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Commercial Applications of Microalgae

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ABSTRACT

The first use of microalgae by humans dates back 2000 years to the Chinese, who used *Nostoc* to survive during famine. However, microalgal biotechnology only really began to develop in the middle of the last century. Nowadays, there are numerous commercial applications of microalgae. For example, (1) microalgae can be used to enhance the nutritional value of food and animal feed owing to their chemical composition, (2) they play a crucial role in aquaculture and (3) they can be incorporated into cosmetics. Moreover, they are cultivated as a source of highly valuable molecules. For example, polyunsaturated fatty acid oils are added to infant formulas and nutritional supplements and pigments are important as natural dyes. Stable isotope biochemicals help in structural determination and metabolic studies. Future research should focus on the improvement of production systems and the genetic modification of strains. Microalgal products would in that way become even more diversified and economically competitive.

INTRODUCTION

Microalgal use by indigenous populations has occurred for centuries. Indeed, edible blue-green algae including *Nostoc, Arthrospira* (*Spirulina*) and *Aphanizomenon* species have been used for food for thousands of years (1). However, the cultivation of microalgae is only a few decades old (2).

In the early 1950's, the increase in the world's population and predictions of an insufficient protein supply led to a search for new alternative and unconventional protein sources. Algal biomass appeared at that time as a good candidate for this purpose (3, 4). Meanwhile, the systematic examination of algae for biologically active substances, particularly antibiotics, began (5).

Interest in applied algal culture continued with studies of the use of algae as photosynthetic gas exchangers for space travel (2). In the USA, environmental technologies aimed at the improvement in the quality of wastewater and the fermentation of the resulting biomass to methane were implemented (6). This use of microalgae for generating renewable energy sources provoked heightened interest during the energy crisis in the 1970's (4, 6, 7).

Commercial large-scale culture started in the early 1960's in Japan with the culture of *Chlorella* by Nihon Chlorella (Taipei, Taiwan) (2, 8, 9). It was followed in the early 1970's by the establishment of an *Arthrospira* harvesting and culturing facility in Lake Texcoco by Sosa Texcoco S.A. (Mexico City, Mexico) (2, 8). The first aquaculture fields also appeared in the 1970's (6).

By 1980, there were 46 large-scale factories in Asia producing more than 1000 kg of microalgae (mainly *Chlorella*) per month. The commercial production of *Dunaliella salina*, as a source of β-carotene, became the third major microalgal industry when production facilities were established by Western Biotechnology (Hutt Lagoon, Australia) and Betatene (Whyalla, Australia) (now Cognis Nutrition and Health) in 1986. These were soon followed by other commercial plants in Israel and the USA. The same as that of these algae, the large-scale production of cyanobacteria (blue-green algae) began in India at about the same time. More recently, several plants producing *Haematococcus pluvialis* as a source of astaxanthin have been established in the USA and India (Biotechnological and Environmental Applications of microalgae (BEAM), an Australian Research Network. Official web page. wwwscieng.murdoch.edu.au/centres/algae/BEAM-Net/BEAM-Appl0.htm, 2005). Thus, in a short period of about 30 years, the microalgal biotechnology industry has grown and diversified significantly. Nowadays, the microalgal biomass market produces about 5000 t of dry matter/year and generates a turnover of approximately US$ 1.25×10^9/year (processed products not included in this figure) (10).

The aim of this study is to summarize the commercial applications of microalgae. As history has shown, research studies on microalgae have been numerous and varied but they have not always resulted in commercial applications. Although recent reviews on microalgal applications exist, they generally mix actual applications and future potential developments. Thus, our purpose is to clarify the actual situation by only discussing real commercial applications and to illustrate them with examples of microalgal manufacturers and commercialized products. Therefore, in the first part, the chemical composition of microalgae, on which a majority of applications are based, is presented in detail. Then, the use of microalgae for human and animal nutrition is presented. Finally, the applications of microalgae in cosmetics and high-value molecules extracted from these microorganisms are reviewed.

CHEMICAL COMPOSITION OF MICROALGAE

Microalgae are able to enhance the nutritional content of conventional food preparations and hence, to positively affect the health of humans and animals. This is due to their original chemical composition. Table 1 presents a comparison of the general compositions of human food sources with that of different algae.

The high protein content of various microalgal species is one of the main reasons to consider them as an unconventional source of protein (4, 11). In addition, the amino acid pattern of almost all algae compares favorably with that of other food proteins. As the cells are capable of synthesizing all amino acids, they can provide the essential ones to humans and animals (12). However, to completely characterize the protein and determine the amino acid content of microalgae, information on the nutritive value of the protein and the degree of availability of amino acids should be given (13). Carbohydrates in microlagae can be found in the form of starch, glucose, sugars and other polysaccharides. Their overall digestibility is high, which is why there is no limitation to using dried whole microalgae in foods or feeds (3). The average lipid content of algal cells varies between 1% and 70% but can reach 90% of dry weight under certain conditions (14). Algal lipids are composed of glycerol, sugars or bases esterified to saturated or unsaturated fatty acids (12 to 22 carbon atoms). Among all the fatty acids in microalgae, some fatty acids of the ω3 and ω6 families are of particular interest. The total amount and relative proportion of fatty acids can be affected by nutritional and environmental factors, nitrogen limitation, for example (15-18). Microalgae also represent a valuable source of nearly all essential vitamins (e.g., A, B_1, B_2, B_6, B_{12}, C, E, nicotinate, biotin, folic acid and pantothenic acid) (3). Vitamins improve the nutritional value of algal cells but their quantity fluctuates with environmental factors, the harvesting treatment and the method of drying the cells (19, 20). Microalgae are also rich in pigments like chlorophyll (0.5% to 1% of dry weight), carotenoids (0.1% to 0.2% of dry weight on average and up to 14% of dry weight for β-carotene of *Dunaliella*) and phycobiliproteins. These molecules have a wide range of commercial applications (as detailed below).

Thus, their composition gives microalgae interesting qualities, which can be applied in human and animal nutrition. However, prior to commercialization, algal material must be analyzed for the presence of toxic compounds to prove their harmlessness (21, 22). In this domain, recommendations have been published by different international organizations and additional national regulations often exist. They concern nucleic acids, toxins and heavy-metal components. Concerning nucleic acid toxicity, the safe level is about 20 g of algae per day or 0.3 g of algae per kg of body weight (3, 13). Finally, many metabolic studies have confirmed the capacities of microalgae as a novel source of protein: the average quality of most of the algae examined is equal or even superior to that of other conventional high-quality plant proteins (3).

MICROALGAE IN HUMAN AND ANIMAL NUTRITION

Human nutrition Microalgae for human nutrition are nowadays marketed in different forms such as tablets, capsules and liquids. They can also be incorporated into pastas, snack foods, candy bars or gums, and beverages (23, 24). Owing to their diverse chemical properties, they can act as a nutritional supplement or represent a source of natural food colorants (2, 11, 25). The commercial applications are dominated by four strains: *Arthrospira*, *Chlorella*, *D. salina* and *Aphanizomenon flos-aquae*.

Arthrospira is used in human nutrition because of its high protein content and its excellent nutritive value (11, 26-28). In addition, this microalga has various possible health-promoting effects: the alleviation of hyperlipidemia, suppression of hypertension, protection against renal failure, growth promotion of intestinal *Lactobacillus*, and suppression of elevated serum glucose level (23, 24, 29). A significant amount of *Arthrospira* production is realized in China and India. The world's largest producer Hainan Simai Enterprising Ltd. is located in the Hainan province of China. This company has an annual production of 200 t of algal powder, which accounts for 25% of the total national output and almost 10% of the world output. The largest plant in the world is owned by Earthrise Farms and streches over an area of 440,000 m^2 (located at Calipatria, CA, USA; Fig. 1). Their production process is presented in Fig. 2. Their *Arthrospira*-based products (tablets and powder) are distributed in over 20 countries around the world. Many other companies sell a wide variety of nutraceuticals made from this microalga. For example, the Myanmar Spirulina Factory (Yangon, Myanmar) sells tablets, chips, pasta and liquid extract, and Cyanotech Corp. (a plant in Kona, Hawaii, USA) produces products ranging from pure powder to packaged bottles under the name Spirulina pacifica. Cyanotech Corp. has developed an orginal process for drying the biomass in order to avoid the oxidation of carotenes and fatty acids that occurs with the use of standard dryers. The patented process employs a closed drying system that is kept at low oxygen concentrations by flushing with nitrogen and carbon dioxide. The process relies on a very cold ocean water crown from a depht of 600 m just offshore to provide dehumidification and actually dries microalgal products in less than 6 s (Fig. 3).

Chlorella is produced by more than 70 companies; Taiwan Chlorella Manufacturing and Co. (Taipei, Taiwan) is the largest producer with 400 t of dried biomass produced per year. Significant production is also achieved in Klötze, Germany (130 – 150 t dry biomass per year) with a tubular photobioreactor. This reactor consists of compact and vertically arranged horizontal running glass tubes with a total length of 500,000 m and a total volume of 700 m^3 (Fig. 4). The world annual sales of *Chlorella* are in excess of US$ 38 billion (23). The most important substance in *Chlorella* is β-1,3-glucan, which is an active immunostimulator, a free-radical scavenger and a reducer of blood lipids (9, Ryll *et al.*, Abstr. Europ. Workshop Microalgal Biotechnol., Germany, p. 56, 2003). However, various other health-promoting effects have been clarified (efficacy on gastric ulcers, wounds, and constipation; preventive action against atherosclerosis and hypercholesterolemia; and antitumor action) (23, 30). *Chlorella* can also be used as a food additive owing to the taste- and flavour-adjusting actions of its coloring agent (23, 31).

D. salina is exploited for its β-carotene content that can reach 14% of dry weight (14). For human consumption, Cognis Nutrition and Health, the world's largest producer of this strain, offers *Dunaliella* powder as an ingredient of dietary supplements and functional foods.

The last major commercial strain application is *A. flos-aquae*. According to many research studies, used alone or in combination with other nutraceuticals and natural food products, *A. flos-aquae* promotes good overall health (1, 32, 33).

Animal nutrition In addition to its use in human nutrition, microalgae can be incorporated into the feed for a wide variety of animals ranging from fish (aquaculture) to pets and farm animals. In fact, 30% of the current world algal production is sold for animal feed applications (3) and over 50% of the current world production of *Arthrospira* is used as feed supplement (23).

In 1999, the production of microalgae for aquaculture reached 1000 t (62% for molluscs, 21% for shrimps, and 16% for fish) for a global world aquaculture production of 43×10^6 t of plants and animals (34). The importance of algae in this domain is not surprising as they are the natural food source of these animals. The main applications of microalgae for aquaculture are associated with nutrition, being used fresh (as sole component or as food additive to basic nutrients) for coloring the flesh of salmonids and for inducing other biological activities.

Microalgae are required for larval nutrition during a brief period, either for direct consumption in the case of molluscs and peneid shrimp or indirectly as food for the live prey fed to small fish larvae (16, 35). The most frequently used species are *Chlorella, Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema* and *Thalassiosira* (23, 25, 35, 36). In order to be used in aquaculture, a microalgal strain has to meet various criteria. It has to be easily cultured and nontoxic. It also needs to be of the correct size and shape to be ingested and to have a high nutritional qualities and a digestible cellwall to make nutrients available (19, 37). Protein content is a major factor determining the nutritional value of microalgae. In addition, highly unsaturated fatty acid (e.g., eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA)) content is of major importance (38). Indeed, some fatty acids are essential for many marine animals (39) and similar requirements exist for the growth and metamorphosis of many larvae (40, 41). However, it should be noted that the ratios of DHA, EPA and AA may be more important than their absolute levels (25). Microalgal vitamin content also has to be taken into account as it may be equally important (19, 23).

To provide more better balanced nutrition and improve animal growth, several reports advise mixing species from the ones listed above. This gives better results than a diet composed of only one algal species (23, 40).

While microalgae provide food for zooplanktons, they also help to stabilize and improve the quality of the culture medium. Indeed, for numerous freshwater and seawater animal species, the introduction of phytoplanktons to rearing ponds (green-water technique) leads to much better results in terms of survival, growth and transformation index than that of the clear-water technique (42-44). The reasons for this are not entirely known but may include (34, 35) water quality improvement and stabilization by algal oxygen production and pH stabilization, the action of some excreted biochemical compounds along with the induction of behavioral processes like initial prey catching, and the regulation of bacterial population, probiotic effects (45), and the stimulation of immunity (suggested but not sufficiently understood).

Microalgae are also used to refine the products of aquaculture. In fact, artificial diets lack natural sources of pigments that give organisms such as salmon and trout their characteristic coloration. Thus, carotenoid pigments like astaxanthin must be supplied in these diets (25, 29, 46). Although a large majority of the astaxanthin market (95%) focuses on the synthetic form, *Haematococcus* algae can also be used as a natural food colorant (47). In fact, microalgal astaxanthin has been approved in Japan and Canada as pigment in salmonid feeds (48). Feeds including 5% to 20% *Arthrospira* (rich in carotene pigments), which enhances the red and yellow patterns in carp, while leaving a brilliant white color. This clarity and color definition increase their value (Resource center for *Spirulina* and microalgae. Official web page. http://www.spirulinasource.com, 2005). Another example is the traditional French technique called the greening of oysters. It consists of creating a blue-green color on the gills and labial palps of oysters using the diatom *Haslea ostrearia*. This increases the product's market value by 40% (35).

Nevertheless, despite the advantages of live microalgae in aquaculture, the current trend is to avoid using them. This is due to their high cost and the difficulty in producing, concentrating and storing them (36, 40). As a result, diets replacements for live algae have been developed (e.g., yeasts, microencapsulated diet, and preserved algae). For example, in Japan, where *Nannochloropsis oculata* is the most important cultured feed for the rotifer *Brachionus plicatilis*, concentrated suspensions and frozen biomass of this microalga are commercially available (49). The partial replacement of live algae with microencapsulated and yeast-based diets is now routine in hatcheries for peneid shrimp (50). The presently available pastes cost at least US$ 160 and more per kg dry weight (personnal communication). In marine fish hatcheries, the tendency is to apply the clear-water technique instead of the green-water technique. Nevertheless, the omission of algae from the larval tanks often results in a less-predictable culture performance. New solutions for totally replacing microalgae in aquaculture diets are not yet sufficiently advanced to enable widespread adoption (35, 36).

Animal feed (pets and farming) Many nutritional and toxicological evaluations have proved the suitability of algal biomass as feed supplement (3). *Arthrospira* is largely used in this domain and concerns many types of animal: cats, dogs, aquarium fish, ornamental birds, horses, cows and breeding bulls. Algae positively affect the physiology (by providing a large profile of natural vitamins, minerals, and essential fatty acids; improved immune response and fertility; and better weight control) and their external appearance (resulting in healthy skin and a lustrous coat) of animals (52). In poultry rations, algae up to a level of 5-10% can be used safely as partial replacement for conventional proteins. Prolonged feeding of algae at higher concentrations produces adverse effects. The yellow color of broiler skin and shanks as well as of egg yolk is the most important characteristic that can be influenced by feeding algae (3). Moreover, the Institut für Getreideverarbeitung (Bergholz-Rehbrücke, Germany) produces a natural feed with the algae *Chlorella* and *Arthrospira* called Algrow.

MICROALGAE IN COSMETICS

Some microalgal species are established in the skin care market, the main ones being *Arthrospira* and *Chlorella* (51). Some cosmeticians have even invested in their own microalgal production system (LVMH, Paris, France and Daniel Jouvance, Carnac, France). Microalgae extracts can be mainly found in face and skin care products (e.g., anti-aging cream, refreshing or regenerant care products, emollient and as an anti-irritant in peelers). Microalgae are also represented in sun protection and hair care products. Here are two examples of commercially available products and their properties claimed by their companies; a protein-rich extract from *Arthrospira* repairs the signs of early skin aging, exerts a tightening effect and prevents stria formation (Protulines, Exsymol S.A.M., Monaco); and an extract from *Chlorella vulgaris* stimulates collagen synthesis in skin, thereby supporting tissue regeneration and wrinkle reduction (Dermochlorella, Codif, St. Malo, France).

Recently, two new products have been launched by Pentapharm LTD (Basel, Switzerland) (51): an ingredient from *Nannochloropsis oculata* with excellent skin-tightening properties (short and long-term effects) (Pepha-Tight) and an ingredient from *D. salina*, which shows the ability to markedly stimulate cell proliferation and turnover and to positively influence the energy metabolism of skin (Pepha-Ctive).

HIGH-VALUE MOLECULES

Owing to their global composition, microalgae are generally used in the field of human and animal nutrition. However, pure molecules can also be extracted when their concentrations are sufficiently high. This leads to valuable products like fatty acids, pigments and stable isotope biochemicals.

Fatty acids Higher plants and animals lack the requisite enzymes to synthesize polyunsaturated fatty acids (PUFAs) of more than 18 carbons (52, 53). Thus, they have to get them from their food. Fish and fish oil are the common sources of long-chain PUFAs but safety issues have been raised because of the possible accumulation of toxins in fish (25). Moreover, the application of fish oil as food additive is limited due to problems associated with its typical fishy smell, unpleasant taste and poor oxidative stability (52, 54, 55). For certain applications, fish oil is not suitable because of the presence of mixed fatty acids (56). As PUFAs are found in fish originating from microalgae consumed in oceanic environments, it is logical to consider microalgae as potential sources of PUFAs (57).

Table 2 presents the microalgal PUFAs of particular interest: however, currently, DHA is the only algal PUFA commercially available. Indeed, even if species have demonstrated industrial production potential of EPA (*Porphyridium purpureum, Phaeodactylum tricornutum, Isochrysis galbana, Nannochloropsis* sp. and *Nitzschia laevis*) (49, 56, 58, 59), no purified algal oil is currently economically competitive with other sources (25, 49, 60). The same problem exist with γ-linolenic acid (GLA) and AA.

DHA is an ω3 fatty acid found in tissues throughout the body. It is a major structural fatty acid in the grey matter of the brain and in the retina of the eye, and is a key component of the heart tissue. DHA is important for correct brain and eye development in infants and has been shown to support cardiovascular health in adults (61, 62). It is found in a limited selection of foods such as fatty fish and organic meat; it also occurs naturally in breast milk but is absent from cow's milk. From 1990 onwards, a number of health and nutrition organizations specifically recommended the inclusion of DHA in infant formula for preterm and fullterm infants. The world wholesale market for infant formula is now estimated to be about US$ 10 billion per annum (62). Martek's DHA oil for this application (DHASCO, Columbia, MD, USA) comes from *Crypthecodinium cohnii* and contains 40-50% DHA but no EPA or other long-chain PUFAs (57, 62, 63). The heterotrophic process uses a number of fermenters, each about 100 m^3, and meets strict manufacturing conditions that follow the US Food and Drug Administration's (FDA) current Good Manufacturing Practice (cGMP) regulations. The production for 2003 was 240 tons (63) and formulas containing Martek's oil are available in more than 60 countries worldwide (e.g., United Kingdom, Mexico, China, United States and most recently, Canada).

Moreover, OmegaTech (USA), also owned by Martek, exploits *Schizochytrium* to produce a low-cost oil formerly known as DHA Gold (10 t in 2003; 63). The oil is currently used as an adult dietary supplement in food and beverages, health foods, animal feeds and maricultural products. Example foods are cheeses, yogurts, spreads and dressings, and breakfast cereals. Other markets include foods for pregnant and nursing women and applications in cardiovascular health (62).

Finally, the Nutrinova process (Frankfurt, Germany) uses *Ulkenia sp.* which grows in 80-m^3 fermenters. The oil is sold under the name of DHActive (63, Pulz, Abstr. Europ. Workshop Microalgal Biotechnol., Germany, p. 35, 2005).

Pigments *Carotenoids* Among the over 400 known carotenoids, only very few are used commercially: β-carotene, astaxanthin (Fig. 5a, b) and, of lesser importance, lutein, zeaxanthin, lycopene and bixin (29, 64). Their most important uses are as natural food colorants (e.g., orange juice) and as additive for animal feed (poultry, fish). Carotenoids also have applications in cosmetics (64). The nutritional and therapeutic relevance of certain carotenoids is due to their ability to act as provitamin A, that is, they can be converted into vitamin A (65, 66). Moreover, carotenoids have intrinsic anti-inflammatory properties owing to their quenching action on relative oxygen species and a therapeutic chemopreventive anticancer effect is sometimes attributed to these molecules (46, 64, 66). However, in vitro and in vivo investigations in animals and humans have not demonstrated this anticancer effect (3). In many markets, microalgal carotenoids are in competition with the synthetic form of the pigments. Although the synthetic forms are much less expensive than the natural ones, microalgal carotenoids have the advantage of supplying natural isomers in their natural ratio (Table 3) (46, 65). It is accepted today that the natural isomer of β-carotene is superior to the synthetic all-trans form (3, 26, 29, 48).

The green halophilic flagellate *D. salina* is the most suitable organism for the mass production of β-carotene since it can produce β-carotene up to 14% of its dry weight (14). It can be cultivated outdoors in open ponds owing to the extreme conditions under which it grows (hypersaline, low availability of nitrogen, and high levels of solar radiation). Several industrial production plants are operational in Australia, Israel, USA and China (65, 67). The major producer of this strain in the world is Cognis Nutrition and Health. Their farms, which cover 800 ha, are located at Hutt Lagoon, Western Australia (Fig. 6) and Whyalla, South Australia. Three categories of products derive from *D. salina*: β-carotene extracts, *Dunaliella* powder for human use and dried *Dunaliella* for feed use. The prices of these products vary from US$ 300 to US$ 3000/kg (68).

Astaxanthin is principally consumed by the salmon feed industry. The annual worldwide aquaculture market of this pigment is estimated at US$ 200 million with an average price of US$ 2500/kg (69). It is dominated by the synthetic form of the pigment which is produced by BASF (Ludwigshafen, Germany) and Hoffman-La Roche (Basel, Switzerland) (70). Regarding natural astaxanthin, it is produced by *H. pluvialis* in a two-stage culture process and its concentration can reach 1.5% to 3% of the dry weight (48). The first stage is optimized for biomass production (green thin-wall flagellated stage); this is followed by an astaxanthin-accumulating stage under intense light conditions and preferably in a nutrient-poor medium (thick-walled resting stage). Because of its price, the astaxanthin of *H. pluvialis* cannot compete commercially with the synthetic form in any markets (46). However, for few particular applications, natural astaxanthin is preferred. These applications include carp, chicken and red sea bream diets. This is due to the enhanced deposition of the natural pigment in tissues, regulatory requirements and consumer demand for natural products (71). Moreover, since the 1990's, human nutraceuticals have appeared as a new market possibility (46, 47) and Algatech Ltd (Kibbutz Ketura, Israel) sells its product (crushed *Haematococcus* biomass rich in astaxanthin) on the pharmaceutical market (Boussiba, Abstr. Europ. Workshop Microalgal Biotechnol., Germany, p. 37, 2003). To lower their costs and compete with the synthetic form, their producers plan to expand their production capacity into locales with lower land, labor and energy costs such as China (46, 70).

Phycobiliproteins The main commercial producers of phycobiliproteins (i.e., phycoerythrin and phycocyanin, Fig. 5c) are the cyanobacterium *Arthrospira* and the rhodophyte *Porphyridium* (72, 73). The primary potential of these molecules seems to be as natural dyes but an increasing number of investigations have shown on their health-promoting properties and broad range of pharmaceutical applications. Thus, the first and most important application of phycocyanin is as food pigment, replacing current synthetic pigments (3). Dainippon Ink & Chemicals (Sakura, Japan) has developed a product called Lina blue which is used in chewing gum, ice sherberts, popsicles, candies, soft drinks, dairy products and wasabi. They also sell another form of this pigment for natural cosmetics like lipstick and eyeliners (23, 72). In addition, phycobiliproteins are widely used in industry and clinical or research immunology laboratories. Indeed, their properties (high molar absorbance coefficients, high fluorescence quantum yield, large Stokes shift, high oligomer stability and high photostability) make them very powerful and highly sensitive fluorescent reagents (Prozyme product literature available from http://www.prozyme.com/technical/pbvrwdata.html#SPECIFICATIONS, 2005). They can serve as labels for antibodies, receptors and other biological molecules in a fluorescence-activated cell sorter and they are used in immunolabelling experiments and fluorescence microscopy or diagnostics (73). The prices of phycobiliproteins products are US$ 3 to US$ 25/mg for native pigment but they can reach US$ 1500/mg for certain cross-linked pigments (with antibodies or other fluorescent molecules, Table 4). Their global market was estimated at more than US$ 50 million in 1997 (personnal communication).

Stable isotope biochemicals Microalgae are ideally suited as a source of stable isotopically labeled compounds. The ability to perform photosynthesis allows them to incorporate stable isotopes (^{13}C, ^{15}N and ^{2}H) from relatively inexpensive inorganic molecules ($^{13}CO_2$, $^{15}NO_3$, $^{2}H_2O$) to more highly valued organic compounds (e.g., amino acids, carbohydrates, lipids and nucleic acids). Stable isotope biochemicals are used for two purposes (25): incorporation into proteins, carbohydrates and nucleic acids to facilitate their structural determination at the atomic level; and metabolic studies exploiting the increased mass of compounds labeled.

Their market is probably higher than US$ 13 million/year (personnal communication). Spectra Stable Isotopes (Columbia, MD, USA), a division of Spectra gases (formerly Martek Stable Isotope) sells its marked amino acids at prices in the range from US$ 260/g to US$ 5900/g and its marked nucleic acids at about US$ 28/mg (Table 4). Moreover, it has recently developed a process for the autotrophic production of labeled PUFAs from microalgae using $^{13}CO_2$, in which $^{13}CO_2$ is directly sparged into the culture as required. Thus, the carbon loss is high and there is a low efficiency of labeled carbon use. In spite of these considerations, this company is manufacturing more than 400 g per year of labeled fatty acids at US$ 38,000/g (Table 4; 74).

CONCLUSION

Some microalgae have been exploited for millenia (*Nostoc* in China and *Arthrospira* in Chad and Mexico). Currently, they have several applications from human and animal nutrition to cosmetics and the production of high-value molecules (e.g., fatty acids, pigments, stable isotope biochemicals, Table 5). However, microalgae are still not a well-studied group from a biotechnological point of view. Indeed, among the 10,000 species that are believed to exist, only a few thousand strains are kept in collections, a few hundred are investigated for chemical content and just a handful are cultivated in industrial quantities (i.e., in tons per year) (70). The development of microalgal biotechnology has been slowed by the limited growth performance of algae in industrial photobioreactors. Currently, the majority of microalgal production occurs in outdoor cultivation. However, closed-system commercialization has begun with *Haematococcus* in Japan and Israel and with *Chlorella* in Germany. Algal production systems need to be further improved in order to become more competitive and more economically feasible. Heterotrophic and mixotrophic cultivation could be a possible avenue of research. The genetic improvement of algal strains is also a present challenge. The use of transgenic microalgae for commercial applications has not yet been reported but holds significant promise. Modified strains could overproduce traditional or newly discovered algal compounds and also serve to express specific genes that cannot be expressed in yeast. This could be of great importance for the production of hydrogen, for example. However, a successful drug discovery is the most promising aspect of microalgal biotechnology because the potential is immense although screening remains limited (75).

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LIST OF FIGURES

FIG. 1. Earthrise Farms *Arthrospira* production plant (Calipatria, CA, USA).

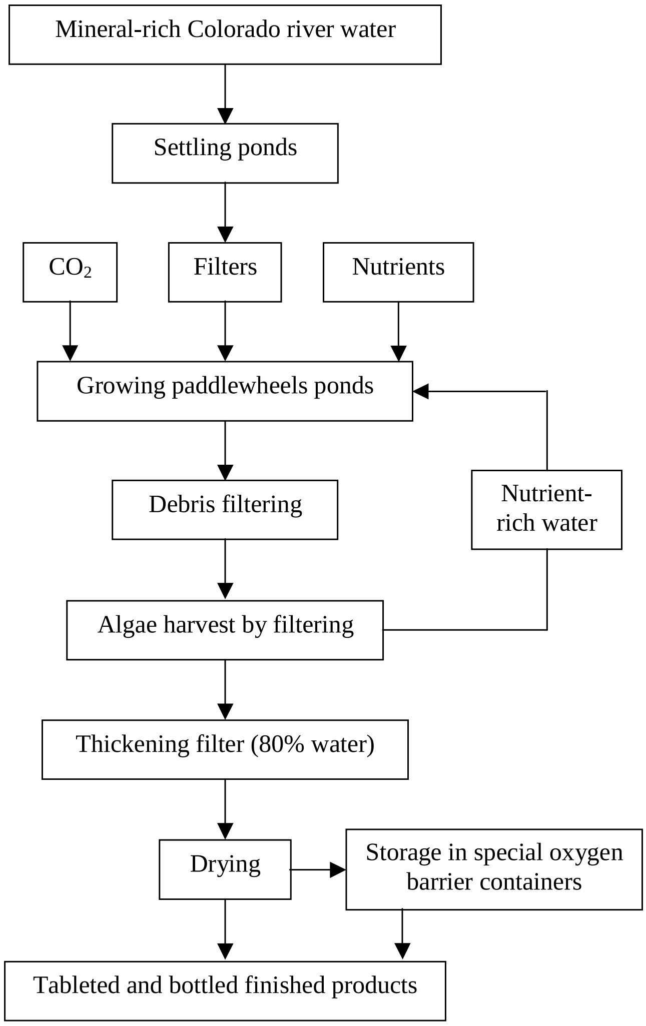

FIG. 2. Earthrise Farms microalgal production process.

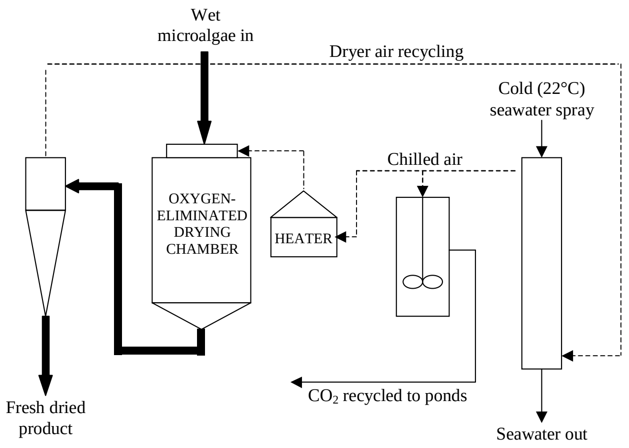

FIG. 3. Cyanotech process for drying microalgae biomass.

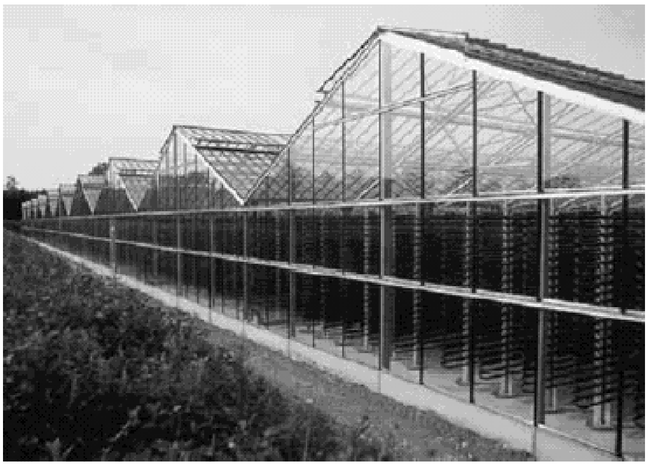

FIG. 4. Glass tube photobioreactor (700 m^3) producing *Chlorella* biomass (Klötze, Germany).

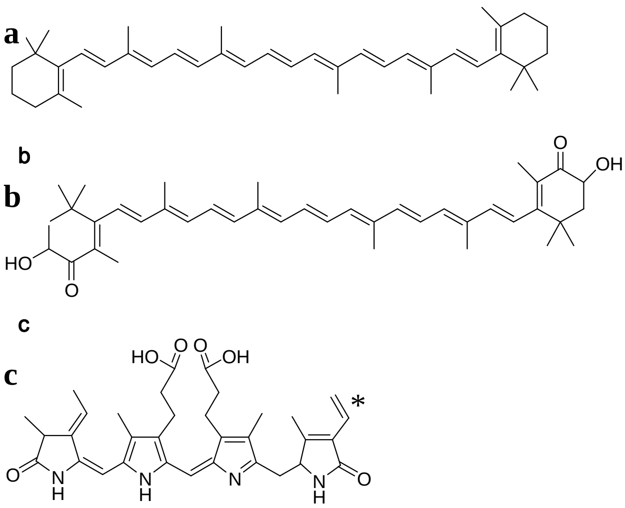

FIG. 5. Chemical structures of microalgal pigments. (**a**) β-Carotene (**b**) astaxanthin (**c**) phycoerythrin (in phycocyanin, the $CH=CH_2$ group noted an asterisk is replaced by $CH_3 - CH_2$).

FIG. 6. Farms producing *D. salina* at Hutt Lagoon, Australia (Cognis Nutrition and Health).

TABLE 1. General composition of different human food sources and algae (% of dry matter) (3)

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

It should be kept in mind that the figures presented in this table are estimaties, since the proportion of individual cell constituents largely depends on environmental parameters.

TABLE 2. Particularly interesting microalgal PUFAs

PUFA	Structure	Potential application	Microorganism producer
γ-Linolenic acid (GLA)	18:3 ω6, 9, 12	Infant formulas for full-term infants Nutritional supplements	*Arthrospira*
Arachidonic acid (AA)	20:4 ω6, 9, 12 ,15	Infant formulas for full-term/preterm infants Nutritional supplements	*Porphyridium*
Eicosapentaenoic acid (EPA)	20:5 ω3, 6, 9, 12, 15	Nutritional supplements Aquaculture	*Nannochloropsis, Phaeodactylum, Nitzschia*
Docosahexaenoic acid (DHA)	22:6 ω3, 6, 9, 12, 15, 18	Infant formulas for full-term/preterm infants Nutritional supplements Aquaculture	*Crypthecodinium, Schizochytrium*

TABLE 3. Comparison between microbial and synthetic sources of carotenoids

Molecule	Origin	Isomer	Market	Price (US$)	Principal producer
β-Carotene	*Dunaliella*	All-trans and 9-cis		300 – 3000/kg	Cognis Nutrition and Health (Hutt Lagoon and Whyalla, Australia), Cyanotech Corp. (Kona, Hawaii, USA), Inner Mongolia Biological Eng. Co. (Inner Mongolia, China), Nature Beta Technologies (Eilat, Israel), Tianjin Lantai Biotechnology (Tianjin, China)
	Synthetic	All-trans	>90%		
Astaxanthin	*Haematococcus*	3S, 3'S			Cyanotech Corp. (Kona, Hawaii, USA), Mera Pharmaceuticals (Kailua-Kona, Hawaii, USA), Bioreal Inc. (Kihei, Hawaii, USA), Parry's Pharmaceuticals (Chennai, India), Algatech Ltd (Kibbutz Ketura, Israel)
	Phaffia yeast	3R, 3'R			DSM (Heerlen, The Netherlands)
	Synthetic	3S, 3'S - 3R, 3'R - 3R, 3'S (meso)	>95%	≈ 2500/kg	Hoffman La Roche (Basel, Switzerland) and BASF (Ludwigshafen, Germany)

TABLE 4. Prices of different products based on microalgal high-value molecules

Product name	Price (US$)	Distributor
R-phycoerythrin	3.25 – 14/mg	Cyanotech Corp.
Allophycocyanin	6 – 17/mg	Cyanotech Corp.
Streptavidin: B-phycoerythrin	145/mg	Martek
Goat anti-mouse IgG: R-phycoerythrin	165/mg	Martek
Sensilight™ PBXL1: anti GST	1500/mg	Martek
Mixed fatty acids	60/g	Spectra Stable Isotopes
^{13}C-mixed free fatty acids	200/g	Spectra Stable Isotopes
^{13}C-DHA (>95%)	38000/g	Spectra Stable Isotopes
^{15}N-alanine	260/g	Spectra Stable Isotopes
$^{2}H_7$, ^{13}C, $^{15}N_4$-arginine	5900/g	Spectra Stable Isotopes
dATP-CN	26000/g	Spectra Stable Isotopes

TABLE 5. Present state of microalgal production (10, 48, 63, 69)

Alga	Annual production	Producer country	Application and product
Arthrospira	3000 t dry weight	China, India, USA, Myanmar, Japan	Human and animal nutrition, cosmetics, phycobiliproteins
Chlorella	2000 t dry weight	Taiwan, Germany, Japan	Human nutrition, aquaculture, cosmetics
Dunaliella salina	1200 t dry weight	Australia, Israel, USA, China	Human nutrition, cosmetics, β-carotene
Aphanizomenon flos-aquae	500 t dry weight	USA	Human nutrition
Haematococcus pluvialis	300 t dry weight	USA, India, Israel	Aquaculture, astaxanthin
Crypthecodinium cohnii	240 t DHA oil	USA	DHA oil
Shizochytrium	10 t DHA oil	USA	DHA oil