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RECENT ADVANCES IN MICROALGAL BIOTECHNOLOGY

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ABSTRACT

In the last ten years, several reviews (Benemann et al, 1987; Goldman, 1979a; Richmond, 1986b; Soeder, 1980) and books (Borowitzka and Borowitzka, 1988b; Richmond, 1986a; Lembi and Waaland, 1988) have been published on the historical background of mass cultivation of microalgal biomass, and its possible commercial applications. This review presents a brief description of the concept of microalgal biotechnology, and describes some of the recent developments, mainly in the application and commercial development of this relatively new biotechnology, Finally, an attempt is made to indicate those areas where current research and development are paving the way for future applications.

KEYWORDS

algae, Spirulina, Dunaliella, Chlorella, Haematococus, biomass

THE CONCEPT

The concept of algal biotechnology is basically the same as in conventional agriculture, namely the utilization of photosynthetic machinery for the production of biomass to be used as a source of food, feed, chemicals and energy.

The main advantages of culturing microalgae as a source of biomass are:

a. Algae are considered to be a very efficient biological system for harvesting solar energy for the production of organic compounds via the photosynthetic process.
b. Algae are non-vascular plants, lacking (usually) complex reproductive organs, making the entire biomass available for harvest and use.
c. Many species of algae can be induced to produce particularly high concentrations of chosen, commercially-valuable compounds, such as proteins, carbohydrates, lipids, and pigments (Cohen, 1986).
d. Algae are microorganisms that undergo a simple cell division cycle, in most cases without a sexual type stage, enabling them to complete their cell cycle within a few hours and making genetic selection and strain screening relatively quick and easy. This also allows much more rapid development and demonstration of production processes than with other agricultural crops.
e. For many regions suffering low productivity due to poor soils or the shortage of sweet water, the farming of microalgae that can be grown using sea or brackish water may be almost the only way to increase productivity and secure a basic protein supply.
f. Algal biomass production systems can easily be adapted to various levels of operational or technological skills, from simple, labor-intensive production units to fully automated systems requiring high investments.

THE PROCESS

The process of producing microalgal biomass is presented in Figure 1, illustrating the major inputs and potential uses of the biomass produced. The process can be divided into two main steps: Growing the algal biomass; and harvesting and processing the biomass.

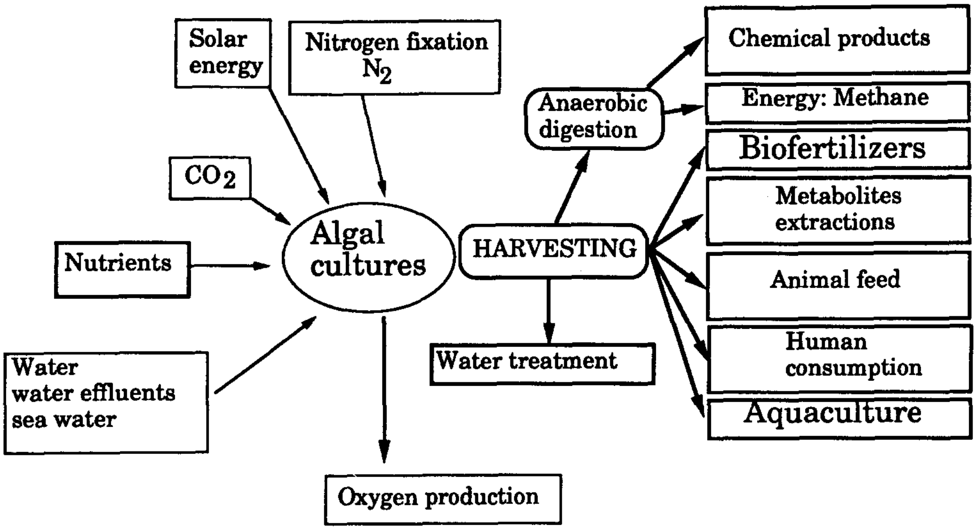

Figure 1. Algal Biotechnology Inputs and Potential Outputs

Growth

The first step in growing the algae combines the biological know-how of growing photoautotrophic microorganisms with the special requirements for designing an appropriate reactor for the process.

The biological know-how involves understanding the interactions of environmental factors -- such as light and temperature, as well as salinity, photoinhibition (Vonshak and Guy, 1988), and dark respiration (Grobolar and Soeder, 1985) -- on algal growth and productivity (Vonshak and Richmond, 1985). These parameters have to be considered in developing an operational protocol for pond management which also includes nutrient levels and pH, in order to establish a continuous culture for sustained production and to avoid development of grazers, predators and contamination by other algae. The parameters have been reviewed in detail for Spirulina (Vonshak, 1987; Vonshak and Richmond, 1988) and for Dunaliella (Ben Amotz and Avron, 1989).

Most commercial reactors used in large scale production of algal biomass are based on shallow raceways in which algal cultures are mixed in a turbulent flow sustained by a paddle wheel (for more information see Dodd, 1986). Another concept involves the use of naturally occurring lagoons or lakes, as in the case of Texcoco, Mexico, where a natural lake and facilities of a sodium bicarbonate production plant were adapted for the production of Spirulina (Ciferi, 1983), or in production sites for Dunaliella biomass in Australia where salt lakes or lagoons near the seashore are used (mixing being provided by the wind), and the production system is an extensive one (Borowitzka, 1991; Schlipalius, 1991). The size of commercial ponds varies from 0.1 to 0.5 ha. in the raceway systems and up to 5-10 ha. in the lagoon system (Borowitzka and Borowitzka, 1988a).

Harvesting and Processing Algal Biomass

The techniques employed in harvesting algal biomass are very important in determining the cost of production. Due to the nature of the growing process, the algal product is relatively diluted as compared to other microbial processes. The maximal concentration of all biomass in the large scale production sites does not exceed more than 500 mg/L and in many cases is only 200-300 mg/L. Applying an efficient harvesting process requires an intensive step of water removal, thus using an efficient device with low energy input is highly desirable.

The different techniques used in algal harvesting were reviewed (Benemann et al, 1980; Mohn, 1980). The specific application of some of those techniques in large scale production was described for Spirulina (Vonshak and Richmond, 1988) and for Dunaliella (Ben-Amotz and Avron, 1989). In general, water removal may be achieved by three different methods:

a. Filtration: This is the preferred method whenever filamentous alga (such as Spirulina) are being harvested, and involves different designs of either vibrating or static nets. As long as no problems in clogging of the filters are encountered, this is a relatively inexpensive and efficient harvesting method.

b. Centrifugation: This technique has been widely used in the harvesting of microalgae, mainly unicellular, such as Chlorella. It is considered to be a very efficient, but relatively expensive, process due to the high investment cost and high energy inputs (Mohn, 1988).

c. Flocculation and Sedimentation: These techniques are used mainly in the removal of algal biomass in waste water treatment (Oswald, 1988), and are usually induced by the addition of chemicals such as aluminum sulphate, ferric sulphate or lime (Mohn, 1988) or by the modification of growth conditions such as pH (Vonshak et al, 1985). These procedures are relatively inexpensive and the main problem is that the flocculated product cannot be directly used as a food since complete removal of the flocculant is required.

The final processing step is highly dependent on the kind of product and its marketing. Whenever the final product is aimed at the health food market, the harvested biomass is dried using a spray drier. If specific chemicals or pigments are being extracted, the biomass is usually used in wet form for the extraction process, as in the cases of Dunaliella and its extract, beta-carotene.

APPLICATION AND COMMERCIAL PRODUCTION

To date, only three species of microalgae have been raised commercially on a large scale. This production is mainly directed at the health food market, and is limited to a very defined and relatively small number of consumers. The following is a summary of the current stage of the commercial production of these algae:

1. Chlorella

This is a unicellular green microalgae that was the first to be used in applied research. In the early 1950's, Chlorella was used as a model organism by the U.S. space program as part of the Closed Environmental Support System (CELLS) (Burlew, 1953). Later, in Taiwan, its potential use as a source for single cell protein was studied. In the late 1960's, the Taiwan Chlorella Company started the large scale production of Chlorella biomass, and ten years later at least thirty different companies were involved in production and marketing of various Chlorella-based products. The total annual production in the late 1970's was estimated to be about 1000 tons, having a potential of at least double that amount (Kawaguchi, 1980; Soong, 1980).

The production process for Chlorella is based on the mixotrophic nature of the Chlorella strains and uses acetic acid as a carbon source. Since the green color of the product is considered to be a measure of its quality, a "greening" stage is incorporated into the production process, which is usually done by exposing the cells to sunlight under low organic carbon concentrations.

Market and Products: Due to commercial secrecy, the information available on market conditions and costs is very limited. Almost all production takes place in Taiwan and only 10-15% of the total production is carried out in greenhouses in Japan. The market for Chlorella products is limited to the Far East, mainly Japan. Two main products are available for the health food market: a dried biomass in powder or pill form; and a Chlorella extract marketed under different brand names. In both cases, the marketing is directed to the health food consumer, with the claim that Chlorella contains a growth factor (CGF). In some early work it was reported that Chlorella extracts may effect the growth and production of lactic acid by lactic bacteria.

The cost of production is estimated to be in the range of US$10-15 per kilogram dry weight. In the last ten years there have been no reports of either significant breakthroughs in the large scale production of Chlorella or new applications and potential uses. The market for Chlorella has declined in the last five years, and indications are that less than 50% of the total production potential of Chlorella biomass is used. Chlorella production is still the largest one in the field of microalgal biotechnology. Nevertheless, without developing new products and reducing the cost of production it is difficult to expect an expansion of this market.

2. Spirulina

Spirulina is a filamentous blue-green algae (cyanobacteria), and has been the subject of recently published reviews on its physiology and biochemistry (Ciferi and Tiboni, 1985), as well as mass production and potential applications (Ciferi, 1983; Richmond, 1988; Vonshak and Richmond, 1988). The algae is cultivated outdoors in open raceway ponds. The growth medium used for the cultivation of the algae is highly alkaline (0.2M $NaH_2 CO_3$) and pH is maintained at 9.5–10.3. These two parameters are of great significance, permitting the maintenance of a monoalgal culture under outdoor conditions.

The history of Spirulina as a staple in human diet is unique. There is evidence from the annals of the Spanish conquest of Mexico, early in the sixteenth century, that the Aztecs harvested mats of algal biomass reminiscent of Spirulina from Lake Texcoco, which they made into dry bricks eaten much as cheese would be eaten today in the West. Likewise, for many generations dried Spirulina has been used as a food by the Kanembu tribe, which lives along the shores of Lake Chad in Central Africa (Furst, 1978).

The large production sites of Spirulina biomass are currently located in the American Southwest, Hawaii, Mexico, and the Far East (mainly Thailand). Although some low grade products are produced in an extensive mode, i.e. using unlined deep ponds, with no mixing or other method to induce turbulent flow, the main production of what is considered to be high grade products is produced in raceway type ponds.

In the past ten years considerable progress has been made in accumulating the knowledge necessary to maintain the continuous production of Spirulina biomass in outdoor cultures. The mode of production is based on the principles of a turbidostate culture, where a constant cell concentration is maintained in the ponds, usually in the range of 400-600 mg/L of dry weight, by daily harvesting. The clarified medium, enriched with nutrients and short Spirulina filaments, is returned to the pond. The harvested biomass, which is a slurry of 8-10%, is further subjected to vacuum filtration and then to the last step of drying. In Table 1 a summary of current production sites, along with their actual productivity and product quality are presented. The figures are based on personal communications and company press releases.

TABLE 1

SPIRULINA PRODUCTION PLANTS

Company Name	Location	Pond Size (ha)	Annual Product. Ton	Dry wt
Earthrise Farm[1]	California, USA	1.5[2]	90[5]	20[6]
Siam Alga[1]	Bangkok, Thail.	1.8[2], 2[3]	70[5]	40[6]
Sasa Texcoco	Lake Texcoco, Mexico	12[3]	300	
Nippon Spirulina	Japan	1.5[2]	30[5]	
Cyanotech	Hawaii, USA	3[2]	80-90[5]	
Blue Continent Chlorella	Taiwan	4-15[4]	up to 300[5]	
--	India		5[6]	
--	Vietnam		5[6]	

Notes:

1 Earthrise Farms and Siam Algae are both owned by Dainippon Ink and Chemicals (Japan). Plans call for increasing production capacity of both sites to 120 t/y and 200 t/y food grade in Siam Algae and Earthrise Farms, respectively.
2 Plastic lined or concrete ponds
3 Native ponds
4 Ponds originally designed for the producton of Chlorella and modified for growing Spirulina according to market demands.
5 Food grade for human consumption, US-FDA approved.
6 Feed grade or relatively low quality.

Market and Application: The biomass produced is mainly sold to the health food market in the form of powder or pills. Attempts have been made by Proteous (a marketing company mainly associated with Earthrise Farms in the USA) to incorporate Spirulina into a variety of food products such as granola bars and various kinds of pasta. In Mexico and China, subsidized by the government, Spirulina powder is added to children's foods such as bisquits, chocolate, etc. (Fox, 1985). Another available product is a protein extracted from

Spirulina, containing mainly the blue pigment phycocyanin and marketed under the "Lima Blue" brand name (Dainippon Ink, 1980, 1981). The product is mainly used as a colorant for the food market, as an edible dye for ice creams and as a natural dye in the cosmetics industry. The main problem is that the pigment is light sensitive and special care has to be taken in handling the dye to protect it from bleaching. Recently, a full account of the applications of Spirulina in human nutrition and various therapeutic effects was given by Henrikson (1989). In Table 2 some of these applications are summarized. It should be pointed out that none of these applications have been permitted by the US-FDA as a proven "claim" for marketing and more experimental work has to be performed before such approval will be given.

TABLE 2
REPORTS ON CLINICAL APPLICATIONS OF SPIRULINA

Application	Subject	Countries
Malnutrition and nutritional deficiencies	Human infants	Mexico, Togo, China
Anti-cancer	Hamsters, mice	USA, Japan
Iron availability and anemia	Rats, humans	USA, Japan
Lowering cholesterol	Humans, rats	Japan

Cost of production is estimated to range from US$6-12 per kg dry weight. The lower figure is based on a "feed" grade product, not for direct human consumption. The product is sun-dried and contains a high ash and low chlorophyll content. The demand for such a product is continually growing, as its relatively low price is making it more attractive to the feed industry as an additive to fish and chicken feed. This demand is reflected in the reports of an increase in the production of feed grade products up to 60 tons per year by Siam Algae and Earthrise Farms. This is compared to only a few tons of feed grade produced just a few years ago. Recently, a starch producing factory in Thailand has started to use its water refuse from an anaerobic digester to grow Spirulina and then sun-dry the product. Cost estimates indicate that the product can be sold for less than US$6 per kg. This is a very important step towards the production of Spirulina, not only as a health food product, but for a much larger markets such as for feed additives.

3. Dunaliella

Dunaliella is a unicellular bi-flagellated green alga known for its unique osmoregulation mechanism, as reported in detail by Ben-Amotz and Avron (1973). The alga was isolated from salt lakes, the Dead Sea in Israel and other salt habitats (Borowitzka and Borowitzka, 1988a). It was first suggested as a commercial source for glycerol since it accumulates glycerol as an osmoregulant and its intracellular concentration may reach a level of up to 50% of total cell mass (Ben Amotz and Avron, 1973). New strains have been isolated and it was found that when Dunaliella is grown under stress conditions (when the growth rate is slowed down and the light per cell cycle is increased [Ben Amotz and Avron, 1983]), the cells accumulate beta-carotene, most likely as a means of protection from photo-inhibition or photo-oxidation. The concentration of beta-carotene in the cells grown under these extreme conditions can reach 8-12% on a dry weight basis. Under outdoor conditions, this value is in the range of 4% per dry weight.

The following unique characteristics of Dunaliella make it an attractive candidate for mass cultivation:

a. Its ability to accumulate relatively high concentrations of beta-carotene, known to be in high demand and have a high commercial value.
b. Its ability to thrive under extreme conditions such as 6-12% NaCl provides a selective advantage, wherein development of other algae or predators in open raceway ponds is prevented.

Indeed, in the last ten years, when the markets for Chlorella and Spirulina seemed to decline, the prospects for Dunaliella products improved. Two approaches are used in the mass production of Dunaliella: One is similar to the type used for Spirulina raceway ponds and the other uses large lagoons. The raceway method, stirred by paddle wheels, is the intensive mode used by Microbio in the USA and Nature Betacarotene Technology (NBT) in Israel. The utilization of lagoons is an approach where growth rate is much slower and the stability of the biological system is more complicated to maintain. Nevertheless, the investment cost is significantly lower.

Recently, a new, closed cultivation system has been applied to the mass cultivation of Dunaliella, based on polyethelene tubes of 1 dm in diameter. The commercial production set-up is based on 5 km length of such tubes, arranged on a vertical fence-type support to a height of approximately 2 m. Flow in the tubes is induced by an air-lift pump. Such a large photobioreactor may represent the future method of choice for cultivation of algae since it seems to have several advantages over open raceway ponds: better temperature control leading to higher productivity; lower susceptibility to infection and contamination; higher stability of the biological system, providing consistent quality.

The main technical problem still to be solved in the mass cultivation of Dunaliella is the harvesting. A variety of methods have been tried, as reviewed by Ben-Amotz and Avron (1989), but all either still require high energy inputs (centrifugation); require the addition of undesirable chemicals (flocculation); or are far from being tested on a large scale.

Application and Market: The Dunaliella biomass may have the potential of becoming the largest market in the micro-algal field, mainly on the basis of its high beta-carotene content. This pro-vitamin A product is widely used in the feed and food industry. Together with recently published reports (Ben Amotz et al, 1986) on the effects of beta-carotene on promoting growth in poultry, its cancer retarding applications may even further increase its potential market (Nagasawa et al, 1989). Today, the product is available mainly in two forms: dried or extracted. Dried Dunaliella, in powder or pill form, is considered to be of the highest quality. The product is harvested by different means of concentration and centrifugation, and thereafter dried by spray-driers. In this form, the product is mainly aimed at the health food market for direct human consumption. The price of this product is based on the beta-carotene content, and can go up to US$2,000 per kg of beta-carotene, i.e. about US$50-80 per kg of dry weight Dunaliella biomass. The second kind of product, mainly obtained from Dunaliella biomass which is harvested by flocculation, is a beta-carotene extract into a vegetable oil. In this case, the concentrated wet biomass is mixed with vegetable oil, and due to the fact that the Dunaliella cell does not possess a cell wall, the beta-carotene is easily extracted into the lipid phase while all the cell debris is removed with the aqueous phase. The product can then be applied as a food dye and a pro-vitamin additive for human consumption, for fish and poultry feed, or in the cosmetics industry as an additive to sun-screen products. The price of this product is significantly lower, and only a little information is available on its current market price. From a few reports it is estimated that the price, on the basis of beta-carotene content, varies in the range of US$1,000-1,200 per kg of beta-carotene.

The size of the market for natural beta-carotene from Dunaliella is still difficult to estimate. From a very modest start-up just a few years ago, all the production facilities around the world are reporting a continuous increase in production and demand. NBT in Israel has just increased its production facility from 1.2 Ha to 5.0 Ha, reaching an estimated annual production of 50-75 metric tons of Dunaliella biomass.

Two American companies, Microbio in California and Cyanotech in Hawaii, are operating larger facilities with a similar annual production rate. The two extensive Australian facilities operated by Western Biotechnology and Betatene are also in the process of intensification of production. The market has now reached a stage where further expansion is a question of costs of production and the ability to demonstrate a significant advantage of using the natural isomers of beta-carotene compared to the artificially synthesized material. If these two conditions can be met, it can easily be foreseen that the market for Dunaliella will constantly increase to a level of more than 1,000 tons per year, building up a multi-million dollar market just for natural beta-carotene from Dunaliella-based products.

OTHER PRODUCTS AND NEW DEVELOPMENTS

Microalgae are widely used as a traditional feed in the aquaculture industry (De Pauw and Persoone, 1988). In some cases it has been demonstrated that in the early developmental stages of molluscs and crustaceans there is a specific requirement for microalgae (Walne, 1974; Webb and Chu, 1982). Microalgae are available as a feed-chain component in the natural habitat of these animals. The constant increase in production of aquaculture products and the intensification of the process have raised the need for a much larger supply of particular microalgae than can be harvested from natural habitats. At present, most hatcheries produce their own microalgae on site and some have developed the procedure for selling algal concentrate to other hatcheries. The main problem is that the hatcheries lack the know-how for mass cultivation of microalgae, and more problematic is the constant need for fresh biomass when the optimal growth conditions for the algae do not coincide with the specific developmental stages of the fish larvae and molluscs, when algae are most required.

Recently, two different companies, Cell Systems Ltd. in the UK and Martek in the USA, have used the same approach to cultivate microalgae under heterotrophic conditions for the production of algal biomass rich in essential fatty acids such as eicosapentaenoic acid (EPA) and docoshexaenoic acid (DHA), or other algae known for their use by the aquaculture industry, such as Tetraselmis. According to the reports of Cell Systems Ltd. (Day et al, 1991), the product sold as "Celsys Algae 161" has been tested in large-scale feeding experiments and may replace up to 70% of the hatchery-grown microalgae. Its major advantages are a constant supply of a storable product with constant and known characteristics. Martek has further applied growing techniques to produce a variety of products marketed under the name of "Aguaro", with different combinations of Isochrysis, Nitschia and Nannochlorophsis, all grown indoors heterotrophically or photoheterotrophically, and designed to meet specific requirements of the aquaculture feed industry. Using almost the same growing facilities, Martek is also producing different microalgae biochemicals for research, such as phycoerythrin, phycocyanin, and amino acids labelled with the stable isotopes ^{13}C and ^{2}H.

Another product for the aquaculture feed industry which was recently introduced is marketed under the brand name "Algaxan Red" and produced by Microbio in the USA (Bubrick, 1991). This product is dried biomass of a microalga known as Haematococcus which, when grown under low light and high nutrient levels, has a green flagelated form. Under high illumination and low nutrient levels, the cells undergo a remarkable morphological change, accompanied by intensive synthesis and accumulation of a carotenoid pigment known as astaxanthin. This pigment is responsible for the red-pink color of salmon flesh, and also for the yellow color of chicken meat. This production is still very limited, and the alga is grown under outdoor conditions in open raceway ponds. Due to the fact that the growth conditions are very common and in a non-selective medium, the cultures are easily contaminated by other organisms. Nevertheless, the high demand for the product and the fact that it reaches relatively high concentrations in the algal biomass (2-4%) as compared to other sources such as yeasts (0.1%), point out its potential to develop as a new product in this quickly developing market of microalgae as feed.

Besides the development of new products and introduction of new strains, considerable effort has been invested in the development of an improved cultivation system (Cohen and Arad, 1989; Lee, 1986; Pirt et al, 1983; Gudin and Chaumont, 1991, Tredici et al, 1991). The realization that in order to be able to cultivate a variety of microalgae one has to come up with a more reliable system where environmental factors such as temperature can be easily controlled and contamination can be avoided, led to the attempt to develop a closed photobioreactor. Many models have been suggested, none of them reaching the stage of large-scale operations under continuous conditions. Nevertheless, the recent development and establishment of large-scale tubular reactors in Spain (as described earlier in this report) and reports on similar plans for demonstration (pilot) plants in Italy and France suggest that such a technology will be available soon, making the introduction of many more algal strains an easier task.

FUTURE

The future of algal biotechnology rests, to a large extent, on two factors: a) The ability to reduce costs of production and thus make algal biomass a commodity traded in large quantities, not limited to the health food market. b) The development of suitable reactors. Closed systems have several advantages over open raceways. In closed systems, cultures are better protected from contaminants and thus the maintenance of monoalgal cultures should be easier. Water loss and the ensuing increase in salinization of the medium are prevented. Areal volumes may be kept much smaller, due to much higher cell densities, reducing harvesting costs. Finally, optimal temperatures may be established and maintained more readily in closed systems ensuring higher output rates. The latter is an essential aspect in the production of a microorganism such as Spirulina, with a growth temperature of 37°C (Vonshak, 1987). These advantages have to be tested on a large scale, set up in order to evaluate whether the higher investment cost is indeed, as expected, compensated for by higher annual yield of algal biomass.

Considerable progress has been made in the past decade in developing the appropriate biotechnology for microalgal mass cultivation aimed at establishing a new agro-industry. This review, besides summarizing recent developments, also indicates the requirements for making this biotechnology economically viable. One requirement is the availability of a wide variety of algal species and improved strains which favorably respond to varying environmental conditions existing outdoors. It is thus just a matter of time and effort before a new methodology like genetic engineering can and will be applied in this field as well.

REFERENCES

Ben-Amotz, A. and Avron, M. (1973) The role of glycerol in the osmotic regulation of the halophilic alga Dunaliella parva. Pl. Physiol. 51:875-8.

Ben-Amotz, A. and Avron, M. (1983) On the factors which determine massive beta-carotene accumulation in the halotolerant alga Dunaliella bardawil. Pl. Physiol. 72:593-7.

Ben-Amotz, A. and Avron, M. (1989) The Biotechnology of mass culturing of Dunaliella for products of commercial interest. In: Algal and Cyanobacterial Technology, (Cresswell, R.D., Rees, T.A.V. and Shah, N., Eds), pp. 90-114, Longman Scientific and Technical Press.

Ben-Amotz, A., Edelstein, S., Avron, M. (1986) Use of the beta-carotene rich alga Dunaliella bardawil as a source of retinol. Br. Poultry Sci. 846, 313-23.

Benemann, J.R., Koopman, B., Weissman, J.C., Eisenberg, D. and Goebel, R. (1980) Development of microalgae harvesting and high rate pond technologies in California. In: Algae Biomass (Shelef, G. and Soeder, C.J., Eds.), pp. 457-95, Elsevier/North-Holland Biomedical Press, Amsterdam.

Benemann, J.R., Tillett, D.M. and Weissman, J.C. (1987) Microalgae biotechnology. Trends in Biotechnology 5, 47-53.

Borowitzka, L.J. (1991) Development of Western Biotechnology's algal beta carotene plant. In: Recent Advances in Algal Biotechnology. Special Issue of Biomass (In press).

Borowitzka, M.A. and Borowitzka, L.J. (1988a) Dunaliella. In: Micro-Algal Biotechnology (Borowitzka, M.A. and Borowitzka, L.J., Eds.), pp. 27-58, Cambridge University Press, Cambridge.

Borowitzka, M.A. and Borowitzka, L.J., Eds. (1988b), Micro-Algal Biotechnology, Cambridge University Press, Cambridge.

Bubrick, P. (1991) Production of astaxanthin from Haematococcus. In: Recent Advances in Algal Biotechnology. Special Issue of Biomass (in press).

Burlew, J.S. (1953) Current status of the large-scale culture of algae. In: Algal culture from laboratory to pilot plant (Burlew, J.S., Ed.). Publ. No. 600, pp. 3-23, Carnegie Institute of Washingon Publication 600, Washington, D.C.

Ciferri, O. (1983) Spirulina, the edible microorganism. Microbiol. Rev. 47, 551-578.

Ciferri, O. and Tiboni, O. (1985) The biochemistry and industrial potential of Spirulina. Ann. Rev. Microbiol. 39, 503-526.

Cohen, Z. (1986) Products from Microalgae. In: Handbook of Microalgal Mass Culture (Richmond, A. Ed.), pp. 421-454, CRC Press, Boca Raton, FL.

Cohen, E. and Arad, A. (1989) A closed system for outdoor cultivation of Porphyridium. Biomass 18, 59-67.

Dainippon Ink and Chemicals Inc. (1980) Production of highly purified alcoholophilic pycocyanin. Japanese Patent 80 77, 890.

Dainippon Ink and Chemicals Inc. (1981) Cosmetics containing water soluble phycocyanin. Japanese Patent 79-138755.

Day, J.G., Edwards, A.P. and Rodgers, G.A. (1991) Development of an industrial scale process for the heterotrophic production of microalga mollusc feed. In: Recent Advances in Algal Biotechnology. Special issue of Biomass (in press).

De Pauw, N. and Persoone, G. (1988) Micro-algae for aquaculture. In: Micro-Algal Biotechnology (Borowitzka, M.A. and Borowitzka, L.J., Eds.), pp. 197-221, Cambridge University Press, Cambridge.

Dodd, J.C. (1986) Elements of design and construction. In: Handbook of Microalgal Mass Culture (Richmond, A. Ed.), pp. 265-263, CRC Press, Boca Raton, FL.

Fox, R.D. (1985) Spirulina, the alga that can end malnutrition. The Futurist 19, 30-35.

Furst, P.T. (1978) Spirulina. Human Nature 60, 60-65.

Goldman, J.C. (1979) Outdoor algal-mass cultures. I. Applications. Water Res., 13,1.

Grobbelar, J.U. and Soeder, C.J. (1985) Respiration losses in green alga cultivated in raceway ponds. J. of Plankton Res. 7, 497-506.

Gudin, C. and Chaumont, D. (1991) Cell fragility: the key problem of microalgae mass production in closed photobioreactors. In: Recent Advances in Algal Biotechnology. Special Issue of Biomass (in press).

Henrickson, R. (1989) Earth Food Spirulina. Ronore Enterprise Inc., USA.

Kawaguchi, K. (1980) Microalgae production systems in Asia. In: Algae Biomass (Shelef, G. and Soeder, C.J., Eds.), pp. 25, Elsevier/North-Holland Biomedical Press, Amsterdam.

Lee, Y.K. (1986) Enclosed bioreactors for the mass cultivation of photosynthetic microorganisms:the future trend. Trends in Biotechnol. 4(7), 186-189.

Lembi, C.A., Waaland, J.R., Eds. (1988) Algae and Human Affairs, Cambridge University Press.

Mohn, F.H. (1980) Experience and strategies in the recovery of biomass from mass cultures and microalgae. In: Algae Biomass (Shelef, G. and Soeder, C.J., Eds.), pp. 547-71, Elsevier/North-Holland Biomedical Press, Amsterdam.

Mohn, F.H. (1988) Harvesting of micro-algal biomass. In: Micro-Algal Biotechnology (Borowitzka, M.A. and Borowitzka, L.J., Eds.), pp. 395-414, Cambridge University Press, Cambridge.

Nagasawa, H., Honishi, R., Sensui, N., Yamamoto, K. and Ben Amotz, A. (1989) Inhibition by Beta-Carotene-Rich algae Dunaliella of spontaneous mammary tumourigenesis in mice. Anticancer Res. 9, 71-76.

Oswald, W.J. (1988) Micro algae in waste water treatment. In: Micro-Algal Biotechnology (Borowitzka, M.A. and Borowitzka, L.J., Eds.), pp. 305-328, Cambridge University Press, Cambridge.

Pirt, S.J., Lee, Y.K., Walach, M.R., Pirt, M.W., Balyuzi, H.H.M. and Bazin, M.J. (1983) A tubular bioreactor for photosynthetic production of biomass from carbon dioxide: design and performance. J. Chem. Tech. Biotechnol. 33B, 35-58.

Richmond, A. (Ed.) (1986a) Handbook of Microalgal Mass Culture, CRC Press, Boca Raton, FL.

Richmond, A. (1986b) Microalgal culture. CRC Critical Reviews in Biotechnology 4, 369-438.

Richmond, A. (1988) Spirulina. In: Micro-Algal Biotechnology (Borowitzka, M.A. and Borowitzka, L.J., Eds.), pp. 83-121, Cambridge University Press, Cambridge.

Schlipalius, L. (1991) The extensive Commercial cultivation of Dunaliella salina. In: Recent Advances in Algal Biotechnology. Special issue of Biomass (in press).

Soeder, C.J. (1980) Massive cultivation of microalgae: Results and prospects. Hydrobiologia 72, 197-209.

Soong, P. (1980) Production and development of Chlorella and Spirulina in Taiwan. In: Algae Biomass (Shelef, G. and Soeder, C.J., Eds.), pp.97-113, Elsevier/North-Holland Biomedical Press, Amsterdam.

Tredici, M.R., Carlozzi, P., Chini, Z. and Matecassi, R. (1991) A vertical alveolar pane (VAP) for outdoor mass cultivation of microalgae and cyanobacteria. In: Recent Advances in Algal Biotechnology. Special issue of Biomass (in press).

Vonshak, A. (1987) Biological limitations in developing the biotechnology of algal mass cultivation. Sc. De L'eau 6, 99-103.

Vonshak, A., Cohen, Z. and Richmond, A. (1985) The feasibility of mass cultivation of Porphyridium. Biomass 8, 13-25.

Vonshak, A. and Guy, R. (1988) Photoinhibition as a limiting factor in outdoor cultivation of Spirulina platensis. In: Algal Biotechnology (Stadler, T., Mollion, J., Verdus, M.C., Karamanos, Y., Morvan, H., and Christiaen, D., Eds.), pp. 365-370, Elsevier Applied Science Pub.

Vonshak, A. and Richmond, A. (1985) Problems in developing the biotechnology of algal biomass production. Plant and Soil 89, 129-135.

Vonshak, A. and Richmond, A. (1988) Mass production of Spirulina – an overview. Biomass 15(4), 233–248.

Walne, P.R. (1974) Culture of bivalve molluscs, 50 years of experience at Conwy. Fishing News (Books) Ltd. Surrey, UK.

Webb, K.L. and Chu, F.L.E. (1982) Phytoplankton as a food for bivalve larvae. In: "Proceedings of the Second International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition." Pruder, G.D., Langdon, C.J. and Conklin, D.E. (Eds.), pp. 272–291, Louisiana State University Press, Baton Rouge, LA..