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MINI-REVIEW

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Valuable products from biotechnology of microalgae

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Abstract The biotechnology of microalgae has gained considerable importance in recent decades. Applications range from simple biomass production for food and feed to valuable products for ecological applications. For most of these applications, the market is still developing and the biotechnological use of microalgae will extend into new areas. Considering the enormous biodiversity of microalgae and recent developments in genetic engineering, this group of organisms represents one of the most promising sources for new products and applications. With the development of sophisticated culture and screening techniques, microalgal biotechnology can already meet the high demands of both the food and pharmaceutical industries.

Introduction

The biotechnology of microalgae is closely related to the biotechnological production, use and application of macroalgae. Macroalgae, represented mainly by a few species of Rhodophyta and Phaeophyta, have an old tradition in the use of biomass for the production of phycocolloids like agar–agar, alginates or carrageenan. Altogether, this macroalgal biotechnology represents a world market of ca. U.S. \$ 6×10^9/year; and more than 7.5×10^6 t/year of macroalgae are harvested.

Microalgal biotechnology is the younger branch of algal biotechnology. The first report referring to macroalgal use dealt with “nori” (*Porphyra*) which has been collected since the year 530. This species, cultivated since 1640, forms today in Asia an industry with a yearly turnover of about U.S. \$ 1×10^9.

The first reports about agar–agar production in Japan can be dated back to the year 1658, although cultivation was only successful from the middle of the last century (Table 1). Brown algae were already being processed for iodine and soda in the eighteenth century; and first attempts at on-site cultivation date back to 1731. In the beginning of the last century, the phycocolloid group of alginates gained industrial value.

Fossile microalgal biomass of diatoms was used by Alfred Nobel for the adsorption of nitroglycerin to create dynamite. Until recently, this was the most striking example of a microalgal application. *Spirulina* (*Arthrospira*) was already collected by the Aztec population and was later cultivated considerably. In comparison, *Chlorella* is a newcomer in microalgal biotechnology and will soon be followed by other promising species for valuable substances.

Macroalgae are to date mainly harvested from natural habitats or cultivated at seashore areas. Microalgae are mainly cultivated in artificial systems called photobioreactors (PBR). These systems were reviewed by Pulz (2001).

The microalgal biomass market has a size of about 5,000 t/year of dry matter and generates a turnover of ca. U.S. \$ 1.25×10^9/year. Processed products are not yet included in this figure and are discussed in this paper.

Successful algal biotechnology mainly depends on choosing the right alga with relevant properties for specific culture conditions and products (Table 2).

Therefore, a basic knowledge of algal physiology, ecology and taxonomy is important. All microscopic algae, usually unicellular or filamentous, are called microalgae, although this term is not related to taxonomy.

By continuing the works and ideas of Dr. Gross, that he could not proceed by himself due a tragic fate in the year 2003, we will keep his place in future not only in the research community but also among all colleagues and other persons who knew him.

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Table 1 Algal biotechnology historical data

Alga	Year of first record		
	Collected	Cultivated	Processed
Macroalgae			
Porphyra	530	1640	–
Chondrus/*Gelidium*/ *Gracilaria*	∞	1950	1658
Laminaria/*Macrocystis*/ *Fucus*	∞	1731	1925
Eucheuma	∞	1971	1965
Microalgae			
Diatoms	∞	1863 (selective use)	1914
Spirulina	∞	1965	1985
Chlorella	–	1975	1994
Dunaliella	–	1982	1985
Odontella	–	2002	2003

Cyanobacteria are considered more and more as a separate group because of their prokaryotic nature.

The biodiversity of microalgae is enormous and represents an almost untapped resource. It has been estimated that between 200,000 and several million species exist (Norton et al. 1996), compared with about 250,000 species of higher plants.

The aim of this paper is to review the most important features of microalgal biotechnology applications. For this purpose, in the first part the taxonomy of microalgae and an estimation of the potentially valuable substances produced by the different taxa are reviewed. In the second part, the industrial application of microalgal biotechnology is discussed, together with a description of past and future developments.

Biotechnologically relevant cyanobacteria and microalgae

Cyanobacteria

About 2,000 species of cyanobacteria are known and can be divided into 150 genera. These colonize almost all habitats, ranging from open oceans to mountain soils and from hot springs to snow fields. Because many cyanobacteria lack distinct morphological characteristics and their phylogeny is poorly understood, the taxonomy of cyanobacteria is very difficult and in almost constant rearrangement. The most accepted system of cyanobacterial taxonomy (Anagnostidis and Komárek 1985) recognizes four orders: the Chroococcales include all unicellular species (Komárek and Anagnostidis 1986), the Oscillatoriales are characterized by simple unbranched filaments (Anagnostidis and Komárek 1988), all species within the Nostocales exhibit heterocysts (Komárek and Anagnostidis 1989) and the Stigonematales have branched filaments with heterocysts (Anagnostidis and Komárek 1990). These

Table 2 Microalgal species with high relevance for biotechnological applications

Species/group	Product	Application areas	Basins/reactors
Spirulina platensis/Cyanobacteria	Phycocyanin, biomass	Health food, cosmetics	Open ponds, natural lakes
Chlorella vulgaris/Chlorophyta	Biomass	Health food, food supplement, feed surrogates	Open ponds, basins, glass-tube PBR
Dunaliella salina/Chlorophyta	Carotenoids, β-carotene	Health food, food supplement, feed	Open ponds, lagoons
Haematococcus pluvialis/Chlorophyta	Carotenoids, astaxanthin	Health food, pharmaceuticals, feed additives	Open ponds, PBR
Odontella aurita/Bacillariophyta	Fatty acids	Pharmaceuticals, cosmetics, baby food	Open ponds
Porphyridium cruentum/Rhodophyta	Polysaccharides	Pharmaceuticals, cosmetics, nutrition	Tubular PBR
Isochrysis galbana/Chlorophyta	Fatty acids	Animal nutrition	Open ponds
Phaedactylum tricornutum/Bacillariophyta	Lipids, fatty acids	Nutrition, fuel production	Open ponds, basins
Lyngbya majuscule/Cyanobacteria	Immune modulators	Pharmaceuticals, nutrition	

heterocysts are specialized cells lacking photosystem II. Thus, the fixation of atmospheric nitrogen is facilitated. Photosynthetic pigments are chlorophyll a, phycocyanins and phycoerythrin, which usually give the cells a blue-green color, although reddish-brown to pink colors also occur in some species. The majority of the cyanobacteria are obligate phototrophs; and only a few species can grow mixotrophically or even heterotrophically.

Some planktonic cyanobacteria can adjust their buoyancy with special gas vesicles, e.g., *Anabena flos-aquae*, in order to receive optimal light incidence. Other species excrete extracellular polysaccharides, sometimes representing 30% of their total polysaccharides, to form floating aggregates. On solid substrates many species, coccoid and filamentous, are capable of gliding movements, e.g., Oscillariaceae.

One reason why cyanobacteria often dominate algal communities is their extremely fast uptake and storage of nutrients: phosphate is deposited in polyphosphate granules, cyanophycin (a polymer of aspartic acid and arginine) serves as a nitrogen, carbon and energy reserve and both carbon and energy are also stored as a highly branched α-1,4-polyglucan (cyanophycean starch). Reproduction occurs by fission and sexual reproduction is absent.

Cyanobacteria produce numerous bioactive compounds. Some of these are strong hepatotoxins or neurotoxins that causes serious problems for public health when cyanobacterial blooms occur in lakes, rivers, or drinking-water reservoirs. Other secondary metabolites are potentially of therapeutical importance, such as antiviral compounds, immunomodulators, inhibitors, or cytostatics (Sivonen and Jones 1999; Skulberg 2000).

Biotechnologically, the most important Cyanobacteria are *Spirulina* (*Arthrospira*) *platensis*, *Nostoc commune* and *Aphanizomenon flos-aquae*.

Prochlorophyta

These cyanobacteria probably represent an artificial group, as judged from genetic fingerprinting. Their completely different pigmentation, however, may justify their separation from other cyanobacteria. The Prochlorophyta are best described as “free-living chloroplasts” because of the absence of phycobiliproteins and the presence of chlorophyll a and b in most cases. This pigmentation and their thylakoid arrangement is very similar to higher plant chloroplasts. Only three genera are currently recognized: *Prochloron*, *Prochlorothrix* and *Prochlorococcus*. While the first two are rather rare species, *Prochlorococcus* was recently found to be the main component of the picoplankton. These very small cells (<1 μm) are the primary producers in open oceans (Blanchot and Rodier 1996) and may even surpass diatoms (see below) in their annual biomass production. Currently, no biotechnological applications are evident.

Eukaryotic algae

The eukaryotic algae can be traced back about 1.9×10^9 years and are much younger than the cyanobacteria with 2.7×10^9 years of phylogenetic history. After the progenitor of the algae arose through an endosymbiosis with a cyanobacterium, two evolutionary lines emerged apparently at the same time—the red and the green algae. These two lines developed many characteristic properties. Other algal groups emerged only much later through the so-called secondary endosymbiosis, when a red or a green alga transformed into a plastid within an eukaryotic host. This event gave rise to the heterokont algae, the dinoflagellates, the cryptophytes and the Euglenida.

The green algae

Based on a number of biochemical and cellular differences, two major groups of green microalgae are recognized: the Chlorophyta and the Conjugaphyta. Although the latter group is almost five times larger than the Chlorophyta, none of the Conjugaphyta has yet been employed for biotechnological applications.

The Chlorophyta are subdivided into four groups. The Prasinophyceae are flagellated unicellular algae, only 10–15 μm in diameter, and covered with organic scales. Most species inhabit marine and brackish environments, while others prefer freshwater. Only 13 genera with about 120 species have been described. *Tetraselmis*, *Pyramimonas* and *Micromonas* exhibit high growth rates and are used in outdoor cultures as food for, e.g., bivalve mollusk larvae (De Pauw and Persoone 1988; Laing and Ayala 1990). Under mixotrophic culture conditions some species can reach considerable cell densities (Xie et al. 2001).

The Chlorophyceae represent the largest group, with about 2,500 species in 350 genera. Most species are unicellular or filamentous freshwater forms. The best known algae, such as *Chlorella*, *Chlamydomonas*, *Dunaliella* and *Haematococcus*, belong to this group. Some species accumulate high concentrations of carotenoids under certain culture conditions. The extraction of β-carotene from *D. salina* has already reached large-scale production (Borowitzka 1998). Another promising carotenoid is astaxanthin, a high-value pigmentation source in aquaculture, especially for trout and salmon. Efforts have been made to produce astaxanthin cost-efficiently from *H. pluvialis*, which accumulates up to 3% astaxanthin (dry weight; Lorenz and Cysewski 2000).

The Ulvophyceae and Charophyceae mainly consist of macroscopic algae. None of the unicellular or filamentous forms are of biotechnological importance, although *Spirogyra* apparently produces bioactive substances (bactericides; Muller-Feuga et al. 2003).

Euglenida

The Euglenida exhibit both plant-like and animal-like characteristics and are related to the protozoan group Kinetoplastida. This group arose from a secondary endosymbiosis between a protozoan-like host and a *Chlorella*-like green alga. Therefore, the euglenoids represent a very diverse and isolated group with roughly 800 species in about 43 genera. All species are flagellated unicells covered by an often highly flexible cell wall, allowing some species to perform crawling movements (e.g., *Euglena*). About 70% of the species contain no photosynthetic pigments and utilize organic matter. Even the pigmented species often combine photosynthesis with organo- or phagotrophic nutrition and are commonly found in eutrophic wastewater or polluted areas. Therefore, most Euglenida require complex growth media and are difficult to grow under axenic conditions. An exception is *E. gracilis*, which can be grown in mineral medium and has successfully been used in large-scale mass cultures (Siegelman and Guillard 1971).

The Euglenida are very interesting for biotechnology because they exhibit a number of unique pathways and metabolites (Ragan and Chapman 1978). Their storage product is a β-1,3-polyglucan, called paramylon starch. A number of bioactive compounds have been reported for euglenoids but have, so far, not been exploited for biotechnology. The accumulation of and resistance towards heavy metals by some euglenoids may also be of biotechnological relevance.

Rhodophyta

The red color of the red algae is due the presence of high amounts of the biliprotein phycoerythrin in addition to the blue phycocyanin and chlorophyll a. In contrast to green algae, rhodophytes accumulate starch in the cytosol and not in plastids. The galactans in their cell wall, with their extraordinary gelling properties, were one of the first substances used in algal biotechnology. Typical rhodophytes are marine macrophytes. Only a few species can be considered microalgae, all of them belonging to the subclass Bangiophycidae. These inhabit marine, freshwater and terrestrial habitats.

Porphyridium cruentum is easily cultivated in artificial seawater medium without the requirement of vitamin B_{12}, which is necessary for most other red microalgae. This is one reason why up to now only *Porphyridium* species are used biotechnologically for the production of arachidonic acid, pigments (phycocyanin, phycoerythrin) and extracellular polysaccharides (Borowizka and Borowitzka 1997).

Prymnesiophyta

The Prymnesiophyta, sometimes called Haptophyta, consist of about 500 species in 50 genera. The yellow-green to brownish color of the flagellated or coccoid unicells derives from xanthophylls, mainly fucoxanthin, and is similar to the color of diatoms and xanthophytes (see Heterokont algae, below). The storage product is chrysolaminarin (a soluble β-1,3-polyglucan). Prymnesiophytes are mostly found in the marine plankton, but freshwater and terrestrial habitats are also known. Some species are phagotrophic. Especially marine species often form massive blooms (e.g., *Chrysochromulina*, *Emiliania*), with negative effects on the ecosystem.

Coccolithophorids, a large group within the Prymnesiophyta, possess calcified structures (coccoliths) on the cell surface. The huge limestone deposits from the Cretaceous period actually consist of coccoliths. Even today, coccolithophorids play an important role in the transfer of carbon from the atmosphere into sediments.

Many prymnesiophytes excrete considerable amounts of extracellular polysaccharides, e.g., up to 64% of total cellular polysaccharides for the marine *Phaeocystis pouchetii*. Other species produce exotoxins which threaten fish farms or natural populations. At present, several species of prymnesiophytes, e.g., *Pavlova lutheri* and *Isochrysis* spp are used as feed for shellfish larvae, especially oysters (Laing and Ayala 1990; Borowitzka 1997).

Heterokont algae

The name heterokonts springs from the unequal length of the flagella—a common feature in this group: This so-called “brown line” within the algal phylogeny, consists of brown algae (Phaeophyta), yellow-green algae (Xanthophyta), golden algae (Chrysophyta), and diatoms (Bacillariophyta). Their generally dominant brown coloring originates from the high content of fucoxanthin, an accessory photosynthesis pigment. Chrysolaminarin and lipids in droplets are common storage products. Despite their very different cellular organization and morphology, these groups form a natural evolutionary branch. The phaeophytes are almost exclusively marine macrophytes and are not considered here.

The Eustigmatophyta and Xanthophyta are apparently closely related and are discussed together. These yellow-green algae consist of about 600 species in more than 90 genera. Most species are unicellular or filamentous and prefer terrestrial or freshwater habitats. Although some species have high growth rates and form late winter blooms in still waters (e.g., *Tribonema*), only *Olisthodiscus* and *Nannochloropsis* are used to some extent as feed in aquaculture (De Pauw and Persoone 1988; Borowitzka 1997).

The Chrysophyta represent a group of about 1,000 species separated into 120 genera. The majority of the species are flagellated unicells living as freshwater plankton. The cells are either naked or covered with elaborate silica scales. Although most species can photosynthesize, mixotrophic growth is very common in this group. A number of species grow mainly phagotrophically. The addition of vitamins, other organic nutrients, or even soil extracts is necessary for most species to achieve good growth under laboratory conditions.

The Bacillariophyta or diatoms represent probably the largest biomass producers on earth and are one of the youngest algal groups. Diatoms colonize nearly all habitats and have developed into a vast number of different species. Conservative estimates count at least 100,000 species in 250 genera. Although some species form pseudofilaments or colonial aggregates, all species are unicellular and unflagellated in their vegetative stage. Diatoms are easily recognized by their cell covering, consisting of two silica valves (like a Petri dish), usually with elaborate fine structure. Taxonomically, the Bacillariophyta are separated into three groups: the Coscinodiscophyceae have centric (pillbox-shaped) cells while, in contrast, the cells of the Fragilariophyceae are pennate (boat-shaped) without a raphe (longitudinal striation) and the pennate Bacillariophyceae exhibit a raphe. Their main reserve products are chrysolaminarin and oil which can, especially under nitrogen-starvation, reach up to 60% dry weight (e.g., *Phaeodactylum tricornutum*). This oil is not only a food reserve; planktonic diatoms regulate their buoyancy by adjusting the oil content in their cell. Because of their high productivity and accumulation of oils, bacillariophytes may represent a future source for fuel and unusual fatty acids. The high concentrations of eicosapentaenoic acid (EPA) in diatoms is of particular biotechnological interest. Both centric and pennate diatoms are also widely used in aquaculture (Borowitzka 1997), mainly as feed for mollusks.

Cryptophyta

About 20 genera with 60 freshwater and marine species form the cryptophytes. Members of this group generally dominate in cool, oligotrophic waters and often form blooms in winter and early spring. Most species are photosynthetically active motile unicells. In addition to chlorophyll, a number of carotenoids and phycobiliproteins are present in the plastids. However, they do not contain phycobilisomes like red algae and cyanobacteria. Thus, their coloring ranges from bluish-green or reddish-brown to yellow-green. The cells are very small and especially marine species are often very fragile. Currently, the biotechnological use of cryptophytes does not go beyond feed for rotifers and clams in aquaculture.

Dinoflagellata

Although the Dinoflagellata represent a rather large group with about 4,000 species in 550 genera, relatively little is known about their physiology and ecology. From current data, it can be deduced that the Dinoflagellata are a very diverse group with little affiliation to other algae. The flagellated unicells inhabit freshwater and seawater. About 50% of the species are non-photosynthetic and utilize organic compounds or feed on bacteria and other small algae. Even in the photosynthetically active species, mixotrophic growth is often dominant. Nevertheless, especially in coastal waters, dinoflagellates contribute significantly to the primary production. Some species are an almost permanent threat to fisheries and aquaculture because they produce extremely harmful toxins. In contrast, *Gymnodinium* species are the preferred feed for fish larvae in aquaculture. In addition to the storage products oil and starch, dinoflagellates contain a large spectrum of unique sterols, fatty acids (e.g., docosahexanoic acid; DHA) and other metabolites with a high potential for biotechnology. However, growing dinoflagellates under laboratory conditions is generally very difficult because most species require complex growth media and special light regimes, or are stressed by turbulence in the medium. Nevertheless, the high value of certain compounds like DHA justify the cost of large-scale cultivation.

Extremophilic algae

Some algae not only tolerate extreme environmental conditions but require such conditions to thrive. For several applications this can be beneficial, even when the growth rates of extremophilic algae are sometimes slower compared with “common” algae. The major advantage of using extremophiles in mass cultures is the minimizing of contamination risk, which is a serious problem in outdoor cultures. Examples are the cultivation of *Dunaliella salina* at very high salt concentrations or the growth of cyanobacteria (e.g., *Spirulina*, *Oscillatoria*) at highly alkaline pH (Gimmler and Degenhardt 2001). Thermophilic cyanobacteria, such as *Mastigocladus laminosus*, *Phormidium laminosum* and *Cyanidium caldarium*, or species growing in diluted sulfuric acid (e.g., *D. acidophila*) may provide similar benefits. The red algae *C. caldarium* and *Galdieria sulphuraria* are both thermo- and acidophilic, having high growth rates at pH 1 and 50°C (De Luca et al. 1981). In addition, *G. sulphuraria* is able to grow heterotrophically on about 50 different carbon sources, unsurpassed by any other organism (Gross and Schnarrenberger 1995). Species found in soda lakes usually combine salt tolerance and alkaliphily (e.g., *Monoraphidium minutum*; Richmond 2004). Another interesting group are the psychrophilic algae, with their very low temperature optimum for growth (Gounot 1986). These slow-growing ice algae contain enzymes with unique kinetic properties (Feller et al. 1996). It can be expected that other extremophilic algae also contain unusual metabolites or enzymes. Because the intracellular pH in acidophilic and alkaliphilic species remains neutral, mainly extracellular enzymes may be of biotechnological interest.

Genetic engineering

The idea to genetically engineer microalgae, e.g., to increase their content of valuable compounds, is very tempting. Because of the usual absence of cell differentiation, microalgae represent a much simpler system for genetic manipulations compared with higher plants. In addition, allelic genes are usually absent because of the haploid nature of most vegetative stages of microalgae. Nevertheless, progress in the genetic engineering of algae was extremely slow until recently. Methods successfully used for transformation in other systems failed when applied to algae, mainly because of the considerable evolutionary distance between algae and other organisms. Thus, the transformation system had to be developed almost from the start, including techniques to introduce DNA into cells (Kindle et al. 1990), suitable promoters, new selectable marker genes and expression vectors (Apt and Behrens 1999). In addition, algae often have an unusual codon usage which requires even further adjustments before a successful transformation is possible. Currently, all these requirements have been fulfilled for the diatom *Phaeodactylum*, the green alga *Chlamydomonas* and the cyanobacteria *Synechococcus* and *Synechocystis*. The development of a functional transformation system can be expected in the near future for other diatoms and cyanobacteria and the red alga *Porphyridium*.

Diatoms are especially interesting for biotechnology because they have the physiological potential to accumulate high proportions of lipid. Improving the oil-producing characteristics of these algae naturally is a prime target of genetic engineering. A major breakthrough was the heterologous expression of a functional glucose transporter in the obligate photoautotrophic diatom *Phaeodactylum*, which enabled the alga to grow on glucose in the dark (Zaslavskaia et al. 2001). Another example of successful genetic engineering of a microalga is the expression of mosquito larvacides in cyanobacteria (Chungjatupornchai 1990; Boussiba et al. 2000).

These very promising advances, however, should be viewed with caution for the following reasons:

– The accumulation of valuable substances in algae via genetic transformation can only increase up to a point where cellular metabolism starts to be negatively affected. This threshold can be rather low and, therefore, the benefits negligible
– Transgenic algae potentially pose a considerable threat to the ecosystem and will most likely be banned from outdoor cultivation systems and otherwise be under strict regulation
– Usually, transgenic cells exhibit less fitness than wild-type cells and, therefore, cells that lose the newly introduced gene quickly outgrow the transformants. To prevent this, a constant selection pressure is necessary, usually by the addition of antibiotics, which is a potential public health hazard

Therefore, the prime field of genetic engineering will be the improved production of valuable products and bioactive compounds in closed culture systems. Genetic manipulations should complement and not substitute the screening of new species.

Application areas of microalgal biotechnology

Enterprises for microalgal products have recently developed numerous new technical systems for biomass production and down-streamed this biomass to very differentiated products. Table 3 gives a review of the main products (Pulz et al. 2000). Completely controllable and closed PBR scaled-up to industrial dimensions are becoming more important (Fig. 1). Nevertheless, the most important product of microalgal biotechnology regarding production amount and economic value is still the microalgal biomass itself.

Algal biomass

The biomass of microalgae as sun-dried or spray-dried powder or in compressed form as pastilles is the predominant product in microalgal biotechnology. This biomass is harvested from natural waters or from cultures in artificial ponds or PBR with subsequent separation from the growth media and drying. The rough estimates in Table 4 show the most important microalgal productions worldwide.

The final product of biomass production is usually a green- or orange-colored powder, which is sold mostly in the human health food market. One can recognize a growing market for microalgal biomass in animal nutrition both in aquaculture and for animal husbandry.

The dynamic growth of microalgal production is shown in Fig. 2, which is based on Borowitzka (1998) and CEVA, Paris (Anon 2000)

Human nutrition

Even today, the consumption of microalgal biomass is restricted to very few taxa, e.g., *Spirulina* (*Arthrospira*), *Chlorella*, *Dunaliella* and (of lesser and regional importance) *Nostoc* and *Aphanizomenon*.

It can be expected that exploitation of the biological diversity of microalgae will be hampered by food safety regulations for human consumption for a long time. Therefore, the successful authorization (following EC regulation 258/97) of the marine diatom *Odontella aurita* as a novel food by the French company INNOVALG in 2002 is a real large advantage for microalgal biotechnology.

Chlorella and *Spirulina* dominate the microalgal market. During the past decades, microalgal biomass was predominately utilized in the health food market, with more than 75% of the annual microalgal biomass

Table 3 Market estimations for microalgal products.*PUFA* Polyunsaturated fatty acids

Product group	Product	Retail value (U. S. $\$ \times 10^{6}$)	Development
Biomass	Health food	1,250–2,500	Growing
	Functional food	800	Growing
	Feed additive	300	Fast-growing
	Aquaculture	700	Fast-growing
	Soil conditioner		Promising
Coloring substances	Astaxanthin	<150	Starting
	Phycocyanin	>10	Stagnant
	Phycoerythrin	>2	Stagnant
Antioxidants	β-Carotene	>280	Promising
	Tocopherol		Stagnant
	Antioxidant extract (CO_2)	100–150	
	ARA	20	Growing
	DHA	1,500	Fast-growing
	PUFA extracts	10	
Special products	Toxins	1–3	
	Isotopes	> 5	

Fig. 1 Comparison of industrial-scale PBR in Germany (Klötze) and Israel (near to Eilat)

Table 4 Worldwide production rates for various algae

Alga	Production (t/year)
Spirulina	3,000
Chlorella	2,000
Dunaliella	1,200
Nostoc	600
Aphanizomenon	500

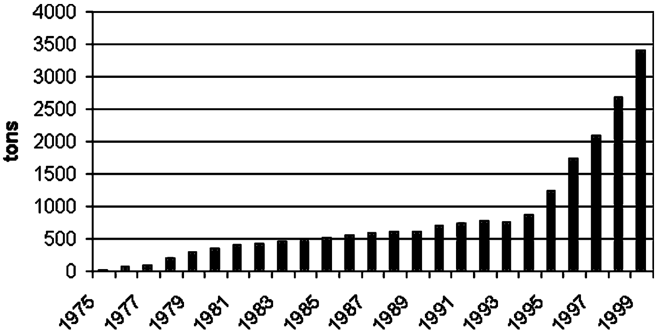


Fig. 2 World production of *Spirulina* biomass between 1975 and 1999 (1,000 tons = 1,016 t)

production being used for the manufacture of powders, tablets, capsules, or pastilles. Countless combinations of microalgae or mixtures with other health foods can be found on the market. Of the numerous attempts to explain the health-promoting effects of microalgal biomass, a general immune-modulating effect is most likely responsible (Belay 1993; Osinga et al. 1999). Therefore, health foods are expected to be a stable market in the future. Currently, most products launched to serve the health food market are supplied as tablets and powder. However, algal extracts in various product forms are creating a new market sector for microalgal products:

- *Chlorella* health drinks (*Chlorella* growth factor)
- *Dunaliella* carotenoid-enriched oily extracts (capsules; Borowitzka 1995; Masjuk 1973)
- *Spirulina* liquid CO_2 extracts (antioxidant capsules)

Compared with algal powders, functional food or nutraceuticals produced with microalgal biomass are sensorily much more convenient and variable, thus combining health benefits with attractiveness to consumers. The market of functional foods is believed to be the most dynamic sector in the food industry and could constitute up to 20% of the whole food market within the next few years. Food supplemented with microalgal biomass might have other positive influences, e.g., prebiotic effects or mineral fortification.

We investigated prebiotic effects with *Spirulina* biomass, both pure and in a functional food application. The results show positive effects on intestinal bacteria, which are regarded as beneficial, by still unknown components of *Spirulina* (Fig. 3).

Spirulina biomass, as extract or processed in pasta, biscuits and other functional food products, supports the function of the digestive tract, e.g., helps maintain healthy intestinal bacteria. All *Spirulina* samples tested stimulated the growth of various lactobacilli species. The addition of *Spirulina* biomass and a derived aqueous extract led to at least a 10-fold increase in the growth rate of the lactobacilli compared with the control. The effect on *Lactobacillus acidophilus* is especially evident.

In Germany, food production and distribution companies have started serious activities to market functional foods with microalgae and cyanobacteria. Examples are pasta, bread, yogurt and soft drinks. Similar developments can be observed, for example, in France, Japan, USA, China and Thailand.

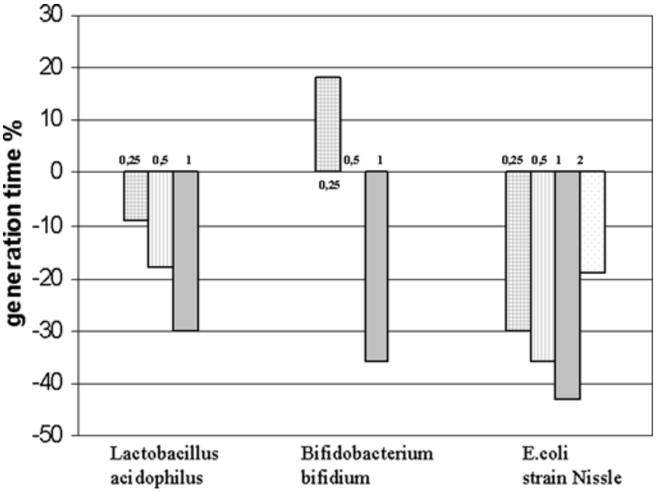


Fig. 3 Influence of *Spirulina* extract (0.25–2.0%) on growth of microorganisms

Animal feed

The survival, growth, development, productivity and fertility of animals are a reflection of their health. Feed quality is the most important exogenous factor influencing animal health, especially in connection with intensive breeding conditions and the recent trend to avoid ”chemicals,” like antibiotics.

After decades of trials where animals were fed with high amounts of microalgae—up to 50% of the common feed—in order to exploit their protein content (Richmond 2004), smaller doses were investigated, especially in eastern Europe (Musafarov and Taubayev 1974). Today, there is evidence that very small amounts of microalgal biomass, almost exclusively of the genera *Chlorella*, *Scenedesmus* and *Spirulina*, can positively affect the physiology of animals. In particular, a non-specific immune response and a boosting of the immune system of the animals was observed (Belay 1993; Table 5).

Such economic effects led to a significant increase in the use of microalgal biomass as feed additives, especially in poultry production. Another very promising application for microalgal biomass or even extracts is the pet food market, where not only the health-promoting effects but also effects on the external appearance of the pet (shiny hair, beautiful feathers) are of consumer importance. Studies on minks and rabbits provide evidence of such effects for pets (Kretschmer et al. 1995).

Aquaculture

The global market for aquaculture products like fish and shellfish ranges over U.S. \$ 40–50×10^9/year (New 1999) with a strongly growing trend (8% p.a.), especially in Asia–Pacific regions. Worldwide, at least two trends emerge with respect to microalgal applications: (1) the increasingly sophisticated production of microalgal species to meet the feeding requirements of invertebrate larvae and vertebrate young and (2) the introduction of commonly produced microalgae into fish feed to achieve positive effects similar to those in animal feeding.

Usually, the production of live microalgal biomass as a starting feed for, e.g., larvae is performed locally. Very different, mostly technically inadequate equipment is used at a high cost level (Muller-Feuga et al. 2003).

Microalgal production is costly, often being the major cost item in aquacultural production. Cost estimates for microalgal production in the aquaculture sector normally range between U.S. \$ 50 and U.S. \$ 150, with peak values of U.S. \$ 1,000/kg dry mass (Spektorova et al. 1997).

As the basis of the natural food chain, microalgae play a key role in aquaculture, especially mariculture, being the food source for larvae of many species of mollusks, crustaceans and fish. In addition, microalgae serve as a food source for zooplankton production (rotifers, copepods), which in turn are used as feed for rearing fish larvae (Lavens and Sorgeloos 1996).

More than 40 species of microalgae are used in aquaculture worldwide, depending on the special requirements of local seafood production.

Some of the most important genera are listed in Table 6.

Apart from feeding larvae and zooplankton, often with special microalgal species, the addition of *Spirulina* and *Chlorella* to common fish feed compositions seems to be a promising market. Initially, the color-enhancing effects of phycocyanin-containing *Spirulina* biomass or carotenoids from *Dunaliella* were exploited in ornamental fish.

In recent years, questions of feed utilization and health status in the dense aquacultural fish populations became

Table 5 Results of *Chlorella* feeding trials with sows and piglets during farrowing at the Regional Research Center (LVA; Iden, Germany; Weber and Grimmer 2001)

Parameter	Trial 1		Trial 2		Trial 3		Total	
	Control	Alga	Control	Alga	Control	Alga	Control	Alga
Sow daily weight gain								
Lactating time (g/day)	290	305	319	318	303	300	304	308
Weight after lactating (kg)	7.5	7.9	8.5	8.5	7.2	7.18	7.8	7.8
End weight (kg)	23.8	24.9	26.9[a]	29.8[b]	24.5	25.7	25.1[a]	26.8[b]
Husbandry (days)	42	42	46.2	45.8	47	46.1	45	44.6
Piglet daily weight gain								
Growth (g/day)	388	404	396[a]	466[b]	369	403	386[a]	424[b]
Feed conversion (kg/kg)	1.67	1.66	1.74	1.66	1.73	1.57	1.71	1.63
Dead animals	0	0	1	0	3	0	4	0

[a,b] Level of significance $P>0.05$

Table 6 Important genera of microalgae used in aquaculture

Taxon	Genera
Bacillariophyta	*Skeletonema*, *Chaetoceros*, *Phaeodactylum*, *Nitzschia*, *Thalassiosira*
Prymnesiophyta	*Isochrysis*, *Pavlova*
Prasinophyceae	*Tetraselmis*
Chlorophyceae	*Chlorella*, *Scenedesmus*, *Dunaliella*
Cyanobacteria	*Spirulina*

more important. Here, the addition of microalgae can, depending on concentration, directly enhance the immune system of fish, as our investigations on carp have shown (Schreckenbach et al. 2001). For some of the results, see Fig. 4.

Furthermore, the addition of microalga-derived astaxanthin to feed formulations enhances the color of the muscles of salmonids. This has a high biotechnological potential and culture techniques for *Haematococcus pluvialis* are well developed for this purpose (Piccardi et al. 1999); and two different types of industrial-scale closed PBR for producing astaxanthin-rich *Haematococcus* are in operation in Japan and Israel. In Israel, a glass tube PBR is used and in Japan, a special spherical thin layer PBR. On the Hawaiian Islands and in China, *Haematococcus* is cultivated in open ponds.

Biofertilizer

Historically, macroalgae are used as soil fertilizer in coastal regions all over the world. The rational background for this interesting utilization of macroalgae or their extracted residues is the increase in water-binding capacity and mineral composition of the soil (Critchley and Ohno 1998). These properties are exploited today, using liquid fertilizers produced from macroalgae during the initial coverage of, e.g., abandoned mining lands in order to avoid erosion and to initiate floral succession. This market segment amounts to approximately U.S. $ 5×10^9/year.

The important role of microalgae in the soil ecosystem has often been neglected. The beneficial effects originate not only from the production of polymers for particle adherence and water storage in soils or nitrogen-fixing, but also from alga-derived bioactive compounds which influence higher plants (Borowitzka 1995; Metting 1996; Ördög et al. 1996). Soil microalgae should be regarded by microalgal biotechnologists as a promising area to find new species with unexpected properties. While nitrogen-fixation with microalgae (*Anabaena*, *Nostoc*) is important for rice production in tropical and subtropical agriculture, surface solidification against erosion processes is also of interest in more arid regions. During the past decade, plant growth regulators from both macro- and microalgae gained increasing attention. Substances or extracts were found which promote germination, leaf or stem growth, or flowering.

A future trend seems to be the use of the biological activity of microalgal products against plant diseases caused by viruses or bacteria. It is likely that microalgae can be a source of a new class of biological plant-protecting substances.

Valuable substances from microalgae

Today, microalgal biomass and extracts from biomass have gained a firm position on the market. There is an increasing demand for sophisticated products from microalgae, which are often closely related to the taxonomic position and physiology of the microalgae. Especially, the phylogenetically archaic cyanobacteria produce numerous substances which exhibit antioxidative effects, polyunsaturated fatty acids (PUFA), heat-induced proteins, or immunologically effective, virostatic compounds. Some of these substances are even excreted by the algae (Cohen 1999).

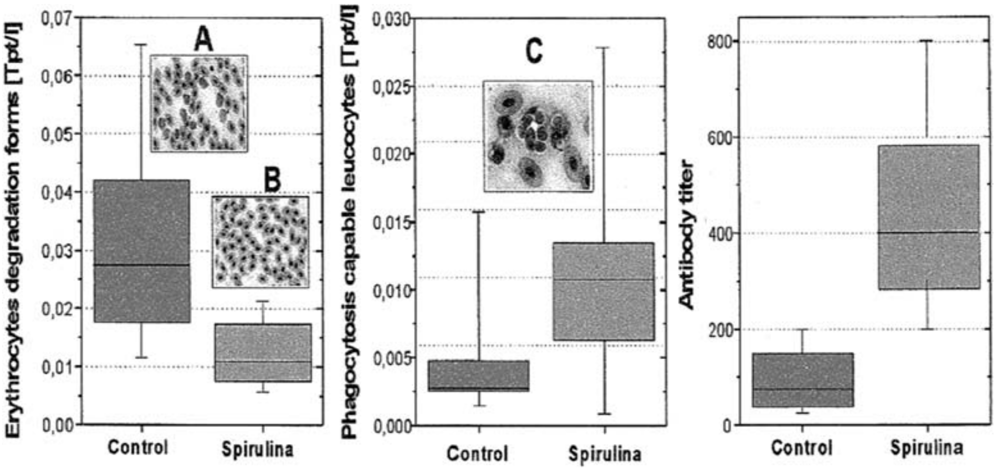


Fig. 4 Influence of adding microalgae to feed for carp. *A–C* Views of cells mentioned in *y*-axis (Schreckenbach et al. 2001)

Polyunsaturated fatty acids

Only plants are able to synthesize PUFA (Fig. 5). Therefore, microalgae supply whole food chains with these vital components. Besides being a primary source of PUFA, these fatty acids from microalgae have further advantages over fish oils, such as the lack of unpleasant odor, reduced risk of chemical contamination and better purification potential.

Therefore, microalgal PUFA have a very promising biotechnological market both for food and feed, e.g., health-promoting purified PUFA are added to infant milk formulas in Europe and hens are fed with special microalgae (like heterotrophically grown *Schizochytrium* resp. *Cryptecodinium*) to produce ”OMEGA” eggs. Both applications have proved to be profitable.

The importance of microalgae as a supplier of γ-linolenic acid was slightly weakened by the use of evening-primrose oil. However, the preparation of EPA and DHA from marine organisms with phototrophic capability, like the dinoflagellate *Crypthecodinium*, for baby food or the health food market is an innovative approach (Apt and Behrens 1999; Radmer 1996). The application of this product line to food products was permitted on the basis of another organism (*Ulkenia* sp.) by EC regulatory boards in 2003. Products from *Odontella aurita* biomass (France) will probably soon be on the market.

First the Martek company (USA) and then Nutrinova (a German company) announced the production of DHA products from microalgal biotechnology for human and other applications (Fig. 6).

For lipid-based cosmetics, like cremes or lotions, ethanolic or supercritical CO_2-extracts are gaining commercial importance because of their provision of both nourishing and protecting effects to the skin. For future developments in skin care, other lipid classes from microalgae, like glyco- and phospholipids, should not be neglected (Muller-Feuga et al. 2003).

Polysaccharides

Macroalgal polysaccharides, like agar, alginates and carrageenans, are economically the most important products from algae. They are used in diverse fields of industry because of their rheological gelling or thickening properties. During recent years, raw material shortages and pollution problems led to an increase in research and development activities to use microalgae, transgenic microalgae, protoplast fusion, or macroalgal cell cultures as a biotechnological source (Cohen 1999; Grobbelaar et al. 1996). Algal polysaccharides are also of pharmacological importance. The results of screening programs to test in vitro immunologically relevant effects of polysaccharides from microalgae have shown that certain highly sulfated polysaccharides can trigger either the cellular or the humoral stimulation of the human immune system (Namikoshi 1996). Effective polysaccharide fractions were found mainly in cyanobacteria; but compounds from the Rhodophyta and Chlorophyta have also shown impressive efficiency. The results correlate with data from studies on animal feeding. Recent literature and the patent situation imply optimistic developments (Cohen 1999).

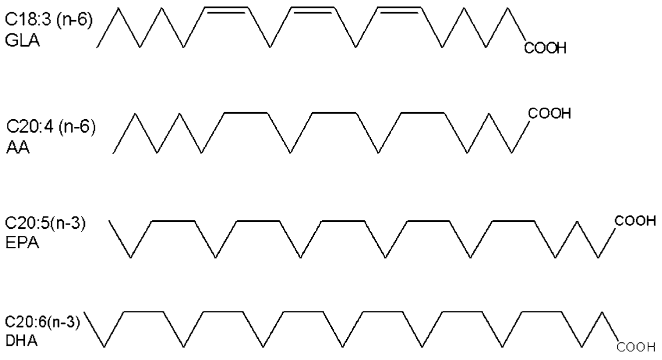
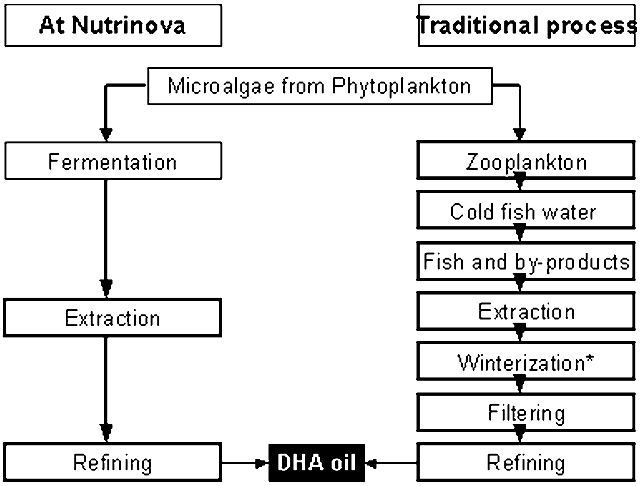


Fig. 5 PUFA of high pharmaceutical and nutritional value



*Precipitation process in the cold

Fig. 6 DHA production processes—a comparison between traditional and Nutrinova processes (Nutrinova 2003)

Antioxidants

Microalgae, as phylogenetically the oldest plants, have adapted uniquely to extreme habitats over billions of years of evolution. Due to their phototrophic life, they are exposed to high oxygen and radical stresses. This has resulted in the development of numerous efficient protective systems against oxidative and radical stressors. The protective mechanisms are able to prevent the accumulation of free radicals and reactive oxygen species and thus to counteract cell-damaging activities. In cultures of photosynthetically active microorganisms of high cell density, molecular oxygen is produced and an oxygen over-saturation is observed. In closed PBR, even at less intensive photosynthetic conditions, oxygen concentrations can be as high as 50 mg/l. Such conditions promote the endogenous detoxification process towards oxidative attack by an accumulation of highly effective antioxidative scavenger complexes, which protect cells from damage by free radicals (superoxide anion, hydroxyl radical). For example, the antioxidative potential of *Spirulina platensis* can increase 2.3-fold during oxygen stress.

Because the antioxidative components originate from a natural source, their application in cosmetics for preserving and protecting purposes is developing rapidly. In combination with other antioxidative or bioactive substances from microalgae, especially sun-protecting cosmetics, they represent an area of high demand. For functional food/nutraceuticals, the radical-scavenging capacity of microalgal products is of growing interest, especially in the beverage market segment and in pharmaceutical applications for the therapy of oxidation-associated diseases, like inflammations.

Colors and food-coloring products

In addition to chlorophyll as the primary photosynthetic pigment, microalgae contain a multitude of pigments which are associated with light incidence. The pigments improve the efficiency of light energy utilization (phycobiliproteins) of plants and protect them against solar radiation (carotenoids) and related effects. Carotenoids from microalgae have a firm position on the market:

- β-Carotene from *Dunaliella* in health food as a vitamin A precursor
- Astaxanthin from *Haematococcus* in aquaculture for coloring muscles in fish
- Lutein, zeaxantin and canthaxantin for chicken skin coloration, or for pharmaceutical purposes

The phycobiliproteins, phycocyanin and phycoerythrin, are unique to algae and some preparations are already being developed for food and cosmetics. This development will certainly go beyond applications in diagnostics and photodynamic therapy and extend to cosmetics, nutrition and pharmacy (Hirata et al. 2000).

Toxins and other substances with biological activity

The most impressive demonstration of the ability of microalgae and cyanobacteria to produce highly effective bioactive compounds are toxins, which in algal blooms become dangerous for animals and humans, especially if such blooms occur in drinking-water reservoirs. There are several freshwater algae which can form toxic blooms, especially the cyanobacteria *Microcystis*, *Anabaena* and *Aphanizomenon*. Marine algal blooms become dangerous via the human consumption of shellfish. Three degrees of poisoning are distinguished here:

- Paralytic shellfish poisoning: caused by the water-soluble neurotoxic substances saxitoxin, neosaxitoxin and gonyautoxin in different derivatives—produced by the dinoflagellate *Alexandrium lusitanicum*—interrupting potential conduction in the neurons
- Diarrhetic shellfish poisoning: induced by the polar polyoxo-substances okadaic acid and dynophysotoxin—produced mainly by *Dinophysis* spp—resulting in strong diarrhetic symptoms
- Amnesic shellfish poisoning: caused by the amino acid domoic acid—produced mainly by the diatom *Nitzschia pungens*—which creates amnesic effects by acting as a glutamic acid antagonist

The therapeutical value of all toxins has not yet been investigated (Luckas 1995). Several, partly extensive, screening programs were performed in the United States, Australia, Germany and France to detect new biologically active substances from microalgae and cyanobacteria, with the following effects:

- Cytotoxic activity is important in anticancer drugs (Sirenko et al. 1999)
- Antiviral activities are found mainly in cyanobacteria but also in apochlorotic diatoms and the conjugaphyte *Spirogyra*, where certain sulfolipids are active, e.g., against the herpes simplex virus (Muller-Feuga et al. 2003)
- Antimicrobial activity is under investigation to find new antibiotics. Although the success rate is about 1% (Muller-Feuga et al. 2003), there seem to be some promising substances from microalgae, e.g., the cyanobacterium *Scytonema*
- Antifungal activity is found in different extracts of cyanobacteria
- Antihelmintic effects are known for *Spirogyra* and *Oedogonium*

Stable isotopes in microalgae

Because phototrophic microalgae can be cultivated under strictly controlled conditions, they are the ideal choice to incorporate stable isotopes from inorganic C-sources, H-sources and N-sources. The various stable isotope-labeled biochemicals can be used not only for scientific purposes (molecular structure, or physiological investigations) but also for clinical purposes, like gastrointestinal or breath diagnosis tests (Radmer 1996).

Environmental applications

Governments and energy companies worldwide have a vested interest in CO_2 fixation via biotechnology. In, for example, Norway, Japan, Italy and the United States, research efforts to find economical feasible processes for microalgal applications in environmental protection and CO_2 fixation are in progress.

In Germany, a project was performed to use both effluent gas and condensed water from this gas to produce microalgal biomass (Fig. 7). The process was scaled-up to

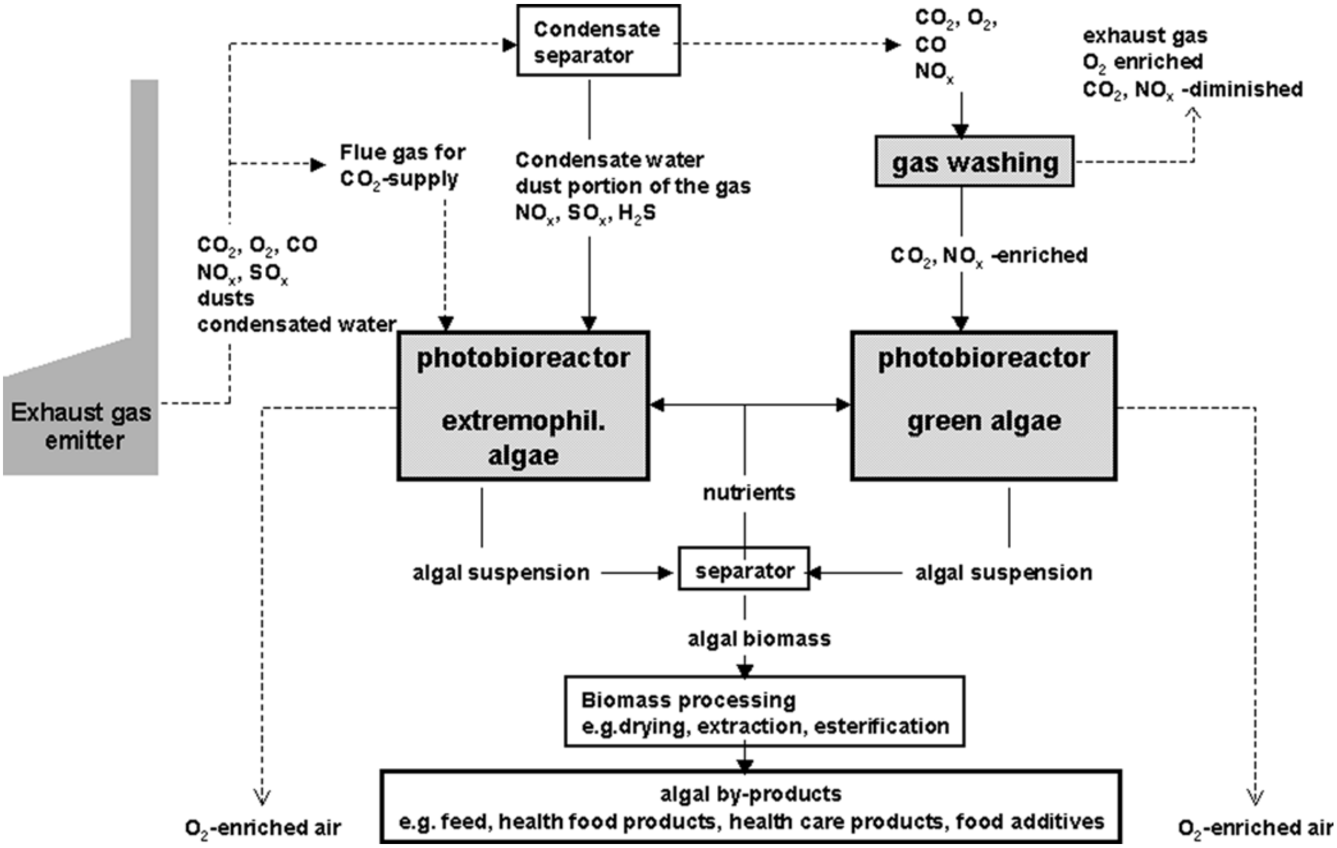


Fig. 7 Scheme for CO_2 fixation from industrial exhaust gas by microalgae

a 6,000-l PBR and was shown to be feasible, but not yet economic.

In addition, we performed pilot-scale experiments to establish closed material cycles combining different effluent fluxes with value-added production. Figure 6 illustrates an example of a material flow including a microalgal production unit, which was actually found to be economically feasible.

Conclusion

Microalgal biotechnology—today still in its infancy—can be seen as a gateway to a multibillion dollar industry. It has just started to tap the enormous biological resource and physiological potential of microalgal species growing in all ecological niches. In recent years, innovative processes and products have been introduced in both macro- and microalgal biotechnology (Table 7).

One can expect that future trends in microalgal biotechnology will lead to a diversity of technical solutions for PBR for cultivating microalgae. These will

Table 7 Algae as the healthy ingredient—recent developments 2003

Country	Company	Alga	Product	Activity
USA	Martek/Omegatec	*Crypthecodinium*	DHA	Brain development
USA	Cyanotec	*Haematococcus*	ASTA	Treating carpal tunnel syndrome
USA	MERA	*Haematococcus*	ASTA	Anti-inflammatory, treats muscle soreness
Canada	OceanNutrition	*Chlorella*	Carbohydrate extract	Immune system, anti-flu
France	InnovalG	*Odontella*	EPA	Anti-inflammatory
Austria	Panmol/Madaus	*Spirulina*	Vitamin B_{12}	Helps immune system
Germany	Nutrinova/Celanese	*Ulkenia*	DHA	Treats brain, heart, mental disorder
USA	Gates Foundation	*Kappaphycus*	Carrageenan	Anti-HIV, microbicide
USA	R&D	*Lobophora*	Macrolides	Anti-fungal
UK	BSV	*Rhodophyta* (mix)	Biomass	Treats irritable bowel candidiasis
Denmark	Danisco	*Macroalga*	Hexose oxidase	Antioxidant

be adapted to the autecological demands of strains and to application aims for biomass, valuable substances and ecology. An exhaustive inventory of species in all regions accompanied by proper taxonomic handling and strain collection could be a basis for future success.

There are some indications to suppose that aquacultural and aquatic applications will be a profitable field for microalgae in the next 10 years. Both ecological applications in the sense of wastewater treatment and the agricultural use of microalgae for nitrogen fixation or as soil conditioners have promising economic potential.

While the use of microalgae in functional foods and animal feed could soon reach the level of mass products, their use in pharmaceutical applications appears to lie more in the future. Nevertheless, some promising candidates already exist.

References

Anagnostidis K, Komárek JA (1985) Modern approach to the classification system of cyanophytes 1—introduction. Arch Hydrobiol Suppl 71:291–302

Anagnostidis K, Komárek JA (1988) Modern approach to the classification systems of cyanophytes 3—oscillatorales. Arch Hydrobiol Suppl 80:327–472

Anagnostidis K, Komárek JA (1990) Modern approach to the classification systems of cyanophytes 5—stigonematales. Algol Stud 59:1–73

Anon (2000) Guide de l'algue alimentaire. CEVA, Paris, p 33

Apt KA, Behrens PW (1999) Commercial developments in microalgal biotechnology. J Phycol 35:215–226

Belay A (1993) Current knowledge on potential health benefits of *Spirulina platensis*. J Appl Phycol 5:235–240

Blanchot J, Rodier M (1996) Picophytoplankton abundance and biomass in the western tropical Pacific Ocean during the 1992 El Niño year: results from flow cytometry. Deep Sea Res 43:877–895

Borowitzka MA (1995) Microalgae as source of pharmaceuticals and other biologically active compounds. J Appl Algol 7:3–15

Borowitzka MA (1997) Microalgae for aquaculture: opportunities and constraints. J Appl Phycol 9:393–401

Borowitzka MA (1998) Company news. J Appl Phycol 10:417

Borowitzka MA, Borowitzka LJ (1988) Micro-algal biotechnology. Cambridge University Press, Cambridge

Boussiba S, Wu X-Q, Ben-Dov E, Zarka A, Zaritsky A (2000) Nitrogen-fixing cyanobacteria as gene delivery system for expressing mosquitocidal toxins of *Bacillus thuringiensis* ssp. *israelensis*. J Appl Phycol 12:461–467

Chungjatupornchai W (1990) Expression of the mosquitocidal-protein genes of *Bacillus thurigiensis* ssp. *israelensis* and the herbicide-resistance gene Bar in *Synechocystis* PCC6803. Curr Microbiol 21:283–288

Cohen Z (1999) Chemicals from microalgae. Taylor & Francis, London

Critchley T, Ohno M (1998) Seaweed resources of the world. JICA, Yokosuka

De Luca P, Masacchio A, Taddei R (1981) Acidophilic algae from the fumaroles of Mount Lawu (Java) locus classius of *Cyanidium caldarium Geitler*. G Bot Ital 115:1–9

De Pauw N, Persoone G (1988) Micro-algae for aquaculture, micro-algal biotechnology. In: Borowitzka MA, Borowitzka LJ (eds) Cambridge University Press, Cambridge, pp 197–221

Feller G, Narinx E, Arpigny JL, Aittaleb M, Baise E, Genicot S, Gerday (1996) Enzymes from psychrophilic organisms, C. FEMS Microbiol Rev 18:189–202

Gimmler H, Degenhardt B (2001) In: Rai LC, Gaur JP (eds) Alkaliphilic and alkali-tolerant algae, algal adaptation to environmental stresses. Springer, Berlin Heidelberg New York

Gounot AM (1986) Psychrophilic and psychrotrophic microorganisms. Experientia 42:1192–1197

Grobbelaar JU, Kroon BMA, Whitton BA (1996) Opportunities from micro- and macroalgae. J Appl Phycol 8:261–464

Gross W, Schnarrenberger C (1995) Heterotrophic growth of two strains of the acidothermophilic red alga *Galderia sulphuria*. Plant Cell Physiol 4:633–638

Hirata T, Tanaka M, Ooike M, Tsunomura T, Sakaguchi M (2000) Antioxidant activities of phycocyanobilin prepared from *S. platensis*. J Appl Phycol 12:435–439

Kindle KL, Richards KL, Stern DB (1990) Engineering the chloroplast genome: techniques and capabilities for chloroplast transformation in *Clamydomonas reinhardtii*. Proc Natl Acad Sci USA 88:1721–1725

Komárek JA, Anagnostidis K (1986) Modern approach to the classification systems of cyanophytes 2-Chroococales. Arch Hydrobiol Suppl 73:157–226

Komárek JA, Anagnostidis K (1989) Modern approach to the classification systems of cyanophytes 4—Nostocales. Arch Hydrobiol Suppl 82:247–345

Kretschmer P, Pulz O, Gudin C, Semenenko V (1995). Biotechnology of Microalgae. (Proceedings of the second European workshop) IGV Institute for Cereal Processing, Potsdam-Rehbrücke

Laing I, Ayala F (1990) In: Akatsuka I (ed) Commercial mass culture techniques for producing microalgae, introduction to applied phycology. SPB, The Hague, pp 447–477

Lavens P, Sorgeloos P (1996) Manual on the production and use of life food for aquaculture. FAO Fish Tech Pap 361:7–42

Lorenz RT, Cysewski GR (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotechnol 18:160–167

Luckas B (1995) Selective detection of algal toxins from shellfishes. Chem Unserer Zeit 29:68–75

Masjuk NP (1973) Morphology, taxonomy, ecology, geographical distribution and utilization of *Dunaliella* (in Russian). Naukowa, Kiev

Metting FB (1996) Biodiversity and application of microalgae. J Ind Microbiol 17:477–489

Muller-Feuga A, Moal J, Kaas R (2003) The microalgae for aquaculture. In: Stottrup JG, McEvoy LA (eds) Life feeds in marine aquaculture. Blackwell, Oxford

Musafarov AM, Taubayev TT (1974). Chlorella (in Russian). FAN, Tashkent

Namikoshi M (1996) Bioactive compounds produced by cyanobacteria. J Int Microbiol Biotechnol 17:373–384

New MB (1999) Global aquaculture: current trends and challenges for 21st century. World Aquacult 3:8–14

Norton TA, Melkonian M, Andersen RA (1996) Algal biodiversity. Phycologia 35:308–326

Nutrinova (2003) http://www.nutrinova.com/easy2web/internet.nsf/directframe/home?OpenDocument

Ördög V, Szigeti J, Pulz O (1996). Proceedings of the conference on progress in plant sciences from plant breeding to growth regulation. Pannon University, Mosonmagyarovar

Osinga R, Tramper J, Burgess JG, Wijffels RH (1999) Marine bioprocess engineering. Proc Prog Ind Microbiol 35:1–413

Piccardi R, Materassi R, Tredici M (1999) Algae and human affairs in the 21st century. (Abstr Int Conf Appl Algol) Universita degli Studi di Firenze, Firenze

Pulz O (2001) Photobioreactors: production systems of phototrophic microorganisms. Appl Microbiol Biotechnol 57:287–293

Pulz O, Scheibenbogen K, Gross W (2000) Biotechnology with cyanobacteria and microalgae. In: Rehm H-J, Reed G (eds) Biotechnology, vol 10, 2nd edn. Wiley-VCH, Weinheim, pp 105–136

Radmer RJ (1996) Algal diversity and commercial algal products. Bioscience 46:263–270

Ragan MA, Chapman DJA (1978) A biochemical phylogeny of the protists. Academic, New York

Richmond A (2004) Handbook of microalgal culture. Blackwell, Oxford

Schreckenbach K, Thürmer C, Loest K, Träger G, Hahlweg R (2001) Der Einfluss von Mikroalgen (*Spirulina platensis*) in Trockenmischfutter auf Karpfen (*Cyprinus carpio*). Fischer Teichwirt 1:10–13

Siegelman HW, Guillard RRL (1971) Large-scale culture of algae. In: Colowick SP, Kaplan NO (eds) Methods Enzymol, vol 23. Academic, New York, pp 110–115

Sirenko LA, Kirpenko YA, Kirpenko NI (1999) Influence of metabolites of certain algae on human and animal cell cultures, Int J Algae 1:122–126

Sivonen K, Jones G (1999) Cyanobacterial toxins. In: Chorus I, Bertram J (eds) Toxic cyanobacteria in water: a guide to public health significance, monitoring and management. Spon, London, pp 41–111

Skulberg OM (2000) Microalgae as a source of bioactive molecules —experience from cyanophyte research. J Appl Phycol 12:341–348

Spektorova L, Creswell RL, Vaughan D (1997) Closed tubular cultivators. World Aquacult 6:39–43

Weber M, Grimmer A (2001) Lohnt sich der Einsatz von Grünalgen im Ferkelfutter? Landwirtsch Wochenbl Rheinland 2001:34

Xie J, Zhang Y, Li Y, Wang Y (2001) Mixotrophic cultivation of *Platymonas subcordiformis*. J Appl Phycol 13:343–347

Zaslavskaia LA, Lippmeier JC, Shih C, Ehrhardt D, Grossman AR, Apt K (2001) Trophic conversion of an obligate photoautotrophic organism through metabolic engineering. Science 292:2073–2075