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Biotechnology of algal biomass production: a review of systems for outdoor mass culture

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Abstract

Microalgae are very efficient solar energy converters and they can produce a great variety of metabolites. Man has always tried to take advantage of these proporties through algal mass culture. Despite the fact that many applications for microalgae have been described in the literature, these micro-organisms are still of minor economic importance. Industrial reactors for algal culture are at present, all designed as open race-ways (shallow open ponds where culture is circulated by a paddle-wheel). Technical and biological limitations of these open systems have given rise to the development of enclosed photoreactors (made of transparent tubes, sleeves or containers and where light source may be natural or artificial). The present review surveys advances in these two technologies for cultivation of microalgae. Starting from published results, the advantages and disadvantages of open systems and closed photobioreactors are discussed. A few open systems are presented for which particularly reliable results are available. Emphasis is then put on closed systems, which have been considered as capital intensive and are justified only when a fine chemical is to be produced.

Introduction

Photosynthesis is one of the basic biochemical processes of photosynthetic micro-organisms which convert solar energy into chemical energy. Man has used this natural process of harvesting the sun in the development of algal cultivation systems for secondary waste water treatment (Oswald, 1988a; De la Noüe *et al.*, 1992), for the production of human food (Becker, 1986), animal feeds (De Pauw & Persoone, 1988; Benemann, 1992), fertilizers (Metting, 1988), chemicals (Chapman & Gollenbeck, 1989; Calvin & Taylor, 1989) and secondary metabolites of pharmaceutical potential (Glombitza & Koch, 1989).

In 1952 the Carnegie Institution of Washington published 'Algal culture from laboratory to pilot plant' (Burlew, 1953), which summarized what had been done on large-scale algal culture before, during and shortly after World War II. In that document, many workers foresaw the great potential of algae as a product different from the fermentation industry and as a potential source

for agricultural and chemical commodities. The first concept adopted immediately after World War II envisioned algal biomass as the principal supplement or even replacement for animal proteins for direct consumption by humans. This work was continued by many research groups during the sixties and the seventies, most notably in the USA, Germany, Israel, Czechoslovakia, Japan, Thailand and France. With the onset of the energy crisis, microalgae were then suggested as a source of biomass for methane. It is only recently that significant attention has been paid to microalgae as a source of feedstock for the production of chemicals.

Several reviews list the main biological possibilities offered by microalgae including those by Cannell (1990), Richmond (1990) and Borowitzka (1992). The present review avoids methods and applications of cell immobilization techniques, which have been reviewed recently by Robinson *et al.* (1986) and Brouers *et al.* (1989). The design of culture vessels for laboratory applications is also not included (see Lee, 1986 and Edmund *et al.*, 1990).

Algal culture systems are generally classified according to their engineering (full or non-confining) and hydraulic characteristics in open systems (including ponds, deep channel, shallow circulating units etc.) and closed or fully hydraulic systems commonly called photobioreactors. Microalgal mass culture technology, and most particularly open systems, have been reviewed by Terry and Raymond (1985), Soeder (1986), Richmond and Becker (1986), Borowitzka and Borowitzka (1989) and Richmond (1990). Because of their technical complexity, photobioreactors have been considered for a long time as the antithesis of open ponds technology. It is only recently, because of the difficulties of overcoming the limitations of open ponds, that closed bioreactors have been considered as a complementary way of algal mass culture. The increasing interest in this technology is apparently leading algal culture to an exponential phase of technical evolution, and a large number of relevant patents continue to be granted (see Patents section in this journal).

The challenge of large-scale algal culture: conclusions of the 1990 Congress on Applied Algology in Tiberias, Israel

Richmond and Vonshak (1991) reported the main points of scientific interests in microalgal culture presented during this Congress:

1. The control of microalgae species is the major unsolved problem in large-scale open systems. Only a few strains are cultured at industrial scale. Much work continues to be done on *Spirulina* (Richmond, 1988), *Dunaliella salina* (Ben Amotz & Avron, 1989) and *Porphyridium cruentum* (Vonshak, 1988). In addition, special emphasis was given to *Haematococcus pluvialis* which is able to produce astaxanthin, a red carotenoid of a particular interest in aquaculture (Bubrick, 1991).
2. All industrial plants are based on open pond technology. These systems seem to have reached their technical limits. The gap between the theoretical biological potential of microalgae and their biomass productivities actually obtained could be narrowed by developing closed photobioreactors.

What culture technology to choose?

Productivity comparison between microalgae mass culture systems are difficult because of different geographic locations, culture strategies (batch or continuous culture), algae species etc. Furthermore, salt concentration in the culture medium are sometimes 10 times higher than dry biomass concentration: a correct estimation of biomass productivities requires reliable methodologies for representative measurements of cell and metabolite concentration (Gudin & Chaumont, 1991). Published data on algal productivity range from 10 to 50 $g\,m^{-2}\,d^{-1}$ (Weissman *et al.*, 1988) with an average rate around 20 $g\,m^{-2}\,d^{-1}$. The maximum practical photosynthetic efficiency is still a problem to be resolved (Pirt, 1983). However, a theoretical photosynthetic yield of 6 to 7% of total solar energy

seems possible (Hall, 1986): this represents, depending on geographic location, a biomass productivity of 35 to 70 g m^{-2} d^{-1}. A photosynthetic efficiency of 10% during a period of 122 days has been reported (Laws *et al.*, 1988). These experimental results are in agreement with Richmond's comments (1986).

Few reliable comparisions between closed and open systems are available in the literature. Torzillo *et al.* (1986) obtained from a closed tubular photobioreactor, an equivalent average output of *Spirulina* biomass of 33 t d. wt ha^{-1} y^{-1}. This value was nearly 90% higher than that obtained from an open pond system under identical culture conditions (location, culture period, algae species). Cohen and Arad (1989) compared growth and polysaccharide production of *Porphyridium cruentum* and *Porphyridium aerugineum* in open plastic containers with closed systems made of polyethylene sleeves sealed at the bottom: in both species biomass and metabolite production were higher in the sleeves than in the pond (approx. 60 to 300% higher biomass).

Several other advantages of closed photobioreactors have been suggested and much debated (e.g. Torzillo *et al.*, 1986; Richmond, 1987, 1990; Richmond *et al.*, 1993; Benemann, 1989; Benemann *et al.*, 1987; Weissman *et al.*, 1988; Kyle, 1989). For identical biomass productivities, the higher cell densities obtained with biophotoreactors result in reduced harvesting volumes and thus in reduced processing (partition solid/liquid) costs. Under continental climatic conditions, photobioreactors allow a considerable extension of the cultivation period. Photobioreactors could also allow the exclusion of atmospheric contaminants. Thus they would increase the acceptability of the algae products, particularly for the health food market, and offer greater flexibility in the choice of the algae species used. Furthermore, continuous culture techniques applied to such systems allow better control of culture parameters and of cell physiology and growth. Finally, photobioreactors prevent water loss through evaporation, which is of particular importance in arid zones.

Closed systems also have some disadvantages. Light penetration is reduced because the materials used are not completely transparent. This phenomenon may be enhanced by dust accumulation, water condensing or biofouling on the inner surface. Chaumont *et al.* (1991a) have patented a system using recycling balls for continuous cleaning of the inner surface of their tubular photobioreactor. The main problems encountered in the operation of photobioreactors are the control of oxygen concentration (Weissman *et al.*, 1988) and overheating in summer which may require special and costly precautions (Torzillo *et al.*, 1986). In order to combine the advantages of both systems, Richmond (1987) proposed a culture concept made of a tubular reactor, where the algae culture would heat quickly at the beginning and the end of the light phase, connected to an open-channel race-way where overheating would be controlled by circulating the culture. Today, photobioreactors are considered as capital intensive approaches: they are only justified when a fine chemical is to be produced. Such technology is not acceptable for a low value material or for very specific applications like waste water treatment. Until now, relatively few examples of economic analysis of microalgae production systems have been published (Benemann *et al.*, 1987; Tapie & Bernard, 1988; Borowitzka, 1992) and not a single one has compared open pond systems with closed photobioreactors as it has been done for biomass productivities.

Microalgae represent a large and unknown resource with the genetic potential to produce valuable compounds. There are probably well over 30000 species of microalgae, only a few hundred of which are cultured in laboratories, and very few of which have been characterized in detail and studied for their economic potential. The development of algal biotechnology depends in particular on the identification of more high value products in algae.

Open culture systems

The evolution of this technology is the reflection of the mixing systems developed in particular to

prevent sedimentation and to enhance light utilization efficiency. In open ponds, mixing the culture is of great significance in terms of costs and particularly of productivity. Biomass productivity is not only dependant on the total amount of solar energy impinging on the culture surface, but also on the quantity of energy available at the cell level: these are the concepts of 'light regime' and 'light per cell' expressed by Richmond (1987). In the early 1960s, circular ponds with large mixing arms were designed but were difficults to scale up over 1000 m^2. Balloni *et al.* (1983) and Cardenas and Markovits (1985) proposed a concept of a board which closes the pond cross section except for a slit of a few centimeters above the bottom and which is 'dragged' through the culture pond. The culture is forced to run through the slit, creating a turbulent flow. Laws *et al.* (1986) described a mixing system consisting of a continuous flume containing arrays of foils similar in design to segments of airplane wings. They achieved average daily biomass production rates over 40 g d wt m^{-2} over periods as long as one month. Many other devices have been proposed to enhance mixing such as air-lift (Persoone *et al.*, 1980), injectors, propellers, pump and gravity flow (Setlik *et al.*, 1970), devices using natural energy sources such as wind, sun and even animals or humans (Becker & Venkataraman, 1982). Today, most of the open growing units are based on the race-way pond design proposed first by Oswald (1969), which consists of long channels arranged in a single or in multiple loops and currently, the most commonly used stirring devices are paddlewheels.

If open ponds are the oldest systems studied, they are until today, the only ones used at the industrial level by Companies such as Sosa Texcoco, Cyanotech, Earthrise Farms, Japan Spirulina, Far East Microalgae, Taiwan Chlorella, Microbio Resources and of course Betatene and Western Biotechnology. But few species of algae are cultured at this level: *Chlorella*, *Scenedesmus*, *Spirulina* for single cell protein health food and dyes and *Dunaliella salina* for β-carotene. If we exclude the exploitation of natural 'blooms' by local populations as in Tchad (for example, the Goranne tribu of Issa Ousmane produce about 80 t yr^{-1} on 4 ha of natural lake; G. Bonnin, pers. comm.), the most extensive types of open cultivation system are used by Betatene Ltd and Western Biotechnology Ltd in Australia for β-carotene production by *Dunaliella salina*. Betatene's facility at Whyalla in South Australia is believed to be the largest area algal cultivation in the world: 300 ha of partly structured evaporation lakes which were designed for solar salt production. The harvesting plant is designed to handle up to 10^6 l h^{-1} of brine through four modular sections (Schlipalius, 1991). The production facility of Western Biotechnology Ltd at Hutt Lagoon, in Western Australia covers more than 50 ha of unlined ponds with earth walls (Borowitzka, 1991). In both cases, the low surface/volume ratio of ponds reduces the salinity dilution effect of rain water run-off but leads to low cell concentrations; mixing and aeration are done by conventional wind movement and wave action. On the other hand, high rate ponds are generally used for the health food product applications (i.e. for *Spirulina* and *Chlorella*). Bonnin (1992) published a technical guide describing step by step engineering of such open pond factories. Elements for design and construction of such ponds have been reviewed by Dodd (1986) and Oswald (1988b). Bottom lining is usually required, made of non-membrane (granular material, asphalt, conventional concrete) or membrane lining (sheet plastic, rubber, spray applied materials). Initially, high rate ponds have been constructed from holes in the ground with a complete plastic lining of the bottom and of the earth banks. The wall design has since been revised and vertical walls are now generally constructed with asbestos-cement roofing panels, concrete blocks or slipformed concrete.

As stated earlier, the control of contaminants in open pond systems is the most important problem raised by this technology. The first attempts to overcome this difficulty was by the use of simple plastic covers or green houses over the open ponds. This allows for an extension of the growing period, facilitates the provision of carbon dioxide and the maintenance of high temperature at night. Covering of open ponds results in an

improvement of the biomass productivities (Richmond *et al.*, 1993) and, as earlier mentioned by Tamiya *et al.* (1953), there is as expected no improvement in contamination prevention. Many other problems (like capital costs, maintenance, overheating etc.) made such covered systems not particularly applicable to large size units.

Four basic approaches are now proposed to maintain monoalgal culture in open ponds (Benemann *et al.*, 1987; Richmond, 1987):

1. Use of highly selective conditions which favour the dominance of the algae strain to be cultured. *Dunaliella* and *Spirulina* are good examples of this approach. In the future, genetic recombination may lead to an increased tolerance of algae to extreme conditions. It is also likely that genetic manipulation will be applied to algae in order to increase production of valuable primary or secondary metabolites but, until now, research has been mainly restricted to cyanobacteria, genetic cloning (Craig *et al.*, 1988; Ciferri *et al.*, 1989) and protoplast production (Roth-Benjerano *et al.*, 1991) or fusion (Canivez, 1988).
2. Culture of algae species which dominate in the open ponds (but which generally do not contain a high value metabolite).
3. Maintenance of sterile or clean conditions of culture as long as possible during the scale up in size, management of the culture in order to prevent contaminant development and for the worst situations, treatment of contaminants through the use of chemicals to destroy fungi, zooplancton etc.
4. Development of the closed photobioreactor technology (Richmond, 1992).

The closed culture systems (photobioreactors) (Fig. 1)

Because they have been less studied, information concerning large-scale closed photobioreactors is scant and several concepts have been proposed. Vertical tubular reactors were among the first real closed systems described in the literature (Cook, 1950). This design has since been developed by several authors. James and Al-Khars (1987) studied the growth and the productivity of *Chlorella* and *Nannochloropsis* in a translucent vertical air-lift photobioreactor and obtained maximum productivities of 20 to 26 g m^{-2} d^{-1} on a dry weight basis. Miyamoto *et al.* (1988) used vertical glass tube (5 cm diameter, 2.3 m high) and obtained, with *Monoraphidium*, productivities of 23 g m^{-2} d^{-1}. This concept of 'bubble column reactor' has as its main advantage good light penetration, although its scale up potential seems difficult. Polyethylene bags (transparent polyethylene sleeves sealed at the bottom in a cone shape to prevent cell settling) are also widely used, mainly in aquaculture operations. Using 50 litres polyethylene bag cultures operated as turbidostats, Trotta (1981) obtained yields of 20 to 30 g m^{-2} d^{-1} for *Tetraselmis*.

During the last ten years, great attention has focussed on tubular closed bioreactors. Several tubular photobioreactors have been studied and developed since the pioneering work of Tamiya *et al.* (1953). These reactors are generally a serpentine in form, made of glass or plastic as the solar receptor, a gas exchange vessel where air, CO_2 and nutrients are added and O_2 removed, and a recirculation of the culture between these two parts by the use of a pump (Gudin & Chaumont, 1983) or an air-lift (Pirt *et al.*, 1983; Chaumont *et al.*, 1991b; Richmond *et al.*, 1993). The most sophisticated is certainly the culture system developed at Batelle (Anderson & Eakin, 1985) for the production of polysaccharides by *Porphyridium cruentum*. The system was a modular design, resembling a solar collector with a photodetector for angular adjustment of the glass surface. The polysaccharide productivities published by the authors ranged from 20 to 25 g m^{-2} d^{-1}.

Chaumont *et al.* (1988) experimented from 1986 to 1989 with a 100 m^2 culture unit (7 m^3 culture) made of a double layer of flexible polyethylene tubes (6 cm diameter) the upper containing the culture (circulating through a pump), the lower containing a variable volume of air. Each section of the culture serpentine incoporated a flexible tube for oxygen degassing. Temperature

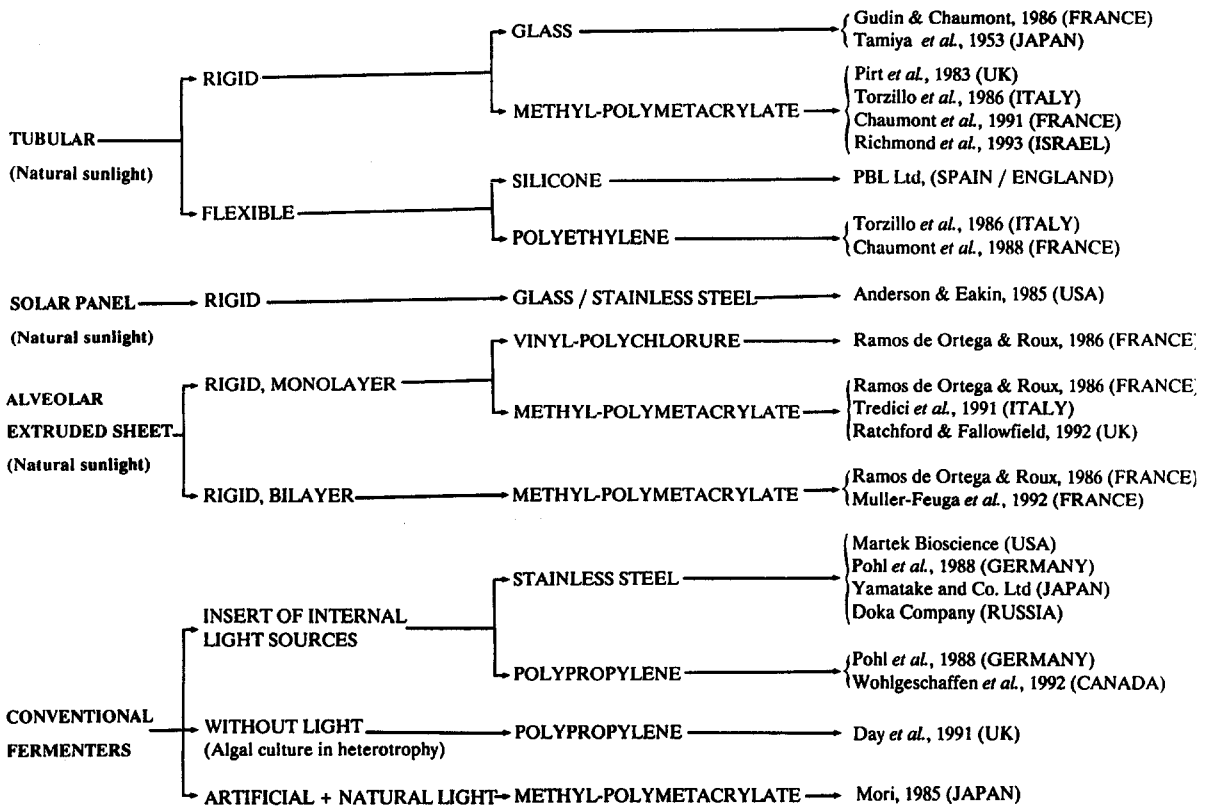

Fig. 1. Main designs of closed photobioreactors for mass culture of microalgae.

control was achieved by floating or submerging the tubular reactor containing the culture on or in a pool of water; the advantage of this technology was to respond not only to daily but also to seasonal air temperature variations. With *Porphyridium cruentum*, these authors achieved steady state continuous culture for about 2 months and obtained productivities of 20 to 25 g m^{-2} d^{-1}. Gudin and Chaumont (1991) have pointed out the drastic influence of pumping on microalgae physiology: biomass productivity may increase up to 75% when the pump is replaced by air-lift for culture circulation. They then set up a new rigid tubular prototype made of methyl-polymetacrylate tubes and using air-lift technology (Chaumont *et al.*, 1991b). A new company named Heliosynthese SA has been recently established to develop this tubular technology in the South of France. A similar culture equipment is under study in Israel (Richmond *et al.*, 1993). The tubes of the solar captor are made either of polyvinylchloride (50 mm in diameter) or of polycarbonate (32 mm in diameter). The rate of flow in the tubes ranges between 30 to 50 cm s^{-1}. These authors conclude that, when the tube diameter is reduced from 50 mm to 32 mm, the optimum population density of *Spirulina platensis* and *Anabaena siamensis* increases, resulting in a higher productivity per culture volume.

Torzillo *et al.* (1986) also studied a comparable closed serpentine bioreactor (100 m^2, 10 m^3 of culture) initially made of flexible polyethylene tubes (14 cm in diameter) and later of methyl-polymetacrylate tubes (13 cm in diameter). Culture pumping was continuous, but the culture circulation was intermittent in order to maintain an adequate culture flow rate inside the tubes: the culture fell continuously into a receiving tank and then was raised to a feeding tank by a siphon that allowed an intermittent discharge into the bioreactor. They obtained maximum productivities of 25 g m^{-2} d^{-1} with *Spirulina*.

Photobioreactor Ltd (PBL), in collaboration with Reading University, set up a vertical tubular serpentine (4 m^3 of culture) made of flexible 12 mm diameter polyethylene tubes in Murcia (Spain). The circulation is by an air-lift and the temperature is maintained at the optimal level by water spray. This company cultured *Dunaliella* but no results are available in the literature. PBL was in operation until the beginning of 1992.

A pilot plant called 'Biocoil' has been set up in the UK and in Australia (Robinson, 1987). The solar collector is arranged as a coil of polyethylene tubes (30 mm diameter) arranged around an open circular framework with a high surface/volume ratio (100 m^2 for 1.2 m^3 of culture). A heat exchanger between the solar collector and the pump allows control of the culture temperature. A large pilot unit operated in Australia for several years (*Spirulina* and *Tetraselmis* cultures). However, the Australian company operating this unfortunately went bankrupt, but operations are continuing in the U.K. by Biotechna-Graesser A.P. Ltd. Small tubular photobioreactors of the Biocoil design (40–100 l) are also used experimentally at Murdoch University (Australia) to study lipid formation and carotenoid production (M.A. Borowitzka, pers. comm.). Biotechna-Graesser A.P. Ltd proposes several biocoil plant sizes (from bench up to 10000 litres), with internal light for *Chlorella* culture. Their biocoil systems are claimed to be used in conjunction with bacterial or algal growth to break down toxic wastes, to extract metals from liquid streams or to remove nitrates from potable water.

Triple wall panels in translucent PVC have been studied by Ramos de Ortega and Roux (1986) to culture *Chlorella*. This double layer panel (8 m^2) is laid on the ground, one of the layers is used for the circulation of the culture, the other for circulation of the temperature-controlled water. Tredici *et al.* (1991) and Tredici and Materassi (1992) have recently developed a vertical alveolar panel (2.2 m^2) based on the same type of material. This panel has a variable orientation with respect to the sun's ray and mixing and deoxygenation of the culture suspension are effected by continuously bubbling air at the bottom of the reactor. Comparable designs but with pump circulation (Muller-Feuga *et al.*, 1992) or with air-lift circulation (Ratchford & Fallowfield, 1992) are also under development. In all cases, high productivities were obtained because of a high surface/volume ratio but biomass output could be limited by photoinhibition and problems with temperature control.

Several authors have suggested production of microalgae autotrophically in conventional type aerobic fermenters modified by the insertion of internal light sources such as fibre optics. Such systems could have controllable illumination for up to 24 hours a day rather than variable duration sunlight exposure. They can utilize artificial light only (Pohl *et al.*, 1988; Javanmardian & palsson, 1991; Wohlgeschaffen *et al.*, 1992), or combined sunlight and artificial light (Mori, 1985), or light source enriched in specific wavelengths (Junter *et al.*, 1990). Several companies are now selling such equipment: e.g. Doka Company (Russia), Yamatake and Co., Ltd (Japan), Apparate und Behähtertechnik Harrislee (Germany). Fully instrumented, the culture could be computer driven. An increased duration of illumination and a much tighter control of growth conditions results in much faster growth rates. About ten patents have been applied for during the last two years on the possibilities of introducing light inside culture systems (Hoeksema, 1991; Fallowfield & Martin, 1991; Meyer *et al.*, 1990). Nevertheless, this culture technology is still at the laboratory scale: the use of artificial light is very expensive and for capacities over 250 l, the control of culture temperature becomes a technical problem. Such a technology is suitable for productions of labelled compounds.

Dialysis culture systems have been proposed in particular for the growth of fragile algae such as Dinoflagelates. In these systems, the algal culture is confined inside a dialysis tube which is maintained in the culture medium. Nutrients and extracellular metabolites can freely diffuse through the dialysis membrane, whereas the algae cells remain inside the tube (Marsot *et al.*, 1981). This could be an attractive system to culture algae

when the metabolite produced could be growth limiting.

When high value products are to be produced, a heterotrophic or mixotrophic growth mode in enclosed bioreactors could be an interesting process (Lee, 1986). Algal strains able to sustain a heterotrophic growth have been listed by Droop (1974). The first commercial venture using heterotrophic cultures to be reported was by the Grain Processing Corp. (Hanson, 1967) who used the green alga *Spongiococcum* as a poultry feed. Heterotrophic production of *Tetraselmis suecica* has been developed more recently to produce algae for the early developmental stages of molluscs and crustaceans, using a prototype production system scaled up from 5 to 50000 l via a number of intermediate-sized vessels (Day *et al.*, 1991).

Another application of algal culture is its use to sustain human life in long period space flight. this has been investigated particularly in the USA, USSR and recently in Japan (Sogokenkyusho, 1991; Fujita Kogyo, 1991). In such a closed environment, the problem to solve is the production of oxygen using CO_2 and human wastes (Fig. 2). The most convenient system to feed CO_2 to algae in weightless conditions seems to be by diffusion through gas-permeable membranes (Lee & Hing, 1989).

Little information is available on the research conducted on algae mass culture in eastern European Countries. The best known are those of the Rupite team (Bulgaria) and of the researchers in the Czechoslovak Academy of Sciences (Dilov *et al.*, 1987). Setlik *et al.* (1970) developed a shallow turbulent and sloped cultivation unit including a series of parallel troughs. After cascading on this step like arrangement, the culture was recycled to the top of the cascade by pumping. Systems of this design were constructed in Trebon with surfaces up to 900 m^2. Based on this design, Vendlova (1969) experimented in Rupite with a culture system where baffles were suspended in the culture in such way as to create a rapid mixing of the culture. The Peruvian-German algal project at Sausal in Peru was a modified version of this principle of sloped algal ponds (Heussler *et al.*, 1978). Large units made of glass tubes are used in the Casastan region to produce algal suspensions for animal husbandry (Dilov *et al.*, 1987). It

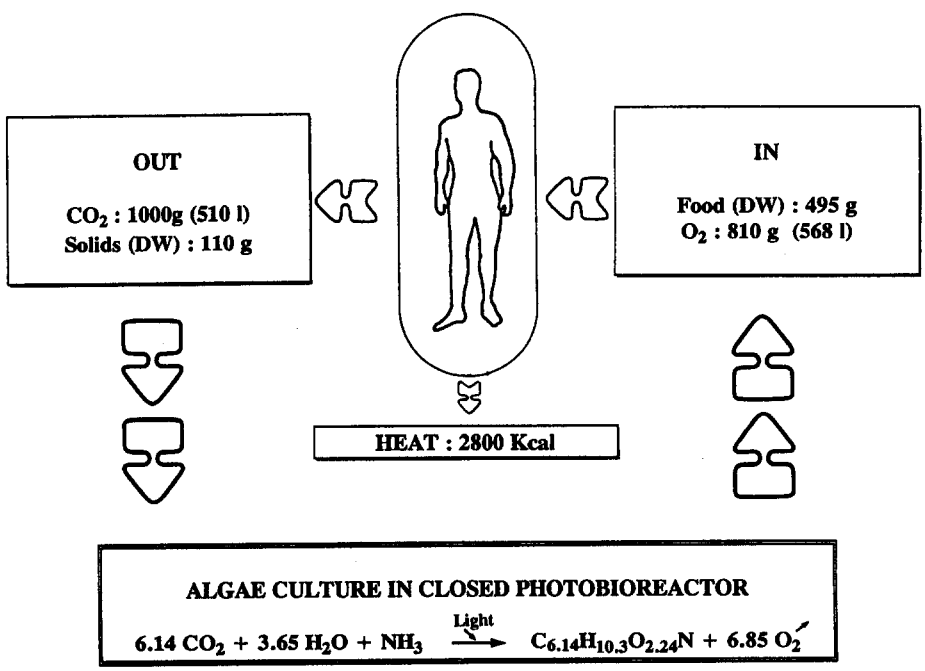

Fig. 2. Diagram of CO_2-recycling through algal culture to sustain life in space flight.

is likely that much more will be known on the subject after the 6th International Conference on Applied Algology in the Czech Republic (6–11 September 1993).

Conclusions

Although algae mass culture is commercially attractive because of the great biological potential of these photosynthetic microorganisms, little industrial and economic succes exists. Microalgae cultivation systems in open ponds are the oldest experimental systems. At the present time, they are used for cultivation of few species of algae: *Chlorella*, *Scenedesmus*, *Spirulina* for single cell protein and health food and *Dunalella* for β-carotene. The two principal advantages of open culture systems are a small capital investment for production of the biomass and the use of a free source of energy. They are the simplest method of algae cultivation and are only intended for algae species growing in selective environments. The productivities obtained are far from the theoretical maximum.

Several authors have concluded that the open race-way systems which are actually used in most commercial plants are not suitable for obtaining high photosynthetic efficiencies. Closed photobioreactors which allow better control of growth parameters may be more suited to reach this biological goal. Photobioreactors have also an essential role to play in increasing the diversity of algae species for culture and the quality of biomass harvested. This technology is a more capital intensive one, but depending on the commercial target, this additional capital cost can be justified. At the present time, closed photobioreactors are limited to high value products such as phycobiliproteins, pharmaceutical or cosmetic products. It is not possible to say that one culture system is better than another: the commercial target, geographic location and metabolite to be produced will determine the choice: axenic or non-axenic, continuous or batch culture, intensive or extensive culture, open ponds or closed photobioreactors.

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