5

Outdoor Mass Production of *Spirulina:* The Basic Concept

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The past ten years have witnessed a burst of activity relating to production of microalgae for commercial purposes. From a modest beginning of *Chlorella* tablets in Japan in the late 1950s, new endeavors have emerged as specialized industries the world over aimed at producing health food, food additives, animal feed, biofertilizers and an assortment of natural products (Richmond 1986a, 1986b; Borowitzka and Borowitzka, 1988). The development of algal biotechnology is reviewed in the introduction to this volume. A more detailed account of the problems and day-to-day maintenance parameters involved in large-scale operation is given in Chapter 8. I have tried to describe briefly the history of *Spirulina* as a staple in human diet in the preface to this volume. The purpose of this chapter is to provide the reader with basic information on outdoor mass cultivation of algae, establishing a scientific ground to methods used in commercial production sites as well as suggesting improvements to obtain a higher output rate from the system, leading presumably to a reduction in production cost, thus making outdoor mass production of *Spirulina* more commercially feasible.

The Concept

The concept of algal biotechnology is basically the same as in conventional agriculture, namely the utilization of the photosynthetic machinery for the production of biomass to be used as a source of food, feed, chemicals and energy. The main advantages of culturing microalgae as a source of biomass are as follows.

1 Microalgae are considered to be a very efficient biological system for harvesting solar energy for the production of organic compounds via the photosynthetic process.

2 Microalgae are non-vascular plants, lacking (usually) complex reproductive organs, making the entire biomass available for harvest and use.

3 Many species of microalgae can be induced to produce particularly high concentrations of chosen, commercially valuable compounds, such as proteins, carbohydrates, lipids and pigments.

4 Microalgae are microorganisms that undergo a simple cell division cycle, in most cases without a sexual type stage, enabling them to complete their cell cycle within a few hours and making genetic selection and strain screening relatively quick and easy. This also allows much more rapid development and demonstration of production processes than with other agricultural crops.

5 For many regions suffering low productivity due to poor soils or the shortage of sweet water, the farming of microalgae that can be grown using sea or brackish water and marginal land may be almost the only way to increase productivity and secure a basic protein supply.

6 Microalgal biomass production systems can be easily adapted to various levels of operational or technological skills, from simple, labor-intensive production units to fully automated systems which require high investments.

The Process

The main components of the production process of microalgae biomass are presented in Figure 5.1, illustrating the major inputs and potential uses of the biomass produced. The process can be divided into two main steps:

1 growing the algal biomass, which involves the biological knowledge and the operational parameters;

2 the engineering aspects dealing with the reactor (pond) design, harvesting and processing of the biomass produced.

Successful algae production combines the biological insights of growing photoautotrophic microorganisms with the special requirements for reactor design appropriate for the process.

The biological understanding required includes the effects of interactions of environmental factors, such as light and temperature, as well as salinity, photoinhibition and dark respiration, on algal growth and productivity. The role of

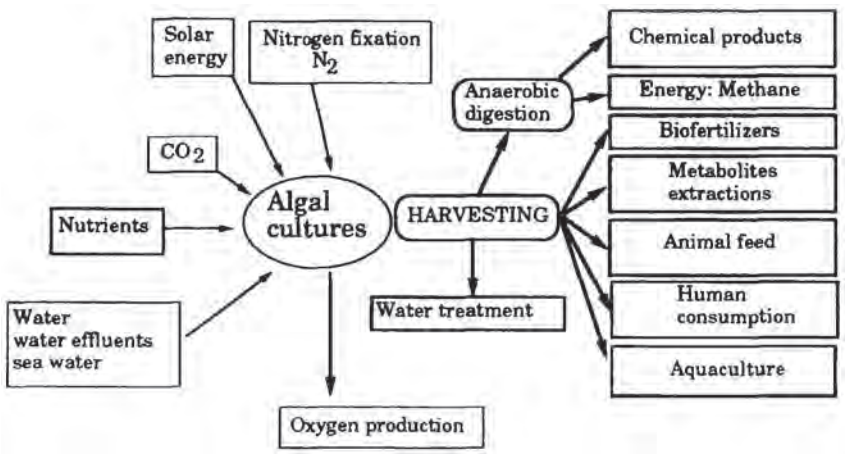

Figure 5.1 A schematic presentation of algal biomass production. Inputs and potential outputs.

these parameters in laboratory grown cultures is discussed in Chapter 3. They have to be considered in developing an operational protocol for pond management which also includes nutrient levels and pH, in order to establish a continuous culture for sustained production and to avoid development of grazers, predators and contamination by other algae.

Biological Problems and Limitations

The major biological limitations for production of *Spirulina* in mass cultures outdoors must be understood in order to obtain maximum output rate. These limitations may also serve as criteria for selecting outstanding strains of *Spirulina* to be used in outdoor production ponds. Assuming that the rate of photosynthesis can be used as an indication of the metabolic activity of outdoor algal cultures, we followed the daytime changes in oxygen concentration in the pond in order to correlate changes in oxygen concentration with diurnal changes in light and temperature. In what may be seen today as a somewhat naive interpretation, we initially concluded from the results presented in Figure 5.2 that in summer the main limiting factor for growth of *Spirulina* in outdoor cultures is light. This derives from the fact that the daily peak in oxygen concentration is reached at the same time that light intensity is maximum. In winter, however, the main limiting factor is temperature because of a shift in the peak of oxygen which follows the peak in the pond temperature rather than light intensity. The effect of temperature and light on growth of laboratory cultures was discussed in Chapter 3. As already stated, dealing with those two

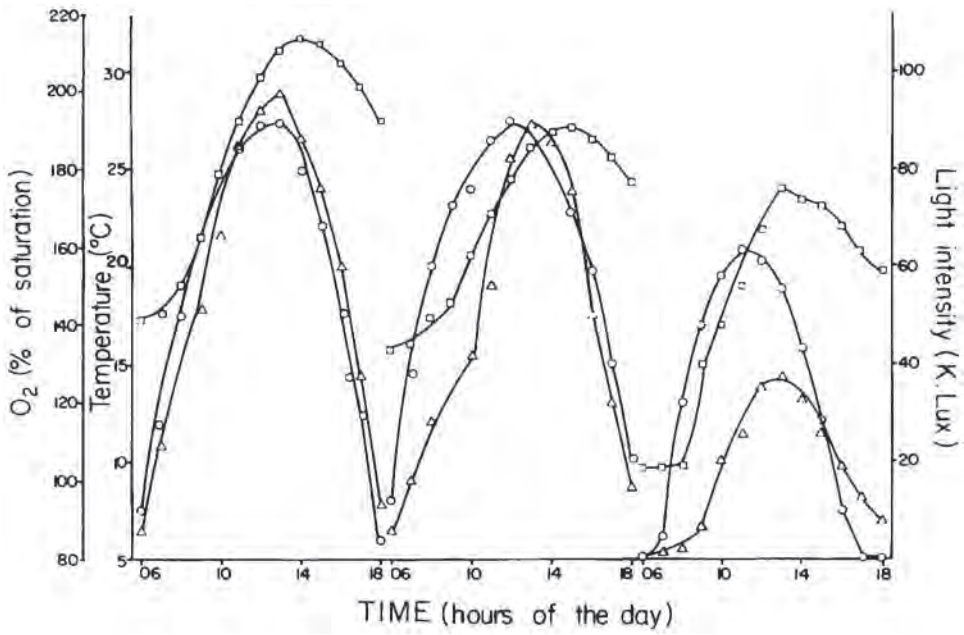

Figure 5.2 Diurnal changes in O_2 concentration, temperature and light during the course of representative days in different seasons. Left to right—summer, spring and winter, □—□ temperature in °C; ○—○ irradiance in klux; Δ—Δ O_2, percentage of saturation.

parameters separately may be somewhat simplistic. Nevertheless, since for many years they were considered as two separate limiting factors in outdoor mass cultivation of *Spirulina* (Vonshak, 1987a; Richmond, 1992b), only in recent studies has the degree of interaction and interrelationship between those factors and productivity been revealed (Vonshak, 1993).

The Effect of Light

Outdoor algal cultures are exposed to two rhythms of the light/dark regime. The first is relatively fast. It is induced by the mixing in the pond which results in a turbulent flow of the culture, dictating the frequency of the light/dark cycle (Laws et al., 1983). In this cycle algal cells are shifted between full solar radiation when located at the upper culture surface and complete darkness when reaching the bottom of the culture, usually at a depth of 12–15 cm. The time scale of such a cycle is measured in fractions of seconds. The other, relatively slower regime, is the change in solar irradiance during the day from sunrise to sunset. These two light cycles impose a unique physiological regime on the adaptation or acclimatization of outdoor algal cells to light.

Light limitation

When growing algae at a depth of 12–15 cm in open raceway ponds, self-shading governs the light availability to the single cell in the culture. Unless one uses a very diluted culture which allows penetration of light throughout the water column, a certain part of the culture will always fail to receive enough light to saturate photosynthesis. Thus almost by definition this kind of culture will be light limited. Indeed, in our very early studies (Richmond and Vonshak, 1978; Vonshak et al., 1982) we demonstrated that increasing cell concentration of the culture, which increases self-shading, results in a decrease of the growth rate. We carried out this kind of experiment during the summer, winter and spring, and findings indicated that the highest response of growth rate to cell concentration, i.e. self-shading, is observed in the summer (Figure 5.3). Our initial interpretation was that, in summer, temperatures are high enough, so the main limitation for growth of *Spirulina* outdoors is light. In winter and spring, however, when the temperature in the outdoor cultures is lower, the effect of self-shading is less pronounced (Figure 5.3).

As in many other microbial systems the important factor to be optimized is the output rate or productivity. The productivity of the system *(Y)* is defined as

$$Y=\mu x \qquad (5.1)$$

where μ is the specific growth rate in units of reciprocal of time and X is the biomass concentration. As demonstrated by our earlier work (Richmond and Vonshak, 1978; Vonshak et al., 1982) and that of others (Richmond and Grobbelaar, 1986), there is an optimal biomass concentration which will correspond to the highest productivity. This concentration is not necessarily the one at which the highest μ is observed, again suggesting that conditions under which the highest output rate is obtained are light limited. This is further demonstrated in Figure 5.4 where the data of Figure 5.3 are replotted so that the output rate of the culture as a function of cell concentration is tested.

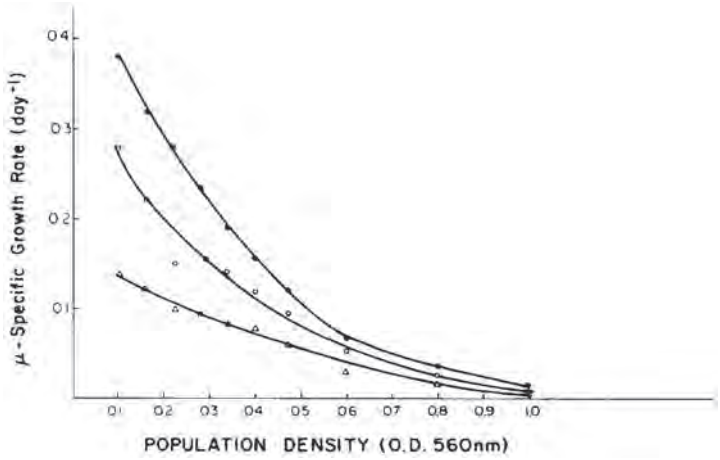

Figure 5.3 The specific growth rate of *Spirulina* as affected by population density and the seasons of the year; ●—● summer (June to September); ○—○ autumn and spring (October to November and April to May); Δ—Δ winter (December to February).

This observation points to one of the most important parameters in the management of outdoor cultures of *Spirulina*: the requirement to maintain an optimal cell concentration or what was defined later as an optimal areal density. In summer, when the main limiting factor is light, it can be seen (Figure 5.4) that any deviation from the optimal biomass concentration resulted in a significant reduction in the output rate of the system. It is also important to remember that the optimal concentration may also be affected by the culture depth, the strain used, and the rate of mixing. The last remains a topic of controversy.

Since the first reports (Richmond and Vonshak, 1978; Vonshak et al., 1982) on the effect of turbulent flow on the productivity of *Spirulina* ponds under outdoor conditions, two effects have been observed:

1 an increase in growth rate at the highest turbulence, resulting in an increase in productivity;

2 an increase in the optimal cell concentration at which the maximal productivity is obtained.

When this phenomenon was observed later by many others (Laws et al., 1983; Richmond and Grobbelaar, 1986; Grobbelaar, 1991), it was still a topic of debate (Grobbelaar, 1989). Interpretation ranged from claims that turbulent flow mimics the flashing light effect reported in the lab (Kok, 1953; Friedrickson and Tsuchiya, 1970), to claims that increased productivity is a result of a mass transfer phenomenon related to better uptake of nutrients and removal of toxic oxygen. We still believe that the effect of turbulent stirring has to do with the role of availability of light and its distribution in dense algal cultures. When stirring is insufficient, only a laminar flow is induced. As is the case for many large-scale commercial ponds, light distribution is unfavorable, leaving a significant part of the culture in complete darkness while another part is overexposed and may even suffer from photoinhibition (this phenomenon will be discussed in the next section). Thus, in a way, increasing the turbulent flow represents the most practical means of improving light distribution

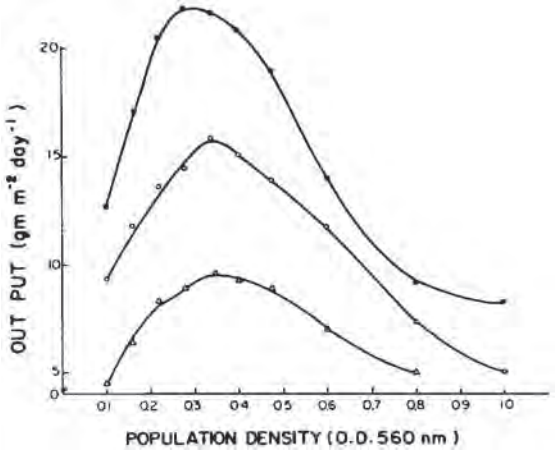

Figure 5.4 The effect of population density on the output rate through the seasons of the year: ●—● summer; ○—○ spring and autumn; Δ—Δ winter.

in outdoor cultures. Further support for our interpretation is suggested by the finding that, as a result of increased turbulence flow, not only is an increase in productivity observed, but also the highest production is achieved at a higher cell concentration (Figure 5.5).

Photoinhibition

Mainly under laboratory conditions, the phenomenon of photoinhibition has been well studied (Kyle and Ohad, 1986; Neale, 1987). For many years it was assumed that outdoor dense cultures, where light penetrates only part way, cannot be photoinhibited. Even when the phenomenon of photoinhibition was discussed as a factor in algal productivity, it related mainly to natural habitats as an ecological factor (Powles, 1984).

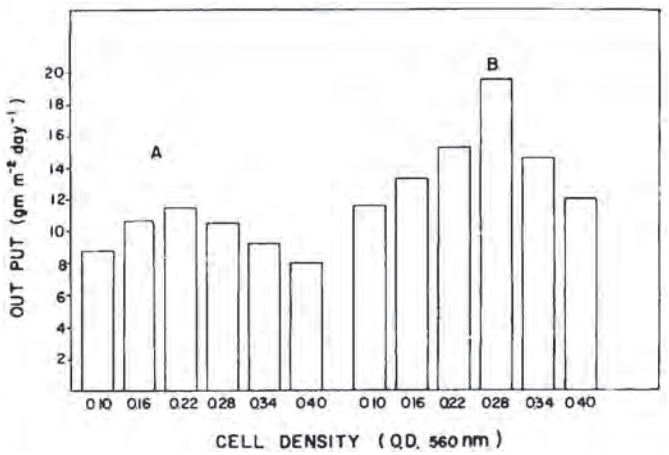

Figure 5.5 The effect of turbulent flow on the output rate of outdoor *Spirulina* cultures grown at different cell concentrations: (A), 7 rpm (slow); (B), 17 rpm (fast).

Vonshak and Guy (1988) were the first to describe the phenomenon of photoinhibition in outdoor-grown *Spirulina* cultures. By following the *in situ* photosynthetic activity of outdoor cultures grown at full solar radiation or under shaded conditions, they observed that shading the cultures resulted in an increase in photosynthetic activity and an increase in productivity.

These findings which seem somewhat to contradict the dogma that outdoor algal cultures are light limited, were then studied further. Using a more advanced methodology of variable chlorophyll fluorescence, it is possible to get a very fast and reliable indication of the quantum efficiency of photochemistry, to which the value of *Fv/Fm* is considered to be directly correlated. Furthermore, a reduction in the *Fv/Fm* ratio in many systems indicates photoinhibitory damage induced in PS II.

We have thus followed the *Fv/Fm* ratio in *Spirulina* cultures grown outdoors in shaded and non-shaded ponds. As demonstrated in Figure 5.6, a marked decline in

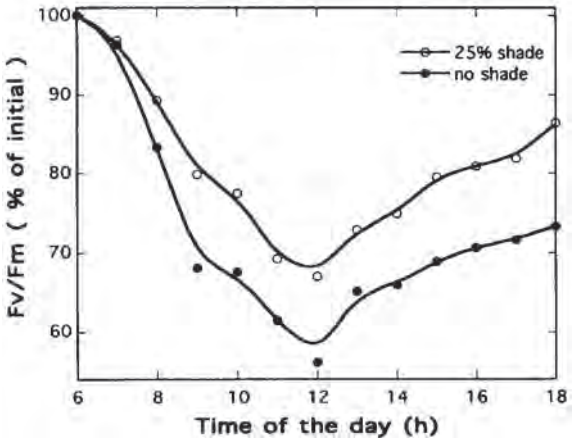

Figure 5.6 Daily changes in *Fv/Fm* in two *Spirulina* cultures grown outdoors, ●—● under full solar radiation, and ○—○ at 25 per cent cut-off by shading.

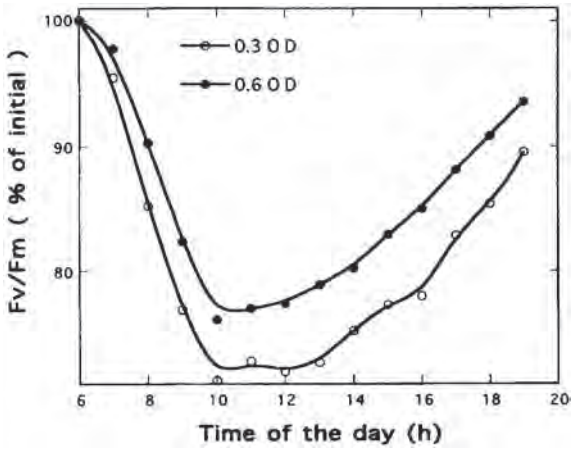

Figure 5.7 Daily changes in *Fv/Fm* in two *Spirulina* cultures grown outdoors at two cell concentrations: ○—○ 0.3 OD and ●—● 0.6 OD.

the ratio, reaching its lowest value at midday, has been observed in the cultures. When the pond was shaded so as to reduce light intensity by 25 per cent, the degree of inhibition was also significantly reduced. It is worth noting that once solar radiation lowers in the afternoon, a recovery in the PS II efficiency occurs. As already pointed out, light availability to the single cell in outdoor cultures is highly dependent on the cell concentration. We have compared the *Fv/Fm* ratio in outdoor cultures maintained at different cell concentrations. As seen in Figure 5.7, diluting the cell concentration of the culture and thus increasing the amount of light to which the cells are exposed, results in a higher degree of inhibition. This observation further supports our interpretation that the decline in *Fv/Fm* ratio is associated with an inhibition process caused by an excess exposure to light.

In the previous section, we suggested that increasing the turbulent flow in outdoor cultures represents a very practical way of improving the light regime. Using the parameter of *Fv/Fm*, we were able to demonstrate that this is indeed the case. When fast- and slow-mixed cultures are compared, the level of PS II efficiency in the fast-mixed culture is higher than that in the slow (Figure 5.8). We suggest that at least part of the reason for the higher productivity obtained at the higher turbulent/mixing system is a result of the prevention of a photoinhibitory stress that occurs in the slow-mixed cultures.

It seems that after almost 15 years of study related to the role of light in productivity of outdoor algal cultures, *Spirulina* in particular, we have reached a better understanding of the complicated light environment to which algal cells are exposed. We know that due to extreme shifts in the level of light intensity, at least in *Spirulina* cultures, photoinhibition may take place. The fact that photoinhibited *Spirulina* cultures have a lower photosynthetic efficiency (Figure 3.3) means that they require more light to reach the same level of activity as non-photoinhibited cells, thus making photoinhibited cultures actually light limited. This finally leads to what may be seen as the paradox of light in outdoor *Spirulina* culture: during a significant part of the day, the outdoor cultures are photoinhibited and light limited at the same time.

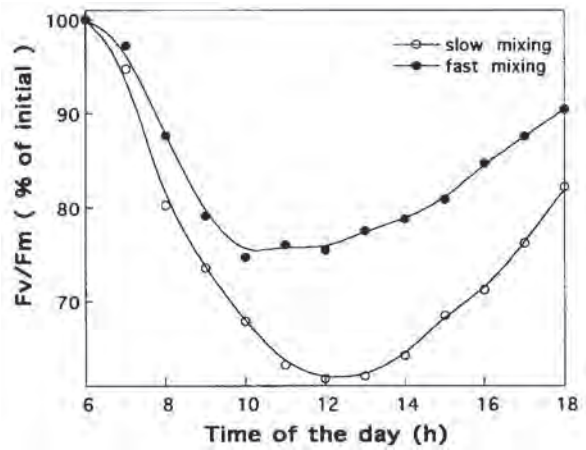

Figure 5.8 Daily changes in *Fv/Fm* of two *Spirulina* cultures grown outdoors at ○—○ slow and ●—● fast mixing rate.

The Effect of Temperature

In many regions of the world, temperature may represent the main limitation for high biomass production rates in outdoor open ponds of *Spirulina* cultures. Although the effect of temperature on growth rate of laboratory algal cultures is well documented, its effect in outdoor cultures is still not fully understood. An outdoor algal culture undergoes a diurnal cycle which in areas out of the tropics may show a difference of 20°C. In the morning, the pond temperature may only be in the range 15–20 °C; an optimal temperature in the range 35–38°C is reached only in the early afternoon. Even in the tropics where the culture does reach the optimal temperature, during a significant part of the day—the early morning hours—the temperature will still be much below the optimum.

One possible advantage to the diurnal cycle may be a low temperature at night. It has been demonstrated that relatively high temperatures at night can increase respiration rate, which may result in the phenomenon described as night loss of biomass. The degree of loss varies as a function of the biomass composition and may reach values of 30 per cent of the previous daily productivity (Torzillo et al., 1991; Guterman et al., 1989; Grobbelaar and Soeder, 1985).

During winter, *Spirulina* cannot be grown in outdoor open ponds, except in the tropics (see Chapter 8). The only way by which cultures can be maintained so as to overcome the low temperature, so that production can be resumed once winter is over, is to put them in greenhouses or to significantly increase the biomass concentration.

Temperature limitation on productivity may represent one of the many drawbacks of open raceway systems. (This is one of the big advantages of closed reactors.) Figure 5.9 illustrates and compares diurnal changes in temperature of three cultures of *Spirulina* in open ponds, a greenhouse, and a tubular reactor. As can be seen in closed systems, the optimal temperature is reached almost 4 h earlier than in open pond cultures. This not only increases the time during which photosynthesis may operate at maximum capacity but also prevents some inhibitory effects due to the interaction of low temperature and high light intensity, as discussed in the following section.

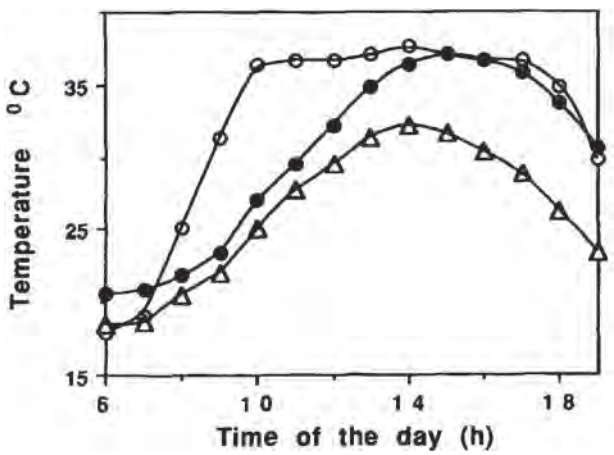

Figure 5.9 Diurnal temperature changes in three *Spirulina* cultures grown outdoors in Δ—Δ open pond, ●—● open pond placed in a greenhouse, ○—○ a tubular reactor.

Interaction of temperature and light

The possible interaction of temperature and light in outdoor algal cultures was overlooked for many years (Vonshak, 1987a; Richmond, 1992b), even though some of the early observations indicated that, when the pond temperature was increased by a few degrees in the morning, an increase in productivity much above what may be expected from just a simple temperature effect was observed. The special interaction of light and temperature stress in higher plants led us to examine the possibility that similar interaction took place in *Spirulina.* A recent paper (Vonshak et al., 1994) proposed that a relatively low morning temperature with rapid increase in light intensity may induce photoinhibitory stress. This was easily demonstrated in tubular reactors where fast heating is possible, and indeed, when the temperature of the outdoor *Spirulina* culture is maintained at 35 °C, the typical reduction in *Fv/Fm* does not occur (Figure 5.10).

In a much more detailed study, we have used *Spirulina* cultures grown in open ponds during the winter in Sede-Boker, Israel. In order to further elucidate the nature of the interaction between light and temperature, four experimental treatments were tested: two cultures were heated (up to 35 °C) during daylight, while in two other cultures the temperature, fluctuating between 5 °C in the early morning and a maximum of 20 °C midday, was not modified. One pond of each temperature treatment was shaded by a net, reducing by 25 per cent the total solar radiation. When following the changes in the *Fv/Fm* ratio in the four ponds during the day (Figure 5.11), a fast decline in *Fv/Fm* is observed, mainly in the non-heated ponds. The fastest decline, which takes place between 8 and 11 am, is slowed down to some extent when the culture is shaded. It has to be pointed out that light intensity at that time of the year is only 50–60 per cent of that measured during summer, reaching a maximum of 1400 $\mu molm^{-2}s^{-1}$ at midday. Whereas heating the cultures significantly prevented the inhibitory effect observed in the non-heated cultures, shading the heated cultures did not provide any further protection. These results clearly indicate that early morning low temperatures in outdoor open cultures may cause photoinhibitory stress. Since the recovery from photoinhibitory stress is slower

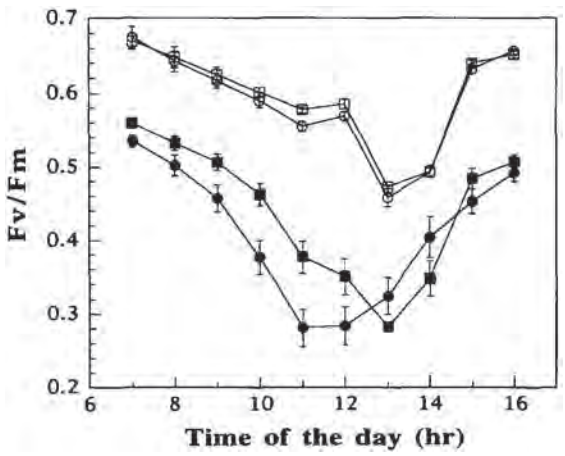

Figure 5.10 Changes in *Fv/Fm* in two *Spirulina* cultures grown outdoors at ○—○ 25 °C and □—□ 35 °C. The diurnal changes in light intensity (PAR) are indicated by △—△.

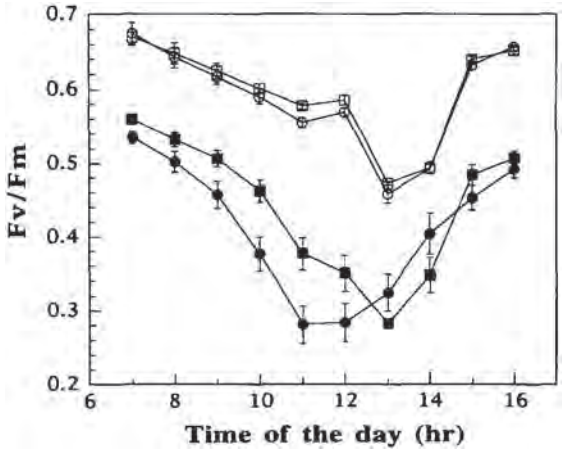

Figure 5.11 The effect of light and temperature on the *Fv/Fm* ratio of *Spirulina platensis* grown in open ponds under (□, ■) 25 per cent shading and (○, ●) non-shaded conditions during the daytime.□, ○ cultures were heated; ■, ● no heating applied.

than the rate at which the stress is induced, even if temperature is increasing later in the day, this early morning inhibition may result in lower productivity during most of the day. Following the daily productivity of the four ponds indicates a good correlation between the PS II efficiency and the daily output rate (Table 5.1). An increase in productivity in the two heated ponds occurs as expected. Shading the heated culture, which did not provide any protection to PS II, resulted in a small reduction in productivity by some 10 per cent. What is more striking is the effect of shading the non-heated cultures. These cultures responded to the 25 per cent reduction of light intensity by a marked increase in productivity of almost 45 per cent. It is thus clear that when *Spirulina* is exposed to suboptimal temperatures, the susceptibility of cells to photoinhibition is significantly increased.

It can be concluded that light and temperature are not separate factors that affect biomass productivity in a simple manner as in laboratory cultures. The fact that cultures in open ponds undergo diurnal and seasonal fluctuations makes this interaction somewhat more complicated to understand. The physiology of outdoor algal cultures is a new field of study, and much more has still to be done in order to fully reveal the interaction between environmental factors and production of outdoor *Spirulina* cultures.

Table 5.1 The effect of light and temperature on the productivity of outdoor *Spirulina* cultures

Experiment conditions	Production ($g\,m^{-2}\,day^{-1}$)
Heated	14.9
Heated + 25% shade	11.1
No heat, no shade	3.7
25% shade	5.3

The Effect of High Oxygen Concentrations

Oxygen evolves in the process of photosynthesis and, as a result, accumulates in the culture. One important role of the turbulent flow, in addition to effecting a proper light-regime, is to remove that oxygen. In relatively small ponds, where high flow rates can be maintained, oxygen concentration can be at levels not higher than 200 per cent of air saturation, i.e. 12–14 mgl^{-1} depending on the temperature and barometric pressure. In large ponds, where the water flow is relatively slow (10 to 20 cms^{-1}), when high photosynthetic rates exist, the O_2 concentration may reach as high as 500 per cent of saturation. High concentrations of O_2 inhibit photosynthesis and growth and may lead to a total loss of the culture. Recently, the ill effects of high O_2 tension in *Spirulina* cultures were studied (Marquez et al., 1995; Singh et al., 1995). Both report that exposing laboratory cultures of *Spirulina* to high oxygen may result in reduced growth rate and bleaching of the pigments. Rather than artificially increasing the oxygen, we have tried to study the effect of high oxygen under outdoor conditions, by letting it accumulate as a result of photosynthesis. By measuring the fluorescence yield we could demonstrate that when the oxygen level is maintained at a range of 20 to 22 mgl^{-1} no significant reduction in the photosynthetic activity is observed. Exposing cultures to a higher level than this results in a rapid decline in the photosynthetic activity (Figure 5.12). This correlates with the daily output rate measured in the cultures, as well as with the observations that large-scale open pond levels of above 300 per cent of saturation also cause inhibitory effects on *Spirulina* productivity.

Maintenance of Monoalgal Cultures

Contamination by different algal species may present a very severe problem for microalgal cultures grown in outdoor open ponds. In a previous paper (Vonshak et al., 1983), we described a set of conditions that is instrumental in preventing

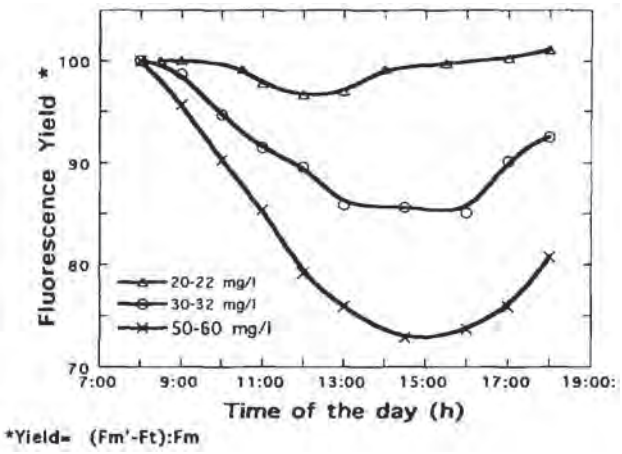

Figure 5.12 Diurnal changes in the fluorescence yield of *Spirulina* cultures grown outdoors at 22 (Δ), 32 (○) and 50–60 (×) mgl^{-1} of oxygen. *Fm'* and *Ft* are maximal and minimal fluroescence intensity of light-adapted cultures, respectively.

contamination of outdoor *Spirulina* culture by *Chlorella*. In most cases, the steps that proved effective in prevention of the *Chlorella* contamination were maintaining a high bicarbonate concentration (e.g. 0.2 M), taking precautions to keep the dissolved organic load in the culture medium as low as possible, and increasing winter temperature by greenhouse heating. Development of grazers in the culture, mainly the amoebae type, presents another problem. Vonshak et al. (1983) noticed amoebae grazing on *Chlorella*, and amoebae grazers of *Spirulina* were also observed in some improperly maintained commercial ponds. Addition of ammonia (2 mM) arrested the development of these grazers. Lincoln et al. (1983) also reported that the population of grazers was significantly reduced when ammonia was used as the main nitrogen source.

When a population of *Chlorella* cells growing in mass cultures of *Spirulina* increased in number (above $10^6 ml^{-1}$), repeating treatments with 1 mM ammonia was sufficient to prevent further proliferation of *Chlorella* (Vonshak and Boussiba,

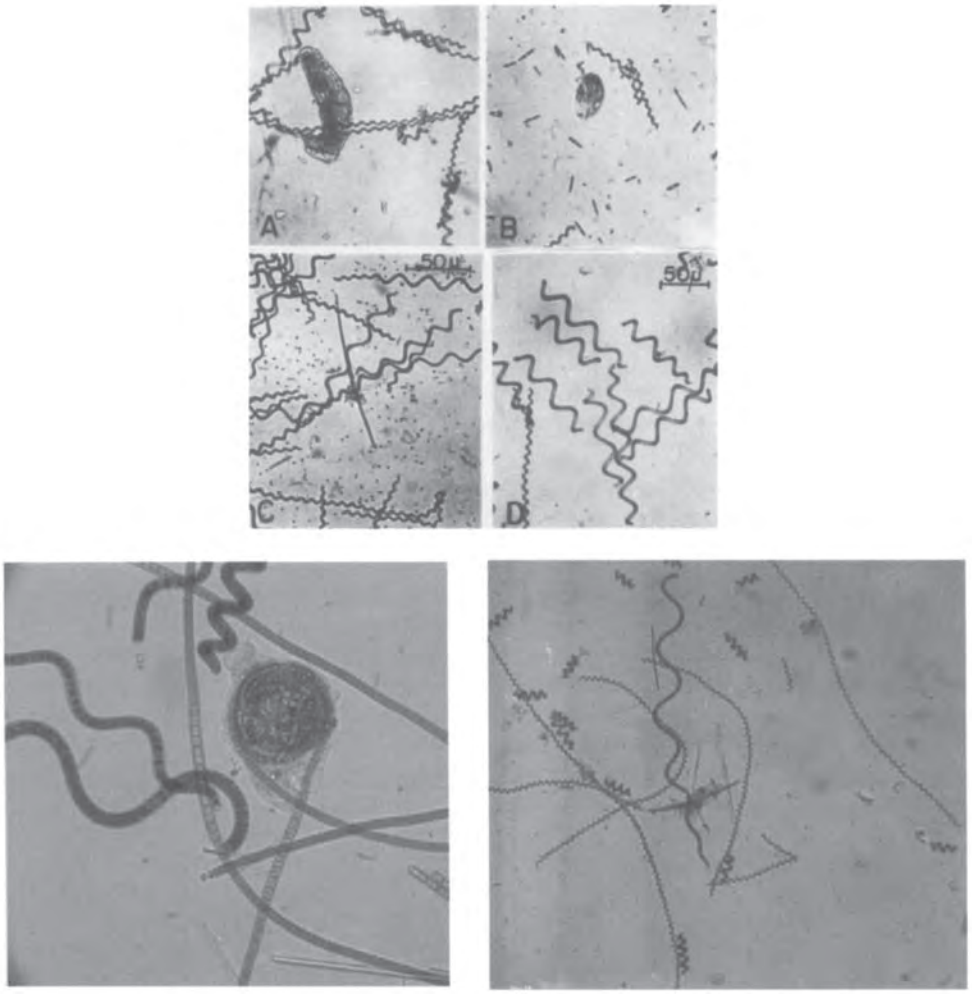

Figure 5.13 Photomicrographs showing different levels of contamination in outdoor cultures of *Spirulina* note the variation in *Spirulina* filaments as well).

unpublished results). Typical contamination and predators that may develop in *Spirulina* cultures are presented in Figure 5.13.

In conclusion, contamination of outdoor *Spirulina* cultures by other organisms can be controlled. Experience indicates that, as a rule, contaminating organisms do not present a serious difficulty as long as good growth is maintained in a monoalgal culture. It is worth noting that no cyanophages attacking *Spirulina* have been observed so far.

Engineering and Design Parameters

Some of the basic engineering and design parameters to be considered for the large-scale operation of algal ponds are summarized in the following section.

Design and Mixing of the Open-channel Raceway Ponds

Almost all commercial reactors for production of *Spirulina* are based on shallow raceways in which algal cultures sustained by a paddle wheel are mixed in a turbulent flow. One exception is Sosa Texcoco (Mexico) where a natural lake and certain facilities of a production plant for sodium bicarbonate were adapted for producing *Spirulina*. (This site has been recently closed down because of a strike, and it is not clear whether it will be re-open.) Recently another semi-natural lake in Myanmar (Burma) has been reported to be used as a production site for *Spirulina*. At other production sites in the USA, China, India, Thailand and Taiwan, two types of open raceway ponds are used: the first, which is more capital intensive, is lined with concrete (Thailand, India); the second is a shallow earthen tunnel lined with PVC or some other durable plastic. The cost and durability of the lining significantly influences the capital costs and thus the economic feasibility of this biotechnology. Benemann et al. (1987) estimated that any durable liner will add up to US $0.5 to the cost of production of each kg of algal biomass produced, demonstrating the need for cheaper lining such as low-cost clay sealing. Such lining has yet to be tested for durability under turbulent flow and periodic cleaning of the pond.

The size of commercial ponds varies from 0.1 to 0.5 ha. They are all stirred by paddle wheels, the design of which varies significantly from large wheels (diameter up to 2 m) with low revolution speed (10 rpm) to small wheels (diameter 0.7 m) with two to three times faster revolution. Culture depth is usually maintained at 15–18 cm. In most places it is determined by technical means, such as ground leveling and the degree of immersion of the paddle wheel.

Although the paddle wheel is the most common device for stirring *Spirulina* in commercial plants, other mixing devices are being tested. One difficulty with paddle stirring lies in the nature of the flow. It is usually insufficiently turbulent to effect an optimal light regime for the single cell (Vonshak et al., 1982; Richmond, 1987). Laws et al. (1983) introduced into the raceway an array of foils, a design similar to an airplane wing, which effected systematic mixing through the vortices created by the foils. These authors report a more than two-fold increase in photosynthetic efficiency. Others (Valderrama et al., 1987), following an idea originally suggested by Baloni et al. (1983), described a novel device for mixing shallow algal ponds. It consists of a board which closes the pond's cross-section,

except for a slit above the bottom of the pond. The board is moved back and forth, creating a turbulent back whirl as the culture is forced through the slit. The authors claim to have achieved 'outstanding' results in growing *Spirulina*. This method of inducing turbulence in shallow raceways has not yet been scaled up, and a comprehensive evaluation of the system has yet to be carried out. Cultivating *Spirulina* in tubular reactors whose flow is induced by a suitable pump seems another promising means of cultivation which has yet to be tested on a large scale. Initial work by Tomaselli et al. (1987) argues for the potential of such a system (see Chapters 6 and 7).

Harvesting and Processing of Spirulina *Biomass*

In all commercial production processes, similar filtration devices are used for harvesting. These are basically of two types of screen: inclining or vibrating. Inclined screens are 380–500 mesh with a filtration area of 2–4 m^2 per unit and are capable of harvesting 10–18 m^3 of *Spirulina* culture per hour. Biomass removal efficiency is high, up to 95 per cent, and two consecutive units are used for harvesting up to 20 m^2h^{-1} from which slurry (8–10 per cent of dry weight) is produced. Vibrating screens can be arranged in double or triple decks of screens up to 72 inches (183 cm) in diameter. Vibrating screens filter the same volume per unit time as the inclining screens, but require only one-third the area. Their harvesting efficiencies are often very high. At one commercial site a combination of an inclining filter and a vibrating screen has been used. Two main problems exist with the systems described above. In the process of pumping the algal culture to be filtered, the filaments of *Spirulina* may become physically damaged, and repeated harvesting leads to an increasingly enriched culture with unicellular microalgae or short filaments of *Spirulina* that pass through the screen readily.

The slurry (8–10 per cent of dry weight) obtained after filtration is further concentrated by filtration using vacuum tables or vacuum belts, depending on the production capacity. This step is also used for washing excess salts from the biomass; it amounts to 20–30 per cent of dry weight. The washed cake is frequently homogenized before being dried. This is accomplished by spray- or drum-drying. The end product should have an ash content of ca. 7 per cent. For good preservation and storage, moisture should not exceed 3–4 per cent.

Production of *Spirulina* by Simple Technology and Locally Available Inputs

Production of *Spirulina* may be greatly simplified, thereby avoiding the high-technology systems described so far. Such a mode of production results in relatively low output rates, which are compensated for by the much-reduced cost of production. Indeed, *Spirulina* culture lends itself readily to simple technology: cultivation may be carried out in unlined ditches through which flow is low (e.g. 10 cms^{-1}). Stirring may be provided by a simple device driven by wind energy or harnessed to humans. Harvesting may be readily performed using some suitable cloth, and the biomass dehydrates in the sun. The quality of the *Spirulina* product obtained in this fashion would not be as high as what is attained in 'clean cultures', but the product could serve very well

as animal feed. In the work of Granoth and Porath (1984), *Tilapia mossambica* was cultivated in artificial ponds with relatively high stocking density and fed with a mixture of solar-dried *Spirulina*, that had been cultivated and processed using low-cost technology and added to groundnut cake. The resulting mean food conversion ratio was lower than that observed using control fish fed with the usual fish ration. Furthermore, the yield of *Tilapia* fed on *Spirulina* mixed with groundnut cake was 41 per cent higher than that of fish fed on groundnut cake alone. (For more on the use of *Spirulina* in aquaculture see Chapter 11.)

Becker and Venkataraman (1982) and Seshadri and Thomas (1979) suggested a *Spirulina* growth medium based on low-cost nutrients obtained from rural wastes such as bone meal, urine or the effluent from biogas digesters. Indeed, this last possibility, an integrated system making use of effluent from a starch production factory using tapioca, was developed and scaled up to a demonstration plant by the team of King Mongkut's Institute of Technology in Bangkok. In this plan, a 160 m^3 digestor is operated for production of biogas with four 200 m^2 algal ponds that produce *Spirulina* biomass which is tested for nutritional value. The system has been scaled up to 14 ponds of 1000 m^2 each and an annual production of 30–50 tons. In another work carried out by Ms Jiamjit Boonsom from the National Inland Fisheries Institute, *Spirulina* strains, mainly from the north-eastern part of Thailand, were isolated. Those strains capable of growing in brackish water were mixed with casava or fish meal and served as a locally produced protein source for fish feed. Elsewhere, Bai (1986) has summarized the know-how developed for production of *Spirulina* biomass in a village in India. According to Chung et al. (1978), *Spirulina* grows well in diluted fermented swine manure, provided that the concentration of ammonia nitrogen is adjusted to 100mgl^{-1} and proper nutrients are supplemented. More detailed information on the use of wastewater for growing *Spirulina* biomass is given in Chapter 9.

Spirulina may also be grown on sea water enriched with urea, after excess Ca^{2+} and Mg^{2+} are precipitated (Faucher et al., 1979). In a recent report (Tredici et al., 1986) the use of sea water for cultivating *Spirulina* without any pre-treatment of the water is described. The bicarbonate concentration was about one-tenth lower than the usual, the pH was maintained at 9.0, and the medium was not recycled. Yields of 10 $gm^{-2}day^{-1}$ were obtained, pointing to the possible use of sea water as a cheap medium for the mass cultivation of *Spirulina*.

Economics and Future Prospective

Cost and Economic Evaluation

Many attempts have been made to predict the production cost of algal biomass produced on a large scale (Benemann et al., 1987; Dynatech, 1978). Rather than present another economic calculation, we mean to elaborate the economic basis of commercial production plants and indicate reasons for the large discrepancy between the actual cost of production and that which was predicted by some early calculations. The main difficulty stems from lack of information caused by commercial secrecy. We estimate that in the intensive production sites (Thailand, USA) the cost of production (excluding capital costs) is in the range of US$7–10 per kg of spray-dried *Spirulina* powder (some of those estimates are summarized in Table 5.2). Manpower represents 20–30 per cent of the running cost, a major

Table 5.2 Major items of investment and production costs in a *Spirulina* production site. Total pond area 50 000 m^2 (intended for high-value food grade production)

Item	Cost (thousand $US)
Investment costs	
Land preparation and development; site operation	138
Water and power network	257
Buildings (labs, offices, shops)	79
Nutrients, storage and stock	50
Pond: including lining, pump, mixing	493
Harvesting: including filtering, drying, packing	541
TOTAL	1558
Annual production costs	
Manpower	320
Repair and maintenance	42
Fixed operation costs	163
Variable operating costs (gas, nutrients, power, etc.)	123
Administration, capital and depreciation	390
TOTAL	1038

component. This reflects one of the main difficulties in attempting to cut down costs. Even in the biggest production sites in the USA, consisting of ten 0.5-ha ponds, the total production area is still too small to realize significant reduction of manpower cost per unit product. According to recent publications this site has doubled the number of ponds in one recent year (1995). According to some published economic analyses, minimal plant size must be no less than 10 to 100 ha, consisting of 1- to 10-ha ponds. Such large production sites are yet to be constructed and tested, so the matter of the effect of size of production on the cost of unit product remains open. Another open question is the effect of increased supply and reduced selling price on consumer demand.

A second major component in the cost of production is the cost of nutrients, particularly carbon. It ranges from 15 to 25 per cent of the total operation costs. The main reason for this high cost is the relatively low efficiency of the conversion of nutrients to algal mass. When nitrogen is supplied in the form of liquid ammonia, a significant part may be lost to the atmosphere in the form of ammonia gas, NH_3. This is due to the high pH (ca. 10.0) used for the cultivation of *Spirulina*. This problem is overcome when nitrate, NO_3-, is used as the nitrogen source, but it is significantly more expensive than ammonia. Loss of nitrate through denitrification may take place especially in large ponds with low mixing rates, in whose still corners anaerobic pockets may be found. In one commercial plant, we calculated a nitrate loss of up to 50 per cent. The carbon requirement is usually supplied with CO_2, following an initial supply of 0.2 M sodium bicarbonate (Vonshak, 1986). The highest efficiency of CO_2 conversion into biomass obtained in commercial reactors of *Spirulina* is about 80 per cent. This high figure can be obtained in relatively small operational units (0.1–0.2 ha) in which exact monitoring and control systems are in place. In most of the production sites where one carbonation system is used for large ponds (0.3–0.5 ha), lower efficiencies are obtained. When growing *Spirulina* at a pH level

higher than 9.6, some of the carbon requirements are met by atmospheric CO_2 (BenYaakov et al., 1985).

Current low efficiency of nutrient utilization also stems from a lack of knowledge concerning the use of recycled medium for long periods of time. Depending upon the production site and local experience, the medium must be changed completely three to six times a year in order to sustain production and to avoid deterioration of the culture. In at least one case, reuse of this 'low-quality' medium for so-called 'feed grade biomass' is practised. Clearly, subsequent information concerning recycling of the culture medium may result in less frequent changes of expensive nutrient medium, cutting costs significantly. In most cases, energy represents some 15 per cent of the operational cost, reflecting the requirement of power for mixing, pumping and drying. A breakthrough in the economic utilization of solar and/or wind energy would have to take place for this cost to decline significantly. In order to make *Spirulina* biomass a widely used commodity, it is clear that the cost of production must be reduced to the range of US$4–6 per kg of dry matter. This figure will be attained only if much higher production rates, about twice as high as at present, are commercially achieved. Recent market information indicates that while the cost of *Spirulina* production has fallen in some sites to about US$8 per kg, the price of powder sold in the market is more than US$20 per kg. One of the reasons for this high price may stem from the increase in demand when Sosa Texcoco, which was supplying about 200–300 tonnes of *Spirulina,* ceased production in 1994 because of management and strike problems.

The economic analysis of low-technology endeavors based on local resources is far more complicated as no exact figures for estimating the cost benefit of locally produced proteins are available. The cost of manpower may become less relevant when one considers social factors such as added jobs, improvement in the standard of living, and prevention of migration of small farmers to urban centers.

Future Prospects

The future of algal biotechnology rests, to a large extent, on two factors: (a) the ability to reduce costs of production and thus make algal biomass a commodity traded in large quantities, rather than limited to the health food market; (b) the development of suitable reactors. Closed systems offer several advantages over open raceways. In closed systems, cultures are better protected from contaminants, and thus the maintenance of monoalgal cultures should be easier. Water loss and the ensuing increase in salinization of the medium are much reduced. This mode of production opens the possibility of using sea water with low bicarbonate concentrations, thus saving on the cost of water and medium. Because of much higher cell densities, areal volumes may be much smaller, thereby reducing harvesting costs. Finally, optimal temperatures may be established and maintained more readily in closed systems, resulting in higher output rates. The latter is an essential aspect in the production of a microorganism such as *Spirulina* whose growth temperature is 37 °C. All of these developments have yet to be tested on a large scale in order to evaluate whether the higher investment cost is indeed compensated for by higher annual yield.

In the last five years many research groups in France, Italy, Israel, Singapore and Australia have reported progress in their attempts to scale up experimental tubular reactors for mass production of different species of algae. In our lab, a

1000 1 tubular reactor for the cultivation of *Spirulina* has been operating. The information gathered so far indicates that *Spirulina* can be grown at a standing biomass of 3–5 gl^{-1} with a daily productivity of 1 gl^{-1}. For more details on closed systems, see Chapters 6 and 7.

Summary

In the past decade, much progress has been made in developing appropriate biotechnology for microalgal mass cultivation aimed at establishing a new agro-industry. This chapter presents a number of basic requirements needed to achieve high productivity and low-cost production with this new agrotechnology. The first is the availability of a wide variety of algal species and strains that will favorably respond to a variety of outdoor conditions. Another essential requirement is an appropriate bioreactor, either by improving an existing open raceway type (Soeder, 1980), or by developing a new type, such as tubular closed systems (Gudin and Chaumont, 1984; Lee, 1986). The latter solution seems more promising. These developments must overcome the main limitation confronted by the industry, i.e. overall low sustainable productivity. The areal yields obtained today fall too short of the theoretical maximum, and attempts must be made to improve efficiency. But even more important than obtaining high efficiency of solar energy conversion is to design a system which will operate at a constant, sustainable rate of production.

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