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MINI-REVIEW

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Photobioreactors: production systems for phototrophic microorganisms

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Abstract Microalgae have a large biotechnological potential for producing valuable substances for the feed, food, cosmetics and pharmacy industries as well as for biotechnological processes. The design of the technical and technological basis for photobioreactors is the most important issue for economic success in the field of phototrophic biotechnology. For future applications, open pond systems for large-scale production seem to have a lower innovative potential than closed systems. For high-value products in particular, closed systems of photobioreactors seem to be the more promising field for technical developments despite very different approaches in design.

Introduction

Both macro- and microalgae have an important role to play in the current world economy with an approximate turnover of US$ 5 billion per year. Microalgae are unique and valuable microorganisms in various respects because they are the initial biological CO_2/O_2 exchangers on this planet, the most important primary producer of biomass, and one of the most variable ecological groups of organisms. For microalgae in particular, recent biotechnological and technical advances call for a reappraisal of their possible future contribution to developments in such areas as food, cosmetics, pharmaceuticals, feed, agriculture, aquaculture and the environment (Soeder 1986; Borowitzka and Borowitzka 1988; Borowitzka 1992; Sirenko and Pulz 2000; Tsoglin and Gabel 2000). Therefore, the biotechnological basis for the most efficient production of microalgal biomass is a key issue for the future impact of these organisms.

Technical systems for the production of phototrophic microorganisms are termed photobioreactors (PBR). These systems have to be evaluated in their various configuration concepts regarding their potential productivity and economic feasibility. The most important and most obvious differences in microalgal production systems are the exposure of the microalgal culture to the environment.

Open systems can be divided into natural waters (lakes, lagoons, ponds) and artificial ponds or containers, erected in very different ways. Regarding the technical complexity, open systems such as the widespread raceway ponds may vary considerably, but they are still much simpler than more recent closed systems for the cultivation of microalgae. Most of these closed systems consist of tubular photobioreactors with tubes of various shapes, sizes, and length as well as the transparent materials used. In all cases the biotechnological solutions for optimum growth, with the main factors being light and turbulence, are key issues for success (Tredici 1999).

Photobioreactor design

Microalgae are found growing within nearly every biotope because of their ecological diversity and their physiological adaptability. This diversity explains why almost everybody who is active in microalgal cultivation has their own technical solutions in mind for mass cultivation. Within this multitude of technical solutions one can basically distinguish between open ponds or reactors, which are open to air, and closed systems (Table 1). In all cases the main efforts have to be made to introduce light energy into the dispersion of microalgae (Ogbonna and Tanaka 2000; Pulz and Scheibenbogen 1998).

Open systems

Open ponds resemble most closely the natural milieu of microalgae. Despite a certain variability in shape, the most common technical design for open pond systems are raceway cultivators driven by paddle wheels and

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Fig. 1 Typical open pond production site using a raceway arrangement

Table 1 Systematics of cultivation equipment

Basic type	Technical variables
1. Open system	
Cuvette, container, stirred vessel	Material (glass, plastics)
Natural water	Turbulence development (pumping, stirring)
Raceway pond	Flow direction (horizontal/vertical)
Inclined surface device	Surface to volume-ratio
2. Closed system	
Plastic sleeves	O_2 removal > CO_2 input
Fermenter-like tank	Type and duration of illumination
Tubular PBR	Temperature control
Laminar PBR	Sterilization

usually operating at water depths of 15–20 cm (Fig. 1). At these water depths, biomass concentrations of up to 1,000 mg/l and productivities of 60–100 mg/(l day^{-1}), i.e., 10–25 g/(m^2 day^{-1}), is possible. Similar in design are the circular ponds which are common in Asia and the Ukraine (Becker 1994).

Significant evaporative losses, the diffusion of CO_2 to the atmosphere. as well as the permanent threat of contamination and pollution, are the major drawbacks of open pond systems. Also, the large area required must not be underestimated. The main disadvantage of this principle in terms of productivity seems to be the light limitation in the high layer thickness. Technically it is possible to enhance light supply by reducing the layer thickness to a few centimeters or even millimeters, using thin layer inclined types of culture systems. Another problem of open systems is the maintenance of the desired microalgal population, which is possible only for extremophilic species and even there some contamination risks remain.

Until recently, open systems were the most important design principle for microalgal production (Richmond 1990). However, the preparation of high-value products from microalgae for applications in pharmacy and cosmetics appears to be feasible only on the basis of closed photobioreactors with the ability to reproduce production conditions and to be GMP-relevant (GMP: good manufacturing practice following ISO and EC guidelines).

Closed systems

Closed PBRs are characterized by the regulation and control of nearly all the biotechnologically important parameters as well as by the following fundamental benefits (Pulz 1992): a reduced contamination risk, no CO_2 losses, reproducible cultivation conditions, controllable hydrodynamics and temperature, and flexible technical design.

The scale-up of simple closed container-based systems (tanks, hanging plastic bags) as a first generation of closed PBRs was soon faced with serious limitations because at a volume of 50–100 l it is no longer possible to effectively introduce the light energy required for successful biomass development. Several technical approaches to underwater lighting, for instance with submersed lamps or light diffusing optical fibers on the one hand, or with pillar-shaped photobioreactors on the other hand, have been tried, but have not been successful in application (Gerbsch et al. 2000; Semenenko et al. 1992). However, this principle seems to be of future relevance only for the aquaculture of certain selected species.

Closed photobioreactors (Fig. 2) are currently tested for microalgal mass cultures in the following configurations: (1) tubular systems (glass, plastic, bags), (2) flattened, plate-type systems, and (3) ultrathin immobilized configurations. Vertical arrangements of horizontal running

Fig. 2 Closed plate type photobioreactor fed with high CO_2 levels from a lime production plant in central Germany

tubes or plates seem to be preferred for reasons of light distribution and appropriate flow.

Since about the 1990s, parameters such as species-efficient light incidence into the photobioreactor lumen, light path, layer thickness, turbulence and O_2 release from the total system volume have gained in importance (Table 2). Closed or almost closed systems based on very different design concepts have already been implemented and tested up to pilot scale. The latest developments seem to be directed toward photobioreactors of a tubular configuration or of the compact-plate type as well as combinations of these main design principles in order to distribute the light over an enlarged surface area (Tredici and Materassi 1992; Gabel and Tsoglin 2000).

Assuming that light for photosynthesis should be continuously available to the receptor in the microalgal cell, a lamination or other enlargement of the reactor surface directed toward the light source seems to be the prime aim. For microalgae this idea may include an appropriate lowering of net light energy supply for the suspension because of the significantly lower level of light saturation needed for these organisms. The basic principle of the laminar concept for thin layer plate or thin diameter tube photobioreactors mimics the leaves of higher plants. For instance a 100-year-old,10 m high lime tree shading an area of 100 m^2 has a leaf surface area of more than 2,500 m^2. Expressed as a surface: volume ratio this amounts to a value of 2.5 m^2/m^3.

On the basis of these considerations, the trend toward developing closed photobioreactors as already described is paralleled by conceptions of the use of relatively thin light-exposed reactor lumina. The tube diameter in tubular photobioreactors is reduced significantly and laminar,

Table 2 Advantages and disadvantages of open and closed algal cultivation plants

Parameter	Open ponds (raceway ponds)	Closed systems (PBR systems)
Contamination risk	Extremely high	Low
Space required	High	Low
Water losses	Extremely high	Almost none
CO_2-losses	High	Almost none
Biomass quality	Not susceptible	Susceptible
Variability as to cultivatable species	Not given, cultivation possibilities are restricted to a few algal varieties	High, nearly all microalgal varieties may be cultivated
Flexibility of production	Change of production between the possible varieties nearly impossible	Change of production without any problems
Reproducibility of production parameters	Not given, dependent on exterior conditions	Possible within certain tolerances
Process control	Not given	Given
Standardization	Not possible	Possible
Weather dependence	Absolute, production impossible during rain	Insignificant, because closed configurations allow production also during bad weather
Period until net production is reached after start or interruptions	Long, approx. 6–8 weeks	Relatively short, approx. 2–4 weeks
Biomass concentration during production	Low, approx. 0.1–0.2 g/l	High, approx. 2–8 g/l
Efficiency of treatment processes	Low, time-consuming, large volume flows due to low concentrations	High, short-time, relatively small volume flows

Table 3 Basic values of various cultivation plants (data obtained from IGV: German cultivation sites at natural illumination)

	Unit	Raceway	Incl. Surface type Open pond, high layer thickness	Tubular Open pond, low layer thickness	Plate	
					Semi-closed tubular system	Semi-closed plate system
Illuminated surface	m^2	500	200	600	500	
Total volume	m^3	75	5	7	6	
Space required	m^2	550	250	110	100	
Layer thickness	cm	10–30	0.5–1	4	3	
Flow rate	cm s^{-1}	30–55	30–45	50–60	120	
Biomass conc.(DW)	mg l^{-1}	300–500	3,000–6,500	5,000–8,000	5,000–8,000	
Productivity (DW)	g l^{-1} d^{-1}	0.05–0.1	0.8–1.0	0.8–1.2	0.8–1.3	

plate-type configurations are strongly favored. The tubular or pipe design principle is the most important basis of completely or partially closed cultivators in plastic ducts and especially in glass or plastic tubes. The development of closed photobioreactors, which had intensified by the end of the 1980s, seems to be of significant future importance. Compared with laminar, plate-type systems, the tubular systems seem to have identical configuration potentials, especially in cases of vertical packing of horizontally oriented tubes (Broneske et al. 2000; Molina Grima et al. 2000; Table 3).

Biotechnological problems and preconditions for PBR design

The normal, i.e., natural life conditions of microalgae which are subject to biotechnological research, are as follows: maximum cell densities of 10^3 cells/ml, average distance between cells of 1,350 µm or 250 times the cell diameter, vertical or horizontal displacements of 5×10^{-3} to 3×10^{-5} m/s, photon flux density (PFD) usually well within the light limited area, light supply subject to daytime rhythm, CO_2 and nutrient conditions, generally far from optimal, and prolonged stability of pH value, ion concentrations and temperature.

In contrast, for cultivation systems, we have to apply very different conditions, namely: cell densities up to 10^8 cells/ml, average distance between cells reduced to 60 µm or 10 times the cell diameter, spatial displacements ranging from 0.3 to 1.2 m/s, turbulence-conditioned PFD variations with frequencies of 0.1–1,000 s superseding the daytime rhythm, generally optimum or surplus nutrient and CO_2 supply, pH values and temperatures undergoing completely nonphysiological variations, and nearly continuous mechanical stress on the cell walls and the cells themselves (Tredici 1999).

Light energy

Light as the energy source for photoautotrophic life is the principal limiting factor in photobiotechnology (Kirk 1994). At illumination intensities above the light compensation point the rate of photosynthesis is directly proportional to light intensity, until at high illumination intensities damage to the photosynthetic receptor system occurs within a few minutes (photoinhibition). In most microalgae, photosynthesis is saturated at about 30% of the total terrestrial solar radiation, i.e. 1,700–2,000 µE/(m^2 s). Some picoplankton species grow with optimal rates at 50 µE/(m^2 s) and are photoinhibited at 130 µE/(m^2 s). Phanerophytes, like most agricultural crops with light limitation values of 900 µE/(m^2 s), are clearly adapted to higher PFD than microalgae are.

Stirred fermenters illuminated with various submersed luminous elements or light pipes facilitate an average productivity in the range of 100–1,000 mg DW/(1 day). This appears to be the upper limit at a surface :volume ratio of 2–8 m^2/m^3 typical for this illumination design. Laboratory bioreactors based on this principle are very well suited to physiological and autecological studies as well as for the establishment and testing of miniature ecosystems, but they cannot be used for scaling up. In tubular or plate-type photobioreactors, surface:volume ratios of 20–80 m^2/m^3 and light incidence values (PAR) up to 1,150 µE/(m^2 s) are achieved. At a layer thickness of up to 5 mm, a productivity of 2–5 g DW/(l day) can be achieved (Chini Zitelli et al. 2000).

Despite growing interest in recent years, there are only a few references in the literature regarding the short-term processes of photoadaptation, on light inhibition or saturation effects in closed photobioreactors. Photoadaptation requires at least 10–40 min, which can explain the discrepancy between the productivity of open-air algal cultures and their light optimum. Photoinhibitory processes, too, are time-dependent; however, in this case irreversible destruction is supposed to occur even after only a few minutes of light stress, exceeding 50% damage after 10–20 min.

CO_2/O_2 balance

For a high photosynthesis rate, the CO_2/O_2-balance has to be adjusted in a way that the prime carboxylating enzyme, Rubisco, furnishes CO_2 for the Calvin cycle but does not use O_2 for photorespiration. Hence, in algal cultures of high cell densities, sufficient CO_2 must be available, while evolved O_2 has to be removed before

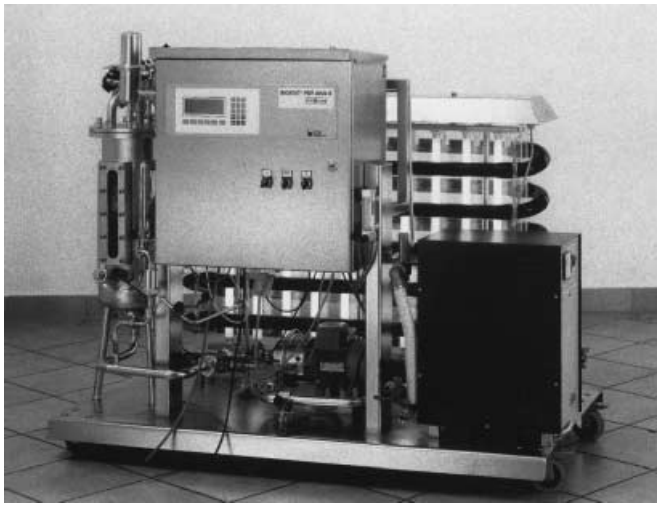

Fig. 3 Small size commercial photobioreactor of 25 l with tubular, sterilizable design

reaching inhibitory concentrations. The complete avoidance of photorespiration, however, remains unsolved.

Oxygen may become a problem in algal cultures of high cell densities not only because of the limitation of the rate of photosynthesis. In cultures of microalgae optimally supplied with CO_2 the O_2 production can easily reach concentrations of up to 40 mg/l. Upon radiation with appropriate energy, oxygen radicals may develop during the respiratory gas exchange and cause toxic effects on cells due to membrane damage. Superoxide dismutases and other O_2 radical neutralizing systems may have a protective effect. In high-cell-density microalgal cultures with optimum growth, species-specific O_2 evolution rates between 28 and 120 mg O_2/(g DW h^{-1}) were recorded. Many algal strains cannot survive in significantly O_2-oversaturated milieu longer than 2–3 h. High temperatures and PFDs, combined with CO_2 limitation, will intensify the physiological inhibitory processes (Fig. 3).

CO_2-concentrations usually have to be kept within narrow margins. While a 0.03% CO_2 content in air is suboptimal for plant growth, most plants will tolerate CO_2 concentrations only up to 0.1%. However, for many strains of microalgae it was observed that they tolerate up to 12% CO_2 at a temperature of 35°C. At present, partial pressure of O_2 (pO_2) in microalgal suspensions inside both open and closed photobioreactors may be reduced only by (1) increasing turbulence, and (2) O_2 stripping with air. Both approaches imply an "unsolved dilemma" in the reactor system: Although an intensive search for membranes suitable for gas exchange is underway, no breakthrough has yet been reported. At present, sufficient CO_2 transitions on membranes can be provided only under pressure, otherwise the transition proceeds slowly along the gradient.

An alternative may be the relatively simple injection of small amounts of pure CO_2 into high-cell-density microalgal cultures with biomass concentrations of 8–10 g dry weight (DW)/l (Straka et al. 2000).

Temperature

Temperature influences respiration and photorespiration more strongly than photosynthesis. When CO_2 or light is limiting for photosynthesis, the influence of temperature is insignificant. With an increase in temperature, respiration will rise significantly, but the flux through the Calvin cycle increases only marginally. Thus, the net efficiency of photosynthesis declines at high temperatures. This effect can worsen in suspension cultures by the difference in decrease of CO_2 and O_2 solubility at elevated temperatures.

Salinity, nutrients, and pH-value

A sufficient nutrient supply for microalgae is a precondition for optimal photosynthesis. Deficiencies will cause disturbances in metabolism and disproportionate production of intermediates of photosynthesis. Deviations from the optimum pH, osmotical conditions and salinity will cause physiological reactions and productivity problems.

Therefore, these easily controllable conditions should be maintained in optimum ratios in photobioreactors. Organic carbon sources seem to be important for some mixotrophic or even pure heterotrophic microalgal biomass production systems. Therefore, organic wastes as well as pure simple organic substances like acetic acid or various sugars have been investigated for their possible use in microalgal production.

Turbulence

Photoautotrophic nano- and microplankters live in their natural environment at a density of 10^3 cells/ml and at distances of more than 1,000 µm between cells. Thus, in high-cell-density microalgal cultures of up to 10^9 cells/ml, the natural conditions are not suitable for high productivity (Borowitzka and Borowitzka 1988; Grobbelaar 2000).

Photobioreactors of pilot to industrial scale

For aquaculture applications, many taxonomically very different species of microalgae are produced, mostly locally. Both advanced open systems in the form of plastic sleeves, plastic pillars and containers are used, in many cases as one-way systems. But because of the high costs of these systems combined with poor productivity, closed systems like tubular PBR (Biocoil, Biofence, ultrathin sheets) are gaining economic importance (Richmond 2000).

Production using conventional fermenters with microalgae-related organisms like *Crypthecodinium* under heterotrophic conditions appears to be of economic interest for the production of polyunsaturated fatty acids. However, this method involves routine techniques of

Fig. 4 Natural lake in Myanmar producing *Spirulina*-biomass

Fig. 5 700 m^3 glass tube photobioreactor producing *Chlorella* biomass

heterotrophic biotechnology and not photobioreactors. Even today the approximately 5,000–6,000 t/a dry biomass of microalgae produced worldwide originates mainly from open systems. These open systems (Fig. 4) include natural waters as well as unstirred but constructed ponds and circular or raceway ponds.

Aphanizomenon and *Nostoc*, two rather uncommon genera, are exclusively harvested from natural waters; *Aphanizomenon* only in the US for health food, *Nostoc* in many Asian countries for food.

The green alga *Haematococcus* has attained a total of approximately 50 t/a for astaxanthin production using a combination of closed and open systems. This alga has great potential as a future crop for aquaculture.

Dunaliella is produced in natural ponds in Australia and in smaller amounts in the Ukraine, in earthwall-lined unstirred ponds in Australia and also in raceway-ponds in Israel and the United States. In all cases carotinoids are the prime products.

In terms of overall production *Spirulina* (*Arthrospira*) seems to be the most important microalgal species and this is produced nearly exclusively in open systems, though there have been some efforts to establish a commercial *Spirulina* culture under temperate climatic conditions (Brouers 2000). The most famous, nearly natural open pond production facility was Lake Texcoco in Mexico with considerable *Spirulina* production in the past. This facility was closed in 1994/95 because of problems with contamination and pollution. In recent years crater lakes in Myanmar (Burma) have gained more and more importance for the production of *Spirulina*. Nevertheless, most *Spirulina* biomass is produced with raceway ponds in the United States (Hawaii, California), China, Taiwan and Japan, altogether with strong growth tendencies. Biotechnical simple open system production of *Spirulina* seems to have great potential because of the biological value in applications of this microalga.

Chlorella has achieved production scale in raceway ponds or in similar circular ponds especially in Asia (Japan, Taiwan, China), in inclined surface open PBRs in the Czech Republic and Bulgaria and most recently in closed tubular PBR systems in Germany (Dilow 1985; Borowitzka and Borowitzka 1988; Apt and Behrens 1999).

After an upscaling period of nearly 3 years, a tubular system on an industrial scale was established in the year 2000 near Wolfsburg, Germany (Fig. 5). This closed system is apparently the largest PBR to have gone into successful production. It consists of compact and vertically arranged horizontal running glass tubes of a total length of 500,000 m and a total PBR volume of 700 m^3. In a glasshouse requiring an area of only 10,000 m^2 an annual production of 130–150 tonnes dry biomass was demonstrated to be economically feasible under Central European conditions.

Conclusion

For the mass culture of microalgae, open pond systems have mainly been the dominating systems up until now. However, closed systems of light-distributing tube or plate design, known as photobioreactors, are now increasingly finding new applications both for high-value products in pharmacy and cosmetics as well as for aqua- and agricultural uses.

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