

Current challenges of microalgae applications: exploiting the potential of non-conventional microalgae species

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Abstract

The intensified attention to health, the growth of an elderly population, the changing lifestyles, and the medical discoveries have increased demand for natural and nutrient-rich foods, shaping the popularity of microalgae products. Microalgae thanks to their metabolic versatility represent a promising solution for a 'green' economy, exploiting non-arable land, non-potable water, capturing carbon dioxide (CO₂) and solar energy. The interest in microalgae is justified by their high content of bioactive molecules, such as amino acids, peptides, proteins, carbohydrates, polysaccharides, polyunsaturated fatty acids (as ω -3 fatty acids), pigments (as β -carotene, astaxanthin, fucoxanthin, phycocyanin, zeaxanthin and lutein), or mineral elements. Such molecules are of interest for human and animal nutrition, cosmetic and biofuel production, for which microalgae are potential renewable sources. Microalgae, also, represent effective biological systems for treating a variety of wastewaters and can be used as a CO₂ mitigation approach, helping to combat greenhouse gases and global warming emergencies. Recently a growing interest has focused on extremophilic microalgae species, which are easier to cultivate axenically and represent good candidates for open pond cultivation. In some cases, the cultivation and/or harvesting systems are still immature, but novel techniques appear as promising solutions to overcome such barriers. This review provides an overview on the actual microalgae cultivation systems and the current state of their biotechnological applications to obtain high value compounds or ingredients. Moreover, potential and future research opportunities for environment, human and animal benefits are pointed out.

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INTRODUCTION

The term 'algae' does not refer to a specific taxonomic group but it is commonly used to indicate microscopic and macroscopic photosynthetic organisms, including three wide groupings: macroalgae, microalgae and cyanobacteria.¹ Actually, the term 'microalgae' lacks a clear taxonomic value, it refers to unicellular, colonial or filamentous organisms, prokaryotic or eukaryotic, which are estimated to be between 200 000 and several millions of species.² Microalgae are considered the most primitive and dominant photosynthetic organisms on the Earth's surface. It has been estimated that they have occupied the Earth's surface for more than 3 billion years, contributing to the creation of the current terrestrial atmospheric composition and being responsible for fixing 40% of carbon dioxide (CO₂).^{3,4} Prokaryotic microalgae include cyanobacteria, traditionally known as blue-green algae (divisions Cyanophyta and Prochlorophyta), which are gram-negative bacteria; whereas eukaryotic microalgae, for which the systemic classification is essentially based on their pigment composition, include Chlorophyceae (green algae), Phaeophyceae (brown algae), Pyrrophyceae (dinoflagellates),

Chrysophyceae (golden brown algae), Bacillariophyceae (diatoms), Rhodophyceae (red algae), Euglenophyta, Cryptophyta, Haptophyta, Dinophyta and Xantophyceae.⁵ Microalgae synthesize a broad range of molecules with different structures and functional roles, a large amount of proteins are produced both for biological and structural functions, whereas secondary metabolites are

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accumulated to interact with external environmental conditions. Moreover, microalgae structural (polar) and reserve (neutral) lipids have a diversified composition of fatty acids, often related to the ecological niches, including polyunsaturated fatty acids (PUFAs) ω -3 and ω -6.⁶ Carbohydrates, obtained through photosynthesis, are a wide category encompassing sugars (monosaccharides) and their polymers (disaccharides, oligosaccharides, and polysaccharides) and the most abundant are glucose, rhamnose, xylose, and mannose.⁷ Regarding pigments, they comply with the light capture ability in the first phases of photosynthesis and chlorophylls (five types: a, b, c, d and e), carotenoids (carotenes and xanthophylls) and phycobilins are the three main classes produced by microalgae.⁸

A key relevant aspect of microalgae is their metabolic versatility. They can grow heterotrophically, autotrophically or photoheterotrophically, namely mixotrophically,⁹ and this makes microalgae as interesting solutions for treatment of wastewater coming from several productive sectors. Contextually, agricultural facilities and agro-industries encounter serious problems due to the co-products and by-products generated during their production processes. The recovery of such products to obtain microalgae biomass would mean to exploit agricultural by-products as growth substrate, being, in many cases quite similar to media for microbial growth. For instance, the vinasse from ethanol distillation (from beet and cane molasses fermentation) is a brown liquid containing mostly organic matter and a high amount of inorganic salts.¹⁰ Indeed, several studies have been performed on microalgal cultivation systems or on treatment of industrial and domestic wastewaters,¹¹ whereby they are widely used for secondary or tertiary treatments. Nevertheless, the contemporary presence of bacteria, fungi and other microorganisms, considered as contaminants, could have adverse results for microalgal performance affecting productivity, or in the worst-case scenario, causing culture crash, as documented in a 10-year comprehensive study in Singapore.¹² An interesting strategy is based on using extremophilic microalgae. The extreme pH, temperature or salinity conditions limit the growth of competitors and predators, as bacteria, improving the efficacy of microalgae-based treatment.¹³

The aim of this review is to explore the use of unconventional microalgae species and their cultivation systems pointing out the current state of their applications, with a view on potential and future research opportunities.

MICROALGAL BIOMASS PRODUCTION

Since 1953, when 'Algae Culture, from Laboratory to Pilot Plant', edited by J.S. Burlew,^{14,15} was published 'where were Brought almost all of the work done including the first larger scale outdoor trials made to date in the USA, Germany, Japan and Israel', many designs have been developed. They can be classified essentially into two categories: traditional open systems and enclosed photobioreactors (PBRs), and their main traits are listed in Table 1. Recently, new designed multi-technology (hybrid) systems have been introduced, sharing the common feature to utilize suspended cultures in an aquatic environment.^{11,21}

The most typical open system, extensively used since the 1950s for algae cultivation, is the raceway pond. The algal culture is constantly mixed and circulated around the raceway track, in either concrete or compacted earth, by paddlewheels, where the flow is driven around bends by baffles placed in the flow channel. During daylight, in front of the paddlewheel, where the flow begins, the culture is continuously fed, while, broth is harvested behind

the paddlewheel, at the end of the circulation loop.¹⁶ The pond is usually about 0.3 m deep to provide sufficient sunlight for photosynthesis. Currently, the most commercial scale algae cultivation systems are open ponds, being relatively inexpensive to build and easy to scale up. Nevertheless, numerous limitations, such as: temperature fluctuations between day and night and among seasons as well as geographic location, evaporative water loss, low CO₂ and high oxygen (O₂) concentrations, large optically dark zones or contaminations with unwanted algae or microorganisms, make the open system much less efficient than PBRs.¹⁷ Nowadays, PBRs are successfully used for producing large quantities of microalgal biomass from single-species culture, thanks to the possibility of maintaining optimal parameters, avoiding contamination, by continuously adjusting light intensity, CO₂ and O₂ concentrations, pH and temperature values, and so forth. There are many available configurations for PBR systems: such as typical closed reactors, that include flat plate reactors, tubular PBRs, and bag systems.²² Tubular PBRs are the most commonly used at industrial scale. A tubular PBR consists of an array of straight transparent tubes, generally 0.1 m or less in diameter, usually in plastic or glass, adapted to capture sunlight. Microalgal culture is kept constantly circulating from a reservoir to the solar collector and back to the reservoir, by highly turbulent flow produced using either a mechanical pump or a gentler airlift pump. Despite biomass yield obtained in PBRs being generally 30 times higher than that obtained in raceways,¹⁶ the cultivations, at commercial scale production, require several crucial considerations, such as: design, cost, risk of contamination and cleaning. An alternative strategy consisting of a hybrid system is obtained by coupling open and closed systems in a two-stage cultivation system. The first stage uses closed PBRs to culture the inoculum and for the second stage algae are cultivated in an open pond. In this way, microalgae are cultivated in optimal conditions for cell growth before being transferred into a growth-limited environment, as open pond which, for instance, can enhance lipid production.^{22,23} However, large-scale applications have been limited by the cost of the first stage.¹¹ Bilad et al. used both a closed PBR and a hybrid system, and the membrane photobioreactor (MPBR) for microalgal cultivation.²⁴ The MPBR consists of an additional filtration tank where a membrane provides the retention of microalgal cells, preventing the wash out and increasing biomass concentration, while the medium passes as permeate. This system achieved nine times higher biomass productivity with a 77% smaller footprint than PBR.²⁴ Overall, microalgae cultivations for commercial biofuel production are usually performed in raceway ponds, whereas PBR designs are suitable for productions of high value products.²⁰

Cultivation strategy

Depending on the selected species and objectives to be achieved a proper cultivation system is required. Despite the disadvantage of CO₂ required, O₂ accumulation, light-growth limitation,^{18,25} the most common strategy for microalgae cultivation is the photoautotrophic way. Alternatively, heterotrophic cultures are commonly performed in conventional fermenters (stirred tank fermenter) where the O₂ required is obtained by intensive aeration.²⁶ Nevertheless, heterotrophic growth has been observed exclusively within few microalgal species, and its productivity is still much less efficient than that obtained by *Candida utilis*, which although presents about the same size, shows a maximum specific growth rate (0.19 per h) about 2–5 times faster than *Chlorella*, which is mainly heterotrophically produced using glucose or acetic acid.^{26,27} Moreover, darkness can lead to reduced pigmentation, limiting

Table 1. Microalgae cultivation: open versus closed systems

Parameters	Open systems	Enclosed photobioreactors (PBRs)	References
Biomass production (kg/m ³)	0.14 (raceway pond)	4 (tubular photobioreactor)	16
Operation costs	Low	High	17
Light utilization efficiency	Poor	Highly efficient	18
Process control	Difficult	Accurate	17
Species control	Difficult	Possible	19
Contamination risk	High	Low	19
Value of produced biomass	Low	High	20

the potential of heterotrophic cultivation for phytochemicals large-scale production. In myxotrophy, the simultaneous presence of two energy sources (light and reduced organic carbon) can significantly increase biomass productivity because of both heterotrophic and autotrophic metabolisms operate concurrently within a single microalgal monoculture, overcoming both autotrophic and heterotrophic limitations.²⁸ Nevertheless, mixotrophic cultivation cannot be adapted in open cultivation systems because the presence of organic carbon improves bacterial contaminant growth, holding closed system the only practical possibility. Recently, Abiusi and coworkers have designed an O₂ balanced mixotrophic process that does not require any gas exchange.^{29,30} *Chlorella sorokiniana*, cultivated in enclosed PBR, as both autotrophic and heterotrophic cultures, was supplied with an optimal rate of acetic acid, showing a doubled biomass production, as the sum of the two metabolisms. Extreme growth conditions aid in preventing contamination and predation of microalgae, therefore facilitating their outdoor cultivation. During the last two decades, particular attentions have been paid toward acidophilic and acid tolerant microalgae and their biotechnological application, for example for production of pigments, as phycocyanin,³¹ and most of the researches on acidophilic microalgae has been focused on *Galdieria* genus. Interesting results were reached using *Galdieria sulphuraria* in mixotrophic cultivation, where at pH 1.7 the biomass productivity was 1.8 times higher than in autotrophic culture, and the culture remained axenic for the whole experiment.³¹

Microalgae harvesting

Microalgae are grown in large water volumes and for the harvesting process a concentration step, a process with intensive energy demand, is required. Therefore, the selection of harvesting technologies depends on microalgae specie and on the economically and energetically suitable process.³² The choice for microalgal harvesting has to take into account the cell structure, the growth rate and the lipid content. Several techniques could be adopted for harvesting and thickening: screening, coagulation–flocculation and sedimentation, flotation, centrifugation, magnetic separation, electrophoresis and dewatering and drying.³³

The screening consists of introducing microalgal biomass onto a screen of given aperture size. The efficiency of the screening operation depends on the size of the screen hole and algal particle dimensions. Microstrainers and vibrating screens are commonly employed as screening devices.³⁴ The harvesting through coagulation and flocculation is based on negative surface charge of microalgal cells, density near to the growth medium, in dispersed state, results in a stable system with a slow natural sedimentation.³³ The coagulation–flocculation of microalgal cells, useful at large scale with

a wide range of microalgal species, can be induced by using chemicals, namely flocculants, causing aggregation of microalgal cells to form larger clumps, which are easier to filter and/or settle.³⁵ In the flotation process, air or gas bubbles are used to move to the top of growth medium the suspended matter that are then collected by a skimming process.³⁶ The filtration culture suspension is based on forcing to flow across filter medium using driving force derived from gravity, vacuum, pressure, or magnets.³⁷

The harvesting through centrifugation is generally characterized by high separation efficiency, > 90% at 13 000 × *g*, as reported by Heasman *et al.*³⁸ However, Dasey and Theegala demonstrated that high biomass separation efficiency could be sacrificed when large volume of culture is processed, resulting in a lower energy intake.³⁹ Indeed, the large energy consumption, the long treatment time make the process very expensive, mainly for large-scale applications.¹⁹

Due to the negative charge on microalgal cell surface, harvesting based on electrical approaches, as electrophoresis, electroflocculation, or electroflotation can be adoptable strategies. Exposing the medium to an electric field by metallic electrodes energized with a DC voltage, microalgal cells can thicken close to the electrode (electrophoresis), to the bottom (electroflocculation) or to medium surface (electroflotation).³² Alternatively, the use of natural coagulant in microalgae harvesting have been studied and proven to exceed the alum.⁴⁰ Biopolymers derived from plant wastes and fruit pieces, as nirmali, moringa and surjana seed, maize seed, Cactaceae, and so forth, have shown significant coagulant capacities, and recently the moringa native to Sudan, has received the greatest level of attention.^{40,41} Proteoglycan coagulant, produced by *Bacillus mojavensis* strain 32A has shown an interesting flocculating activity, as 96% at pH 10.⁴² Lastly, chitosan-based compounds, derived from marine crustaceans, are also potentially eco-friendly coagulants and flocculants in the harvesting process. Generally, the mechanism involved in the harvesting process of chitosan is based on bridging and chitosan is commonly used in laboratory for harvesting, for example, *Chlorella* sp. from cultivation medium.⁴³

BIOTECHNOLOGICAL APPLICATIONS

Microalgae are fast-growing organisms able to survive in several environmental conditions. The biomass production is faster and higher than that of high plants, with a less seasonal variation, producing abundant raw materials characterized by easy biomolecule extraction processes. As well as for the biofuel production, for which microalgae are a potential renewable source, different commercial applications are possible, such as: wastewater treatment and CO₂ capturing, human nutrition, feed for animal and aquatic life, active ingredients for cosmetic industry, high-value

compounds, pigments like astaxanthin, β -carotene, and phycobiliproteins, stable isotope production, biofertilizer, or pharmaceutical means, as antimicrobial, antiviral, antibacterial and anticancer drugs. In Table 2 an overview on microalgae biotechnological applications is shown.

Production of nutraceutical compounds

Microalgae, according to the belonging species, are a source of several biological molecules, such as proteins, PUFAs, peptides, minerals and pigments with high nutraceutical value.¹ Since the early 1950s, microalgae have been explored as an alternative protein source to face global food demand, and their large-scale production has been successfully established since the 1980s, in several countries.⁵³ As already established by the World Health Organization (WHO) and Food and Agriculture Organizations of the United Nations (FAO), microalgae are eligible as substitutes of animal proteins.⁵⁴ The Cyanobacterium *Arthrospira platensis* presents a calcium content higher than 180% than milk, proteins higher than 670% than tofu, β -carotene higher than 3100% than carrot, and iron higher than 5100% than spinach, reasons why microalgae has been promoted as 'superfood' by WHO.⁵⁵ Both *A. platensis* and *Arthrospira maxima* are the species most commonly and intensively investigated. They are rich in PUFAs, such as γ -linolenic acid (18:3 ω -6), arachidonic acid (ARA, 20: ω -6) eicosapentaenoic acid (EPA, 20:5 ω -3), and docosahexaenoic acid (DHA, 22:6 ω -3).⁵⁶ Many microalgae species are reported to be producers of edible oil. *Isochrysis galbana*, *Nannochloropsis* sp., *Tetraselmis* sp. and *Phaeodactylum tricornutum* are EPA-producers, while *Porphyridium cruentum* is an ARA producer.^{57,58} Furthermore, as largely reported by Spoloare et al.,⁵⁹ for the high content of B vitamins and phycobiliproteins these species are considered as healthy promoters with antioxidant, cholesterol-lowering and other beneficial effects. Moreover, *Arthrospira* has gained significant popularity in the health and food industry, as primary food source in Asian countries, mainly in China, Korea, and Japan, while in other parts of the world it has been used as a nutrition supplement. The green algae *Chlorella vulgaris* is the second most relevant species for human nutrition, to be rich in proteins (48% of dry weight) and phosphorous (1761.5 mg/100 g of dry weight biomass). Furthermore, its nutraceutical benefits are related to β -1,3-glucan, macromolecules with immunostimulant effects.⁶⁰ *Dunaliella salina*, containing carotenoids (9-*cis*- β -carotene) known to prevent intracellular oxidative damage, has been consumed as dietary supplements for human health in the form of pills, capsules, and fortified nutritional mixtures, or as natural food and beverages.⁶¹ Other species have been investigated for their nutraceutical value, as the halophilic *Picochlorum* sp. for its folate content, that appeared higher than that detected in *Chlorella*.⁶² The biomass of *Picochlorum* sp. showed a total folate content of 6470 μ g/100 g, which is currently the highest total folate content detected in algae, reported as 1700 and 2600 μ g/100 g.⁶³ Similar results were reported by Fujii et al. that, in microalgae collected from Japanese ponds, found total folate contents in the range between 1500 and 3600 μ g/100 g in dry biomass.⁶⁴ Within the halophilic *Dunaliella tertiolecta* species, known for production of carotenoid from natural seawater, the strain *Dunaliella tertiolecta* mp3 was found able to accumulate zeaxanthin, under different growth conditions.⁵⁰

Furthermore, a recent study, carried out by Fields et al., revealed that consumption of *Chlamydomonas reinhardtii* mitigated weight loss in a murine model of acute colitis and positively affected gastrointestinal health in humans.⁶⁵

Production of active compounds for cosmetic applications

If macroalgae are already widely exploited in the cosmetic industry, microalgae are still less used. Among the different pharmacological activities, some microalgae compounds could be applied in cosmetics.^{66,67} Pigments, such as β -carotene, astaxanthin, lutein, have been largely described as natural ingredients to be incorporated into moisturizing, antiaging, photoprotection, and skin lightening products.^{68,69} β -Carotene is known for its provitamin A activity and it is largely used in antiaging products. In particular, the halotolerant *Dunaliella salina* species is described as the main producer of β -carotene, up to 10% of its dry weight.⁶⁸ Astaxanthin is also applied in antiaging products because of its remarkable antioxidant properties, which are much greater than that detected for tocopherol.⁷⁰ *Haematococcus pluvialis* is the richest source of natural astaxanthin (it can accumulate more than 3 g of astaxanthin per kilogram of dry biomass) and nowadays it is produced on an industrial scale.⁶⁸ Lutein has been used for skin combating sunburn, reducing wrinkles, and for other cosmetic benefits. Within the product-type segment, the lutein segment accounts for the largest value, sharing around 19%. Furthermore, lutein produced by *Scenedesmus* sp. has been successfully used to slow eye macular degeneration. The specific composition and quantity of lipids are species-dependent and the most common oleaginous microalgae are *Chlorella* sp., *Nannochloropsis* sp., *Scenedesmus* sp., and *Dunaliella* sp.⁷¹ A fundamental aspect in pigment production is downstream processing, in particular their extraction from microalgal cells. The high consumption of toxic solvents can be a burden to the environment and longer processing time result on lower profit.⁷² The downstream processing technique, proposed by Chia et al.,⁷³ to extract C-phycoyanin from *Arthrospira* spp., based on a sonication treatment coupled to a liquid biphasic system (to purify the phycobiliproteins), was described as able to achieve a purification fold of 6.17 and a recovery yield of 94.89%. At the same time, for extraction of astaxanthin from *H. pluvialis*, an alternative solution, to overcome the criticisms explained earlier, has been proposed by Khoo et al.⁷⁴ In particular, the use of CO₂-based alkyl carbamate ionic liquids has been proposed and results stated that DIMCARB (dimethylammonium dimethylcarbamate) gave the highest yield of astaxanthin (27.99 \pm 1.01 mg/g of astaxanthin) under the optimized extraction conditions, namely 100% (w/w) of DIMCARB, 75 min of incubation, at 45 °C.

Production of food ingredients

Microalgae have a great potential to be used as ingredients in innovative and sustainable food products, improving protein content, valuable nutrients, such as phenolic compounds, vitamins and minerals, or as pigments in food dyes, such as astaxanthin (red), lutein (yellow), chlorophyll (green), or phycocyanin (bright blue). *Chlorella vulgaris* has been used as food coloring or antioxidant agent, while *Isochrysis galbana* as a ω -3 PUFAs provider.⁷⁵ As reported by Hossain et al.,⁷⁶ when 15% (w/w) of astaxanthin from *H. pluvialis* was added to cookies, there was a significant reduction in glucose released in *in vitro* digestion, and an increase in total phenolic content and antioxidant capacity was observed. Furthermore, when *I. galbana* and *Diacronema vlkianum* biomass were added to pasta a significant increase of PUFAs, in particular EPA and DHA, both in raw and cooked pastas were detected.⁷⁷ In 2016, a study explored the effect of adding 10% of *Arthrospira platensis* biomass to bread and an increase, from 7.40% to 11.63%, in protein and mineral contents, especially calcium, magnesium, and iron was noted.⁷⁸ In 2019, supplementation of 2%, 6%, and 10% of

Table 2. A comprehensive overview on microalgae biotechnological applications

Application	Main genera or species	Valuable compounds	Weakness	Strengths	Reference
Food and nutraceutical	<i>Arthrospira</i> spp.; <i>Chlorella</i> spp.; <i>Scenedesmus</i> spp.; <i>Dunaliella</i> spp.; <i>Haematococcus</i> spp.	Proteins; β -carotene; iron; acid γ -linolenic (ω -3 fatty acid), B vitamins	Adverse effects on food taste and texture High amount of nucleic acid if used directly as food	High nutritional value Several sale formats	44,45
Animal feeding in aquaculture	<i>Chlorella vulgaris</i> ; <i>Isochrysis galbana</i> ; <i>Phaeodactylum tricomutum</i> ; <i>Chaetoceros</i> spp.; <i>Nannochloropsis oceanica</i> ; <i>Skeletonema</i> spp.; <i>Haematococcus pluvialis</i> .	Proteins; β -carotene; lutein; ω -3 fatty acid	High production cost Low digestibility of cell walls for some microalgae	Better growth rate and health of fishes High protein feed for animals	5,46
Wastewater treatment	<i>Chlorella</i> sp.; <i>Anabaena</i> sp.; <i>Arthrospira</i> sp.; <i>Botryococcus</i> sp.; <i>Chlamydomonas</i> sp.; <i>Dunaliella</i> sp.; <i>Haematococcus</i> sp.; <i>Isochrysis</i> sp.; <i>Nannochloropsis</i> sp.; <i>Neochloris</i> sp.; <i>Microcystis</i> sp.; <i>Oscillatoria</i> sp.; <i>Phormidium</i> sp.; <i>Scenedesmus</i> sp.; <i>Synechococcus</i> sp.	Fresh biomass; treated wastewater	Expensive harvesting method Low quality biomass due to contaminant	Cheap nutrient sources Two products obtained: Treated wastewater and microalgae biomass	21,47
Plant growth promoting	<i>Scenedesmus quadricauda</i> ; <i>Arthrospira plantensis</i> ; <i>Chlorella</i> spp.	Phytohormones; amino acids; vitamins; polysaccharides; carbohydrate; polyamine; polyphenols	Unclear biomechanisms of microalgae in plant physiology	Improved plant growth	88
Biofuels	<i>Chlorella</i> spp.; <i>Scenedesmus</i> spp.; <i>Dunaliella</i> spp.; <i>Porphyridium</i> spp.; <i>Euglena</i> ; <i>Microcystis</i>	Polysaccharide; lipids	Few microalgal genus exploited Several pretreatment processes needed	Bio-fertilizers Clean energy	48,49
Biorefinery and cosmetic	<i>Dunaliella salina</i> ; <i>Haematococcus pluvialis</i> ; <i>Spirulina</i> spp.; <i>Porphyridium</i> spp.; <i>Nannochloropsis</i> spp.	β -Carotene; astaxanthin; fucoxanthin; phycobiliproteins; zeaxanthin; lutein.	Industrial production scale not yet economically convenient Low production efficiency Highly specialized refinement processes	Highest socio-economic significance High-value products, potential microalgal production, scaled-up to industrial level	50-52

Arthrospira biomass in crostini, a bakery product, was evaluated and results showed higher protein and antioxidant content along with the increase of microalgal addition.⁷⁹ Nevertheless, digestibility of microalgal biomass is still a crucial aspect because the robust cell walls, composed of peptidoglycan or cellulose, or the high amount of nucleic acid or neurotoxins, could represent a risk for human health.^{44,80,81} To overcome this aspects, numerous pretreatments are available, such as bead milling, high pressure homogenization, heat treatment and many others, however further studies are required to find higher efficient and cost-effective technologies to increase digestibility without hindering quality of the high value compounds.⁸² Furthermore, consumer and government acceptance play a key role in developing and marketing food products containing microalgae. Moreover, application of microalgae in functional foods is still restricted since limited data are available about allergenic compounds or effect on human health.⁸³ Contextually, due to the considerable amount of microalgae biomass added to explicate their benefits, often adverse effects on food taste and texture occurred.⁸⁴ Different microalgal properties affect their potential use as food ingredients, such as gelation, emulsification, and miscibility.^{85,86} Incorporation of *Chlorella* in processed cheese led to increased hardness and reduced meltability,⁸⁷ while addition of *Arthrospira* into pasta enhanced the firmness and the cohesiveness, without affecting cooking properties.⁸⁸ Nevertheless, sensorial data revealed that consumers are generally positive about both green color and marine taste. Moreover, it is interesting to highlight that the addition of *Arthrospira platensis* and *Chlorella vulgaris* into probiotic fermented milks resulted in an enhancing of probiotic viability.⁸⁹ Currently, many of these products are present on the global market. New and unconventional food, such as vegetable creams (with higher protein content), are becoming very popular thanks to the increased demand for healthy products, and some new recipes meet the criteria to be labeled as 'high-protein content' following the current European Union (EU) legislation.⁹⁰ Unfortunately, commercial companies do not clarify the used microalgae species and, in most cases, the label only describes the microalgae genus.

Safety concerns and legislation

Like other microorganisms, including yeast and bacteria, some species of microalgae are safe for human consumption and have obtained the GRAS (Generally Recognized as Safe) status from the US Food and Drug Administration (FDA). In such a case the purification costs are significantly reduced and the applications as valuable food and/or feed ingredients are potentially expanded. The few microalgae that have obtained the GRAS status are: *Arthrospira platensis*, *Chlamydomonas reinhardtii*, *Auxenochlorella protothecoides*, *Chlorella vulgaris*, *Dunaliella bardawil*, and *Euglena gracilis*. In the EU, the European Food Safety Authority (EFSA), following the 'precautionary principle' approach, stated that foods that have been consumed within the EU before May of 1997 are deemed safe to be consumed, whilst any other food have to be labeled as 'novel food' and must undergo a safety assessment by the EFSA, before being marketed.⁹¹ In the EU the approved species are only *Arthrospira platensis*, *Chlorella pyrenoides*, and *Chlorella vulgaris*.⁹¹

Nevertheless, among the thousands of existing microalgae species, around 200 showed concerns about safety traits and about 100 species have been proven to produce toxins.^{92,93} A comprehensive data on toxic species can be found at www.marinespecies.org, an updated list of microalgae species

producing or suspected to produce toxins or toxic effects (IOC Harmful Algal Bloom Program and the World Register of Marine Species). In the last years, important advances have been achieved towards the development of more specific, sensitive, and rapid methodologies that allow the identification of different microalgae species and toxins.⁹⁴ In addition, legislations and regulatory aspects on the commercialization of carotenoids from microalgal biomass are recently described for food and cosmetic products in the United States, Japan, China, and Europe.⁹⁵ Thus, microalgal-derived astaxanthin, β -carotene, and chlorophyll are regulated and approved by the FDA, based on their non-toxic and non-carcinogenic properties.⁹⁶ Moreover, astaxanthin from *H. pluvialis* has been approved as a color additive in Europe, the United States, and in Japan. In details, the EFSA Panel on Nutrition, Novel Foods, and Food Allergens concluded that an intake of 8 mg of astaxanthin through food supplements is safe for adults⁹¹ and the FDA approved it for direct human consumption.⁹⁷

Livestock and aquaculture feed

Aquaculture is an important sector as terrestrial agriculture that provides food for the human population. Fishmeal is usually supplied as feed in fish farming and it is produced from small fishes or fish waste which are cooked, pressed, dried and ground to form a solid.⁹⁸ An environmentally and economically sustainable alternative for replacing fishmeal can be the microalgae-based feed, which showed significant results in production of zooplankton, mollusks, crustaceans, shrimp and fish farming,⁹⁹ providing a high value nutrition, improving the color of aquatic organisms and disease resistance.¹⁰⁰ However, only some microalgal species are proven species to be used as feed in aquaculture, in particular microalgae belonging to the genera *Isochrysis*, *Pavlova*, *Nannochloropsis*, *Arthrospira*, *Chlorella*, *Scenedesmus*, *Dunaliella*, *Haematococcus*, and *Schizochytrium*.^{60,101} For instance, the carotenoids, as astaxanthin from *H. pluvialis*, or β -carotene from *Dunaliella salina*, phycocyanin from *Arthrospira*, are used as sources of natural pigments for culturing salmonid fish, prawns, and ornamental fish.¹⁰² Besides aquafeed, livestock feed for pets, horses, broilers and ruminant animals are other potential microalgal applications. At low percentages, microalgal biomass, has been recommended to be included in feed for animals such as pigs, cows, sheep, chicken and other domestic animals, in order to improve their immune systems, lipid metabolism and gut functions.¹⁰³ However, ruminants are the most suitable animals to feed on algae because they are even able to digest unprocessed microalgal biomass.¹⁰³ However, the technology to produce microalgae is still immature and the main drawbacks and challenges are the high production costs,¹⁰² the low digestibility of cell walls, for some microalgae, and the presence of contaminants.¹⁰⁴

Wastewater treatment

Wastewater treatment by means of microalgal systems is a technology that has been applied for longer than 60 years. Microalgae-based wastewater treatment has been recently intensively studied, with a focus on the production of algal biomass and the associated removal of inorganic nutrients from a wide variety of wastewaters.¹⁰⁵ The pioneering studies of Oswald, in California, set the fundamentals of wastewater treatment in the so-called 'high-rate algal ponds' (HRAPs), originally focused on removing organic matter and nutrients.^{106,107} Nowadays, the bio-transformation of pollutants from wastewater, as xenobiotics, nutrients and CO₂ from polluted air by macroalgae or microalgae is known as phycoremediation. The main aim of the phycoremediation is to depurate wastewater for irrigation or other aims

and concurrently, exploiting wastewater as growing medium based on the high concentration of nitrogen and phosphorus.¹⁰⁸ Also the organic-rich anaerobic digestate, which is difficult to be purified by conventional techniques, is appropriate to be used as a low-cost nutrient source for the economic viability and sustainability of microalgae production.¹⁰⁹ *Chlorella vulgaris* has been extensively exploited for biomass production from food waste, sludge extracts, corn steep liquor, cheese whey, vinasse, tofu wastewater, and industrial dairy effluents.¹¹⁰⁻¹¹²

Recently the ability of *Arthrospira platensis* to accumulate lipids during mixotrophic growth on dairy wastewater has been evaluated,¹¹³ obtaining about 5 g/L of total biomass concentration with about 30% of lipid content. *Arthrospira platensis* has also been applied to treat piggery wastewater, confectionary effluent, composite media containing minerals, beet vinasse, and distillery wastewater.¹¹² Typically, treatment of municipal and agricultural wastewaters by microalgae is performed in outdoor systems, without any adjustment of temperature and pH. However, the wastewater parameters can be widely different, from highly acidic pH values and high temperatures (spanning from 10 to 40 °C), high organic loads (> 100 g/L) and a high load of contaminant population. An interesting strategy to successfully treat a wide type of wastewaters can be the extremophile microalgae. *G. sulphuraria*, known as *Cyanidium caldarium*, has been exploited for its interesting extremophilic growth properties, being able to grow both in neutral and highly acidic conditions, down to pH value of 1.8,¹¹⁴ acidifying the medium by an active proton efflux, reducing the costs of pH control and, in turn, the risk of contamination.¹¹⁵ Moreover, the versatile metabolism, able to grow autotrophically, heterotrophically and mixotrophically, exploiting more than 50 different sugars and alcohols, makes *G. sulphuraria* a promising candidate for treating wastewaters.^{116,117} As recently reported, *G. sulphuraria* showed to grow heterotrophically and mixotrophically on cultivation medium containing a by-product of the dairy industry (buttermilk) as carbon source.¹¹⁸ A further promising acidophilic microalgal species is *Chlamydomonas acidophila*, isolated from acidic river in a mining area, at pH values ranging from 1.7 to 3.1.¹¹⁹ It has been shown that *Chlamydomonas acidophila* can grow mixotrophically without CO₂ addition by using different carbon sources, such as glucose, glycerol or starch, at acidic conditions (pH 2.5) removing ammonia.^{119,120} *Chlorella sorokiniana*, a well-studied thermophilic green microalga, has revealed high photoautotrophic growth rates, up to 43 °C.¹²¹ Despite microalgal feedstock for biofuel use being considered an ideal scenario, many disadvantages must be solved, as for example the expensive harvesting process required in a high-rate algal ponds (HRAP), for microalgal biomass, avoid the fact that the biomass settles to the bottom of the ponds, where it decomposes, releasing methane and degrading water quality.¹²²

Bio-fertilizers and bio-stimulants for promoting plant growth

Microalgae can be utilized for sustainable agriculture by partial substituting chemical fertilizers. Recently detailed insights on algal biochar as a potential fertilizer for sustainable agriculture have been produced.¹²³ The mechanism responsible for biofertilization is still unclear, biomass is provided to soil, but it should be available for plants and their roots. The most accredited theory, explained by Perin and Morosinotto,¹²⁴ is that microalgal biomass could be degraded by soil microbiota present in rhizosphere, thus releasing nutrients over a prolonged period. Alternatively,

symbiotic interactions could be established, as in cyanobacterium nitrogen fixators case, where bioavailable forms of nitrogen are released in return for carbonic compounds from plants. Furthermore, after providing biomass in soil, the nutrient status, water retention capacity, pH and electrical conductivity results improved.¹ Among the most reported responses, an increased content in proteins, carbohydrates and photosynthetic pigments has been registered in plants treated with microalgal extract, in particular from *Scenedesmus quadricauda*.¹²⁵⁻¹²⁷ However, the microalgae biomechanisms in the plant physiology and the different effects for each bioactive compound remain still unclear, since the effect of the microalgal biomass is considered a complex of reactions.^{128,129} In addition, biochemical composition of microalgal cells, rich in micro- and macro-nutrients, makes their biomass a promising source for biofertilizer.

Unfortunately, only a few microalgal genera are industrially exploited as microalgae-based commercial products,^{130,131} confirming how young the sector is, and of how little is known on microalgal species, considering they are several million.

Biofuel production

Many efforts have been done to find biofuel production technologies, but both the first and the second generations have been discovered as not suitable for environmental incompatibility for requiring more arable agricultural lands and modern innovations, respectively.¹³² For this reason, the attention is now shifting to the third generation technology which uses defined species of microalgae as feedstocks, thanks to the high oil content and biodiesel yield, the low land area needed and absence of lignin, that is recalcitrant and needs several pretreatment processes for carbohydrates to be realized.^{48,133}

Many microalgae species can supply several different types of renewable biofuels such as biomethane, produced by anaerobic digestion of algal biomass; bioethanol, produced via fermentation and anaerobic digestion of the remaining algal biomass.⁴⁹ Microalgal species widely investigated belong to the green algae genera *Chlorella*, *Scenedesmus*, *Dunaliella*, *Porphyridium* and *Euglena*, that show particular attitudes for bioethanol production, thanks to their high polysaccharide content.⁴⁸

Microcystis aeruginosa, a freshwater blue green alga (cyanobacterium), and *Scenedesmus obliquus* (green alga), with lipids content as 28% and 40%, respectively,⁴⁹ were considered the most promising specie for biodiesel production. To produce biodiesel, as reported by Leong *et al.*,¹³⁴ it seems very promising to use a microalgal-bacterial consortium. Also, biohydrogen, currently produced by techniques such as steam reforming or electrolysis and not entirely free from the involvement of fossil fuels,¹³⁵ can be produced by cultivation of Cyanobacteria and green algae, through direct and indirect pathways, as explained by Show *et al.*¹³⁶ Unlike other well-established biofuels, as biodiesel and bioethanol, biohydrogen from microalgae is still at its preliminary stage of development. Criticisms in microalgal biohydrogen centered on its practicality and sustainability.¹³⁶ There are still various difficulties in biofuel production from microalgal biomass feedstock. Current data indicate that the cost of biofuel production from microalgal biomass is still higher than that of different other sources, and actually, it is not yet proven to be an economically sustainable source of biofuel.¹³² A great challenge is to reduce the harvest cost, which is estimated as 40% of the whole production cost. Furthermore, designing efficient and innovative oil extracting methods could improve the biodiesel yield from microalgal cells. Nguyen *et al.* showed the highest fatty acid ester yield

(96.0% w/w) under wet microalgae conditions with 650 mol/mol and 10 000 mol/mol of sulfuric acid and methanol concentrations.¹³⁷ Furthermore, employing transgenic strains to produce high-value products and using residual by-products to boost production economics, microalgal production can be scaled-up to an industrial level.¹³²

CONCLUSION AND FUTURE PERSPECTIVES

According to the FAO report on 'The future of Food and Agriculture: trends and challenges',¹³⁸ about one-third of global food produced is still lost or wasted along the food chain, from production to consumption highlighting an inefficiency of current food systems. At the same time, increasing healthcare spending, growing geriatric population, food innovations, changing lifestyles, and medical discoveries have benefited demand for superfoods. Furthermore, the increasing awareness regarding superfoods as natural, nutrient-rich agro-foods containing vitamins and minerals are some of the key aspects shaping the popularity of microalgae products in the world. In this scenario, microalgae represent a promising candidate for both food/feed and energy production as well as for valorization of by-products aimed to create a virtuous recycling system, in accordance with the United Nation 2030 Agenda goals.¹³⁹ As Pikaar et al.¹⁴⁰ theorized in a model simulation, microbial sources of food and feed hold great promise for achieving a future food production system that is both more sustainable and resilient. In particular, it would be feasible to replace 10–19% of conventional crop-based protein feed with microbial biomass by 2050, with significant reductions in global cropland area, nitrogen leakage and agricultural emission. Despite several species being already commercially used, they are still not produced in high-enough quantities or in a cost-effective manner, required for fuels and feeds. Nowadays, total soy oil and meal production, estimated to be around 200 million t/yr, with a current price below 0.5 €/kg, is far away from the microalgae oil and meal production, which amount to about 25 000 t/yr with a market price of 20–50 €/kg. Although, it has been estimated that, if production reached 10 000 t of biomass per year, the cost price will fall below 5 €/kg, and further industrialization could reduce it below 1 €/kg.^{103,141,142} Hence, optimization initially of the manufacturing and then commercialization of microalgae products is required. In this context, several strategies can be adopted to overcome these limits: open pond cultivation systems based on poly-extremophile microalgae can be a strategy to cut down production costs; innovative and natural methods to harvest, extract and process microalgae represent opportunities to develop the most promising sectors such as food, energy and cosmetic productions. Finally, microalgae can be a great opportunity to develop new production systems to complement or improve traditional agriculture in order to satisfy the world's food and feed demand.

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AUTHOR CONTRIBUTIONS

Conceptualization, CC and CLR; methodology, CC; software, AP; validation, FVR, CLR; investigation, PSO and IMZ; resources, FVR; data curation, AP; writing – original draft preparation, PSO;

writing – review and editing, CC; visualization, NR; supervision, CC and CLR; project administration, CC; funding acquisition, CLR. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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