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# Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: a predictive model of algal yield

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**Abstract**

*Spirulina* is one of the most extensively used microalgae for animal and human nutrition; its main interest is centered in its high protein content, 60–65% on a dry weight basis. In this study, *Spirulina* was grown in open raceway ponds, and several physicochemical (e.g., pH, dissolved oxygen concentration, temperature, conductivity and irradiance) and biological (e.g., biomass concentration and yield) variables were studied. The variables were correlated in order to implement a mathematical model to predict algal yield. Dissolved oxygen concentration in the cultivation ponds ranged between 10 mg $l^{-1}$ in winter (115% of $O_2$ saturation) and 30 mg $l^{-1}$ in summer (375% of $O_2$ saturation); a clear decrease of biomass concentration was found when dissolved oxygen was >25 mg $l^{-1}$. Neither biomass concentration nor productivity was saturated at the maximum temperature achieved in the open pond during this study (approximately 28 °C). The pH seemed to control both the maximal algal density in the pond and the productivity that were found to be maximum at pH values below 10.5. Finally, all the variables were positively correlated with irradiance. Principal component analysis (PCA) allowed recognition of different sets of samples characterized by a combination of temperature, dissolved oxygen concentration, pH, biomass, productivity, irradiance and conductivity. This method helped to predict a significant loss of productivity in the open ponds in mid-summer due to high pH and high-dissolved $O_2$ concentration.


*Keywords:* *Spirulina*; *Arthrospira*; Mass culture; Open ponds; Productivity

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## 1. Introduction

The growing demand for natural products for the health food and beauty products markets has attracted the interest for microalgal biotechnology during the last two decades. This burst of activities related to microalgae production has led to develop many aspects of their ecology, physiology and biochemistry. Mainly, three species of microalgae have attracted, until now, the attention of scientists and companies, i.e., *Dunaliella*, *Chlorella* and *Spirulina*, but many others are currently under research for the production of high-value compounds (see Tredici et al., 2000). The major concern in microalgal biotechnology is the low efficiency and yield of mass cultures outdoors, so there is a need for improvements in the growth performance of microalgae in these conditions (Richmond, 2000). Current yields obtained in industrial facilities range from 10 to 30 tonnes dry biomass ha$^{-1}$ year$^{-1}$ (Sasson, 1997), but production costs are still high.

*Spirulina* is nowadays produced commercially by several companies around the world, and the product is mainly sold as a food supplement and animal feed (Belay et al., 1993). Its commercial production is based almost exclusively on open raceway ponds (Tredici et al., 1993). The two principal advantages of open culture systems are a small capital investment and the use of a free source of energy, the solar irradiance (Chaumont, 1993). They implement the simplest method of algae cultivation and are only intended for algae species growing in selective environments. However, the productivities obtained are far from the theoretical maximum.

Apart from long natural light periods, most microalgae produced commercially require high temperature for optimal growth, thus their cultivation is restricted to tropical and subtropical regions. In other areas, e.g., temperate latitudes, high temperature to support mass cultivation of microalgae are only achieved in the summer. *Spirulina* has an optimal growth temperature of around 35 °C and no net growth occurs outdoors below 18 °C (Richmond, 1986).

Several previous attempts have been taken to cultivate *Spirulina* under Mediterranean climate (Belay, 1997), but all of them have failed (although nowadays the correct generic name for most species and strains cultivated with industrial purposes seems to be *Arthrospira*, historically and commercially the name *Spirulina* has been extensively used. For this reason, we will use this last generic name throughout this work). Recently, Chini-Zitelli et al. (1996) demonstrated the viability of small-scale outdoor cultivation of *Arthrospira* (*Spirulina*) *platensis* during autumn and winter in temperate climates in tubular bioreactors, and Fornari et al. (1999) reported an initiative in Southern Italy for the production of microalgae, including *Spirulina*, using the same technology. In a previous work (Jiménez et al., 2003), the viability of industrial production of *Spirulina* in southern Spain was evaluated. To develop the project, a pilot plant consisting of several raceway open ponds of increasing surface area up to 450 m$^2$ was built and tested.

In this work, we have studied the relationships between several physicochemical variables (e.g., pH, dissolved oxygen concentration, temperature, conductivity and irradiance) and those related to microalgal productivity (e.g., biomass concentration and yield) in open raceway ponds for the production of *Spirulina*. A model to predict algal yield has been developed in order to know the major biological limitations for the production of *Spirulina* in this area.

## 2. Material and methods

### 2.1. Site

Málaga is located in southern Spain (36°42′N, 4°28′W), on the Mediterranean coast. This is one of the sunniest areas in Europe, with average temperature during day-time usually exceeding 30 °C during the summer, while in winter it is above 15 °C. Irradiance is also high (Fig. 1), averaging >1.5 × $10^7$ J $m^{-2}$ $day^{-1}$ during the summer and 0.4 $10^7$ J $m^{-2}$ $day^{-1}$ in winter (irradiance was measured by means of specific sensors in the range 280–700 nm; see below).

### 2.2. Microorganisms and culture conditions

*Spirulina platensis* strain Laporte M132-1 was used for this study. It was grown in Zarrouk's (1966) medium. The preindustrial unit consisted in four ponds of 1350 l of capacity, two of 13,500 l and one of 135,000 l (450 $m^2$), with a culture depth of 30 cm. These ponds were lined with food-grade polyvinylchloride (PVC). Cultures were circulated at a speed of 30 cm $s^{-1}$ by means of paddle-wheels. Several physicochemical variables (e.g., pH, temperature, dissolved $O_2$ concentration and conductivity) were recorded daily at mid-day with a WTW Pocket Meter MultiLine P3 pH/OXI.

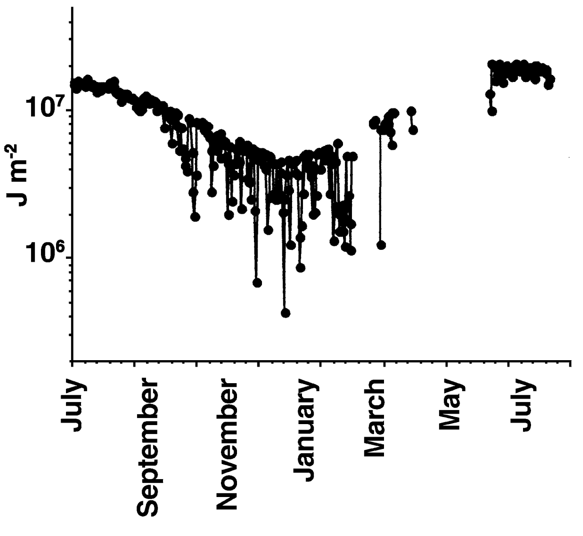


Fig. 1. Integrated daily irradiance in the waveband 280–700 nm at the location of the study. Irradiance averaged >1.5 $10^7$ J $m^{-2}$ $day^{-1}$ in summer and 0.4 $10^7$ J $m^{-2}$ $day^{-1}$ in winter.

### 2.3. Harvesting and drying

Before harvesting, the biomass concentration was estimated by sampling and filtering 500 ml of the culture through a 62-μm mesh. The filter was weighed to obtain fresh weight. Dry weight was estimated after drying the filters at 105 °C for 24 h.

Harvesting was performed by pumping the cultures into a Sweco LS24S555 vibrating screen (Sweco, Belgium), and filtered through two membranes of 1.5 mm (to remove insects and other undesirable material) and 62 μm. The filtrate was pumped back into the ponds, while the algal slurry was pumped to a Niro minor spray-drier (Niro, Denmark) without further washing.

### 2.4. Measurement of solar radiation and air temperature

Solar irradiance and air temperature were continuously measured using a dosimeter (ELDONET, Real Time Computer, Möhrendorf, Germany) that takes readings in three wavelength bands (UV-B, 280–315 nm; UV-A, 315–400 nm; PAR, 400–700 nm) at 1-s intervals, storing them in a computer.

## 3. Results and discussion

In a previous work (Jiménez et al., 2003), we have reported the results of the pilot study in which we estimated the viability of *Spirulina* industrial production in southern Spain. Several trials were made in open raceway ponds of increasing area, from 4.5 to 450 $m^2$. The results of the study showed that outdoors cultivation of *Spirulina* in southern Spain was satisfactory at temperature above 15 °C and that commercial production would be feasible at this location during at least 9 months per year. Potential average productivity for this period would be in the order of 10.3 g DW $m^{-2}$ $day^{-1}$, while averaged 12 months would be 8.2 g DW $m^{-2}$ $day^{-1}$ with an expected yield of 30 Tn $ha^{-1}$ $year^{-1}$. Nevertheless, a partial loss of productivity in mid-summer was detected.

### 3.1. Evolution of the physicochemical and biological variables

A 450-$m^2$ open raceway pond was inoculated on September 23rd, 1997 and it was operative until July 29th, 1998. Fig. 2 shows the evolution of the temperature, dissolved oxygen concentration, pH and conductivity in the pond. All data were recorded at 12:00 h local time. Maximal temperature of the culture at mid-day was 28 °C in July, while the minimal recorded temperature was 9 °C in December (Fig. 2a). Average winter temperature of the culture at mid-day was about 12 °C, while in spring and autumn it was around 20 °C. Although *Spirulina* is considered a thermophilic microorganism with an optimal growth temperature of 35–37 °C, Vonshak (1997a) reported that the optimum range of temperature is variable, and different strains will differ both in their optimal growth temperature and their extreme ranges. It has been previously reported that, for outdoor cultivation, the minimal temperature that allows growth is around 18 °C and that

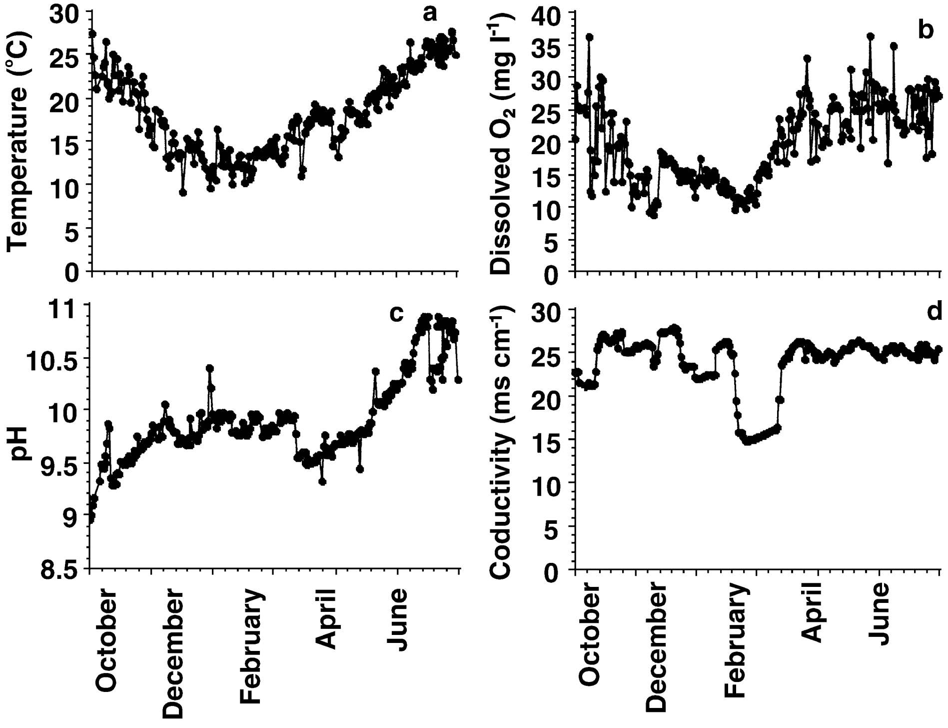


Fig. 2. Evolution of the temperature, dissolved oxygen concentration, pH and conductivity in the 450 $m^2$ pond during the whole period of operation (September 23rd, 1997 to July 29th, 1998). Drop in conductivity in the month of February was due to heavy local rains.

cultures deteriorate quickly when maximum day-time temperature is below 12 °C (Richmond, 1986). However, different low-temperature limits may be expected for the different species, strains or isolates of *Spirulina*. Vonshak and Tomaselli (2000) have shown that several strains may have different optimal growth temperature, ranging from 24 to 42 °C.

Dissolved oxygen (Fig. 2b) also showed a marked variability over the year, with minimum figures of 10 mg $l^{-1}$ in winter (115% of $O_2$ saturation) and maximum around 30 mg $l^{-1}$ (375% of $O_2$ saturation) in summer. It has been previously reported (Vonshak, 1997a) that, in large ponds with small water circulation and turbulence, $O_2$ concentration may reach as high as 500% of saturation, inhibiting photosynthesis and growth, and eventually leading to culture death (Márquez et al., 1995; Singh et al., 1995).

Initial pH of the cultures after inoculation was close to 9.0, increasing rapidly after few days to values around 9.8–9.9 (Fig. 2c). The pH remained stationary, below 10.0, during autumn, winter and spring, increasing to values close to 10.9 in mid-summer. It must be pointed out that no system for pH control was established during this pilot study. *Spirulina* is an alkaliphile (Grant et al., 1990), with an optimum pH range of 9.5–10.5, and it does not grow well at pH below 8.0. Maintaining the pH above 9.5 is mandatory in large open ponds in order to avoid contamination with other microalgae (e.g., diatoms, *Chlorella* and cyanobacteria). However, a decline of the growth rate has been detected at pH above 10.5 (Fox, 1996: Belay, 1997).

Finally, conductivity of the culture (Fig. 2d) did not show significant variations during the culture period, ranging between 22 and 28 mS $cm^{-1}$, due to daily refilling with freshwater to compensate evaporation. During the first days of February 1998, a marked dilution of the culture occurred due to heavy local rains. In order to recover the normal conductivity, part of the culture was pumped to the vibrating screen and the growth medium discarded. The algae were pumped back to the ponds and nutrient concentration was adjusted to initial level.

Together with these variables, biomass concentration and productivity were recorded daily (Fig. 3). Biomass concentration (also called "areal density") (Fig. 3a) rapidly increased after inoculation of the pond, rising from 15 g DW $m^{-2}$ to >140 g DW $m^{-2}$ in 13 days. During summer, areal density was kept at values around 140 g DW $m^{-2}$, that were progressively decreased during autumn to get a concentration below 100 g DW $m^{-2}$

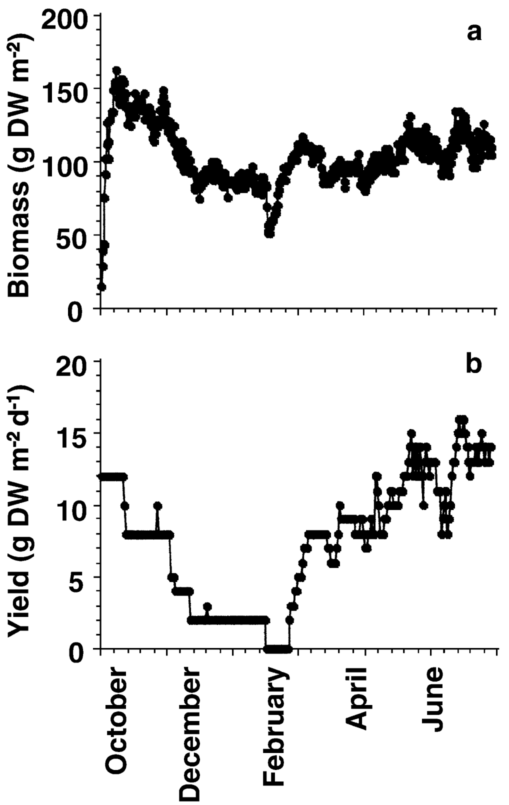


Fig. 3. Biomass concentration (a) and productivity (b) of the 450 $m^2$ pond during the operative period. The pond was inoculated on September 23rd, 1997 and biomass harvesting started on October 7th, 1997 until July 29th, 1998. The pond was never emptied during this period. Harvesting was stopped in February due to dilution of the cultures by rain.

Table 1
Average monthly productivity of the growth pond during the period of operation, expressed in g DW $m^{-2}$ $day^{-1}$

| Month | Yield |
|---|---|
| October | 9.54 |
| November | 6.86 |
| December | 2.62 |
| January | 2.00 |
| February | 2.18 |
| March | 7.35 |
| April | 8.37 |
| May | 11.00 |
| June | 11.73 |
| July | 13.95 |
| August | n.a. |
| September | 10.70 |

No realistic data for the month of August are available (n.a.).

in winter. During spring, algal concentration started to rise again to values above 120 g DW $m^{-2}$. Algal yield (Fig. 3b) showed a clear decrease during autumn, from initial values of 12 g DW $m^{-2}$ $day^{-1}$ during October to a minimum of 2 g DW $m^{-2}$ $day^{-1}$ in December and January. Biomass harvesting had to be stopped for several days during

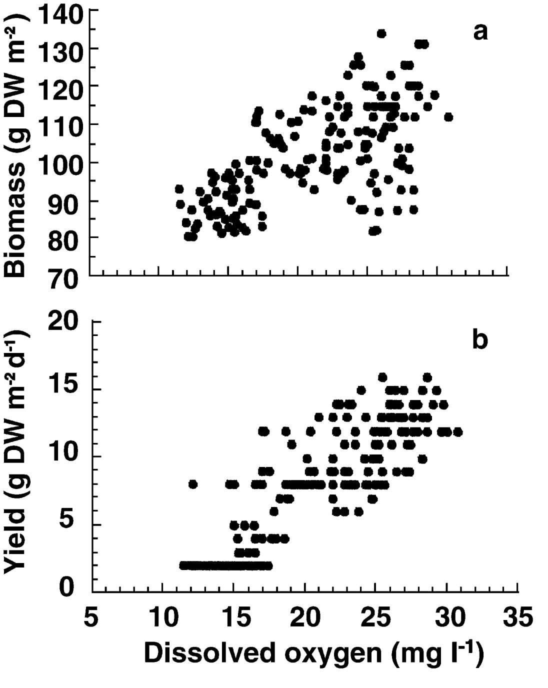


Fig. 4. Plot of biomass concentration (a) and of algal yield (b) in function of the dissolved $O_2$ concentration in the cultivation pond. A decrease of biomass concentration and a partial loss of productivity were detected at high $O_2$ concentration.

February due to rain. By the end of that month, a rapid increase in water temperature allowed biomass yield to increase to values around 8 g DW $m^{-2}$ $day^{-1}$. As the spring season progressed, algal yield increased to values around 12 g DW $m^{-2}$ $day^{-1}$, followed by a further increase in early summer to values around 14 g DW $m^{-2}$ $day^{-1}$. Average productivity is summarized in Table 1. Minimum productivity occurred in December (2.62 g DW $m^{-2}$ $day^{-1}$), January (2.0 g DW $m^{-2}$ $day^{-1}$) and February (2.18 g DW $m^{-2}$ $day^{-1}$). In spring and autumn, average productivity was 8.9 and 9.0 g DW $m^{-2}$ $day^{-1}$, respectively, while in summer an algal yield of 13.5 g DW $m^{-2}$ $day^{-1}$ might be expected.

### 3.2. Interrelations between areal density, productivity and physicochemical variables

Concentration of *Spirulina* in the cultivation pond and its daily yield was highly influenced by the values of the physicochemical variables in the ponds (e.g., dissolved $O_2$ concentration, pH, temperature, irradiance, etc.). Also, areal density determined the efficiency of algal growth, thus controlling productivity (Richmond, 1987). As it is seen in Fig. 4, both biomass concentration as well as algal yield were saturated at dissolved $O_2$ concentration above 25 mg $l^{-1}$, and even a partial loss of biomass and yield occurred, in agreement with Richmond (1986). According to Vonshak (1997a), when $O_2$ concentration was maintained in values as high as 20–22 mg $l^{-1}$, no significant reduction in photo-

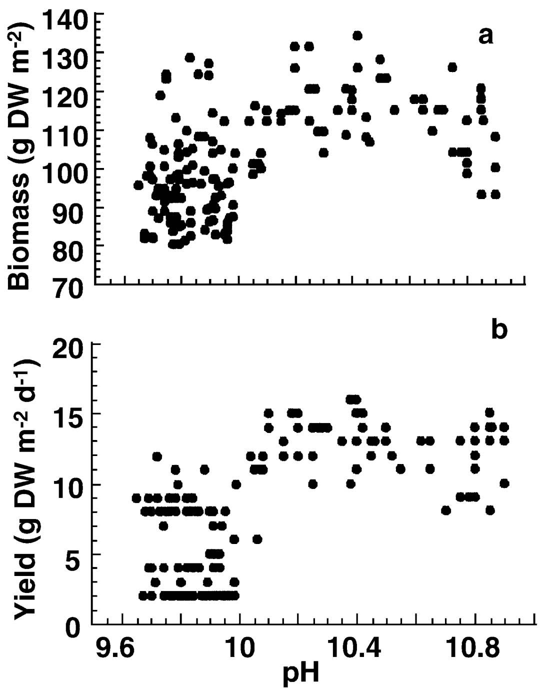


Fig. 5. Relationship of the areal density (a) and the productivity (b) with the pH of he cultures. A clear decrease of both biomass and productivity were detected at the highest values of pH in the pond.

synthesis occurred in *Spirulina* cultures and an approximate limit of 300% for $O_2$ concentration in large-scale cultivation ponds of *Spirulina* does not negatively affect algal growth. However, at 30–32 mg $O_2$ $l^{-1}$, a significant decrease in chlorophyll fluorescence yield of 15% was detected (Vonshak, 1997a). High pH (Fig. 5) exerted an even more strict control on *Spirulina* growth, and it is seen that, at pH values above 10.2–10.4, there was a clear decrease of biomass and yield, in agreement with previous reports (see Fox, 1996; Vonshak, 1997a; Vonshak and Tomaselli, 2000, for a review). Cossío et al. (2002) reported that growth rate of *Spirulina* in laboratory experiments was significantly reduced (15–20 % decay) at pH above 9.5. As for temperature, both biomass concentration and yield did not seem to be saturated in the range of temperature found in the cultivation pond during the study period (Fig. 6). Algal yield increased linearly in the range 10–28 °C, thus higher productivity might be expected at higher temperature. As for the irradiance (Fig. 7), there was not a clear saturation of biomass content and yield at the highest level of irradiance found at the surface of the cultures during the study period. Irradiance is a limiting factor in dense open-air cultures of *Spirulina* due to strong light extinction in the ponds and higher yield might be reached at higher irradiance. However, irradiance values higher than $2.1 \times 10^4$ kJ $m^{-2}$ are not expected to be reached in this area. Finally, areal density also influenced the productivity of *Spirulina* (Fig. 8). Maximal yield was found when biomass concentration was in the range 120–140 g DW $m^{-2}$, in agreement with previous reports

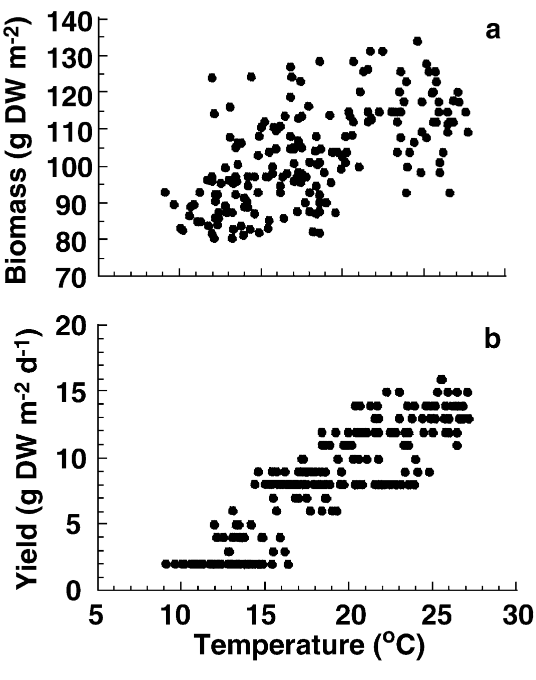


Fig. 6. Variation of the algal density and yield as a function of the temperature of cultivation pond during the study period. A linear increase of both variables was found when temperature of the culture increased, and no saturation or inhibition of growth were detected at the highest temperature found in the pond.

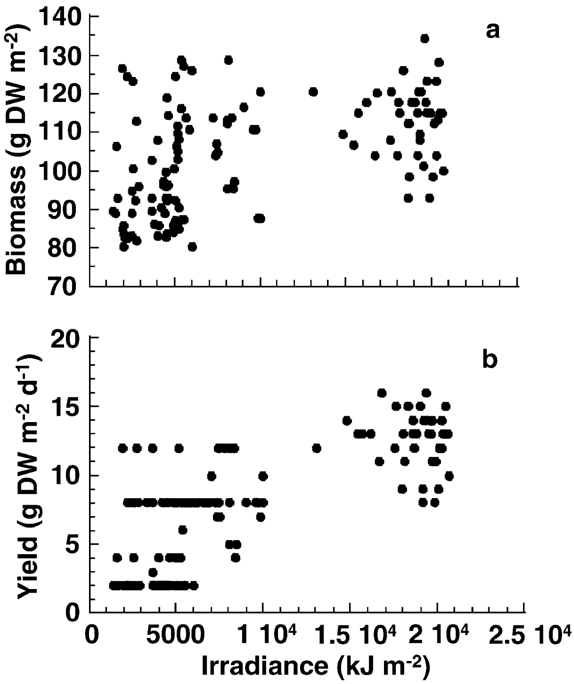


Fig. 7. Plot of the biomass concentration and of the algal yield as a function of the irradiance at the surface of the cultivation pond.

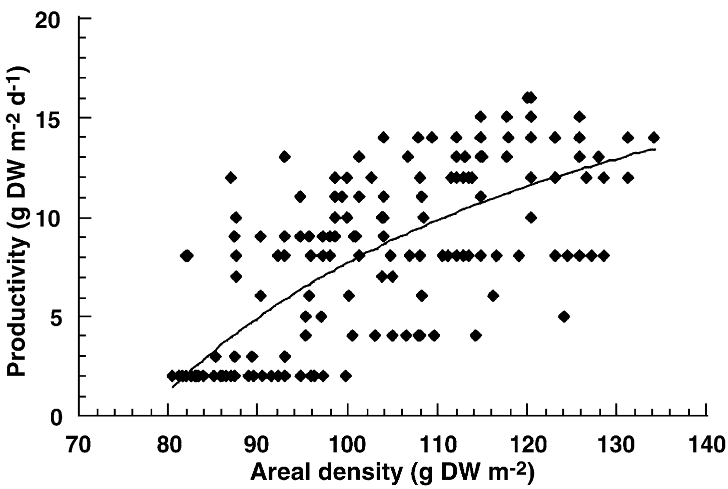


Fig. 8. Productivity vs. areal density of *Spirulina*. Algal productivity seemed to be saturated at biomass concentration >120 g DW $m^{-2}$.

(Richmond, 1987). Several authors have concluded that light is the main limiting factor for *Spirulina* growth outdoors in summer, while temperature limits growth in winter (Vonshak, 1997b). However, as seen before, excess of both dissolved $O_2$ and pH in the ponds significantly reduced biomass concentration and productivity, especially in mid-summer. No inhibition of growth by excess irradiance and temperature are expected in this area.

### 3.3. Prediction of Spirulina productivity

A multiple linear regression analysis was performed in order to predict the productivity in our cultivation system, in function of the values of the physicochemical variables and of the areal density in the ponds. However, as shown above, the relationship between productivity and the rest of the variables was not always linear. Thus, in order to get linear regressions between the dependent variable (productivity) and the independent ones (e.g., dissolved $O_2$ concentration, temperature, pH, irradiance and biomass concentration), double-inverse plots were constructed. Table 2 summarizes the results of the multiple regression, in which the inverse of the productivity ($1/P$) was calculated as a function of the inverse of the rest of variables (e.g., $1/[O_2]$, $1/T$, 1/pH, $1/I$ and $1/B$). The multiple regression coefficient was high ($r^2 = 0.899$), while the $F$-ratio was 270, indicating that the regression was highly significant. The obtained linear equation was used for calculating the expected productivity in our cultivation system and the theoretical algal yield was compared with the experimental data obtained during the cultivation period (Fig. 9). The model significantly predicted the productivity of our ponds. Regression coefficient between experimental and predicted values was highly significant ($r^2 = 0.756$, $n = 157$), being the slope of the lineal function 0.87.

Table 2
Multiple linear regression for prediction of *Spirulina* productivity. Two hundred and forty-four cases were included in the analysis, of which 87 were missing in at least one variable

| Source | Sum of squares | *df* | Mean square | *F*-ratio |
|---|---|---|---|---|
| Regression | 3.314 | 5 | 0.663 | 270 |
| Residual | 0.37 | 151 | 0.002 | |

| Variable | Coefficient |
|---|---|
| Constant | − 1.361 |
| $1/B$ | 54.095 |
| $1/[O_2]$ | 4.018 |
| $1/T$ | 1.676 |
| 1/pH | 7.638 |
| $1/I$ | 73.491 |

Dependent variable was 1/productivity (see text). Independent variables were 1/biomass, 1/temperature, $1/[O_2]$, 1/pH and 1/irradiance.

$1/P = -1.361 + (54.095*1/B) + (4.018*1/O_2) + (1.676*1/T) + (7.638*1/\text{pH}) + (73.491*1/I)$.

$P$ = Productivity (g DW $m^{-2}$ $day^{-1}$), $B$ = biomass concentration (g DW $m^{-2}$), $O_2$ = dissolved oxygen concentration (mg $l^{-1}$), $T$ = culture temperature (°C), pH = culture pH, $I$ = daily irradiance at the culture surface in the range 280–700 nm (kJ $m^{-2}$ $day^{-1}$).

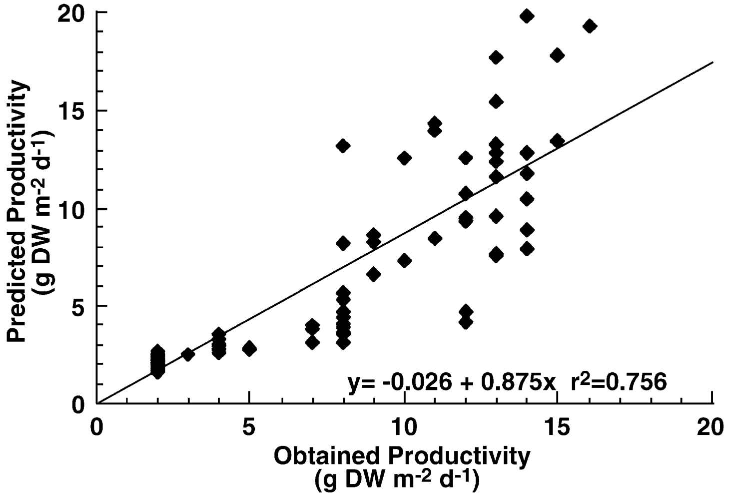


Fig. 9. Regression between the experimental and the theoretical algal yield predicted by the multiple regression model. Our model significantly predicted the productivity obtained in the pond during this study.

### 3.4. Principal component analysis (PCA)

A PCA was performed in order to ordinate the samples in two dimensions, allowing to recognize and classify different sets of samples characterized by a combination of temperature, dissolved oxygen concentration, pH, areal density, productivity, irradiance and conductivity.

Table 3
Results of the principal component analysis

| Eigenvalues | | | |
|---|---|---|---|
| | Values | Variance proportion | |
| e1 | 4.100 | 58.6 | |
| e2 | 1.579 | 22.6 | |
| e3 | 0.679 | 9.7 | |
| **Loading factors** | | | |
| | F1 | F2 | F3 |
| Productivity | 0.931 | 0.158 | 0.088 |
| Biomass | 0.587 | 0.708 | 0.242 |
| Oxygen | 0.871 | − 0.031 | 0.139 |
| Temperature | 0.952 | − 0.003 | 0.143 |
| pH | 0.560 | − 0.720 | − 0.298 |
| Irradiance | 0.871 | − 0.416 | − 0.051 |
| Conductivity | 0.388 | 0.600 | − 0.694 |

Two hundred and forty-four cases were included in the analysis, of which 87 are missing in at least one variable. Only the loading factors for the three first components are shown.

The loading factors of each variable in the definition of the new axis are shown in Table 3. The first component was positively related mainly to the productivity, dissolved oxygen concentration, temperature and irradiance. Thus, those samples with high oxygen concentration, temperature, productivity and irradiance would be located in the positive part of the first axis. The second axis was positively related to the biomass concentration and negatively to the pH. Thus, samples with high biomass and low pH would be located at the positive part of axis II. In Fig. 10, it is shown the position of the 157 samples included in the analysis. As it is seen, the plot of axis I vs. axis II ordinates the samples according to the season, from winter (negative part of both axis) to spring and autumn (positive part of both axis), indicating the temporal trend of the productivity of the system. However, samples from mid-summer were located in the negative part of axis II, evidencing that some inhibition of the productivity had happened during that season.

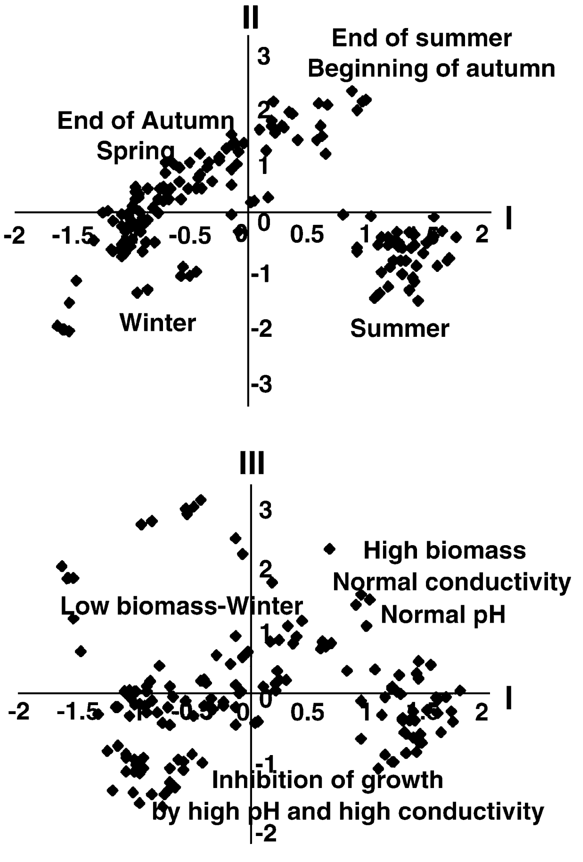


Fig. 10. Principal component analysis. Plots of axis I vs. axis II and axis I vs. axis III, representing the position of the 157 samples included in the analysis. Samples from mid-summer formed a cluster apart from those from spring and end of summer (axes I–II). The loading factors helped us to detect a loss of potential productivity due to high pH, $O_2$ and conductivity in mid-summer.

The explanation for this decrease in productivity was found in the plot of axis I vs. axis III. Here, it can be seen that in mid-summer there was a partial inhibition of growth (areal density and algal yield) at high pH, high conductivity and high $O_2$ concentration. Thus, in summer, our cultures were not developing their maximal capacity for production, due to partial inhibition by excess pH, $O_2$ concentration and conductivity.

## 4. Conclusions

From the results of this study, it can be concluded that pH and dissolved oxygen concentration make a strict control of the productivity of *Spirulina* in large-scale ponds. Principal component analysis is a powerful tool to detect partial loss of productivity induced by inappropriate operation of the cultures.

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