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Problems in developing the biotechnology of algal biomass production

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Summary The effects of environmental conditions (solar irradiance and temperature) and population density on the production of Spirulina biomass with brackish water are reported for cultures grown in outdoor ponds. Higher specific growth rates were observed at lower population densities. Lower growth rates were associated with limitation by light in dense cultures under optimum conditions in the summer. Seasonal variation in productivity was observed. In summer, light was the limiting factor, whereas in winter the low daytime temperature appeared to constitute the major limitation. The oxygen concentration in the culture can serve as a useful indicator of limiting factors and can also be used to estimate the extent of such limitations.

Introduction

The unique environment of many arid and desert areas imposes severe limitations on conventional agriculture, yet offers important advantages for the cultivation of algae, *i.e.* saline water resources, high temperatures, abundant solar irradiation, and large areas of currently unused land.

Saline water

Arid zones are deficient in fresh water. However, saline aquifers are found under many desert areas all over the world. The saline aquifers of the Negev in Israel, which vary in salinity from 2500 to 6000 ppm total dissolved solids, could supply $30 \cdot 10^6$ m^3 of water annually with no depletion[1], and up to three times that amount with only minor depletion. In addition to brackish water, seawater can also be considered for use in algal culture.

Temperature

Algal growth increases exponentially with temperature until an optimum temperature is reached. Daytime temperatures in many desert areas approach an average of 40–50°C in the summer, although the water temperature in the ponds would be a few degrees cooler due to evaporation. The optimum temperature for growth of many warm-water algae is about 35°C. Hence, normal daytime temperatures for hot deserts, which subject many conventional plant species to severe stress, fall in a range that permits maximum yields of algae.

Solar radiation

The average annual levels of solar radiation in hot desert regions are considerably higher than in other areas of the world because of their latitudes and the characteristic absence of cloud cover. High radiation levels offer two advantages for algal cultivation. First, the intensity of solar radiation in warm regions is a major factor that determines the rate of growth of algae, unlike with conventional agricultural crops, where water, soil nutrients, and carbon dioxide are usually the major limiting factors. Second, the high radiation levels should allow the use of solar energy for processing the harvested algae.

Large tracts of land

For maximal absorption of solar radiation, algae must be cultivated in shallow, 10–20 cm deep ponds, which requires large level stretches of vacant land. These are abundant in most desert areas.

Technological problems

Three major technical aspects must be considered in developing commercial systems for the mass cultivation of algae. The first relates to pond construction: its shape and depth, the lining material, and the system for mixing the algae-laden water. The second concerns separation of the algal mass from the medium, and the third relates to dehydration and storage of the harvested algae.

Biotechnological problems

Algae are among the most efficient plants in utilizing solar energy, having the highest output rate in terms of dry weight and protein per unit area[2] when the sole limiting factor for growth is light. Thus, the most important single issue in the practical understanding of biomass production in outdoor cultures is the study of light as the major factor limiting the output rate. Because of self-shading, the significant parameter in studying the effect of illumination is the integrated radiant flux incident on each algal cell. Outdoors, this parameter depends on three factors: (1) the light intensity, (2) the algal population density, and (3) the dark/light cycle to which the average cell in the culture is

Table 1. Percent distribution of incident light throughout the depth of a pond of *Spirulina platensis*. Incident light (100%) = 2300 $\mu E\, m^{-2}\, s^{-1}$

Depth (cm)	Cell concentration (O.D. 560 nm)					
	0.10	0.16	0.22	0.28	0.34	0.40
1	57	53	47	41	37	35
2	48	41	33	29	20	16
3	43	33	28	16	10	3
4	38	38	18	8	3	0
5	29	18	2	14	0	0
6	18	12	0	0	0	0
7	2.4	2	0	0	0	0
15	0	0	0	0	0	0

exposed. The latter is affected by the turbulence, the depth of the medium, and the population density, as will be discussed below.

The interrelationship between light irradiance and population density is presented in Table 1, which shows a typical pattern of distribution of incident light throughout the depth of a pond of *Spirulina platensis*. When the cell density was relatively high (optical density of 0.40 at 560 nm, or 0.5 g dry weight per liter), only the upper 3 cm of the pond, or about 20% of the cells, received light. Accordingly, about 80% of the cells were in almost complete darkness at any given moment. Even when the cell concentration was halved, solar radiation did not penetrate beyond 5 cm, and over 60% of the cells were in complete darkness or at a light intensity below the compensation point at any given instant. Even in cultures of very low cell densities (which exhibit the maximum specific growth rate but cannot be economically maintained in the pond), light penetrated to only about half the pond's depth, leaving half the cell population unilluminated at any given time. Thus in outdoor ponds, the extent of mutual shading, which is a function of the population density and the pond depth, is the major factor determining the amount of solar radiation available to the cells in the culture.

Turbulent flow should be maintained in the pond, so that each cell in the culture is exposed to a light/dark cycle. The cycle may take a few seconds to many minutes to complete as each cell travels back and forth from the upper, illuminated layer of the pond to the lower and much thicker unilluminated layer. The light/dark regime to which each cell in the culture is thus exposed has hardly been investigated. However, when light is the factor limiting growth, the effective light/dark cycle ought to have a crucial effect on the growth rate and the photosynthetic efficiency. The nature of the light/dark regime depends on the intensity and duration of solar irradiance, the depth

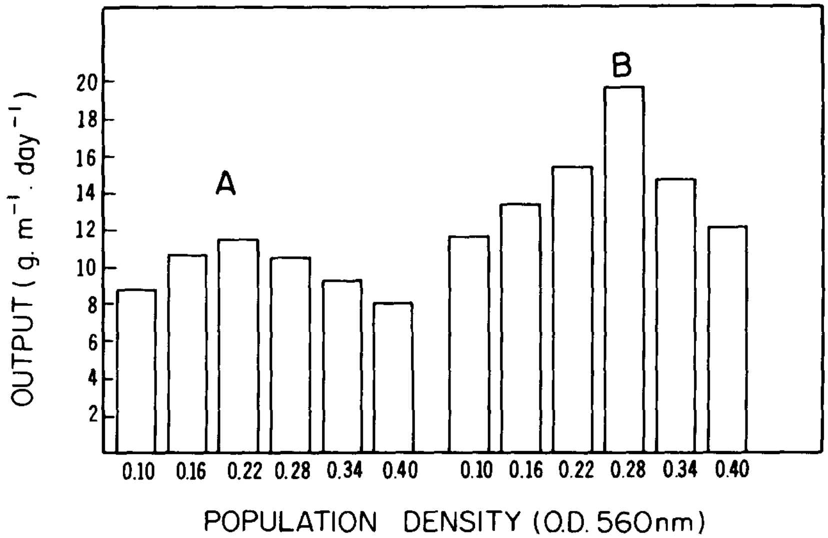

Fig. 1. Effects of cell density and turbulence on output rate in cultures of *Spirulina platensis*. **A**, paddle speed, 15 rpm; **B**, paddle speed, 30 rpm.

of the pond, the population density, and the extent and type of turbulence.

Since the net output of biomass is a product of both the cell density and the specific growth rate, and since these parameters are negatively related (see Fig. 3), then when the system is only light-limited, maximum output may be achieved at some optimum cell density (Fig. 1). The output rate is also clearly affected by the extent of turbulence in the pond (Fig. 1). Increased mixing may impose a more favorable light/dark cycle on the average cell in the culture, resulting in improved photosynthetic efficiency per unit area. The greater the turbulence, the shorter should be the duration of one complete light/dark cycle. In addition, when irradiation is very high, e.g. 250 microeinsteins m^{-2} s^{-1}, cells located in the uppermost layer of the pond may suffer from overexposure to light. Intense stirring would decrease the duration of this overexposure. Increasing the turbulence clearly increases the growth rate (Fig. 2). The finding that, when stirring is enhanced, the maximum output rate is shifted to a higher cell density (Fig. 1) is consistent with our thesis that greater turbulence improves the light regime for the cells.

The complexity of optimizing outdoor biomass production is shown by the effect of population density on μ (the average specific daily growth rate) throughout the year (Fig. 3). Since μ is substantially affected by temperature, this relationship varies greatly with the

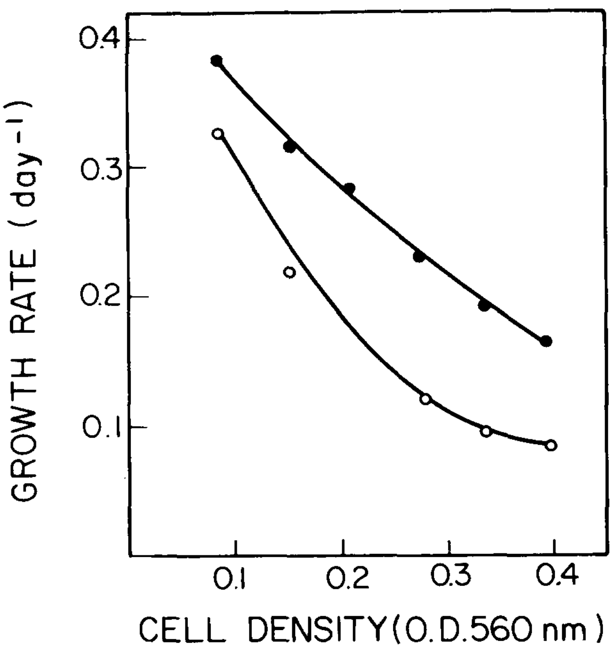

Fig. 2. Influence of cell density and turbulence on the specific growth rate. Upper curve, paddle speed, 30 rpm; lower curve, paddle speed, 15 rpm.

seasons. The more severe the temperature limitation on μ, the smaller is the dependence of μ on the population density. This dependency becomes very low in mid-winter, when the specific growth rate is low (open triangles, Fig. 3). In winter, temperature becomes the main factor limiting the output rate.

A major biological question relevant to the mass production of algae is to what extent is it possible to maintain a monoalgal continuous culture outdoors. In nature, there are some examples of algal species such as Spirulina, which predominate in a body of water as the major photosynthetic species. In small-scale experiments, we found that a continuous culture of Spirulina was readily maintained throughout the summer when the cell density was kept constant by continuously filtering the excess biomass, returning the effluent to the pond, and keeping the volume of the medium constant by adding tap water daily. Carbon dioxide was added to maintain the pH between 9.5 and 9.8, and the nutrient level was maintained by analyzing for PO_4^{3-} and NO_3^- three times weekly and adding appropriate amounts of the entire mineral nutrient mixture, as needed. As long as the temperature in the pond did not fall below 20°C, Spirulina cultures could be kept

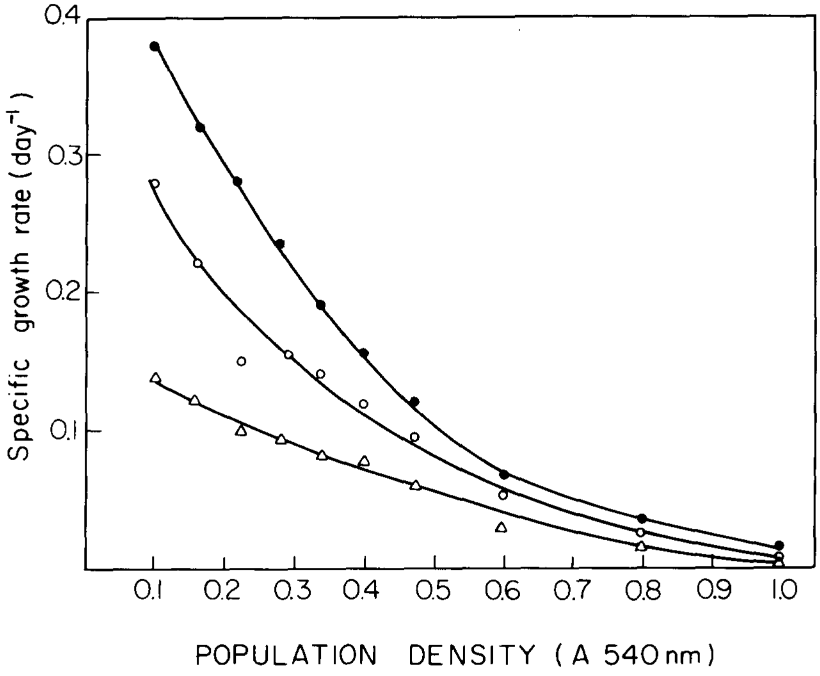

Fig. 3. Effect of cell density on the specific growth rate in August–September (top), May–June (middle), and December–January (bottom).

essentially clean of other organisms. The number of bacterial cells did not increase above $1 \cdot 10^4\ ml^{-1}$. In addition, analysis of the daily specific growth rate did not reveal any signs of self-limitation in the pond throughout its continuous operation from April to October.

A crucial requirement for pond maintenance is a way to constantly and readily evaluate the relative performance or 'well-being' of the culture in the pond. We, as well as others, have found that the partial pressure of oxygen in the pond during the daytime serves as a useful tool. Table 2 shows the effects of the intensity of solar irradiance and of temperature on pond oxygen. Data were recorded hourly throughout the year. Most figures represent the average of many scores of observations, 10% of which were deleted from both extremes. All readings were made at 1 PM, when the concentration of oxygen in the pond was usually increasing. The highest oxygen content was recorded at the highest values of temperature and irradiation, thus further illustrating that production of this warm-water algal species depends on both of these environmental parameters.

Table 2. Effects of temperature and the intensity of solar irradiance on oxygen concentration in *Spirulina platensis* ponds

Temperature (°C)	Pond oxygen content (% saturation)					
	Incident light (klux)					
	0–5	5–20	20–40	40–60	60–80	80–100
6–12	71	–	–	–	–	–
12–18	87	95	109	115	145	–
18–24	88	101	113	122	140	157
24–30	94	108	120	131	142	181
30–36	107	–	135	125	155	208

Conclusion

Consistent efforts towards optimization of algal biomass production, harvesting, and product processing, as well as genetic improvement and basic research will lead in time to improved biotechnologies of significant economic importance. This seems especially useful in arid lands, where agricultural productivity is particularly low and cultivation of conventional crops is severely handicapped. In these areas, algaculture in brackish or sea water has distinct advantages.

References

1 Issar A 1981 The role of non-replenishable aquifers in development projects in arid regions. *In* Settling the Desert. Eds. L Berkofsky, D Faiman and J Gale. pp 117–128. Gordon and Beach, London.

2 Richmond A 1983 Phototrophic microalgae. *In* Biotechnology, Vol. 3. Eds. H J Rehm and G Reed. pp 109–143. Verlag Chemie, Weinheim.