1 Introduction

The large-scale cultivation of microalgae and the practical use of its biomass as a source of certain constituents (for instance, lipids) was probably first considered seriously in Germany during World War II. This initial research was taken up by a group of scientists at the Carnegie Institution of Washington, who summarized their experiences in the classic report *Algal culture from laboratory to pilot plant* (Burlew, 1953). The aim of this project was the use of the green alga *Chlorella* for large-scale production of food, based on the uncertainty of whether the yields obtained under laboratory conditions could be maintained outdoors on a larger scale.

In the course of time, the continuous cultivation of algae under partially or fully controlled conditions has become an important development, with various economic possibilities. The past decades have witnessed great strides in the production and utilization of algae through the efforts of many scientists in several countries. Techniques for the cultivation of algae on a large scale and proccesses for their utilization have been successfully developed in several countries and an attempt in this direction is worthwhile in some of the developing countries (Venkataraman & Becker, 1985).

During this early period of algal mass cultivation, technological advances and nutritional evaluations have received major attention. However, widespread criticism on the exploitation of unconventional protein sources in general, and microbial proteins in particular, has affected the aspirations relating to the use of algae as supplementary food for human consumption. The great expectations promised initially in the use of algae as food have subsided for several reasons, and today researchers seem to be more cautious in expressing any opinion about the practicability of algal production on a commercial scale for use in foods.

This change in attitude is mainly a result of the high cost of algal production, acceptability problems, and safety criteria. Consequently, extensive research on different fundamental and applied aspects of phycology were carried out that demonstrated that algal biomass can be used for various other applications such as animal feed, biofertilizer, soil conditioner, and as feed in aquaculture, and that it may help to solve public health problems by means of biological purification of waste waters of a fast-developing society. Latest developments have established the potentiality of algae for the production of a variety of compounds such as polysaccharides, lipids, proteins, carotenoids, pigments, vitamins, sterols,

Table 1.1. *Projected applications of commercially produced algae*

I. Food	Protein supplement/fortification in diets for malnourished children and adults.
II. Feed	Protein/vitamin supplement in feeds for poultry, cattle, pigs, fish and bivalves.
III. Health food	Algal powder as ingredient and supplement in health food recipes and products.
IV. Therapeutics	β-Carotene as possible anti-skin-cancer treatment. Algal antibiotics as wound treatment, enzymatic hydrolyzates to promote skin metabolism. Prostaglandin stimulation by γ-linolenic acid. Regulation of cholesterol synthesis. Isotopic compounds in medical research.
V. Pigments	β-Carotene as food color and food supplement (provitamin A). Xanthophylls in chicken and fish feeds. Phycobilins as food color, in diagnostics, cosmetics and analytical reagents.
VI. Source of fine chemicals	Glycerol used in foods, beverages, cosmetics, pharmaceuticals. Fatty acids, lipids, waxes, sterols, hydrocarbons, amino acids, enzymes, vitamins C and E. Polysaccharides as gums, viscocifiers and ion exchangers.
VII. Fuel	Long-chain hydrocarbons and esterified lipids as combustible oil. Hydrogen, biogas.
VIII. Hormones	Auxins, gibberllins and cytokines.
IX. Others	Biofertilzer, soil conditioner. Waste treatment.

enzymes, antibiotics, pharmaceuticals and several other fine chemicals (or their precursors), as well as hydrogen, hydrocarbons and other biofuels (methane, alcohol). These projected applications are summarized in Table 1.1.

Furthermore, the past years have witnessed the use of algae cultures for the assay of biological and physiological studies and proved a convenient medium for testing the effects of various chemical agents on living organisms.

At present, however, commercial algae production is still restricted to very few plants producing high-value health food or pigments, most located in the Far East and the USA. Health food production systems mainly cultivate the filamentous cyanobacterium *Spirulina* and the unicellular green alga *Chlorella* and – to a lesser extent – the green alga *Scenedesmus*, whereas for the production of pigments *Dunaliella* and *Spirulina* are preferred. In basic research, algae, by their structural simplicity and functional complexity, have become the vehicles for important discoveries and experiments. As a result, the cultivation of algae as a research tool is expanding rapidly as modern research becomes interested in such diverse fields as taxonomy, morphology, physiology, biochemistry, genetic engineering, tissue culture, food production, agriculture, waste disposal and medicine.

During the 1970s urgent efforts were made in finding alternative energy sources in view of the diminishing and ever more expensive supply of petroleum products. Thus the biological application of solar energy has attracted global interest. As algae constitute an efficient system for harnessing solar energy, there is continued interest in the technology of algae production as a source of renewable energy. Coupled with this development is the increased awareness of environmental pollution and waste disposal hazards. In this context, again, algae have a vital role to play in transforming sewage and waste water into valuable biomass.

As a result of the problems encountered with the popularization of microalgae as food or feed, different approaches for overcoming economic difficulties of algal production gained increasing attention. Instead of producing proteinaceous commodities of low commercial value, the priority of research was the utilization of high-value algal constituents, metabolites and fermentation products.

Although there are at present only a few chemicals isolated from microalgae that have gained commercial success, there is still a large number of algae species that are waiting to be screened for their ability to produce specific commodities.

Interest in commercial production of microalgae is not lacking and their economic potential is becoming widely recognized, yet little industrial effort in the mass production of microalgae is presently evident, several decades after the first large-scale algal ponds were constructed. In other words, a discrepancy clearly exists between the well-recognized commercial potential of many microalgal species and the actual industrial development. Two of the major reasons for the slow progress in industrial algal culture are the prohibitively high cost of production, which to a large extent is due to the relatively low areal outputs, and the acceptability difficulties linked with the popularization of algal biomass in any form.

Although some of the problems and critical factors encountered and identified during the 1950s could have been solved, it cannot be denied that several of them still await their solution and that several new problems have come up. Even at that time it was realized that the various problems associated with large-scale algae production are beyond microbiology and require co-operation with other scientific disciplines such as engineering, economy, medicine, toxicology, analytical chemistry, etc.

In this book, any economics for the production of algae or algal constituents are deliberately omitted. During the past 20 years, too many impressive calculations have been published based on extrapolations of algal yields obtained in small experimental ponds to large plants of several hectares, using costs of harvesting and processing techniques that have never been practically tested, and steps for the isolation of algal constituents or products – which presently are produced much more cheaply by other processes – leaving out no detail (including laser-guided levelling of

the site up to the salary of the general manager). None of these designs and calculations have ever been fully or partly realized. They simply reflect the wealth of ideas and are no more than castles in the air. Algal biotechnology, however, is more than a playground for would-be ecologists or entrepreneurs. These futuristic calculations do not help to propagate algal biotechnology; on the contrary, they do harm to this field of research. Perhaps all these attempts may be excused by the fascination which emanates from the algae and the wishful thinking to 'domesticate' these microorganisms.

This book is not intended as a scientific review – although sufficient references are cited for almost any interested reader – but envisages the compilation of the major results published so far on various aspects of applied phycology. It aims at presenting the available information on the methods and applications of algal cultures in a simple and concise form, having in mind the interests of students and working microbiologists.

The enormous expansion of research using algal cultures and the constant development of new techniques and devices make it futile to claim any comprehensiveness. Effort has, however, been made to include the important references and also to give illustrative material as far as possible with the aim of providing a working guide on the various aspects of algal cultivation based on available information and on the author's own experience. It is hoped that the technical data presented here are of relevance and value for further development in the field of algal technology, and will generate additional interest in those who are committed to work on algae and their applications.

2 Algae production systems

For almost 50 years, numerous attempts have been made to exploit microalgae on a technological scale as a source of food, feed, lipids, vitamins, pigments, fertilizers, pharmaceuticals and other speciality chemicals. This common interest has arisen as a result of the need for additional food supplies, increasing problems of waste disposal and the shortage of raw materials and energy resources.

The cultivation of algae, especially under outdoor conditions, involves a very complex system, depending on the interaction of various external and internal factors. An overall, generalized schematic flow diagram of the various inputs and requirements involved in algae mass cultivation is given in Fig. 2.1. Although the individual parameters may differ from case to case, the basic elements will be similar in almost all cultivation units: an algal growth pond of varying shape and size which must receive all the necessary nutrients, harvesting and concentrating equipment, and a provision to process (extract, dry) the harvested algal biomass.

It has been stated repeatedly that algae cultivation is a special form of agriculture but is easier to handle and to manipulate than the conventional form. Indeed, at a first glance this judgement seems to be correct since some of the factors involved in the production of algae (such as fertilization, control of contamination or time of harvesting) can be operated more precisely. However, practice has shown that this is not true; there are many wealthy farmers but as far as we know nobody who became a rich man by the cultivation of algae (or writing about it). The several abandoned algal projects worldwide taught us that the successful growth of algae is 'high technology'; it is more or less an art and a daily tightrope act with the aim of keeping the necessary prerequisites and the various unpredictable events involved in algal mass cultivation in a sort of balance (Fig. 2.2). If at all, algae cultivation may be considered as an intermediate between that of agriculture and fermentation technology. Although the contrary has been reported repeatedly, it seems not to be true that microalgae have higher photosynthetic efficiencies than conventional crop plants. Nevertheless, algae production units can be more productive in terms of product output than conventional agriculture.

The research on applied algology in the past has led to the design and construction of several types of cultivation plants capable of producing quantities of algae ranging from kilograms to tons. Two main approaches

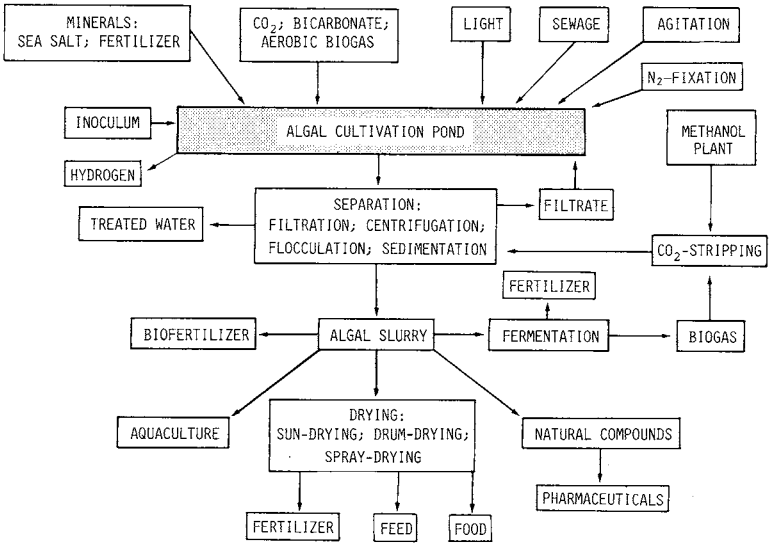

Fig. 2.1. Flow diagram of algae mass-cultivation system.

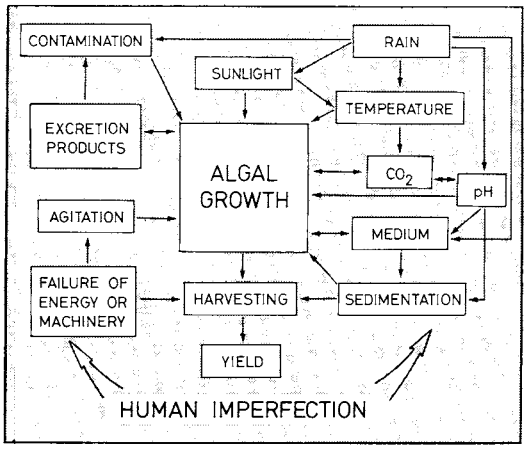

Fig. 2.2. Schematic diagram of the main interactions of algae cultures growing on sewage.

to the design of such plants have emerged from this work. They have been dictated primarily by the nature of the desired end-product, namely plants for the production of protein or food additives, which must be cheap to build and easy to maintain, and therefore suitable for developing countries, in order to compete effectively with other modes of food production; and more sophisticated biotechnologies with a high input of equipment and energy for the growth of specialized algal strains intended for the produc-

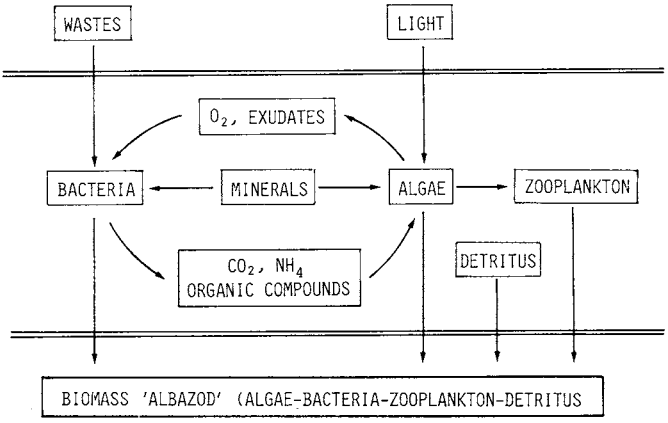

Fig. 2.3. Interaction of main factors influencing outdoor algae cultivation.

tion of specific biochemicals such as enzymes, pigments, therapeutic agents, etc. In such cases, the product quality justifies the large initial investment costs. For these purposes, three different systems can be distinguished, depending on the raw materials used and the utilization of the obtained algal biomass.

a) Systems in which a selected algal strain is grown in a so-called clean process, using fresh water, mineral nutrients and additional carbon sources. The algae in such systems are intended to be utilized mainly as food supplement.
b) Systems using sewage or industrial waste waters as the culture medium without the addition of mineral and external carbon. In such units the algal population consists of several species in the presence of high amounts of bacteria. The plant operation is based on a symbiotic nutritional circuit between algae and bacteria; i.e. the oxygen, photosynthetically produced and liberated by the algae, is utilized by the bacteria for aerobic biodegradation of organic compounds. During this process, organic carbon compounds are partially oxidized to CO_2, which in turn is assimilated by the algae. In addition, the algae utilize soluble nitrogen and phosphorus compounds from the medium and convert them into biomass, whereby more N and P can be consumed than are absolutely necessary for cell metabolism ('luxury metabolism') thus enhancing the waste water treatment (Fig. 2.3). Under limited or insufficient light conditions, certain algae adapt their metabolism from autotrophy to heterotrophy so that they are able to utilize both inorganic and organic compounds from the medium. The biomass thus produced consists of a mixture of algae, bacteria and zooplankton.

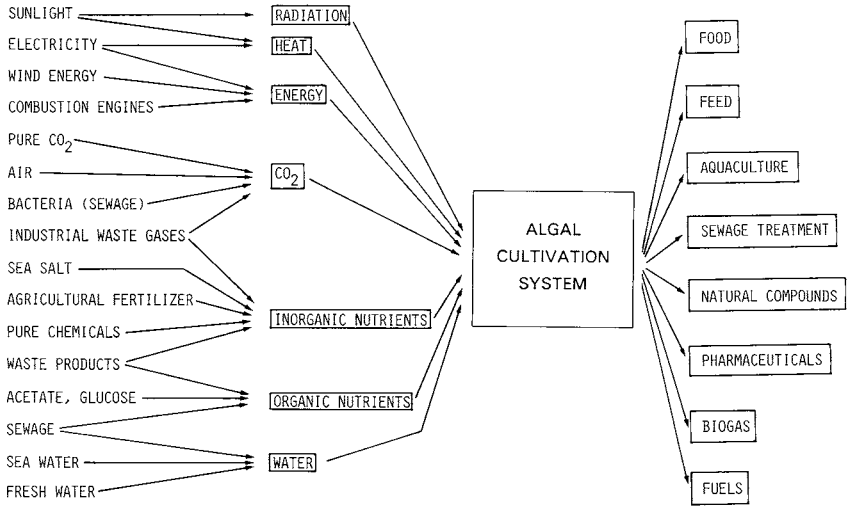

Fig. 2.4. Potentials of algal biomass: raw material, energy sources, products and applications.

c) Cultivation of algae in enclosed systems under sunlight or artificial light, with cells being grown preferably in autotrophic media.

It is obvious that algae production plants for mass cultivation have to be located in areas with suitable climatic conditions. Unfortunately, places where all the involved parameters are optimal are very rare. Some locations which offer favourable climatic conditions have to be excluded because of the absence of the required infrastructure.

In the early work of algal mass cultivation, a variety of different algae were investigated for their growth characteristics, particularly for fast multiplication rate and high protein content. The species that are suitable for this purpose in the 'clean' system are *Chlorella*, *Scenedesmus*, *Uronema*, *Coelastrum*, *Dunaliella*, *Porphyridium* and *Spirulina*; and in sewage or waste water, *Micractinium*, *Oocystis*, *Oscillatoria*, *Chlamydomonas*, *Euglena* and *Ankistrodesmus*. Among these, the most common species used nowadays for mass cultivation are *Chlorella* with its strains *C. pyrenoidosa*, *C. ellipsoidae* and *C. vulgaris*, *Scenedesmus* with its strains *S. obliquus (acutus)* and *S. quadricauda*, *Dunaliella bardawil*, *Porphyridium cruentum*, and the only cyanobacterium *Spirulina*, with its not always clearly defined strains *S. maxima*, *S. platensis* and *S. geitleri*. Almost all of the results, data and experiences described here are based on studies performed with these algae.

The various different potential inputs and applications that have emerged from the past research activities on the production and utilization of microalgae are summarized in a simplified form in Fig. 2.4.

3 Culture media

Under natural conditions, most algae grow as mixed communities, which include various species and genera. In order to study and cultivate individual species, the required form must be cultivated without other species present. The isolation of the desired species depends on the provision of a suitable environment for its growth. Forms of algae which grow more rapidly under the conditions provided will outnumber the other species after a certain period of time. If not replaced or fortified, the composition of the medium will gradually change thus limiting the growth of the originally predominant species, and other species, also introduced into the medium, will come up. If such cultures are maintained for longer periods of time, successive development of many different algal species will take place in a single culture.

For most of the ecological and physiological studies performed nowadays with algae, pure algal cultures are a basic requirement. Such cultures have greatly contributed to many physiological discoveries and are still being used in various morphological, biochemical and genetic researches. Many studies on antibiotic resistance, variability, teratology, cytology etc., have been made possible with the help of authentic algal cultures. Several terms can still be found in the literature for pure cultures, namely unispecific, unialgal or axenic culture. Whereas the first two terms refer to cultures which contain only a single species of alga in the presence of other organisms, the latter is used for cultures which contain only one species of alga, free from any other organism.

The development of synchronous cultures is another important example of the usefulness of culture methods and has facilitated the understanding of various biochemical reactions at cellular level. Since the early 1940s, when the possibility of utilizing fast-growing unicellular algae as a source of food or feed was suggested, algal cultures have assumed an industrial dimension.

For the successful growth of algae in a culture, the environment must be conditioned to meet as many of the intrinsic requirements of that organism as possible. The environmental factors may be either physical, such as temperature and light, or chemical, which provides all the raw materials used for the structural and protoplasmic synthesis of the algal cell.

Studies on the nutritional requirements of algae, using the replacement technique as employed for higher plants, have revealed that the require-

ments of algae are similar to those for the phanerogams. In 1896, Molisch had observed that the mineral nutrition of the algae was not different from that of higher plants. The major absolute requirements include carbon, phosphorus, nitrogen, sulfur, potassium and magnesium. Elements like iron and manganese are required in small amounts; sodium is not essential for many forms and the specific requirement for calcium is contradictory. Various other elements like cobalt, zinc, boron, copper and molybdenum are essential trace elements. In addition to these basic minerals, several algae (heterotrophs, see below) require additional organic substrates (vitamins, nucleic acids, growth factors) for their growth.

Two major forms of algal nutrition can be distinguished, i.e. autotrophy and heterotrophy. Autotrophs are all those organisms that obtain all the elements they require for growth from inorganic compounds only, whereas heterotrophs are organisms that need organic substrates synthesized by other organisms. The term amphitrophy is used sometimes to describe organisms able to live either auto- or heterotrophically.

Among the autotrophs two groups can be classified, namely photoautotrophs and heteroautotrophs. Photoautotrophs are those organisms that obtain the energy for their metabolism from light; chemoautotrophs obtain this energy from the oxidation of inorganic compounds or ions. Most algae are phototrophs; they obtain their energy through the absorption of light and electrons used for the reduction of CO_2 by the oxidation of inorganic substrates, predominantly water, coupled with the evolution of oxygen. If the organisms cannot grow in the dark, which is the case with most algae, they are called obligate phototrophs. Several intermediate variations can be found among the heterotrophs. For organisms whose energy is derived from the oxidation of organic compounds that also serve as a carbon source, the term chemoheterotrophy is used.

Photoheterotrophy is the term for those organisms that require light as an energy source to utilize organic carbon. Auxotrophy is a form of heterotrophy in which organisms require only very small quantities of essential organic compounds (vitamins, amino acids, etc.). Mixotrophy designates a nutritional mode in which the energy is derived either through photosynthesis or by chemical oxidation, but CO_2 and organic carbon sources are necessary to sustain growth.

Phagotrophy describes the uptake of solid organic particles by the organism (phagocytosis) and digestion in food vacuoles. Many media have been devised for culturing different algae, but no single medium can be said to be the best one. The main considerations in developing nutrient recipes for algal cultivation can be summarized as follows (Vonshak, 1986).

a) Total salt concentration, dependent on the original biotope of the alga.
b) Carbon source. Since about 50% of the algal biomass consists of carbon, a sufficient supply of carbon is of vital importance for