Production of *Spirulina* Biomass: Maintenance of Monoalgal Culture Outdoors

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Summary

The effects of sodium bicarbonate concentration, population density, and temperature on the maintenance of an outdoor monoculture of the cyanobacterium *Spirulina platensis* were studied. A clear response by *Spirulina* to the concentration of bicarbonate was evident, with 0.2*M* bicarbonate representing the lowest concentration in which a monoculture could be maintained. When the temperatures fell during the winter period to some 20–25°C below the optimum for *Spirulina*, *Chlorella* sp. gradually increased and became the dominant species in the culture. Raising the temperature by covering the pond with transparent polyethylene resulted in a sharp decline in the population of *Chlorella*, and a gradual resumption of species dominance by *Spirulina*. In winter, there was an inverse relationship in the pond between the population density of *Spirulina* and the extent of contamination by *Chlorella* sp.; but no such effect was observed under field conditions at temperatures higher than 25°C.

INTRODUCTION

Cultivation of algal monoculture outdoors is hampered by contamination with other algae and with zooplankton. Scientists therefore seek appropriate conditions which will selectively promote the growth of the alga of interest and/or inhibit the growth of the alien organisms.

Different environmental factors were suggested as the selective forces in different algal systems. Nutrients were shown by Schantz and co-workers[1] to affect the selective growth of two strains of *Anabaena*. In a system containing *Anabaena* and *Chlorella*, light was suggested to control their selective growth.[2] The light optima for the two algae were distinctly different, the former being light saturated at one-half of the saturating light intensity of the latter. In this system, temperature and pH optima were also significantly different. Mur and co-workers[3] also indicated that light may serve as a selective force in the competition between green and blue-green algae. They suggested that mutual shading of the algae should diminish the average intensity of the available light, thus providing conditions more favorable for a number of blue-green algae than for green algae. A distinct temperature effect on the dominance relations between different marine phytoplankton species was found by Goldman.[4] Below 19°C, the diatom *Phaeodactylum tricornutum*

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was dominant, whereas above 27°C, the blue-green *Oscillatoria* became progressively dominant. The initial relative abundance of the different species in a culture seems also to affect the outcome of the competition. In a mixed culture, *Anabaena* dominated *Chlorella* except when the initial concentration of *Chlorella* was high relative to that of *Anabaena*.[5]

How the algae exert their effects on one another is not understood yet. A filtrable substance capable of suppressing the growth of *Chlorella* was found to be produced by *Microcystis* and *Anabaena*. *Chlorella*, on the other hand, was not found to exert by influence on blue-green algae by means of soluble materials.[5]

In the framework of our long-term study on *Spirulina* biomass production, we investigated the effect of various environmental factors on the growth of the alga.[6] In the present work, we attempted to elucidate the conditions controlling the competition between *Spirulina* and *Chlorella* in outdoor ponds.

MATERIALS AND METHODS

The Organism and Growth Conditions

The blue-green alga *Spirulina platensis* was cultivated in Zarouk's medium[7] indoors and outdoors as described in a previous work.[6] Under laboratory conditions, *Spirulina* was cultivated in 250-mL flasks placed on a shaking rotator at 30°C. Illumination was provided by cool white lamps providing altogether 60–70 μeinstein m^{-2} s^{-1}. Under outdoor conditions, the daily maximal light intensity fluctuated between 600 μeinstein m^{-2} s^{-1} on a cloudy winter day and 2000 μeinstein m^{-2} s^{-1} on a bright summer day. The maximal daily fluctuations in temperature varied from 10°C in winter to 36°C in summer in the pond.

Estimation of Contamination

Three different methods were used for the determination of the extent of contamination of the *Spirulina* culture.

1) The first utilized was the ratio of methanol-extracted chlorophyll to the dry weight. This ratio essentially indicates the extent of contamination by zooplankton. Total chlorophyll and dry weight were determined according to Vonshak and co-workers.[6]

2) The second technique was differential extraction of chlorophyll by acetone and methanol. Methanol extracts chlorophyll from blue-green and green algae, while acetone extracts chlorophyll from blue-green algae primarily. Total chlorophyll was calculated after Holden,[8] as follows:

$$\text{Chlorophyll (Chl)}\ (a + b) = 22.5E_{650} + 4.0E_{665}\ \text{(for methanol and acetone)}$$

The percent of blue-green algae in the sample was derived according to the following equation:

$$\frac{\text{acetone-extracted Chl } (a + b)}{\text{methanol-extracted Chl } (a + b)} \times 100 = \text{percent blue-green algae in the sample}$$

The efficiency of the method in determining the extent of contamination of the blue-green *Spirulina platensis* by the green alga *Chlorella* is illustrated in Table I.

3) The last method used was microscopic measurement of relative volumes in a hemocytometer. The reproducibility of this method is very good, the variability of counts in a given sample being not greater than $\pm 1\%$. A somewhat complicating factor in this method stems from the fact that *Spirulina platensis* is a polymorphic organism, i.e., two forms are observed most frequently. In one, the filament is relatively short, and the spirals are wide and large. In other form, the filament is much longer, and the spirals are much narrower and smaller than in the first form (Fig. 1). A single long filament may be composed of both forms. To measure the relative purity of the *Spirulina* culture, an aliquot was taken from the pond and placed in a hemocytometer with a cell volume of 10^{-4} mL. The number of turns in the filament was counted. The volume of each turn, which included the cells as well as the outer envelope, was found to be 2.390 μm^3 in a filament of the first type and 525 μm^3 in the second type. Summing up the number of large and small turns and multiplying by their respective volumes yields the overall volume of *Spirulina* filaments in the counting chamber. The average volume of a *Chlorella* cell in our culture was found to be 28 μm^3. By multiplying this number by the number of cells, the total volume was obtained. On the basis of these figures, the percent of each algal species in the total algal population could be calculated. This method, albeit time-consuming, is by far the most accurate method for quantitative determination of the extent of deviation from a monoculture of *Spirulina*.

RESULTS AND DISCUSSION

Bicarbonate Effect

The effect of bicarbonate on the growth rate of *Spirulina* was tested in the laboratory at the concentration range 1-30 g/L. Under all bicarbonate concentrations, the logarithmic phase of growth began some 21 h after the inoculum was introduced. Twenty-three hours later, a slight decrease in the growth rate was already evident in cultures grown in a solution of 1 or 2 g bicarbonate/L. At the 44th hour, the growth rate of cultures grown in a solution of 4 or 8 g Na-bicarbonate/L was clearly smaller than that manifested by cultures grown at 15 or 30 g/L (Fig. 1). In contrast, no decrease in the growth rate of *Spirulina* was detectable in the field experiments even in cultures maintained at a bicarbonate concentration as low as 4 g/L, i.e., one-quarter of the bicarbonate concentration in the Zarouk formula (not shown). Under these conditions, however, a significant increase in the extent of con-

TABLE I
The Relative Chlorophyll Extraction Efficiencies by Methanol and Acetone

Alga	Chlorophyll (mg/L)		$A/B \times 100$	Theoretical ratio
	A acetone extraction	*B* methanol extraction		
Spirulina	16.38	17.24	97.9	100
Chlorella	0.40	5.17	7.7	0
Spirulina and *Chlorella* mixture (1:1 v/v)	8.51	11.84	71.8	75.9

tamination of the *Spirulina platensis* culture by *Chlorella* species was observed. Repeated experiments indicated that it was impossible to maintain a clean, monoalgal culture of *S. platensis* outdoors even with 8 g/L of bicarbonate [Fig. 4(c)]. When 10% of *Chlorella* (w/w) was added to a batch culture of *S. platensis* grown with 16 g/L bicarbonate under laboratory conditions, the population of *Chlorella* declined steadily as soon as the cells were introduced, and eight days later the population of *Chlorella* disappeared completely. In a *Spirulina* culture grown with 8 g/L bicarbonate, the population of *Chlorella* added to the culture also declined gradually, but it nevertheless stabilized at 1% of the total algal biomass [Figs. 2 and 4(d)].

A different pattern emerged in cultures grown outdoors, where a continuous culture of *S. platensis*, grown with 4 g bicarbonate/L, was harvested se-

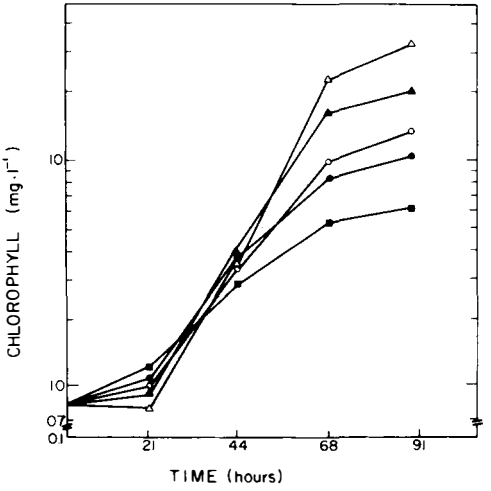

Fig. 1. Effect of bicarbonate concentration on the growth rate of *Spirulina platensis* grown in batch culture in the laboratory at 30°C: (■—■) 2 g/L $NaHCO_3$, (○—○) 8 g/L $NaHCO_3$, (●—●) 16 g/L $NaHCO_3$, (▲—▲) 32 g/L $NaHCO_3$, (△—△) 50 g/L $NaHCO_3$.

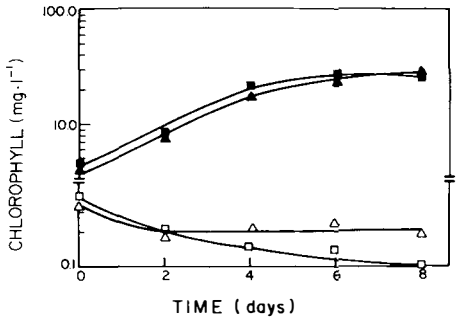

Fig. 2. Growth rate of *Spirulina platensis* and *Chlorella* grown in batch cultures in the laboratory in full Zarouk medium (16 g/L $NaHCO_3$) or Zarouk medium containing 8 g/L $NaHCO_3$ only: (■—■) *Spirulina* in full Zarouk medium, (□—□) *Chlorella* in full Zarouk medium, (▲—▲) *Spirulina* in Zarouk medium containing 8 g/L $NaHCO_3$, (△—△) *Chlorella* in Zarouk medium containing 8 g/L $NaHCO_3$.

lectively by filtration on a 400-mesh vibrating screen (Sweco, Los Angeles, CA). The screen did not retain the *Chlorella* cells, and continuous enrichment of the culture with *Chlorella* sp. took place so that, after several harvests, over 50% of the algal biomass in the pond was identified as belonging to some species other than *Spirulina*. At 16 g/L of bicarbonate, however, the dominance of *Spirulina* was maintained, as indicated by the stability of the chlorophyll ratio (Fig. 3).

The population shift in the pond to dominance by *Chlorella* sp. under a low bicarbonate regime resulted in the development of a food chain, which consisted of several *Chlorella* grazers belonging to a variety of ciliates as well as their predators (e.g., copepodes, termatodes), some of which are shown in Figures 4(a) and 4(b). Indeed, in cultures maintained in 4 g/L bicarbonate solution, in which heavy contamination with *Chlorella* was evident, total chlorophyll, as percent of total organic dry weight, underwent great fluctua-

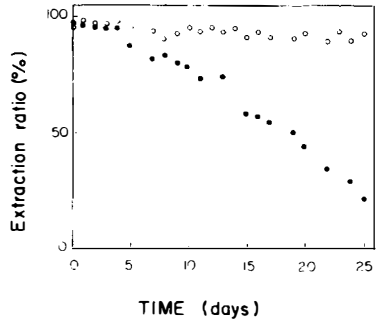

Fig. 3. Effect of bicarbonate concentration on the extraction ratio of chlorophyll in outdoor cultures grown in 100-m^2 ponds: (○) full Zarouk medium, (●) Zarouk medium containing 4 g/L $NaHCO_3$ only.

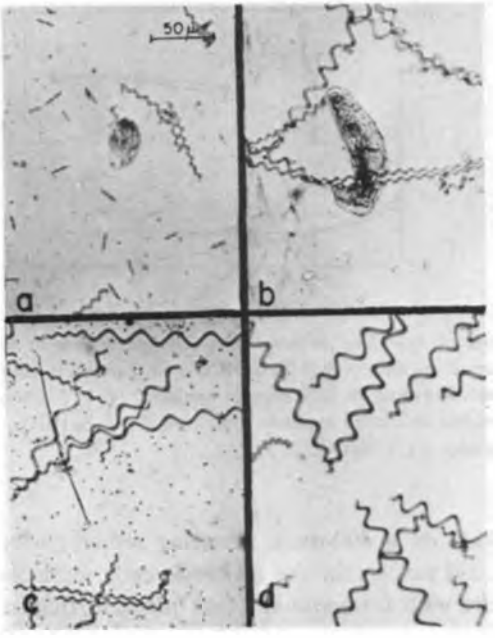

Fig. 4. Effect of bicarbonate concentration on the purity of *Spirulina* outdoor cultures grown in 100-m^2 ponds: (a) Zarouk medium containing 2 g/L $NaHCO_3$, (b) Zarouk medium containing 4 g/L $NaHCO_3$, (c) Zarouk medium containing 8 g/L $NaHCO_3$, (d) full Zarouk medium.

tions, reflecting rapid shifts in the populations of both *Chlorella* and its predators (Fig. 5). In contrasts, in a pond containing a monoculture of *Spirulina*, this ratio was kept steady at about 1.5%.

Population Density Effect

Whereas high bicarbonate concentrations (16 g/L) caused *Chlorella* grown under laboratory controlled conditions to disappear within a few days, it did not have the same effect on an outdoor culture, i.e., *Chlorella* did not disap-

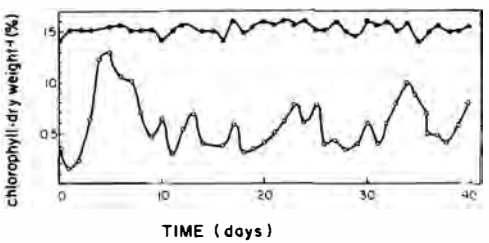

Fig. 5. Effect of bicarbonate concentration on the chlorophyll dry weight ratio in a *Spirulina* outdoor culture: (●—●) full Zarouk medium, (○—○) Zarouk medium with 4 g/L $NaHCO_3$.

pear completely. Our interpretation of this observation was that field conditions fell short of the optimal requirements for *Spirulina* growth. Indeed, the population density of *Chlorella* in such *Spirulina* ponds stabilized, albeit at a low percentage—1-5% (v/v)—of the total algal biomass. Within this range, the population density of *Spirulina* was observed to affect that of *Chlorella* in that, the lower the density of the *Spirulina* population, the larger became that of *Chlorella* (Table II). Thus, the percent of contaminating *Chlorella* biomass in heavily populated *Spirulina* cultures (1200 mg dry wt./L) was between one-half and one-quarter of that present in the less dense *Spirulina* cultures of 660 mg dry wt./L. This could perhaps indicate the existence, at least under certain conditions, of a slight antibiotic effect exerted by *Spirulina* on *Chlorella*. Alternatively, it could reflect the possibility that with decreased availability of light irradiance—as would be caused by increased mutual shading at high densities of *Spirulina*—the blue-green *Spirulina* has an added advantage over the green *Chlorella*.

Temperature Effect

The effect of temperature on the growth of *Spirulina* became prominent with the advent of winter. At this time, the maximal daily temperature became increasingly lower, reaching about 10-15°C in midwinter, and the minimal diurnal temperature reached close to freezing point. The net growth of *Spirulina platensis* ceased altogether at such temperatures, and the overall biomass in the pond declined steadily. Under these circumstances, a steady takeover of the pond by *Chlorella vulgaris* was clearly evident and, at the start of the experiment summarized in Table III, the population of *Chlorella* consisted of nearly half of the pond biomass.

Covering the pond with a 0.2-mm-thick polyethylene sheet raised the temperature of the medium by 5-7°C above that in the uncovered pond. This temperature increase had a very marked effect on the culture. The growth of *Spirulina* was resumed, as evidenced by a steady threefold net increase in chlorophyll during the 28-day period that followed the installation of the cover. Concomitantly, a substantial steady decrease in the *Chlorella* popula-

TABLE II
Effect of the Population Density of *Spirulina* on the Relative Contamination (Percent of the Total Population) by *Chlorella* in 1-m^2 Outdoor Ponds during Fall and Early Winter; Mean Daily Temperature in the Ponds was 19-20°C

Spirulina mg dry wt./L	Month			
	September	October	November	December
660	3.2	4.1	2.8	2.6
930	2.9	1.1	1.4	0.7
1200	1.7	0.7	1.5	0.8

TABLE III
Effect of the Temperature on the Relative Abundance of *Chlorella* in a *Spirulina* Culture Growing in a 100-m^2 Pond

Date, 1982	Time after pond was covered (days)	Covered pond				Uncovered pond			
		Diurnal temperatures		Population density (mg chl a/L)	*Chlorella* [% of biomass (v/v)]	Diurnal temperatures		Population density (mg chl a/L)	*Chlorella* [% of biomass (v/v)]
		maximum	minimum			maximum	minimum		
13 Jan	0	22	14	4.96	35.0	16.0	8.0	5.44	40
20 Jan	7	21	12	6.80	30.0	14.0	4.5	4.48	40
27 Jan	14	23	15	10.67	15.0	20.0	10.0	4.82	35
2 Feb	21	22	14	11.70	4.0	14.0	8.0	5.65	34
10 Feb	28	22	13	15.70	5.0	15.0	6.0	6.01	40

tion was evident upon covering the pond, the population diminishing from 35% to 4% of the total algal-cell volume in the culture. Evidently, when increased temperature effected the resumed growth of *Spirulina* grown in a high-bicarbonate medium, *Chlorella* could not compete, and a continuous decrease in its population ensued.

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