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Effect of temperature on yield and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors

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Abstract

Outdoor experiments carried out in Florence, Italy (latitude 43.8° N, longitude 11.3° E), using tubular photobioreactors have shown that in summer the average net productivity of a *Spirulina platensis* culture grown at the optimal temperature of 35 °C was superior by 23% to that observed in a culture grown at 25 °C. The rates of night biomass loss were higher in the culture grown at 25 °C (average 7.6% of total dry weight) than in the one grown at 35 °C (average 5%). Night biomass loss depended on the temperature and light irradiance at which the cultures were grown, since these factors influenced the biomass composition. A net increase in carbohydrate synthesis occurred when the culture was grown at a low biomass concentration under high light irradiance or at the suboptimal temperature of 25 °C. Excess carbohydrate synthesized during the day was only partially utilized for night protein synthesis.

Introduction

Because of seasonal and diurnal fluctuations, temperature represents one of the major biological limitations for biomass production of *Spirulina*. As pointed out by Vonshak and Richmond (1988), high net biomass output may not be achieved in open ponds due principally to the difficulties in maintaining the optimal temperature throughout the day and around the year. Indeed, even during summer when the day temperature reaches maximum values, the morning temperature of the culture, almost 10 °C below the optimum, prevents full exploitation of the photosynthetic capacity of the culture for a few morning hours. In a comparative study on the yield of *Spirulina* achieved in open ponds and in photobioreactors, the better yield in the latter was attributed to the better temperature profile maintained inside the photobioreactors (Torzillo *et al.*, 1986). Laboratory experiments have shown that the maximum biomass yield is obtained when *Spirulina* is grown at the optimal temperature of 35 °C (Tomaselli *et al.*, 1987; Payer *et al.*, 1980). To enhance yields of *Spirulina* biomass it seems important to maintain the culture temperature as close as possible to the optimum during the day.

Night temperature of the culture represents another important factor which may influence net biomass productivity since it affects night loss due

to respiration. Studies carried out on microalgae grown both in the laboratory and outdoors have shown that the overall loss during twelve hours of dark was 2–10% of the biomass prior to darkening (Grobbelaar & Soeder, 1985). Preliminary measurements of night biomass loss in outdoor cultures of *Spirulina* have shown that up to 35% of the biomass produced during the day may be lost during the night (Vonshak & Richmond, 1988). However, there is a lack of information concerning the magnitude of the night biomass loss in outdoor culture of *Spirulina* and about the factors influencing it. In order to obtain more data on the influence of temperature on productivity and night loss we have conducted a five-month experiment in outdoor thermostated photobioreactors.

Materials and methods

The culture equipment

The study was carried out in Florence, Italy (latitude 43.8° N, longitude 11.3° E). The design and instrumentation of the culture equipment have been described in detail elsewhere (Bocci *et al.*, 1987). It was composed of eight thermostated photobioreactors and four air-conditioned metallic shelters housing the control equipment and analysis instruments. The system was built and assembled by Carlo Erba Strumentazione, Milan. Each reactor consists in a loop made of ten parallel Pyrex tubes (length 2 m, i.d. 4.85 cm) connected to PVC (polyvinylchloride) U-bends with watertight flanges. The reactor is placed in a stainless steel basin containing thermostated demineralized water. The culture is recycled by a PVC pump having three flat PVC blades at 120° to each other on the propeller shaft; the distance between blades and casing is 1.3 cm. The working volume of each reactor is 51 litres.

Organism and culture conditions

S. platensis, strain M2, of the culture collection of the Centro di Studio dei Microrganismi Autotrofi of Florence was used. The culture medium has been described elsewhere (Bocci *et al.*, 1987). A pH close to 9.6 was maintained by automatic addition of CO_2. The dissolved oxygen concentration was maintained within the 8–20 mg l^{-1} range by bubbling air through porous candles in the culture as it flowed into the receiving vessel. The circulation speed of the culture was kept at 0.46 m s^{-1} during the day and 0.2 m s^{-1} during the night, for the whole duration of the experiment. Ammonia was used as the nitrogen source and supplied to the culture through a peristaltic pump. The ammonia concentration was checked at three-hour intervals during the day and maintained within the 1–2 mM range. From May to September we used two reactors, one thermostated at 35° C ± 1 °C and another at 25 °C ± 1 °C, by day and by night. A third reactor, thermostated at 35 °C ± 1 °C by day and 25 °C ± 1 °C by night, was set up during the month of July. The cultures were operated in a semicontinuous regimen. Daily dilution was performed at sunset using fresh medium and in such a measure as to restore the biomass concentration to 1100 ± 50 mg l^{-1}.

During another four-day experiment, we used three photobioreactors, thermostated at 35 °C ± 1 °C by day and 20 °C ± 1 °C by night, in which the culture speed was constant at 0.2 m s^{-1} by day and by night. Nitrate was used as the nitrogen source. The biomass concentrations tested were 710 ± 28, 1290 ± 80 and 2169 ± 398 mg l^{-1}.

Analytical procedures

Dry weight was determined in duplicate or triplicate 10 ml samples. The cells were washed twice with distilled water and dried at 105 °C for 3 h. In all the experiments night biomass loss was calculated as the difference between the dry weight measured at sunset and that measured before sunrise on the following morning. Elemental analysis of the biomass (C, H, N, O) was performed on triplicate samples with an elemental analyzer (model 1106, Carlo Erba Strumenta-

zione, Milan). The carbohydrate content was measured using the phenol-sulphuric acid method (Dubois *et al.*, 1956) (3 replicates) with D + glucose as a standard. Calculated ash was 100 – (C + H + N + O). Lipid was calculated as follows: lipid = 100 – carbohydrate-protein (N x 6.25)-ash. The combustion heat of the biomass was calculated according to Spoehr and Milner (1949).

Results

Temperature had a significant influence on the productivity and biochemical composition of *Spirulina* grown in outdoor thermostated photobioreactors. During a five-month experiment performed from May to September, the average net biomass synthesis during the daylight period in the culture grown at the optimal temperature of 35 °C was superior by 14% to that in the culture grown at 25 °C (Fig. 1A). It was also observed that the average biomass loss during the night was significantly higher in the culture grown at 25 °C (7.6% of total dry weight reached in the evening) than in the culture grown at 35 °C (5% of dry weight). This difference in biomass loss accounts for the more pronounced differences in the net productivity of the two cultures (23% average); i.e. productivity over 24 hours (Fig. 1B).

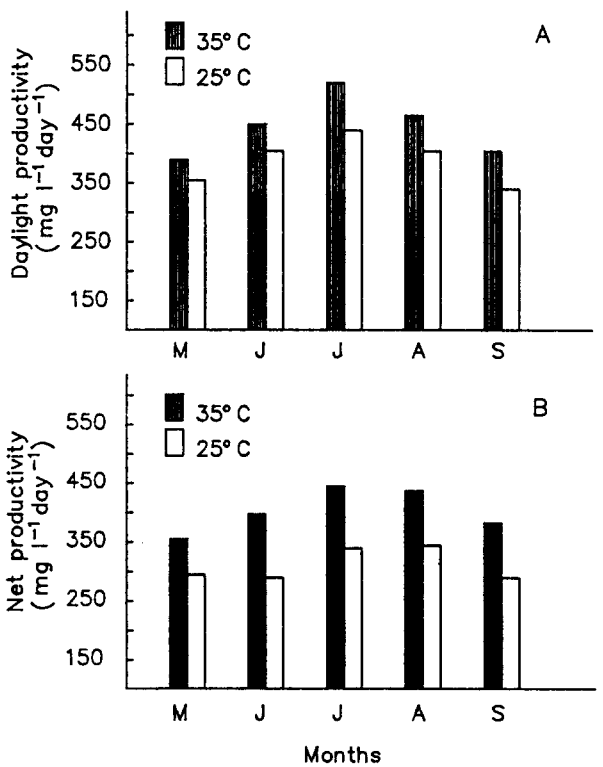

Fig. 1. Influence of temperature on the productivity of *Spirulina platensis* during the daylight period (A) and over 24 h (B). Ammonia was used as the nitrogen source.

Table 1 summarizes the monthly average values of the night biomass loss from May to September in two cultures grown at a constant temperature of 25 °C or 35 °C. As mentioned above, night biomass loss did not depend directly on the night temperature at which the cultures were maintained. This unexpected behaviour became more evident during the second month of the experiment: in June, the night biomass loss amounted to 10% of dry weight in the culture grown at 25 °C and 6% of dry weight in the one grown at 35 °C; during that month, the night biomass loss represented 31.5% and 16.0%, respectively, of daylight production. In order to determine whether this behaviour could be influenced by the growth temperature, in July we set up a third photobioreactor in which the culture was maintained at 35 °C during the day and at 25 °C during the night. The average night biomass loss in this culture was lower by 52% and 32% to those observed respectively in the cultures grown at the constant temperatures of 25 °C and 35 °C (Table 1). This experiment confirmed that the growth temperature had an influence on biomass loss during the night. Table 1 also shows that the amount of biomass lost during the night was not directly linked to the length of the night period. Indeed, in June we observed that the night biomass loss in the culture grown at the constant temperature of 25 °C was 1.75 times that observed in September in the same culture. In contrast, the night biomass loss appeared to be influenced by the quantity of light irradiance received by the cells in the preceding day: indeed, higher rates of night biomass loss were observed, especially in the culture grown at 25 °C, during the months of June and July when total light

Table 1. Influence of temperature on the night biomass loss in *Spirulina platensis* grown outdoors in thermostated photobioreactors: A = as % dry weight reached at the end of the daylight period; B = as % daylight productivity. Data are the mean of 26 determinations recorded over each month.

Month	Sunlight irradiance ($MJ\ m^{-2}\ d^{-1}$)	Mean length of the night (h)	Temperature regime (°C) Light : Dark	Night biomass loss	
				A	B
May	18	9.3	25 : 25	6.5	22.0
			35 : 35	5.3	19.6
June	22	8.6	25 : 25	10.3	31.5
			35 : 35	6.0	16.0
July	24.5	8.9	25 : 25	8.6	24.3
			35 : 35	6.0	14.8
			35 : 25	4.1	10.4
August	20	10.0	25 : 25	6.7	19.2
			35 : 35	3.8	10.0
September	16	11.5	25 : 25	5.9	19.8
			35 : 35	3.6	11.8

irradiance was higher (22 and 24.5 MJ $^{-2}$ d^{-1} respectively).

The influence of the daily light irradiance received by the cells on the night biomass loss became even more evident in another experiment performed in August. We used three cultures, maintained at 35 °C during the day and at 20 °C during the night, with three different biomass concentrations (710, 1290, 2169 $mg\ l^{-1}$). Night biomass loss ranged from 14% of the total dry weight reached in the evening in the culture grown at the lowest biomass concentration to 5.1% in the one grown at the highest biomass concentration (Table 2).

Chemical analysis of the biomass harvested in the morning and in the evening showed that the photosynthetic activity of the culture grown at the constant temperature of 25 °C was directed to a greater extent towards carbohydrate synthesis than towards protein synthesis. On average, protein and carbohydrate represented, respectively, in the morning 67.5% and 18.7% of dry weight; in the evening, 59.9% and 26.3% (Table 3). The reverse happened in the culture grown at the constant temperature of 35 °C in which the biomass composition was scarcely modified throughout the day. The night biomass loss entailed higher carbohydrate loss in the culture grown at 25 °C;

Table 2. Influence of biomass concentration on the productivity and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors: A = as % of the dry weight reached at the end of the daylight period; B = as % of the daylight productivity. Data are mean values ± standard deviation of a four-day experiment. Sunlight irradiance throughout the experiment was 21.35 ± 1.3 $MJ\ m^{-2}\ d^{-1}$.

Biomass concentration ($mg\ l^{-1}$)	Daylight productivity ($mg\ l^{-1}\ d^{-1}$)	Net productivity ($mg\ l^{-1}\ d^{-1}$)	Night biomass loss	
			A	B
710 ± 28	492 ± 24	323 ± 48	14.0 ± 2.6	34.4 ± 7.2
1290 ± 80	496 ± 25	396 ± 56	6.6 ± 1.7	22.0 ± 7.9
2169 ± 398	434 ± 36	302 ± 62	5.1 ± 1.3	31.0 ± 8.5

Table 3. Influence of temperature on crude protein and carbohydrate contents of *Spirulina platensis* biomass harvested at the end of the daylight period.

Month	Temperature: 25 °C		Temperature: 35 °C	
	Protein	Carbohydrate	Protein	Carbohydrate
May	59.58	28.17	66.76	22.75
June	57.00	28.83	65.34	22.40
July	60.00	27.18	64.45	24.58
August	60.98	24.32	66.71	20.81
September	62.26	23.24	67.85	17.98

Table 4. Changes in total biomass, carbohydrate and protein during a complete light-dark cycle in *Spirulina platensis* grown outdoors in a thermostated photobioreactor. Data, in mg l^{-1} of ash-free dry weight, are mean values ± standard deviation of four determinations throughout the respective days.

Component	Sunrise	Sunset	Sunrise
Total biomass	686 ± 27	1165 ± 42	998 ± 50
Protein	486 ± 30	666 ± 26	707 ± 22
Carbohydrate	133 ± 9	394 ± 19	195 ± 24
Lipid	66 ± 6	105 ± 6	96 ± 13

consequently, the biomass composition of the two cultures turned out to be very similar in the morning (mean of 67.7% protein and 18.8% carbohydrate in both cultures).

Table 5. Composition of ash-free biomass of *Spirulina platensis* harvested at sunrise and sunset. Data are mean values ± standard deviation of four determinations throughout the respective days.

Component	Percentage of ash-free dry weight	
	Sunrise	Sunset
Protein	70.90 ± 2.37	57.14 ± 0.69
Carbohydrate	19.48 ± 1.48	33.81 ± 0.66
Lipid	9.61 ± 0.9	9.05 ± 0.61
C	50.91 ± 0.29	49.53 ± 0.32
H	7.67 ± 0.13	7.52 ± 0.18
N	11.34 ± 0.38	9.14 ± 0.11
O	30.07 ± 0.29	33.85 ± 0.31
Reduction degree	41.72 ± 0.003	39.573 ± 0.21
Heat of combustion (kcal g^{-1} ash-free biomass)	5.632 ± 0.008	5.342 ± 0.028

Table 4 shows the time course of biosynthesis and the consumption of biomass, carbohydrate, protein and lipid during a complete light-dark cycle observed in the *Spirulina* culture grown at a low biomass concentration (710 mg l^{-1}) under high light irradiance (21.4 MJ m^{-2} day^{-1}) and having nitrate as the nitrogen source. Under such conditions, more carbohydrate than protein was produced during the daylight period: carbohydrate represented 19.5% of ash-free biomass in the morning and rose to 33.8% in the evening, while protein represented 71% in the morning and fell to 57.1% in the evening (see also Table 5). During the night there was a 14% dry weight loss as a result of decline in carbohydrate. Lipid content did not change significantly during the night. Carbohydrate loss during the night exceeded biomass loss: the difference accounted for by significant protein synthesis during the night. Under these conditions, night loss of biomass was about 35% of daylight production. When the biomass concentration was increased from 710 to 2169 mg l^{-1}, reducing the light energy available per cell, the carbohydrate content in the evening fell to 25.4% (Table 6) and the biomass loss

Table 6. Influence of biomass concentration on crude protein and carbohydrate contents of *Spirulina* harvested at sunset. Data are mean values ± standard deviation of four determinations throughout the respective days.

Biomass concentration (mg l^{-1})	Protein (%)	Carbohydrate (%)
710 ± 28	55.43 ± 0.67	32.8 ± 0.64
1290 ± 80	61.3 ± 0.49	27.8 ± 0.87
2169 ± 398	64.3 ± 0.63	25.4 ± 1.7

decreased to 5.1% of the dry weight reached at the end of the daylight period (Table 2).

Discussion

The data indicate that net yield of *S. platensis* grown in outdoor cultures depends on the photosynthetic production and night biomass loss. Our observations on night biomass loss in *Spirulina* agree with those of Grobbelaar and Soeder (1985) who reported that biomass loss due to dark respiration in *Coelastrum*, grown under field conditions and indoors, depended on the day temperature and light history to which the algae were subjected. Indeed, when *Spirulina* cultures were grown at the suboptimal temperature of 25 °C or at a low biomass concentration under high light irradiance, we observed both a net increase in carbohydrate content and a decrease in protein synthesis during daylight. The excess of carbohydrate was then lost during the following night.

Previous field and laboratory studies with *Oscillatoria redekei* (Gibson, 1975) have shown that the respiration rate and carbohydrate content are closely related. A similar pattern was observed in *Spirulina*. We found higher biomass losses in cultures which, either because they were grown at a low temperature or under high light irradiance, had a higher carbohydrate content. *Vice versa*, a lower biomass loss was associated with a relatively low carbohydrate content of the cells. The carbohydrate analysis performed at the end of the day and in the morning seems to indicate the existence of a labile pool of carbohydrate which increases during the day and disappears during the night.

The fate of the excess carbohydrate stored in *Spirulina* was analyzed during a four-day experiment performed in August (Table 4). By analyzing the biomass composition of a culture with nitrate as the nitrogen source, at sunset and in the morning of the following day, we found that carbohydrate breakdown was correlated with a synthesis of protein. We observed that 18.5% of total protein synthesized by the culture was synthesized during the night. The mean value for carbon flow, i.e. the carbon increase in protein divided by the carbon loss in carbohydrate in the dark period, was 0.27, indicating that only part of the carbohydrate stored during the day was utilized for night protein synthesis, the majority being respired. The protein yield from consumption of an endogenous store of carbohydrate in our outdoor culture of *Spirulina* was about 36% lower than that found in *Oscillatoria redekei* (Foy & Smith, 1980). However, protein degradation may have accounted for the lower efficiency of net protein synthesis in *Spirulina*.

A 23% increase in biomass productivity was observed during the five-month experiment performed from May to September when the culture temperature was raised from 25 °C to 35 °C. However, only 14% of the difference was due to an effective increase in photosynthetic activity during the day in the culture grown at the optimal temperature of 35 °C: the difference in net biomass productivity rose to 23% because of the greater night loss observed in the culture grown at 25 °C.

With regard to the biomass concentrations we tested, the highest net productivity was reached at the biomass concentration of 1.290 $g\,l^{-1}$ i.e. at the growth rate (0.23 d^{-1}) at which the amount of biomass lost during the night was the lowest compared with that synthesized during the daylight period. Indeed, with respect to the amount of biomass synthesized during the daylight period, night biomass loss was higher when the cultures were grown at the lowest and highest biomass concentrations (Table 2). In the first case, it was due to a high content of carbohydrate which was lost during the night and, in the second case, to a higher biomass load reached in the evening.

Our results show that when *Spirulina* is grown at optimal day-night temperatures and optimal biomass concentration, the overall night loss is 4–6% of the total dry weight reached at the end of the daylight period in summer; that is, 10–16% of the daylight biomass production is lost during the night. This rather low value confirms the general assumption that cyanobacteria have low respiration values (van Liere & Mur, 1979). Higher rates of night biomass losses are expected

either when the culture temperature falls below the optimum during the day or when the culture is grown at a low biomass concentration under high light irradiance: both lead to an increase in carbohydrate synthesis. As the culture temperature can hardly be modified in outdoor cultures, the use of closed systems that can maintain the temperature as close as possible to the optimum values throughout the day and around the year can be advantageous for obtaining higher biomass outputs.

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