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Review: Biotechnology Applications of Microalgae in the Context of EU “Blue Growth” Initiatives

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Abstract

Microalgae biomass is in great demand for many prospective applications, most of which are currently subject to on-going research. Given their potential for resolving many major problems, generated mainly by human activity, including greenhouse gas emission, water contamination, fossil fuel depletion and the need for novel therapies for many diseases, microalgae are being widely cultivated using a variety of different processes. This interest has placed microalgae at the center of efforts to develop new biotechnological tools driven by the “Blue Growth” initiative of the EU. The purpose of this review is to present an overview of research advances in biotechnology applications for microalgae including biofuels, environmental protection, aquaculture, and nutraceuticals. Molecular biology studies particularly important for the latest microalgae research approaches are also considered.

Keywords: Blue Growth; Circular economy; Microalgae

Introduction

This review brings together current information related to the development of microalgae biotechnology. The corresponding fields of research are closely related, and feedback between them is continually taking place. This review will provide valuable information for future decisions that must be made to ensure the continuity of productive research on microalgae.

The study of microalgae is a recent and rapidly growing research field (Figure 1), due to the substantial range and scope of potential applications, reflected the amount of current research and its growing evolution. Microalgae research ranges widely, from alternative energy, production to cancer treatment [1] and covers areas such as the agri-food sector [2], the removal of diverse contaminants from the environment [3] and developments in the aquaculture sector. In many of these fields microbiology studies at the molecular level provide new insights, increase knowledge and contribute additional value [4]. Other two fields, which are basic to impulse the microalgae possibilities, Improve the condition culture [5] and genetic engineering based in proteomics [6]. Given their importance and current relevance, all these approaches and research fields will be described in this review.

Many research groups are actively interested in these microorganisms, and the interest is shared and supported by many companies. A key theme common to this interest sustainability for the future [7]. The increasing interest in microalgae is reflected in the statistics of references including the term “microalgae” listed in NCBI databases (Web) (Figure 1). The bulk of this review article deals with the possible applications of phytoplankton and microalgae covered by all fields of microbiology research.

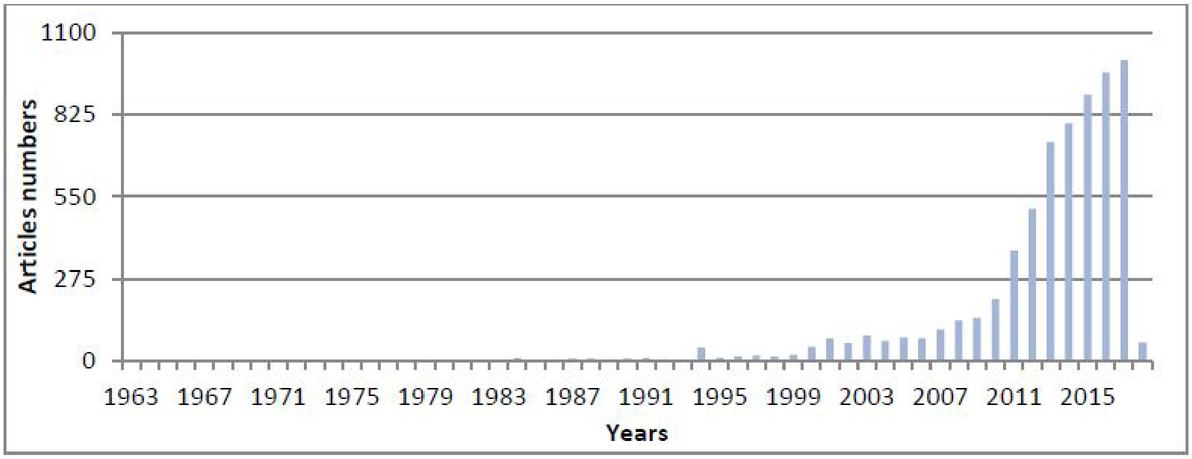

Figure 1: Evolution of number of published articles reporting microalgae studies. The graph is based on NCBI data.

The accumulation of knowledge on microalgae in recent decades makes a review related to microalgae biotechnology now essential, to present an up-to-date picture of relevant research work. The production of microalgae has been increasing globally mainly because the interest of consumers in the nutritional and health-care products obtainable from microalgae has been stimulated, making them attractive for manufacturing and marketing companies active in these sectors. Another significant reason for increasing interest is the growing public awareness and concern about environmental protection, the "green movement", because of the known capacity of microalgae to capture the main greenhouse gas, carbon dioxide, present in the atmosphere. Many companies in diverse sectors have been attracted by the potential profitability of "green" marketing.

The use of fossil fuels for many human activities is having serious repercussions on the planet's climate, which is changing in very worrying ways. Over the last 200 years, emissions of greenhouse gases have increased exponentially, and now the situation is beginning to cause widespread serious concern. It is essential to find solutions to reverse this trend. The global population is expected to increase from 6.3 billion in 2015 to more than 9 billion by 2050. The worst problems for humanity over the next 50 years will be caused by rapid population increase: these relate to the demand for energy (depletion of fossil fuels sources), access to fresh water, the emergence new human, animal and plant diseases and the increase of existing diseases, loss of natural diversity and ecosystems, contamination and the generation of waste, and the myriad more specific problems derived from these [8].

Oceans, seas and freshwater resources are global assets that have demonstrated great potential for stimulating and supporting innovation and growth, and these assets are the focus of the Blue Growth initiative of the European Union. Blue Growth is the EU's long-term strategy for supporting sustainable growth in the marine and maritime sectors. Oceans, seas and rivers have long been drivers of the European economy, offering great potential for innovation and growth; they represent the maritime contribution towards achieving the overall goals of the Europe 2020 strategy for smart, sustainable and inclusive growth ("Commission to the European: "Blue Growth" opportunities for marine and maritime sustainable growth,"). In Europe, the 'blue' economy is estimated to account for approximately 5.4 million jobs and the generation of gross added value of around €500 billion a year. The Blue Growth strategy represents one of the most ambitious plans of humanity, a plan in which microalgae have an important role. The objective is to secure the implantation of the "circular economy" model [9].

The many potentially-valuable possibilities provided by microalgae have been brought to light during studies aimed at finding new ways to generate alternative energy, ways not dependent on fossil fuels, particularly oil. These studies have opened many other fields of interest. Microalgae have certainly emerged as offering novel potential solutions for human diseases, to address the global challenge of sustainable healthy lives for all people, and contributing to what has become known as the circular economy [10].

Phytoplankton represent the autotrophic components of the plankton community and play a key role in vital ecosystems in the world's oceans, seas and freshwater basins. They obtain the energy needed for growth by photosynthesis, converting sunlight and carbon dioxide into biomass and oxygen. The potential of microalgae is very considerable, especially when it is known that there are several million-different species of algae and microalgae, compared to around 250,000 species of terrestrial plants [11]. As commented previously, the study of microalgae frequently leads to the discovery of possibilities for resolving many serious problems. Microalgae are groups of microorganisms of especially great biodiversity and with significant differences between them. Some of them are already extensively cultivated, processed, used in industry and already extensively commercialized. Some of them have begun to be very popular [12] because of their content in components valuable for health reasons: polyunsaturated fatty acids [13]; pigments [14]; vitamins; and phenolics [15]. One example is *Arthrospira* spp., commonly known as Spirulina, which is used for the extraction of phycocyanin, a blue photosynthetic pigment. Some of the species produce organic metabolites, such as sporopollenin, scytonemin and mycosporine-like amino acids, to protect themselves from UV light; some, like the genus *Chlorella,* produce pigments that are extracted for use by cosmetic companies in skin care products [16]; other highly-valued bioactive compounds are obtained from species of the *Tetraselmis* genus. *Pyrocistis lunula* is a bioluminescent alga of interest for derivative possibilities and applications in gastronomy. Some species produce the antioxidant and pigment astaxanthin which is extracted and used as a food supplement. Other microalgae, such as *Nannochloropsis gaditana*, have a composition similar to petroleum with an oils content of about 50% [17]. Phytoplankton are finding uses in almost every sector of industry and are helping to rediscover the concept of the circular economy.

The focus of this review is the state of current research in the main areas of application, with the object of revealing solutions, and providing information to guide future research.

Aquaculture

Aquaculture is the first industrial sector in the developed economies of the world in which cultivated microalgae have been widely used, as fish feed. It has grown to become an industry that currently produces more than half of the protein of animal origin for human consumption worldwide, and its contribution is expected to continue growing in the future. However, despite a large investment of money and effort, and despite the level of sophistication achieved, the industry still depends mainly on fishmeal and fish oil to meet the demand for concentrated feed for use in aquaculture. For this reason, given the degree of exploitation to which the fishery resources from which these products are derived have been subjected, and given the reality of a global population growing exponentially, it is necessary to find alternative sources of protein that will help meet this need [18] In a world in which more than 800 million people continue to suffer from chronic malnutrition, in which the global population is expected to increase by another 2 billion to reach 9.6 billion by 2050, we face the immense double challenge of feeding the population of our planet and, at the same time, protecting our natural resources for future generations. In this regard, it is important to highlight the major role aquaculture play in the elimination of hunger. Never in the history of the world has such a vast quantity of fish been consumed and never has so much depended on the fisheries industries, and on aquaculture, to ensure the welfare of so many people. Aquaculture is currently the fastest growing animal-protein food producing sector; it now supplies over 50% of the world’s fish and seafood for human consumption [18]. If developed and practiced responsibly, aquaculture can generate lasting benefits for global food security and economic growth Fish is a vital source of essential proteins and nutrients, especially for many poorer members of our global community. Aquaculture is a source not only of food, but also of income. Employment in the sector has grown faster than the world population. The sector employs tens of millions of people and is the basis for the livelihoods of hundreds of millions more. In volume and value, fish has always been one of the most widely marketed products in the world. It is especially important for the developing countries, because sometimes it accounts for half of the total value of all the products brought to market in these countries. However, besides the economic importance, it is necessary to ensure that fish stocks and their natural environment are protected to ensure sustainable long-term exploitation and hence the welfare and prosperity of those dependent on these resources. To this end, fostering the responsibility and sustainability of fisheries and aquaculture is fundamental [18]. Another option, for the future, will be to process and use microalgae directly as a food for human consumption, substituting the meat, fish and other sources of protein currently consumed by the human population.

As with humans, cold water marine finfish are inefficient at converting ALA, the metabolic precursor, into appreciable levels of the beneficial omega-3 fatty acids EPA and DHA; these, therefore, must be obtained from their diet [19]. The increasing use of vegetable oil as a replacement for fish oil in aquafeeds has subsequently resulted in the decline of the beneficial long-chain n-3 PUFA content in farmed fish, particularly salmon, thereby reducing the nutritional value to the final consumer and putting in question the current dietary guidelines with respect to fish intake [20]. With gaps in the actual versus recommended intake of EPA and DHA for the majority of the world’s population [21], and the mismatch between the supply of n-3 LC-PUFA and the population’s needs [22], there is a pressing need for novel *de novo* sources of n-3 LC-PUFA. In 2015 the Global Salmon Initiative, an evolving collaboration among the world’s leading farmed salmon-producing companies, requested commercial organizations to supply its members with between 25000 and 200,000 tons annually of novel omega-3 rich oils to support the sustainable use of marine oils in aquafeeds [23].

Microalgae, along with other single cell microbes, are the primary producers of n-3 LC-PUFA in the aquatic environment; they provide a continuous supply of EPA and DHA that is then concentrated up through the trophic food chain where there is limited capacity to synthesize these beneficial fatty acids. Accordingly, microbial sources offer a natural way of increasing the supply of n-3 LC-PUFA for farmed fish and have already been used by the aquaculture industry, microalgae are also being used directly in formulated feeds for larval and juvenile fish [24]. Within the last year several algae-based and animal nutrition companies have announced novel product lines specifically intended for use in aquaculture as sustainable alternatives to fish oil. This was largely stimulated by the Global Salmon Initiative referred to above [25].

Researchers are developing strains of microalgae that contain more lipids, nutrients and bioactive compounds [26]; and "biocrude" oil and residual protein-rich fractions are co-products of cultivated microalgae [27]. Thus, biofuel and the defatted biomass that is rich in protein will be available in large amounts soon. *Nannochloropsis* is a candidate that is being exploited for biofuel production because of its high lipid content [28]. In *Nannochloropsis*, EPA is the dominant fatty acid [29], and this characteristic makes this microalga a potential partial fish oil replacement in fish feeds [30].

Microalgal biomass can provide potential feed ingredients for carnivorous fish [30] investigated the growth, feed-intake: gain ratio and health parameters in Atlantic salmon fed with defatted *Nannochloropsis oceania* as a fishmeal replacement. These fish were compared with groups that consumed traditional feeds with no alga content. The fish that received feed with 20% alga content tended to show reduced weight gain and specific growth rate. Hepatosomatic and viscerosomatic indices, whole body and fillet proximate composition were not affected by the dietary treatments. Digestibility of dry matter, protein, lipid, ash and energy, as well as retention of lipid and energy, of the fish that received feed with 20% alga meal content were also significantly different from those of the control group [30]. Although alga feeding did not cause any distal intestinal inflammation, the intestinal proteins that were altered after feeding with 20% algal meal might indicate systemic physiological disturbances. However, the defatted *Nannochloropsis oceania* can safely be used at lower inclusion levels, around 10%, without negative effects on the growth and health of Atlantic salmon.

Aquaculture is one of the fastest growing areas within the overall food production industry. Microalgae are an important source of food and additives in the commercial breeding of many aquatic species, especially larvae and rotifers; these latter are employed in the breeding of both crustaceans and fish. The cultivation of microalgae for this purpose is a high-value industry serving a market already of substantial size [31].

Improving the Condition of Cultures

The condition of the culture in microalgae production is one of the most important aspects for study, because a better condition leads to increased efficiency and economic returns, in all the applications subject to study. Obtaining improvements and increases in the production of microalgae depends on many factors, particularly physico-chemical conditions; this encompasses numerous variables (water composition, pH, temperature, …), photoperiod, and feeding (autotrophs, heterotrophs or mixotrophic culture) [5].

Optimization of culture conditions has become almost a required element for all microalgae research studies [32]. Research aimed at improving the condition of microalgae culture is based on making changes in physical and chemical culture parameters, and on establishing the optimal nutrients required by each strain of microalga. Studies are also aimed at resolving problems deriving from the type of photobioreactor used. Since it is known that diverse compounds and components of possible commercial interest can be derived from microalgae, many researchers are seeking to increase the fraction of such components of interest obtainable from microalgae. It is not only the total biomass that is of research interest; well-known examples of valuable fractions are chlorophyll, Polyunsaturated Fatty Acids (PUFA) and carbohydrates.

Strategies reported in the literature to promote the chlorophyll content in microalgae include variation in light intensity, culture agitation, and changes in temperature and nutrient availability, including nitrogen, phosphorus, carbon source, micronutrients and other parameters. In the literature reviewed many authors underline the importance of identifying optimal chlorophyll-inducing conditions in the species of interest, and chlorophyll can be taken as an example of the many fractions of interest whose production researchers are seeking to increase [32]. A similar topic investigated is the optimal culture parameters to obtain both exopolysaccharides and biomass from microalgae [33]. In this case the difficulty is that the culture condition in which microalgae accumulate large amounts of exopolysaccharides does not coincide with the best condition for cultivating microalgae biomass: apparently a greater proportion of exopolysaccharides can be segregated as polyunsaturated fatty acids when the microalgae are under conditions of stress. That is a common difficulty that is found in most of studies aiming to obtain the maximum amount of a fraction of interest from the total microalgae [34].

Another possibility currently under study is to cultivate microalgae using urban or industrial waste water as both the medium and the source of nutrients, effectively obtaining biomass and purification treatment at the same time, more cost-effectively [35]. It is having been demonstrated that environmental conditions, particularly temperature, light intensity, photoperiod and other physico-chemical factors - in fact, every possible variable - have an influence on biomass growth and the composition of that biomass [36]; the photoperiod, light intensity and temperature were varied, and different growing curves were observed. By modifying these conditions, different lipid content in the microalgae was obtained, but the best conditions for maximum lipid content are not the same as those for biomass production [36]. It is, however, necessary to continue with research on increasing microalgae biomass production even though the importance of the physical-chemical factors is known; the characteristics of the water, the components that make up the culture in which the microalgae are being grown, as well as the photoperiod since these are microorganisms that use photosynthesis, are factors requiring study.

The kind of bioreactor, the advantages and disadvantages of agitation and aeration, and how to avoid interference with the exposure to light of the accumulated biomass, have also been studied. Observing the effects of airlift reactor hydrodynamics on the growth of microalgae species has revealed a favorable effect of the inlet gas flow rate on biomass productivity under continuous light illumination; higher inlet gas flow rates resulted in increased biomass productivity. The Beer-Lambert model was found to predict successfully the light profile inside the reactor. Microalgae cultivated under higher light intensities, and continuous light supplementation yielded desirable attributes for biodiesel [37]. These results that increase the production for one species may, however, be inappropriate for another; this makes optimization of the culture a complex task. Proper methods of achieving such adaptation need to be developed for every species of microalgae and every fraction of interest.

The effects of the magnetic treatment of microalgae has recently been studied [38]. Magnetic treatment on the metabolism of algae and the possible use of this treatment for the optimization of biotechnological processes, such as cultures using wastewater [39], protein production [40], and the concentration of pigments and total biomass production [41].

The capacity for culture growth has been improved, as well as the yield of essential amino acids and trace elements, antioxidant fractions, pigments and carbohydrates. The studies with magnetic treatment get improve the results of the microalgae cultures. Continuing with the information show, remarks the importance of improve the biomass or interest fraction obtaining, for get even more cost effectiveness from microalgae biomass. These interests about optimization condition culture is reflected in the bioreactors evolutions in the last time [32].

To summarize, the three main approaches for increasing the efficiency and economic viability of microalgae production in the future will be: 1. To improve culture conditions so that the quantity of microalgae biomass produced is increased; 2. To improve culture conditions so that one or more fractions of interest are obtained more cost-effectively; and 3. To develop new or improved cultivation, processing and extraction methods to increase efficiency and/or reduce cost. The last approach is likely to involve seeking to combine, in one process plant or stream, the requirements of two or more different sectors of industry or areas of application, in other words, looking for appropriate synergies. One example of this approach is to base the microalgae culture on using domestic or industrial waste water as the medium and the nutrient source; the potential benefits sought are a reduced cost of wastewater treatment, the recovery of the purified water for other productive uses, and the production of microalgae cost-effectively, together with protection of the natural environment. Algal blooms are abundant in the natural environment due to increasing eutrophication of the waters in certain parts of the world. To date, these blooms have not been exploited commercially, so they could be studied and developed at laboratory scale, with the objective of obtaining methods capable of being scaled up to commercially-feasible levels [42].

Biofuels

Various concepts of alternative or sustainable energy are under development in response the inexorable rise of global temperature already under way, attributable to the accumulation of greenhouse gases in the atmosphere, and to compensate for the predicted scarcity of fossil fuels, regulatory restrictions imposed on the continued use of those fuels, and arbitrary limitations on supply and price variability, among various negative considerations. Currently, in global terms, energy generated from fossils fuels comprises about 90% of total energy, and only 10% is produced from renewable energy sources [43]. Given the ever-increasing demand for energy there are predictions that global reserves of readily-accessible oil and gas will be exhausted after 2050 [44]. There is therefore an urgent need to develop alternative energy sources to mitigate the approaching shortage of energy the world probably faces.

Since these organisms capture and store solar energy as chemical energy in various forms, microalgae are a promising source to produce biofuels, while at the same time helping to mitigate the greenhouse effect. As feedstock for biofuels microalgae are superior to terrestrial plants in many positive respects, and their biomass is naturally rich in lipids, carbohydrates, proteins, pigments and other valuable compounds [8]. Certain individual strains of microalgae have already been identified as good sources for biofuel production, particularly biodiesel [45].

Although the possibility has been known for some time, in recent years the use of microalgae as an alternative biodiesel feedstock has gained renewed interest from researchers, companies, and the public. Algae offer many potential advantages: algae can potentially produce 1000 - 4000 gallon/acre/year, significantly a higher productivity than soybeans and other oil crops. They can be grown in a wide variety of climate and water conditions; they can sequester and utilize CO_2 from the atmosphere and other sources. The importance attached to microalgae by researchers in this field is because they have high storage capacity for the lipids they contain. Neutral lipids, for example triacyl glycerides, can be converted relatively easily to biodiesel (fatty acid methyl esters)

[46]. One of the ways to reduce costs in microalgae cultivation is to take advantage of the CO_2 present in the atmosphere to increase the microalgae biomass by photosynthesis. The results of studies on this use of CO_2 from the atmosphere are satisfactory, although the methods have only been proven at laboratory scale; the profitability of this approach by comparison with diesel from fossil fuels has not yet been checked. It is also possible to use the CO_2 emitted by waste water treatment plants to increase the yield from microalgae cultivation [47]. If this technology proves feasible it will be possible to solve two of the most important problems facing humanity - the end of fossil fuels and the greenhouse effect.

As discussed in the preceding section, selection of the most appropriate culture conditions is essential, as is the selection of the species of microalgae most suitable for the commercial application of interest; in this case, for biofuels generation, a species with a high storage capacity of fatty acids [47]. Lipid accumulation is triggered in microalgae when cell division is blocked by the depletion of certain nutrients like Sulphur or nitrogen, whereas carbon is continuously fixed by cells leading to lipid accumulation. As a result, high lipid accumulation usually cannot be achieved during rapid microalgae division and growth, thereby severely limiting the lipid productivity of microalgae in the exponential growth phase. It was also observed that some microalgae species with fast growth rate have a very low lipid content [48]. Thus, it seems very difficult to achieve high cell growth rate and high cellular lipid content simultaneously. To overcome the intrinsic limitations associated with production of lipids or other functional compounds from microalgae or cyanobacteria, a series of studies have focused on the possibility of genetically modifying these organisms to enhance carbon fixation [49], lipid accumulation [50] and the formation of high added-value chemical compounds [8]. Given these circumstances it is necessary to select a suitable mid-point in the culture conditions between conditions favoring growth and conditions favoring lipid accumulation, prior to developing microalgae genetically modified for lipid accumulation.

In the context of the circular economy, microalgae can play a significant role in biodiesel, biomethane, biohydrogen and other biofuel systems. The unicellular algae species are a potential feedstock for sources of bioenergy [51], and in the context of biofuel production, microalgae have been used primarily for the transesterification of lipids for biodiesel production [52]. However, use of microalgae as a feedstock for biogas production may be more feasible than for biodiesel, for a number of reasons: the low dry solids content are suitable for digestion systems, obviating the need for drying, as would be the case with biodiesel production; the species needed for biogas is not restricted to one particular distinctive species; and the degree of contamination with higher trophic life forms, as would be found in open algal ponds, is not an issue for anaerobic digestion [53]. Even if microalgae are used for biodiesel production, the remaining residues post lipid extraction can still be utilized in a biohydrogen or biomethane system [54], thus improving the energy balance of the overall biorefinery process [55]. Producing biohydrogen from microalgae, with cyanobacteria as well, is attractive to industry due to its potential as a reliable and renewable alternative energy source. Progress in genetic/metabolic engineering may significantly improve the prospects for the photobiological production of hydrogen from microalgae.

The use of certain species of microalgae as a non-conventional source of food and pharmaceuticals seems promising. Some members of green algae and cyanobacteria species are considered an excellent source of renewable biofuels such as bio-diesel, biogas, bio-oil and bio-hydrogen [56]. It is possible to generate H_2 by different methods, such as steam reforming, electrolysis, photolysis or biohydrogen production and several other methods, but these methods are not completely clean, like photo-biological hydrogen production [57]. The various photosynthetic and non-photosynthetic microorganisms that exist have very diverse physiology and metabolism that allow them to generate hydrogen using different metabolic pathways. The production of hydrogen by microorganisms has attracted public interest due to its potential as a renewable energy carrier which can be produced using nature's most plentiful resource, solar energy [58]. Obtaining a clean energy using H_2 from microorganism metabolism, in this case from a heterotrophic microorganism, generates desirable products. However, with microalgae or cyanobacteria, the photosynthesis process generates only H_2. Photo-biological hydrogen production is considered a more efficient and less energy-intensive process. Now photobiological H_2 is not profitable due to high costs, and it is necessary to improve the method to make it profitable. By means of genetic engineering, for example, modified microorganisms will yield more H_2 with an accelerated metabolism [58]. Biologically produced hythane, known as Biohythane, is a fuel of great potential; it is a mixture of hydrogen and methane from different kinds of residual algae biomass, with better characteristics than other biofuels, and is currently considered the best alternative to fossil fuels. Hythane, comprising CH_4 and H_2, has been described as most the important alternative energy carrier with a wide range of commercial uses [59]. The addition of hydrogen and methane increases the H:C ratio, which reduces the emission of greenhouse gases on combustion, widens the narrow flammability range of methane, leading to increased combustion efficiency and flame speed; thus, the duration of combustion is reduced, and heat efficiency is improved [59]. Production of hydrogen from renewable sources is currently the major bottle-neck in the sustainable production of hythane [42].

It is evident that future EU legislation will encourage the use of advanced biofuels to further the reduction of greenhouse gas emissions in the energy sector. The most recent EU directive proposals have suggested a progressive reduction in first-generation (terrestrial plant-based) biofuels by 2030; renewable-source, low-carbon transport fuels (including electric vehicles) are predicted to increase their share of the total used in Europe from 1.5% in 2021 to 6.8% by 2030 [60], with advanced biofuels expected to account for at least 3.6% by that time (Parliament of the European Union.). This will require a significant overhaul of the current energy system which is predominantly fossil fuel-based. Sustainability will become a more significant issue in terms of biofuels contributing towards Renewable Energy Supply (RES) targets set for the EU. At present, on a whole-life-cycle analysis basis, greenhouse gas emissions must be reduced by 60% with respect to the fossil fuel displaced to count as a renewable-source transport fuel, with a further increase of this proportion to 70% scheduled for 2021 [9]. Thus, biofuels must not only be a renewable energy source but must also be truly sustainable in future energy systems [60]. These decisions taken by the Europe Union should considerably reinforce efforts and investment in obtaining energy from microalgae.

Many research groups are working on improving the efficiency of methods for obtaining clean bioenergy from microalgae. In combination with techniques such as using sunlight and CO_2 from the atmosphere and using waste water for as a nutritious medium for microalgae culture, these efforts should reduce the costs of the process. These efforts are bringing us closer to making biofuels obtained from microalgae sufficiently cost effective and profitable.

Removal of Pollution from Various Systems

Microalgae are being used to recover contaminated waters and contaminated atmosphere. The capacity of different microalgae species to capture CO_2 from the atmosphere and to eliminate diverse contaminants from various kinds of water-based effluent flows, e.g. from industrial processes, biological reactors, agricultural run-off, and many more, are being exploited [61]. It is being found that microalgae offer potential solutions to many problems generated by human economic activity.

Bioremediation is the term used to describe the use of organisms (including animal, plant and bacteria species), individually or in combination, to minimize the contaminating load carried by effluent flows from any productive activity (including aquaculture). This practice takes advantage of the natural or modified abilities of those organisms to reduce and/or transform waste products [62]. The two different systems currently treatable by means of microalgae are water (domestic, urban, agricultural and industrial waste water) and the atmosphere (contaminated mainly by emissions and treated indirectly). The idea is to use microalgae to either eliminate or assimilate the polluting substances from affected waters and from the air.

The pollution of water and air are two parts of an enormous problem facing for humanity, a problem that is rapidly getting worse and is expected to continue increasing. The failure of existing natural systems to prevent the proven increase in the concentration of carbon dioxide (CO_2) in the atmosphere, and in waters of the oceans and seas (where the effect is to increase the acidity), due to the various large-scale anthropogenic interventions in natural global systems, is leading to significant alteration in the global carbon cycle; this has now attracted the worldwide attention of the public-at-large, and has been the subject of massive research efforts in the last few decades. In this alarming scenario, microalgae have emerged as a scientifically and technologically-attractive means capturing the excess CO_2 present in the atmosphere, generated by emissions from diverse sources such as power plants, automobiles, volcanic eruptions, decomposition of organic matter, and forest fires. This CO_2 captured by means of cultivating microalgae could be used as a potential source of carbon to produce lipids for the generation of biofuel (principally for replacing petroleum-derived transport fuels) without diminishing the supply of crops and food [63].

The capacity of microorganisms for removing pollutants in different ecosystems is a widely-studied topic [64]. They have been used for a relatively long time in water purification applications (in biological reactors), but in this review, the focus is on research into the capacities and feasibility of employing microalgae to eliminate contaminants of different origin. The large-scale production of microalgae biomass poses challenges due to the requirements for large quantities of water and nutrients for cultivation. Using wastewater for microalgae cultivation has emerged as a potential cost-effective strategy for large-scale microalgae biomass production [65]. This method of cultivating microalgae offers an efficient means to remove contaminants from wastewater, since it makes wastewater treatment more sustainable and energy-efficient. Much research has been devoted in the recent years to utilizing a variety of different wastewater streams for microalgae cultivation, and the biomass produced can be further processed to yield a wide range of value-added products.

Cultivation of microalgae in wastewater has long been recognized as a viable option for sustainable biomass production and wastewater treatment [66]. The latest research has demonstrated the potential of microalgae for reduce effectively the pollution present in waste waters, reducing the carbon, phosphorus, nitrogen inclusive radon content of these waters [65,67]. Currently, most experiment are at laboratory scale, and results from domestic waste water show biomass productivities are increased while the dissolved P and N are reduced at close to 90% efficiency, depending on the species [65]. In the case of agricultural waste water, the method is shown to be highly effective with waste water of vegetable origin, but some problems are encountered with waste water of animal origin, especially waste water from poultry farms. Waste water of industrial origin presents completely different problems, because the toxicity and composition of the water will vary considerably, together with the resistance of the microalgae species selected. The capacity for removing the contaminants depends on how the microalgae can assimilate or degrade the pollution present in the waste water [68]. Recently the capacity of microalgae for removing pharmaceutical contaminants from water has been studied [68]. That study demonstrated the broad range of capacities of microalgae for removing many of these kinds of contaminant, through three biochemical pathways: bioadsorption, bioaccumulation and biodegradation (intracellular and extracellular). In fact, it can be stated that every contaminant present in water can be eliminated, provided that the microalgae species can incorporate the contaminant by bioadsorption, bioaccumulation or biodegradation in its metabolism or in its cells.

In the same way that microalgae are used to restore waste water to usable quality, they can also be used to improve the condition in drinking waters. A case studied by many researchers is how to remove nitrates from potable water, because nitrates cause serious diseases. The application of algae-based water treatment is also being introduced as a nature-inspired approach that may broaden the future horizons of nitrate removal technology [3]. Removing nitrates from drinking water using conventional treatment methods, such as coagulation and filtration, is almost impossible owing to the high stability and solubility of nitrate and its limited potential for precipitation or adsorption in water [69]. The elimination of nitrates from water is, however, possible with heterotrophic or autotrophic microorganisms. Heterotrophic microorganisms need organic carbon to be able to remove the nitrate. Therefore, this process requires additional carbon and causes secondary pollution. The autotrophic technology attracted the attention of many researchers because this process is profitable, does not generate contaminants, but yields a lower biomass compared with heterotrophic microorganisms. Among autotrophic methods, sulfur-based and hydrogen-based denitrification processes have attracted substantial attention in respect of microalgae [3]. Many researchers have shown that *Thiobacillus denitrificans* and *T. thioparus*, both autotrophic microorganisms, can effectively remove nitrate from drinking water when a reduced sulfur compound is used as the electron donor and nitrate acts as the electron acceptor [3]. The benefits of autotrophic denitrification compared with heterotrophic denitrification can be classified as follows: it does not need organic carbon as the carbon and energy source, but instead uses inorganic carbon dioxide as the carbon source; and it uses inorganic minerals or light as the energy source. In anaerobic conditions, chemoautotrophic organisms oxidize inorganic minerals while reducing nitrate to nitrogen gas [70]. The method has been demonstrated to be more beneficial than physic-chemical methods. Of the two types of biological denitrification studied, autotrophic nitrate reduction is preferable, and hydrogenotrophic denitrification may be the best choice, owing to the clean nature of hydrogen and the absence of any unwanted byproducts [3]. It should be possible to optimize the process to obtain better results; however, the operational parameters of the autohydrogenotrophic process, such as hydrogen and CO_2 flow rates, pH, and temperature, as well as the nutrient concentration, need to be studied to increase both the nitrate removal efficiency and safety [3].

The best-known case, considered to have a major environmental impact for the future, is the removal of CO_2 from the world's atmosphere. CO_2, the greenhouse gas with by far the greatest volume, is readily dissolved by the world's oceans, lakes and seas but is largely released again quite soon: it is not permanently sequestered. However, certain species of microalgae, and especially cyanobacteria, known as "blue-green algae", are present in countless numbers and have an exceptionally high capacity to capture CO_2 from the atmosphere by photosynthesis; they then convert it into carbon as their own body mass, and oxygen which is released back into the atmosphere. This capacity greatly exceeds that of any terrestrial plants [56]. If CO_2 removal can be achieved on a sufficiently large scale to check global warming, land that is scarce in some parts of the world will not need to be allocated to natural plant-cover, like forests, steppes, prairies, etc., and can be used for other human needs as the population continues to grow.

To summarize, the cultivation of microalgae makes it possible to resolve both small, local-scale problems like cost-effective waste-water treatment and global-scale pollution problems like the greenhouse effect and consequent global warming. Using these microorganisms to remove CO_2 from the atmosphere could vastly increase the production of useful biomass as well as increasing the growth rate. The many other applications of microalgae would also benefit, with the derived products becoming more profitable.

Nutraceuticals (Healthy Foods and Biomedicine)

Microalgae contain high levels of carbohydrates, proteins, enzymes, pigments and fiber. Many vitamins and minerals important for health, including vitamins A, C, B1, B2, B6, niacin, iodine, potassium, iron, magnesium and calcium, have been found in relatively high concentrations [71]. Microalgae are a rich source of essential nutrients, and are already consumed in large volumes as human food, principally in Asia [71]. Algal biomass also has recently received considerable attention due to its high carbohydrate content [51,53].

Green micro-algae have been used as a nutritional supplement or food source in many countries of Asia for hundreds of years, and now they are consumed throughout the world for their nutritional value. Some of the microalgae species that are of most biotechnological relevance are the green algae (Chlorophycea) *Chlorella vulgaris*, *Haematococcus pluvialis*, *Dunaliella salina* and the cyanobacteria *Spirulina maxima*; these are all widely commercialized and used, mainly as nutritional supplements for humans, and as animal feed additives [71]. This is part of a worldwide trend towards reducing the currently high levels of meat consumption, in the interests of a healthier diet - a wide-ranging social change that is leading to an increased demand for plant protein. Traditionally, microalgae such as *Spirulina* and *Chlorella* are sold directly to the public as dietary supplements, without any kind of processing except drying.

Microalgae contain several bioactive compounds that can meet the nutrition and energy needs of the population, promote health and prevent chronic disease [72]. Microalgae including the genera *Spirulina, Botryococcus, Chlorella, Dunaliella, Haematococcus,* and *Nostoc* have been recognized as valuable sources of these bioactive compounds [72] (Table 1,2).

Microalgae	**Bioactive compounds present in microalgae**
Arthrospira sp., *A. platensis*, *S. fusiformis*,	phenolic acids, tocopherols (vitamin E),
S. maxima	neophytadiene, phytol, PUFAs (n-3) fatty acids, oleic acid, linolenic acid, palmitoleic acid, diacylglycerols, terpenoids, alkaloids, flavonoids
Chlorella sp., *C. vulgaris*, *C. minutissima*, *C.*	Carotenoids, sulfated polysaccharides, sterols,
ellipsoidea, *C. protothecoides*	Eicosapentaenoic Acid (EPA), zeaxanthin, PUFAs (n-3) fatty acids, canthaxanthin, astaxanthin, peptide, oleic acid, violaxanthin, lutein, phenolic, terpenoids, alkaloids, phytol, phenol
Haematococcus pluvialis	Astaxanthin, lutein, zeaxanthin, canthaxanthin, lutein, β-carotene, oleic acid
Dunaliella salina	All-trans-β-carotene, all-trans-zeaxanthin, all-trans-lutein, cis-betacarotene, β-carotene, oleic acid, linolenic acid, palmitic acid, diacylglycerols, sterols
Botryococcus braunii	Linear alkadienes (C25, C27, C29, and C31), triene (C29)
Nostoc sp., *N. muscorum*, *N. humifusum*, *N.*	Borophycin, cryptophycin, phycocyanin,
linckia, *N. spongiaeforme*	phenolic, terpenoids, alkaloids, phycobilins

Table 1: Modified from [72]. Main bioactive compounds extracted from microalgae.

Product	***Algae species used***
Hepatitis B Antigen Protein (HBsAg)	*Dunaliella salina*
Human Growth Hormone (HGH)	*Chlorella vulgaris, Chlorella sorokiniana*
Erythropoietin; Human fibronectin 10FN3 and 14FN3; Interferon β; Proinsulin; Human Vascular Endothelial Growth Factor (VEGF); High Mobility Group Protein B1 (HMGB1)	*C. reinhardtii*
Bovine Lactoferricin (BLF)	*C. reinhardtii*
Avian and human metallothionein type II; Antigenic peptide P57; Antigenic proteins VP19,24,26,28; Foot and mouth disease virus VP1 protein; Anti-glycoprotein D of herpes simplex virus; Anti-rabbit IgG; Human tumor necrosis factor; Bovine mammary-associated serum amyloid; Classical swine fever virus E2 viral protein; Human glutamic acid decarboxylase 65; Human erythropoietin; Anti-anthrax protective antigen 83 antibody; D2 fibronectin-binding domain	*C. reinhardtii*
Flounder Growth Hormone (FGH)	*Synechocystis*

Table 2: Many components of considerable interest for disease control have been found in different microalgae species. This finding makes many applications of microalgae biomass potentially more beneficial and valuable.

Natural pigments are important for the metabolism of photosynthetic algae and present several beneficial biological properties, such as antioxidant, anti-carcinogen, anti-inflammatory, anti-obesity, anti-angiogenic, skin health and neuroprotective activities [73] inclusive have been detected anti-cancer properties in marine microalgae [74]. Nutritional and toxicological evaluations have demonstrated that microalgal biomass is beneficial as a food supplement or substitute for conventional protein sources [2]. Microalgae do not require arable land and can be grown in regions where no more land for conversion to agricultural use is available; they would thus protect many terrestrial ecosystems and their biodiversity in the face of traditional agricultural practices [75]. Thus, given the growing demand for microalgae biomass as a substitute food, as well as a food supplement, it is an Microalgae have opened the pathway towards a healthier diet, without provoking alterations in wild ecosystems and derived threats to biodiversity [76]. Microalgae are the source of many products widely used in diverse fields, ranging from biomedicine to nutrition, and industrial and commercial interest is continually evolving.

Genetic Engineering Based in Proteomics, for Developing Specific Applications of Microalgae

In the context of molecular techniques is essential to know the proteome of the microalgae and the facilities to produce "something" (biological product of interest), this information is getting through proteomics assays. The proteomics is the responsible of the organism behavior (phenotype) [77]. This information about proteome show a widely information, transforming the proteome in information with high value for all investigation fields with the information of proteomics research can be design the bests strategies in genetic engineering [6].

Genetic manipulation in microalgae is a field of study in constant evolution. Important advances have been reported, such as the efficient expression of transgenes [78]; a novel mechanism for gene regulation in algae using riboswitches [79]; inducible nuclear promoters and luciferase reporter genes [34]; and inducible chloroplast gene expression [80]. A molecular toolkit or genetic tool information are also essential, along with metabolites, metabolic pathways and bioinformatic analysis. Sequence analysis will include information on metabolomics, which opens the way for the analysis of metabolic flux, and the development of metabolic networks [81]. RNAi technology can also be used to downregulate the expression of some gen mechanism [82]. It is known that microalgae are an attractive source to produce diverse proteins and other metabolites [83]. In several studies, microalgae have been reported to be a valuable source to produce fatty acids, biohydrogen, etc. [84]. Recent applications of gene editing, novel platform designs for proteins and computational modeling will also be helpful toward increasing production [84]. Recent applications of gene editing, novel platform designs for proteins and computational modeling will also be helpful toward increasing production supported by proteomics studies, for understanding the best way for proteins or metabolites production [84].

Genome editing is typically performed using the CRISPR/Cas9 system (CRISPR stands for "clustered regularly-interspaced short palindromic repeat"); this is a ribonucleoprotein complex consisting of Cas proteins (a bacteria-derived DNA endonuclease) and small processed CRISPR RNAs. Instead of trusting DNA-binding proteins to guide the targeting of nuclease activity, the system uses a ~20-bp short guide RNA sequence (sgRNA or gRNA), which fixes onto its DNA target by means of base complementarity. Moreover, the CRISPR/Cas9 system can be used to enable simultaneous editing of multiple target sites on the genome by using multiple sgRNAs in a single CRISPR array, e.g. in the plant genus *Arabidopsis* [85].

There are four main methods being used for the transformation of microalgae: agitation with glass beads, electroporation, particle bombardment, and Agrobacterium-construction. Each method has its own advantages and disadvantages based on efficiency, integration, or stability of the transgene. Alternatively, the selection system can be based on antibiotic resistance or reporter gene selection. Different host and promoter strength would affect the selection efficiency. In the genetic engineering of microalgae, two important steps are involved: the genetic delivery tools, and selectable and screen able markers [8]. In unicellular microalgae, each cell contains a single chloroplast, the main function of which is to perform photosynthesis. The chloroplast genomes of more than 20 species are now accessible to genetic modification [86]. Since the chloroplast is the site of major anabolic pathways (e.g. carotenoid and fatty acid biosynthesis), from an engineering perspective the ability to engineer this cellular compartment is of significant biotechnological importance, and the methodology is well-established for several higher plants [79]. Compared with nuclear transformations, transformation of the chloroplast genome for transgene expression provides unique advantages, most importantly the ability to target transgene insertion via homologous recombination. In addition, high-level expression of transgenes and compartmentalized over-accumulation of proteins containing disulphide bonds occur readily, and undesired glycosylations are prevented [87].

Discovered in 2013, the CRISPR-Cas9 system, belonging to the bacterial adaptive immune system, has been receiving a lot of attention. A simplified variant of the type-II CRISPR-Cas9 system from *Streptococcus pyogenes* relies on CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA) or single synthetic guide RNA (sgRNA) located before the protospacer adjacent motif (PAM), to lead the Cas9 nuclease for triggering double-strand breaks (DSBs) in genomic DNA [88]. In comparison with ZFNs and TALEN, CRISPR/Cas9 showed greater applicability but more whole genome data is needed to prevent off-target sgRNA design. The CRISPR interference (CRISPRi) system uses the same design of guide-RNA but with nuclease-deficient Cas9 (or dead Cas9), which lacks the ability to cleave DNA, and functions only as a DNA-binding complex for gene interference, instead of gene modification for gene regulation [8]. The first reported study to demonstrate CRISPR-Cas9-based gene modification in *C. reinhardtii* provided clear evidence that Cas9 and sgRNA can successfully express functions in algae, but lack efficiency and have a low survival ratio due to the toxicity of vector-driven Cas9 [89]. This huge problem has been solved by delivering Cas9 protein-gRNA Ribonucleoproteins (RNPs) directly into *C. reinhardtii* to induce mutations at three loci; performance was improved by many orders of magnitude, compared to the earlier study [90].

Genome sequencing and a set of "omics" technologies which include genomics, transcriptomics, metabolomics, lipidomics, and highlighting prtoeomics are some of the recent technologies that have had a major impact on the modification and manipulation of microalgae. When these tools are used together with the object of transformation, and as molecular genetics toolboxes for algal strains, ample opportunities are provided for researchers to redesign or construct new algal metabolism methods to produce oils or any other chemical molecules which are useful for industrial and other applications. The many applications of genomic models have proved to be relevant in development and hypothesis-based research in algal metabolic engineering. "Omics" approaches can be used to characterize diverse bio-molecules such as DNA, RNA, protein, and other relevant metabolic entities from one sample of a source of interest.

The most efficient strategy to obtain the information of how microalgae work is the proteomics, giving us the information of the "software" of the Microalgae supported by the "hardware", genomics [77]. Proteomic studies are a fundamental tool to verify the expression of the genes of interest sought in genomics, corroborating the presence of by-product searched from microalgae. This expression of the genome is given by the environment which is changing in each of the sections previously discussed in this review. Therefore, a Microalga accumulates more PUFA under culture conditions or multiplies rapidly in other culture conditions, because the set of proteins expressed are being different [91]. In this way proteomics gives us the opportunity to know the behavior and relationships with the environment of microalgae. By the other hand, proteomic produce a huge amount of information from where we can find biological molecules with interest for a multitude of research fields. Since proteins or systems responsible for the accumulation of PUFA, restore contaminated water, until produce an anti-cancer protein or to accumulate a greater quantity of pigments of interest phycocyanin. The proteomics analyzes have revolutionized the way of understanding the behavior of organisms, based not only on genomics, the expression of this genes depends on the proteome, in certain conditions [92]. It is in this way that the true value of the microalgae will be obtained, remarking in these studies and the great and important information obtained from them using "omics" working together.

Modern experimentation tools help fill in the research gaps and provide a better understanding of genomics-based approaches in microalgae [84] based in more supported by more approaches, as proteomics. Synthetic biology is the re-adapting of biological systems for objectives and applications of interest. Through the coordinated and balanced expression of genes, both native and those introduced from other organisms, resources within an industrial chassis can be siphoned off for the commercial production of high-value substances. This developing interdisciplinary field has the potential to revolutionize natural product discovery, by providing a diverse array of tools, technologies, and strategies for exploring the large chemically-complex space of natural plant-based products using unicellular organisms [93].

Understanding the relationships between bio-molecule structure and function allows the design of novel nucleic acid and protein sequences, incorporating non-canonical building blocks and thus assembling fully artificial systems with customized properties based on the principles of synthetic biology [94]. There is no doubt the application of molecular techniques to microalgae will allow the custom design of microalgae, selecting desirable characteristics and discarding undesirable characteristics [95], to obtain microalgae completely custom-designed to the specification required by a sector of industry.

Conclusion

As can be appreciated from this review, each chapter, each area of application or approach described, is inter-related with the rest, and each can potentially contribute valuable input and feedback to the others. When such feedback takes place, the development of each application or approach can evolve faster and can be improved more than it would in isolation, separately. It should soon be possible to evaluate rapidly the many new potential applications for microalgae that are continually being envisaged or proposed, leading to the discovery of many feasible new applications to resolve the serious problems we face, and to facilitate a better life for all. Microalgae are positioned as an important future food for humans due to its composition and the advantages it offers as a cultivated crop: it can help to counter global warming and restore the atmosphere and water resources of the planet; it does not require large areas of scarce land; and it helps to protect the natural environment. Microalgae are currently the source of many interesting products not just in biomedicine and healthy food but also in technological applications, such as phycocyanin. The exchange of research results between the various fields of investigation will lead to an expansion of its possibilities in the coming years; new applications will be found in all sectors of industry. Microalgae cultivation and processing is becoming an important element in efforts to resolve serious global problems created by human activities in the past that continue virtually unabated into the present and future.

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