The Biotechnology of Cyanobacteria

26

Claudia B. Grewe and Otto Pulz

Contents

Summary

This chapter gives an overview of the range of cyanobacterial materials being harvested from nature and grown in culture, increasingly on a large scale. *Arthrospira*, which is usually marketed as Spirulina, is the most important, but studies are also underway on developing methods to grow *Nostoc* commercially; at present colonies of several species are harvested for local use in a number of countries in Asia, Africa and South America. Although *Aphanizomenon flos-aquae* has been harvested and sold, the costs of the quality control needed to avoid long-term risks of material including toxins makes its large-scale cultivation in photobioreactors preferable. The various approaches to mass culture are considered and the ways in which cyanobacteria are now being used are described. These include food, phycobiliproteins for pigment and antioxidant, animal feed, cosmetics, biofertilizers and treatment of wastewater and exhaust gas. Promising products for the near future include some of the huge range of bioactive molecules produced by cyanobacteria and most important of all, biofuel.

26.1 Introduction

Cyanobacteria are among the oldest and most successful life forms on earth (Chap. 2; Sharma et al. 2010) and their importance in the production of oxygen and fixation of CO_2 has often been stressed (DeRuyter and Fromme 2008). At the same time they are one of the most important primary producers and part of the beginning of the food chain in almost all aquatic habitats; cyanobacterial growth early in the earth's history made a substantial contribution to present-day supplies of crude oil (Chap. 16). However, despite their long evolutionary history, the involvement of cyanobacteria represents one of the newest trends in biotechnology, since much of the focus during the past century has been on bacteria, yeasts and fungi.

The high demand for food, feed and pharmaceuticals has led the development of heterotrophic production processes to

C.B. Grewe (✉) • O. Pulz
Research and Development, Salata GmbH, An der Salzbruecke, 98617 Ritschenhausen, Germany
e-mail: CGrewe@Salata-Salzbruecke.de

B.A. Whitton (ed.), *Ecology of Cyanobacteria II: Their Diversity in Space and Time*,
DOI 10.1007/978-94-007-3855-3_26, © Springer Science+Business Media B.V. 2012

a high level. Extensive screening programmes have been carried out with heterotrophs in the search for bioactive metabolites, so that the re-discovery rate of compounds is already above 90% (Olaizola 2003). At present, energy-consuming processes using heterotrophic organisms are exploited to a much higher degree than autotrophic production processes. Interest is therefore shifting towards other organisms, which are able to produce valuable products in a more sustainable way. Cyanobacteria present a rich resource of biotechnologically important organisms; they can be used both to produce specific molecules and for industrial processes. The biodiversity of cyanobacteria is enormous and represents an almost untapped resource.

The biotechnology of cyanobacteria has gained considerable importance in the last decades, with applications ranging from simple biomass production for food and feed to valuable products. The market size for most of these products continues to increase and the biotechnological use of cyanobacteria will extend into new areas. Considering the vast biodiversity of cyanobacteria and developments in genetic engineering, they represent one of the most promising sources for new products and applications.

The chapter reports on the cyanobacteria which have become relevant in terms of economic applications, their markets as well as the biotechnology behind their production. Only brief mention is made of studies prior to 2000.

26.2 Economically Relevant Cyanobacteria

26.2.1 Overview

Three cyanobacterial genera represent most of the commercially relevant products at present: *Arthrospira*, *Nostoc* and *Aphanizomenon*. These are being produced and/or collected for different purposes, mostly as health food and dietary supplement. All these applications are related to their valuable components and their gross biochemical composition is summarized in Table 26.1. For *Arthrospira* and *Aphanizomenon* a protein content of over 50% dry weight, with a high proportion of essential amino acids is characteristic, though values are lower for *Nostoc*, because of the large amount of extracellular polysaccharide. The lipid content of cyanobacteria is much lower, typically 5–8% (Griffiths and Harrison 2009).

In the case of *Arthrospira*, the average values are above 60% for proteins and 6–8% for lipids, with free fatty acids forming about 50% of these lipids (Gershwin and Belay 2007). β-carotene constitutes 0.14–0.23% of its dry weight, a content 20 times that of carrots, equaling 375,000 IU of vitamin A. Its vitamin B_{12} content is higher than in beef liver, on average 2.5 μg g^{-1} dry weight. The calcium content exceeds the proportion in milk by factor 2.5 (3 mg g^{-1}).

Table 26.1 Gross biochemical composition of commercial relevant cyanobacteria (Schreckenbach et al. 2001; Danxiang et al. 2004; Capelli and Cysewski 2010)

	Arthrospira spp. (%)	*Nostoc* spp. (%)	*Aphanizomenon flos-aquae* (%)
Protein	58–73	10–23	60–75
Lipids (fat)	6–8	5–6	2–8
Carbohydrate	15–25	56–57	20–30

Iron, which is the most common mineral deficiency worldwide, is present in contents up to 2.17 mg g^{-1} exceeding the iron content in spinach by more than 6 times based on dry weight (Capelli and Cysewski 2010). However, any realistic comparison has to acknowledge that people do not consume cyanobacterial biomass in the range of 100 g day^{-1}. The ingredients more relevant to health are of much more interest, such as polysaccharides and antioxidants (e.g. carotenoids). *Aphanizomenon flos-aquae* has a broadly similar composition to *Arthrospira*, being rich in proteins, carotenoids, phycobiliproteins, vitamins and minerals, while *Nostoc flagelliforme* has a higher carbohydrate content but lower protein content (Danxiang et al. 2004), although fewer data are available for the latter. The concentrated nutritional profile makes cyanobacteria in general and *Arthrospira* in particular a valuable nutrient source; *Arthrospira* seems particularly suited to counteract malnutrition (Henrikson 1989).

The exact chemical composition of the biomass depends on environmental conditions, including the source of nutrients and the mode of nutrition (autotrophic, mixotrophic or heterotrophic). Nevertheless considerable consistency has been found in the biochemical composition of *Arthrospira* during the production season, which is remarkable in view of the fact that production is in open systems (Belay 2007). However, production procedures differ between the *Arthrospira* producers and, in addition to cultivation, drying methods and conditions. Packaging and storage can all have an impact on the gross chemical composition of the product, so that the final products of *Arthrospira* biomass can differ markedly in their characteristics (Grobbelaar 2003). Investigations on the chemical composition of *Chlorella* products have shown similar quality differences depending on the production procedures (Görs et al. 2010).

Their interesting product characteristics have stimulated large-scale production of cyanobacterial biomass in the past decade (Fig. 26.1). The amount of *Arthrospira* produced has consistently increased and probably reached 5,000 t dry weight in 2010. Although China joined the producing countries later than many others, it soon became the largest producer worldwide. Over 3,500 t biomass is being produced annually in China by many different companies, with 20% of this on an area of over 500,000 m^2 in Inner Mongolia (Lu et al. 2010). The annual production of the biomass of the

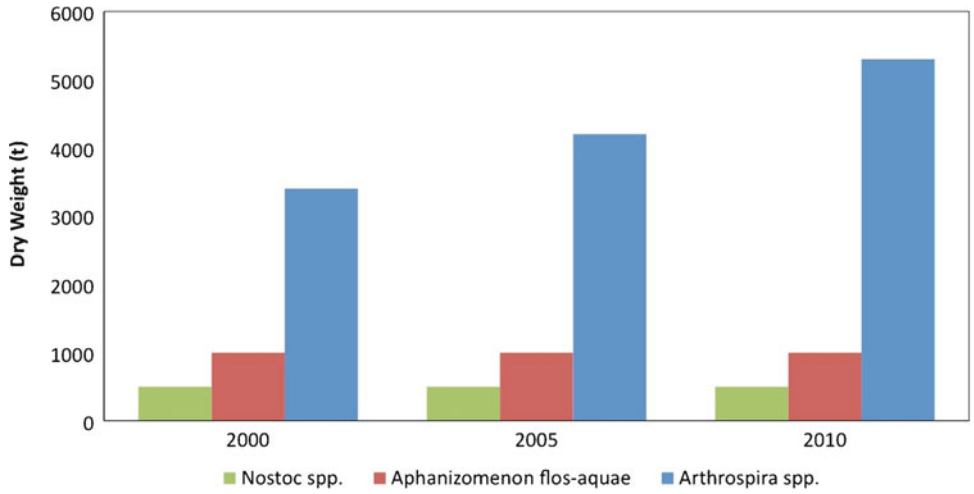

Fig. 26.1 Worldwide production of commercially most relevant cyanobacteria (Available data 2011, Carmichael et al. 2000; Lu et al. 2010)

three cyanobacteria accounts for at least 6,800 t dry weight per year (Fig. 26.1) and thereby 68% of the total worldwide microalgal biomass production of 10,000 t (Rosello Sastre and Posten 2010). The production costs in China of US$ 3–4 per kg (Lu et al. 2010) are lower than those at other production facilities in tropical or subtropical climates. In addition to the large commercial producers, there are efforts to support *Arthrospira* production in Chad: in 2007 the European Union was funding a US$ 1.4 million project run by the UN Food and Agriculture Organization (FAO) in order to support *dihé* production.

Aphanizomenon flos-aquae was harvested in the range of 1,000 t dry weight per year and sold with a market volume of US $100 million (Carmichael et al. 2000). Dried colonies of *Nostoc verrucosum* are consumed in the range of 100 t per year in Asia, especially Myanmar, and are being sold for less than US$ 1 per kg in the local markets in Myanmar (Min Thein, personal communication 2011). In China, prices for *N. flagelliforme* can be as high as US$ 125 per kg (Roney et al. 2009), but other species sell locally for far less in some regions of the country.

For most of the products the whole biomass is used. Increasingly, however, particular ingredients like phycobiliproteins and polysaccharides are being extracted and further purified. These are the subject of some of the following sections of this chapter.

26.2.2 *Arthrospira* (Spirulina)

Arthrospira ("Spirulina") is both the most popular microalga as well as the most extensively studied. *A. platensis* has gained worldwide popularity as a food supplement (Gershwin and Belay 2007), being one of the most protein-rich foods known. As with nearly all microalgae cultivated at present, *A. platensis* is an extremophile, which has many advantages for mass culture (Chap. 25). The alkalophilic organism has the largest stake of cyanobacteria biomass produced worldwide and its unique position will be maintained for the next decades. Although it is not the only cyanobacterial biomass on the market today, it represents the only cyanobacterium that is being extensively cultivated in artificial systems for different applications during the past decades.

Arthrospira shows a cylindrical, loosely or tightly coiled trichome in a regular helix (John et al. 2002). *Arthrospira* is, as many cyanobacteria, a cosmopolitan, being found in many different habitats. Nevertheless alkaline as well as salt containing habitats are preferred. Blooms are observed in bicarbonate-rich environments as well as in high salt concentrations or brackish waters. Trichome breakage depends on necridium formation (Tomaselli 1997; Hu 2004). Its gram-negative, soft cell wall is composed out of four layers, with a major layer of peptidoglycan (Chap. 25).

Arthrospira is regarded as a rich source of vitamins, essential amino acids, minerals, essential fatty acids like y-linolenic acid (GLA, a ω6-polyunsaturated fatty acid) and antioxidant pigments like phycobiliproteins and carotenoids. *A. platensis* and *A. maxima* are apparently the only producers of GLA so far reported for cyanobacteria. This fatty acid is a precursor of arachidonic acid, which is required for the synthesis of important metabolic mediators. The proportion of linoleic, γ-linolenic acid and palmitic acid seem to be species specific within *Arthrospira* (Mühling et al. 2005b) and can aid strain identification. The composition of fatty acids is affected by cultivation conditions: lower temperature, higher light intensity and a change to heterotrophic nutrition favour a higher proportion of PUFAs (Mühling et al. 2005b). GLA is bound to over 94% to glycolipids in the lipid fraction of *A. platensis* (Sajilata et al. 2008a). The carotenoids of *A. maxima* consist mainly of zeaxanthin (25%),

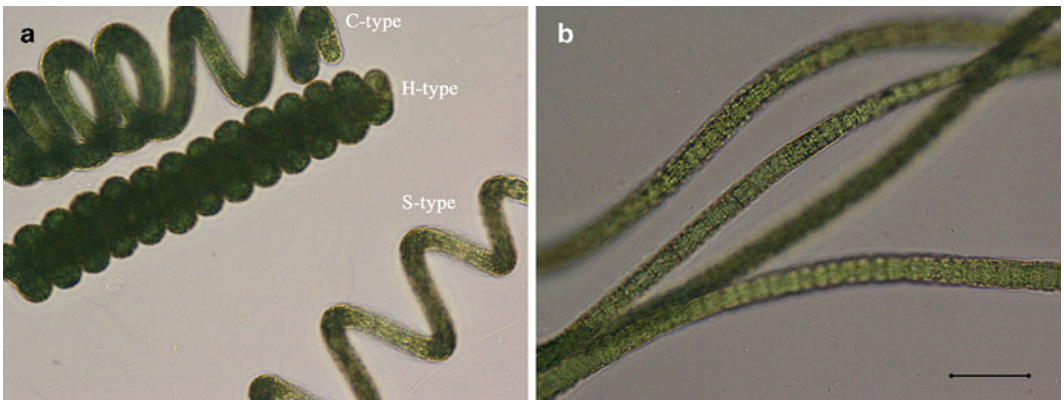

Fig. 26.2 Laboratory culture of *Arthrospira platensis*, (**a**) coiled trichome forms, S-type: loosly coiled, C-type: intermediatly coiled, H-type: tightly coiled; (**b**) straight trichome. Cultures both isolated from Myanmar, Lake of Twin Taung; scale bar = 20 µm

myxoxanthophyll (13–17%), β-carotene (15%), echinenone (11–13%), β-cryptoxanthin (7%) and 3′-hydroxyechinenone (7–11%) (Miki et al. 1986). Studies by Wilson et al. (2008) have shown that high light induces structural changes in a recently identified orange carotenoid protein. This photoactive protein senses blue-green light and triggers photoprotection in cyanobacteria.

Laboratory experiments under autotrophic conditions showed that light saturation in *A. platensis* at 150–200 µmol photon m^{-2} s^{-1}. The original or modifications of the medium of Zarrouk (1966) is widely for culture. The optimal growth temperature is strain dependant, being in the range of 35–38°C, and with a minimum temperature required growth about 15°C in the strains reported by Belay (1997). Its salt amplitude is in the range of a few millimolar up to 0.75 M NaCl. In the latter concentration growth is strain dependant and already inhibited up to 70% (Vonshak and Tomaselli 2000). *Arthrospira* spp. are considered as obligatory alkalophilic organisms, with optimum pH ranges between pH 9.5–12 (Hu 2004). The high pH represents the ecological niche that permits successful cultivation of quite homogeneous cultures of *Arthrospira* in open cultivation systems.

Growth of some strains can occur under mixotrophic and heterotrophic conditions, with a range of substrates reported for various strains: glucose (Chojnacka and Noworyta 2004; Lodi et al. 2005), fructose (Mühling et al. 2005a), acetate (Chen et al. 1996), glycerol (Narayan et al. 2005), propionate (Lodi et al. 2005) and peptone (Vonshak 1997b). Mühling et al. (2005a, b) assayed strains freshly isolated from nature on their heterotrophic growth on different carbon sources, reporting that at least one strain subcultured under autotrophic conditions for a further 2 years had lost the ability to use fructose, emphasizing the influence of strain origin. In addition, mixotrophic tests with ten of the strains from heterotrophic strains showed differences in their response: all made use of glucose and maltose, but none used fructose, even those able to do so under heterotrophic conditions. Mixotrophic tests with sucrose showed rapid lysis of many trichomes, but subsequent recovery of short lengths, followed by continued growth of healthy cultures. Marquez et al. (1993) concluded from studies on *Arthrospira platensis* that in mixotrophic cultivation autotrophic and heterotrophic growth functioned independently during mixotrophic growth.

Use of organic substrates for mixotrophic growth can lead to considerable increases in growth rate compared with autotrophic growth (Mühling et al. 2005a). Mixotrophic cultivation with glucose enhanced growth of *Arthrospira platensis* by a factor of 5.1 (Chen and Zhang 1997), while Lodi et al. reported a 33% higher volumetric cell productivity for *Arthrospira platensis* (Lodi et al. 2005). Not only is the growth rate faster, but the final biomass concentration is higher, enhancing the efficiency of harvesting. In spite of these successes, an upscaling step towards mixotrophic production in commercial systems does not at present seem feasible. This is mainly due to higher costs and microbiological problems; including the likelihood of heterotrophic bacteria and fungi outcompeting *Arthrospira* in the utilization of the organic carbon source.

In general cultivation parameters are influencing the metabolism and the morphology of the algal cells, in the case of *Arthrospira* the change of helix orientation or even the straightening of the trichomes have been observed (Vonshak and Tomaselli 2002; Mühling et al. 2003), see Chap. 25. Nevertheless, this phenomenon occurs both in nature as well as in culture (Fig. 26.2). It has not been satisfactorily explained yet, neither from the taxonomic, nor from the biochemical point of view. For a longer period it was believed that the straightening is irreversible (Tomaselli 1997), but later Wang and Zhao (2005) proved that straight trichomes of *Arthrospira platensis* can revert their morphology to the usual helical structure.

Cultivation of *Arthrospira* began in France and Mexico in the 1970s (Durand-Chastel 1980; Shelef and Soeder 1980) using *A. platensis* and *A. maxima*. Ripley D. Fox did much

Fig. 26.3 *Arthrospira* production in volcanic crater Twyn Taung in Sagaing Province, Upper Myanmar (22°21′50.79″ N 95°01′28.31″ E)

to publicize the cyanobacterium in the next two decades, including his own work on integrated systems for village production (Fox 2001). Other researchers who helped to develop mass culture methods include Richmond and Vonshak (1978) on practical methods for developing large-scale cultivation in Israel and Soeder (1992) on raceway pond technology. The commercial large scale cultivation of *Arthrospira* as food and feed ingredient was established in the late 1970s in Thailand by Dainippon Ink & Chemicals, Japan, (DIC) and in the early 1980s by Proteus Coorporation in the USA, which was later incorporated into DIC. Today numerous companies are producing *Arthrospira* worldwide in an estimated output of over 5,000 t of dry weight per year (Lu et al. 2010; Rosello Sastre and Posten 2010). *Arthrospira* is cultured in constructed outdoor ponds in Africa, USA, Thailand, China, Taiwan, Myanmar and India (Chap. 25).

One of the oldest production facilities is in Calipatria, California, USA, maintaining a total of 300,000 m² pond surface in 2011 for the production of *Arthrospira* for food and feed. Production, which started in summer 1983 at "Earthrise Farms" is predominantly carried out in open raceway ponds each with an area of 1,000 to 5,000 m² and a depth of 0.15–0.3 m. A paddle-wheel mixes the suspension continuously, not exceeding velocities of 0.3 m s^{-1} in order to avoid shear stress and damage to the trichomes. The optimal biomass concentration for production lies between 0.4 and 0.5 g L^{-1}. Due to its location in southern California evaporation in the order of magnitude of several hundred m^3 day^{-1} for the whole plant has to be replenished by water from the Colorado River (Belay 1997). Raw water with a high calcium ion concentration leads to precipitation of calcium phosphate and probably iron phosphate, and hence a reduction in P and Fe available for the organism. Pretreatment of the water, CO_2 addition and removal of detritus are measures help to minimize problems associated with use of the river water. Due to the low temperatures during winter, production is restricted to April–October. There has been a tendency observed for increased coiling of trichomes throughout the cultivation period, resulting in a decrease in trichome length of about 34%. Higher temperatures as well as mechanical stress during harvesting seem to be responsible for the changed morphology (Belay 1997).

Another large production facility located in Kona, Hawaii (Cyanotech Corporation) has operated since 1984; in 2010 it had a total pond area of 116,000 m² and an average pond size of about 2,900 m² (Cysewski 2010). Here, consistent temperatures and sunlight allow production all the year. However, China has now become the world's largest *Arthrospira* producer. Part of the success is due to adaptation of the cultivation strategy in Inner Mongolia for growth in a much colder climate, yet one with high radiant energy during summer. A significant proportion of the produced biomass now comes from this region, where average temperatures of 6.4°C strongly reduce growth of mesophilic organisms. Thermo-proof greenhouses are placed over the raceway ponds in order to prevent growth inhibition or even culture deterioration. Under these conditions productivity is relatively low (5–9 g m^{-2} day^{-1}) and production is limited to about 5.5 months a year (Lu et al. 2010).

Besides many local or regional producers in India, South America and Africa a single mass producer from Myanmar is operating on the world market. The biomass is produced in natural crater (Fig. 26.3) lakes where the pH is high. The majority of the biomass is produced in the Twyn Taung crater, a lake of 200 ha area. Its salinity is 4 ppt and the pH at 9.5. It is operated for biomass production since 1988. In order to enhance growth combined nitrogen is replenished in regular intervals. The production capacity lies in the range of 200 t per year (Thein 2011). During summer month a maximum of 5 t per d is harvested from boats, while in 'off season' the production is much lower (below 1 t per d). In March, daily water temperatures range from 23–27°C.

Harvesting of the biomass is carried out via different, partially automated filtration techniques: inclined gravity screens, horizontal vibration sieves and vacuum filters are combined in order to dewater and desalinate the biomass to a solid content of 8–12%. The configuration of the different solid liquid separation steps needs to be tuned depending upon the amount of biomass that needs to be removed and upon the trichome size of *Arthrospira*. Vibro screens with a mesh size of about 100 μm are being used (Grobbelaar 2009a). The input of energy can be problematic for the cultures, since shear forces can lead to breakage of the trichomes. This results in reduced harvest efficiency, because small fragments pass the screens. In addition, bacterial contamination is increased due to the release of organic compounds by broken trichomes. In general, separation of solids from liquids requires more effort if cell densities are low.

Subsequently the biomass is dried and this may be done by technically easy procedures such as sun or oven drying or the more demanding use of drum and spray drying. The decision about the method largely depends on the investment capital available to the company. The resulting *Arthrospira* powder contains a residual moisture of 3–5%. It is certainly possible to obtain stable powders using low cost methods (Tiburcio et al. 2007). However, the conditions during drying influence the quality of the final products: hot temperatures or long drying times increase degradation of valuable ingredients such as carotenoids, enzymes and polyunsaturated fatty acids. Oliveira et al. (2010) found for both drying temperature and layer thickness a significant effect on the product quality of *Arthospira platensis* employing convective drying. Packaging is carried out directly after the drying process, preferably under vacuum and in non-transparent oxygen barrier bags rather than in polyethylene, in order to prevent oxidation of ingredients during storage (Gershwin and Belay 2007).

Beyond the nutritional value of the biomass, the numerous health benefits of *Arthrospira,* assessed by various studies, are of growing interest and are facilitating the market size. Amongst others anti-inflammatory, exhaust relief, immune system boosting, assisting in digestion and improvement of well-being have been claimed (Jensen et al. 2001). A therapeutic value in animal models and/or in humans was observed in the context of lowering hypertension, regulating hypercholesterolemia and hyperglycerolemia, enhancing the immune system, contributing to the stimulation of intestinal lactobacilli, reducing nephrotoxicity caused by heavy metals and drugs, protecting against radiation damages and being active against some cancer types, e.g. oral leukoplakia (Blinkova et al. 2001; Belay 2002; Gershwin and Belay 2007; Deng and Chow 2010). The enhancement of the immunity against different infections was often reported in pre-clinical studies (Capelli and Cysewski 2010). Those effects were connected to the enhanced production of antibodies and cytokines, as well as to the activation of macrophages, T and B cells (Blinkova et al. 2001). The cardiovascular benefits of *Arthrospira* are primarily resulting from its hypolipidemic, antioxidant, and antiinflammatory activities (Deng and Chow 2010).

High molecular weight polysaccharide preparations were isolated, e.g. “Immulina” from *A. platensis* that showed a high immunostimulatory activity, between 100 and 1,000 times more active for in vitro monocyte activation than polysaccharide preparations that are currently used clinically for cancer immunotherapy (Pugh et al. 2001). Moreover, in vitro a strong action against *Candida albicans* and tetanus toxoid was measured. In a human clinical trial the immune markers tumor necrosis factor alpha (TNF-α), interferon gamma (INF-γ) and interleukin-6 (IL-6) were significantly enhanced after administration of a high-molecular-weight polysaccharide extract from *Arthrospira platensis* (Løbner et al. 2008). Parages et al. (2012) investigated the immunostimulating effect of an acidic polysaccharide isolated and purified from a laboratory culture of *Arthrospira platensis*. The proinflammatory activity of the received fraction suggests that the polysaccharides could stimulate the immune response of the cells by inducing the production of the cytokine TNF-α in macrophages. The polysaccharide fractions, mainly sulfated, show anti-viral properties that are of great interest for the development of therapeutic drugs. Hernandez-Corona et al. (2002) detected also a high antiviral activity against HSV-2, in a hot water extract of *A. maxima*. Further purification of these fractions led to the identification of calcium-spirulan (Ca-Sp), a sulphated polysaccharide composed mainly of rhamnose, from *A. platensis* by Japanese researchers. It inhibited replication of HIV-1, human cytomegalovirus, measles, mumps, influenza A and HSV-1 (Hayashi et al. 1996). Its antiviral effect was found to be superior to that of dextrane sulfate against HIV-1 and HSV-1. The action is based on the selective inhibition of virus penetration to the host cells.

Sandau and Pulz (2009) also found a high activity of Ca-Sp against HSV-1, measured superior above Acycloguanosin, an active agent against HSV that is currently on the market.

Interestingly, the *in vitro* antiviral activity was already linked to the dietary consumption of *Arthrospira* by humans *in vivo*; the HIV infection rate in Chad is low compared to the rest of Africa, where *Arthrospira* is a traditional ingredient in the diet (Teas et al. 2004).

Ca-Sp was also found to be active against tumor invasion and metastasis of B16-BL6 melanoma cells by inhibiting the tumor invasion probably through the prevention of the adhesion and migration of tumor cells to laminin substrate and of the heparanase activity (Mishima et al. 1998).The invasion of carcinoma, melanoma and fibrosarcoma was inhibited by Ca-Sp (Capelli and Cysewski 2010). Water extracts of

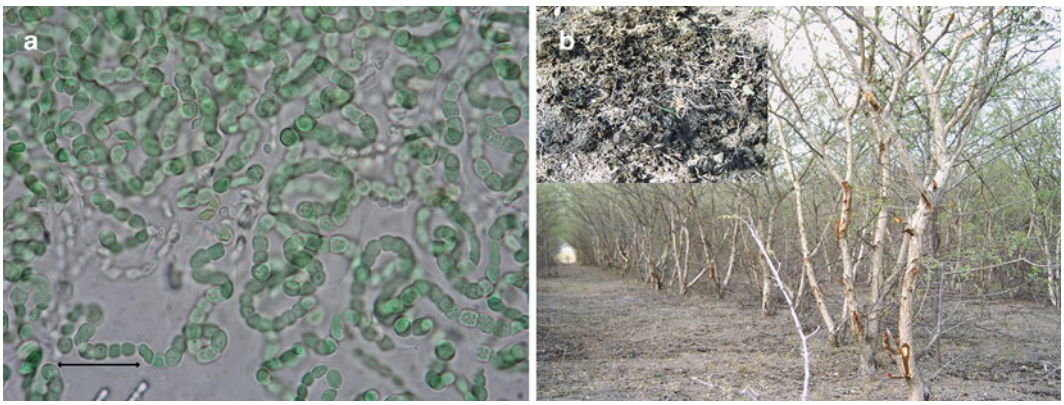

Fig. 26.4 (**a**) *Nostoc ellipsosporum* from liquid laboratory culture (SAG 1453-7), scale bar = 20 μm; (**b**) growth of *Nostoc flagelliforme* in its habitat on soil in Myanmar

Arthrospira have been reported to be cause regression of cancer progression in rodents (Grawish 2008; Akao et al. 2009; Grawish et al. 2010).

Anwer et al. (2012) reported the presence of insulin as a hypoglucemic agent in the range of 2 to 33 μg*g^{-1} within the biomass of 16 out of 23 investigated *Arthrospira* strains cultured under laboratory conditions. Its content was positively connected to the log phase of growth and influenced by the nitrate, phosophate, sulfate and bicarbonate concentration in the medium in *Arthrospira platensis*. The prebiotic effects of *A. platensis* biomass, both pure and in functional food application (biomass and aqueous extracts in processed in pasta, biscuits and others), were investigated on intestinal bacteria (Pulz and Gross 2004). An up to tenfold increase of growth rate of various lactobacilli was found, especially on *Lactobacillus acidophilus*. The measured effects were regarded as beneficial, although the components responsible and their mode of action have still not been explained satisfactorily.

The historical use of the biomass as food as well as safety studies imply that human consumption is generally safe. However, rare cases of side-effects in humans have been reported (Mazokopakis et al. 2008). The accumulation of heavy metals by the cells grown in open photobioreactors may be the highest risk, though this should be limited if there are stringent quality control measures. Moreover the produced biomass can contain alien cyanobacteria such as *Anabaena*, which may produce the neurotoxin anatoxin-A, so that neurological reactions can occur after consumption of *Arthrospira* (Grobbelaar 2003). Stringent quality control and maintenance measures will need to be applied in order to avoid damage of the industry.

The strong evidence that the intake of a few grams of *Arthrospira* (in the range of 2–13 g day^{-1}) leads to an array of therapeutic benefits will most certainly result in its still wider use as a nutraceutical food supplement worldwide. However, although the available data are many and coherent, further clinical research is needed to solidify the case for its use.

26.2.3 *Nostoc*

Nostoc colonies have been used as a food in Asia, especially China, for more than 2,000 years (Gao 1998; Qiu et al. 2002). Both their herbal and their pharmaceutical value contribute to their economic importance (Khaing 2004). *Nostoc commune*, *N. sphaeroides, N. verrucosum* and *N. flagelliforme* are the main species, with the last being probably the best known. Due to its appearance *N. flagelliforme* is called 'Fa cai' (hair vegetable) and grows on soil in China and Myanmar throughout the year (Fig. 26.4).

In Myanmar the organism reported as *N. verrucosum* grows attached to cliffs or on the soil, though in the latter case only during rainy season (Min Thein, personal communication 2011). (The original description of this species and almost all other subsequent reports are for flowing water, so it seems possible this may be another species.) Clouds have to prevent direct illumination for at least 5 days after the rain occurs to promote its growth. *N. sphaeroides* is collected from rice fields in China, where its colonies comprise dark green, subspherical colonies up to 25 mm diameter (Helblin et al. 2006). This is probably the same as the Ge-Xian-Mi reported by Qiu et al. (2002) from many parts of China, including Hefeng County, the location of their study. In this case recent agricultural changes, such as addition of fertilizer to the rice-fields, had led to a marked decrease in the *Nostoc* population, which in economic terms may not have been replaced by the increased rice yield.

In the natural habitat described by Danxiang et al. (2004), *N. flagelliforme* is about 0.5 m long, 0.2–1 mm in diameter and usually unbranched. In China the species grows in arid or semi-arid steppes of the west and north-west, where it usually occurs in the altitude range 980–2,800 m, often together with *N. commune* (Danxiang et al. 2004). There are also records for the species in dry regions of many other parts of the world, including Africa, Europe and USA. *N. flagelliforme* is physiologically adapted to both

drought and heat. Temperature extremes ranging from −29°C to 66°C have been reported from China (Gao 1998). The optimum temperature reported by Diao (1996) for growth was in the range of 15–25°C and for nitrogenase activity 21–28°C (Zhong et al. 1992); the heat tolerance is restricted to dry conditions (Danxiang et al. 2004). Among many protective mechanisms involved in desiccation tolerance are the presence of a high molecular weight extracellular polysaccharide that prevents phosphytidyl choline membrane fusion, and also the presence of a water stress protein (Hill et al. 1997). In order to understand the physiological processes during drought stress Liu et al. (2012) investigated genes of *Nostoc flagelliforme* exposed to sorbitol and reported a differential regulation of drought tolerance-associated genes providing an insight into molecular mechanisms connected to its drought adaptation. During rehydration colonies expand as water is taken up. Yoshimura et al. (2012) recently investigated the role of the extracellular polysaccharide from a terrestrial Nostoc sp. and linked its function to salinity tolerance. Under salt stress the amount of capsular polysaccharides increased up to 65% of dry weight, and a modified composition of monosaccharides was measured in *Nostoc* HK-01. Shi et al. (1992) reported on salinity tolerance, with values ranging from 0.05 to 0.9 M NaCl and maximum photosynthetic activity at 0.15 M. Light saturation occurred between 700 and 900 µmol photon m^{-2} s^{-1} (Zhong et al. 1992). As nitrogen sources both inorganic N (nitrate, nitrite and ammonium), organic N (amino acids) and N_2 can be utilized (Danxiang et al. 2004).

N. flagelliforme grows very slowly in its natural environment. Due to the reduced available area and an increasing market demand, attempts are being made to establish a cultivation technology. The first such attempts focused on solid media, due to its terrestrial habit (Cui 1983; Cheng and Cai 1988; Su et al. 2005). Cells divided 3–4 times in 10 days (25°C, low light conditions) when *N. flagelliforme* was cultured on solid medium (Cheng and Cai 1988), with growth in this case being enhanced with a soil solution extract obtained from its habitat. The maximum elongation rate was 43% in 12 days, with an average of about 20% for a similar period on a wheat field soil. Under aquatic conditions the best colonial development of the strain used by Gao and Ye (2003) occurred at 60 µmol photon m^{-2} s^{-1} and 25°C. On synthetic mats daily increases in dry weight of 0.6–6.1% have been observed (Qiu and Gao 2002); the procedure involved soaking the mats of *N. flagelliforme* with BG11-medium once or twice per day. A strategy to use liquid-grown cultures on sand bed materials has been developed by Chen et al. (2009, 2011). This approach can be used as a tool against soil erosion and desertification (Chap. 12).

A higher moisture content in the *N. flagelliforme* mats also encourages bacterial growth, which resulted in disintegration of the filaments after 7–10 days (Gao 1998). Pre-sterilizing filaments with 75% ethanol was effective against bacterial growth (Su et al. 2008), enhancing the elongation of filaments to 40% in 14 days at 30°C. Periodic desiccation seems to be important to prevent *N. flagelliforme* from being disintegrated by bacteria, indicating that drought is not simply an environmental stress, but of physiological and ecological significance (Gao and Ye 2003).

The few studies on the cultivation of *N. flagelliforme* in suspended culture have mostly been done in shaking flasks (Gao and Ye 2003; Liu and Chen 2003), but attempts at laboratory scale photobioreactors have been reported by Su et al. (2008). Trichomes from natural colonies were surface sterilized and then grown in a 20-L stirred photobioreactor with different agitation rates. Colony morphology, volumetric biomass productivity and EPS production were all affected by agitation speed. The highest volumetric biomass productivity (0.07 g L^{-1} day^{-1}) was reached at an impeller speed of 0.8 m s^{-1} and aeration rate of 0.8 vvm. Morphology in liquid culture changed from a compact colony to a thin slime formed around the cells. Another cultivation approach involving mixotrophic conditions showed comparable biomass productivity (0.04 g L^{-1} day^{-1}), but even higher biomass productivity of (0.23 g L^{-1} day^{-1}) (Yu et al. 2009). This yield was achieved at 25°C and continuous illumination using BG11-medium supplemened with 14 mM glucose; the initial pH was 8.0. Biomass productivity under both autotrophic and mixotrophic conditions was best at 60 µmol photon m^{-2} s^{-1}. This is the most efficient production method so far, but the work was done in shaking flasks (500 mL), so its scalability remains questionable. The results indicate that biomass yields are highly influenced by the nutrient supply, with the highest values under mixotrophic conditions, followed by heterotrophic and autotrophic growth, as it has been reported for several other cyanobacteria, including *Arthrospira* (Chen and Zhang 1997).

The physiological state of *Nostoc* colonies influences their carotenoid composition. Actively growing ones usually contain zeaxanthin, β-cryptoxanthin, myxoxanthophyll and β-carotene as primary carotenoids. However, the composition of desiccated cultures or ones exposed to UV is dominated by secondary carotenoids such as echinenone and canthaxanthin (Ehling-Schulz et al. 1997; Scherzinger and Al Babili 2008).

Early reports from China mention the use of *Nostoc* spp. to treat diarrhea, hypertension and hepatitis. More recent investigations have shown that a hot water extract from *N. flagelliforme* has anti-tumour activity, and an acid polysaccharide, Nostoflan, isolated from the *N. flagelliforme*, has anti-HSV-I activity (Kanekiyo et al. 2005; Kanekiyo et al. 2007). To the best of our knowledge no oral acute and subacute toxicity tests have been reported to establish the safety of *N. flagelliforme* for human consumption (Takenaka et al. 1998). Nevertheless the genus *Nostoc* is capable of producing

the neurotoxic amino acid β-N-methylamino-L-alanine. (BMAA) (Cox et al. 2005). Products of *N. commune* and *N. flagelliforme* traded in Hawaii, Switzerland and Peru have been investigated and some were found to contain BMAA (Johnson et al. 2008; Roney et al. 2009), a fact that needs to be considered as a safety concern.

The over-exploitation of *N. flagelliforme* in China (Qiu et al. 2002) show the need for further investigationson on the growth *Nostoc* spp. This is not only in order to establish and optimize a production process, but also to identify the conditions which favour toxin production with the aim of minimizing or avoiding any risk.

26.2.4 *Aphanizomenon flos-aquae*

In the early 1980s *Aphanizomenon flos-aquae* (AFA) was introduced to the US market as a health food supplement, and is therefore a relatively new food source, possessing a similar chemical composition as *Arthrospira*. The biomass was produced at Upper Klamath Lake, a shallow lake system in Oregon, USA. The production differs from *Arthrospira* in being harvested exclusively from a natural lake rather than from constructed ponds. According to Carmichael et al. (2000) blooms occur between late May and October or November with biomass concentrations of 3–50 mg L^{-1}. During this time a small bloom of *Anabaena flos-aquae* could occur (< 1% biomass), whereas *Microcystis aeruginosa* and *Coelosphaerium* (probably *Woronichinia*) appear in July and persist.

A laboratory study of two populations of *Aph. flos-aquae* from Japan showed that the organism could not grow below pH 7.1 and a temperature of 11°C, while growth tended to be suppressed under a light dark cycle of 10:14 (Yamamoto and Nakahara 2005).

Harvesting of the biomass from Lake Klamath is carried out via large harvesting nylon screens in different canals using the flow direction of the water and additionally pumping. One harvesting system is functioning via an aqueduct like system off lake the other one with on-lake barges. The biomass screens are guarded by debris screens in order to separate fish and floating material from the biomass and dewater the cyanobacterial biomass with a flow of up to 28.3 m^3 s^{-1} (Carmichael et al. 2000). A second vibrating screen concentrates the biomass to 1 g L^{-1}. Due to the variability of the environmental conditions that influence composition of the biomass, all settings need seasonal adjusting. Subsequently the biomass is pumped to a series of three slow- speed horizontal centrifuges, which remove sand etc. followed by a vertical high speed centrifuge that yields a product of 6–7% dry matter. Afterwards algae are chilled to 2°C and shock frozen in a flake freezer. Subsequently the biomass is freeze dried and the resulting product is used for the production on tablets or capsules.

Strict quality control procedures are necessary in order to avoid cyanobacterial toxins (Chap. 24), which have been reported for two strains of *Aph. flos-aquae* itself (Preußel et al. 2006; Wood et al. 2007). Assays such as mouse bioassays, ELISA and protein phosphatase inhibition assay (PPIA) are employed. The enzyme assays used are about 1,000 times more sensitive than the HPLC methods. Although the literature reports that *Aphanizomenon* can produce neurotoxins including saxitoxins and anatoxin-a, all tests on Klamath Lake cyanobacteria and the time of the review by Carmichael et al. (2000) failed to detect any cyanobacterial neurotoxins when examined by mouse bioassay, HPLC or mass spectrometry. The only cyanotoxin found in the phytoplankton during this testing period was microcystin from *Microcystis*, backed by the detection of microcystin syntase genes in health food supplements containing AFA by Saker et al. (2005). A risk assessment for the microcystin content of *Aph. flos-aquae* containing products has been carried out by Schaeffer et al. (1999), calculating 10 μg microcstin LR per g dietary supplement as safe based on a mouse feeding trial conducted in 1984.

Moreover anatoxin-a was found in *Aph. flos-aquae* strains isolated from toxic blooms of lakes in Finland (Rapala et al. 1993), who carried out a laboratory batch culture study. The toxin content of these strains was strongly influenced by growth conditions such as temperature, light intensity, nitrate and phosphate concentration; up to 19% of total toxins were released into the growth medium. Moreover PSP toxins have been identified in *Aph. flos-aquae* isolated from a river in Northern Portugal and of PSP toxins (neoSTX, dcSTX, STX and GTX5) in *Aphanizomenon* sp. (LMECYA31) after cultivation under laboratory conditions (Dias et al. 2002). One can summarize that of *Aph. flos-aquae* is capable of producing numerous toxins under several environmental conditions. Presumably strains differ in their toxicity and the ones assayed may differ from the one blooming at Klamath Lake. Although the production of *Aph. flos-aquae* at Klamath Lake benefits from the lack of costs during the growth stage, the numerous required toxin analyses reverse this advantage.

The quality assurance and related safety issues remain a problem for the marketing in other countries besides the USA. There is neither a food approval status for the *Aph. flos-aquae* biomass in the EU, nor a GRAS (generally regarded as safe) status in the USA. The detection of microcystins in commercial AFA products was published by German health protection officials in 2011, unsettling potential customers as well as biomass producers. The food industry insists on certified production processes for their products, which cannot be issued for the 'wild harvested' biomass. The label 'wild harvest' which had been a distinct marketing advantage in the health food market earlier (Carmichael et al. 2000) is no longer valid. In summary, we conclude that the ability of some *Aph. flos-aquae* strains to synthezise harmful toxins makes its consumption possibly

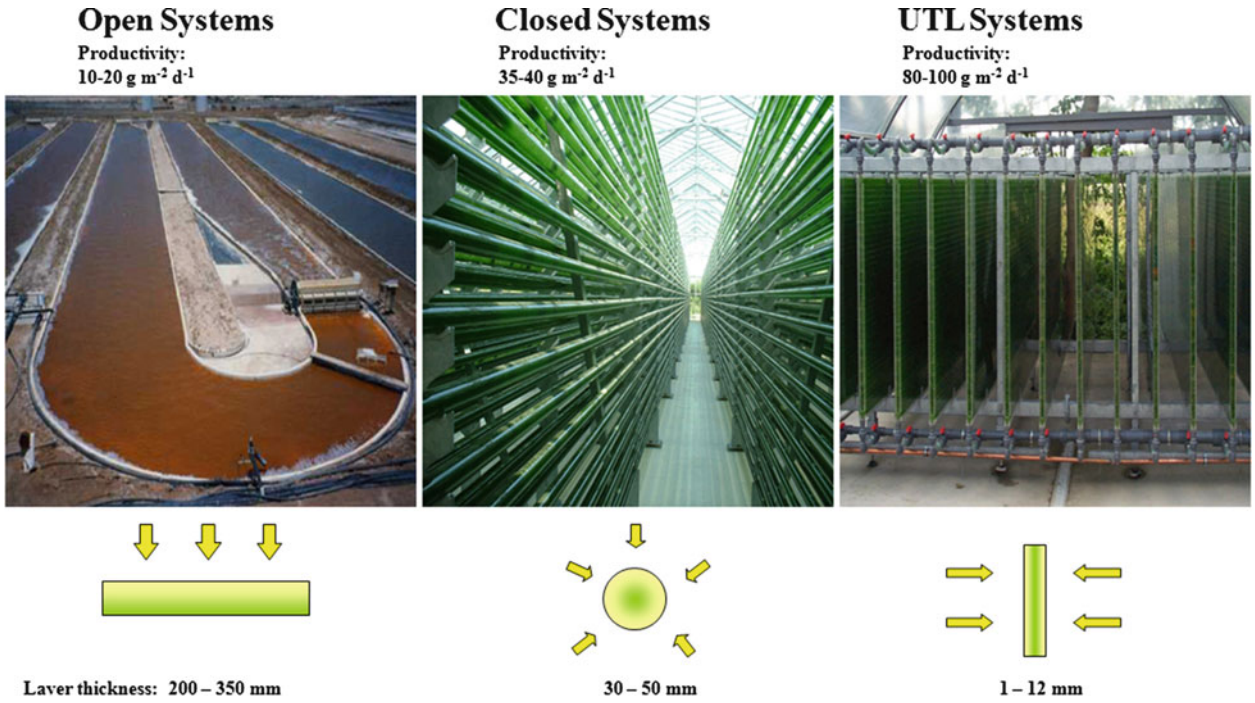

Fig. 26.5 Area productivity of various algae cultivation systems depending upon photobioreactors (*PBR*) geometry and layer thickness of photosynthetically active parts, *arrows* show light input : open system: raceway pond, closed system: tubular PBR, designed by Pulz (Pulz and Scheibenbogen 1998), ultrathin layer system: designed by Pulz for improved PBR performance

unsafe and thus conflicts with the initial purpose of a health food supplement.

Moreover the cause of *Aph flos-aquae* blooms in the lake is problematic. High P input is probably the main factor responsible for the blooms, which can lead to low oxygen concentrations when breakdown of the bloom occurs. In order to avoid hypoxic conditions, efforts are being undertaken by the State authorities to lower the load of total P within the next few years (Simon et al. 2009) to reduce the amount of bloom. In order to maintain the annual biomass output, open pond cultivation strategies would have to be applied. If successful, their application could enhance quality, yield and toxicological safety of the product.

26.3 Mass Culture of Cyanobacteria

In contrast to fermentation using heterotrophs, in photobioreactors the ability of phototrophic organisms to use photosynthesis as energy source is employed. The most important condition for the algal growth in the suspension is therefore the optimal provision with light, which cannot be stored in excess, like nutrients. The sufficient light supply can only be achieved by very thin layers of the culture suspension, given that by the algal growth and by the increased clouding of the suspension a rapidly decrease of the available light for the algal cells occurs. On the other hand only high cell densities in the culture assure high growth rates per volume unit. Just as with land plants, the culture area required to produce biomass plays a very important role.

Microalgae, including cyanobacteria, can be cultivated in open, closed or ultrathin layer systems (UTL) (Fig. 26.5). Open cultivation systems comprise natural or artificial ponds, raceway ponds, or so-called inclined surface systems. They present the classic method for the production of algal biomass and require large areas. If land utilization costs are low and climatic conditions favourable, the investment costs for sizes up to 100 ha are also relatively low. The greatest advantage of this approach to production is the low investment cost, which can be almost none if pond construction occurs in natural waters. Lake Chad is the best known natural system for *Arthrospira* production (Chap. 25). However, during the past decade a considerable *Arthrospira* production has also been developed in several alkaline crater lakes in central Myanmar. Natural and artificial ponds are generally used for the cultivation of marine, naturally predominant or extremophilic species, where there is a relatively low risk of contamination. Open pond systems dominate industrial scale algal biotechnology in general (Grobbelaar 2009b) and cyanobacterial biotechnology cultivating *Arthrospira* in particular, both in terms of annual output and their distribution worldwide.

The open ponds usually consist of cement or plastic basins, no deeper than 0.2–0.35 m, in order to maintain light conditions for optimal growth. The areas range from 25 to 5,000 m². The problem of stirring is of fundamental importance, because large amounts of energy are required to prevention

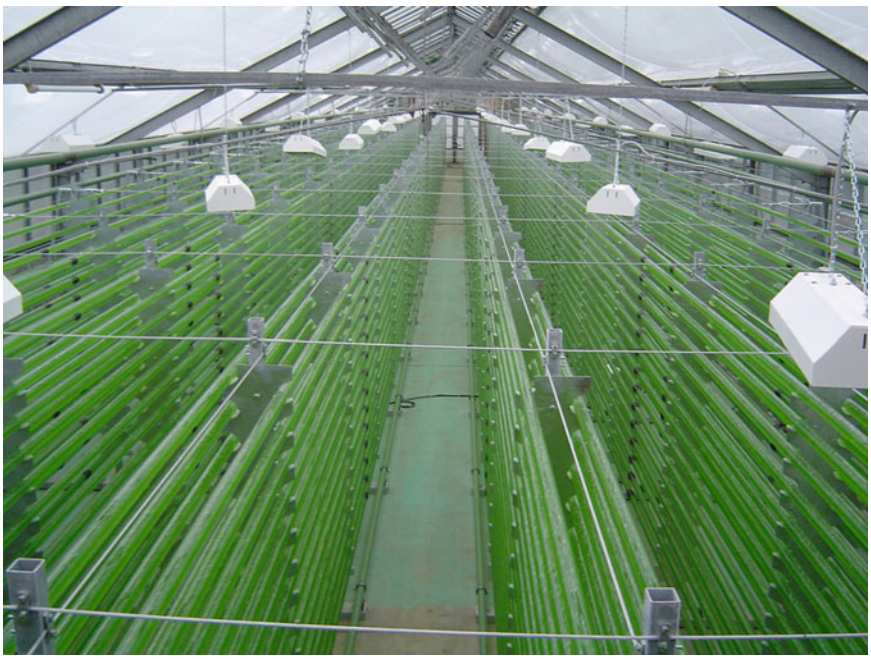

Fig. 26.6 A closed tubular photobioreactor plant at Salata GmbH, Ritschenhausen, Germany, installation by IGV GmbH, total volume 15,000 L, cultivation of *Oscillatoria* sp.

concentration gradients and algal sedimentation. Paddle wheels combined with aerating units incorporated into parallel, loop-like channels several km in total length is the common technique for the plants of several hectares, resulting in suspension velocities of 0.01–0.3 m s^{-1} and requiring relatively low operating expenses. The growth of biomass in raceway ponds is also dependent on the prevailing regional climate, the mean growth rates for *Arthrospira platensis* ranging from 5 to 20 g m^{-2} day^{-1}. Weather conditions like heavy rain, aridity and thunderstorms can influence the morphology and productivity of the cultures.

This cultivation method is restricted to tropical or subtropical climate zones, where the light input that is directly linked to the prevailing temperatures is sufficient for the ecological demands of the algae. Semiarid and arid climates mean less danger of flooding, but pose a higher water demand. Modifications have been introduced to permit growth in some mid-latitude maritime or continental locations, using greenhouses with foil in order to raise the temperature and therefore the production period per year (Lu et al. 2010). In such cases production is interrupted during the winter months, as occurs for *Arthrospira* production in China and France. Another advantage of the greenhouse is the possibility to introduce CO_2, increasing the CO_2 concentration in the culture suspension and thus the growth rate.

The low productivity and the vulnerability of open systems to contamination of various kinds has led to the development of closed reactors, in which photo-biological processes are less dependent on interfering environmental influences. Such closed reactors have a range of principal advantages: (1) *low CO_2-loss*; (2) *low water losses*; (3) *reduced contamination risk*; (4) optimal *temperature* regulation; (5) *controllable hydrodynamics*; (6) *reproducable cultivation conditions*; (7) considerable *flexibility regarding* environmental *influences*; (8) *low space requirements*. These photobioreactors allow introduction of light into the culture suspension by their light transparent reactor walls (tubes, plates); about 90% of the incident light can reach the cells in this system. In general the closed photobioreactor consists of photosynthetically active modules (glass or plastic tubes, or extruded profile plates), a compensation tank, distribution pipes and pumps. The reactor is characterized by its limited gas exchange with the surroundings. There is a relatively low contamination risk and the process is technically well controllable. In response to the various microalgal cultivation tasks and the specific requirements of particular species, many different reactor types have been developed, which are mainly used for research and development work. Extensive summaries of design principles are given by Ugwu et al. (2008) and Posten (2009).

Today the principal use of tube reactors is established for large-scale production. The largest European industrial algae production plant was built in Klötze, Saxony–Anhalt, Germany in 2001. The total production is realized in 20 plant sections, which work independently from each other. Each of these plant sections, which consist of a total volume of 35,000 L and a photosynthetic active tube surface of 3,500 m², is equipped with an independent control system. With such jointly connected bioreactor modules a maximum production capacity of 150 t per year can be produced on an area of 12,000 m^2. In 2005 a 85,000-L production plant was established in Ritschenhausen, Thuringia, Germany (Fig. 26.6). Here a single photobioreactor has a volume of 42,000 L and *Arthrospira* is produced with higher productivities than in the open systems. The biggest photobioreactor of the IGV Institut für Getreideverarbeitung GmbH was built with a total volume of 85,000 L as one module in Jerez, Spain, in 2011.

Fig. 26.7 Ultra-thin Layer PBR installation at APS Red Hawk Power Plant, Phoenix, AZ, USA; continous cultivation of a cyanobacterium for CO_2-sequestration using stack gas out of a gas fired power plant

Neither the open systems nor the described closed glass tube reactors meet the requirements of mass production of algal biomass for the production of bioenergy, such as biodiesel. Both systems fail to meet the efficiencies of biomass production and production costs required. Whereas in open systems productivities between 10 and 20 g m^{-2} day^{-1} can be reached, in the closed system described they are about 35–40 g m^{-2} day^{-1}. The biomass produced in the investment and operation cost intense closed photobioreactors, like the ones in Klötze and Ritschenhausen, is marketed successfully to the food and feed additive sectors. For bioenergy applications the production costs are too high.

The production principle has been developed further by IGV GmbH to produce a new design, the Ultra Thin Layer (UTL). This photobioreactor has been tested and showed positive results for functionality in a pilot plant in Arizona, USA (Fig. 26.7). The UTL-technology has led to an increase in productivity to 80–100 g m^{-2} day^{-1}. The yield for the UTL-system is 5 times higher than in an open system, mainly due to the high surface to volume ratio and improved gas exchange. This is the most productive photobioreactor reported so far (Posten 2009).

26.4 Present Uses

26.4.1 Food

In non -western civilizations algae have been used as food in the human diet for millennia. First records report about the consumption of macroalgae in coastal regions 6,000 years ago. Cyanobacteria, with the first records from *Arthrospira*, have been utilized by the Aztecs (Lake Texcoco, near Tenochtitlan) in Mexico by the Aztek population since 1300 AD (Pulz and Gross 2004) and in Africa (Lake Chad) even earlier (Abdulqader et al. 2000). *Arthrospira* is traded in Africa as *dihé* after collecting and sun drying (Ciferri 1983) and is consumed in soups at up to 60 g per meal (Delpeuch et al. 1975). In Mexico, the dried cake of *Arthrospira* called “tecuitlatl” was commonly eaten with maize, different cereals or in a sauce of tomatoes and spices. *Aphanothece sacrum* (Fujishiro et al. 2004), *Nostoc muscorum* and *N. commune* have been used as side dishes in Japan since ancient times (Lee 2008). Human consumption of *Nostoc* has been reported from Mongolia (China), Japan and Peru and also occurs in other Asian countries such as Myanmar (see Sect. 26.2.2). Dried and stir-fried *Nostoc* balls available on Asian markets are used mainly as soup ingredients. Since rising demands were driving the prices and massive collection of *N. flagelliforme* led to grassland degradation, desertification and social problems, all further collection, sale and exportation was banned by Chinese authorities (Roney et al. 2009). In order to follow the law an artificial *fa cai* was developed consisting of sepia-dyed starch noodles. However, similar material had been sold in Hong Kong long before that, even though many buyers at this time probably did not realize they were paying a lot of money for dyed starch (But et al. 2002). Other uses of cyanobacteria, such as *Aphanizomenon flos-aquae*, have been discussed above.

After the cyanobacterial biomass has been harvested and separated from the medium, it is processed to a sun- or

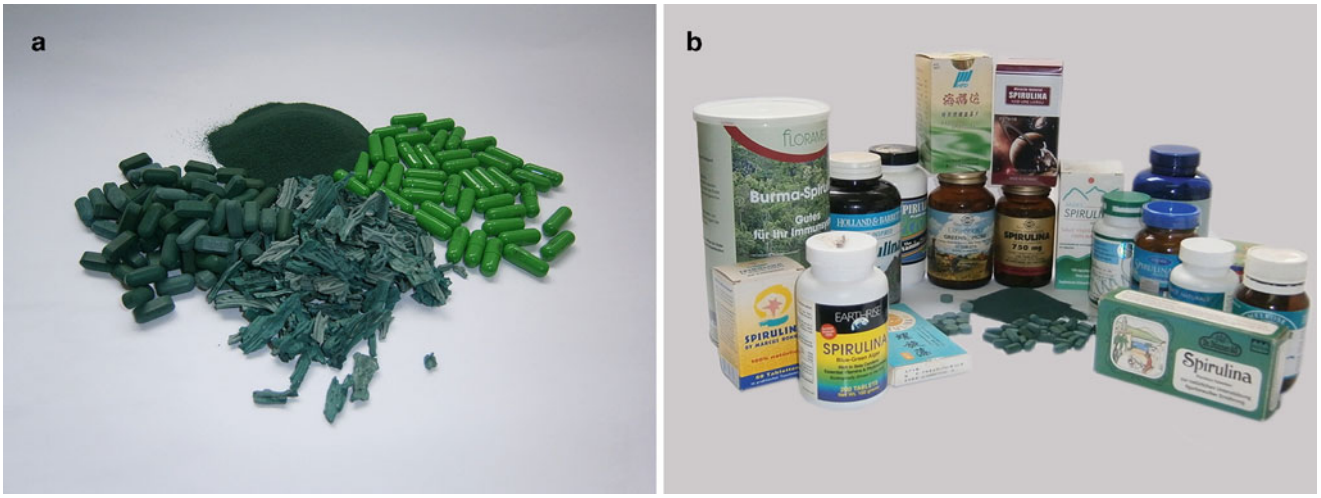

Fig. 26.8 (**a**) *Arthrospira platensis* processing forms (powder, flakes, capsules, tablets); (**b**) examples of *Arthrospira platensis* dietary supplement products

spray-dried powder of blue-green colour. In China it is almost always sold in normal shops and markets, but outside China mostly in health food stores. More than 95% of the annual microalgal biomass production is used for the manufacture of powders, tablets, capsules and pastilles (Fig. 26.8). Numerous combinations of microalgae or mixtures with other health foods can now be found on markets all over the world. The structure of the cell wall permits an easy digestion by humans and animals, which in turn accounts for the very high bioavailability of the valuable components of the biomass (Raja et al. 2008). This is an advantage over eukaryotic cells, which often need a separate cell disruption process due to their thick polysaccharide-containing cell walls hindering bioavailability.

Marketing and sales of algal biomass depend to a great extent on obtaining approval by the authorities. The market for dietary supplements is dominated by *Arthrospira*, the only cyanobacterial biomass that is food approved in the EU. *Spirulina* received GRAS in 1981 by the Food and Drug Administration of the USA, whereas *Aphanizomenon flos-aquae* has never been granted this status.

The idea to use microalgae as dietary supplements to cure malnutrition and in addition help close the protein gap in world nutrition due to its high protein content can be traced back to the 1970s. Important papers include (Soeder et al. 1971) and then ones by other pioneers of algal biotechnology such as (Richmond and Vonshak 1978) working with *Arthrospira*. Even today the consumption of microalgal biomass is restricted to a very few taxa, *Arthrospira*, *Nostoc* and *Aphanizomenon*, and *Chlorella*, *Dunaliella* and *Haematococcus* among the green algae. Cyanobacteria play the major role in terms of biomass for both traditional and current use. The exploitation of phototroph diversity was hampered by food safety regulations for human consumption for a long time. The successful authorization (following EC regulation 258/97) of microalgae or their extracts, such as the marine diatom *Odontella aurita* or the astaxanthin-containing *Haematococcus pluvialis* extract as novel food is a real advance for microalgal biotechnology. Facing the food regulations in the western countries there will soon be another problem – the fact that traditional collecting and trading of *Nostoc* and other microalgae for food purposes cannot keep up with the demands of an increasing population, particularly in Asia. The harvest is often exceeding the growth in natural habitats, exploitation of land decreases the production area and moreover the use of herbicides and pesticides in agriculture is negatively affecting the algal growth. The only promising solution for this challenge is the development of straightforward biotechnological production processes.

Studies promoting the use of *Arthrospira* as nutraceuticals are facilitating the development of extraction techniques in order to develop functional, new neutraceuticals. Therefore downstream processing techniques, such as supercritical fluid extraction (SFE) are optimized on high yields of valuable compounds, such as carotenoids, vitamins and fatty acids. For the purification of γ-linoleic acid from *A. platensis* two strategies are possible: column chromatography and urea crystallization of saturated fatty acids (Sajilata et al. 2008a) and supercritical carbon dioxide extraction (Sajilata et al. 2008b). A major advantage of CO_2-SFE is that the solvent is generally recognized as safe (GRAS), while a drawback is that the biomass needs to be dried prior to extraction. The extraction yields can be significantly enhanced using ethanol as an entrainer (Sajilata et al. 2008b). Mendiola et al. (2008) optimized extraction of tocopherol from *A. platensis*, reaching final contents of 2.9% in the extract.

Currently most products launched to serve the health food market are supplied as tablets and powder. Nevertheless, functional food or nutraceuticals produced with microalgal biomass or algal extracts are sensorically much more convenient than algal powders. In Germany, food production and distribution companies have started serious activities to market functional food with microalgae and cyanobacteria. Examples are pasta, bread, yogurt, sweets and soft drinks. Similar developments can be observed, for example, in Japan, USA, China and Thailand. New product developments will combine health benefits with attractiveness to consumers and create a stable market in the future. 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.

26.4.2 Special Ingredients – Phycobiliproteins

In addition to chlorophyll a, as the primary photosynthetic pigment, microalgae contain a multitude of pigments which are associated with light harvesting. Over 100 different carotenoids are synthesized by microalgae of all divisions and classes (Liaaen-Jensen and Egeland 1999), but the biosynthesis of phycobiliproteins is restricted to cyanobacteria, rhodophytes and cryptophytes (Eriksen 2008). They are divided according to their absorption characteristics to the main classes phycocyanin (PC, λ_{max} 610–620 nm), allophycocyanin (APC, λ_{max} 650–655 nm) and phycoerythrin (PE, λ_{max} 540–570 nm) (Bermejo Román et al. 2002). Phycobiliproteins are multi-chain proteins consisting of apo-proteins and phycobilins (linear tetrapyrrols) covalently bound to specific cystein residues of the protein. The three dimensional structure of c-phycocyanin (C-PC), with minor species-dependent variations has been elucidated in various cyanobacteria (Dobler et al. 1972; Stec et al. 1999; Padyana et al. 2001; Contreras-Martel et al. 2007). In cyanobacteria phycobiliproteins are incorporated into phycobilisomes located in the outer thylakoid membrane and improve the efficiency of light energy utilization by broadening the absorption spectrum of light, transferring the excitation energy by radiation less processes to the reaction centres (Eriksen 2008). In addition to their role in light absorption, they can serve also as a N store that is mobilized in case of N depletion in their environment (Boussiba and Richmond 1980).

Phycobiliproteins are water-soluble, scavenge free radicals and are strongly fluorescent. Among several potentially useful effects of phycocyanins which have been shown are antioxidant and anti-inflammatory properties (Benedetti et al. 2004). C-PC is a selective inhibitor of cytochrome oxidase 2, resulting in hepatoprotective action and a reducion in leucotriene B4 levels, a reaction responsible for its anti-inflammatory properties. It has also shown to have therapeutic value by immunomodulating activity and anticancer activity (Rasool and Sabina 2009).

Table 26.2 Commercial application of phycobiliproteins

Industrial sector	Product/application
Food industry	Candy, chewing gums, ice creams, dairy products, soft drinks, wasabi
Cosmetic industry	Eye shadow, eye liner, lip sticks
Analytics	Flow cytometry, fluorescent activated cell sorting, fluorescence immunoassay and fluorescence microscopy

An important application for phycobiliproteins is their use as natural dyes in foods and cosmetics, thus replacing synthetic colourants that are often toxic, carcinogenic or otherwise unsafe (Bermejo Román et al. 2002). The phycocyanin extract produced from *Arthrospira* is market under the name 'lina blue' and used for different products in food and cosmetic industry (Table 26.2). In the analytical sector both PC and PE are being used as fluorescent tags.

The industrial production of phycocyanin (C-PC) is based on open pond cultures of *Arthrospira platensis*, that of phycoerythrin on *Porphyridium cruentum* (Bermejo Román et al. 2002). Although C-PC is presently extracted from *Arthrospira*, other cyanobacteria have been used for downstreaming method development as well (Table 26.3). The establishment of C-PC production with *Arthrospira* is mainly due to its availability from outdoor cultivation in Asia. The C-PC yield depends mainly on volumetric biomass productivity and the C-PC content of the cells. Table 26.4 summarizes the organisms, their biomass productivity in different photobioreactor types used as well as the C-PC content in the biomass.

Although autotrophic, mixotrophic and heterotrophic cultivation have been investigated for the production of *Arthrospira* (Chojnacka and Noworyta 2004), the highest productivity was achieved under mixotrophic conditions. Both biomass and pigment production was enhanced in the range of 1.5–2-fold compared to photoautotrophic growth (Marquez et al. 1995), but this has not been established in commercial cultivation, mainly due to quality issues. The total bacterial count cannot be controlled in open systems, as organic C sources lead to a much faster growth of obligate heterotrophs. Comparisons of heterotrophic growth of various axenic *Arthospira* strains have identified glucose and fructose as suitable C sources in concentrations of 20 mM (Chojnacka and Noworyta 2004; Mühling et al. 2005a). Both growth rate and C-PC productivity are lower than with autotrophic and mixotrophic growth (Eriksen 2008) and greater effort is required for sterilization of culture equipment. Heterotrophic production of C-PC with the rhodophyte *Galdieria sulphuraria* may have greater potential, since biomass productivity of 50 g L^{-1} day^{-1} and C-PC productivity of 0.9 g L^{-1} day^{-1} have been measured

Table 26.3 Downstream processing methods for purification of phycocyanin from cyanobacteria (Based on Eriksen 2008)

Cell disruption (CD): Liquid nitrogen (CD1), sonication (CD2), freeze thaw cycles (CD3), osmotic shock (CD4), enzymatic treatment (CD5)
Extraction (E): Phosphate buffer (E1), aqueous two phase extraction (E2), aqueous extraction (E3)
Precipitation (P): $(NH_4)_2SO_4$ (P1), rivanol (P2)
Impurity adsorption (A): Activated charcoal (A1), chitosan (A2)
Concentration (C): Dialysis (C1), ultrafiltration (C2)
Chromatography (CH): Anion exchange chromatography (CH1), gel filtration chromatography (CH2), expanded bed chromatography (CH3), hydroxyapatite chromatography (CH4), hydrophobic interaction chromatography (CH5)

Species	Purification method	Purity C-PC	Yield	References
Arthrospira platensis	E2 (PEG 4000/potassium phosphate)	4.05	85%	Patil and Raghavarao (2007)
	P1, C4, CH1	4.15	–	Boussiba and Richmond (1979)
	CD3, P1, C1, CH1 (DEAE-Sepharose, fast flow)	5.59	67%	Yan et al. (2011)
	P1, CH1, CH2	5.06	–	Zhang and Chen (1999)
	E2, CH1	6.69	–	Patil et al. (2006)
	CD1, E1, P1, C1, CH2	4.98	–	Bhaskar et al. (2005)
	CD2, E1, A1 & A2, C2, CH1 (DEAE Sephadex A-25)	4.3	42.3%	Liao et al. (2011)
	CH3 (Phenyl-Sepharose)	2.87	3.1%	Niu et al. (2007)
	CH1	3.2	0.77%	
	CH4	3.2	0.45%	
Arthrospira maxima	A1, C2	0.74	14.1%	Herrera et al. (1989)
	A1, P1, C1, CH2 (G200), CH3 (DEAE 100)	3.91	3.6%	
	E2, C2, P1	3.8	29.5%	Rito Palomares et al. (2001)
Arthrospira fusiformis	P1 & P2, CH2 (Sephadex G-25), P2	4.3	46%[a]	Minkova et al. (2003)
Aphanizomenon flos-aquae	P1, CH4	4.78	–	Benedetti et al. (2006)
Microcystis aeruginosa	CD3, E1, CH1 (DEAE cellulose)	–	–	Padgett and Krogmann (1987)
Synechocystis aquatilis	CD4, CH1 (DEAE-cellulose)	–	69%	Ramos et al. (2011)
Synechococcus sp. IO9201	CD3, CH5, CH1 (Q-sepharose)	4.85	–	Abalde et al. (1998)
Phormidium fragile	CD1, P1, CH5	4.52	62%[a]	Soni et al. (2008)
Phormidium ceylanicum	CD3, C2, CH1	4.15	63.5%[a]	Singh et al. (2009)
Oscillatoria quadripunctulata	CD3, P1, CH2 (Sephadex G-150), CH1 (DEAE cellulose)	3.31	68%[a]	Soni et al. (2006)
Calothrix sp.	CD5 (lysozyme), CH1 (Q-Sepharose, fast-flow), CH5	–	–	Santiago-Santos (2004)

[a]Referring to the crude extract

Table 26.4 Overview of biomass and phycocyanin productivity in two cyanobacteria and the rhodophyte *Galdieria* sulphuraria according to production and cultivation conditions (Based on Eriksen 2008)

Species	PBR	Volume (L)	Conditions	P_x (g L^{-1} d^{-1})	P_{C-PC} (% of dm)	References
A. platensis	Raceway	135,000	Autotrophic	0.05	6.1	Jiménez et al. (2003)
	Raceway	300	Autotrophic	0.18	6.7	Pushparaj et al. (1997)
	Tubular	11	Autotrophic, ID 0.01 m	1.32	7.0	Carlozzi (2003)
	Bubble column	12	Autotrophic, ID 0.47 m	1.05	7.0	Zitelli et al. (1996)
	Fermentor	2.5	Mixotrophic, fed batch	0.82	12.5	Chen and Zhang (1997)
	Fermentor	2.5	Autotroph		13.5	Chen and Zhang (1997)
Anabaena sp.	Raceway	300	Autotroph	0.24	5.6	Moreno et al. (2003)
Galdieria sulphuraria	Fermentor	2.5	Heterotroph	50	1.8	Graverholt and Eriksen (2007)

P_x volumetric biomass productivity, ID inner diameter

(Graverholt and Eriksen 2007); however, the absolute C-PC content is only about one-third that of *Arthrospira*.

The C-PC content in *A. platensis* ranges from 6.1% to 13.5% biomass, it being easier to obtain high values in small-scale laboratory fermentors because adequate light supply can be realized. Glucose has no or only minor effects on the C-PC content of *Arthrospira* (Chen et al. 1996; Eriksen 2008). The light supply can be considered the most critical factor for biomass productivity in autotrophic cultures (Pulz and Scheibenbogen 1998). In industrial scale raceway ponds an average value of 6% C-PC is reached with a biomass productivity of 14–23.5 g m^{-2} day^{-1}

(Pushparaj et al. 1997; Jiménez et al. 2003; Moreno et al. 2003). As soon as closed photobioreactors are applied for biomass production, the lower light path and higher cell densities result in higher productivities of biomass and C-PC. Volumetric productivity values above 1 g L^{-1} day^{-1} have been reported for small-scale photobioreactors, corresponding to 47.7 g m^{-2} day^{-1} (Carlozzi 2003). The absolute C-PC content has not been found to be significantly higher in closed photobioreactors, although its productivity was enhanced by a factor nine compared to value reported by Pushparaj et al. (1997). However, no data on C-PC production in large-scale closed photobioreactors are available, probably because of the high production costs, that are not compensated by the higher productivity. The C-PC content in *Anabaena* sp. was about 5.6%, and thus in the same range as *Arthrospira* (Moreno et al. 2003), whereas that of *Aphanizomenon flos-aquae* can be as high as 15% dry matter (Benedetti et al. 2004).

Several researchers have investigated the possibility of recombinant production of C-PC in *E. coli* (Cai et al. 2001; Tooley et al. 2001; Ge et al. 2005; Guan et al. 2007). The work is being hampered by the fact that the α- and β- chain of those multi-chain proteins needs to be expressed simultaneously together with the synthesis and correct insertion of the chromophores (Eriksen 2008).

For the downstream processing of the C-PC, methods with varying purities and yields of the pigment have been developed for different *Arthrospira* spp. (Boussiba and Richmond 1979; Abalde et al. 1998; Sarada et al. 1999; Zhang and Chen 1999; Rito Palomares et al. 2001; Bermejo Román et al. 2002; Doke Jr 2005; Niu et al. 2007; Oliveira et al. 2008; Patil et al. 2008; Soni et al. 2008; Ramos et al. 2011), *Aphanizomenon flos-aquae* (Benedetti et al. 2006), *Microcystis aeruginosa* (Padgett and Krogmann 1987), *Synechococcus* (Abalde et al. 1998), *Phormidium fragile* (Soni et al. 2008), *Phormidium ceylanicum* (Singh et al. 2009), *Calothrix* (Santiago-Santos 2004), *Oscillatoria quadripunctulata* (Soni et al. 2006), *Synechocystis aquatilis* (Ramos et al. 2011) and the rhodophyte *Porphyridium cruentum* (Bermejo Román et al. 2002).

Differences in the methods are needed because of the differing morphology of the various organisms, such as the structure of cell walls and membranes; no standard technique exists (Sekar and Chandramohan 2008). For high pigments yields, the cell wall needs to be broken prior to PC extraction. In the case of wet biomass processing, freezing-thawing-cycles have been found to be very effective for the rhodophyte *P. cruentum* (Abalde et al. 1998) and this method has been employed successfully for *Arthrospira* (Zhang and Chen 1999) and *Phormidium* (Soni et al. 2008). For cell disruption, mechanical methods such as ball mills, high pressure homogenization, French press, liquid nitrogen, mortars or sonication are used (Eriksen 2008). The application of diluted buffers for an osmotic shock or the use of enzymes such as lysozyme has also been reported for the extraction of phycocyanins (Boussiba and Richmond 1979; Sekar and Chandramohan 2008). Unfortunately many of the methods used in the laboratory are non-scalable (Bermejo Román et al. 2002). If dried biomass is extracted, it has been reported that a high drying temperature during the processes (flow drying, spray drying, sun drying and oven drying) are responsible for a significant yield reduction of C-PC (up to 50%) in *Arthrospira* (Sarada et al. 1999; Doke Jr 2005; Oliveira et al. 2008). After cell disruption, purification of the C-PC is done, usually in a two-step process involving extraction and purification of the raw extracts. Although different methodologies have been proposed for purifying phycobiliproteins, only some of them are useful for scale-up. The extraction from phycobilisomes and precipitation is mainly carried out by a $(NH_4)_2SO_4$ solution (overnight), whereas high ionic strength (0.5 M) leaves the phycobilisomes intact. Another frequently used extraction method is the resuspending of the biomass in phosphate buffer (0.1 M, pH = 7) and incubation at 4°C for 24 h (Doke Jr 2005). C-PC is thereby stable in the range of pH 5–7.5 at temperatures of 9–40°C, temperatures of above 40°C led to instability (Sarada et al. 1999). Another approach to C-PC purification was introduced by Rito Palomares et al. (2001), an aqueous two phase system (ATPS) using polymers (polyethylene glycol) and salts. This method in combination with ion exchange chromatography resulted in the highest C-PC purity reported so far (Patil et al. 2006) (Fig. 26.4).

Typically, phycocyanins are purified after extraction by a combination of several chromatographic techniques such as anion-exchange chromatography (Bermejo Román et al. 2002; Liao et al. 2011; Yan et al. 2011) with a pH gradient elution, hydrophobic interaction chromatography (Soni et al. 2008), gel filtration chromatography, column chromatography with hydroxyapatite (Benedetti et al. 2006), expanded bed adsorption chromatography (Ramos et al. 2011), as well as combinations of these (Abalde et al. 1998). Bermejo Román et al. (2002) developed a one-step scalable chromatographic method that provides B-PE solutions in hexameric aggregation state, as well as pure fractions of R-PC from *P. cruentum*. Dehydration is carried out by freeze drying, the gentlest method available. Liao et al. (2011) applied an additional adsorption step with chitosan and activated charcoal in order to remove impurities, which is cheap and effective and yields food grade purity.

The purity of the phycocyanins is expressed as ratios of A_{620}/A_{280} for C-PC and A_{650}/A_{280} for APC, respectively, while absorption at 650 or 620 nm accounts for the phycocyanobilin content which is specific for the protein, and the absorption at 280 nm, which represents all aromatic amino acid residues of the proteins. Throughout the purification methods A_{620}/A_{280} values between 0.7 (Herrera et al. 1989) and 6.69 have been recorded (Patil et al. 2006). A ratio greater than 0.7 is

recognized as food grade quality, while A_{620}/A_{280} of 3.9 is considered reaction grade and A_{620}/A_{280} of above 4.0 is analytical grade (Herrera et al. 1989). The overall yields of the downstream process vary with the method applied; being about 46% based in crude extract (Minkova et al. 2003) and go up to 85% total yield as reported by Patil and Raghavarao (2007).

In the development of the purification methods the focus is clearly laid on methods that comprise one chromatographic step; furthermore they need to be scalable, inexpensive and time saving. Both purity and yield serve as quality parameters.

Depending upon the purity and application required, the price for phycobiliproteins ranges between US$ 3 and 25 per mg for the pigment and up to US$ 1,500 per mg for antibody-complexes (Spolaore et al. 2006). Currently, numerous companies are producing PC, while 297 patents have been found by Sekar and Chandramohan (2008) in this connection. This documents the high degree of commercialization of cyanobacterial biotechnology in this field. Development will certainly go beyond applications in diagnostics and photodynamic therapy and extend to cosmetics, nutrition and pharmacy.

26.4.3 Animal Feed

Survival, growth, development, productivity and fertility of animals are basically determined by their health. Feed quality is the most important exogenous factor influencing this, especially in connection with intensive breeding conditions and the trend to reduce or to avoid antibiotics. The feeding trials of the past employing algal biomass were dominated by the use of high proportions of the common feed (up to 50%), aiming to replace the raw protein source. Now that research results have shown that very small amounts of microalgal biomass can have a positive effect on the physiology of some animals, lower amounts (0.1–10%) of *Arthrospira* are being used. Besides the positive effects of vitamins, minerals and PUFAs, an unspecific immune response and boosting of the immune system of animals has been observed and are considered to contribute to the positive results (Khan et al. 2005). The enhancement of growth, fertility, survival rate, live weight, feed conversion efficiency, resistance to bacterial and viral infections and enhanced colour have been reported in chickens, buffalos, prawns, salmon, carp and tilapia (Belay et al. 1996).

Arthrospira has proved very effective in the poultry industry for the colouration of egg yolk (Vonshak 1997a), reaching a maximum after 7 days diet. A content of 21% *Arthrospira* in the diet led to the colour score being 37% higher than indigenous eggs and 2.6 times more effective than dehydrated berseem (*Trifolium alexandrinum*) meal and 1.9 times more than 40% yellow maize. At all used proportions *Arthrospira*-fed birds produced egg yolks with a deeper colour than the two conventional carotenoid sources, clover and maize. The investigation of different processing methodologies in the preparation of quail feed revealed that freeze drying of the biomass is preferable to an extrusion process, since the latter degrades the carotenoid content of the cyanobacterial biomass by heat. Due to high processing costs freeze drying is not dominating feed production. The carotenoid content in the extruded *Arthrospira*-feed was also influenced by the raw material used and were higher for cassava than for corn or barley products (Ross et al. 1994). Sufficient colour scores of quail eggs were obtained with comparably low *Arthrospira* contents (0.5–4%) with no adverse effects on egg production, egg weight, final body weight or mortality. The enhancement of meat colour in muscle of broiler chickens with the use of 40 g kg^{-1} *Arthrospira* in the diet was positively correlated with the zeaxanthin content in the flesh (Toyomizu et al. 2001).

There are a number of reports of increased animal production in addition to improved quality. Supplemention of corn-based diet for hens with 1% *A. platensis* (1 g biomass per hen per day) led to an average increase of 51.7% in eggs laid over a trial period of 30 days (Fig. 26.9) compared to the control group (Fig. 26.9) (Storandt et al. 2000), whereas the egg weight increased by 2.4%. Moreover the firmness of the eggshell, the appearance of the plumage and general health were all influenced positively. The positive effect on egg productivity was still measurable in the subsequent period, when feeding was carried out without *A. platensis* addition. After another 10 days the number of produced eggs was identical between the cyanobacterial-fed group and the control. In a feeding trial on broiler hens a diet containing 0.1% *A. platensis* was shown to result in increased nutrient uptake and final slaughter weight (Pulz et al. 2008). The reduced percentage of *A. platensis* in the diet compared to previous work is an important cost factor for the farmer; economic analysis has shown that the increased slaughter weight pays for the increased feeding costs.

Aquaculture holds a specific position in the use of algal biomass as feed, since algae are the basis of the natural food chain in almost all aquatic systems. Beside the direct nutritional use for molluscs, zooplankton, crustaceans and fish larvae, they are being used as an addition for the enhancement of colouration, growth and immunity. More than 40 species of microalgae are being used in aquaculture worldwide depending on the special requirements for production. Key features needed are adequate cell size for ingestion by the particular animal, the absence of toxins and the nutritional profile, including ω3 fatty acids. Marine aquaculture is an important economic sector worldwide with a predicted growth trend of 8% p.a., which will lead to an increase in growth systems using artificial ponds. As feed production contributes a major cost input in aquacultural production, biotechnological production of algae is increasingly the focus of interest for

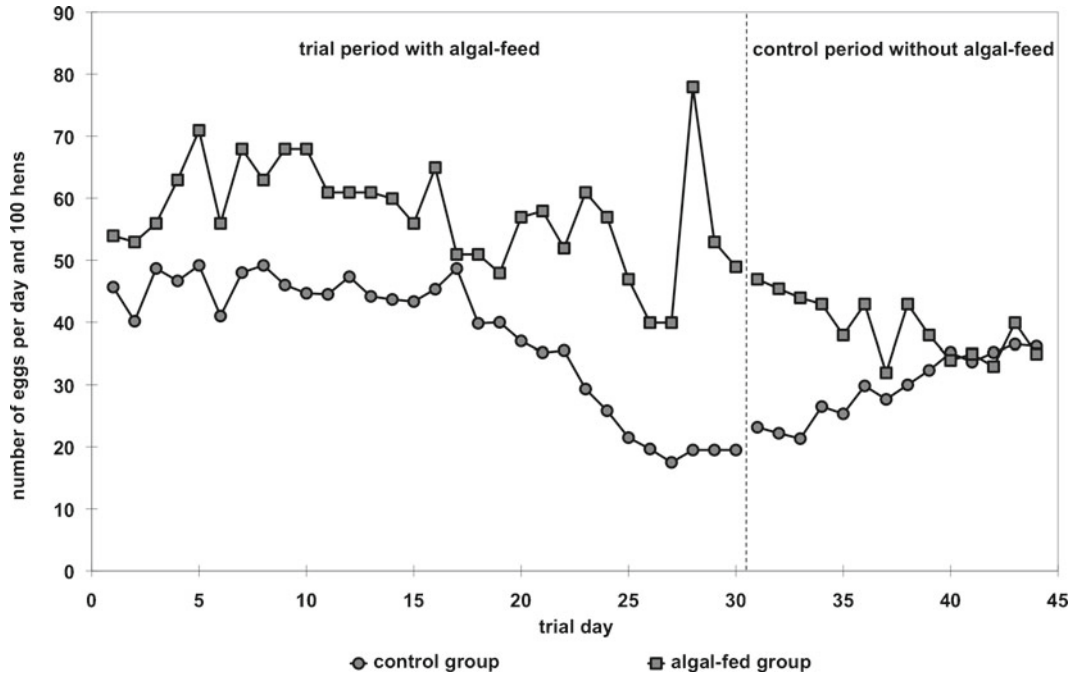

Fig. 26.9 Feeding trial for the investigation of the effect of 1% *Arthrospira* diet on the egg-laying productivity of laying hens in a free range breeding company in Brandenburg, Germany, compared to laying hen feed over a period of 30 days (*Arthrospira* uptake of 1 g per hen per day)

aquaculturists. The worldwide production of long chain polyunsaturated fatty acid containing eukaryotic algae, such as *Nannochloropsis*, *Tetraselmis* and *Isochrysis*, was estimated to be 1,000 t (Muller Feuga et al. 2003). Diverse, mostly technically inadequate equipment is used, resulting in low quality biomass, low yields and a high cost level. Cost estimates for microalgal products in the aquaculture sector normally range between US$ 50 and 200 per kg dry weight.

Among cyanobacteria *Arthrospira* is currently mainly used for aquaculture feed (Muller Feuga 2004), with most experience in the Japanese region. Its benefits have been investigated by several authors. El-Sayed (1994) reported that silver seabream utilized *Arthrospira* biomass more efficiently than either soy bean meal and chicken offal meal. The examination of *A. platensis* in the diet of tilapia (*Oreochromis mossambicus*) showed that it can replace up to 40% of the fish meal protein in tilapia diets (Olvera Novoa et al. 1998). Another species of tilapia (*O. niloticus*) fed solely on raw *Arthrospira* maintained its normal reproductive performance (fertilization rate, hatching rate of the fertilized eggs, survival time of larvae) throughout three generations (Lu and Takeuchi 2004). Other cyanobacteria have also been tested for their suitability as the sole source of nutrition. Cultured *Phormidium valderianum* was used successfully for tilapia production in India (Thajuddin and Subramanian 2005). The utilized strain tolerated high salinities and the biomass was incorporated into feed pellets in order to reduce handling at the production site. In view of the need for water quality control in aquaculture, *Arthrospira* has been successfully co-cultured with black tiger shrimp (*Penaeus monodon*), resulting in reduced N concentrations in the tanks and enhanced shrimp survival rate (Chuntapa et al. 2003).

Apart from feeding larvae and zooplankton the addition of *Arthrospira* to common fish feed compositions seems to be a promising strategy. Initially, the colour-enhancing effects of *Arthrospira* biomass were exploited for ornamental fish (Benemann 1992) and crucian carp (Min et al. 1999). The inclusion of *Arthrospira* into the diet of pond-reared prawn (*Fenneropenaeus indicus*) eliminated the pigment deficiency syndrome (PDS) at a level of 30 g kg^{-1} in the diet after a 4-week period (Regunathan and Wesley 2006). This confirmed the bioavailability of carotenoids from *Arthrospira* for shrimp broodstock and its regular use in the diet was recommended to avoid carotenoid deficiency-related problems in shrimp hatcheries.

With increasing use of *Arthrospira*, questions of feed utilization and health status in dense aquacultural fish populations became more important. Therefore, the immunomodulatory effects of *A. platensis* have been investigated by its inclusion in the diet of the carp *Cyprinus carpio*. For instance, immunostimulant effects were demonstrated by Watanuki et al. (2006). In an earlier study by Schreckenbach et al. (2001) both the unspecific cellular immune defense and the humoral specific immune immune response of the carp population were enhanced. The content of *Arthrospira* in the diet and the processing form of the biomass were identified as the main factors influencing how effective the response

Table 26.5 Examples of active substances from algae (utilized and potential sources) in marketed and potential cosmetic products (Modified after Sandau 2010)

Cosmetic activities	Active substances	Algae division	Utilized algae/potential sources
UV protection	Phlorotannins	Phaeophyta	*Ascophyllum nodosum*
	Colorless carotenoids	Chlorophyta	*Dunaliella salina*
	Mycosporin-like amino acids	Cyanobacteria	*Anabaena, Oscillatoria, Nostoc commune, Scytonema*
		Rhodophyta	*Porphyra umbilicalis*
	Photolyase	Chlorophyta	*Dunaliella salina*
	Scytonemin	Cyanobacteria	*Scytonema*
Skin protection	Radical scavenger: tocopherols, superoxid dismutase, polyphenols, β-carotene, carotenoids	Chlorophyta	*Dunaliella salina* *Haematococcus pluvialis*
	Phytohormones: auxins, gibberellins, cytokinins, abscisic acid and betaines	Phaeophyta	*Ascophyllum nodosum* *Stypocaulon scoparium*
Hydration/moisturizers skin protection	Polysaccharides, mucopolysaccharides, sulphated polysaccharides	Eustigmatophyta Rhodophyta Cyanobacteria	*Nannochloropsis oculata* *Porphyridium cruentum* *Arthrospira platensis*
Skin smoothing/skin regeneration	Essential amino acids	Cyanobacteria Chlorophyta	*Arthrospira platensis* *Chlorella vulgaris*
	Polyunsaturated fatty acids	Eustigmatophyta Rhodophyta	*Nannochloropsis oculata* *Porphyra umbilicalis*
Skin lightening/ skin whitening	Phlorotannins, phloroglucinol and its oligomers	Phaeophyta	*Ascophyllum nodosum* *Undaria pinnatifida*
Stimulating collagen synthesis	Low molecular weight fucans	Phaeophyta	*Fucus vesiculosus*

was. A similar immunomodulatory effect was reported for tilapia (*Oreochromis nilotocus*), but in this case correlated with the additional β-1,3-glucan content in an *Arthrospira*-containing diet (Cain et al. 2003).

In terms of cost effectiveness the immune enhancement and antibacterial and antiviral activities are much more desirable than the use of the biomass in high proportions as a partial substitute for higher plant or animal proteins. *Arthrospira* can help protect against the many pathogens which diminish the yields in aquaculture and agriculture industry. The use of antibiotics to control pathogens is costly and has undesirable health consequences for consumers. Today more studies on its immunomodulatory, antiviral and anti-cancer effects on various animals are available than on humans. Supplementation with *Arthrospira* may offer an alternative to common strategies relying on chemicals. Nevertheless the potential for incorporating cyanobacteria in feed is not utilized today. To the best of our knowledge not more than 1% of the worldwide produced biomass is utilized for different feed applications.

26.4.4 Cosmetics

With an aging world population and increasing per capita income, we see a growing market share for cosmetics in general and for anti-aging products in particular, especially in Europe, the USA and Asia. Along with their valuable nutritional ingredients many algae contain active dermogenic substances. Currently numerous products containing cyanobacterial extracts have been formulated and are being marketed, such Protulines® and Aquaflor®, both of which contain *Arthrospira* extracts with proven moisturizing and anti-wrinkling effects. Water extracts of *Arthrospira* with a high magnesium salt content were found to facilitate both ATP and matrix protein synthesis, resulting in a stimulation of keratinocyte differentiation (Schlotmann et al. 2005). The proportion of those extracts is typically in the range of 2–10% of the final formulation.

Amino acids represent 40% of the group of natural moisturizing substances and contribute to the hydration of the corneous layer cells by holding water. In cosmetic products they are being used for regulating softness, flexibility and elasticity of the skin. Our investigations have shown that hot-water extracts of *Arthrospira* contain particularly high amounts of amino acids, since during extraction the water soluble proteins are being degraded. These extracts are therefore frequently used as moisture regulating products. In recent years mainly aqueous and aqueous-ethanolic extracts have been applied in different cosmetic products such as creams, lotions, sun and hair care. For lipid-based cosmetics, like creams or lotions, supercritical CO_2-extracts are gaining commercial importance, because toxic solvents can be avoided. Mendiola et al. (2007) described an antibacterial and antifungal CO_2 extract from *A. platensis*, and also a tocopherol- enriched extract (Mendiola et al. 2008), both of which are of potential interest for cosmetic preparations. Table 26.5 compares

marketed and potential products containing active ingredients from *Arthrospira* with those from eukaryotic algae.

Aphanizomenon and *Arthrospira* produce high molecular weight polysaccharides, which have been reported to have in vitro higher immunostimulatory activities than commercially available immunotherapeutica (Pugh et al. 2001). As an example calcium spirulan (Ca-Sp) is of particular interest for its use in cosmeceuticals. A 3-step purification process for the polysaccharide from *Arthrospira* has been developed and high anti-HSV activities detected (Sandau and Pulz 2009). Ca-Sp stimulates the metabolic activity of human scin fibroblast cell lines (NHDFc), which are responsible for collagen synthesis and firmness of the skin. With increasing age the collagen synthesis drops significantly, so a main target of cosmetic research is the development of anti-aging products capable of enhancing the metabolic activity of fibroblasts, such as shown for Ca-Sp. A 36% enhancement of collagen synthesis was found by applying Ca-Sp at 10 μg mL^{-1} (Sandau and Pulz 2009). It was also found that UV-A exposed fibroblasts showed a higher vitality, if Ca-Sp had been added prior to or even after radiation. Although the protective mechanisms have not yet been studied, the application of *Arthrospira* extracts or purified Ca-Sp seems promising for different cosmetic products, with an emphasis on anti-aging and sun screens, as well as on anti-HSV lipsticks.

Cyanobacteria are exposed to high oxygen and radical stresses, especially in extreme environments. This has resulted in the development of numerous efficient protective systems against oxidative and radical stressors (Whitton and Potts 2000). The protective mechanisms are able to prevent the accumulation of free radicals and reactive oxygen species and thus to counteract cell damaging activities. Because the antioxidative components originate from a natural source, their application in cosmetics for preserving and protecting purposes is developing rapidly. Since exposure of the skin to UV light is one of the main reasons for premature skin aging and also for skin cancer, sun-protecting cosmetics represent an area of high demand. Many cyanobacteria are capable of overcoming the toxicity of ultraviolet radiation by synthesizing UV-absorbing compounds (Chap. 19). The strongest UV-A-absorbing compounds in nature are the water-soluble mycosporine-like amino acids (MAAs) e.g. shinorine. They are small (<400 Da) molecules, consisting of cyclohexinimine or cyclohexenone, their synthesis being induced by UV-B radiation (Sinha et al. 2001). Other powerful UV-absorbing natural compounds are the scytonemins. These are lipid-soluble indole alkaloids of yellow brown colour found exclusively in cyanobacteria and accumulate in the polysaccharide sheath. The conjugated double bond system absorbs UV-A radiation so that they act as photo-protectants (Rastogi and Sinha 2009). These compounds are biotechnologically exploited by the cosmetic industry for the development of sunscreens.

In general the cosmetic market segment is changing rapidly and new products with skin- protecting characteristics are welcome to the industry. The almost untapped potential of the cyanobacteria with their vast adaptation mechanisms is of particular interest in this connection.

26.4.5 Biofertilizers

Macroalgae are used as soil fertilizer in coastal regions all over the world (Critchley and Ohno 1998; Zemke-White and Ohno 1999). The role especially of cyanobacteria in the soil ecosystem has thereby often been neglected. The main beneficial effects are numerous: increased water-binding capacity and water storage, particle adherence and decreased soil erosion, improvement of mineral composition of the soil, the production and secretion of bioactive compounds such as phytohormones, which stimulate the growth of agricultural crops (Stirk et al. 2002). These properties can also be used in liquid fertilizers produced from the macroalgae, such as in the development of cover for abandoned mining lands to avoid erosion and to initiate floral succession.

In the last years numerous studies have been carried out in order to include strain identification, isolation and culture, analyzing their N_2-fixing activity and related physiology, biochemistry, and energetic as well as the structure and regulation of nitrogenfixing (nif) genes and nitrogenase enzyme (Vaishampayan et al. 2001). Due to their wide tolerance of adverse environmental conditions like desiccation, hot temperatures etc. cyanobacteria appear to be particularly suitable for the use as fertilizers.

There have also been many studies on the use of cyanobacteria, but in this case usually as inocula to encourage the growth of particular species rather than effects due to the whole biomass. Various potentially other useful effects have been shown for the cyanobacteria, such as antifungal substances (Kim 2006). Pre-soaking rice seed with cyanobacterial cultures or extracts has been reported to enhance germination and growth, although consistent investigations are still lacking (Sharma et al. 2010). Overwhelmingly, however, it has been the ability of some cyanobacteria to fix N_2 which has the main interest. Some of the many accounts of the properties of N_2-fixing organisms and isolates from soils, especially rice-fields, were reviewed by Whitton and Potts (2000) and Vaishampayan et al. (2001). The study of N_2 fixation in rice fields of north-east Spain by Quesada et al. (1997) provides an example of the importance of cyanobacterial fixation; they estimated that N_2 fixed on a per crop basis was in the range of 5–>80 kg ha^{-1} N, the value beingly strongly influenced by environmental conditions. Vaishampayan et al. (2001) quoted an average of 20–30 kg N $crop^{-1}$ ha^{-1}. This can lead to a reduction in the need for N fertilizer. Using *Aulosira fertilissima* and *Anabaena*

doliolum with or without the combination of urea, the chemical N demand for a rice field in north India could be reduced by 25% (Dubey and Rai 1995). While cyanobacterial N_2 fixation to enhance crop yields and reduce use of N fertilizers, cyanobacteria can also be used in more arid regions to reduce erosion processes, because of their formation of EPS, which improves water-binding capacity and soil structure (Chap. 12). Some of the organisms used for this are also N_2-fixers.

Among the numerous studies on the use of cyanobacterial inocula, perhaps the most important aspect is whether or not they make use of indigenous strains. The study in Chile by Pereira et al. (2009) did incorporate *Anabaena iyenganii* and *Nostoc* spp. indigenous to the region in their biofertilizer for trials on local rice fields. The use of the fertilizer decreased the amount of synthetic nitrogen fertilizer (50 kg N ha^{-1}) required by as much as 50%, while still resulting in the same yield of 7.4 t ha^{-1} rice.

An alternative approach is to make use of *Azolla* with its symbiotic *Anabaena* (Chap. 23). This has been done for green manuring rice fields in China and Vietnam for centuries (Watanabe 1982). There are two principle methods used in various locations for manuring with *Azolla* (Sharma et al. 2010). It can be grown as a monocrop and incorporated to the paddy prior to the rice being planted; it can also be grown together with the rice. The rice yield is thereby enhanced by 0.5–0.75 t per ha. Nevertheless it has been shown that free-living cyanobacteria release ammonium into the water, where it can be utilized by the crop, whereas with *Azolla* the ammonium is not as directly available.

Although phytohormones and growth regulators have been recognized in cyanobacteria for a long time, they have gained increasing attention during the past decade (Tarakhovskay et al. 2007). More recent accounts include indole-3-acetic acid (IAA) in *Nostoc* sp. (Sergeeva et al. 2002) and gibberellin-like plant growth regulators in *Scytonema hofmanni* (Rodríguez et al. 2006); the latter reduced NaCl-induced growth inhibition in rice. IAA and cytokin were released to the growth medium by *Chroococcidiopsis* sp. Ck4 and *Anabaena* sp. Ck1 under both axenic and field conditions (Hussain and Hasnain 2011). Germination, shoot length, tillering, number of lateral roots, spike length, and grain weight were significantly enhanced in wheat. The authors concluded that cyanobacterial phytohormones are a major tool for improved growth and yield in wheat.

The results available indicate the strong potential for cyanobacterial biofertilizer technology in rice-growing countries, and applied biotechnology should contribute its part to increase biomass production so that the requirement for inorganic N can be reduced. A future trend seems to be the use of cyanobacteria against plant diseases caused by fungi, viruses or bacteria.

26.4.6 Wastewater and Exhaust Gas Treatment

The protection and preservation of the natural basis of life are not only ethical demands, but also essential for durable economic and social development. They create technological progress and jobs. Further development and improvement of existing systems for wastewater treatment, the reduction of problematic emissions and the need for water recycling are important objectives.

Micro- and macroalgae, sometimes in combination with other microorganisms, are utilized to treat wastewater and other effluents. Current uses include: removal of nutrients from circulating process water, use of CO_2 from industrial exhaust gas; disposal of contaminants from agricultural wastewater; purification of wastewater from biogas production; tertiary wastewater purification.

Governments and energy companies worldwide are showing an increasing interest in CO_2-fixation biotechnology, especially due to the introduction of CO_2 certificates. For example, Germany together with the EU, USA and Australia are conducting research efforts to find economically feasible processes for applying microalgae in environmental protection and CO_2-fixation (Pedroni et al. 2001). The cement industry, as one of the largest CO_2-emitting branches, is undertaking several investigations on how to use algae for the fixation of CO_2 (Ferey et al. 2010; Borkenstein et al. 2011).

In Germany, several projects have been completed to use both stack gas and condensed water out of this gas to produce microalgal biomass. The process has been scaled up to photobioreactor volumes of 2–6 m^3 and shown to be feasible, but not yet economic (Pulz and Gross 2004; Ferey et al. 2010). At a cement plant, both salt and freshwater algae can be cultivated using the CO_2 in the stack gas as the sole C source. Growth rates in terms of volumetric biomass productivity are high and comparable to cultures grown on pure CO_2. No harmful effects on growth or cell death could be detected within an experimental period on a cement plant in Southern France cultivating the eukaryotic algae *Nannochloropsis* sp. and *Scenedesmus* sp. (Ferey et al. 2010).

Several cyanobacteria have been used in the past decade for the treatment of agricultural or industrial wastewater. *Arthrospira* was used for pig wastewater treatment in Mexico (Olguín et al. 2003) after dilution with sea water, while *Nostoc muscorum* and *Anabaena subcylindrica* were used for industrial wastewater effluents in Egypt (El-Sheekh et al. 2005). In the latter case the growth rates were higher than in standard synthetic media. The main problem is the sterilization step prior to cyanobacterial cultivation, which hampers volumetric flow and economic feasibility. Markou and Georgakakis (2011) reviewed the utilization of cyanobacteria for the reduction of organic and inorganic load with an emphasis on *Arthrospira*. As the biogas production by

anaerobic fermentation of crops and wastes or sludges is established, cyanobacteria can be used in a second biological stage for the purification of the biogas while utilizing CO_2 for their growth and enhancing hereby the methane content in the biogas. This approach has been sucessful for *Arthrospira platensis* (Converti et al. 2009; Travieso et al. 1993) with high C utilization efficiencies. The combination of anaerobic digestion and the cultivation of microalgae seems promising and will support development of large-scale cultures (Sialve et al. 2009).

Micro- and macroalgae both have considerable ability to adsorb metals and there is considerable potential for their use in treating wastewater polluted by heavy metals. In general non-viable cells are able to adsorb more heavy metal ions than living cells, while the sorption capacity can be enhanced by pretreatment of the biomass with $CaCl_2$ (Mehta and Gaur 2005) or NaOH (Nagase et al. 2005). There are two mechanisms as basic steps responsible for the heavy metal removal effect: passive adsorption to the cell surface and the active uptake into the cytoplasm. For the adsorption process negatively charged groups (e.g. carboxyl-COOH) of the cell wall as well as functional groups of extracellular polysaccharides are available (De Philippis et al. 2003), a fast process where equilibrium is reached within minutes. The biosorption inside of the cell happens by binding of the cations to ligands, phytochelatins and metallothioneins, taking several hours before maximum uptake rate is reached. Whereas the adsorption processes are dependent upon temperature and pH, biosorption is influenced by the sum of the environmental factors that impair the metabolism of the cell. Compared with other biosorbents such as bacteria and fungi, algae show high sorption capacities and efficiencies to remove heavy metal cations as well as favourable sorption kinetics (Langmuir or Freundlich adsorption isotherms), indicating a chemosorption rather than physical adsorption (Chojnacka et al. 2005). The advantage of cyanobacteria in the field of biosorption of heavy metals is the fact that the biomass is locally and available cheaply in developing countries like China. Throughout the literature *Arthrospira* has been reported to be a very efficient biosorbent for lead, cadmium, chromium and copper cations. Gong et al. (2005) investigated the lead biosorption capacitiy of *A. maxima*; removal rates of 92% were obtained at pH 5.5. Investigation on Cr (VI) loaded wastewaters with *Lyngbya putealis* by Kiran and Kaushik (2008) and *Nostoc muscorum* by Gupta and Rastogi (2008) showed high removal ability and regeneration efficiency. *Oscillatoria laetevirens* and *Oscillatoria trichoides* were studied for their Cr^{6+} removal efficiency and comparably high sorptive capacities of 21.88 mg g^{-1} and 38.7 mg g^{-1} have been found. Living biomass showed a higher sorption capacity as dead biomass, tolerating Cr^{6+} concentrations of 30 mg L^{-1} (Miranda et al. 2012). Cd-selective adsorption ability was investigated in *Tolypothrix tenuis*, *Anabaena variabilis* and *Microcystis aeruginosa* and alkaline treatment was found to be a useful technique for producing biosorbents with highly specific binding abilities for heavy metals (Nagase et al. 2005). *Lyngbya taylorii* exhibited high uptake capacities of 1.47 mmol Pb, 0.37 mmol Cd, 0.65 mmol Ni and 0.49 mmol Zn per g dry biomass (Klimmek et al. 2001). Phosphorylation of the biomass enhanced uptake of metal ions by a factor of 2.1–6.8 by enhancing the anion density of the raw material. This additional step led to 637 mg Pb g^{-1} dry biomass, the highest value for any of the metals.

The EPS produced by many cyanobacteria are of special interest for wastewater treatment. Those anionic heteropolymers have been shown to remove heavy metals very effectively. In *Cyanothece* CE 4 and *Cyanospira capsulata* a very fast recovery of Cu^{2+} was observed, based on the anion density (De Philippis et al. 2001). The high removal capacity probably relates to the high proportion of uronic acid monomers in the polysaccharide fraction that is binding cations to its carboxyl moieties and which can reach 80% in *Cyanothece* (De Philippis and Vincenzini 1998).

Research dealing with heavy metal decontamination of aqueous or organic-aqueous solutions by hybrid biofilters, consisting of an inorganic oxide matrix and an algae biomass led to a patent (Böttcher et al. 2006) and by polyurethane foam, containing inactivated algae biomass or extraction residues to another (Falke et al. 1999). For the hybrid biofilter the combination of the two materials enhanced removal time so that shorter residence times could be realized. Good removal rates for lead and uranium have been achieved by the use of different micro- and macroalgae biomasses e.g. *Arthrospira*, *Scenedesmus*, *Fucus*, *Chlorella* and *Porphyridium*. Zhang et al. (2012) investigated the biodegradation of γ-hexachlorocyclohexane (lindane), a persistent toxic pesticide by *Anabaena azotica* isolated from Chinese paddy soil. Concentrations of 0.2 mg L^{-1} were well tolerated by the cultures and a removal of 48.8% was measured after 5 days of cultivation when grown on nitrate. The capability of *Anabaena azotica* to degrade, most likely by dechloriniation, opens the potential use in the bioremediation of contaminated soils.

The application of algal biosorbents is applicable if conventional processes of heavy metal disposal at relatively low heavy metal concentrations are uneconomic. The use of immobilized microalgae in these processes offers significant advantages with respect to solid liquid separation and accelerated reaction rates (Mallick 2002; Mehta and Gaur 2005) and is therefore applicable, as shown for *Arthrospira* by Patnaik et al. (2001). Especially in countries, where ternary wastewater treatment facilities have not been fully established yet, and cyanobacterial biomass is available cheaply, this application shows great potential and should be developed further in the near future. Additionally, they may

be added to conventional treatment facilities as "safety filters". Mobile plants for the treatment of contaminated surface, ground and wash waters would also be feasible.

26.5 Future Potential

26.5.1 Perspective

The increasing world population challenges our handling of available resources and the techniques for the production of food, feed and energy. There is little future potential for cyanobacterial biotechnology, unless the following are achieved: (1) an increase of annual biomass output in the range of at least 100-fold; (2) lowering the production costs; (3) development of new products. Valuable parts of algal biomass need to be incorporated in new, functional food and feed products in order to create markets that do not yet exist. There will be a need to adopt multidisciplinary approaches for high yielding biorefinery steps and a multi-product production strategy for cyanobacterial biomass.

The production of *Arthrospira* started with the claim to provide a cheap source of nutrition in order to feed the hungry. However, both cost and availability counteract this initial idea. The price of *Arthrospira* biomass is at least 14 times higher than for conventional plant based nutrition sources (Fig. 26.10) and 3 times higher than fish meal. Moreover the acceptance of the biomass by people that do not regularly consume algal biomass is very poor. It should be noted, however, that production of this biomass is not competing with land for food supply, which soon will become a major political question.

Nevertheless, the biotechnology of cyanobacteria is gaining momentum, especially in the preparation of valuable substances and in the field of bioenergy. Cyanobacteria have some potential for the production of biopolymers since some accumulate large amounts of poly-β-hydroxybutyrate (PHB) under certain cultivation conditions (e.g. *Nostoc muscorum* up to 21.5% of dry weight) (Haase et al. 2012). A quite new approach is the use of cyanobacteria for the intracellular bioconversion of metal ions to nanorods that are applicable in the in the development of biosensors and bio-imaging tools and have even potential as therapeutic agents (Parial et al. 2012). The nanoparticle synthesis of auric ions (Au3+) to gold nanorods was recently demonstrated in growing filaments of *Nostoc ellipsosporum*. The biotechnological production of renewable raw materials as an alternative to fossil resources or new bioconversion strategies will contribute to the enhanced cultivation of cyanobacterial biomass in the future. Both, the literature and patent situation imply optimistic developments for the future, some of which are highlighted in this section.

26.5.2 Bioactive Metabolites

Cyanobacteria are widely distributed in habitats ranging from aquatic to terrestrial environments as well as extreme habitats such as hot springs, hypersaline waters, deserts, and polar regions (Whitton and Potts 2000). As one adaptation strategy they produce a wide variety of chemically unique secondary metabolites with biological activities that include antiviral, antibacterial, antifungal, antimalarial, antitumoral or anti-inflammatory properties. Chemically, these compounds represent a wide range of drugs, including peptides, alkaloids and indole alkaloids, polyketides and terpenes (Table 26.6). The cyanobacteria have proved to be one, if not the, richest source of such bioactive metabolites (Sivonen and Börner 2008). Sharma et al. (2010) summarize

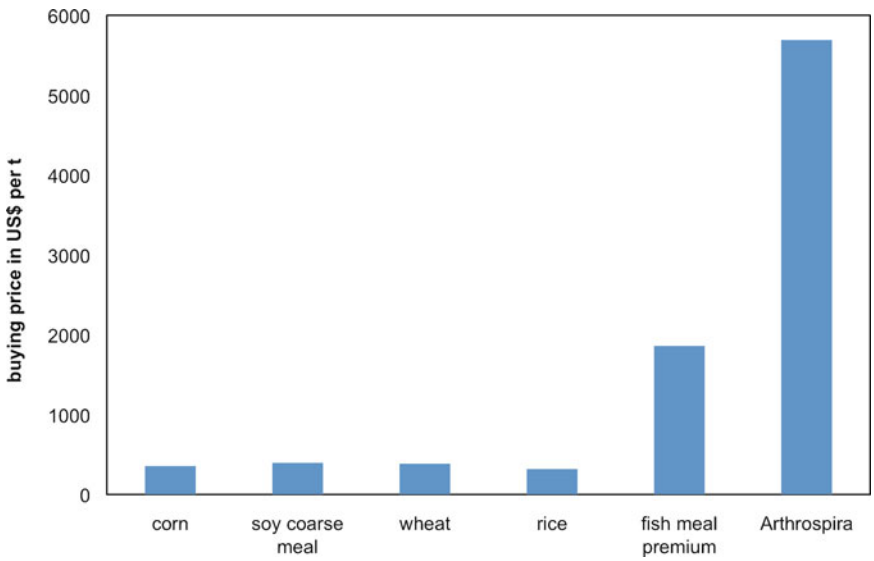

Fig. 26.10 Current buying prices for feeding stuff commodities compared to *Arthrospira platensis* biomass (W, Lehmann, 11.5.2011; Spezialfuttermittelwerk Beeskow, Germany; personal communication)

Table 26.6 Bioactive secondary metabolites isolated from cyanobacteria

Substance class	Chemical class	Examples	Biological activity	Mode of action	Organism	References
Depsipetide/polyketide	Cryptophycin	Cryptophycin 1	Anticancer	Inhibition of tubulin polymerization	*Nostoc* spp.	Trimurtulu et al. (1994)
		Cryptophycin 24	Antifungal			
		Cryptophycin 54				
Depsipeptide	Cyanopeptolins	Cyanopeptolin 1067A	Cytotoxic	Protease inhibitors	*Scytonema hofmanni* PCC 7110	Grewe (2005) and Gademann and Portmann (2008)
Depsipeptide	Microviridin	Microviridin J	Toxic to *Daphnia*	Protease inhibitors	*Microcystis viridis*	Rohrlack et al. (2003)
Depsipeptide		Largazole	Cytotoxic antiproliferative	HDAC inhibitor	*Symploca* sp.	Taori et al. (2008)
Depsipeptide	Lyngbyastatins	Lyngbyastatin 1	Cytotoxic	Serin protease inhibitor	*Lyngbya* spp.	Harrigan et al. (1998a)
			Anti-inflammatory			
			Anti-arthritic			
			Anticancer			
Cyclic peptide	Cyanobactins	Lyngbyatoxin	Tumor promotion inflammatory	Protein kinase C activation	*Lyngbya majuscula*	Jones et al. (2009)
Polyketide-peptide	Jamaicamides	Jamaicamide A	Cytotoxic	Sodium channel blocking	*Lyngbya majuscula*	Edwards et al. (2004)
Peptide	Microcystins		Antifungal	Inhibition of protein phosphatase	*Anabaena* spp.	Gupta et al. (2012)
Peptide	Symplostatins	Symplostatin 1	Anticancer	Antimitotic, inhibits cell proliferation	*Symploca hydnoides*	Harrigan et al. (1998b)
Peptide	Symplostatins	Homodolastatin 16	Anticancer	Antimitotic	*Lyngbya majuscula*	Davies-Coleman et al. (2003)
Peptide	Symplostatins	Dolastatin 10	Anticancer	Antimitotic	*Symploca* sp. VP642	Luesch et al. (2001)
Lipopeptide	Dragonamide	Dragonamide C, D	Anticancer		*Lyngbya polychroa*	Gunasekera et al. (2008)
		Dragonamide E	Antileishmanial		*Lyngbya majuscula*	Balunas et al. (2009)
Lipopeptide		Dragomabin	Antimalarial		*Lyngbya majuscula*	McPhail et al. (2007)
Lipopeptide		Spiroidesin	Anti-cyanobacterial		*Anabaena spiroides*	Kaya et al. (2002)
Thiazoline containing lipopeptide	Curacins	Curacin A	Anticancer (antiproliferative/ antimitotic)	Inhibitor of tubulin polymerization	*Lyngbya majuscula*	Gerwick et al. (1994)
Protein	Cyanovirins	Cyanovirin N	Antiviral (HIV-1, HIV-2, HSV-6, measle)	Inhibit fusion to host cells	*Nostoc ellipsosporum*	Boyd et al. (1997)
Polyketide		Borophycin	Antibiotic, anticancer		*Nostoc linckia*	Hemscheidt et al. (1994)
					Nostoc spongiaeforme	
Indole alkaloids		Hapalindole, Welwitindolinone Ambiguine	Anti-algal, antifungal and insecticidal		*Fischerella musciola, Hapalosiphon fontinalis*,	Gademann and Portmann (2008)
					H. weltwitschi	
Indole alkaloids	Bauerines, b-carboline	Bauerine A-C	Anti HIV 2		*Dichothrix baueriana* GO-25-5	Larsen et al. (1994)
Indole alkaloids	b-carboline	Norharmane	Anticyanobacterial		*Nodularia harveyana*	Volk (2005)

b-carboline alkaloid	b-carboline	Nostocarboline	Antiplasmodial	Cholinesterase inhibition	*Nostoc* 78-12A	Becher et al. (2005)
Indole alkaloide		Nostodione	Antifungal cytotoxic	Antimitotic	*Nostoc commune*	Kobayashi et al. (1994)
Indolocarbazole alkaloids	Tjipanazole	Tjipanazol A1	Antifungal		*Tolypothrix tjipanensis*	Bonjouklian et al. (1991) and Falch et al. (1995)
		Tjipanazol D	Antibacterial		*Fischerella ambigua*	
Alkaloids, Pyrrolidin-Diine	Fischerillins	Fischerellin A	Allelopathic herbicidal, antialgal antifungal	Photosystem II inhibition	*Fischerella muscicola*	Hagmann and Jüttner (1996)
Alkaloids, Indolophenanthridine	Calothrixins	Calothrixin A	Antiplasmodial anticancer		Calothrix sp.	
					Fischerella sp.	
Macrolide/lacton	Scytophycins	Tolytoxin	Anticancer, cytostatic, antifungal	Inhibition of actin polymerization	*Scytonema* sp.	Patterson and Carmeli (1992) and Patterson et al. (1993)
Sulfolipids	Sulfoquinovosyldi-acyglycerol		Anti HIV	Inhibition of reverse transcriptase	*Lyngbya lagerheimii* *Phormidium tenue*	Gustafson et al. (1989)
			Anticancer			
Porphyrin		Tolyporphin	Anticancer reversing multi drug resistance	Photosensitising	*Tolypothrix nodosa*	Prinsep et al. (1992)
Sulphated polysaccharide		Calcium Spirulan	Antiviral (HSV, cytomegaloviruses, measles, mumps, Influenza A, HIV-1) anticancer	Inhibition of penetration to host cells, inhibition of membrane invasion by tumor cells	*Spirulina* sp.	Hayashi et al. (1996) and Lee et al. (2001)
HDAC Histone Deacetylase Inhibitor	Pyrazolotriacine	Nostocine A	Antialgal cytotoxic		*Nostoc spongiaeforme*	Hirata et al. (2003)

24 novel bioactive compounds isolated from genera such as *Symploca*, *Lyngbya*, *Nostoc*, *Oscillatoria*, *Anabaena*, *Microcystis* and *Nodularia* during the decade up to the time of their review. The structurally varying metabolites are derived from mixed biosynthetic pathways and are active in the concentration range of pico- to nano-molar. More than 300 N-containing bioactive metabolites in cyanobacteria have been isolated and reported on, with the largest number being from *Nostoc* and *Symploca* (Tan 2007) details of more than 120 cyanobacterial alkaloids were published between 2001 and 2006. This research led to the identification of curacin A and dolastatin 10, which are being evaluated as anti-cancer agents or have triggered the creation of analogues (Harvey 2008). It can be summarized that cyanobacterial metabolites target specific enzymes or macromolecules related to processes that are malfunctioning in illnesses that involve cell proliferation, such as in the case of cancer. Those can be targets for the development of pharmaceuticals.

The great increase in publications in this field shows the large potential and the capability of cyanobacteria to synthesize complex metabolites with useful properties. The potential of many other compounds for clinical applications is currently under investigation. Although many of the substances isolated have potential therapeutic uses, none have yet reached clinical use. Several reasons are responsible for this. *In vivo* the activity is mostly lower or absent and any synergistic effects in raw extracts are no longer shown. At the same time toxicity may be higher. Nevertheless it seems very promising to screen and culture cyanobacteria that have not been investigated so far. New species will no doubt contribute to the finding of new substances. Neither the numerous culture collections nor more than a small number of likely habitats have been examined in depth, there is an untapped potential that awaits exploration. Cyanobacteria can serve as a prime source both for novel bioactive compounds and for leads of drugs or analogues with improved characteristics (lower toxicity, higher solubility).

A new approach is metagenomics, where synthetic abilities of organisms can be evaluated by cloning their DNA into host organisms like *E. coli*; the resulting recombinant bacteria are cultured and tested for the expression of bioactive metabolites. Molecular screening techniques can then be applied in order to identify the presence of secondary metabolite genes in species that either were not investigated or had not show bioactivity so far. Investigations on the distribution of peptide synthetase genes and polyketide synthase genes have been carried out in Nostocales, Chroococcales and Oscillatoriales. Gupta et al. (2012) investigated 28 *Anabaena* strains and identified the toxic ones by amplification of a microcystin synthase gene, linking them to previously assayed antifungal activity (see Table 26.6). Cyanobacteria can also be used for the production of recombinant proteins, such as for the treatment of diseases like HIV or to combat mosquito larvae (Sharma et al. 2010), and for the production of recombinant compounds of medicinal and commercial value. The advances in culture, screening and genetic engineering techniques have opened new ways to exploit the potential of cyanobacteria.

26.5.3 Bioenergy

The rising energy demand and the limitation of traditional sources of energy, unsettled safety issues and concerns about ecological impacts are all encouraging the development of renewable, environmental friendly energy sources. However, most studies so far have concentrated on the production of biofuel or biodiesel, for which cyanobacteria appear less suitable than eukaryotic algae, since their lipid content is much lower (on average 8%) compared to, for instance, green algae, with an average of 16–25% (Griffiths and Harrison 2009; Sialve et al. 2009). For biofuel production a high lipid productivity has been identified as a key characteristic. Nevertheless, cyanobacteria show higher solar energy conversion factors than crop plants and their easy cultivation methods, the absence of non-usable parts and comparably easy genetic manipulation protocols justify their being considered as an option for bioenergy production. The genetic transformation of *Synechococcus* PCC 6803 and *Synechococcus* PCC 7942 with pyruvate decarboxylase (*pdc*) and alcohol dehydrogenase II (*ad*h) genes from the bacterium *Zymomonas mobilis* for ethanol production was successfully carried out by Deng and Coleman (1999) and Dexter and Fu (2009). Fermentation of agricultural crops and residues, the common sources of bioethanol today, could be replaced by direct production of ethanol from cyanobacteria on land that is not competing with agricultural production. However, in general ethanol has some major drawbacks: a low energy density, volatility, difficulties in piping owing to its corrosive properties, hygroscopic properties necessitating energy consumptive distillation steps. Moreover the restrictive genetic engineering regulations by many state authorities will hamper scaling up this process.

Hydrogen is a possible alternative energy source that can be derived from cyanobacteria via two main biochemical pathways: mediated by bidirectional hydrogenase and by nitrogenase. A third way would be the transformation with an efficient hydrogenase from non-cyanobacteria (Angermayr et al. 2009). Hydrogen is produced within at least 14 cyanobacteria genera and under a vast range of culture conditions (Lopes Pinto et al. 2002). However, the hydrogen yields achieved today are fairly low. Problems result from hydrogen-consuming methanogens and acetogenic bacteria associated with the cyanobacteria, limited duration of production and difficulty in collecting the gas; all this is troubling its commercialization (Sharma et al. 2010).

The production of biofuel or hydrogen from algae in general, including cyanobacteria is not yet commercially feasible. The high investment costs for plants large enough to produce reasonable amounts of lipids and the subsequent high production and downstreaming costs represent the largest drawbacks. Based on the existing knowledge and technology, much future improvement and progress needs to be done before bioenergy from cyanobacteria will become a commercial commodity. The need for cost reduction in the production processes is essential. Even open pond cultivation is not yet economically feasible for commodity markets like fuel or human nutrition. Major drawbacks besides the high production cost are the high costs for downstreaming.

26.6 Conclusion

Although many ideas have been considered and much research work carried out, cyanobacterial biotechnology must be considered to be still in its infancy. Nevertheless, application of biotechnological processes to cyanobacteria is already established industrially, so academic and applied scientists should both be aware of their responsibility to make better use of their knowledge to promote this resource. We have only just started to tap the enormous biological resource of cyanobacterial species growing in all ecological niches.

Acknowledgement The authors thank the editor for critical comments and careful revision of the manuscript.

References

Abalde J, Betancourt L, Torres E, Cid A, Barwell C (1998) Purification and characterization of phycocyanin from the marine cyanobacterium *Synechococcus* sp. IO9201. Plant Sci 136(1):109–120

Abdulqader GL, Barsanti L, Tredici MR (2000) Harvest of *Arthrospira platensis* from Lake Kossorom (Chad) and its household usage among the Kanembu. J Appl Phycol 12(3):493–498

Akao Y, Ebihara T, Masuda H, Saeki Y, Akazawa T, Hazeki K, Hazeki O, Matsumoto M, Seya T (2009) Enhancement of antitumor natural killer cell activation by orally administered *Spirulina* extract in mice. Cancer Sci 100(8):1494–1501

Angermayr SA, Hellingwerf KJ, Lindblad P, Teixeira de Mattos MJ (2009) Energy biotechnology with cyanobacteria. Curr Opin Biotechnol 20(3):257–263

Anwer R, Khursheed S, Fatma T (2011) Detection of immunoactive insulin in *Spirulina*. J Appl Phycol. doi:10.1007/s10811-011-9757-1:1-9

Balunas MJ, Linington RG, Tidgewell K, Fenner AM, Urena LD, Togna GD, Kyle DE, Gerwick WH (2009) Dragonamide E, a modified linear lipopeptide from *Lyngbya majuscula* with antileishmanial activity. J Nat Prod 73(1):60–66

Becher PG, Beuchat J, Gademann K, Jüttner F (2005) Nostocarboline: isolation and synthesis of a new cholinesterase inhibitor from *Nostoc* 78-12A. J Nat Prod 68(12):1793–1795

Belay A (1997) Mass culture of *Spirulina* outdoors: the Earthrise Farms experience. In: Vonshak A (ed) *Spirulina platensis (Arthrospira)*: physiology, cell-biology and biotechnology. Taylor & Francis, London, pp 131–142, 233 pp

Belay A (2002) The potential application of *Spirulina* (*Arthrospira*) as a nutritional and therapeutic supplement in health management. J Am Nutr Assoc 5(2):27–48

Belay A (2007) Production and quality assurance. *Spirulina* in human nutrition and health. In: Gershwin ME, Belay A (eds) *Spirulina* in human nutrition and health. CRC Press, Boca Raton, pp 1–26, 312 pp

Belay A, Kato T, Ota Y (1996) *Spirulina* (*Arthrospira*): potential application as an animal feed supplement. J Appl Phycol 8(4): 303–311

Benedetti S, Benvenuti F, Pagliarani S, Francogli S, Scoglio S, Canestrari F (2004) Antioxidant properties of a novel phycocyanin extract from the blue-green alga *Aphanizomenon flos-aquae*. Life Sci 75(19):2353–2362

Benedetti S, Rinalducci S, Benvenuti F, Francogli S, Pagliarani S, Giorgi L, Micheloni M, D'Amici GM, Zolla L, Canestrari F (2006) Purification and characterization of phycocyanin from the blue-green alga *Aphanizomenon flos-aquae*. J Chromatogr B 833(1):12–18

Benemann JR (1992) Microalgae aquaculture feeds. J Appl Phycol 4(3):233–245

Bermejo Román R, Alvarez-Pez J, Acién Fernández F, Molina Grima E (2002) Recovery of pure B-phycoerythrin from the microalga *Porphyridium cruentum*. J Biotechnol 93(1):73–85

Bhaskar SU, Gopalaswamy G, Raghu R (2005) A simple method for efficient extraction and purification of C-phycocyanin from *Spirulina platensis* Geitler. Indian J Exp Biol 43(3):277

Blinkova L, Gorobets O, Baturo A (2001) Biological activity of *Spirulina platensis*. Zh Mikrobiol Epidemiol Immunobiol 2:114–118

Bonjouklian R, Smitka TA, Doolin LE, Molloy RM, Debono M, Shaffer SA (1991) Tjipanazoles, new antifungal agents from the blue-green alga *Tolypothrix tjipanasensis*. Tetrahedron 47(37): 7739–7750

Borkenstein CG, Knoblechner J, Frühwirth H, Schagerl M (2011) Cultivation of *Chlorella emersonii* with flue gas derived from a cement plant. J Appl Phycol 23(1):131–135

Böttcher H, Franke H, Kallies KH, Kiessig Pompe W, Sandau E, Soltmann U (2006) Heavy metal decontamination by hybrid biofilters. WO00/2006/081932, 10.08.2006 (Patent application)

Boussiba S, Richmond AE (1979) Isolation and characterization of phycocyanins from the blue-green alga *Spirulina platensis*. Arch Microbiol 120(2):155–159

Boussiba S, Richmond AE (1980) C-phycocyanin as a storage protein in the blue-green alga *Spirulina platensis*. Arch Microbiol 125(1):143–147

Boyd MR, Gustafson KR, McMahon JB, Shoemaker RH, O'Keefe BR, Mori T, Gulakowski RJ, Wu L, Rivera MI, Laurencot CM (1997) Discovery of cyanovirin-N, a novel human immunodeficiency virus-inactivating protein that binds viral surface envelope glycoprotein gp120: potential applications to microbicide development. Antimicrob Agents Chemother 41(7):1521

But PP-H, Cheng L, Chan PK, Lau DT-W, But JW-H (2002) *Nostoc flagelliforme* and faked items retailed in Hong Kong. J Appl Phycol 14:143–145

Cai YA, Murphy JT, Wedemayer GJ, Glazer AN (2001) Recombinant phycobiliproteins 1: recombinant C-phycocyanins equipped with affinity Tags, oligomerization, and biospecific recognition domains. Anal Biochem 290(2):186–204

Cain KD, Grabowski L, Reilly J, Lytwyn M (2003) Immunomodulatory effects of a bacterial derived 1, 3 glucan administered to tilapia (*Oreochromis nilotocus* L.) in a *Spirulina* based diet. Aquac Res 34(13):1241–1244

Capelli B, Cysewski GR (2010) Potential health benefits of *Spirulina* microalgae. Nutr Food 9(2):19–26

Carlozzi P (2003) Dilution of solar radiation through "culture" lamination in photobioreactor rows facing south–north: a way to improve the efficiency of light utilization by cyanobacteria (*Arthrospira platensis*). Biotechnol Bioeng 81(3):305–315

Carmichael WW, Drapeau C, Anderson DM (2000) Harvesting of *Aphanizomenon flos-aquae* Ralfs ex Born. & Flah. var. *flos-aquae* (Cyanobacteria) from Klamath Lake for human dietary use. J Appl Phycol 12(6):585–595

Chen F, Zhang Y (1997) High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. Enzyme Microb Technol 20(3):221–224

Chen F, Zhang Y, Guo S (1996) Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnol Lett 18(5):603–608

Chen X, Smith GD, Waring P (2003) Human cancer cell (Jurkat) killing by the cyanobacterial metabolite calothrixin A. J Appl Phycol 15(4):269–277

Chen XF, Jia SR, Yue SJ, Wang N, Li CT, Wang Y (2009) Effect of solid bed-materials on vegetative cells of *Nostoc flagelliforme*. J Appl Phycol 22(3):341–347

Chen XF, Jia SR, Yue SJ, Wang Y, Wang N (2011) Biological crust of *Nostoc flagelliforme* (cyanobacteria) on sand bed materials. J Appl Phycol 23:67–71

Cheng Z, Cai H (1988) A preliminary study on the early-stage development of three species of *Nostoc*. J Northwest Norm Univ 3:41–52

Chojnacka K, Noworyta A (2004) Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme Microb Technol 34(5):461–465

Chojnacka K, Chojnacki A, Gorecka H (2005) Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue-green algae *Spirulina* sp.: kinetics, equilibrium and the mechanism of the process. Chemosphere 59(1):75–84

Chuntapa B, Powtongsook S, Menasveta P (2003) Water quality control using *Spirulina platensis* in shrimp culture tanks. Aquaculture 220(1–4):355–366

Ciferri O (1983) *Spirulina*, the edible microorganism. Microbiol Mol Biol Rev 47(4):551–578

Contreras-Martel C, Matamala A, Bruna C, Poo-Caamano G, Almonacid D, Figueroa M, Martinez-Oyanedel J, Bunster M (2007) The structure at 2 Å resolution of phycocyanin from *Gracilaria chilensis* and the energy transfer network in a PC-PC complex. Biophys Chem 125(2–3):388–396

Converti A, Oliveira R, Torres B, Lodi A, Zilli M (2009) Biogas production and valorization by means of a two-step biological process. Bioresour Technol 100(23):5771–5776

Cox PA, Banack SA, Murch SJ, Rasmussen U, Tien G, Bidigare RR, Metcalf JS, Morrison LF, Codd GA, Bergman B (2005) Diverse taxa of cyanobacteria produce -N-methylamino-L-alanine, a neurotoxic amino acid. Proc Natl Acad Sci USA 102(14):5074–5078

Critchley A, Ohno M (1998) Seaweeds resources of the world. Japan International Cooperation Agency, Yokosuka

Cui Z (1983) Culture trial of facai in soil-soaked solution. Sci Technol Lett Inner Mongol 4:10–38

Cysewski GR (2010) Commercial Production of *Spirulina*. 2nd Algae World, Brussels, Belgium, 31th May–1st June

Danxiang H, Yonghong B, Zhengyu H (2004) Industrial production of microalgal cell-mass and secondary products – species of high potential: *Nostoc*. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Oxford, pp 304–311, 566 pp

Davies-Coleman MT, Dzeha TM, Gray CA, Hess S, Pannell LK, Hendricks DT, Arendse CE (2003) Isolation ofomodolastatin 16, new cyclic depsipeptide from a Kenyan collection of *Lyngbya majuscula*. J Nat Prod 66(5):712–715

De Philippis R, Vincenzini M (1998) Exocellular polysaccharides from cyanobacteria and their possible applications. FEMS Microbiol Rev 22(3):151–175

De Philippis R, Sili C, Paperi R, Vincenzini M (2001) Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. J Appl Phycol 13(4):293–299

De Philippis R, Paperi R, Sili C, Vincenzini M (2003) Assessment of the metal removal capability of two capsulated cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936. J Appl Phycol 15(2):155–161

Delpeuch F, Joseph A, Cavelier C (1975) Consumption as food and nutritional composition of blue-green algae among populations in the Kanem region of Chad. Ann Nutr Aliment 29:497–516

Deng R, Chow TJ (2010) Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae *Spirulina*. Cardiovasc Ther 28(4): e33–e45

Deng MD, Coleman JR (1999) Ethanol synthesis by genetic engineering in cyanobacteria. Appl Environ Microbiol 65(2):523–528

DeRuyter YS, Fromme P (2008) Molecular structure of the photosynthetic apparatus. In: Herrero A, Flores E (eds) The cyanobacteria: molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 217–270, 484 pp

Dexter J, Fu P (2009) Metabolic engineering of cyanobacteria for ethanol production. Energy Environ Sci 2(8):857–864

Diao Z (1996) Study of natural conditions and ecological physiology characteristics for growth of *Nostoc flagelliforme* in Qinhai province. Chin J Ecol 15:8–13

Dias E, Pereira P, Franca S (2002) Production of paralytic shellfish toxins by *Aphanizomenon* sp. LMECYA 31 (Cyanobacteria). J Phycol 38(4):705–712

Dobler M, Dover S, Laves K, Binder A, Zuber H (1972) Crystallization and preliminary crystal data of C-phycocyanin. J Mol Biol 71(3): 785–787

Doke JM Jr (2005) An improved and efficient method for the extraction of phycocyanin from *Spirulina* sp. Int J Food Eng 1(5):1

Dubey A, Rai A (1995) Application of algal biofertilizers (*Aulosira fertilissima* Tenuis and *Anabaena doliolum* Bhardawaja) for sustained paddy cultivation in northern India. Isr J Plant Sci 43(1):41–52

Durand-Chastel H (1980) Production and use of *Spirulina* in Mexico. In: Shelef G, Soeder CJ (eds) Algal biomass, production and use. North Holland Biomedical Press, Amsterdam, pp 51–64, 852 pp

Edwards DJ, Marquez BL, Nogle LM, McPhail K, Goeger DE, Roberts MA, Gerwick WH (2004) Structure and biosynthesis of the jamaicamides, new mixed polyketide-peptide neurotoxins from the marine cyanobacterium *Lyngbya majuscula*. Chem Biol 11(6):817–833

Ehling-Schulz M, Bilger W, Scherer S (1997) UV-B-induced synthesis of photoprotective pigments and extracellular polysaccharides in the terrestrial cyanobacterium *Nostoc commune*. J Bacteriol 179(6):1940–1945

El-Sayed AFM (1994) Evaluation of soybean meal, *Spirulina* meal and chicken offal meal as protein sources for silver seabream (*Rhabdosargus sarba*) fingerlings. Aquaculture 127(2–3):169–176

El-Sheekh MM, El-Shouny WA, Osman MEH, El-Gammal EWE (2005) Growth and heavy metals removal efficiency of *Nostoc muscorum* and *Anabaena subcylindrica* in sewage and industrial wastewater effluents. Environ Toxicol Pharmacol 19(2):357–365

Eriksen NT (2008) Production of phycocyanin – a pigment with applications in biology, biotechnology, foods and medicine. Appl Microbiol Biotechnol 80(1):1–14

Falch BS, Koenig GM, Wright AD, Sticher O, Angerhofer CK, Pezzuto JM, Bachmann H (1995) Biological activities of cyanobacteria: evaluation of extracts and pure compounds. Planta Med 61:321–328

Falke P, Hendreich R, Knorr G, Güttes B, Sandau P (1999) Verfahren zur Herstellung von Polyurethan-Schaumstoffen für die Adsorption von Schwermetallionen. DE000019958702

Ferey F, Walenta G, Grewe C (2010) Microalgae culture on a Lafarge cement plant. 8th workshop on microalgal biotechnology, June 7–10, Nuthetal, Germany

Fox RD (2001) The spiral of *Spirulina*. In: Trevidi PC (ed) Algal biotechnology. Pointer Publishers, Jaipur, 398 pp

Fujishiro T, Ogawa T, Matsuoka M, Nagahama K, Takeshima Y, Hagiwara H (2004) Establishment of a pure culture of the hitherto uncultured unicellular cyanobacterium *Aphanothece sacrum*, and phylogenetic position of the organism. Appl Environ Microbiol 70(6):3338–3345

Gademann K, Portmann C (2008) Secondary metabolites from cyanobacteria: complex structures and powerful bioactivities. Curr Org Chem 12(4):326–341

Gao K (1998) Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. J Appl Phycol 10(1):37–49

Gao K, Ye C (2003) Culture of the terrestrial cyanobacterium, *Nostoc flagelliforme* (Cyanophyceae) under aquatic conditions. J Phycol 39(3):617–623

Ge B, Tang Z, Zhao F, Ren Y, Yang Y, Qin S (2005) Scale-up of fermentation and purification of recombinant allophycocyanin overexpressed in *Escherichia coli*. Process Biochem 40(10):3190–3195

Gershwin ME, Belay A (2007) *Spirulina* in human nutrition and health. CRC Press, Boca Raton, 328 pp

Gerwick WH, Proteau PJ, Nagle DG, Hamel E, Blokhin A, Slate DL (1994) Structure of Curacin A, a novel antimitotic, antiproliferative and brine shrimp toxic natural product from the marine cyanobacterium *Lyngbya majuscula*. J Org Chem 59(6):1243–1245

Gong R, Ding Y, Liu H, Chen Q, Liu Z (2005) Lead biosorption and desorption by intact and pretreated *Spirulina maxima* biomass. Chemosphere 58(1):125–130

Görs M, Schumann R, Hepperle D, Karsten U (2010) Quality analysis of commercial *Chlorella* products used as dietary supplement in human nutrition. J Appl Phycol 22(3):265–276

Graverholt OS, Eriksen NT (2007) Heterotrophic high-cell-density fed-batch and continuous-flow cultures of *Galdieria sulphuraria* and production of phycocyanin. Appl Microbiol Biotechnol 77(1):69–75

Grawish ME (2008) Effects of *Spirulina platensis* extract on Syrian hamster cheek pouch mucosa painted with 7, 12-dimethylbenz [a] anthracene. Oral Oncol 44(10):956–962

Grawish ME, Zaher AR, Gaafar AI, Nasif WA (2010) Long-term effect of *Spirulina platensis* extract on DMBA-induced hamster buccal pouch carcinogenesis (immunohistochemical study). Med Oncol 27(1):20–28

Grewe JC (2005) Cyanopeptoline und Scytocyclamide: zyklische Peptide aus *Scytonema hofmanni* PCC7110; Struktur und biologische Aktivität. Fakultät für Biologie. Ph D thesis, Albert-Ludwigs-Universität Freiburg im Breisgau, Freiburg

Griffiths MJ, Harrison STL (2009) Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J Appl Phycol 21(5):493–507

Grobbelaar JU (2003) Quality control and assurance: crucial for the sustainability of the applied phycology industry. J Appl Phycol 15(2):209–215

Grobbelaar JU (2009a) From laboratory to commercial production: a case study of a *Spirulina (Arthrospira)* facility in Musina, South Africa. J Appl Phycol 21(5):523–527

Grobbelaar JU (2009b) Factors governing algal growth in photobioreactors: the "open" versus "closed" debate. J Appl Phycol 21(5):489–492

Guan X, Qin S, Su Z, Zhao F, Ge B, Li F, Tang X (2007) Combinational biosynthesis of a fluorescent cyanobacterial holo-α-phycocyanin in *Escherichia coli* by using one expression vector. Appl Biochem Biotechnol 142(1):52–59

Gunasekera SP, Ross C, Paul VJ, Matthew S, Luesch H (2008) Dragonamides C and D, linear lipopeptides from the marine cyanobacterium brown *Lyngbya polychroa*. J Nat Prod 71(5):887–890

Gupta V, Natarajan C, Chaudhary V, Kumar A, Sharma E, Sharma J, Bhatnagar, AK, Prasanna R (2012) Analyses of diversity among fungicidal *Anabaena* strains. J Appl Phycol. doi:10.1007/s10811-012-9793-5:1-11

Gupta V, Rastogi A (2008) Sorption and desorption studies of chromium (VI) from nonviable cyanobacterium *Nostoc muscorum* biomass. J Hazard Mater 154(1–3):347–354

Gustafson KR, Cardellina JH, Fuller RW, Weislow OS, Kiser RF, Snader KM, Patterson GML, Boyd MR (1989) AIDS-antiviral sulfolipids from cyanobacteria (blue-green algae). J Natl Cancer Inst 81(16):1254

Haase SM, Huchzermeyer B, Rath T (2012) PHB accumulation in *Nostoc muscorum* under different carbon stress situations. J Appl Phycol 24(2):157–162

Hagmann L, Jüttner F (1996) Fischerellin A, a novel photosystem-II-inhibiting allelochemical of the cyanobacterium *Fischerella muscicola* with antifungal and herbicidal activity. Tetrahedron Lett 37(36):6539–6542

Harrigan GG, Yoshida WY, Moore RE, Nagle DG, Park PU, Biggs J, Paul VJ, Mooberry SL, Corbett TH, Valeriote FA (1998a) Isolation, structure determination, and biological activity of dolastatin 12 and lyngbyastatin 1 from *Lyngbya majuscula/Schizothrix* calcicola cyanobacterial assemblages. J Nat Prod 61(10):1221–1225

Harrigan GG, Yoshida WY, Moore RE, Nagle DG, Park PU, Biggs J, Paul VJ, Mooberry SL, Corbett TH, Valeriote FA (1998b) Isolation, structure determination, and biological activity of dolastatin 12 and lyngbyastatin 1 from *Lyngbya majuscula/Schizothrix calcicola* cyanobacterial assemblages. J Nat Prod 61(10):1221–1225

Harvey AL (2008) Natural products in drug discovery. Drug Discov Today 13(19–20):894–901

Hayashi T, Hayashi K, Maeda M, Kojima I (1996) Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. J Nat Prod 59(1):83–87

Helblin EW, Gao K, Ai H, Ma Z, Villafañe VE (2006) Differential responses of *Nostoc sphaeroides* and *Arthrospira platensis* to solar ultraviolet radiation exposure. J Appl Phycol 18(1):57–66

Hemscheidt T, Puglisi MP, Larsen LK, Patterson GML, Moore RE, Rios JL, Clardy J (1994) Structure and biosynthesis of borophycin, a new boeseken complex of boric acid from a marine strain of the blue-green alga *Nostoc linckia*. J Org Chem 59(12):3467–3471

Henrikson R (1989) Earth food *Spirulina*. Ronore Enterprises, Inc, Laguna Beach, 174 pp

Hernandez-Corona A, Nieves I, Meckes M, Chamorro G, Barron BL (2002) Antiviral activity of *Spirulina maxima* against herpes simplex virus type 2. Antiviral Res 56(3):279–285

Herrera A, Boussiba S, Napoleone V, Hohlberg A (1989) Recovery of c-phycocyanin from the cyanobacterium *Spirulina maxima*. J Appl Phycol 1(4):325–331

Hill DR, Keenan TW, Helm RF, Potts M, Crowe LM, Crowe JH (1997) Extracellular polysaccharide of *Nostoc commune* (Cyanobacteria) inhibits fusion of membrane vesicles during desiccation. J Appl Phycol 9(3):237–248

Hirata K, Yoshitomi S, Dwi S, Iwabe O, Mahakhant A, Polchai J, Miyamoto K (2003) Bioactivities of nostocine A produced by a freshwater cyanobacterium *Nostoc spongiaeforme* TISTR 8169. J Biosci Bioeng 95(5):512–517

Hu Q (2004) Industrial production of microalgal cell mass and secondary products major industrial species: *Arthrospira (Spirulina) platensis*. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Oxford, pp 264–272, 566 pp

Hussain A, Hasnain S (2011) Phytostimulation and biofertilization in wheat by cyanobacteria. J Ind Microbiol Biotechnol 38(1): 85–92

Jensen GS, Ginsberg DI, Drapeau C (2001) Blue-green algae as an immuno-enhancer and biomodulator. J Am Nutr Assoc 3:24–30

Jiménez C, Cossío BR, Labella D, Niell FX (2003) The feasibility of industrial production of *Spirulina (Arthrospira)* in Southern Spain. Aquaculture 217(1–4):179–190

John DM, Whitton BA, Brook AJ (eds) (2002) The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae. Cambridge University Press, Cambridge, UK, 904 pp

Johnson HE, King SR, Banack SA, Webster C, Callanaupa WJ, Cox PA (2008) Cyanobacteria (*Nostoc commune*) used as a dietary item in the Peruvian highlands produce the neurotoxic amino acid BMAA. J Ethnopharmacol 118(1):159–165

Jones AC, Gu L, Sorrels CM, Sherman DH, Gerwick WH (2009) New tricks from ancient algae: natural products biosynthesis in marine cyanobacteria. Curr Opin Chem Biol 13(2):216–223

Kanekiyo K, Lee JB, Hayashi K, Takenaka H, Hayakawa Y, Endo S, Hayashi T (2005) Isolation of an antiviral polysaccharide, Nostoflan, from a terrestrial cyanobacterium, *Nostoc flagelliforme*. J Nat Prod 68(7):1037–1041

Kanekiyo K, Hayashi K, Takenaka H, Lee JB, Hayashi T (2007) Anti-herpes simplex virus target of an acidic polysaccharide, nostoflan, from the edible blue-green alga *Nostoc flagelliforme*. Biol Pharm Bull 30(8):1573–1575

Kaya K, Mahakhant A, Keovara L, Sano T, Kubo T, Takagi H (2002) Spiroidesin, a novel lipopeptide from the cyanobacterium *Anabaena spiroides* that inhibits cell growth of the cyanobacterium *Microcystis aeruginosa*. J Nat Prod 65(6):920–921

Khaing MK (2004) A study on the edible cyanobacteria (blue green algae) *Nostoc* species in Upper Myanmar. Ph D thesis, Department of Botany, Mandalay University, Myanmar

Khan Z, Bhadouria P, Bisen P (2005) Nutritional and therapeutic potential of *Spirulina*. Curr Pharm Biotechnol 6(5):373–379

Kim JD (2006) Screening of cyanobacteria (blue-green algae) from rice paddy soil for anti-fungal activity against plant pathogenic fungi. Korean J Mycol 34(3):138–142

Kiran B, Kaushik A (2008) Cyanobacterial biosorption of Cr (VI): application of two parameter and Bohart Adams models for batch and column studies. Chem Eng J 144(3):391–399

Klimmek S, Stan HJ, Wilke A, Bunke G, Buchholz R (2001) Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. Environ Sci Technol 35(21):4283–4288

Kobayashi A, Kajiyama S, Inawaka K, Kanzaki H, Kawazu K (1994) Nostodione A, a novel mitotic spindle poison from a blue green alga *Nostoc commune*. Z Naturforsch 49(7–8):464–470

Larsen LK, Moore RE, Patterson GML (1994) β-Carbolines from the blue-green alga *Dichothrix baueriana*. J Nat Prod 57(3): 419–421

Lee RE (2008) Phycology, 4th edn. Cambridge University Press, Cambridge, UK, 560 pp

Lee JB, Srisomporn P, Hayashi K, Tanaka T, Sankawa U, Hayashi T (2001) Effects of structural modification of calcium spirulan, a sulfated polysaccharide from *Spirulina platensis*, on antiviral activity. Chem Pharm Bull 49(1):108–110

Liaaen-Jensen S, Egeland ES (1999) Microalgal carotenoids. In: Cohen Z (ed) Chemicals from microalgae. Taylor & Francis Ltd., London, pp 145–172, 417 pp

Liao X, Zhang B, Wang X, Yan H, Zhang X (2011) Purification of C-phycocyanin from *Spirulina platensis* by single-step ion-exchange chromatography. Chromatographia 73:291–296

Liu XJ, Chen F (2003) Cell differentiation and colony alteration of an edible terrestrial cyanobacterium *Nostoc flagelliforme*, in liquid suspension cultures. Folia Microbiol 48(5):619–626

Liu Y, Liu K, Ai Y, Jiang H, Gao X, Qiu B (2012) Differential display analysis of cDNA fragments potentially involved in *Nostoc flagelliforme* response to osmotic stress. J Appl Phycol. doi:10.1007/s10811-012-9806-4:1-8

Løbner M, Walsted A, Larsen R, Bendtzen K, Nielsen CH (2008) Enhancement of human adaptive immune responses by administration of a high-molecular-weight polysaccharide extract from the cyanobacterium *Arthrospira platensis*. J Med Food 11(2):313–322

Lodi A, Binaghi L, De Faveri D, Carvalho JCM, Converti A, Del Borghi M (2005) Fed-batch mixotrophic cultivation of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyceae) with carbon source pulse feeding. Ann Microbiol 55(3):181

Lopes Pinto FA, Troshina O, Lindblad P (2002) A brief look at three decades of research on cyanobacterial hydrogen evolution. Int J Hydrog Energy 27(11–12):1209–1215

Lu J, Takeuchi T (2004) Spawning and egg quality of the tilapia *Oreochromis niloticus* fed solely on raw *Spirulina* throughout three generations. Aquaculture 234(1–4):625–640

Lu YM, Xiang WZ, Wen YH (2010) *Spirulina* (*Arthrospira*) industry in Inner Mongolia of China: current status and prospects. J Appl Phycol 23(2):265–269

Luesch H, Moore RE, Paul VJ, Mooberry SL, Corbett TH (2001) Isolation of dolastatin 10 from the marine cyanobacterium *Symploca* species VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. J Nat Prod 64(7): 907–910

Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 15(4): 377–390

Markou G, Georgakakis D (2011) Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review. Appl Energy 88(10):3389–3401

Marquez FJ, Sasaki K, Kakizono T, Nishio N, Nagai S (1993) Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. J Ferment Bioeng 76(5):408–410

Marquez FJ, Nishio N, Nagai NS, Sasaki K (1995) Enhancement of biomass and pigment production during growth of *Spirulina platensis* in mixotrophic culture. J Chem Technol Biotechnol 62(2): 159–164

Mazokopakis EE, Karefilakis CM, Tsartsalis AN, Milkas AN, Ganotakis ES (2008) Acute rhabdomyolysis caused by *Spirulina* (*Arthrospira platensis*). Phytomedicine 15(6–7):525–527

McPhail KL, Correa J, Linington RG, González J, Ortega-Barría E, Capson TL, Gerwick WH (2007) Antimalarial linear lipopeptides from a Panamanian strain of the marine cyanobacterium *Lyngbya majuscula*. J Nat Prod 70(6):984–988

Mehta S, Gaur J (2005) Use of algae for removing heavy metal ions from wastewater: progress and prospects. Crit Rev Biotechnol 25(3):113–152

Mendiola JA, Jaime L, Santoyo S, Reglero G, Cifuentes A, Ibanez E, Señoráns F (2007) Screening of functional compounds in supercritical fluid extracts from *Spirulina platensis*. Food Chem 102(4):1357–1367

Mendiola JA, García-Martínez D, Rupérez FJ, Martín-Álvarez PJ, Reglero G, Cifuentes A, Barbas C, Ibañez E, Señoráns FJ (2008) Enrichment of vitamin E from *Spirulina platensis* microalga by SFE. J Supercrit Fluid 43(3):484–489

Miki W, Yamaguchi K, Konosu S (1986) Carotenoid composition of *Spirulina maxima*. Bull Jpn Soc Sci Fish 52(7):1225–1227

Min HEP, Zhang Yin Jiang H, Hui W (1999) Effect of the *Spirulina* feed on the growth and body color of Crucian carp. J Fish Chin 2:162–168

Minkova K, Tchernov A, Tchorbadjieva M, Fournadjieva S, Antova R, Busheva MC (2003) Purification of C-phycocyanin from *Spirulina (Arthrospira) fusiformis*. J Biotechnol 102(1):55–59

Mishima T, Murata J, Toyoshima M, Fujii H, Nakajima M, Hayashi T, Kato T, Saiki I (1998) Inhibition of tumor invasion and metastasis by calciumspirulan (Ca-SP), a novel sulfated polysaccharide derived from a blue-green alga, *Spirulina platensis*. Clin Exp Metastasis 16(6):541–550

Miranda J, Krishnakumar G, Gonsalves R (2012) Cr^{6+} bioremediation efficiency of *Oscillatoria laete-virens* (Crouan & Crouan) Gomont and *Oscillatoria trichoides* Szafer: kinetics and equilibrium study. J Appl Phycol. doi:10.1007/s10811-012-9800-x:1-16

Moreno J, Vargas MA, Rodriguez H, Rivas J, Guerrero MG (2003) Outdoor cultivation of a nitrogen-fixing marine cyanobacterium, *Anabaena* sp. ATCC 33047. Biomol Eng 20(4–6):191–197

Mühling M, Harris N, Belay A, Whitton BA (2003) Reversal of helix orientation in the cyanobacterium *Arthrospira*. J Phycol 39(2):360–367

Mühling M, Belay A, Whitton BA (2005a) Screening *Arthrospira* (Spirulina) strains for heterotrophy. J Appl Phycol 17(2):129–135

Mühling M, Belay A, Whitton BA (2005b) Variation in fatty acid composition of *Arthrospira* (Spirulina) strains. J Appl Phycol 17(2):137–146

Muller Feuga A (2004) Microalgae for aquaculture: the current global situation future trends. In: Richmond A (ed) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Publishing, Oxford, pp 352–364, 566 pp

Muller Feuga A, Robert R, Cahu C, Robin J, Divanach P (2003) Uses of microalgae in aquaculture. In: Stottrup J, McEvoy L (eds) Live feeds in marine aquaculture. Blackwell Publishing Co, Oxford, pp 253–299, 336 pp

Nagase H, Inthorn D, Oda A, Nishimura J, Kajiwara Y, Park M, Hirata K, Miyamoto K (2005) Improvement of selective removal of heavy metals in cyanobacteria by NaOH treatment. J Biosci Bioeng 99(4):372–377

Narayan M, Manoj G, Vatchravelu K, Bhagyalakshmi N, Mahadevaswamy M (2005) Utilization of glycerol as carbon source on the growth, pigment and lipid production in *Spirulina platensis*. Int J Food Sci Nutr 56(7):521–528

Niu JF, Wang GC, Lin X, Zhou BC (2007) Large-scale recovery of C-phycocyanin from *Spirulina platensis* using expanded bed adsorption chromatography. J Chromatogr B 850(1–2):267–276

Olaizola M (2003) Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomol Eng 20(4–6):459–466

Olguín EJ, Galicia S, Mercado G, Pérez T (2003) Annual productivity of *Spirulina* (*Arthrospira*) and nutrient removal in a pig wastewater recycling process under tropical conditions. J Appl Phycol 15(2):249–257

Oliveira E, Rosa G, Moraes M, Pinto L (2008) Phycocyanin content of *Spirulina platensis* dried in spouted bed and thin layer. J Food Process Eng 31(1):34–50

Oliveira EG, Duarte JH, Moraes K, Crexi VT, Pinto LAA (2010) Optimisation of *Spirulina platensis* convective drying: evaluation of phycocyanin loss and lipid oxidation. Int J Food Sci Tech 45(8):1572–1578

Olvera Novoa M, Domínguez Cen L, Olivera Castillo L, Martínez Palacios CA (1998) Effect of the use of the microalga *Spirulina maxima* as fish meal replacement in diets for tilapia, *Oreochromis mossambicus* (Peters), fry. Aquac Res 29(10):709–715

Padgett MP, Krogmann DW (1987) Large scale preparation of pure phycobiliproteins. Photosynth Res 11(3):225–235

Padyana AK, Bhat VB, Madyastha K, Rajashankar K, Ramakumar S (2001) Crystal structure of a light-harvesting protein C-phycocyanin from *Spirulina platensis*. Biochem Biophys Res Commun 282(4):893–898

Parages ML, Rico RM, Abdala-Díaz RT, Chabrillón M, Sotiroudis TG, Jiménez C (2012) Acidic polysaccharides of *Arthrospira* (Spirulina) *platensis* induce the synthesis of TNF-α in RAW macrophages. J Appl Phycol. doi:10.1007/s10811-012-9814-4:1-10

Parial D, Patra HK, Roychoudhury P, Dasgupta AK, Pal R (2012) Gold nanorod production by cyanobacteria—a green chemistry approach. J Appl Phycol 24:55–60

Patil G, Raghavarao K (2007) Aqueous two phase extraction for purification of C-phycocyanin. Biochem Eng J 34(2):156–164

Patil G, Chethana S, Sridevi A, Raghavarao K (2006) Method to obtain C-phycocyanin of high purity. J Chromatogr A 1127(1–2): 76–81

Patil G, Chethana S, Madhusudhan M, Raghavarao K (2008) Fractionation and purification of the phycobiliproteins from *Spirulina platensis*. Bioresour Technol 99(15):7393–7396

Patnaik S, Sarkar R, Mitra A (2001) Alginate immobilization of *Spirulina platensis* for wastewater treatment. Indian J Exp Biol 39(8):824–826

Patterson GML, Carmeli S (1992) Biological effects of tolytoxin (6-hydroxy-7-O-methyl-scytophycin b), a potent bioactive metabolite from cyanobacteria. Arch Microbiol 157(5):406–410

Patterson GML, Smith CD, Kimura LH, Britton BA, Carmeli S (1993) Action of tolytoxin on cell morphology, cytoskeletal organization, and actin polymerization. Cell Motil Cytoskel 24(1):39–48

Pedroni P, Davison J, Beckert H, Bergman P, Benemann J (2001) A proposal to establish an international network on biofixation of CO_2 and greenhouse gas abatement with microalgae. J Energy Environ Res 1(1):136–150

Pereira I, Ortega R, Barrientos L, Moya M, Reyes G, Kramm V (2009) Development of a biofertilizer based on filamentous nitrogen-fixing cyanobacteria for rice crops in Chile. J Appl Phycol 21(1):135–144

Posten C (2009) Design principles of photo bioreactors for cultivation of microalgae. Eng Life Sci 9(3):165–177

Preußel K, Stüken A, Wiedner C, Chorus I, Fastner J (2006) First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (Cyanobacteria) isolated from two German lakes. Toxicon 47(2):156–162

Prinsep MR, Caplan FR, Moore RE, Patterson GML, Smith CD (1992) Tolyporphin, a novel multidrug resistance reversing agent from the blue-green alga *Tolypothrix nodosa*. J Am Chem Soc 114(1):385–387

Pugh N, Ross SA, ElSohly HN, ElSohly MA, Pasco DS (2001) Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. Planta Med 67(8):737–742

Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65(6):635–648

Pulz O, Scheibenbogen K (1998) Photobioreactors: design and performance with respect to light energy input. In: Scheper T (ed) Bioprocess and algae reactor technology, apoptosis, vol 59. Springer, Berlin, pp 123–152, 259 pp

Pulz O, Storandt R, Boback A (2008) Mikroalgen in der Broileraufzucht. DGS 9:15–18

Pushparaj B, Pelosi E, Tredici MR, Pinzani E, Materassi R (1997) An integrated culture system for outdoor production of microalgae and cyanobacteria. J Appl Phycol 9(2):113–119

Qiu B, Gao K (2002) Daily production and photosynthetic characteristics of *Nostoc flagelliforme* grown under ambient and elevated CO_2 conditions. J Appl Phycol 14(2):77–83

Qiu B, Liu J, Liu Z, Liu S (2002) Distribution and ecology of the edible cyanobacterium Ge-Xian-Mi (*Nostoc*) in rice fields of Hefeng County in China. J Appl Phycol 14(5):423–429

Quesada A, Leganés F, Fernández-Valiente E (1997) Environmental factors controlling N_2 fixation in Mediterranean rice fields. Microb Ecol 34(1):39–48

Raja R, Hemaiswarya S, Kumar NA, Sridhar S, Rengasamy R (2008) A perspective on the biotechnological potential of microalgae. Crit Rev Microbiol 34(2):77–88

Ramos A, Acien FG, Fernandez-Sevilla JM, Gonzalez CV, Bermejo R (2011) Development of a process for large-scale purification of C-phycocyanin from *Synechocystis aquatilis* using expanded bed adsorption chromatography. J Chromatogr B 879(7–8):511–519

Rapala J, Sivonen K, Luukkainen R, Niemelä SI (1993) Anatoxin-a concentration in *Anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-*toxic Anabaena*-strains—a laboratory study. J Appl Phycol 5(6):581–591

Rasool M, Sabina EP (2009) Appraisal of immunomodulatory potential of *Spirulina fusiformis*: an in vivo and in vitro study. J Nat Med 63(2):169–175

Rastogi RP, Sinha RP (2009) Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnol Adv 27(4):521–539

Regunathan C, Wesley S (2006) Pigment deficiency correction in shrimp broodstock using *Spirulina* as a carotenoid source. Aquac Nutr 12(6):425–432

Richmond A, Vonshak A (1978) *Spirulina* culture in Israel. Arch Hydrobiol Beih Ergebn Limnol 11:274–280

Rickards R, Rothschild WJM, Willis AC, de Chazal NM, Kirk J, Kirk K, Saliba KJ, Smith GD (1999) Calothrixins A and B, novel pentacyclic metabolites from *Calothrix* cyanobacteria with potent activity against malaria parasites and human cancer cells. Tetrahedron 55(47):13513–13520

Rito Palomares M, Nuñez L, Amador D (2001) Practical application of aqueous two phase systems for the development of a prototype process for c phycocyanin recovery from *Spirulina maxima*. J Chem Technol Biotechnol 76(12):1273–1280

Rodríguez A, Stella A, Storni M, Zulpa G, Zaccaro M (2006) Effects of cyanobacterial extracellular products and gibberellic acid on salinity tolerance in *Oryza sativa* L. Saline Syst 2(1):7

Rohrlack T, Christoffersen K, Hansen PE, Zhang W, Czarnecki O, Henning M, Fastner J, Erhard M, Neilan BA, Kaebernick M (2003) Isolation, characterization, and quantitative analysis of microviridin J, a new *Microcystis* metabolite toxic to *Daphnia*. J Chem Ecol 29(8):1757–1770

Roney BR, Renhui L, Banack SA, Murch S, Honegger R, Cox PA (2009) Consumption of fa cai *Nostoc* soup: a potential for BMAA exposure from *Nostoc* cyanobacteria in China? Amyotroph Lateral Scler 10(S2):44–49

Rosello Sastre R, Posten C (2010) Die vielfältige Anwendung von Mikroalgen als nachwachsende Rohstoffe. The variety of microalgae applications as a renewable resource. Chem Ing Tech 82(11):1925–1939

Ross E, Puapong D, Cepeda F, Patterson P (1994) Comparison of freeze-dried and extruded *Spirulina platensis* as yolk pigmenting agents. Poult Sci 73(8):1282–1289

Sajilata M, Singhal R, Kamat M (2008a) Fractionation of lipids and purification of γ-linolenic acid (GLA) from *Spirulina platensis*. Food Chem 109(3):580–586

Sajilata M, Singhal RS, Kamat MY (2008b) Supercritical CO_2 extraction of γ-linolenic acid (GLA) from *Spirulina platensis* ARM 740 using response surface methodology. J Food Eng 84(2):321–326

Saker ML, Jungblut AD, Neilan BA, Rawn DFK, Vasconcelos VM (2005) Detection of microcystin synthetase genes in health food supplements containing the freshwater cyanobacterium *Aphanizomenon flos-aquae*. Toxicon 46(5):555–562

Sandau P (2010) Examples for successfully marketed active ingredients of algae in cosmetic products. Institue for Cereal Processing, Nuthetal

Sandau P, Pulz O (2009) Untersuchungen zu bioaktiven Wirkungen des Algenpolysaacharids Calcium-Spirulan aus *Arthrospira platensis*. OM & Ernährung 131:F40–F45

Santiago-Santos M (2004) Extraction and purification of phycocyanin from *Calothrix* sp. Process Biochem 39(12):2047–2052

Sarada R, Pillai MG, Ravishankar G (1999) Phycocyanin from *Spirulina* sp: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. Process Biochem 34(8):795–801

Schaeffer DJ, Malpas PB, Barton LL (1999) Risk assessment of microcystin in dietary *Aphanizomenon flos-aquae*. Ecotoxicol Environ Saf 44(1):73–80

Scherzinger D, Al Babili S (2008) In vitro characterization of a carotenoid cleavage dioxygenase from *Nostoc* sp. PCC 7120 reveals a novel cleavage pattern, cytosolic localization and induction by highlight. Mol Microbiol 69(1):231–244

Schlotmann K, Waldmann-laue M, Jassoy C, Kaeten M, Koehler E, Pulz O, Kurth E (2005) Extract of blue-green alga *Spirulina* and the use thereof in cosmetic skin-care and body care agents, EP Patent 1,239,813

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

Sekar S, Chandramohan M (2008) Phycobiliproteins as a commodity: trends in applied research, patents and commercialization. J Appl Phycol 20(2):113–136

Sergeeva E, Liaimer A, Bergman B (2002) Evidence for production of the phytohormone indole-3-acetic acid by cyanobacteria. Planta 215(2):229–238

Sharma NK, Tiwari SP, Tripathi K, Rai AK (2010) Sustainability and cyanobacteria (blue-green algae): facts and challenges. J Appl Phycol 23(6):1059–1081

Shelef G, Soeder C (1980) Algal biomass, production and use. North Holland Biomedical Press, Amsterdam

Shi D, Zhou G, Fang S, Qiu Y, Zhong Z, Cui Z (1992) Studies on photosynthesis, respiration and morphology of *Nostoc flagelliforme*. Acta Bot Sin 34:507–514

Sialve B, Bernet N, Bernard O (2009) Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 27(4):409–416

Simon NS, Lynch D, Gallaher TN (2009) Phosphorus fractionation in sediment cores collected In 2005 before and after opnset of an *Aphanizomenon flos-aquae* bloom in Upper Klamath Lake, OR, USA. Water Air Soil Pollut 204(1):139–153

Singh NK, Parmar A, Madamwar D (2009) Optimization of medium components for increased production of C-phycocyanin from *Phormidium ceylanicum* and its purification by single step process. Bioresour Technol 100(4):1663–1669

Sinha RP, Klisch M, Walter Helbling E, Häder DP (2001) Induction of mycosporine-like amino acids (MAAs) in cyanobacteria by solar ultraviolet-B radiation. J Photochem Photobiol B Biol 60(2–3): 129–135

Sivonen K, Börner T (2008) Bioactive compounds produced by cyanobacteria. In: Herrero A, Flores E (eds) The cyanobacteria. Molecular biology, genomics and evolution. Caister Academic Press, Norfolk, pp 159–197, 484 pp

Soeder C (1992) Mass cultures of microalgae – achievements and perspectives. 1st European workshop on microalgal biotechnology, Potsdam Rehbrücke, Germany

Soeder C, Muller H, Payer H, Schulle H (1971) Mineral nutrition of planktonic algae: some considerations, some experiments. Int Ver Theor Angew Limnol 19:39–58

Soni B, Kalavadia B, Trivedi U, Madamwar D (2006) Extraction, purification and characterization of phycocyanin from *Oscillatoria quadripunctulata* isolated from the rocky shores of Bet-Dwarka, Gujarat, India. Process Biochem 41(9):2017–2023

Soni B, Trivedi U, Madamwar D (2008) A novel method of single step hydrophobic interaction chromatography for the purification of phycocyanin from *Phormidium fragile* and its characterization for antioxidant property. Bioresour Technol 99(1):188–194

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial Applications of Microalgae. J Biosci Bioeng 101(2):87–96

Stec B, Troxler RF, Teeter MM (1999) Crystal structure of C-phycocyanin from *Cyanidium caldarium* provides a new perspective on phycobilisome assembly. Biophys J 76(6):2912–2921

Stirk W, Ördög V, Van Staden J, Jäger K (2002) Cytokinin-and auxin-like activity in Cyanophyta and microalgae. J Appl Phycol 14(3):215–221

Storandt R, Franke H, Pulz O, Loest K, Ecke M, Steinberg KH (2000) Algae in animal nutrition. Tierernährung – Ressourcen und neue Aufgaben, Hannover, Germany, Landbauforschung Völkenrode

Su J, Jia S, Qiao C, Jung G (2005) Culture of *Nostoc flagelliforme* on solid medium. Korean J Environ Biol 23:135–140

Su J, Jia S, Chen X, Yu H (2008) Morphology, cell growth, and polysaccharide production of *Nostoc flagelliforme* in liquid suspension culture at different agitation rates. J Appl Phycol 20(3):213–217

Takenaka H, Yamaguchi Y, Sakaki S, Watarai K, Tanaka N, Hori M, Seki H, Tsuchida M, Yamada A, Nishimori T (1998) Safety evaluation of *Nostoc flagelliforme* (Nostocales, Cyanophyceae) as a potential food. Food Chem Toxicol 36(12):1073–1077

Tan LT (2007) Bioactive natural products from marine cyanobacteria for drug discovery. Phytochemistry 68(7):954–979

Taori K, Paul VJ, Luesch H (2008) Structure and activity of largazole, a potent antiproliferative agent from the Floridian marine cyanobacterium *Symploca* sp. J Am Chem Soc 130(6):1806–1807

Tarakhovskay E, Maslov YI, Shishova M (2007) Phytohormones in algae. Russ J Plant Physiol 54(2):163–170

Teas J, Hebert JR, Fitton JH, Zimba PV (2004) Algae – a poor man's HAART? Med Hypotheses 62(4):507–510

Thajuddin N, Subramanian G (2005) Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci India 89(1):47–57

Thein M (2011) The use of natural resource for sustainable production of Spirulina in Myanmar crater lakes. 5th International Algae Congress, Dec 1–2, Berlin

Tiburcio P, Galvez FCF, Cruz L, Gavino V (2007) Optimization of low-cost drying methods to minimize lipid peroxidation in *Spirulina platensis* grown in the Philippines. J Appl Phycol 19(6):719–726

Tomaselli L (1997) Morphology, ultrastructure and taxonomy of *Arthrospira (Spirulina) maxima* and *Arthrospira (Spirulina) platensis*. In: Vonshak A (ed) *Spirulina platensis* (*Arthrospira*): physiology, cell biology, and biotechnology. Taylor & Francis, London/San Francisco, pp 1–19, 233 pp

Tooley AJ, Cai YA, Glazer AN (2001) Biosynthesis of a fluorescent cyanobacterial C-phycocyanin holo- subunit in a heterologous host. Proc Natl Acad Sci USA 98(19):10560–10565

Toyomizu M, Sato K, Taroda H, Kato T, Akiba Y (2001) Effects of dietary *Spirulina* on meat colour in muscle of broiler chickens. Br Poult Sci 42(2):197–202

Travieso L, Sanchez E, Benitez F, Conde J (1993) *Arthospira* sp. intensive cultures for food and biogas purification. Biotechnol Lett 15:1091–1094

Trimurtulu G, Ohtani I, Patterson GML, Moore RE, Corbett TH, Valeriote FA, Demchik L (1994) Total structures of cryptophycins, potent antitumor depsipeptides from the blue-green alga *Nostoc* sp. strain GSV 224. J Am Chem Soc 116(11):4729–4737

Ugwu C, Aoyagi H, Uchiyama H (2008) Photobioreactors for mass cultivation of algae. Bioresour Technol 99(10):4021–4028

Vaishampayan A, Sinha R, Hader DP, Dey T, Gupta A, Bhan U, Rao A (2001) Cyanobacterial biofertilizers in rice agriculture. Bot Rev 67(4):453–516

Volk RB (2005) Screening of microalgal culture media for the presence of algicidal compounds and isolation and identification of two bioactive metabolites, excreted by the cyanobacteria *Nostoc insulare* and *Nodularia harveyana*. J Appl Phycol 17(4):339–347

Vonshak A (1997a) *Spirulina platensis (Arthrospira)*: physiology, cell-biology and biotechnology. Taylor & Francis, London/Bristol, 233 pp

Vonshak A (1997b) *Spirulina*: growth, physiology and biochemistry. In: Vonshak A (ed) *Spirulina platensis (Arthrospira)*: physiology, cell-biology and biotechnology. Taylor & Francis, London/Bristol, pp 43–65, 233 pp

Vonshak A, Tomaselli L (2000) *Arthrospira (Spirulina)*: systematics and ecophysiology. In: Whitton BA, Potts M (eds) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publihsers, Dordrecht, pp 505–522, 669 pp

Wang ZP, Zhao Y (2005) Morphological reversion of *Spirulina (Arthrospira) platensis* (Cyanophyta): from linear to helical. J Phycol 41(3):622–628

Watanabe I (1982) *Azolla – Anabaena symbiosis* – its physiology and use in tropical agriculture. In: Dommergues YE (ed) Microbiology of tropical soils and plant productivity. Kluwer Academic Publishers, Dordrecht, p 169

Watanuki H, Ota K, Tassakka ACMAR, Kato T, Sakai M (2006) Immunostimulant effects of dietary *Spirulina platensis* on carp, *Cyprinus carpio*. Aquaculture 258(1–4):157–163

Whitton BA, Potts M (2000) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, 669 pp

Wilson AC, Punginelli C, Gall A, Bonetti C, Alexandre M, Routaboul JM, Kerfeld CA, Van Grondelle R, Robert B, Kennis J (2008) A photoactive carotenoid protein acting as light intensity sensor. Proc Nat Acad Sci 105(33):12075

Wood SA, Rasmussen JP, Holland PT, Campbell R, Crowe ALM (2007) First report of the cyanotoxin anatoxin A from *Aphanizomenon issatschenkoi* (Cyanobacteria). J Phycol 43(2):356–365

Yamamoto Y, Nakahara H (2005) The formation and degradation of cyanobacterium *Aphanizomenon flos-aquae* blooms: the importance of pH, water temperature, and day length. Limnology 6(1):1–6

Yan SG, Zhu LP, Su HN, Zhang XY, Chen XL, Zhou BC, Zhang YZ (2011) Single-step chromatography for simultaneous purification of C-phycocyanin and allophycocyanin with high purity and recovery from *Spirulina (Arthrospira) platensis*. J Appl Phycol 23(1):1–6

Yoshimura H, Kotake T, Aohara T, Tsumuraya Y, Ikeuchi M, Ohmori M (2012) The role of extracellular polysaccharides produced by the terrestrial cyanobacterium *Nostoc* sp. strain HK-01 in NaCl tolerance. J Appl Phycol 24:237–243

Yu H, Jia S, Dai Y (2009) Growth characteristics of the cyanobacterium *Nostoc flagelliforme* in photoautotrophic, mixotrophic and heterotrophic cultivation. J Appl Phycol 21(1):127–133

Zarrouk C (1966) Contribution à l étude d une cyanophycée: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima*. Paris, University de Paris, Ph.D.

Zemke-White W, Ohno M (1999) World seaweed utilisation: an end-of-century summary. J Appl Phycol 11(4):369–376

Zhang YM, Chen F (1999) A simple method for efficient separation and purification of c-phycocyanin and allophycocyanin from *Spirulina platensis*. Biotechnol Tech 13(9):601–603

Zhang H, Hu C, Jia X, Xu Y, Wu C, Chen L, Wang F (2012) Characteristics of γ-hexachlorocyclohexane biodegradation by a nitrogen-fixing cyanobacterium, Anabaena azotica. J Appl Phycol 24(2):221–225

Zhang YM, Chen F (1999) A simple method for efficient separation and purification of c-phycocyanin and allophycocyanin from *Spirulina platensis*. Biotechnol Tech 13(9):601–603

Zhong Z, Shi D, Wang F, Cui Z (1992) Effects of temperature, water content and light intensity on nitrogenase activity of *Nostoc flagelliforme*. Acta Bot Sin 34:219–225

Zitelli GC, Tomasello V, Pinzani E, Tredici MR (1996) Outdoor culture of *Arthrospira platensis* during autumn and winter in temperate climate. J Appl Phycol 8:293–301