poultry. The genera *Spirulina*, *Nostoc*, and *Porphyra* are harvested as food stuff. 60% of crude protein in cyanobacteria biomass of *Spirulina platensis* and *Arthrospira maxima* underlines the nutritional value of microalgae. Also *C. vulgaris*, perhaps the best investigated representative of green algae, contains protein portions exceeding 50% of its cell mass (Becker, 2007).

2.2 Micro algal pigments

The major groups of micro algal pigments found, namely **carotenoids** (among them, carotenes provide an orange coloration, and are responsible for yellowish shade), **phycobilins** (red or blue coloration), and **chlorophylls** (green coloration). The pigment fraction of algae can be applied as nutrient supply due to their high contents of pro-vitamin A (E160a) and vitamin E (E306, E307, E308) (Lozano et al, 2007) and for other pharmaceutical, veterinary and medical purposes (anti-inflammatory effects, anti-oxidative effect, cancer prevention (Wu et al, 2007), as well as in cosmetic industry and food technology. Additionally, β -carotene and lutein are needed for poultry feeding because of its importance for the yellow-orange coloration of egg yolk.

a. Chlorophylls

Various applications of chlorophylls are found in the kitchen (John et al, 2011). These pigments are used as food additive (E140) for coloration. Famous “Chefs de cuisine” use chlorophyll to provide a green colouring to a variety of beverages and foodstuffs and, such as pasta, pesto or absinthe. A study reports the benefit of chlorophyll derivatives (chlorophyllin) against colon cancer cells if provided as dietary supplement. This study examined the response of HCT116 human colon cancer cells to treatment with a chlorophyll derivative (chlorophyllin), demonstrating its high effectiveness as a chemo-preventive agent (Díaz et al, 2003). Due to its high deodorant capacity, chlorophyll a is an important ingredient in various product of personal hygiene, such as deodorants, or, as pastilles. It is also commercialized in formulations against bad breath (Montgomery et al, 1950).

b. Carotenoids

Carotenoids (carotenes and xanthophyll's) display so called “secondary light harvesting pigments”, supporting the “primary pigment” chlorophyll in capturing light energy. Carotenoids can be stored in oil droplets, in the cytosol or in stroma of the chloroplast depending on the type of microalgae. Carotenoids in microalgae are usually present in low concentrations (0.5%_g1 dry weight), although in some Chlorophyta they can reach up to 10% dry weight when cultivated under adverse conditions, as is the case for *Dunaliella salina* (Mulders, et al, 2013). These pigments absorb electromagnetic energy in spectral ranges where chlorophylls are not able to absorb light energy, mainly at wave lengths between 400 nm and 500 nm. Furthermore, because they have a great potential as antioxidants by "catching" free radicals, these pigments prevent algal cells from the harmful effects of excessive solar radiation, a process known as "light inhibition." (Cardozo et al, 2007). The main carotenoids of microalgae are **carotene**, **lycopene**, **astaxanthin**, **zeaxanthin**, **violaxanthin** and **lutein**. Among them, carotene, lutein and astaxanthin are the most studied (Alessandro et al, 2016). **β -Carotene**, also known as provitamin A, is mainly applied for nutritional purposes as a vitamin Supplement. In addition to β -carotene, other carotenoids display high importance as colorants in food industry (Wu et al, 2007). A technologically significant example is displayed by the production of astaxanthin (E161j), Astaxanthin, formerly also known as hematochrome, is considered the most powerful natural antioxidant, hence a highly efficient scavenger of free radicals. In human metabolism, astaxanthin is of importance for protection of the skin against UV-induced photo-oxidation, for antibody production, as well as for cancer prevention and anti-tumor therapy. Food additive bixin (E160b), an example of carotenoids, provides a yellowish to peach-colour shade. Also, bixin is used as colorant in cosmetics. The xanthophylls lutein and zeaxanthin are technically applied for coloration of chicken skin and, regarding human health, of high importance to prevent macular degeneration and cataract (Cardozo et al, 2007). Violaxanthin and canthaxanthin are the members of the xanthophylls. Violaxanthin has an orange colour pigment that makes it another food colorant (E161e).

c. Phycobilins

Phycobilins (Phycocyanin and Phycoerythrin) are mainly found in the stroma of chloroplasts of cyanobacteria, rhodophyta (red algae), glaucophyta and some cryptomonads. Green algae including chlorophyta are not natural producers of chromophores. Chemically, they constitute open-chain tetrapyrroles structurally related to the pigments of mammalian bile. Phycocyanin is a blue pigment primarily found in cyanobacteria, while phycoerythrin is a pigment occurring in red algae and responsible for its characteristic red coloration.

Phycobilins are unique among various photosynthetic pigments because they are interlinked with certain water-soluble proteins, phycobili proteins. They forward the energy from the harvested light to chlorophylls for photosynthesis. Therefore, similar to carotenoids, they serve as “secondary light harvesting pigments (Parmar et al, 2011). Phycobilins fluoresce at a particular wavelength, and act by binding phycobili proteins to antibodies in immunofluorescence techniques (Bindu et al, 2007). Such phycobili proteins are the algae-derived products probably of highest market value. Due to their intense colour, phycobilins are

often utilised as food colouring and in cosmetics in addition to these very complex as chemical indicators. They are highly soluble in water and are widely used in the food industry as dyes and in molecular biology as fluorescent markers (Koller et al, 2014). Microalgae also represent a precious source of vitamins A, B1, B2, B6, B12, C, E, biotin, folic acid, pantothenic acid, etc. Vitamin B12 refers to water soluble vitamin and is synthesized in animal products, but absent in plants. Deficiency of Vitamin B12 is common in people following a vegan or vegetarian diet. Some types of microalgae may contain or synthesize vitamin B12, for example, *Chlorella* sp. and *Pleurochrysis carterae* (Kumudha et al, 2015). Vitamin E is synthesized in many microalgae, for e.g., *Dunaliella tertiolecta*, *Tetraselmis suecica*, *Nannochloropsis oculata*, *Chaetoceros calcitrans* and *Porphyridium cruentum*. Numerous studies suggest that microalgae may contain substantially more vitamin E than plants do. In this regards, microalgae are an important source for the generation of vitamin E. (Santiago-Morales et al, 2018).

Table 2.2 Chlorophyll a and b content in different microalgae
(Koller et al, 2014, Kumudha et al, 2015, Mulders et al, 2013, Alessandro et al, 2016).

Microalgae	Content, %	
	Chlorophyll a	Chlorophyll b
<i>Chlorella vulgaris</i>	4.18 ± 0.12	2.53 ± 0.07
<i>Chlamydomonas reinhardtii</i>	3.48 ± 0.09	3.61 ± 0.10
<i>Botryococcus braunii</i>	3.72 ± 0.09	2.17 ± 0.07
<i>Neochloris cohaerens</i>	6.13 ± 0.18	1.57 ± 0.05
<i>Nannochloropsis gaditana</i>	3.71 ± 0.10	1.72 0.04

It was found (Mulders et al, 2013, Alessandro et al, 2016) that in the microalgae *Botryococcus braunii*, *Neochloris cohaerens*, *Chlamydomonas reinhardtii*, *Nannochloropsis gaditana* and *Chlorella vulgaris*, the content of chlorophyll a exceeds the quantitative content of chlorophyll b. Therefore the microalgae *Botryococcus braunii* contains 6.58 - 0.19 mg/g and 2.17 - 0.07 mg/g of chlorophyll a and b, respectively. Microalgae cells of *Neochloris cohaerens* contain 3.9-times less chlorophyll b (6.13 - 0.18 mg/g and 1.57 - 0.05 mg/g).

2.3 Micro algal lipids

Triacylglycerols, phospholipids, glycolipids, or phytosterols are some of the different types of lipids that microalgae can produce ranging from C12 to C24, often with mono- and poly-unsaturated fatty acids C16 and C18. Microalgae range in lipid content from 20% to 50% of dry weight. According to Bellou et al, (2014) these lipids can be employed for a variety of metabolic processes, including energy storage, energy substrates, cell membrane structure, signal transduction, transcriptional and in translational regulation, intercellular connections, secretion, and the movement of vesicles. The production of neutral lipids like triacylglycerols might rise under unfavourable conditions whereas the conversion of fatty acids to glycerol-based membrane lipids can be facilitated under favourable conditions. Typically, many microalgae contain polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid arachidonic acid, and docosahexaenoic acid. The main saturated fatty acid is palmitic (Lopez et al, 2017). The commercial importance of lipids generated from algae as a substitute source for obtaining PUFAs like eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as its precursor linolenic acid (Mendes et al, 2006). The majority of marine species are rich in omega-3 microalgae, like *Schizochytrium* sp. and *Nannochloropsis* sp. However, freshwater species have also been investigated as a source of omega-3 long-chain PUFA, EPA, and DHA acids (Sprague et al, 2017, Ferreira et al, 2019). Since the human body is not able to produce some essential fatty acids, they required to be obtained from food or using various food additives, often obtained from fish and fish oil. However, due to the increased interest in the modern world for vegan diets and vegetarian, microalgae can become an alternative source of these nutritional supplements.

Table 2.3.a an overview of Micro algal fatty acids and potential fields of application

Fatty acid (lipid)	Micro algal representatives	Application of fatty acid (lipids)	References
(5Z,8Z,11Z,14Z,17Z)-eicosa-5,8,11,14,17- pentenoic acid (EPA)	<i>Isochrysis galbana</i> , <i>Nannochloropsis oculata</i> , <i>Nitzschia laevis</i> , <i>Phaeodactylum cornutum</i> , <i>Porphyridium cruentum</i> , <i>Porphyridium purpureum</i> , <i>Phaeodactylum tricornutum</i>	Nutritional supplements: heart diseases, blood clotting, blood pressure, lowering plasma levels of cholesterol and other lipids, anti-thrombosis and anti-arthrosclerosis. Psychotherapeutic medication, Aquaculture	(Jude et al, 2006, Peet et al, 2001)
(4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenoic acid (DHA)	<i>Cryptocodinium cohnii</i> , <i>Pavlova lutheri</i> , <i>Schizochytrium limacinum</i>	Nutritional supplements: important for brain and eye development at fetus and Children, Adult dietary supplement in food and Beverages, Significant for cardiovascular health, Anti-colon cancer, anti-breast cancer, Aquaculture	(Spolaore et al, 2006, Trappmann et al, 2011, Yaakob et al, 2011)
5Z,8Z,11Z,14Z)-5,8,11,14-eicosatetraenoic acid (arachidonic acid, ARA)	<i>Porphyridium</i>	Nutritional supplements; anti-inflammatory, muscle anabolic formulations (body builder), Aquaculture	(Trappe et al, 2001, Mendes et al, 2006)
All-Z-6,9,12-octadecatrienoic acid (γ -linolenic acid, GLA)	<i>Arthrospira</i>	Nutritional supplements Anti-inflammation and auto-immune diseases, potential to suppress tumor growth and metastasis (anti-cancer)	(Milledge et al, 2012, Papadaki et al, 2017)
In addition Mono- and polyunsaturated and even branched hydrocarbons (up to 75%) n-Alkadienes and trienes; triterpenoid botryococenes and methylated squalenes, lycopadiene (a tetraterpenoid)	<i>Botryococcus braunii</i>	Biofuel production	(Rao et al, 2007, Kato et al, 2007)

The composition of fatty acids for the similar species of microalgae could vary depending on the cultivation parameters and the nutrient medium's composition. For eg., Scharff et al, (2017) evaluated the effect of the photoperiod on the biochemical profile of microalgae *Chlorella vulgaris* and *Scenedesmus obliquus* and found that longer photoperiods (24:0, 22:2, 20:4) can reduce the synthesis of linolenic acid and can stimulate the synthesis of linoleic acid, which is more distinctly observed for *C. vulgaris* than *S. obliquus*.

On the other hand, Chandra et al, (2017) established that the content of both linoleic and linolenic acids increases together with an increase in light intensity, which is a continuous lighting condition for an optimal photoperiod. Ramirez-Lopez et al, (2016) proposed a fresh culture medium to encourage the microalga *Chlorella vulgaris*' biomass and lipid accumulation. The concentrations of sodium nitrate, ammonium bicarbonate, heptahydrate of magnesium sulphate, potassium dihydrogen phosphate, dipotassium phosphate, and diammonium phosphate were modified in the process of optimising the component composition of the medium. It was possible to enhance biomass productivity by 40% and lipid concentration by 85% using a new culture medium. The concentration of some elements of the nutrient media was reduced to 50%. In order to boost lipid production, Josephine et al, (2015) investigated the role of growth factors and the ideal time to collect *Chlorella vulgaris*. An exogenous supplement named the *Chlorella* growth factor (CGF) significantly increased lipid levels and biomass, which determined its potential as a biomass growth promoter in lab and large-scale production. The production of more unsaturated fatty acids than saturated ones was discovered to be favoured by nitrogen deficiency.

In order to sequentially isolate various biologically active compounds from the *Chlorella vulgaris* biomass (a nucleotide-peptide complex abundant with vitamins, minerals, polysaccharides, lipids, and carotenoids), Anthony et al, (2018) devised a column chromatography approach. The suggested procedure (Josephine et al, 2015) made it possible to improve the yield of lipids (18%) and lutein (9%) while removing any external chlorophyll impurities. Micro cultures of the microalgae *Haematococcus pluvialis*, *Scenedesmus obliquus*, and *Chlorella vulgaris* were found to have the maximum lipid content. These microalgae cultures contain 19.61 0.58%, 17.13 0.51%, and 16.24 0.48% of lipids, respectively.

Table 2.3.b Fatty acids composition of microalgal lipids
(Bellou et al, 2014, Sprague et al, 2017, Ferreira et al, 2019, Scharff et al, 2017, Chandra et al, 2017)

Fatty Acids	<i>Chlorella Vulgaris</i>	<i>Botryococcus Braunii</i>	<i>Neochloris Cohaerens</i>	<i>Chlamydomon as reinhardtii</i>	<i>Nannochloropsis gaditana</i>
Myristic acid	1.15 ± 0.03	2.21 ± 0.06	0.72 ± 0.01	-	-
Palmitic acid	13.65 ± 0.47	-	20.48 ± 0.61	17.25 ± 0.51	16.18 ± 0.48
Palmitoleic acid	1.23 ± 0.03	5.04 ± 0.15	2.79 ± 0.08	-	1.71 ± 0.05
Stearic acid	38.51 ± 1.21	-	42.97 ± 1.31	42.81 ± 1.28	-
Oleic acid	16.79 ± 0.55	14.68 ± 0.44	-	-	22.26 ± 0.66
Linoleic acid	7.02 ± 0.20	-	8.03 ± 0.23	6.29 ± 0.21	8.27 ± 0.25
Linolenic Acid	1.47 ± 0.04	6.42 ± 0.12	3.06 ± 0.09	1.73 ± 0.04	3.78 ± 0.10

Neochloris cohaerens cell culture possesses a fat content of 6.61 0.19%, which is 1.35 times larger than the lipid content of *Chlamydomonas reinhardtii* microalgae's dry biomass (4.90 0.14%), but lower. *Chlorella vulgaris*, *Botryococcus braunii*, *Neochloris cohaerens*, *Chlamydomonas reinhardtii*, and *Nannochloropsis gaditana*'s microbial lipid profiles were further studied using gas chromatography.

2.4 Micro algal Polysaccharides

In order to activate natural killer cells (NKs) and cause T helper cells to differentiate into Th1 cells, it has been suggested that the polysaccharides of the microalgae *Chlorella sorokiniana* can trigger the release of interleukin 12 (IL12). This effect is important in antiviral and antitumor therapies (Chou et al, 2012).

Naggar et al, (2020) identified and extracted water-soluble polysaccharides from the microalga *Chlorella vulgaris* to use them as plant growth stimulants. Liu et al, (2018) isolated a water-soluble polysaccharide from *Haematococcus pluvialis* using Sephacryl S400 chromatography and DEAE-52 anion exchange column. The resulting polysaccharide has showed immunomodulatory activity. Gaignard et al, (2019) examined 166 species of microalgae marine cyanobacteria in order to identify strains producing exopolysaccharides. Forty-five strains with the various characteristics were isolated. Eight new genera of microalgae producing exopolysaccharides, including polymers with original composition were also discovered.

3. Biological Activity of special valued Substances from Microalgae

This section provides a brief analysis of the works aimed at various substances showing biological effects from microalgae.

a. Antitumor Activity

Tumors are one of the major causes of death in the modern world. In recent years, studies on the new substances with antitumor properties have become increasingly relevant. Micro algal enzymes, fucoidans, zosterol, certain sulfated polysaccharides, alginates, and peptides possess antitumor activity (Galasso et al, 2019). Somasekharan et al, (2016) evaluated the antitumor activity of an aqueous extract of microalgae against various human cancer cell lines, including lung, prostate, stomach, breast, pancreas cancer and osteosarcoma. The ability of microalgae extract to prevent cancer cells from forming colonies has been studied by the authors. A micro alga extract tested in vitro shown strong anticlonal-forming properties. Chen et al, (2019) extracted sulfated polysaccharides from filamentous microalgae *Tribonema* sp. and tested their activity on liver cancer cells, HepG2. Samarakoon et al, (2014) studied the antitumor activity of the alcoholic fatty acid ester synthesized by the microalgae *Phaeodactylum tricornutum* Bohlin. Antitumor activity was evaluated for three different cancer cell lines (human lung cancer (A549) and mouse melanoma (B16F10) human leukemia (HL-60).

b. Antimicrobial Activity:-

Numerous metabolites with antibacterial, antiviral, and antifungal activities can be produced by microalgae. Some microalgae have the ability to grow in extremely harsh environments, which stimulates the synthesis of unique compounds for adaptability to changing environmental conditions. As a result, the environmental conditions of cultivation have direct impact on the content of biologically active compounds. Many algae are known to be able to create chemical defence system in order to live in adverse environment. Antimicrobial activity observed in lutein and ferulic acid, polyunsaturated fatty and organic acids, active metabolites, and other particular biologically active compounds isolated from microalgae⁷³. The microalgae *Dunaliella salina* produced physiologically active compounds that were evaluated to have antibacterial action by Krishnakumar et al, (2013). The antibacterial and antifungal activity of two green microalgae (*Chlorella vulgaris* and *Scenedesmus* sp.) against four Gramme positive bacteria (*Staphylococcus aureus*, *Sarcina lutea*, *Bacillus subtilis*, and *Bacillus megaterium*), one Gramme negative bacteria (*Klebsiella pneumonia*), and five fungal strains (*Fusarium oxysporum*) was tested by Salem et al, (2014).

c. Antioxidant Activity

An antioxidant is a biological substance that protects the body or essential components against oxidative reactions caused by radicals (Scaglioni et al, 2018). Numerous natural antioxidants are frequently used as an active ingredient and to guard various cosmetics' constituents from oxidative processes. Antioxidants are also actively used in the production of functional foods (Patel et al, 2019, Joshi et al, 2018). Microalgae, as photosynthetic organisms, are often exposed to reactive oxygen species, as a result of which they accumulate antioxidant complexes and have developed a mechanism for protecting cells from the action of free

radicals. According to Sathasivam et al, (2018), carotenoids, dimethyl sulfoxide, special phenolic, and nitrogen compounds isolated from microalgae all have antioxidant properties.

Agregan et al, (2018) assessed the antioxidant potential of extracts obtained using ultrasound from microorganisms *Chlorella* and *Spirulina*. It was found that extracts of the investigated microalgae are more suitable for application as phenolic antioxidant sources in human food. Microalgae extracts aren't appropriate for commercial use, in contrast to macro algae, due to their comparatively poor antioxidant potential (measured in terms of polyphenols). Antioxidant and cytotoxic activity of carotenes produced by *Dunaliella salina* under stress examined by Pritika Singh et al, (2016). Sansone et al, (2017) analyzed the biological activity of the aqueous-alcoholic extract of the microalgae *Tetraselmis suecica* containing high concentrations of carotenoids. The studied extract had a high antioxidant and reparative activity.

d. Anti-Inflammatory Activity

Micro algal compounds with a distinctive structure and characteristics, such as phycocyanin, polysaccharides, monosaccharides, enzymes, polyunsaturated fatty acids, peptides, and polyphenols have anti-inflammatory properties (Chen et al, 2018). A highly nutritious blue-green microalgae known as spirulina (*Arthrospira platensis*) is used as a nutraceutical food supplement all over the world. It has nutritional benefits as well as medicinal properties such as anti-inflammatory activity. Spirulina contains a unique component, phycocyanin, which inhibits the formation of pro-inflammatory cytokines such as TNF, reduces the production of prostaglandin E(2) and inhibits the expression of cyclooxygenase-2 (COX-2) carotene (Chen et al, 2018), accumulation of which suppresses the transcription of IL-1, IL-6 and IL-12, inflammatory cytokines, in the macrophage cell line stimulated by lipopolysaccharide or IFN⁸⁵. Spirulina exhibits prebiotic properties such as polysaccharides in their structure, monosaccharides, enzymes, PUFAs, peptides and polyphenols (Andrade et al, 2018). It promotes the growth of probiotic bacteria such *Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus thermophilus*, and *Bifidobacteria*. Along with various analgesics and antioxidants, extracts of *Phaeodactylum tricorutum* and *Chlorella stigmatophora* also have anti-inflammatory effects.

4. Removal of pollutant by micro algal action

In addition to CO₂ ablation, microalgae are considered as potential source which easily remove various heavy metals such as lead, chromium, cadmium and others from diverse aquatic environments, especially from industrial waste water, by "bioleaching" (Raposo et al, 2016) carbon compounds present in waste water can also be converted by the algae during heterotrophic phases of cultivation, thus lowering the chemical oxygen demand of the water body (Balaji et al, 2014).

5. Biofuels

A biofuel is a fuel produced through contemporary biological processes, such as agriculture and anaerobic digestion, rather than geological processes involved in the formation of fossil fuels. They are derived from biological material, mainly from microorganisms, plants, animals and wastes (Demirbas, 2007). Fourth generation biofuels have been also evolved which makes use of novel synthetic biological tools but still emerging at the basic research level. Microalgae derived biofuels are:

a. Bioethanol

Bioethanol is ethyl alcohol or ethanol derived from a biological source. It can be used as a substitute or an additive to petrol (Nahak et al, 2018). Bioethanol is manufactured by breaking down of starch or other sugars from first and second generation feed stocks such as corn, lignocellulosic biomass (sugarcane waste), wheat, etc. (John et al, 2011). Algae, a third-generation renewable source, are one of the most pronounced after sources for the production of bioethanol. Bioethanol can be produced mainly by fermentation of either starch, which is a storage component or cellulose which is a component of the cell wall (Ullah et al, 2015). Algae are considered as beneficial source for bioethanol since they contain a good amount of carbohydrates. Some of the commonly used algae for bioethanol production are *Sargassum Glacilaria*, *Prymnesium parvum*, *Euglena gracilisporphyridium*, *Chlorella Dunaliella*, *Chlamydomonas*, *Scenedesmus*, and *Spirulina* (Chaudhary et al, 2014).

b. Biogas

By anaerobic degradation in biogas plants, residual algal biomass can be used for the generation of methane, an energy carrier of negligible carbon footprint (Oslaj, 2018).

c. Biodiesel

Biodiesel, like bioethanol, is also a highly sought alternative to fossil fuels. Biodiesel is produced by the trans esterification of lipids obtained from algae, to form methyl esters of long chain fatty acids. As first and second Generation biofuel lead to the fuel versus food conflict, high usage of arable land and poor economy, thus making algae as one of the most feasible sources (Demirbaş 2018). Also, from the environmental point of view, biodiesel from algae is more preferable as it has lesser emission of carbon dioxide, NO₂ and other greenhouse gases, also conducted a similar study with microalga *Chlorella protothecoides* (Rizwan et al, 2018).

Conclusion

Since microalgae are regarded as a significant biological resource, it has drawn a lot of interest in recent years. An extensive range of microalgae uses, including in the food business, in medical field, biofuels, immunostimulants, and in cosmetics, are linked to the economic importance globally. Proteins, polysaccharides, lipids, polyunsaturated fatty acids, vitamins, pigments, enzymes, phycobiliproteins, and other substances can all produced by microalgae having Antioxidant, antibacterial, antiviral, anticancer, regenerative, antihypertensive, neuroprotective, and immunostimulating effects are all displayed by these biologically active compounds from microalgae.

The only microalgae that can be consumed by humans are *Arthrospira*, *Spirulina platensis*, *Chlorella* or *Chlorella vulgaris*, *Dunaliella*, *Aphanizomenon*, and *Nostoc*. These microalgae have a high concentration of physiologically active compounds and a very simple cultivation procedure, making them an attractive for large-scale growth. Even though other microalgae species like *Chlamydomonas sp.*, *Chlorococcum sp.*, *Scenedesmus sp.*, *Tetraselmis chuii*, and *Nanochloropsis sp.* have made a name for themselves as a source of beneficial ingredients in aquaculture, feed, fertilizers, and cosmetics, they do not yet have GRAS (Generally Recognized as Safe) status.

Due to the wide variety of microalgae, various cultivation conditions, and high metabolic flexibility the real potential of microalgae has not yet been fully examined. Researchers working with Microalgae are facing the following problems:-

1. Improvement in photo bioreactors for better cultivation condition.
2. Improvement of strain for higher productivity (selection and genetic engineering methods).
3. Search for new properties.
4. Study effects of different cultivation conditions on the content of biologically active substances in cells.

5. Optimization of cultivation processes (lower costs and increased product yield).

6. Assessment of environmental, economic and medical risks of scaling up production.

The joint work and multifaceted efforts on these problems will make it possible to replace the currently unstable production processes with alternative, less destructive ones. Future advancements in microalgae production optimisation technologies will make its utilisation more desirable and economically viable.

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