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The Feasibility of industrial production of *Spirulina* (*Arthrospira*) in Southern Spain

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Abstract

The viability of the industrial production of three strains of *Spirulina platensis* was tested in Malaga, Southern Spain. In a pre-industrial trial using raceway ponds from laboratory-scale to 450 m^2, all three strains displayed satisfactory growth. In a 10-month industrial trial in 450 m^2 ponds, production was equivalent to 30–32 metric tons of dry powder per hectare per annum. In conclusion, intensive industrial production of *Spirulina* is viable in certain Mediterranean climates, a region previously thought to be outside its geographic limits.

Keywords: *Spirulina*; Mass culture; Temperate latitude; Commercial production; Microalgae

1. Introduction

The microalga *Spirulina* is produced commercially all over the world, and the dried product is a valuable food supplement (Cifelli, 1983; Belay et al., 1993). It is rich in proteins (60–70% by weight), vitamins (especially B_{12} and β-carotene), and minerals. It contains many essential amino acids and fatty acids, being one of the sources of dietary γ-linolenic acid (GLA). Pre-clinical and clinical studies suggest it has certain therapeutic effects (see Fox, 1996), such as reduction in blood cholesterol, protection against some cancers, enhancement of the immune system, increase of intestinal lactobacilli, reduction of nephrotoxicity by heavy metals and drugs, radiation protection, reduction of hyper-

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lipidemia, and obesity (see Belay et al., 1993). Ayehunie et al. (1998) reported that an aqueous extract of *S. platensis* partially inhibited HIV-1 replication in human T-cell lines, peripheral mononuclear cells, and Langerhans cells.

Consequently, there has been increasing interest in the production of microalgae for commercial use. Since some of the first studies at the Carnegie Institution of Washington in the 1950s (Burlew, 1953) many aspects of the biotechnology have been developed which have led to increased efficiency in mass culture (see Richmond, 1986, 1987; Benemann et al., 1987; Chaumont, 1993). Three species now attract major commercial interest, namely *Chlorella*, *Spirulina* and *Dunaliella*. *Spirulina* was probably the most widely used in outdoor cultivation trials and there has been over four decades of intensive ecological and physiological research and development using large-scale production units.

In addition to prolonged periods of natural light, this microalga requires high temperatures for optimal growth, thus commercial cultivation has been restricted to tropical and subtropical zones. In some temperate latitudes, where high temperatures are achieved during the day in summer but there are marked falls in the night, the overall diurnal temperature range may be a suitable range for growth but the prolonged cold morning temperature is 10–15 °C below optimum (Richmond, 1987). Thus, there is little or no production of cultures in morning hours. However, several reports have shown that *S. subsalsa*, a estuarine/marine species, could be developed for commercial production in temperate latitudes (Hargraves and Viquez, 1982; Shimada et al., 1989).

The objective of these pilot-scale trials was to produce *Spirulina* in a selected part of the Mediterranean region using traditional open ponds or raceways. The location chosen was Málaga, in Southern Spain. There have been several previous attempts to cultivate *Spirulina* in Mediterranean climates but most of them have failed (Belay, 1997). In Southern Italy, Chini-Zitelli et al. (1996) successfully demonstrated the viability of small-scale outdoor cultivation of *Arthrospira* (*Spirulina*) *platensis* during autumn and winter in temperate climates but using tubular bioreactors, as did Fornari et al. (1999) for the production of *Spirulina*.

Commercial production of *Spirulina* is today based almost exclusively on open ponds of the raceway type (Tredici et al., 1993), although some companies use closed tubular bioreactors (Specktorova et al., 1997). According to Chaumont (1993), the two principal advantages of open culture systems are a small capital investment and the use of free solar energy. However, it is simple technology and only practical for certain species in select environments, but productivity is far below the theoretical maxima. Closed photobioreactors allow better control of growth and have higher photosynthetic efficiencies, but the technology is capital-intensive even though it may be justified with economies of scale.

2. Materials and methods

2.1. The production site

The city of Málaga was selected for the pilot study for production of *Spirulina*. It is located in Southern Spain (36°42′ N, 4°28′ W), about 100 km east of Gibraltar Strait, on

the Mediterranean coast. The area is one of the sunniest in Europe. Average daytime temperatures normally exceeds 30 °C in summer, and in winter are above 15 °C. The average number of sunny days per year is 328, with >10 h of sun per day for the whole year. Mean daily temperatures are shown in Fig. 1A. Irradiance is also high, averaging >1.5 10^7 J m^{-2} day^{-1} in summer and 0.4 10^7 J m^{-2} day^{-1} in winter. Mean daily irradiance, measured by sensors in the range 280–700 nm, is shown in Fig. 1B. From 1996 to 2000, the average annual rainfall was <400 mm, and the average concentration of dust particles (>0.8 μm) in atmosphere in the vicinity of the cultivation area was 0.04–0.05 mg m^{-3}.

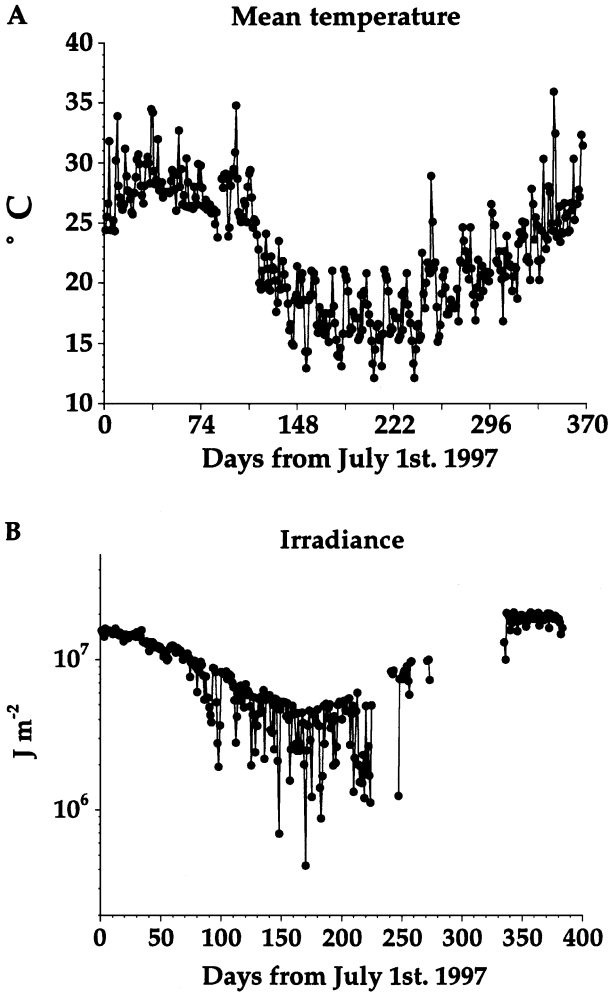

Fig. 1. Mean daily temperature (A) and integrated daily irradiance in the waveband 280–700 nm (B) at the location of the study.

2.2. The microorganisms

Three strains of *S. platensis* were provided by Prof. M. Brouers from the culture collection of the Department of Botany of the University of Liège. These were Laporte M132-1 (strain A), Compère 1968/3786 (strain B) and Laporte 1963/8579 (strain C). All three strains were isolated from alkaline lakes in Chad. Each had a different morphology as well as different temperature and irradiance optima. Strain A had short filaments (0.2–0.25 mm long) with low spiralization, and a spiral width of 44.4 μm. Strain B consisted of very long filaments (1–5 mm long) with very low spiralization, and a spiral width of 18 μm. Strain C also had very short filaments (0.2–0.25 mm), which were tightly coiled, and had a spiral width of 38.4 μm. These morphological differences influenced the harvesting performances.

2.3. Experimental design

The work was developed in several phases. The first phase involved production of large volumes of the inocula in the laboratory. This required scaling-up the volumes from 10 ml to 20 l. The selected growth medium was that prescribed by Zarrouk (1966). Agitation of the cultures was performed either by orbital shaking or by bubbling air (only for culture volumes >5 l). Temperature control was set at 25 °C, and light was provided continuously by fluorescent lamps.

The second phase was performed outdoors using a series of raceway ponds of increasing area, up to a maximum of 450 m^2. Inoculation of the raceways followed the scaling-up sequence described by Borowitzka and Borowitzka (1989). In these outdoors cultures Zarrouk's original growth medium was simplified by replacing the micronutrients with technical grade fertilizers, thus micronutrients could be avoided. All raceways had a length/width ratio of approximately 4–4.2:1. Each had a single paddle wheel located at one end. The depth of culture in the pond was 30 cm, and the contents were circulated at a speed of 30 cm s^{-1}.

2.4. Harvesting and drying

Before harvesting the biomass concentration was estimated by sampling and filtering 500 ml of each culture through a 62-μm mesh. The filter was dried at 105 °C for 24 h, and weighed.

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.

2.5. Microbiological analysis

Mesophylic aerobic microorganisms were evaluated at 31° ± 1 °C in nutrient agar (Oxoid). Total coliforms were calculated after incubation in brilliant green bile lactose

broth (BGBL); positive tubes were incubated in Levine agar for the estimation of *Escherichia coli*. Presence of *Staphylococcus aureus* was evaluated by using the Giolitti–Cantoni broth; for *Clostridium perfringens* sulfite polymixine sulfadiazine agar (SPS) was used. Estimation of *Salmonella* was performed using Rappaport–Vassiliadis soya peptone (RVS) broth; positive tubes were confirmed by means of selenite–cystine broth. Finally, molds and yeasts were estimated through the Sabouraud dextrose agar method.

2.6. Climatic measurements

Solar irradiance and temperature were measured on a permanent basis using a dosimeter (Eldonet, Real Time Computer, Möhrendorf, Germany). For solar irradiance the instrument took readings in three wavelength bands (UV-B, 280–315 nm; UV-A, 315–400 nm; and PAR, 400–700 nm) at intervals of 1 s. The data were continuously stored on the computer. The dosimeter also had temperature sensors and the data were also collected and stored.

3. Results

3.1. Laboratory trials

During the laboratory phase, the growth of each of the three strains was monitored by measuring the optical density at 680 nm. As shown in Fig. 2, strain B (Compère 1963/8579) grew faster than the other two. Intrinsic growth rate (r_{max}) was calculated for the

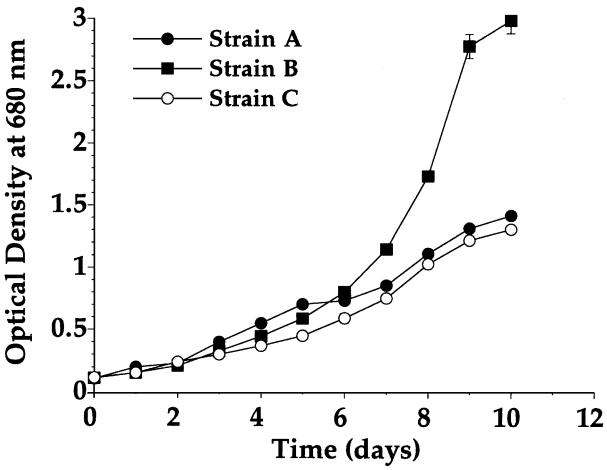

Fig. 2. Evolution of the optical density at 680 nm of the three strains of *S. platensis* tested during the laboratory phase of this study. Details of the culture conditions may be found in the text.

exponential phase of growth, which gave figures of 0.28, 0.36, and 0.26 doublings day $^{-1}$ for strains A, B, and C, respectively.

However, variation of the optical density of the cultures was not a good estimation of the biomass for two reasons. First, as culture density increased cells acclimated to reducing irradiance by increasing their pigment content. This was valid for an estimation of biomass through variation of the O.D. at 565 and 680 nm, due to the absorption of phycobilins and chlorophyll *a*, respectively. Second, due to the different morphology of the strains some showed higher light absorption even if the biomass content was low. In particular, the high light absorption at 680 nm by strain Compère 1963/8579 did not correlate with a high biomass content.

3.2. First outdoor trial

The open raceway ponds had increasing surface areas of 4.5 m^2 (1350 l), 45 m^2 (13,500 l) and 450 m^2 (135,000 l). All three strains were inoculated in the open raceway ponds of 1,350 l of capacity in August 1997. Harvesting commenced after 12–15 days (Fig. 3) and continued until October. During this period, the growth rate averaged 0.18, 0.09, and 0.15 doublings day $^{-1}$ for strains A, B and C, respectively, and productivity was 9.4, 6.3, and 6.6 g DW m $^{-2}$ day $^{-1}$.

Strain B did not support the same level of production as the other two and there was a continuous decrease of biomass concentration in those particular ponds. Strain C suffered an initial decrease of biomass concentration when harvesting first took place but it

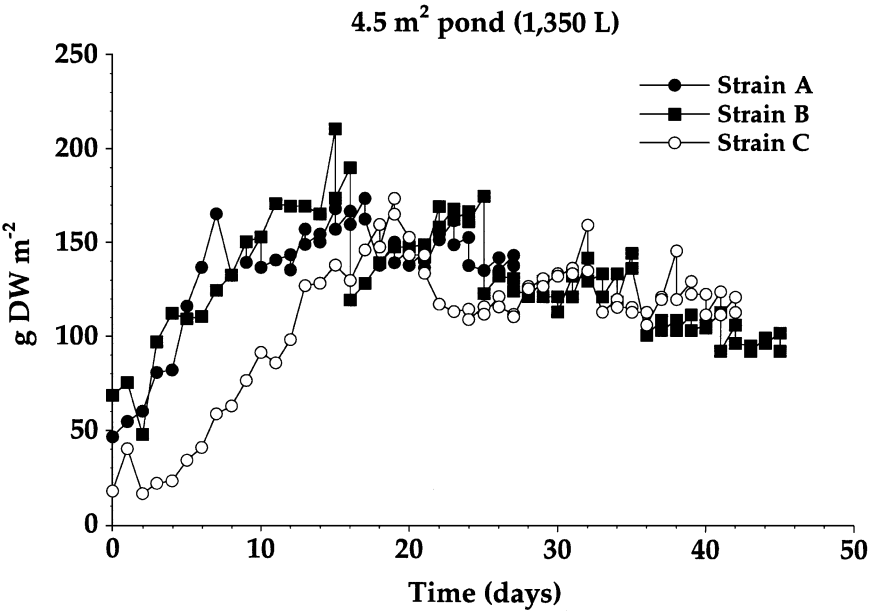

Fig. 3. Biomass concentration of *S. platensis* in 1350-l open raceway ponds, operated at a depth of 30 cm from August 1997.

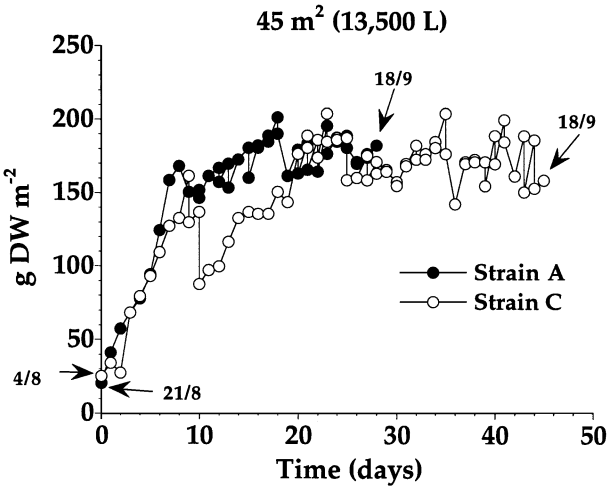

Fig. 4. Growth and biomass concentration of *S. platensis* in 13,500-l open raceway ponds operated at a depth of 30 cm. Details of biomass yield are shown in Table 1.

subsequently stabilized with values around 130 g m^{-2} for the rest of the harvesting season.

Two of the larger raceway ponds, of 13,500-l capacity, were also inoculated with strains A and C in August, and their biomass concentrations are shown in Fig. 4. Both displayed satisfactory growth, with an intrinsic growth rate of 0.19 doublings day^{-1} for strain A and 0.17 doublings day^{-1} for strain C. Both ponds were harvested daily and the results showed productivities of 10.7 g m^{-2} day^{-1} for both strains in September. Table 1 shows the results of the biomass yields during the different months of operation.

3.3. Second outdoor trials

The second trial used a single open raceway pond of 450 m^2. Based on the previous data, harvesting performance, and yield, strain A was selected for extended cultivation in this pond. Inoculation took place on September 23, 1997 and the pond was in continuous operation until July 29, 1998. During this period the biomass concentration in the pond ranged from 85 g DW m^{-2} in winter to 140 g DW m^{-2} in summer. Growth rate for the first 13 days after inoculation was 0.13 doublings day^{-1}, and daily harvesting com-

Table 1
Biomass yield of the *Spirulina* strains in the 45 m^2 open raceway ponds

Month	Strain A	Strain B	Strain C
September	10.7		10.7
October	9.6	7.3	
November	7.03		
December	2.81		

All cultures were operated at a depth of 30 cm (13,500 l).

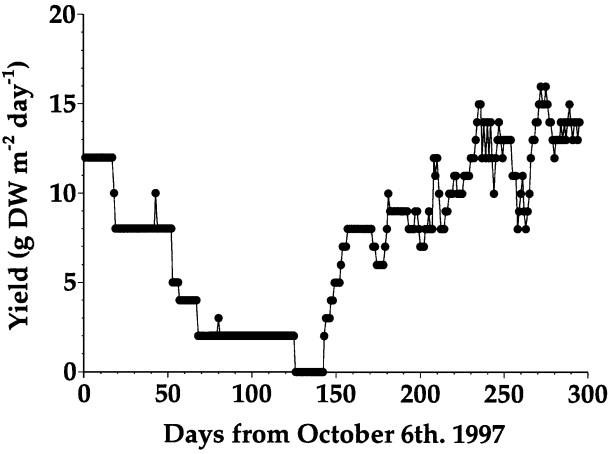

Fig. 5. Daily biomass yield (in g DW m^{-2} day^{-1}) of *S. platensis* Laporte M132-1 in a 450 m^2 open raceway pond.

menced. For 10 months, the pond was never emptied or renewed. In October and November, the biomass concentration averaged ca. 120 g DW m^{-2}; and during the winter months the concentration fell to around 85–90 g DW m^{-2}. In the spring and summer months it reverted back to about 120 g DW m^{-2}. Harvesting the biomass was only stopped for 17 days in February when there was a period of local heavy rains.

Temperatures of the culture ranged from a minimum of 12 °C in winter to a maximum of 28 °C in summer.

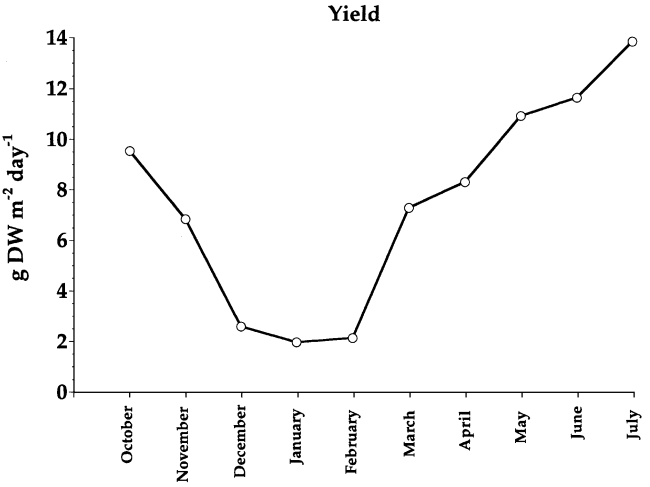

Fig. 6. Monthly average productivity of *S. platensis* Laporte M132-1 in a 450 m^2 pond during the feasibility study.

Fig. 5 summarizes daily biomass harvesting from a pond 450 m^2 in surface area. Peak productivity occurred in July, with values over 15 g DW m^{-2} day^{-1}. The lowest productivity occurred in January and February, averaging 2 g DW m^{-2} day^{-1}. Fig. 6 shows the average productivity of the pond in the 10-month trial period.

Extrapolating the data from the successful operation of a 450 m^2 pond, with an operational depth of 30 cm (135,000 l) and average production of 8.2 g DW m^{-2} day^{-1} in a 365-day cycle, the annual productivity would be 30 metric tons of dry weight product per hectare of surface area.

3.4. Analysis of the algal powder

Several samples of the dry biomass of *Spirulina* harvested during the period of operation of the 450 m^2 pond were analysed for cell composition and for microbiological standards. The results are given in Table 2. The protein content was low, averaging only 47% on a dry weight basis. The moisture accounted for 6–7% and the ash content was high, ca. 20%. Phycocyanin concentration was below the standards, averaging only 58.8 g kg^{-1}. The total carotenoid and chlorophyll *a* concentrations averaged 6 and 7.9 g kg^{-1}, respectively.

Microbiological analysis gave low concentration of mesophylic bacteria (7.5–9 10^3 bacteria g^{-1}), estimated through the standard plate count at 31 °C (Table 2). For other

Table 2
Results of the analysis of the dry powder of *Spirulina*

	Strain A	Strain C	Standards
Composition			
Phycocyanin (g kg^{-1})	60.7	56.9	140
Chlorophyll (g kg^{-1})	6.6	9.2	6.1–10
Carotenoids (g kg^{-1})	5.9	6.0	3.7
β-Carotene (g kg^{-1})	1.9	2.1	1.5–1.9
Proteins (%)	47.4	47.0	55–70
Moisture (%)	6.0	6.9	4–7
Ash (%)	19.5	21.1	6–13
Microbiology			
Mesophylic microorganisms			
SPC ($\times 10^3$/g)	9	7.5	50
Total coliforms MPN/g	<3	<3	<10
Escherichia coli MPN/g	<3	<3	Neg.
Staphylococcus aureus MPN/g	<3	<3	Neg.
Clostridium perfringens MPN/g	<3	<3	Neg.
Salmonella MPN/g	<3	<3	Neg.
Mold (#/g)	40	30	<100–<1000
Yeast (#/g)	<3	<3	

SPC—standard plate count.
MPN—most probable number.
All values <3 were below the detection limit of the MPN method. They could be considered as negative.
Composition standards have been taken from several sources (Fox, 1996; Belay, 1997). Microbiological standards resume the regulations of several countries (e.g., Japan, U.S.A., France, Sweden and Spain).

analyses, all results were in the detection limit of the most probable number (MPN) method with the exception of the molds, which had concentrations of 40 colonies g^{-1} for strain A and 30 colonies g^{-1} for strain C.

4. Discussion

Spirulina is a thermophilic microorganism with an optimal growth temperature of 35–37 °C. Outdoors the minimal temperature that permits growth is around 18 °C and the culture deteriorates quickly when maximum daytime temperatures are lower than 12 °C (Richmond, 1986). These practical requirements mean that commercial cultivation is restricted to tropical and subtropical areas around the world.

This work describes the results of a pilot study for commercial production of *Spirulina* in open raceways in Southern Spain, at a latitude of 36°N. Several previous attempts have been made to cultivate *Spirulina* in Mediterranean climates in open raceways but without success. Belay (1997) reported two unsuccessful attempts in Israel and another in Southern Spain. Another large unit of 5000 m^2 in Southern Spain is also reported to be undergoing some difficulty.

The results of these trials at Málaga indicate that outdoor cultivation of *Spirulina* is satisfactory at temperature above 15 °C. Winter temperature of the cultures in fact averaged 12 °C, which supported a biomass yield of 2 g DW m^{-2} day^{-1}. At 15 °C, the yield averaged 6 g DW m^{-2} day^{-1}.

Temperature in the outdoor cultivation ponds never exceeded 28 °C at midday, and the cultures did not deteriorate at ambient winter temperatures. Richmond (1986) concluded that minimal temperatures for production of *Spirulina* was 18 °C. In these trials *S. platensis* was successfully cultured at temperatures as low as 9 °C, and ca. 8 g DW m^{-2} day^{-1} was produced at 18 °C by one of the three strains tested. However, different strains of *Spirulina* differ in their optimal growth temperature, according to Vonshak and Tomaselli (2000), who presented data on several *Spirulina* isolates with optimal growth temperatures ranging from 24 to 42 °C.

In the analysis of the chemical composition of *Spirulina* dry powder produced in the pilot study, several figures differed from those of the standard composition (see Fox, 1996; Belay, 1997). However, the small deviations can be explained. For example, ash content was around 20%, which is high (typical values are 5–7%), and the protein content was low (47%; typical values 60–65%). However, the algal slurry obtained from the filtering unit was introduced directly into the spray drier without acid washing. As the growth medium is highly enriched with bicarbonate, acid washing is necessary to eliminate them. This process normally reduces the ash content. The above normal high value of ash content must be considered an artefact, and proper acid washing of the slurry would produce the more typical values of 5–7%. The calculation of the protein content as ash-free dry weight would give values of 60%. Similarly, when estimating humidity, several temperatures are used, for example, 65 °C to constant weight, 105 °C for 24 h, and 110 °C for 2 h, etc., leading to a wide range of values. According to the methodology adopted by the food industry, it is more appropriate to determine humidity at 65 °C to constant weight, which, in this case, gives an average humidity content of the dry powder of 4–5%.

Therefore, in the analysis of the dry powder (Table 2), the high ash content, together with the high humidity make the protein content lower than expected. However, the only component lower in concentration than expected was phycocyanin (typical values 140 g kg^{-1}). All the analyses carried out gave values averaging 6% by weight (60 g kg^{-1}). There is no obvious explanation for this lower value but again it is found to vary highly between different strains of *Spirulina* and more research at a production level may be necessary to help understand variations in phycocyanin content.

Microbiological analyses confirmed that the dry powder passed the necessary sanitary control regulations to qualify the product as food for human consumption. All micro-organisms were below the norms required by the strict regulations for members of the European Union, and Japan (Belay, 1997). Most concentrations were in fact below the level of detection of the approved microbiological techniques.

5. Conclusion

From a scientific and technical point of view, mass production of *Spirulina* in open ponds is viable under the climatic conditions of Southern Spain for a period of 9 months per year, from March to November. The potential average productivity for this period is of the order of 10.3 g DW m^{-2} day^{-1}. The average 12-month productivity is about 8.2 g DW m^{-2} day^{-1}, which would yield a production of 30 metric tons Ha^{-1} $year^{-1}$.

Acknowledgements

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