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Commercial applications of algae: opportunities and constraints

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Abstract

Microalgae have three fundamental attributes that can be converted into technical and commercial advantages:

(1) They are genetically a very diverse group of organisms with a wide range of physiological and biochemical characteristics; thus they naturally produce many different and unusual fats, sugars, bioactive compounds, etc.
(2) They can cost-effectively incorporate the stable isotopes ^{13}C, ^{15}N and ^{2}H into their biomass, and thus into various compounds they produce.
(3) They comprise a large, unexplored group of organisms, and thus provide a virtually untapped source of products.

The challenge in the application of microalgae to commercial ends is to focus only on those products with large market and/or profit potential, where the use of microalgae leads to clear competitive advantages. This requires a clear understanding of the practical use of the microalgal product, the market it addresses, and its advantages and cost relative to competitive products or processes. Several examples are presented.

Introduction

As a prelude to the following 17 papers and 5 abstracts, we shall present some general considerations concerning the commercial applications of microalgae. More specifically, we shall describe some potential opportunities, some constraints, and provide a few examples of microalgal products currently produced in closed systems.

Opportunities

Microalgae have at least three fundamental attributes that can be exploited to useful ends: They are (1) very diverse, (2) often phototrophic, and (3) virtually unexplored. These attributes can provide significant technical and commercial advantages.

(1) Genetic diversity of microalgae

The great genetic diversity of microalgae is implied from their polyphyletic origins. Indeed, the algae have long been recognized as a heterogenous group of organisms, ranging from prokaryotic blue-greens (Cyanophyceae) to numerous, only distantly related eukaryotic lines. Figure 1 illustrates the taxonomic diversity of the various classes of algae based on molecular biological data from a limited selection of taxa (Hecht, 1993; Wainright *et al.*, 1993). In addition to three widely spaced algal classes (the Cyanophyceae, Euglenophyceae and Rhodophyceae), algae also are members of three other groups in this scheme, namely the Plantae (which contains the several classes of the Chlorophyta), the Alveolates (including the Dinophyceae), and the Stramenopiles (containing the heterokont algae). Three major groups of algae (Chlorarachniophyceae, Cryptophyceae, Haptophyceae) have been omitted because of their uncertain systematic position. Yet their existence as algae adds

further to the great genetic diversity potential. Since nearly all of these groups contain at least some microalgae (unicells, colonies, filaments), efforts to exploit the microalgae as a group will result in the study and development of organisms with a very broad taxonomic and evolutionary scope.

Microalgae also exhibit more obvious and tangible diversity. Their growth modes range from phototrophy, through photoheterotrophy, to heterotrophy – each growth mode in turn can be either obligate or facultative (Kaplan *et al.*, 1986). The genetic diversity of microalgae is also manifest in their structural diversity. Microalgae occur as unicells, colonies, filaments (branched or unbranched); the membranous types for the most part are macroalgae. Internally, cells can be uninucleate, coenocytic or siphonous, and they can have phycoplast, phragmoplast or other types of cytoskeletons, as well as other unusual cytological features. Finally, some algae are prokaryotes (the blue-green algae, which have no histones associated with the DNA and no chloroplast or nuclear membranes); some are mesokaryotes (the euglenoids and dinoflagellates, which have condensed nuclear DNA and a nuclear membrane, but do not have histones); still others are eukaryotes (which have histones and a membrane-bound nucleus) (Lee, 1989; Bold, 1985).

The genetic diversity of microalgae is evident still further in their ecological diversity and ubiquitous distribution in our biosphere. Different species can grow in water ranging from freshwater to hypersaline environments (e.g. Great Salt Lake); from the water surface to the limits of the photic zone, which is often 200–300 meters; either free-floating (plankton) or attached (periphyton or Aufwuchs) (Lee, 1989). Microalgae grow in soils ranging from rich humus to desert sands, and even inside rocks (Friedmann *et al.*, 1988). More exotic habitats in which algae have been reported include clouds, tree bark, caves, sloth fur and polar bear hair. Microalgae can be either free-living or exist in association with other organisms; within these associations they can exist as saprophytes, parasites or symbionts (Lee, 1989). An interesting example of the last case is the association of algae and coral animals. Coral reefs represent some of the most highly productive marine ecosystems characterized to date. The hundreds of species of hermatypic coral animals which build coral reefs are totally dependent on one or more endosymbiotic dinoflagellates (e.g. *Symbodinium microadriaticum*) for 90% of their energy; hermatypic corals quickly die if kept in the dark (Nybakken, 1982).

The great genetic diversity of algae cannot but lead to a great variety of products. Microalgae have a wide range of physiological and biochemical characteristics, many of which are rare or absent in other taxonomic groups. For example, unlike higher plants and animals and most prokaryotic microorganisms, some algae synthesize long-chain polyunsaturated fatty acids ('LC-PUFAs'), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Pohl, 1982). An interesting consequence of this situation is that the fish oils currently being promoted for their beneficial health effects are, in reality, algal oils that have been accumulated through the food chain (Lands, 1986). Like humans, fish cannot efficiently synthesize these LC-PUFAs, and obtain them primarily from their diet.

Some species of microalgae can efficiently synthesize internal or extracellular polysaccharides, some of which contain unusual monomer sugars. For example, some algae synthesize extracellular polysaccharides containing substantial quantities of D-galactose, while others use L-galactose or a mixture of the two isomers, probably for the same purpose (O'Colla, 1962).

Microalgae also synthesize a wide variety of bioactive compounds. An interesting example of this category is okadaic acid and its derivatives, which are the toxic principles of an affliction called diarrheic shellfish poisoning. Although these compounds were first isolated from sponges, subsequent work has shown that they are in reality produced by dinoflagellates closely associated with the sponges (Shimizu, 1993).

(2) Cost-effective incorporation of stable isotopes by microalgae

Because of their phototrophic growth capability, many algae can be used to generate specific compounds universally labeled with ^{13}C, ^{15}N and/or ^{2}H (deuterium) according to the following schematic equations:

$$^{13}CO_2 + H_2O + KNO_3 \longrightarrow [^{13}CH_2O] + O_2$$

$$CO_2 + ^{2}H_2O + KNO_3 \longrightarrow [C^{2}H_2O] + O_2$$

$$CO_2 + H_2O + K^{15}NO_3 \longrightarrow [CH_2O^{15}N] + O_2$$

Specific examples of such compounds are universally-labeled (UL) sugars, such as ^{13}C-D-xylose and ^{13}C-D-glucose, and $^{2}H_7$-D-glucose (D-glucose-1, 2, 3, 4, 5, 6, 6-d). The uses of such compounds are discussed below.

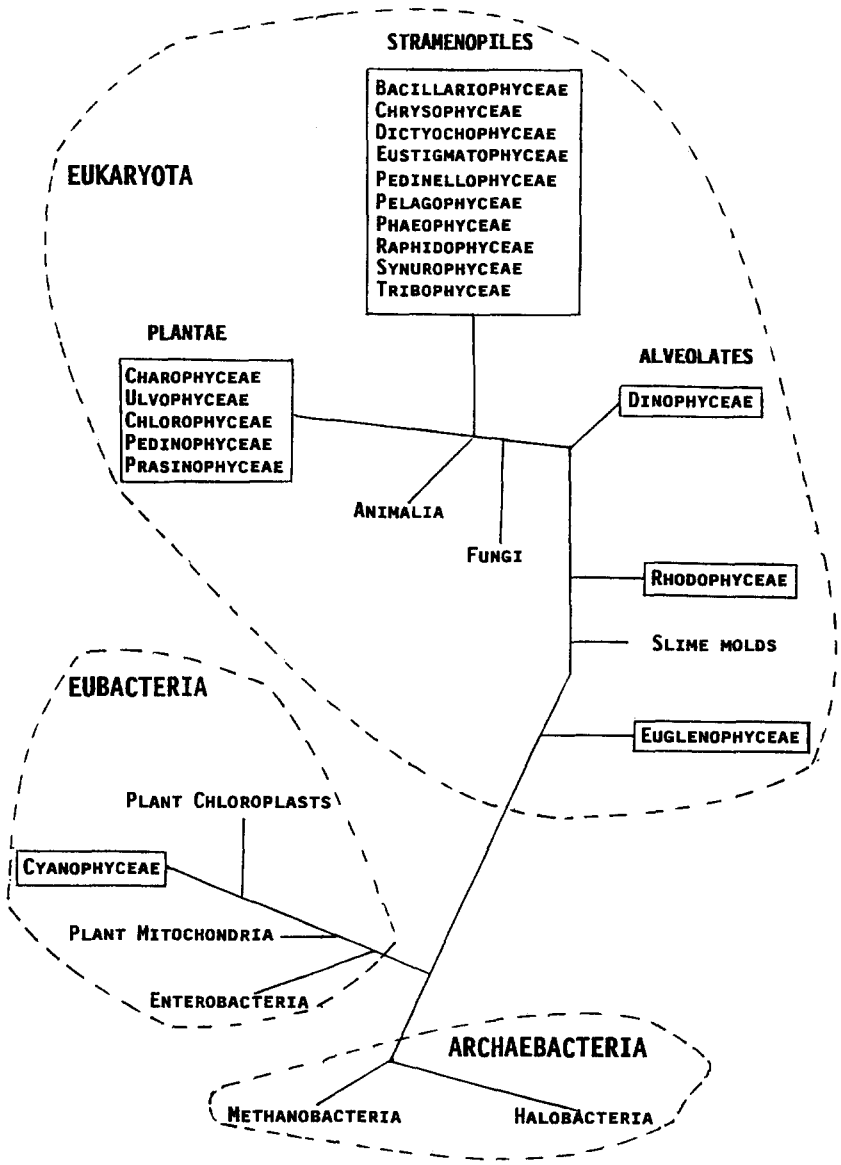

Fig. 1. Partial reconstruction of phylogenetic affinities of organisms based on genetic approaches used by Wainwright *et al.* (1993) and discussed by Hecht (1993). The 19 classes which comprise most of the algae are located in boxes.

(3) Numerous species and strains of microalgae unexplored

Approximately 40,000 identified species of algae exist on earth (Raven *et al.*, 1992), and new species are being identified at a rate of about one per week. However, unlike the bacteria (ca. 2500 known species) or the fungi (ca. 77,000 known species), algae have been virtually unexplored with respect to the production of useful products. For example, soil bacteria and fungi have been so thoroughly screened for pharmaceutical activities that most so-called active leads identified in screening programs prove to be rediscoveries of already-described compounds (G. Patterson, pers. comm.). In contrast, very few algae (probably less than 1% of known species) have been similarly tested. This may be due, in part, to the relative ease of culturing and testing the heterotrophic bacteria and fungi compared to phototrophic algae. As a result, algae (and particularly microalgae) provide a large and untapped reservoir of potential new products and applications.

Production systems

Most biotechnological applications of microalgae will require the use of closed culture. Three reasons for this are:

(1) The alternative open-culture systems (ponds, raceways and the like) can only maintain algal monocultures under unusual conditions. For example, *Dunaliella* can be grown at very high salt, while *Spirulina* can be grown at very alkaline conditions. In both of these cases, other algal species generally cannot compete, but these pond cultures do contain other organisms.

(2) Closed culture systems are amenable to the process control systems, procedures and technologies that have been well-developed for the fermentation industry. Thus, closed culture algal systems can benefit from a half-century of prior experience in the science and art of producing useful and valuable products using microbial systems.

(3) Regulatory requirements normally can be more easily satisfied using closed culture systems. Indeed, for many applications one might expect that closed production systems will be required. For most high-value applications, GMP (Good Manufacturing Practices) facilities will be mandated; this will probably require closed culture systems.

The well-controlled closed culture of microalgae will require the use of either photobioreactors (closed light-driven culture vessels designed for phototrophic growth) or fermenters (closed vessels designed for heterotrophic growth). The choice between these two production modes will depend upon the type of product desired and the economic constraints involved. Photobioreactors are required (directly or indirectly) whenever the production process involves the incorporation of stable isotopes, and can be economically used whenever high-valued products are being produced. The use of fermentation systems is required whenever large amounts of lower-value product is required. A brief comparison of the costs of these two production modes will illustrate.

The minimum cost for closed phototrophic growth can be estimated as follows: Assume that we wish to grow a *Chlorella* species in a photobioreactor illuminated with fluorescent lights in a metropolitan area of the eastern USA. Since the heat of combustion of *Chlorella* is 5.77 kcal $gram^{-1}$ (Kok, 1960) and one kcal is equivalent to 0.00116 kw h, one kg of *Chlorella* biomass is equivalent to 6.69 kw h. If we assume an electrical cost of \$0.05/kw h (typical of the eastern USA), a maximum photosynthetic efficiency of 20% (Radmer & Kok, 1977) and a lighting efficiency of 20% (Sylvania Engineering Bulletin 0-341), we conclude that the minimum cost to generate one kg of *Chlorella* biomass is \$8.36. One can expect that the actual costs will be substantially higher than this value.

Fermentation is a much more well-developed process with a vast experience base at a variety of production scales. Thus, typical costs for closed heterotrophic growth using these systems can be estimated quite accurately. The cost of producing simple products, such as single-cell protein, can be less than \$1 kg^{-1} of biomass on a dry weight basis (Crueger & Crueger, 1989). This is at least an order of magnitude less than the cost of phototrophic growth. Equally important is the availability of large scale production systems. Large fermentation facilities, with capacities of hundreds of thousands of liters, are available virtually world-wide, while photobioreactor facilities of even hundreds of liters are quite rare. Finally, the cell densities attainable in fermentation is about an order of magnitude higher than that generally attained in phototrophic systems; cell densities of greater than 100 g L^{-1} are attained in some fermentation processes (Suzuki *et al.*, 1985).

Constraints

A comparison of the production costs described above with some real-world numbers can serve as a guide to the kind of products and businesses that might be favorably impacted through the use of algal biotechnology. Table 1 provides approximate selling prices for 10 typical biological products. Note that even products manufactured in large-scale fermentation systems would not compete favorably with bulk products such as soybean meal and oil. It is only when we consider higher-value products, such as vitamins C and E, that closed microalgal culture systems, which for the lower value products must be heterotrophic, can begin to compete.

Thus, the challenge in the application of microalgal biotechnology to commercial ends is to focus only on those products for which one can identify a significant market, with good profit potential, where the use of algae leads to clear competitive advantages. This, in turn, requires a clear understanding of the practical use of the product, the market it addresses, and its advantages and cost relative to competitive prod-

Table 1. Approximate current prices of 10 typical products of interest.

Product	$ kg^{-1}
Soybean meal	0.19
Soybean oil	0.50
Yeast (brewers)	2.40
Sodium alginate	14.00
Vitamin C (Ascorbic Acid)	17.00
Agar	35.00
Vitamin E (alpha-tocopherol)	67.00
β-carotene (chemical, all-trans)	600.00
β-carotene (biological, "natural")	1400.00
Biotin	7000.00

Source: Chemical Marketing Reporter, 30 August 1993 and 11 October 1993 issues, Schnell Publishing Company, Inc., New York.

ucts or processes. Operationally, this means that market research should lead the technical research-and-development effort. The goal is to develop a product that fills a need; one should avoid developing a product in search of a use.

Examples of products from microalgae in closed systems

Nutritional products

Certain microalgae produce large quantities of oils and fats containing long-chain omega-3 and omega-6 fatty acids (LC-PUFAs, see above). LC-PUFAs are essential to human nutrition and health, and recent studies have indicated that certain of these LC-PUFAs may be associated with physical, mental and visual development in infants (see e.g. Neuringer & Conner, 1987). Technology for the production of oils containing specific LC-PUFAs has been developed by discovering, modifying and culturing microalgae which produce large quantities of certain LC-PUFAs (Kyle *et al.*, 1992; Barclay *et al.*, 1994). The production costs of these oils is approaching economic feasibility for several applications, and clinical tests are in progress.

Stable isotope biochemicals (SIBs)

Rational drug design has recently emerged to improve the efficiency of new pharmaceutical development (Appelt *et al.*, 1991). Many drugs work by mimicking the interaction between two molecules, a 'ligand' and a 'receptor'. Rather than rely solely on the chance synthesis of an active chemical compound, practitioners of rational drug design seek to study the interaction between the ligand and receptor, and to design a specific drug prototype on the basis of that interaction. The ability to determine the structure of the ligand, the receptor, and ideally, the complex between the two is advantageous in rational drug design.

Microalgae can produce SIBs, such as UL-^{13}C-glucose (Behrens *et al.*, 1989), which enable three-dimensional structures of proteins of pharmaceutical interest to be determined in solution. The SIBs are used as growth media to feed organisms which generate proteins comprised of stable isotopes. The presence of stable isotopes allows for the determination of the three-dimensional structure of these proteins using a technique called nuclear magnetic resonance spectroscopy (NMR). Proteins incorporating stable isotopes such as ^{13}C and/or ^{15}N have been obtained by growing organisms on these SIBs, and their three-dimensional structures have been deduced from information provided by NMR (Kay *et al.*, 1990). In addition, the incorporation of the NMR-invisible isotope deuterium into the protein allows for the structure of either the ligand or the receptor to be determined while bound to its NMR-invisible partner (Hsu & Armitage, 1992).

Breath test diagnostics

Microalgae may provide the means to develop a family of gastrointestinal diagnostic tests that are based on measuring the amount of ^{13}C in breath CO_2. These breath tests are simple to administer and are only minimally invasive (Schoeller *et al.*, 1977). They are designed to replace invasive, sometimes difficult and higher-risk procedures, such as biopsy of the liver, or the insertion of instruments into a patient's throat through to the stomach or small intestine. Specific examples include the use of labeled galactose to monitor liver function (Shreeve *et al.*, 1976) and the use of labeled xylose to determine the extent of bacterial overgrowth of the small intestine (King & Toskes, 1986). These breath tests all make use of the same general procedure:

(1) a capsule containing a specific ^{13}C labeled compound is ingested;
(2) breath samples are periodically collected; and

(3) the breath samples are analyzed for their ^{13}C content.

Clinical research on several of these diagnostic tests is in progress.

Conclusion

Numerous opportunities are available to use microalgae for the production of useful and valuable products. Furthermore, we have just scratched the surface. As the knowledge of this diverse and intriguing group of organisms grows, new and even more exciting opportunities will emerge. Work of the type and quality manifest in the following 17 communications will serve to advance the nascent field of microalgal biotechnology.

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