Algal Biotechnology

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ABSTRACT

The review gives a general outline of macro- and microalgal biotechnology. The main methods by which algae are cultivated and harvested are described. The first section deals with the environmental factors affecting mass culture and the principles governing the design and operation of mass cultivation systems. The second section gives the main current and potential uses of algae: in wastewater treatment, a source of food and feed, an energy source, and in the production of common and fine chemicals, such as polysaccharides, lipids, glycerol, pigments, and enzymes. Pharmaceutical uses of algae are described, and their potential as a source of novel biologically-active compounds is discussed. Future developments and the great potential of algae are considered.

Index Entries: Algae; lipids, enzymes; biotechnology.

INTRODUCTION

Algae are photosynthetic, nonvascular plants that contain chlorophyll *a* and have simple reproductive structures. They are a very large and heterogeneous group, containing about 30,000 known species. These range from unicellular forms to large multicellular plants, forming parenchymatous thalli. They are almost all aquatic or, if terrestrial, they inhabit damp environments. They exhibit great heterogeneity and, as a group, are only loosely bound together by virtue of being the "simplest" plants, with very little differentiation and, specifically, no specialized reproductive structures.

The Algae were described succinctly by Lewin (1976): "Apart from a general inclination to occupy wet places, almost the only feature the algae have in common is their ability to fix carbon from carbon dioxide and to evolve oxygen when suitably illuminated. The algae are thus more of a guild than a clan, united more by common aptitude than common ancestry."

Historically, and for the purposes of this review, cyanobacteria (blue green algae) are included within this group though these prokaryotic organisms are now considered to be more closely related to the bacteria. The heterogeneity of algae means that their taxonomic classification often undergoes revision.

PRODUCTION OF ALGAE

Macroalgae

Collection

Historically, utilized macroalgae were simply harvested from the naturally-growing populations on the shore, or coastal regions. This is still often the case, but is now increasingly combined with more effective management that takes into account, harvesting strategies, effects of pruning, dynamics of populations, and so on (Poblete & Inostroza, 1987; Westermeier et al., 1987; Santelices & Norambuena, 1987; Ang, 1987) as well as more effective marketing techniques that can involve grading and sorting material and improving processing techniques to upgrade material (Luxton & Courtney, 1987; Rotmann, 1987).

Cultivation

Macroalgae are also cultivated, most notably in China and Japan, where demand for seaweed has always been relatively high. The first recorded cultivation of seaweed was in Japan in 1717 (Nisizawa et al., 1987). The most important genera cultivated are *Porphyra, Laminaria, Undaria, Gracilaria,* and *Eucheuma.* It is estimated that Japan, China, Korea, and the Philippines cultivate about 2.4×10^6 tons of wet seaweed annually, in an industry worth hundreds of millions of dollars and involving 670,000 people in these four countries. China alone harvests about 1.4×10^6 tons (wet wt) of *Laminaria,* making it the most important marine plant crop. After *Laminaria, Porphyra* is the most exploited seaweed, with Japan the main producer (Tseng & Fei, 1987).

Offshore cultivation usually takes place by creating new seaweed beds by direct planting of thallus fragments into the seabed, or providing an artificial substratum to which sporelings or thallus fragments are attached. The former method can involve planting simply by pushing thalli into the sand or placing them in plastic bags filled with sand, which then are deposited on the bottom. The plastic bag eventually disintegrates leaving an established plant (Santelices & Ugarte, 1987). The more usual method of cultivation involves raising sporelings in artificial conditions, such as the case for the *Porphyra* conchospores and the *Laminaria* sporelings that are regularly used in Japan and China. These are then attached to a suitable substratum consisting of suspended ropes, nylon lines, or nets. These can be bottom-anchored or attached to raft structures. Alternatively, seaweed can be cultivated in open-topped cages anchored to the sea floor; this has the advantages that there is less stipe breakage, less material is lost to predators, and the structures could stabilize sand losses in areas where beach erosion is a problem (Beavis & Charlier, 1987). In this way the seaweed bed can be better controlled in terms of plant densities and growing depths, and the area available for cultivation can be extended by placing the artificial substrata at new locations or further offshore, where the alga would not normally grow.

The most labor intensive feature of macroalgal cultivation is harvesting. For some systems, harvesting takes place annually, but often it occurs more frequently. This is obviously more effective in countries where labor is cheap (Rotmann, 1987). For harvesting the great natural kelp beds of *Macrocystis* in California, specially-designed barges have been used (Whitney, 1987).

Problems in seaweed farming can be owing to fouling and disease. These can reduce both the quality and quantity of the harvest. Various physiological diseases of *Laminaria,* such as white and green rot, can be prevented and/or treated relatively easily, whereas some pathogenic diseases of *Laminaria* and *Porphyra* are more of a problem. One possibility under investigation is the control of epiphytes by marine microherbivores (Tseng & Fei, 1987).

Studies have also taken place on macroalgal cultivation in onshore systems. These have the advantage that the seaweed is not subject to pollution, effects of currents, climatic changes, e.g., storms that can cause great damage, and growth conditions can be controlled. *Gracilaria* growth in circulating seawater tanks has been studied both outdoors (Edding et al., 1987) and in a greenhouse (Edelstein et al., 1987). In Taiwan, *Gracilaria* is cultivated in about 300 hectares of land-based ponds (Robinson, 1985). Another method under development and reported to give high yields, as well as avoiding the problem of epiphytes, is spray cultivation, where the seaweeds are placed on the ground or on multitiered nets and sprayed with seawater as a fine mist (Lignell & Pederson, 1986). This land-based technology is particularly amenable to countries where there is plentiful sunshine and seawater that cannot be used for irrigation. Through inbreeding, selection, and X-ray treatment, the Chinese have produced several new varieties of *Laminaria* and *Porphyra* with improved characteristics. Hybridization has also led to novel strains that are being employed commercially (Tseng & Fei, 1987). Tissue and cell culture work has led to the isolation of single cell protoplasts and plant regeneration, in a few cases, and callus formation. (Polne-Fuller & Gibor, 1987; Liu & Gordon, 1987). Most of this work has been done on *Porphyra.* At some point in the future, genetic manipulation techniques also are likely to be applied to the exploitation of macroalgae.

Microalgae

Environmental Factors of Mass Culture

Macroalgae have always been of greater commercial value than microalgae, but the latter hold greater potential mainly because they have far higher specific growth rates.

The development of systems for the large-scale cultivation of microalgae has been under way in many parts of the world since the 1940s. There are several different processes whereby particular microalgae may be successfully cultured on a large scale, but the difficulty lies in making them economically viable.

Most attention has been focused on open systems, but there are still many problems to be addressed (Richmond, 1987; Benemann et al., 1987). The main challenge is to achieve the optimal balance between the factors affecting growth in the algal pond: These include light, temperature, nutrient supply, mixing, and pH.

In order to achieve maximum biomass, it is necessary that light is the limiting factor. The theoretical value of maximum light energy conversion efficiency is reported to be 6.6%, but in practice, acceptable levels are likely to be lower than this (de la Noue & de Pauw, 1988). For example, large scale production of *Spirulina* converts solar energy with an efficiency of about 1% (Richmond, 1987).

Nutrient addition is necessary for optimal growth. These can include macronutrients, trace elements, and vitamins and can vary according to species and product desired. Biomass yields are often increased by adding an organic carbon source (Borowitzka, 1988a). Mass culture nutrients can be added in a strictly controlled, defined manner, or on a larger scale, in the form of sewage, wastewater, nutrient-rich seawater, saline groundwater, and agricultural fertilizer. There is also some interest in heterotrophic (as opposed to mixotrophic) growth of microalgae on a large scale (Kawaguchi, 1980). The type of carbon source used is related to the pH (Richmond, 1986).

Ideally, the daytime temperature of the culture would be as close as possible to optimum for growth (usually between 15–30 °C), followed by a fast and fairly large reduction in temperature during darkness in order to avoid respiratory loss of biomass. Several models have been developed for predicting algal productivities as functions of these major parameters of growth (Markl, 1980). Systems have been developed that employ computer technology to monitor and control factors, such as carbon dioxide and nutrient additions (Harvey, 1988).

Though conditions may be established for achieving maximum biomass, these may be different to those required for achieving maximum amounts of specific algal products, such as lipids or pigments, and particularly when that product is the result of secondary metabolism. Growth conditions, therefore, must be optimized separately for each individual system (Goldman, 1980). This might involve a two-stage process whereby maximum biomass was obtained, which would then be subjected to some form of stress, e.g., nutrient limitation, which would lead to biosynthesis of the desired product.

Bioreactors

Cultivation systems can be divided into two major types: large, open outdoor systems and closed systems. Most attention has been focused on the latter.

Open Systems

Early commercial algal ponds tended to be circular, with large mixing arms, but these were replaced with the general design favored to date, the raceway pond of about 0.5 hectare, mixed by paddlewheels.

Most ponds are plastic lined since this prevents loss of culture and nutrients and allows for greater control of the factors affecting the culture. The disadvantage is the cost and alternatives, such as clay sealing, have been suggested (Benemann et al., 1987). The depth of the pond depends on the method of harvesting.

Mixing the culture is of the greatest significance, both in terms of costs and productivity. Mixing helps to prevent thermal stratification, photoinhibition, and bottom anaerobiosis (Persoone et al., 1980). Owing to mutual shading, each cell in the culture is irradiated intermittently. Benemann et al. (1987) report that there is little evidence to suggest that increased mixing leads to increased productivity, but as Richmond (1987) points out, the important factor governed by mixing is not just the duration of light received, but the light regime of each cell. It is important to find the optimal relationship between light regime, population density, extent of mixing, and depth of culture (Richmond, 1986). Apart from paddlewheels, other mixing mechanisms include air-lift pumps, mixing boards, propellors, or centrifugal pumps. It might also be practical to harness wind energy to pond stirring (Dubinsky et al., 1980).

The addition and availability of carbon dioxide are also important considerations in the design of an efficient pond. The deeper the pond, the greater the carbon dioxide storage capacity and the lower the carbon dioxide loss (Benemann et al., 1987).

The use of open ponds means that there is little or no temperature control and, therefore, climatic conditions will have a great effect on the algal growth rate (Payer et al., 1980; Lee et al., 1985). Outside tropical regions, limited control can be exerted by covering ponds with transparent sheets in colder weather (Richmond, 1987). The problems of maintaining a pure culture are also of major importance. Species control is generally maintained by using highly selective conditions to favor a particular species, cultivating species that tend to dominate naturally and developing systems for preventing or treating contamination. This latter policy may involve sterilization of culture medium and the use of chemicals to destroy fungi and zooplankton. Larger contaminants, such as insects, can be removed by filtration. Productivities in outdoor cultures obviously vary, but a few reported examples of peak productivities in terms of dry mass are: 25–30 $g/m^2/d$ (Kawaguchi, 1980), 35–40 $g/m^2/d$ (Benemann et al., 1987), and in excess of 50 $g/m^2/d$ (Grobbelaar, 1988), with potential yields in excess of 60 $g/m^2/d$ being suggested (Pirt et al., 1980).

CLOSED SYSTEMS

These have several advantages over open systems: They allow for greater control of growth conditions, resulting in greater population densities. This reduces the cost of the medium and harvesting. They also prevent contamination by other algal species, insects, and dirt, making the algae more acceptable for human consumption. Closed systems lead to a reduction in loss of water and serve to increase the temperature of growth—important in cooler parts of the world. In fact, this saving may be negated by the energy required to lower the temperature of closed reactors (Richmond, 1987). They also allow for the possibility of harnessing released oxygen. Since capital and running costs are far higher for these systems, they will be limited to high-value products.

Harvesting

One of the most expensive aspects of commercial microalgal production is harvesting. Centrifugation is one of the main traditional methods. Chemical flocculants are also used to aggregate the algae, which then can be collected by flotation or centrifugation. Traditional flocculants, such as lime and alum, are expensive in the large amounts required, and cheaper, synthetic positively-charged polymers have been developed, but some of these may be toxic or carcinogenic. Much interest has been shown recently in the utilization of bioflocculation—the tendency of algae to aggregate owing to the extracellular production of polysaccharides (Benemann et al., 1980).

Processing may involve simply drying algae in the sun, as in the case of many seaweeds. More complex drying techniques include drum-drying, freeze-drying, and spray-drying, of which the latter two appear to be the most useful. The processing method will depend on the intended use of the biomass; if intended for human consumption, for example, the final material must possess acceptable toxicity levels and be of suitable (i.e., marketable) appearance, texture, odor, and so on. The form of drying will also affect the digestibility of the biomass (Becker, 1980).

Immobilization

Algae can be immobilized either by active entrapment or invasive adsorption. In the former method, the cells are included in a solution of a matrix precursor that then forms an insoluble matrix, entraping the cells. Natural polymers, such as agar, alginate, and carageenan, and synthetic polymers, such as polyacrylamide and polyurethane, are generally used as matrices. Filamentous algae, in particular, can be immobilized by invasive adsorption whereby the algae colonize preformed matrices, usually in the form of polyurethane or polyvinyl foam blocks or glass fiber mats.

The advantages of immobilization of algae include separation of cell from the released product, particularly when the product is released during the stationary phase, and immobilization increases longevity of the cells in this phase. In the same way, immobilized algae might be used to carry out specific biotransformations, in the production of energy (hydrogen and ammonia), for the bioaccumulation of waste materials, as part of biosensors, coimmobilized with other heterotrophs, to provide them with oxygen and NADPH. The main disadvantage is high cost; the system must almost certainly be applied to the production of high-value compounds if it is to be used on a commercial scale.

Work has been carried out in all the areas mentioned above, e.g., on the production of sulfated capsular polysacharride by immobilized *Porphyridium*, on the release of ammonia from *Anabaena*, on the release of glycerol from *Dunaliella*, and on the coimmobilization of *Chlorella* and *Providencia*, with a subsequent increase in keto acid production. This area has been reviewed by Robinson et al. (1986) and Trevan & Mak (1988).

CURRENT AND POTENTIAL USES OF ALGAE

Wastewater Treatment

One of the most promising areas of algal biotechnology lies in the field of waste treatment, whereby the algae grown on wastes produce oxygen, which is used by bacteria to oxidize biodegradable matter. Concomitant with this is the production of biomass that can be used as food or a feed supplement. This process is particularly amenable to cyanobacteria since they are able to fix nitrogen (Balloni et al., 1980). In many cases, water treatment by algae is one of the least expensive methods of purification (Shelef et al., 1980). Also of interest is the treatment of animal manure with algae to produce both biomass and biogas (Benemann et al., 1980). Algae can also be used to specifically accumulate, and thereby remove, toxic compounds from industrial wastes.

Therefore, because it can be beneficial on two or more fronts, waste treatment by algae has particular potential. The main drawback to the widespread use of these processes is the cost of harvesting the biomass. There are also problems regarding the levels of contaminants, such as heavy metals, pesticide residues, and pathogenic organisms, in the resulting biomass if it is to be used for food or feed (Edwards, 1980). However, it is to be expected that, with growing awareness of environmental problems and the need for recycling, work will continue to make this process more reliable and less expensive.

Human Food

Many species of marine macroalgae, such as *Porphyra, Laminaria,* and *Undaria,* have regularly been used as food in the Far East, particularly coastal areas of Japan, China, Korea, and the Philippines. There is evidence that seaweeds, including *Eisenia* and *Sargassum,* were used as food by people in Japan dating back to 6000 BC, and there are records of 21 species of red, green, and brown seaweeds being used as food in Japan around 900 AD (Nisizawa et al., 1987). The world market value of *Porphyra* as a food crop is $\$540 \times 10^6$ (Arad, 1987). In Europe, general use of seaweed as food virtually ceased about 50–100 years ago, except in Wales and Iceland where small quantities are still eaten.

Species of freshwater filamentous green algae, e.g., *Spirogyra* and *Oedogonium* are eaten in Burma, Thailand, and India. The freshwater green algae, *Prasiola yunnanica* and *Prasiola japonica,* are consumed in China and Japan, respectively, and the freshwater red alga, *Lemanea mamillosa,* is eaten in India (Khan, 1973).

A number of cyanobacteria have been used as food for many centuries. *Spirulina* is eaten in Central America, e.g., around Lake Texcoco and around Lake Chad in Africa. It is collected from dense blooms and dried as cakes (Durand-Chastel, 1980). A number of other cyanobacteria, such as *Phormidium, Chroococcus,* and *Nostoc commune* are also eaten in Mexico. *Nostoc commune* often grows thickly on calcareous soils and is used as food in Mongolia, China, Ecuador, Fiji, and Okinawa. *Nostoc edule* is eaten in Tartary, Mongolia, China, and the Peruvian Andes, whereas *Nostoc verrucosum* is eaten in Thailand. *Phylloderma sacrum* is considered a delicacy in several areas of Japan (Soeder, 1980). At present, most commercial production of microalgae as food is restricted to the health food market (about 2000 tons/year) (Bemenann et al., 1987). This results in the cultivation of *Spirulina* and *Chlorella* in Japan, the US, Taiwan, Mexico, Israel, and Thailand (Soong, 1980).

Although microalgae have not been used as widely as macroalgae in this respect, their much higher growth rate and nutrient profile makes them potentially a valuable source of food protein. Microalgae generally have a higher protein content than soybean and are also rich in vitamins and minerals. Particular attention has been paid to *Spirulina,* especially in relation to third world development (Fox, 1987). *Spirulina* has a protein content up to 60% (Ciferri & Tiboni, 1985). The cultivation of algae primarily for their vitamins is another commercial possibility (Klausner, 1986; Borowitzka, 1988b).

The main problems lie in cost effectiveness, but with developing technology and the rising cost of other sources of single-cell protein, such as soybean and cashews, these may yet be overcome. Fears of toxicological problems have been largely countered by various tests (Becker, 1980; Payer et al., 1980).

Poor digestibility of algae is a problem that may be improved by processing techniques that break the cell wall (Becker, 1980). Marine algae, in particular, contain large amounts of carbohydrates that are not easily assimilated by humans, thus limiting their general use as foods, but suggesting a role in low-calorie diets (Takagi, 1975). It may be more efficient to prepare from the biomass, by acid or enzyme hydrolysis, protein concentrates that are better digested.

Feed and Fertilizer

The importance of microalgae as a source of high protein feed is also increasing. *Chlorella, Scenedesmus,* and *Spirulina* are among the algae that have already proven useful as feed supplements for poultry, swine, fish, molluscs, and even silkworm caterpillars (Aaronson et al., 1980). Microalgae can be used in various forms of aquaculture, such as live food for molluscs, oysters, and shrimps, and also as food for zooplankton, such as rotifers and copepods, that are themselves food for larvae of marine fish and crustaceans (De La Noue & De Pauw, 1988). This appears to be a particularly successful application of algae. One advantage is that mechanical harvesting steps are not generally required. Again, toxicity does not appear to be a major problem with these systems (Yannai et al., 1980).

Seaweeds, such as *Laminaria, Macrocystsis,* and *Ascophyllum,* have been used, to varying extents, as coarse fodder, fed to livestock in the proportion of about 10% of the total volume by weight, and fertilizers in coastal areas for many centuries (Robinson, 1985; Kain & Dawes, 1987). Brown seaweeds are valuable in this respect, because they contain cytokinins (Harvey, 1988). Liquid seaweed fertilizers are used in several parts of the world (Luxton & Courtney, 1987). Particularly effective fertilizers are the nitrogen-fixing cyanobacteria that have been used to increase rice yields in Japan (Robinson, 1985).

Energy

Apart from the energetically-unfeasible process of incineration of algae to produce electricity, there are three main ways in which energy may be obtained from algae. One is the production of hydrogen: cyanobacteria are able to evolve free hydrogen in the absence of nitrogen, and this could be used as a valuable source of energy (Benemann et al., 1980).

Algal biomass can also be used as a fermentation substrate for bacteria to produce biogas, in particular—methane, alcohols, and longer-chain hydrocarbons. This has been done successfully with several species of eukaryotic and prokaryotic algae, particular success having been achieved with the digestion of *Spirulina maxima* (Samson & LeDuy, 1982). Algae can also be used to produce liquid fuels more directly, by controlling the supply of nitrogen and silicon, so that the algae produce unusually high quantities of lipids that can be extracted and chemically converted to diesel or petrol. The algae used for this include *Navicula, Chaetoceros* and *Monoraphidium* (Emsley, 1988).

Algal immobilization (*see above*) may have a role to play in the production of energy by algae. One future use of algae might be to supply oxygen for astronauts on space flights or on long-term space stations, an idea that has long been under consideration (Soeder, 1980).

Polysaccharides

One of the major current uses of algae is provided by the gel-forming polysaccharides obtained mainly from the red and brown seaweeds. These cell wall components constitute a unique group of polymers that, apart from food, have provided the main commercial base for seaweed farming for several decades. The sulfated phycocolloids, such as agar, porphyran, and carageenan, have a wide range of uses as viscosifiers, emulsifiers, and lubricants in the food, paper, textile, drug, and cosmetic industries (Borowitzka, 1986; Glicksman, 1987). Agar and agarose are particularly valuable in molecular and microbiology and emerging areas of biotechnology; alginic acid is used to immobilize cells.

Phaeophyceae, particularly *Laminaria, Macrocystis, Ascophyllum,* and *Ecklonia,* are the main source of alginate, fucoidan, and laminaran. Most of the world's supply of alginate derives from *Macrocystis pyrifera* found around the coasts of North and South America, Australia, and New Zealand (Whitney, 1987). Rhodophyceae are the source of agar, carageenan, and porphyran. Agar is extracted mainly from *Gracilaria* and *Gelidium* species found around Japan, Chile, Mexico, and the US, and also in New Zealand from *Pterocladia.* Carageenan is derived from *Eucheuma* species in Korea and the Philipines and *Chondrus, Gigartina,* and *Iridaea* in Canada, the US, Korea, and parts of Europe (Glicksman, 1987). Owing to their higher specific growth rates, some work has also been carried out into the possibility of using microalgae—notably *Porphyridium, Rhodella,* and *Rhodosorus,* as sources of polysaccharides (Canivez, 1988).

Pigments

Algae produce a number of pigments that are extracted on a commercial scale. These include carotenoids, notably β-carotene, which is used as a food colorant, mainly in margarine. It is a vitamin A precursor, and its potential as a possible anticancer agent has also established it as a health food. Some strains of *Dunaliella bardawil* can produce up to 10% of their dry weight as β-carotene. Xanthophylls, such as lutein and canthaxanthin, can be metabolized and, therefore, are used as chicken feed additives to color chicken skin and egg yolks.

Pigments unique to algae are the phycobilins and phycobiliproteins. These chromoproteins occur in the Rhodophyceae, Cryptophyceae, and Cyanophyceae and have great potential in the food, drug, and cosmetic industries as replacements for synthetic pigments suspected as being carcinogens. The blue biliprotein, phycocyanin, is extracted from *Spirulina platensis* in Japan, where it is sold for use in health foods and cosmetics (Borowitzka, 1986). Biliproteins from Rhodophyceae are also used as highly sensitive fluorescent dyes in diagnostic tests (Arad, 1987). Chlorophylls can also be used industrially as colorants, and a preparation of *Spirulina* chlorophyll has even been patented as a strong deodorant (Cohen, 1986).

Lipids

Under certain environmental conditions, many algae produce particularly large amounts of polyunsaturated fatty acids that, with the exception of linoleic acid, are generally rare in higher plants and animals. (*See also* the ''Energy'' section.)

Essential fatty acids (EFAs) have been reported to reduce serum cholesterol levels, possess antihypertensive activity, inhibit blood platelet aggregation, and be potentially useful in the treatment of atopic disorders. γ-Linoleic acid (GLA) and eicosapentaenoic acid (EPA) are of particular commercial interest (Klausner, 1986).

GLA is currently extracted from evening primrose seeds, of which it constitutes about 8% of the total fatty acids. *Spirulina platensis,* however, contains about 20–30% of its total fatty acid content as GLA. Preliminary reports have suggested that GLA might be useful in the treatment of heart disease, Parkinson's disease, multiple sclerosis, and premenstrual tension (Cohen, 1986). EPA is usually obtained from fish, which, in turn, obtained it from the consumption of algae, such as diatoms.

Some EFAs, e.g., arachidonic acid, are the precursors of prostaglandins, and these have potential clinical application in the treatment of hypertension and also asthma, ulcers, and so on. Prostaglandins themselves have actually been isolated from one species of alga, *Gracilaria lichenoides* (Gregson et al., 1979).

Sterols are also found in algae. The highest concentration (1% of dry weight) was found in *Ochromonas danica* (Patterson, 1982). Chondrilasterol from *Scenedesmus* or *Navicula* species might be a suitable precursor for the synthesis of pharmacologically-important hormones, e.g., cortisone. Algae are also useful in the production of radiolabeled fatty acids, which are valuable research tools. Cyanobacteria, particularly *Lyngbya majuscula*, contain a number of lipids not found in eukaryotic algae (Moore, 1981).

Enzymes

Algae can also be utilized as a source of enzymes. Phosphoglycerate kinase (PGK) is used for the determination of ATP. Commercial PGK is not specific for ATP, but also acts on GTP and ITP. PGK from *Spirulina*, however, is specific for ATP (Krietsch & Kuntz, 1978). Dinoflagellates contain bioluminescent systems that can also be used for ATP determination.

Restriction endonucleases are, of course, vital molecular biology tools, and these have been identified from a number of cyanobacteria, such as *Anabaena* and *Aphanizomenon* species (Cohen, 1986). Cyanobacteria are also known to produce ion chelators, such as siderochromes, for the uptake of iron, by *Anabaena* spp. (Murphy et al., 1976). Some algae exhibit amino acid oxidase activity, and these can be coimmobilized with certain bacteria to produce relatively high amounts of keto acids, which are useful as supplements in protein-restricted diets (Wikstrom et al., 1982).

Superoxide dismutase has been purified from *Spirulina* and *Porphyridium*, and microalgae, therefore, could be an important source of this enzyme, which appears to act as a free radical scavenger and, as such, could play a role as a therapeutic agent for cancer (Cohen, 1986). A number of microalgae and cyanobacteria have been found to release enzymes extracellularly. These include proteinases from several *Chlamydomonas* and *Chlorella* species, glycosidases from *Anabaena* and *Microcystis* species, and nucleases from a number of eukaryotic and prokaryotic species (Cannell, 1988). Other excreted enzymes include acid and alkaline phosphatases from *Amphidinium carteri*, *Chlamydomonas reinhardii*, and *Ochromonas* species, as well as β-glucosidase, amylase, invertase, proteinases, and lipases from the latter (Patni et al., 1977). These have been shown to be excreted and not just the result of cell leakiness (Meyer, 1976).

Miscellaneous

In the 17th and 18th centuries, various seaweeds were utilized for the production of soda and used in the manufacturing of glass, but this industry declined in the mid-19th century and has now disappeared. (In Mexico, *Spirulina* is a byproduct of soda manufacture from carbonate brine (Soeder, 1980)).

Macroalgae have also been the source of iodine for several centuries, and although most iodine is now extracted from mineral deposits, there is still an active iodine industry in the Soviet Union, China, and Japan (Robinson, 1985). Glycerol is obtained commercially to a limited extent from several *Dunaliella* species, with some strains reported to produce up to 50% of their dry weight as glycerol under suitable conditions (Ben-Amotz

& Avron, 1981). Other compounds that also act as osmoregulators, such as sorbitol and mannitol, might also be amenable to exploitation.

Pharmaceutical Uses

The pharmaceutical use of algae—almost always marine algae—has extended over many centuries. The earliest recorded use comes from the Chinese 'Materia Medica' around 2700 BC. The ancient Indian system of medicine—Ayurveda, dating back to the 2nd millenium BC—also utilizes algae.

Many different treatments for intestinal worms have been described. These include *Ulva, Chondria, Corallina,* and *Codium* species. *Alsidium, Grateloupia,* and *Hypnea* species are used in Greece, Turkey, and Indonesia, and *Rhodomenia indica* is used in East Asia. The Maoris used *Durvillea* spp. as a vermifuge and as a treatment against scabies (Hoppe, 1979). One of the most famous algal vermifuges, known for over a thousand years in China and India, is *Digenea simplex,* from which the active principle—kainic acid—has been isolated and marketed as a drug, one of the very few drugs to derive from algae.

Another widespread use of algae, particularly in mountainous areas where soils have been heavily leached, is in the prevention and treatment of goiters caused by iodine deficiencies. Marine algae generally have a high iodine content, and their beneficial effects have been recognized by the Russians, who consume *Fucus* and *Laminaria;* Himalayan peoples, who eat *Laminaria saccharina;* and the peoples of the Andes and the government of Chile, where seaweed is added to children's food for this reason (Michanek, 1979).

Lung diseases and scrofula are treated with *Dictyopteris polypodioides* and *Stilophora rhizoides* in Mediterranean countries and *Laminaria saccharina* in India (Misra & Sinha, 1979). In Asia, *Gelidium, Chondrus, Eucheuma,* and *Ceramium* species are utilized to treat colds, coughs, and chest diseases; burns have been treated with *Corallina* spp. and syphilis and skin diseases with *Laminaria* (Hoppe, 1979). Gastric ulcers have been relieved by *Chondrus crispus* and *Cutleria multifida,* and other intestinal troubles, such as dysentery, are treated with *Hypnea nidifica* (in Hawaii) and *Corallina* spp. The opposite condition of chronic constipation is relieved by the Maoris using fermented *Porphyra columbina* (Brooker & Cooper, 1961).

Various brown algae, such as *Laminaria* and *Sargassum,* are incorporated into Chinese and Japanese folk medicine to lower blood pressure, and *Ascophyllum nodosum* and *Fucus vesiculosus* have been reported as remedies for obesity (Trease, 1936). *Sargassum* spp. have been used in various parts of the world to treat urinary and renal trouble; in Russia, *Fucus esculentus* and *Laminaria* spp. are used as prophylactics for sclerosis; in China, *Laminaria* is used for menstrual problems; in Southeast Asia, gallstones are treated with *Acetabularia;* North American Indians used

Nereocystis luetkeana as a drug, and in other parts of the world, gout is treated with *Ulva* (Hoppe, 1979).

Recently, there has been a return to the use of the *Laminaria* "tent," i.e., dried, sterile stipes of *Laminaria digitata,* in the field of obstetrics and gynecology. This is inserted in the cervix, and liquid is absorbed by the stipe, causing gradual dilation of the cervix (Feochari, 1979).

Algal polysaccharides are used in the drug industry, and agar is used as a blood anticoagulant (Chapman, 1979; Chapman & Chapman, 1980). Reports of freshwater algae being utilized as medicines are more limited, mainly because of their size and dispersal. The green alga, *Rhizoclonium,* has been used as a treatment of wounds and as an antihelminthic agent. Indian medicine employs some freshwater species, such as *Spirogyra* and *Oedogonium* (Misra & Sinha, 1979). Many centuries ago, the cyanobacterium, *Nostoc,* was used as a treatment for cancer and gout.

These are just some examples of the pharmaceutical prowess that has been attributed to algae, without the use of modern scientific methods (Stein & Borden, 1984). A few of the active constituents of the algae listed above have been isolated, but this is an area of algal utilization that demands more research.

Antibiotic and Pharmacological Activity

Marine algae have been extensively screened for, and a large proportion of them appear to exhibit, antimicrobial activity (Hornsey & Hide, 1974; Pesando & Caram, 1984; Reichelt & Borowitzka, 1984; Caccamese et al., 1985; Kellam et al., 1988). Antibiotic activity has been detected from every algal class.

Many of the active agents have been isolated and their structures fully or partially determined (Baker & Murphy, 1976; Faulkner, 1977; 1986; Kaul & Daftari, 1986). Though many types of compound are produced by a taxonomically wide range of organisms, certain classes of algae tend to produce certain classes of compound.

For example, Rhodophyceae produce a range of halogenated compounds, including aliphatics, terpenes, and phenols. The Bonnemaisoniaceae family, notably the genera *Asparagopsis* and *Bonnemaisonia,* are particularly productive. Two of the most active antibiotics of the halogenated phenolics are the phenolic sesquiterpenes, including laurinterol and isolaurinterol from *Laurencia* spp. *Chondria californica,* and some *Dictyopteris* species, produce a range of cyclic polysulfides, fairly simple molecules, but relatively rare in nature (McConnell & Fenical, 1977). The Pheaophyceae produce a number of compounds, including a large number of phenolic derivatives, a common constituent of which is phloroglucinol in various polymerized forms (Glombitza & Grosse-Damhues, 1985). Some Chlorophyceae produce brominated and nonbrominated hydroquinones, as well as unsaturated fatty acid derivatives, which also possess antibiotic activity (Pesando, 1972).

Relatively little work has been carried out regarding antibiotic activity from freshwater algae. However, the first algal antibiotic reported—chlorellin—was discovered from a freshwater *Chlorella*. Some freshwater algae have been found to exhibit antibacterial and antifungal activity (Cannell et al., 1988a; Kellam et al., 1988). The only antibiotics characterized unambiguously from freshwater algae are cyanobacterin—a chlorinated compound from *Scytonema hofmanni* (Mason et al., 1982) and penta-galloylglucose from *Spirogyra varians* (Cannell et al., 1988b). An algal inhibitor has been partially characterized from a *Chlamydomonas* (McCracken et al., 1980).

Algae have also been shown to possess antiviral activity (Blunden et al., 1981; Deig et al., 1974). The polyanionic sulfated polysaccharides, such as agar, exhibit some antiviral activity, the degree of which is related to the degree of sulfation (Ehresmann et al., 1979). Some marine algae, such as *Spatoglossum schmitii*, possess antineoplastic (Patterson et al., 1984; Harvey, 1988) and inotropic (Barwell, 1980) activity. Interesting and useful lectins that act as specific haemagglutinins have also been found from algae (Blunden et al., 1975). For example, a lectin from *Ptilota plumosa* has been found that agglutinates blood group B erythrocytes preferentially and does not react with P-system antigens (Blunden & Gordon, 1986). Some algae have also been found to possess specific enzyme-inhibitory activity (Cannell et al., 1987; 1988b,c). *Dunaliella tertiolecta* produces compounds that possess anticonvulsant, hypotensive, bronchodilatory, and antiinflammatory properties (Baker, 1984).

Plant growth regulators have been found from several cyanobacteria, but none have yet been isolated and characterized. The brown alga, *Sargassum tortile*, yielded crinitol—an insect growth inhibitor (Kubo et al., 1985). Various algal metabolites act against pests, such as citrus nematode, black bean aphid, saltmarsh caterpillar, and the spider mite (Harvey, 1988).

Many algae, particularly cyanobacteria, dinoflagellates, and chrysophytes, produce toxins. Many of the freshwater cyanobacteria responsible for algal blooms, such as *Microcystis aeruginosa*, *Anabaena flos-aquae*, and *Aphanizomenon flos-aquae*, have been studied with respect to toxin production since these blooms have often caused deaths of fish, livestock, and humans (Metting & Pyne, 1986). These blooms are important because increasing eutrophication, caused by pollution, has led to recent increases in toxic blooms (Skulberg et al., 1984). Marine cyanobacterial toxins include aplysiatoxin and debromoaplysiatoxin from *Lyngbya majuscula*, which is responsible for "swimmer's itch." The latter compound has also shown antileukemia activity (Mynderse et al., 1977). Cyanobacteria are also known to produce a class of lipid—cyanobacterial lipid-A—not present in other bacteria and which is the probable origin of a number of lipopolysaccharide endotoxins that are believed to give rise to gastrointestinal disorders.

Outbreaks of red tide in the Gulf of Mexico and other areas are associated with fish and marine animal death. These outbreaks are caused by blooms of dinoflagellates, notably *Gymnodinium breve*, from which several endotoxins have been isolated. *Gonyaulax* species produce at least 12 different exotoxins, and these cause paralytic shellfish poisoning (PSP) by accumulating in various shellfish that are then consumed (Collins, 1978). The *Gonyaulax* toxins include saxitoxin, the best studied and most potent algal toxin known and with similarities to tetrodotoxin. The dinoflagellates, *Amphidinium* spp. and *Noctiluca scintillans,* produce icthyotoxins, as does *Prymnesium parvum,* which produces prymnesin, consisting of several components, including acidic polar phospholipoproteins. Toxin production by algae is strongly affected by environmental factors.

These represent just a few of the many examples of antibiotic and pharmacological activity exhibited by algae and give some idea of the wide range of interesting molecules produced by these organisms. It is clear, therefore, that algae produce a great many biologically-active compounds, some of which might one day find use as novel drugs, but none that are yet of any commercial significance. However, with further investigation, some of these metabolites could turn out to be the high-value compounds that are required to boost algal biotechnology.

FUTURE

Algae are an extremely diverse group of organisms and constitute a large proportion of the world's organic matter. However, they are of relatively minor economic importance. This is despite the fact that many applications for algae have been described. They offer the most efficient means of fixing solar energy in the form of biomass and an ideal way of treating wastewater. They produce a wide range of useful chemicals and the potential for many more. However, the difficulty lies in transferring these advantages to economically-efficient systems, owing to problems, such as species control in large-scale processes, harvesting costs, and the low price of products.

The successful utilization of algae in the future will almost certainly be through means that combine two or more of the uses described above. Present examples of this include the use of algae for aquaculture, coupled to wastewater treatment.

Also, it is very likely that the development of algal biotechnology will depend on the discovery of more high-value products from algae, such as pharmaceuticals. To some extent this is already the case, with much of the current algal industry aimed at production of compounds, such as pigments and glycerol, but more high-value products need to be found in order to boost algal biotechnology. Further studies must be carried out on the characterization of algal metabolites and the growth conditions that affect their production.

The techniques of genetic manipulation are likely to be applied to algal biotechnology at some point in the future. Little gene cloning so far has been carried out with algae and has been restricted mainly to the prokaryotic cyanobacteria (Craig et al., 1988). Genetic recombination has been achieved through algal protoplast fusion (Canivez, 1988). Results of genetic manipulation may lead to increased production of valuable primary or secondary metabolites, faster growth rates, tolerance of extreme growth conditions, and so on (Lewin, 1983).

There is a need for continuing research on the biological and engineering problems associated with large-scale algal cultivation, harvesting, and processing. It is also necessary to win the support of long-term financial investment, be it public or private, in order to fulfill the potential of algal biotechnology. Algae represent an ecologically-sensible form of technology, and it is to be hoped that, with an increasing awareness of environmental problems and continuing research and development, algae will soon begin to properly fulfill their enormous potential.

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