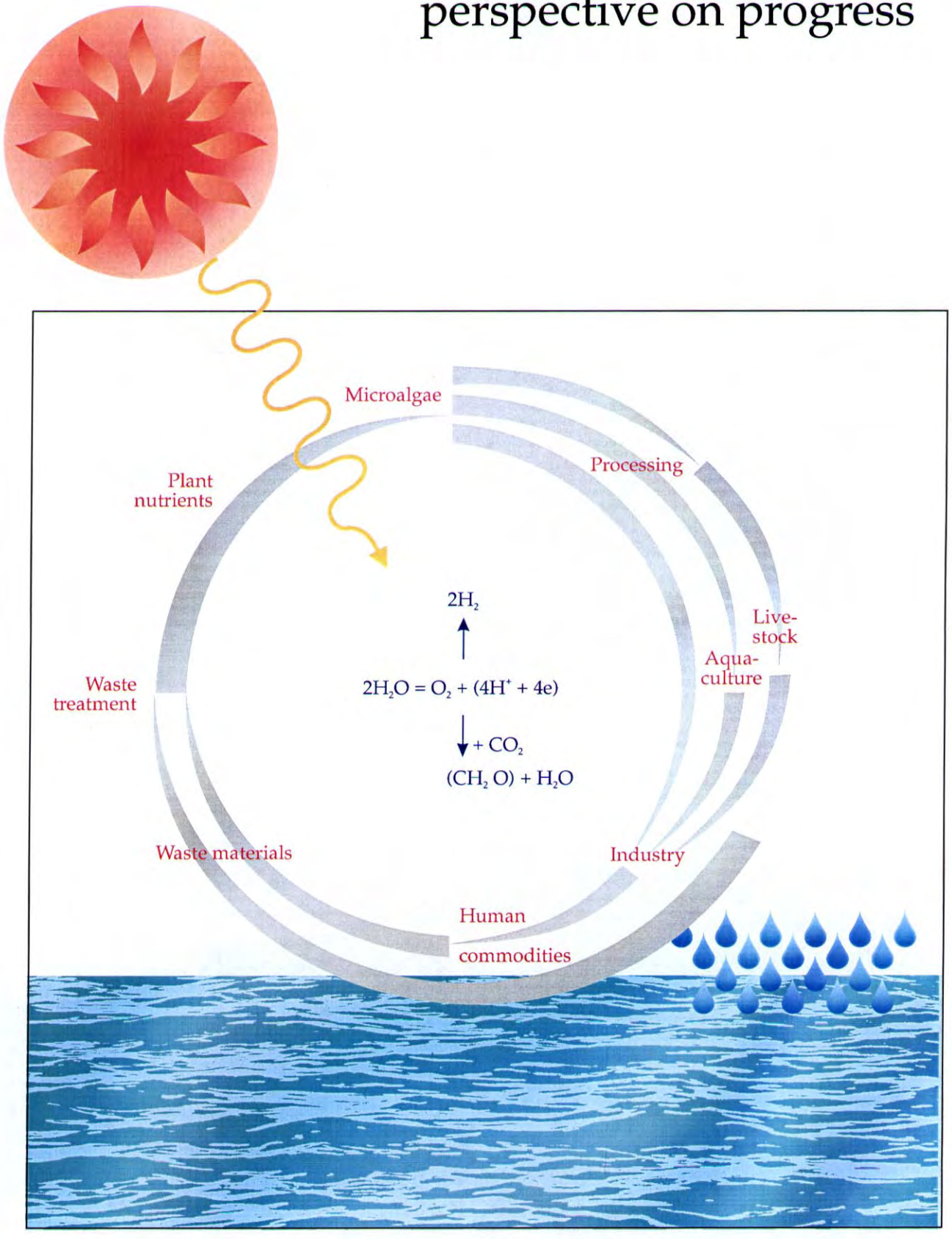
Professional background for development of Algal Culture Technology - a perspective on progress



Professional background for development of algal culture technology - a perspective on progress

Norwegian Institute for Water Research
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"Sustainable development requires better scientific understanding of the problems. Nations should share knowledge and innovative technologies to achieve the goal of sustainability."

Rio Declaration on Environment and Development. June 1992.

Preface

The water quality problems caused by dense populations of microalgae are intricate and manifold. In consequence their negative characteristics have usually gained primary research attention, as well as the practical means how to control their growth where they are undesirable. However the same specific properties making some microalgae of general undesirable practical significance, may be just the qualifications involving possibilities for their positive economic utilization.

As a modern research institute, it is required that NIVA constantly develops and responds on new challenges. Algal culture technology involves an outlook towards a future where photosynthetic microorganisms gain importance for practical application.

Substantial progress will depend on success in achieving new products, isolating the relevant strains and developing effective systems for cultivation and harvest. In this perspective attention will be directed towards the innovative uses of the microalgae for a multipurpose generating of important market commodities and managing environmental problems.

Oslo, November 1995

Haakon Thaulow

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1. Introduction

Norwegian research has contributed significantly to turning coastal kelps and other seaweeds into a successful international export commodity (Jensen 1995). Alginates containing the mannuronate and guluronate monomers compose a family of biopolymers with unique chemical and physical qualities. Their different properties of viscosity suits e.g. the manifold applications as thickeners or stabilizers in the food industry. There are more than half a thousand uses of alginates in present food manufacturing. Carrageenan and agar - also harvested from seaweed forests of macroalgae - are likewise widely utilized and have similar large economic interest on global scale.

But in contrast to macroalgae, the microscopic photosynthetic organisms of oceans and inland waters - the microalgae - have so far scarcely been commercially exploited. This implies indeed that microalgae make out one of the largest untapped biological resources on earth. Microalgae are really a primary challenge in biotechnology for the next millennium. Phycological research has an important task to perform in the development of the emerging manufacturing industry we may designate **algal culture technology.**

Algal culture technology (ACT) is here defined as the practical utilization of prokaryotic and eukaryotic microalgae for the harvesting of biomass and production of special commodities with economic importance (Skulberg 1994).

2. Historical perspective

The scientific utilization of pure cultures of algae is no more than about a hundred years (Oltmanns 1904). Among the scientists who prepared the way, biologists like A. Faminzyn [1835-1918], H. Molisch [1856-1937], M.W. Beijerinck [1851-1931] and R. Chodat [1885-1934] may be mentioned. The historical development is well described in the classic publication by E.G. Pringsheim: Pure cultures of algae. (1949).

The progress in cultivation of algae is closely connected with the use of algae as research objects for scientific work. The modern rise of biochemistry was promoted by the research of A.J. Kluyver [1888-1956] and O. Warburg [1883-1970] stimulating the search for the key reactions governing the chemical activities of life. Cultures of algae supplied a useful basis for the experimental-orientated physiological investigations. The fruitfulness of the unified biochemical and taxonomical approach is illustrated by the scientific work of C.B. van Niel. His comprehensive theory of photosynthesis was an essential element in the natural classification of chemosynthetic and photosynthetic microorganisms versus other autotrophic organisms (Murray 1974). Several other important contributions in the development of algal cultures are represented by publications of phycologists like A. Lwoff (1951), S.H. Hutner (1951), L. Provasoli (1968) and G.E. Fogg (1965).

Norwegian biologists have helped making the use of algal cultures successful. Research work by H.H. Gran [1870-1955], B. Føyn [1898-1985] and T. Braarud [1903-1985] are among the pioneering contributions. This scientific tradition is fruitfully advanced - to mention a few - by E. Paasche (1978), G. Knutsen (Knutsen et al. 1974), D. Klaveness (1988) and J. Throndsen (1973).

The practical and economical exploitation of algae has promoted the use of algal cultures and culture collections very much (Soeder 1980). A short retrospective examination is presented. Industrial production of *Chlorella* was introduced in Japan during the fifties. A historical landmark was the famous Carnegie Institution Report: Algal Culture, from Laboratory to Pilot Plant (Burlew 1964). Microalgal large scale culturing was performed in Czechoslovakia in the sixties (Šetlík et al. 1970). In the seventies algal cultures were involved in the research and development of wastewater treatment (Oswald 1988). Microalgal culturing offers an important potential for production of food and organic chemicals. Several species are used in the modern biotechnology - e.g. strains of *Spirulina, Dunaliella, Isochrysis, Haematococcus, Euglena, Porphyridium* (Gudin & Chaumont 1984, Borowitzka & Borowitzka 1988).

Progress over the past three decades has moved microalgal cultures from the pilot plant to a commercial reality. A number of experimental and full scale facilities has been operated in several industrial countries. In Japan, Taiwan and Australia commercial microalgal production plants have been in action for more than twenty years. Several new plants are established in recent years in the US, South Africa, Israel and other countries (Chapman & Gellenbeck 1989).

3. The resource opportunities

In a world increasingly aware of the finite nature of its natural resources, biological production is a pressing concern for anyone dealing with the shape of the future (Keating 1993). The human controlled cultivation and harvest of aquatic species - aquaculture - offers one means for increasing production of food and organic commodities. Proper managed and controlled cultivation systems, by permitting optimal use of input materials such as nutrients and energy, can provide greater yield exceeding far the unmanaged natural systems.

The use of primary producers in aquaculture and

related commercial applications is still on a small scale, compared with the extensive culturing of marine and freshwater animals. However, in the future growth and development of aquaculture, the microalgae will have a large contribution and possibly providing the greatest benefits. This is based on the recognized need to procure future sustainability of the human industrial society (World Commision on Environment and Development 1987).

The two essential requirements for survival of humans on earth are food and fuel, both are products of photosynthesis with the sun as a primary energy source. Techniques and new developments in photosynthetic research on prokaryotic and eukaryotic microalgae have turned the attention to the potentials of using these organisms for the production of vital human goods and harvesting of energy. This technology will play a major role in the development of the sustainable human society (Keating 1993).

The strategies for a sustainable future of mankind have to include "clean technology" (Kirkwood & Longley 1995). That is an approach for protecting the environment based on the use of clean energy supply, preventing pollution at source and minimizing waste production. By the standards of sustainable development, the consumption of natural resources is long ago too intensive, and are related to a multitude of pollutants and other adverse effects at unacceptable levels (Hille 1995). The carrying capacities related to human activity seem to be beyond their limit whithin present modes of technology. In other words our technology and lifestyle today are not sustainable.

The choice is either to accept limited carrying capasity, or to seek other forms of technology. Algal culture technology stands out among the practical options beeing explored allowing sustainability, and not depriving future generations of necessary resources. In this connection the utilization of microalgae is a challenge for creative scientific contributions and economic interactions.

4. Production of microalgae

The cultivation of microalgae both in the laboratory and industry is based on the same theoretical principles (Pirt 1975).

In a batch culture photosynthetic microorganisms grow exponentially as long as all substrates for growth are present in excess. The conditions in cultures of this type are however changing as the biomass density increases and the culture passes several phases of successively slower growth until, finally, the growth ceases because of resource depletion. As a result of the changing conditions, the attributes of the cells, such as mean size and composition do not remain constant. To obtain cell material of a certain composition from a batch culture the biomass has to be harvested in the appropriate growth phase.

For industrial operation, continuous production of microalgae of defined quality is desirable. This can be achieved by adopting continuous or semi-continuous culture techniques. In a continuous culture a steady supply of growth medium and outflow of produced microalgae creates constant conditions in the culture. The growth rate of the algae is adjusted by the supply rate of growth medium, keeping the biomass density constant at the optimum level (Veldkamp 1976).

In a semi-continuous culture the same effect is created by withdrawing a defined portion of the culture at periodic intervals, and replacing this with fresh medium to the original volume. Essentially, the industrial production of microalgae is accomplished by holding the cultures at some chosen phase in their growth cycle by the regulated addition of a new medium.

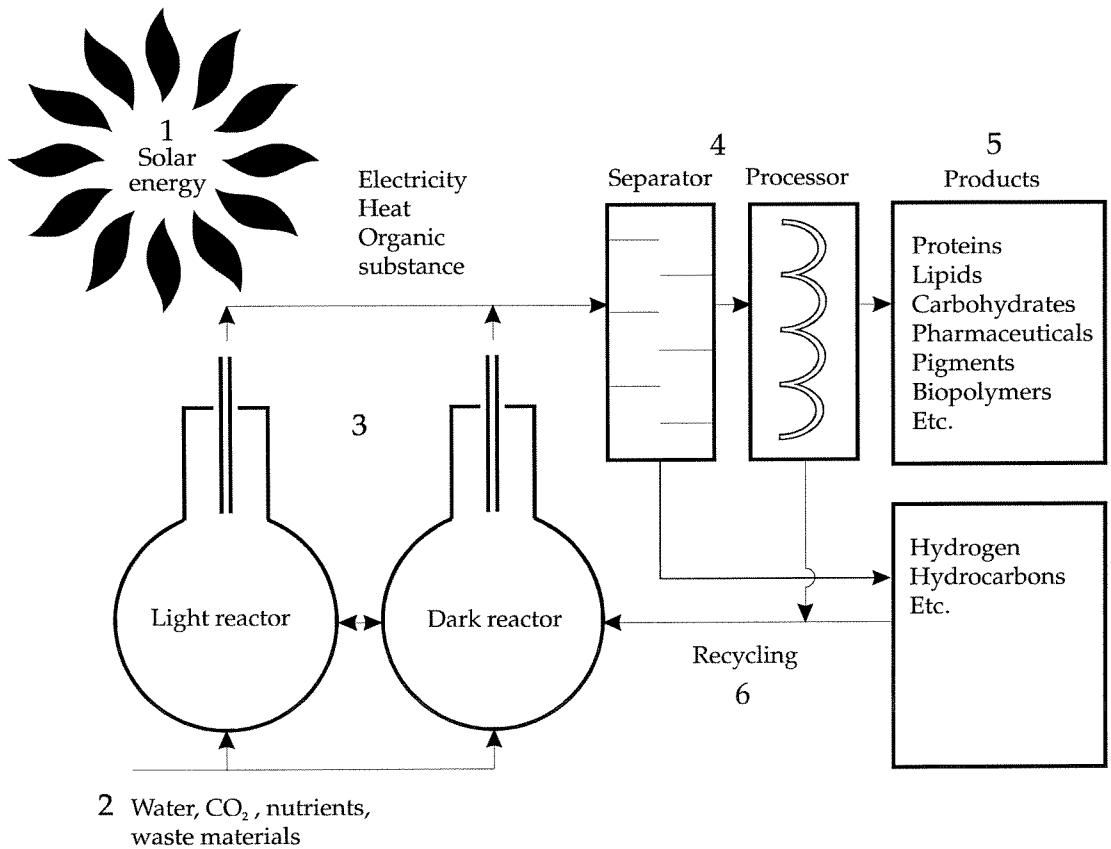
Automatic continuous culture methods enable precise and running control of algal population density. Two types of apparatus are used. In **turbidostats** dilution is controlled by a photometric device to keep the population density (turbidity) of the culture constant, and thus balance the rate of growth. The other kind of apparatus is the **chemostat**. It depends on the addition of fresh medium to the culture at a constant rate. The population density will then adjust itself to a maximum rate, determined by the rate of supply of the limiting nutrient. Constant volume is maintained by an overflow device, at the same time used for the harvesting of cells.

Mass culture of microalgae has different industrial objectives. Among the most common are production of large amounts of a particular algal species, production of a separate metabolite or algal biomass. The construction of a culture system and the choice of equipment have to consider the essential growth requirements of microalgae (light, carbon dioxide, temperature, turbulence, media etc.).

The productivity of mass cultures of microalgae depends primarily on the available light irradiance and the efficiency of the photosynthetic conversion of absorbed energy by the algae. The theoretical problems of photosynthetic efficiency has not yet been completely solved (Pirt 1983, 1986), but the practical maximum efficiency is probably in the range 5-6 % of the total irradiation (Geider & Osborne 1992). With this conversion the yield of daylight-based microalgal production would be 70-80 g m^{-2} day^{-1} at maximum global irradiation (800 cal cm^{-2} day^{-1}). In practice, the long term yields obtained in commercial mass cultures are rarely more than 20 g m^{-2} s^{-1}.

PLATE 1

Proposed scheme for an industrial ACT system of multiple product utilization.



1 By photosynthesis in microalgae, light energy is converted into stored chemical energy by the production of cellular constituents from carbon dioxide and water.

2 Growth of algal cultures requires a medium of water, mineral nutrients and carbon dioxide. Continuous production of biomass with defined quality is the basis for industrial operation.

3 Several kinds of microorganisms are included in the practical implementation. The main selection will be photosynthetic prokaryotic and eukaryotic microalgae.

4 Integrated processes are used for separating and extracting primary and secondary metabolites with possible economic potentials.

5 Two categories of products of vital human need and commercial interests are aimed at:

- costly substances needed in small quantities (e.g. pharmaceuticals)
- cheap substances used in large quantities (e.g. hydrogen gas).

6 Superfluous components (e.g. plant nutrients) are recycled to the bioreactors.

The energy requirement for production based on artificial light from fluorescent tubes has been estimated at approximately 170 kWh per kg of dry algal biomass (Radmer & Parker 1994). The cost of electric energy often prevents the use of artificial light for commercial production of microalgae.

Most production units based on natural light irradiance are geographical located to tropical or subtropical latitudes, making use of the high insolation and high growth temperatures. The temperate and boreal regions seem less attractive in comparison. However, during the period March-September the total irradiation is sufficient in these regions for a substantial production of microalgae. In midsummer the total daily irradiation is similar to the tropics, although the sunlight is distributed over a longer period. This creates more favourable conditions for mass production of microalgae, since photoinhibition at high irradiation levels will be less severe (Van Liere & Walsby 1982). Moreover, the short dark period and cool night temperature will tend to reduce the loss caused by respiration, which may be substantial in tropical and subtropical latitudes. Locally adapted strains of microalgae may therefore have an interesting potential for mass production under the prevailing conditions in the temperate region. Based on the light utilization efficiencies obtained in outdoor mass cultures the potential long-term productivity in the North Sea-area is e.g. approximately 15-20 g dry weight m^{-2} day^{-1}. Proper design and management of the cultivation system and better understanding the physiological processes in dense algal suspensions could be the key to further improvement of the production yield (Pirt 1975).

High production costs are currently the main obstacle for development of industrial activities based on microalgae. A meaningful cost reduction would be achieved if the yields of production per bioreactor unit could be much increased (Borowitzka 1992). Although the areal production in existing mass culture plants exceeds the yield of agricultural crops, further improvement up to some 100 metric tons of dry weight per hectar and year is theoretically available at low latitudes (Richmond 1992). Such a substantial increase in productivity requires the development of tailor-made algal species coupled with an optimal bioreactor design.

5. Technological achievements

Substantial progress has been made during the past decade in devloping appropriate technology for microalgal mass cultivation.

Improvements have been obtained in the management of open outdoor culture systems (Borowitzka & Borowitzka 1988). This is based partly on a better understanding of the physiology of dense cultures grown on large scale, partly on refined technology concerning pond design, mixing systems and methods of harvesting (Vonshak & Richmond 1985). High maximum values of production in outdoor reactors have been reached (e.g. 50-60 g dry weight m^{-2} day^{-1}). However long-term average production is usually ca. 10 g m^{-2} day^{-1}. This corresponds to a net photosynthetic efficiency of approximately 1% which is significantly less than the theoretical limit (Bassham 1977).

Almost all commercial bioreactors for outdoor production of microalgae are based on shallow raceways in which the cultures are mixed in a turbulent flow sustained by mixers of various designs (Ben-Amotz & Avron 1989). Although operating failures are frequently recorded in commercial plants, it seems to be no particular obstacle to cultivate prokaryotic or eukaryotic microalgae using these systems, even on a large scale. Commercial production ponds have usually an average surface area of 1000-4000 m^2 (Dodd 1985).

Open raceways or ponds are relatively simple to operate and will probably remain the most frequently used reactor type for commercial production for a long time. In these plants a turbulent flow is created by paddle weels or aeration. This turbulence is important to achieve optimum utilization of light, CO_2 and nutrients, and to keep the algae in suspension. When sufficient nutrients and CO_2 are supplied, the production is mainly dependent on irradiation and temperature. To achieve maximum production at a given level of irradiation, the optimum combination of pond depth and biomass density has to be established. Since it is desirable to have a high biomass density in order to reduce the cost of harvesting, the culture must be shallow, in practice less than 30 cm. If the depth of the culture is increased above the optimum depth, a greater portion of the culture will, because of self-shading, receive too little light for net production and the respiration loss will decrease the areal production. Therefore, from a productivity point of view, deeper cultures will not result in higher yield. Experiments carried out with small outdoor cultures of phytoflagellates e.g., indicated that the productivity - expressed as g algae m^{-2} day^{-1} - was identical when the depth of the culture medium was varied between 5 and 30 cm (Ben-Amotz & Avron 1989).

The pond or raceway design is not suitable for very high cell densities, which require shallow depth. An alternative technique based on large scale sloping culture systems has been developed and used in Central Europe (Šetlík et al. 1970). With this system the problems to obtain an efficient mixing at low depth have been partly overcome. The plant operated is a large-scale open culture system of more than 1000 m^2. By using cascades of falling films of culture medium, turbulent flow is ensured at a low culture depth (3.5 cm). High

PLATE 2

Processes and techniques involved in mass culture of microalgae.

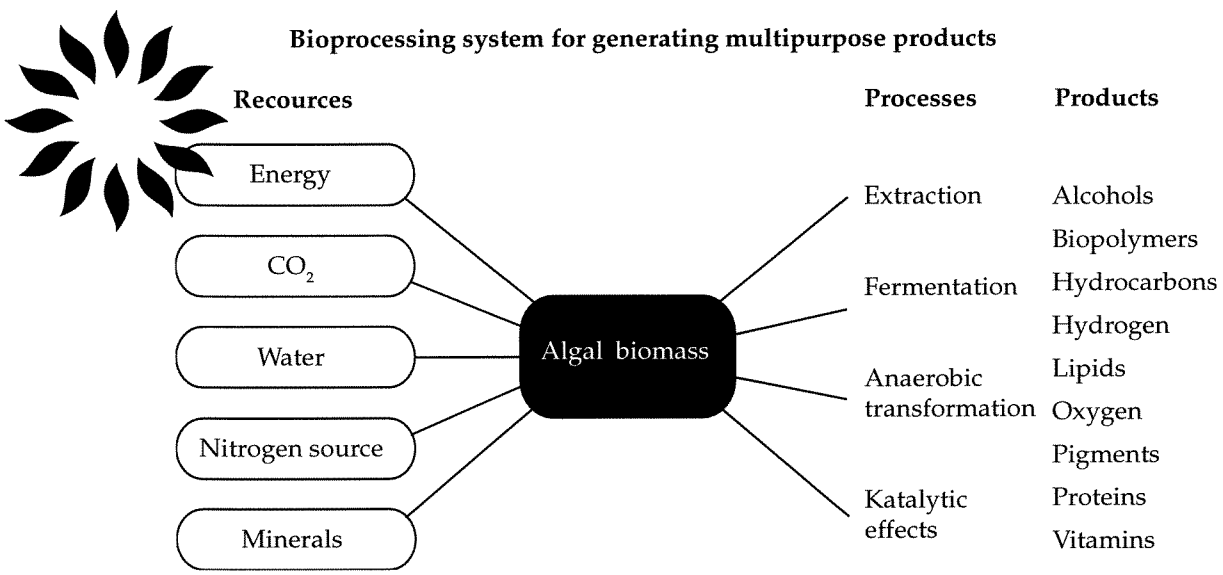
The primary processes of photosynthesis involve the harvesting of light by certain antennae molecules which become excited by absorption of solar photons. The excitation energy is transferred by resonance effects to a reaction centre, where a charge transfer process is initiated.

This energy-rich state is the basis for the overall reactions of photosyntesis.

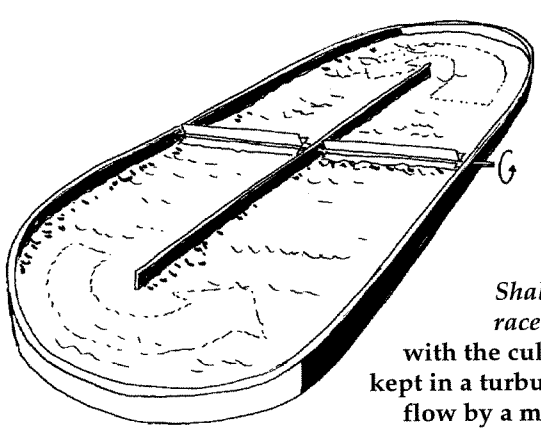
$$n\,CO_2 + n\,H_2O \xrightarrow{hv=n\ 2821\ kJ} (CH_2O)\,n + n\,O_2$$

Photosyntesis is a two stage process. The first produces a voltage, while the second is driven by this potential, and resulting in the formation of high energy molecules such as adenosintriphosphate (ATP) and nicotinamide adenine dinucleotide phosphate ($NADPH_2$).

Bioprocessing system for generating multipurpose products

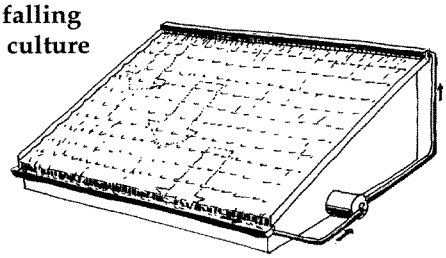


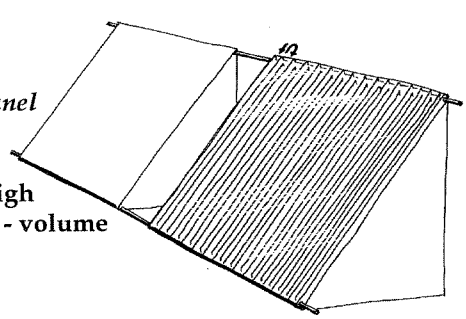
Equipment for cultivation of microalgae designed for mass production is adopted for industrial purposes. The technical solutions include developments of different types varying from photobioreactors based on closed systems to open raceways for outdoor production.



Shallow raceway **with the culture kept in a turbulent flow by a mixer.**

Sloping culture system **using cascades of falling films of the culture medium.**





Alveolar panel bioreactor **designed to obtain high surface - to - volume ratio.**

productivities of green algae are obtained (average 25 g m^{-2} day^{-1}, personal communication).

Open culture systems as those described above have some disadvantages. Because of the large open surface the culture volume may be severely affected by evaporation and rainfall. The open systems also offer limited possibilities to control culture temperature and supply of CO_2 at optimum levels. Furthermore open cultures are vulnerable to contamination with weeds (microalgae) and predators. Consequently open culture systems are most suitable for fast growing algae, which are less susceptible to competition, and for microalgae that can grow in highly selective media, such as the cyanophyte *Spirulina platensis*.

The drawbacks with open cultures for mass production of microalgae have stimulated new developments of photobioreactors based on closed systems. These may have the form of tubular reactors, where the culture is circulated through transparent tubes (Pirt et al. 1983, Gudin & Thepenier 1986). The circulating algal culture is passed through a tower for de-oxygenation and supply of CO_2. Temperature control can be obtained by cooling or heating in the gas-exchange unit or by having the tubes immersed in a temperature controlled water basin. Use of tubular reactors allows an effective utilization of light energy since the unit can be oriented so that optimal light conditions are created. This is particularly the case with the reactors of the alveloar type (Tredici & Materassi 1992, Tredici et al. 1993). In the alveloar reactors the algal cultures are circulated through the channels of double transparent Plexiglas plates. The thickness of the culture is only one or a few centimeters, which gives a very high surface to volume ratio, and the plates can be arranged so that they receive light from both sides. With this culturing technique very high biomass density (5-10 g l^{-1}) can be achieved, which is important for the reduction of operating costs. The reason for this is lower energy consumption, higher volumetric productivities and lower contamination risk. In view of the future application the capability of being vertically oriented appears a most relevant feature of the system. Comparative studies of the output rate in open raceways and vertical tubular photobioreactors revealed a consistent positive increase of about 10-30% for the tubular system (Richmond 1992).

A promising development in algal culture technology is the use of immobilization techniques (Hall & Rao 1989). The method consists of maintaining microalgae in a defined microenvironment where control of growth factors and product removal can easily be obtained. By securing a stable cell entrapment and high concentration of the biocatalyst, a continuous flow photobioreactor can be operated. Particularly for the production of exocellular compounds has the immobilized algal culture system advantages, compared to production systems using suspended cultures.

6. Benefits of microalgae

Photosynthesis by microalgae converts in nature large quantities of solar radiation into chemical energy in the form of carbohydrates, lipids, proteins, ammonia, adenosinetriphosphate, pyridine nucleotides etc. The practical importance of microalgae as energy converters and organic chemical producers lies in the fact that the substrates they use such as water, carbon dioxide, nitrogen, are ubiquitous and inexpensive. The commercial potential of microalgae is widely recognized, and their applications for economic purposes have increasing interest with the advances in algal culture technology (Richmond 1990). Some examples of the exploitation possibilities for the relevant bioprocessing will be considered connected to the metabolic diversity of microalgae.

Food and feeding

The qualities of selected microalgae as raw material for foodstuff are well documented (Borowitzka & Borowitzka 1988). Their endocellular storage of polysaccharides and protein-rich material makes them valuable as producers of animal and human nutrition. Polysaccharides are of the glycogen type in the prokaryotic microalgae, and of starch type in the eukaryotic ones. Under certain culture conditions cells of chlorophytes can accumulate more than 30% of their dry weight in form of starch (Harris 1989). The contents of crude proteins of microalgae vary greatly both with respect to quality and quantity. Species cultivated for commercial purposes usually have a chemical composition high in proteins (e.g. chlorophytes 47-56%, cyanophytes 46-71% of dry matter). The variations in protein content stem mainly from the growth conditions and the percentage of ash (Richmond 1988).

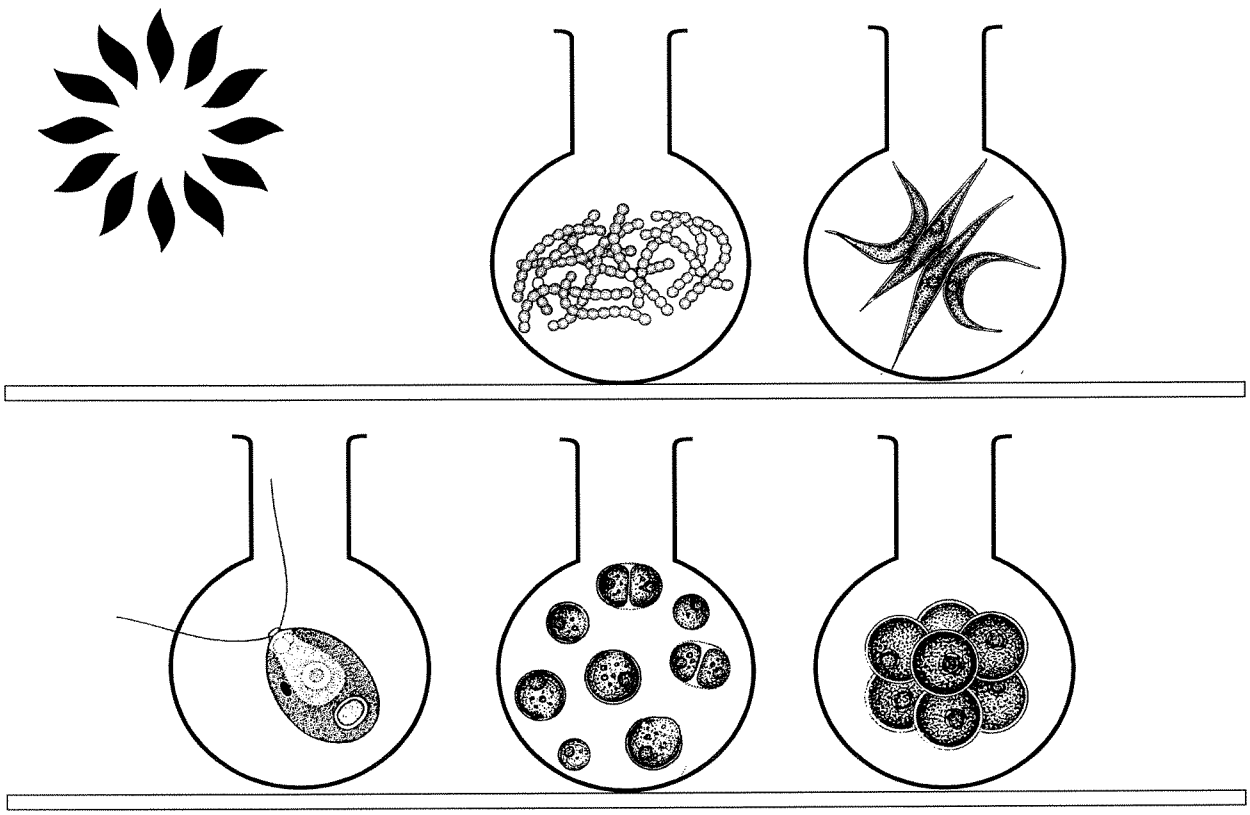
A growing concern for the acute food needs of the exploding human population has led to the examination of a variety of unusual food sources as potential expander of the world's food supply (Brown 1994). Among these the photosynthetic prokaryotes present the best chance for the development of a unique microalgal based food supply. The cyanophyte species belonging to the genus *Spirulina* are for example today the only microalgae to be extensively grown to get protein-rich material used for human consumption (Becker 1994).

The nutritive value of protein is generally related to its amino acid composition, digestibility and bioavailability of its essential amino acids. The essential amino acids comprise 47% of the protein contents of *Spirulina*. In human nutrition *Spirulina* will probably be used most often as a protein supplement improving the nutritive qualities of staple food in the underdeveloped parts of the world (Dillon & Phan 1993).

The most abundant protein in cyanophtyes are phycobiliproteins. These mainly blue-coloured

PLATE 3

Commodities and commercial applications of microalgae and their metabolites.



Microalgae are capable producers for a wide spectrum of natural products of vital human need and interest.

Nutrition	Pharmacy	Cosmetics	Agriculture	Energy
Antioxidants	Adaptogenics	Antioxidants	Ammonia	Biomass
Carbohydrates	Analgetics	β-Carotenoids	Antibiotics	Hydrocarbons
β-Carotenoids	Antibiotics	Biomass	Biomass	Hydrogen
Biomass	Anti cancer agents	Fatty acids	Flocculants	
Enzymes	Anti caries agents	Pigments	Growth regulators	
Lipids	Antioxidants	Sensorics	Minerals	
Nucleic acids	Antiviral agents			
Nutriceuticals	β-Carotenoids			
Pigments	Bioenergetics			
Polyphenols	Enzymes			
Proteins	Immunomodulators			
Vitamins	Isotopic agents			
	Pigments			
	Radioprotectors			
	Toxins			

substances account for about 30% of the biomass (dry matter). Phycocyanin and allophycocyanin belong to a large array of natural products of economic potential which may be produced from cyanophytes. They are applied as pigments or colourants in the food industry as well as for cosmetics. Governmental regulations restricting the artificial colourings provide an added incentive to develop natural pigments from microalgae.

Microalgae are effective synthesizers of several interesting carotenoids (Foss 1985). The practical use of carotenoids is extensive, e.g. food colouring, feed additives to enhance flesh colour of salmonid fish, as well as the colour of egg yolks. The health and fertility of lot-fed cattle and fish are improved by application of selected carotenoids in their feeding (Borowitzka & Borowitzka 1988). Prominent carotenoid producers are for example *Dunaliella salina* and *Haematococcus pluvialis*. The last mentioned phytoflagellate produces significant quantities of the carotenoid astaxanthin in the aplanospore stage of the life cycle (up to 2% of biomass, dry matter).

The fatty acid contents of lipids are commercially interesting properties of microalgae (nutriceuticals). Relevant species used in algal culture technology are characterized by high levels of the long-chain poly-unsaturated fatty acids (PUFA) belonging to the omega-6 series, including the essential fatty acid (EFA) linoleic 18:2 omega-6. Some cyanophytes are relatively rich in the rare fatty acid gamma-linolenic acid (GLA), reported to have wide therapeutic properties (Richmond 1992).

Secondary metabolites

The search for bioactive chemicals from microalgae has resulted in a range of substances with possible pharmaceutical potentials.

Several species of cyanophytes have been known to produce substances with antibiotic activity (Cresswell et al. 1989). The chemicals involved are mostly unidentified, but comprise fatty acids, other organic acids, bromphenols, polysaccharides and alcohols. The relevant activities are described including e.g. antibacterial, algicidal, antifungal effects. Many of the substances are secreted extracellularly, others can be released from the cells by influence from cultivation factors.

Toxin production by freshwater and marine oscillatorias has long been recognized (Østensvik et al. 1981, Moore 1984). The bioactive compounds include among others alkaloids and low-molecular weight peptides (Skulberg et al. 1984). The alkaloid toxins - neurotoxins - target the neuromuscular system. They paralyze the skeletal and respiratory muscles and cause death from respiratory arrest within minutes. The peptide toxins - hepatotoxins - target the liver. They cause extensive necrosis of this organ, resulting in death by hemorrhagic shock after a few hours (Charmichael 1988).

The potential of pharmaceuticals from toxins of cyanophytes is under investigation (Cresswell et al. 1989). The oscillatorias are promising candidates for the bioprocessing of required substances. Fortunately they are by nature given the superior ability to synthesize the stereospecific compounds with the relevant bioactivity (Amato 1992). The increased understanding of the basic metabolic processes combined with the development of instrumentation for purification and separation of the biomolecules concerned, has recently led to an ability to undertake a biorational approach to screening and selection of interesting products.

The newly detected homoanatoxin-a (Skulberg et al. 1992) may serve as an example. The potent neurotoxin is produced by a strain of *Oscillatoria formosa* Bory ex Gom. The familiar alkaloid anatoxin-a is a nicotinic agonist that is valuble for the study of nicotinic receptors. Homoanatoxin a is the homologue in which the sidechain is extended by one methylene unit from methyl to an ethyl ketone. Homoanatoxin-a allows the generation of a tritiated product (Wonnacott et al. 1992). Homoanatoxin-a has the necessary characteristics that enable it to be exploited in the investigations of high affinity nicotinic sites in the human brain.

Cyanophytes are known to contain glycolipids and sulpholipids. They can be cultivated in ways to significantly increase their lipid content and presumably also the content of sulpholipids. Sulphonic acid-containing glycolipids extracted from certain clones of *Oscillatoria* have been found to be remarkably active agains the AIDS virus *in vitro* (Gustafson 1989).

Energy crop/biophotolysis

Supply of energy from the sun offers fascinating perspectives for the future of mankind. The sun radiates to the earth in half an hour that quantity of energy which is annually used in the form of primary energy throughout the world (Gatzka et al. 1982). The total solar energy falling on earth in two weeks is equivalent to all stored fossil fuels.

The amount of solar radiation reaching the earth's surface depends upon both the time of year and the geographical location. On a surface inclined at 30° to the horizontal, in the North Sea-area the values range from 80-90 megajoules $m^{-2}month^{-1}$ in winter to ca 500 megajoules $m^{-2}month^{-1}$ in summer. It is interesting to note that the annual insolation from the sun in the North Sea-area is still half as high as in the areas which have the greatest amount of insolation. In practise, however, effective magnitude of the solar radiation fit for use by any light-driven process depends upon the surface area over which that radiation is collected. Decisive is also what proportion of the absorbed energy that is converted into useful output. But it is quite clear that the size of the solar energy resource, even in the temperate and boreal climate zones of Europe, is very large. At the same time it is necessary to underline that a photobiological microalgal reactor

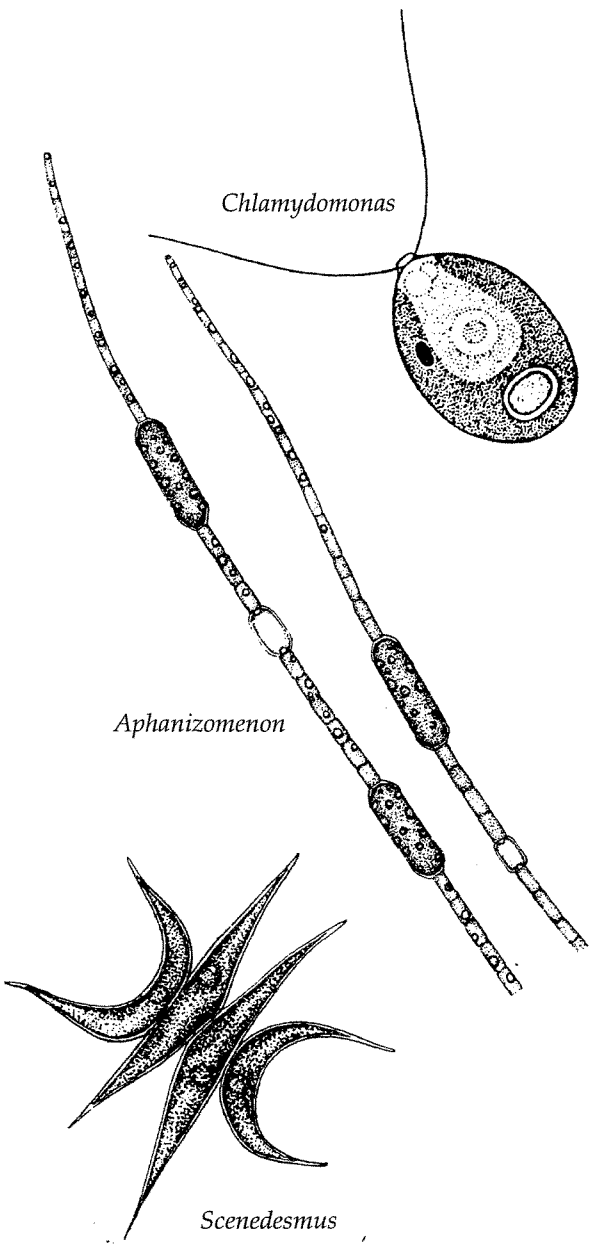
PLATE 4

Photoproduction of molecular hydrogen by biophotolysis.

ORGANISMS

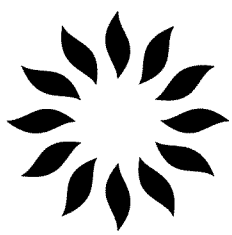
A variety of photosynthetic microorganisms can evolve molecular hydrogen by the reaction:

$$2H^+ + 2e^- \rightleftharpoons H_2$$

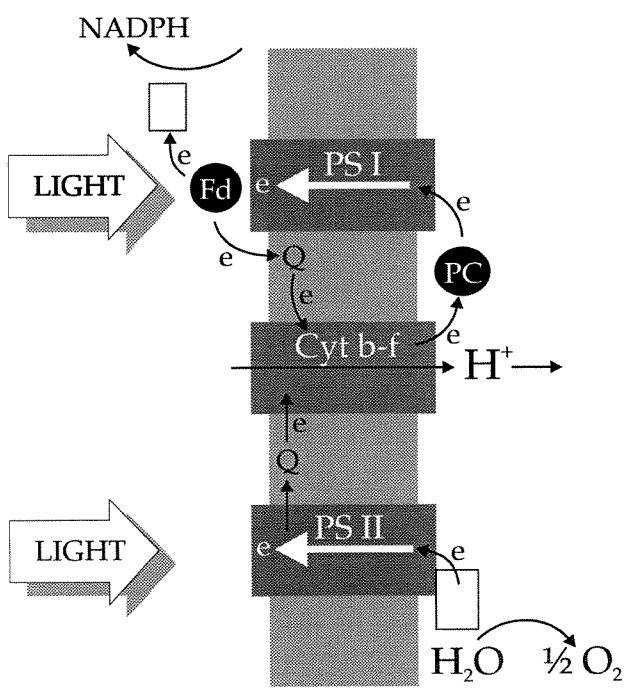


BIOPHOTOLYSIS

Biophotolysis is the process resulting in breaking the stable bond between hydrogen and oxygen in the water molecule by radiant energy in the primary reaction of photosynthesis.



Thylacoid membrane in the photosynthetic apparatus of microalgae



The hydrogen-producing systems accept electrons from donors which are thereby oxidized in the process. For any commercial application a cheap electron donor is essential. Water is ideal, but also various industrial waste products with negative cost are suitable (e.g. organic wastes, sodium sulphide).

needs an extensive surface area as a light-driven device.

There are several main methods of conversion of solar energy for production of fuel and other vital human interests. They are all closely related. All use an absorber of photons which utilizes electronic excitation to move electrons to higher potential.

Possibilities and theoretical efficiences of the processes are based on the same thermodynamic considerations. The thermodynamic restrictions set an upper limit of 27% for the conversion efficiency of solar radiation energy by microalgae to stored chemical potential. But there is clearly no theoretical reason why efficiencies greater than those of natural photosynthesis should not be attained. Chemical models of photosynthesis and genetic engineering of photosynthetic microorganisms are among the realistic solutions (Hunter & Mann 1992).

The biophotolysis of water is achieved by two biochemical processes carried out by the activity of chlorophyll containing reaction centres coupled to hydrogenase and nitrogenase. Microalgae belonging to chlorophytes and cyanophytes can produce molecular hydrogen by the decomposition of water using solar energy (Skulberg 1995). Among Anoxyphotobacteria some organisms of the families *Chromatiaceae* and *Chlorobiaceae* are also used for the bioengineering development of biophotolysis.

Two types of enzymes form the basis for the production of hydrogen at the expense of light energy. They are respectively hydrogenase and nitrogenase (Cammack et al. 1985, Schlegel 1985). Hydrogenase activity is present in most prokaryotic and eukaryotic microalgae and anoxyphotobacteria. Nitrogenase activity is present in e.g. prokaryotic microalgae (cyanophytes) and photosynthetic bacteria.

The hydrogen-producing systems accept electrons from electron donors which are thereby oxidized in the process. For any commercial application a cheap electron donor is essential. Water is ideal, but also various industrial waste products with negative cost are suitable (e.g. organic wastes, sodium sulphide).

Solar energy drives the light-harvesting, photochemical and electron transport systems of the photosynthetic reaction in green algae (e.g. species of *Chlamydomonas* and *Scenedesmus*). Under normal, aerobic conditions, $NADPH_2$ transports reducing equivalents into the Calvin cycle for the enzymatic reduction of atmospheric carbon dioxide. But in a carbon dioxide-free anaerobic atmosphere, green algae are capable of synthesizing hydrogenase and evolving molecular hydrogen (Gaffron & Rubin 1942). In this case the water molecule is split into H^+ and O_2, and the electrons are carried through the two photosystems involved. The reduced electron carriers are then reoxidised by hydrogenase, and hydrogen gas is formed.

Nitrogenase is structurally composed of a larger protein moiety and a smaller one (Schlegel 1985, Van Baalen 1987), both of which are essential for the activity. An electron donor, ATP and Mg^{2+} are required for the reduction of N_2 or other appropriate substrate under anaerobic conditions. Nitrogenase is particularly sensitive to oxygen. Preparations of nitrogenase are inactivated in the presence of oxygen even though they originate from aerobic organisms (e.g. *Azotobacter*).

The production of hydrogen occurs through the action of nitrogenase (Ormerod et al. 1961). In the absence of nitrogen can the enzyme reduce several compounds including protons. In the latter case hydrogen gas is produced.

The use of nitrogenase for hydrogen production by cyanophytes has been investigated for applied purposes. Relevant photosyntethic prokaryotes can utilize solar energy over a wide spectral range to produce the energy for nitrogenase activity. Fortunately the nitrogenase reaction is not subject to end-product repression by molecular hydrogen. Thus hydrogen gas evolution can continue even at one atmosphere hydrogen or more. However some sources of fixed nitrogen (e.g. NH_3) severely inhibit nitrogenase and so hydrogen production.

The cost effectiveness of using nitrogenase for commercial production of hydrogen will depend above all on the selection of suitable mutants of relevant organisms, and the construction of genetic modifications, based on new insights into prokaryotic gene organization and expression (Hunter & Mann 1992, Smith et al. 1992).

Agriculture and aquaculture

Algal culture technology has an important part to play in connection with new developments in agriculture and aquaculture.

The use of cyanophytes - enabling an effective utilization of biological nitrogen fertilizer - can illustrate the point. Many tropical rice fields receive no chemical fertilizer or natural manure, yet they remain productive and capable of supporting large populations with basic food (Tsunoda & Takahashi 1984). The fertility of paddy soil is maintained by activities of heterocystous cyanophytes which grow spontaneously and often luxuriantly in the water-logged field (Whitton 1992). They provide fixed nitrogen to rice plants through secretion of nitrogenous substances and on their decay and subsequent mineralization of organic substances in the soil. Cyanophytes are cultured on a large scale in several East Asian countries (e.g. India, Thailand, China, the Philippines) for promoting the production of rice. The starter culture is a mixture of the most efficiently nitrogenfixing cyanophyte strains which grow well in the particular region, and is used to inoculate outdoor nursery plots. In turn these cultures provide the cyanophytes disseminated in the flooded rice field. Field trails in the Philippino rice fields indicate that cyanophyte growth added annually up to 40 kg nitrogen per hectar to the soil (Fogg et al. 1973). The beneficial

PLATE 5

Microalgae and the protection of our environment.

Basic interactions in organic waste treatment.

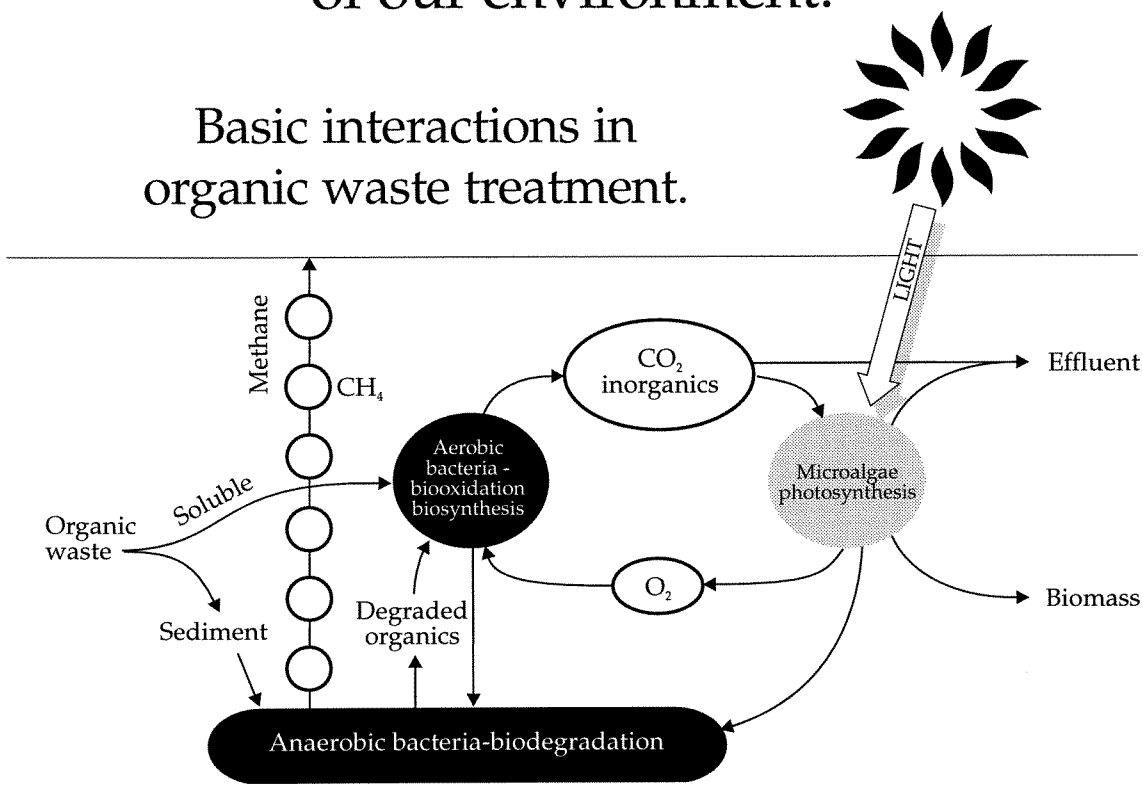
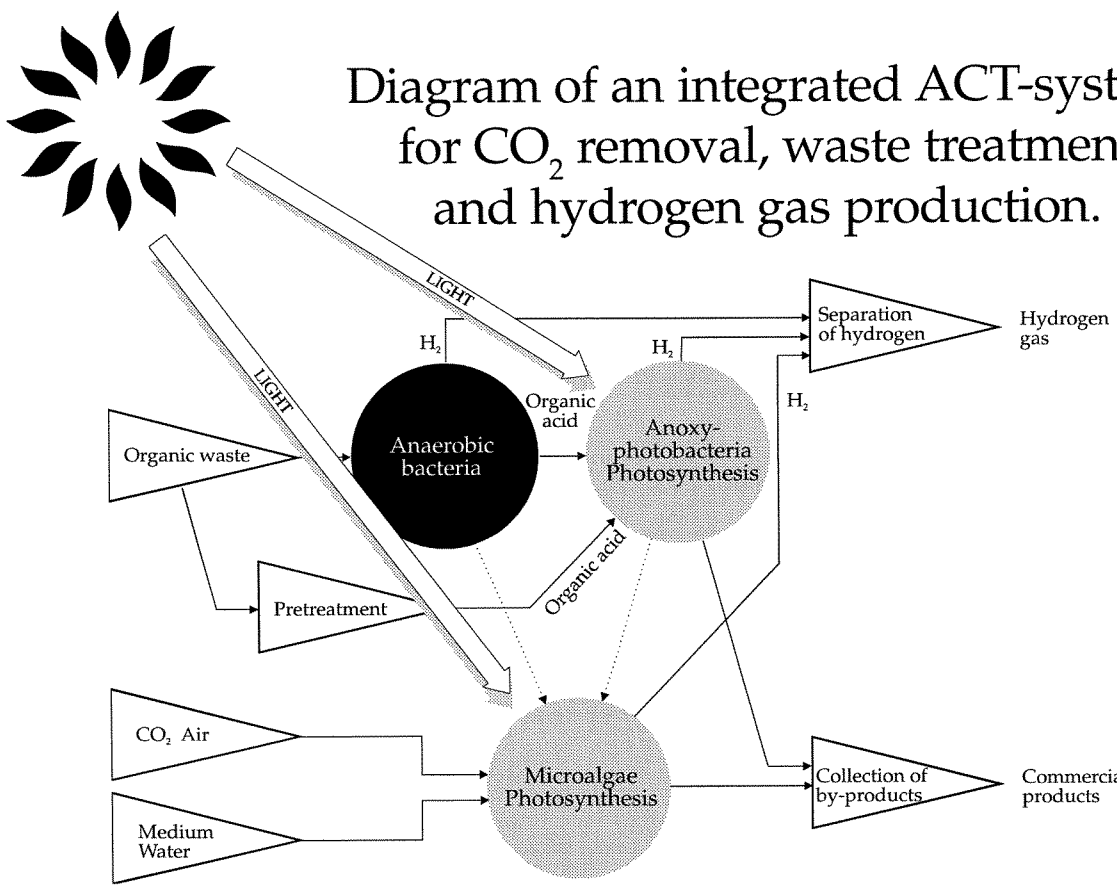


Diagram of an integrated ACT-system for CO_2 removal, waste treatment and hydrogen gas production.



effect to cyanophytes for crop production is even more apparent in paddy fields which are populated by the water fern *Azolla* during the waterlogged period. The agricultural utilization of the symbiosis between cyanophytes and the fern is estimated to add annual quantities of 120-312 kg nitrogen per hectar (Rai 1990).

Manure in dilute water suspensions provide a suitable growth medium for various species of microalgae (e.g. chlorophytes, chrysophytes and cyanophytes). During growth the algal cells convert dissolved nitrogen, phosphorus and carbon to algal protoplasm. The produced algal biomass is high in protein, and can with proper treatment be used for feed supplement. The management of animal wastes has become a serious environmental problem in many parts of the world. In the US for example the amount of manure produced surpasses human sewage by an order of magnitude. Engineered algal culture systems are being developed for the recycling of nutrients contained in the wastes from feedlots, poultry houses and piggeries (Lincoln et al. 1993).

Microalgae can be functional as a nutritional supplement in rearing conventional foods. The use of algal culture technology in aquaculture is one example. An application is feeding of shellfish on marine or brackish water algae in hatcheries (Davis 1969). A dependable method for culturing sufficient quantities of phytoplankton foods for different stages of development is essential for commercial rearing of shellfish. Continuous and semi-continuous culture systems of microalgae are adopted being suitable for fulfilling practical requirements. The methods developed are used for production of microalgae - e.g. *Dunaliella tertiolecta, Isochrysis galbana, Pavlova gyrans, Phaeodactylum tricornutum* - as food in rearing bivalves (Ukeles 1976, Borowitzka & Borowitzka 1988).

Fisheries products are the world's largest source of animal proteins. Overfishing has partly reduced natural stocks to critically low levels. In the past decades, the most rapid increases in fisheries production have come from aquaculture. To meet the demand, the aquaculture will be required to double its production by the year 2000. This will require large increase of feed per year. Traditional fish feed comes from grains, soya beans and fish meal, the production of which is stable or declining. Microalgae are the food that the entire, natural marine food chain depends on. Microalgae-based feeds produced by algal culture technology will continue increasing in importance for aquaculture.

Waste water treatment and waste recycling

Algal culture technology provides important innovation and commercially attractive solutions for the treatment and recycling of wastes.

Carbon dioxide is a dominant product from industrial combustion of fossil fuels. The primary contributors of this prime cause of global warming are power stations, which in several industrial countries produce more than 30% of total carbon dioxide emissions (Gatzka et al. 1982). Geopolitical forces to limit carbon dioxide production are increasing (Hille 1995), and environmental mitigation technology, and alternative energy have to be developed (e.g. renewable energy sources, hydrogen energy system). Microalgae applied in algal culture technology provide the most effective biological means known to remove carbon dioxide from the atmosphere. The relevant systems are operated at the site of fossil-fuel burning power plants, and carbon dioxide is removed from the stack gases before it enters the environment. Efforts have to be directed to adapt these culturing systems to increase the biomass yields and their economical potential value.

Treatment of wastewater with microalgae is based on the algal recycling concept under hypereutrophic conditions (Oswald & Gotaas 1957). The engineered algal systems can be most efficient, and production of microalgal biomass through wastewater treatment has an economic long term potential, particularly in urbanizing regions (Lincoln & Earle 1990). Major beneficial effects of microalgae subject to enhancement, are for example oxygen production in amounts equal to or greater than the wastewater BOD, and creation of useful biomass from the recycled nutrients. Experience indicates that in suitable climates, microalgal based treatment plants produce effluents equal or superior to other wastewater systems at less cost for construction and maintenance, and with minimum environmental nuisance.

Miscellaneous

The contribution of microalgae to man's world is diverse and large. Algal culture technology cover numerous aspects of utilization with emphasis on the special properties and products of economic interest. To describe the potential applications of microalgae is a very broad task. Readers are referred to recent books which comprehensively cover the various aspects of industrial advancements and research interests (Akatsuka 1990, Mann & Carr 1992, Bryant 1994).

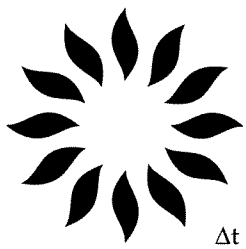
7. The role of culture collections of microalgae

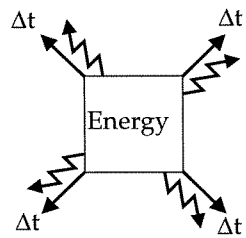
Advances in algal culture technology have highlighted the role of microalgae and lead to growing demand for culture collections (Skulberg & Skulberg 1990).

Present-day needs for professionally organized culture collections are increasing. The primary functions of culture collections are collecting, preserving and distributing authenticated material

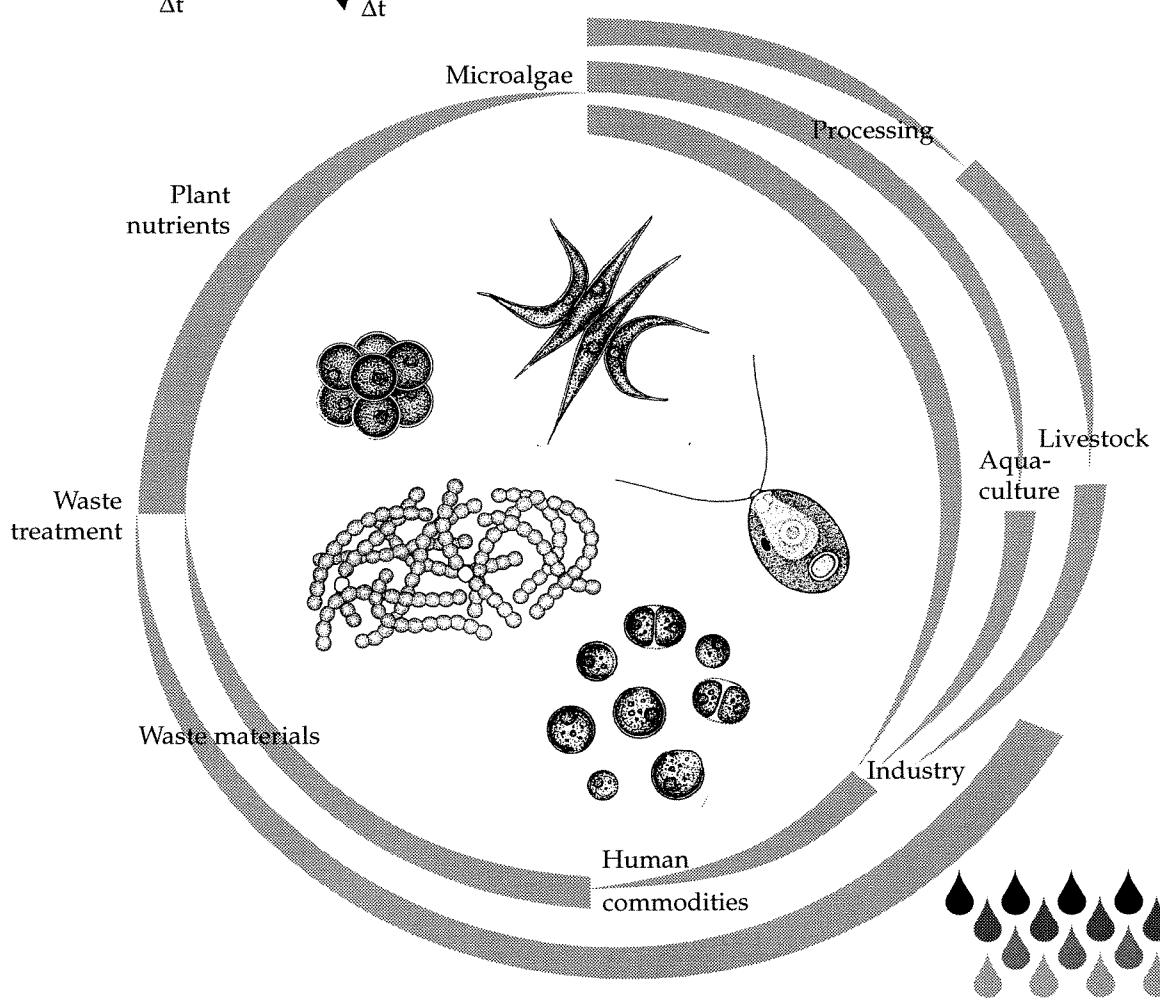
PLATE 6

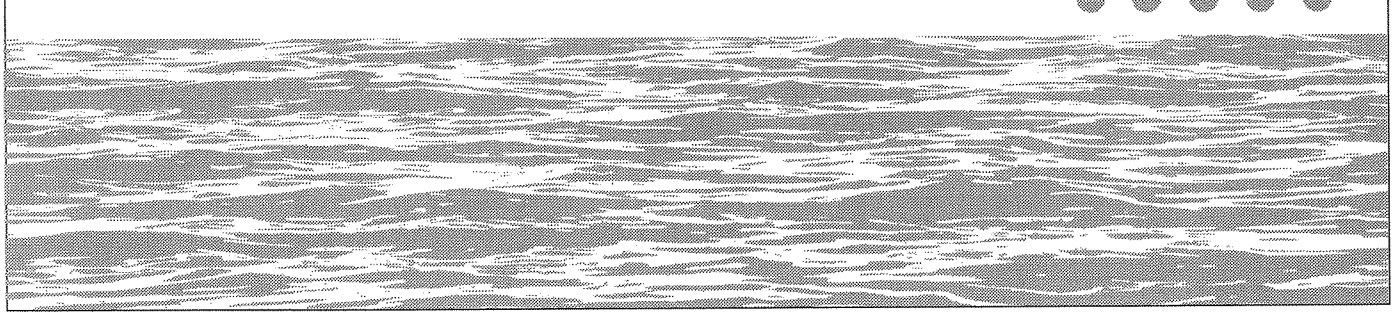
Industrial photobiological production program for a sustainable future of mankind.





$$2H_2$$
$$\uparrow$$
$$2H_2O = O_2 + (4H^+ + 4e) \qquad \Delta G = 470\ kJ/M$$
$$\downarrow + CO_2$$
$$(CH_2O) + H_2O$$





and serving as taxonomic reference centres. Most collections organized in regional or global federations (European Culture Collections' Organization, World Federation for Culture Collections), offer professional consultancy and services as primary sources of expertise in identification and preservation. They give specialized support in a growing number of areas such as isolation, screening and industrial testing. By providing an element of stability and continuity in scientific work and experimental research, the culture collections are a *sine qua non* for the progress of algal culture technology.

8. Multiple purpose utilization

Microalgal cultures in both laboratory and industry were for the most part of this century treated more as a botanical art and scientific tool than a commercial enterprise. As the above discussion indicates, there have recently been numerous achievements in the field of algal culture technology, and there exist many more potential opportunities. One critical factor, however, is the formulation of achieveable objectives to aid the rational improvement of microalgal production and the relevant processing technology, and to reach desired goals for product quality. What is necessary is a rational program for algal culture technology that has a structure based on identification of essential research needs. Utilization of microalgae will then in turn bring economic benefits at both macro- (national) and micro- (individual industries) levels.

Although exploring the optimization of the production of the several above mentioned useful products of microalgae is important, economically feasible industrial systems should be developed through the integration of different production procedures. A fruitful concept is for example the integration of system for biomass production of nitrogenfixing photosynthetic prokaryotes, the hydrogen production by these organisms (Skulberg 1995) and their use as food and feed producers. In this way a potentially beneficial and economic program of multiple utilization may be realized. Uniformity of reactor equipment and methods for culturing and harvesting can be adopted. Access to a wide spectrum of products of vital human and commercial interest offers economic advantages for the total industrial operation of the photobiological production system based on microalgae.

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"Knowledge is not enough,
you need also imagination."

Albert Einstein

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