



HAL
open science

Improvement of the growth of *Arthrospira* (*Spirulina*) *platensis* from Toliara (Madagascar): Effect of agitation, salinity and CO₂ addition

Pierre H. Ravelonandro, Dominique H. Ratianarivo, Claire Joannis-cassan,
Arsène Isambert, Marson Raherimandimby

► To cite this version:

Pierre H. Ravelonandro, Dominique H. Ratianarivo, Claire Joannis-cassan, Arsène Isambert, Marson Raherimandimby. Improvement of the growth of *Arthrospira* (*Spirulina*) *platensis* from Toliara (Madagascar): Effect of agitation, salinity and CO₂ addition. *Food and Bioproducts Processing*, 2011, 89 (3), pp.209-216. 10.1016/j.fbp.2010.04.009 . hal-03537403

HAL Id: hal-03537403

<https://hal.science/hal-03537403>

Submitted on 20 Jan 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Open Archive Toulouse Archive Ouverte (OATAO)

OATAO is an open access repository that collects the work of Toulouse researchers and makes it freely available over the web where possible

This is an author's version published in: <http://oatao.univ-toulouse.fr/21573>

Official URL: <https://doi.org/10.1016/j.fbp.2010.04.009>

To cite this version:

Ravelonandro, Pierre H. and Ratianarivo, Dominique H. and Joannis-Cassan, Claire  and Isambert, Arsène and Raherimandimby, Marson *Improvement of the growth of Arthrospira (Spirulina) platensis from Toliara (Madagascar): Effect of agitation, salinity and CO2 addition.* (2011) *Food and Bioproducts Processing*, 89 (3). 209-216. ISSN 0960-3085

Any correspondence concerning this service should be sent to the repository administrator: tech-oatao@listes-diff.inp-toulouse.fr

Improvement of the growth of *Arthrospira (Spirulina) platensis* from Toliara (Madagascar): Effect of agitation, salinity and CO₂ addition

Pierre H. Ravelonandro^b, Dominique H. Ratianarivo^a, Claire Joannis-Cassan^{d,*}, Arsène Isambert^c, Marson Raherimandimby^a

^a Laboratoire de Microbiologie Biotechnologie du Département de Biochimie Fondamentale Appliquée, Faculté des Sciences, Université d'Antananarivo, BP 906 Tana-101 Madagascar, France

^b Laboratoire de Chimie Minérale et de Chimie Appliquée du Département de Chimie Minérale et de Chimie Physique, Faculté des Sciences, Université d'Antananarivo, BP 906 Tana-101 Madagascar, France

^c Laboratoire de Génie des Procédés et Matériaux, Ecole Centrale Paris, Grande Voie des Vignes, 92295 Châtenay-Malabry cedex, France

^d Université de Toulouse, Laboratoire de Génie Chimique, UMR-CNRS 5503, 4 allée Emile Monso, BP44362, 31432 Toulouse cedex 4, France

A B S T R A C T

Arthrospira (Spirulina) platensis Toliara isolated from alkaline and salt lakes in the south-western area of Madagascar is a potential source of proteins that could efficiently fight against food deficiency in developing countries like Madagascar. Up to now, productivity in this country has been low, so a better understanding of the growth conditions of this species is needed to improve its production. Growth experiments were undertaken in bubble columns at laboratory scale. The influence of agitation of the culture, medium salinity (ranging from 13 to 35 gL⁻¹) and CO₂ addition (ranging from 0 to 2%, v/v) on growth and protein content was examined. Because *Arthrospira* cells are fragile, a bubble column without additional mixing gave the best growth. *Arthrospira (Spirulina) platensis* showed higher specific growth rate (μ_{max}) and protein content for lower salinity. Addition of 1% of CO₂ improved the productivity by near 60%. The feasibility of semi-continuous culture was demonstrated and optimal culture conditions led to a mean productivity of 0.22 ± 0.03 gL⁻¹ d⁻¹, a mean specific growth rate of 0.015 ± 0.002 h⁻¹ and a protein content of $53 \pm 2\%$ of total dry weight.

Keywords: *Arthrospira (Spirulina) platensis*; Salinity; CO₂ addition; Photobioreactor; Light

1. Introduction

Cultivation of *Arthrospira (Spirulina) platensis* could be an alternative process for the production of proteins for human and animal food, and a possible way to obtain other products like vitamins, lipids and pigments (Henrikson, 1989; Spolaore et al., 2006). For developing countries like Madagascar, which have natural *Arthrospira* resources, its cultivation could constitute a sustainable opportunity to fight against malnutrition and food deficiency in this country. *Arthrospira platensis* is a blue-green filamentous cyanobacterium adapted to the envi-

ronment of alkaline lakes (Vonshak, 1997). In Madagascar, *A. platensis* is found in salt and alkaline lakes near Toliara in the south west. Recently, the feasibility of cultivation on a pilot scale was investigated in open ponds using modified sea water but low growth and productivity were observed (Jarisoa et al., 2003). Thus, improvements in culture conditions are needed to obtain adequate productivity and protein content. It is well known that numerous parameters influence the growth or protein content of microalgae: light, temperature, salinity, CO₂ addition, nutrient addition, inoculation size, stirring, pH, etc. (Zarrouk, 1966; Richmond, 1988; Chojnacka and Noworita,

* Corresponding author.

E-mail address: claire.joanniscassan@ensiacet.fr (C. Joannis-Cassan).

2004). However, there is a lack of agreement on their influence for *A. platensis*. An understanding of the biotechnological characteristics of *A. platensis* from Toliara over a wide range of parameters is necessary if production of this microalga is to be improved.

With this object, laboratory-scale studies were undertaken in an air-lift column. This system was preferred as it was simple, well mixed to ensure good light availability to cells and effective for gas exchange (Borowitzka, 1999; Travesio et al., 2001). Some parameters, such as medium composition, temperature, pH, light intensity and wavelength, age and concentration of inoculum have already been optimised and were used in this study (Sarada et al., 1999; Pelizer et al., 2003; Ravelonandro et al., 2008). The present work focused on three parameters of particular interest that could improve *Arthrospira* production. The first was culture mixing as it could influence shading of photosynthetic cells and gas transfer. *A. platensis* is sensitive to shear stress, so a compromise should be found between good and gentle mixing. The air-lift system was compared to medium recirculation by means of a pump and mechanical stirring. The second parameter was medium salinity. As *A. platensis* from Toliara was isolated from salt lakes and as Madagascar lacks fresh water resources, the first growth assays were undertaken in modified sea water (Jarisoa et al., 2003). However, these authors showed that the strain grew better in a medium having a salinity of 14 gL⁻¹ than in sea water with an average salinity of 44 gL⁻¹. The literature reports contradictory results concerning the influence of medium salinity on *Arthrospira* growth (Dhiab et al., 2007; Kebede, 1997; Lu and Zhang, 2000; Lu et al., 1999; Schlesinger et al., 1996; Verma and Mohanty, 2000; Zeng and Vonshak, 1998), so further investigations are needed.

The last parameter was CO₂ addition to the medium. In most *Arthrospira* cultures in closed reactors, CO₂ has only been added as a pH regulator to prevent excessively alkaline conditions (pH should be lower than 11) (Gordillo et al., 1999; Soletto et al., 2008), so growth may have been carbon limited (Borowitzka, 1999). Thus, CO₂ addition could improve the growth rate (Gordillo et al., 1999).

In the present work, a bubble column system was used to cultivate *A. platensis* from Toliara (Madagascar). The effects of agitation, salinity and CO₂ addition to the medium on growth and protein content were examined successively. An experiment in semi-continuous mode with the optimal conditions found was then performed.

2. Methods and materials

2.1. Microorganism and culture medium

A. platensis was provided by the Institut Halieutique des Sciences Marines (IHSM), University of Toliara, Madagascar. The strain was maintained in a modified Zarrouk medium (Zarrouk, 1966) in 500 mL flasks containing 200 mL of culture at room temperature. The composition of the medium was per liter of distilled water: 10 g NaHCO₃, 0.5 g K₂HPO₄, 2.5 g NaNO₃, 1.0 g K₂SO₄, 1.0 g NaCl, 0.20 g MgSO₄·7H₂O, 0.01 g FeSO₄·7H₂O, 0.08 g EDTA and 0.04 g CaCl₂ (Ravelonandro et al., 2008). The medium was autoclaved for 20 min at 121 °C. The salinity of the medium was 13 gL⁻¹. Inoculum was cultivated at room temperature, mixed by bubbling sterile air at a flow-rate of 25 mL min⁻¹ and continuously illuminated with white fluorescent tubes with an average intensity of 600 lx.

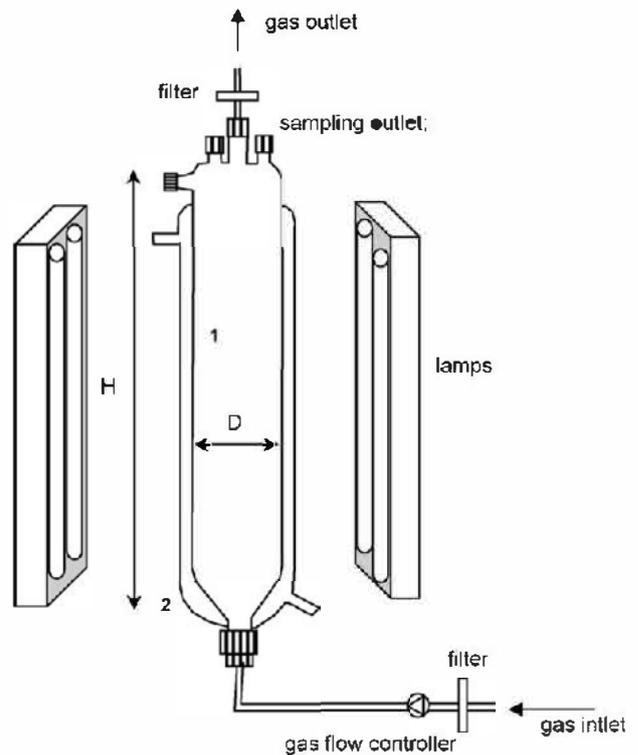


Fig. 1 – Photobioreactor made of cylindrical glass column (1) and a double-jacket (2); inlet diameter $D = 9$ cm; height $H = 41$ cm, surface/volume ratio: 44 m^{-1} .

2.2. Growth kinetics experiments

The experiments were carried out in a 2.5 L double-jacked column made of glass (Fig. 1). The temperature was maintained at 30 ± 1 °C. When necessary, sterile air or air/CO₂ mixture was introduced at a flow-rate of 4 mL s⁻¹. The reactor was continuously illuminated from two sides by vertical white fluorescent tubes (Fluotone™TD'L 18W/54-765, Philips) placed parallel to the reactor. For some experiments, the light used was made green by wrapping the light tubes in green polyethylene films. The light intensity was varied by changing the number of lamps.

For batch experiments, the inoculation density was 50 mgL⁻¹ (Pelizer et al., 2003). The initial pH was adjusted to 9.5 ± 0.1 (Sarada et al., 1999). Samples (5 mL) were withdrawn daily for optical density (OD) and pH measurements. At the end of growth, the protein content of the biomass was analyzed.

For semi-continuous experiments, inoculation and initial culture conditions were the same as for the batch mode. Periodically, samples (70% of the total volume) were taken and replaced by an equal volume of fresh medium.

2.3. Effect of mixing

Three ways of culture mixing were tested: mixing with a magnetic agitator inside the column, recirculating through a pump, and bubbling air into the column.

2.4. Effect of salinity

The salinity of the medium was varied by addition of sodium chloride NaCl. Five medium salinities were tested (13, 20, 25, 30, 35 gL⁻¹).

2.5. Effect of CO₂ addition

The variation of CO₂ percentage in the inlet feed was obtained by addition of pure CO₂ (SOAM, Madagascar). Four percentages of CO₂ were tested: 0, 0.5, 1 and 2% (v/v). The concentration was measured using a gas chromatograph (INTERSMAT, IGC 120 ML, column: PORAPAK Q 80/100 2m; detector: catharometric; carrier gas: H₂; injector temperature: 90 °C; column temperature: 60 °C; detector temperature: 150 °C).

2.6. Analytical methods

The physicochemical parameters (pH and salinity) were measured using a multiparameter analyser (CONSORT C535 version 3.0).

Both incident light (*I*_{in}) and outgoing light (*I*_{out}) intensities were measured by a luxmeter (LI-COR®, Biosciences LI-250A Meter Light).

To determine the dry weight concentration, the optical density (OD) of the microalgal suspension at 665 nm was measured using a spectrophotometer (Biochrom CO7500). The OD readings were converted to dry cell concentrations with a pre-established correlation (Ravelonandro et al., 2008). Filaments and whorls were counted on Malassez cells under an optical microscope (Motic® MICROSCOPES) with a magnification of 40× (Jarisoa et al., 2003). The protein concentration was determined from the dried biomass by the Kjeldahl method, with a catalyst using potassium sulphate, copper sulphate and selenium (Anaga and Gideon, 1996). Chlorophylls from the fresh biomass were studied quantitatively by spectrophotometry after extracting pigments using 80% acetone (Olaizola and Duerr, 1990).

2.7. Statistics and growth parameters calculation

Analyses of dry weight, protein content, chlorophyll content and number of spires were made in duplicate so the results were expressed as mean ± 95% confidence interval, i.e. $m \pm ts/\sqrt{n}$ where *m* is the mean of the *n* values (*n*=2), *s* is the estimated standard deviation and *t* is given by the Student–Fisher distribution for 95% of confidence interval and a degree of freedom of *n* – 1 (for *n*=2, *t*=12.706) (Wonnacott and Wonnacott, 1990).

The maximum specific growth rate (μ_{max}) was calculated from biomass (*X*) during the logarithmic phase values by an exponential regression (Bailey and Ollis, 1986). So the 95% confidence interval was: $\mu_{max} \pm t's'$ where *t'* is given by the Student–Fisher distribution for 95% confidence interval and a degree of freedom of *n'* – 1 (*n'* is the number of values used for μ_{max} estimation) and *s'* the standard error for μ_{max} .

The maximum productivity was obtained using: $P_{X_{max}} = (X_{max} - X_0)(t_{max} - t_0) - 1$ where *X*_{max} is the maximum biomass concentration achieved in the photobioreactor at *t*_{max}, and *X*₀ is the biomass concentration at inoculation (*t*₀).

3. Results and discussion

3.1. Preliminary study: influence of light intensity

As the three parameters studied could be influenced by light availability, a preliminary study of the influence of light intensity was undertaken. Previous results showed that the best conditions for growth of *A. platensis* from Toliara were green

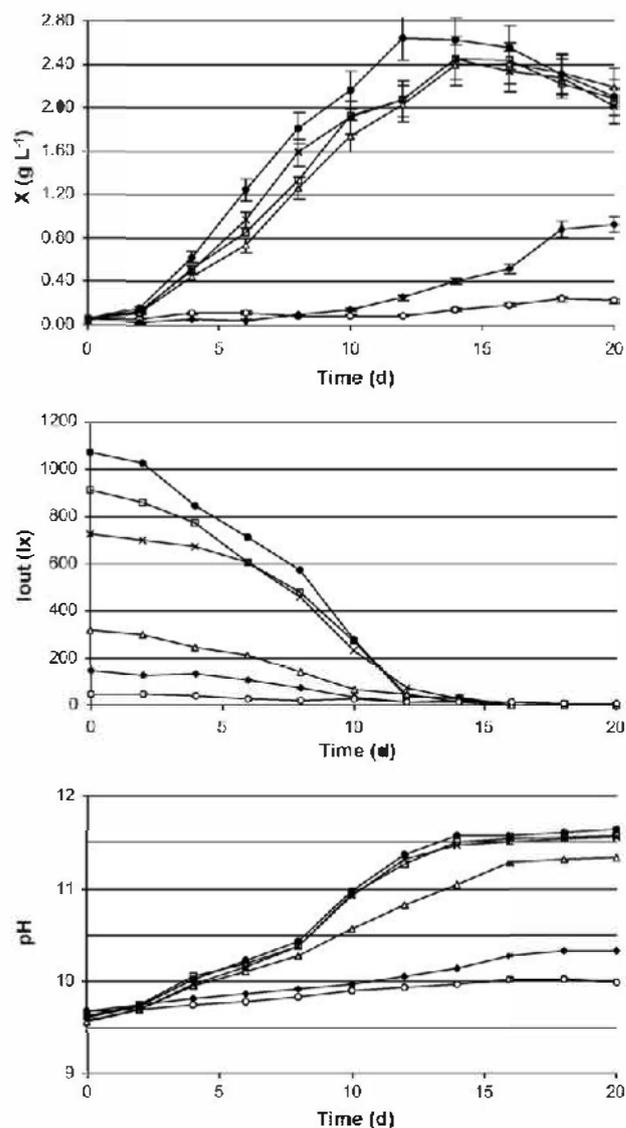


Fig. 2 – Biomass concentration *X* (g L⁻¹), outgoing light and pH during cultivation of *Arthrospira platensis* from Toliara for different green light intensities: 100 lx (○), 200 lx (◈), 400 lx (△), 800 lx (×), 1000 lx (□) and 1200 lx (●). Bars represent 95% confidence intervals.

light and 1200 lx (Ravelonandro et al., 2008). In this paper, we present the influence of green light intensity on growth (from 100 to 1200lx). Growth kinetics were obtained for six intensities: 100, 200, 400, 800, 1000 and 1200 lx (Fig. 2). The growth kinetics showed different phases: a very short phase of latency indicating that the microalgae were already acclimated to the culture medium; a short exponential phase (4 days); a linear phase (8–10 days) probably due to light or carbon limitation inside the reactor (Ravelonandro et al., 2008; Binaghi et al., 2003) and then a stationary phase. It can be seen that growth was improved by increasing light intensity. The highest specific growth rate, named $\mu_{max,opt}$, was obtained for 1200 lx ($\mu_{max,opt} = 0.023 \pm 0.003 \text{ h}^{-1}$). Fig. 3a presents the variation of μ_{max} with light intensity in comparison with $\mu_{max,opt}$. For values above 400 lx, values of μ_{max} were near 90% of $\mu_{max,opt}$. The same result was obtained with white light (data not shown). In this range (400–1200 lx), protein content was greater than 90% of its maximal value obtained for 1000 lx, i.e. $59 \pm 2\%$ of dry weight (DW) (Fig. 3b). The same result was observed for *X*_{max} (*X*_{max,opt} for 1200 lx was $2.6 \pm 0.1 \text{ g L}^{-1}$). Thus, for large scale

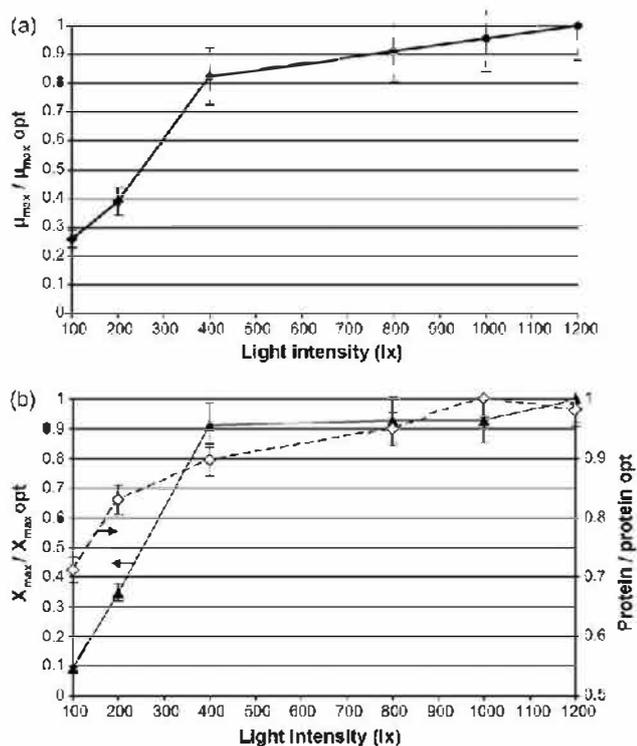


Fig. 3 – Influence of light intensity on: (a) specific growth rate compared to optimal specific growth rate ($\mu_{max}/\mu_{max, opt}$), and (b) ratio of protein content to optimal protein content (\diamond) and ratio of X_{max} to optimal X_{max} (Δ) for *Spirulina platensis* from Toliara. Bars represent 95% confidence intervals.

application, light of 400–1200 lx could be applied to *Arthrospira* of good nutritional quality (protein content greater than 55%).

The variation of pH and outgoing light (I_{out}) is presented in Fig. 2. The pH increase with biomass growth was principally due to the consumption of the carbon source, which shifted the bicarbonate–carbonate equilibrium towards bicarbonate (Binaghi et al., 2003). For 800, 1000 and 1200 lx, the stationary phase observed after 14 days could be explained by pH values up to 11.5 (Binaghi et al., 2003). However, the experiment for 400 lx showed a growth stop at the same time whereas the pH was 11. This suggested that reduced light availability could prevent growth when the biomass reached 2.5 g L⁻¹. However, the light seemed not to be the limiting factor responsible for the linear phase observed as early as 4 days for all the experiments with light intensities above 400 lx. This suggested that, in this linear phase, growth was carbon limited.

3.2. Influence of agitation of the culture

Improving the mixing of a photobioreactor affects both the mass transfer and the light–dark cycling of cells. An intensity of 600 lx was used in order to observe any effects of mixing on light transmission. For the three mixing methods tested, biomass growth is presented in Fig. 4. The bubble column provided the best growth rate for *Arthrospira*: μ_{max} was $0.0122 \pm 0.0006 \text{ h}^{-1}$. Stirring and pumping gave similar values of 0.009 ± 0.001 and $0.010 \pm 0.001 \text{ h}^{-1}$ respectively. The maximal biomass was achieved with a bubble column ($1.8 \pm 0.1 \text{ g L}^{-1}$).

The experiments did not distinguish among the influence of hydrodynamics, light transmission in the reactor and CO₂ transfer. However, the difference in growth appeared from 4

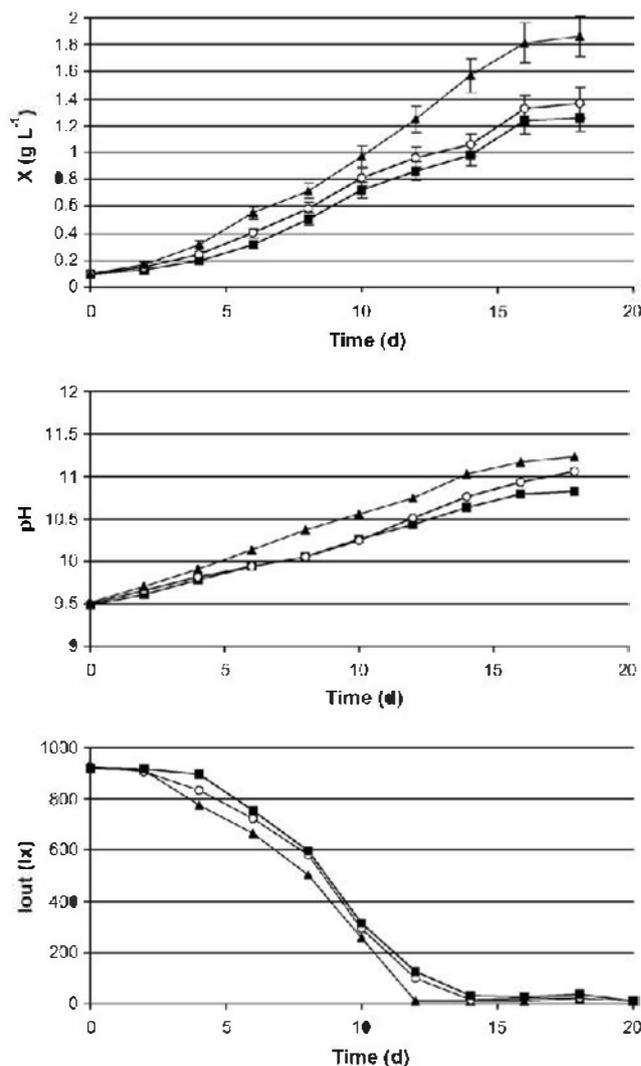


Fig. 4 – Biomass concentration X (g L⁻¹), pH and outgoing light during cultivation of *Arthrospira platensis* from Toliara for different types of agitation: magnetic agitator (■), recirculation (◇), and bubbling (▲). Bars represent 95% confidence intervals.

days of growth, before light limitation. So shear stress appears to be the principle effect. This was supported by the mean number of spires, which was highest for the bubble column (9 ± 1) and recirculated reactor (8 ± 1) than for the stirred reactor (5 ± 1). These results confirmed that *A. platensis* was sensitive to shear stress (Vonshak et al., 1988). Weissman et al. (1988) found that enhanced mixing did not improve productivity for low biomass densities. For higher densities, other authors have indicated that excessive mixing can be counter-productive because of shear forces (Richmond, 1996; Thomas and Gibson, 1990). So the bubble column seemed to be the most suitable system for the study of *Arthrospira* growth on a laboratory scale as well as on a large scale.

3.3. Influence of the medium salinity

Five cultures were grown in batch mode with salinities of 13, 20, 25, 30, 35 g L⁻¹ using a 600 lx light intensity in the bubble column. The growth kinetics are presented in terms of biomass concentration, pH variation and salinity variation in Fig. 5. A decrease in salinity was observed during growth because of the assimilation of ions by the microalgae but

Table 1 – Comparison of *Arthrospira platensis* cultivation for different medium salinities ($P_{X_{max}}$: productivity; μ_{max} : maximal growth rate; X_{max} : maximal biomass concentration).

	Medium salinity (g L ⁻¹)				
	13	20	25	30	35
Lag phase (day)	0	2	2	2	2
Exponential phase (day)	4	4	4	4	4
Linear Phase (day)	8	8	8	8	8
$P_{X_{max}}$ (g L ⁻¹ d ⁻¹)	0.08 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
μ_{max} (h ⁻¹)	0.0101 ± 0.008	0.009 ± 0.001	0.009 ± 0.001	0.0091 ± 0.0008	0.0076 ± 0.0003
X_{max} (g L ⁻¹)	1.6 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Protein content (% of DW)	50 ± 2	46 ± 1	47 ± 1	43 ± 1	38 ± 1
Maximum number of whorls	21 ± 2	19 ± 2	16 ± 2	13 ± 2	12 ± 2

Values are expressed as mean ± 95% confidence interval.

the variation was not very large (less than 9%) and did not influence the growth. The kinetic parameters are presented in Table 1. No lag phase was observed for 13 g L⁻¹, and that for other salinities was very short (2 days), indicating that *Arthrospira* was rapidly able to adapt to salinity stress. The influence of salinity has no significant effect on μ_{max} except

for the higher salinity for which the specific growth rate was minimum (0.0076 ± 0.0003 h⁻¹). However, the range of salinity was not high enough to stop growth. Other growth parameters decreased significantly with increasing salinity: $P_{X_{max}}$ and X_{max} decreased by 50% and 44% respectively when salinity increased from 13 to 35 g L⁻¹. The optimum microalgal growth was obtained with salinity of 13 g L⁻¹ even though *A. platensis* from Toliara was isolated from a salt lake. As reported in the literature, cyanobacteria are able to adapt to the alkaline habitat, but high salinity could become limiting (Mohanty et al., 1997; Vonshak, 1997).

The decrease in growth with increasing salinity has frequently been reported in the literature (Rosales et al., 2005; Vonshak et al., 1988; Zeng and Vonshak, 1998; Kebede, 1997). It is accompanied by a decrease in photosynthetic efficiency, phycobilin/Chla ratio and PSII activity and an increase in carbohydrate metabolism (Vonshak et al., 1988; Warr et al., 1985). The enhanced respiration indicates that the response to salinity stress is an energy consuming process (Zeng and Vonshak, 1998) but the mechanisms have not yet been elucidated. It is probable that salinity stress affects light utilization and metabolism (particularly carbohydrates involved in osmoregulation) to counteract ionic and osmotic stresses (Kebede, 1997; Rosales et al., 2005). Some authors have reported an increase in growth with salinity (Dhiab et al., 2007) but they measured *Arthrospira* growth by following the chlorophyll content, which was not the case in our study. These authors explained their contradictory results by the difference in genetic and environmental factors, so different strains of *A. platensis* could use different strategies to respond to salt stress.

Morphological variation was also observed as the number of whorls decreased with the increase in salinity (Table 1). This was an expression of the physiological stress to which the cells were subjected. Some studies have already observed that addition of NaCl inhibits growth of the helicoidal morphone (Lewin, 1980; Jeeji Bai, 1985).

Protein content was also influenced by the salinity of the medium (Table 1). The protein content decreased from 50 ± 2% for a salinity of 13 g L⁻¹ to 38 ± 1% for a salinity of 35 g L⁻¹. Results for protein are contradictory in the literature. Most authors have found results in accordance with this study (Vonshak et al., 1988; Zeng and Vonshak, 1998) whereas some found an augmentation of protein contents with salinity (Rosales et al., 2005). However, strains and culture conditions were very different for all these studies so results are difficult to compare. In this work, it could be suggested that stressed cells had a lower protein synthesis capacity linked to the higher carbohydrate metabolism (Kebede, 1997; Rosales

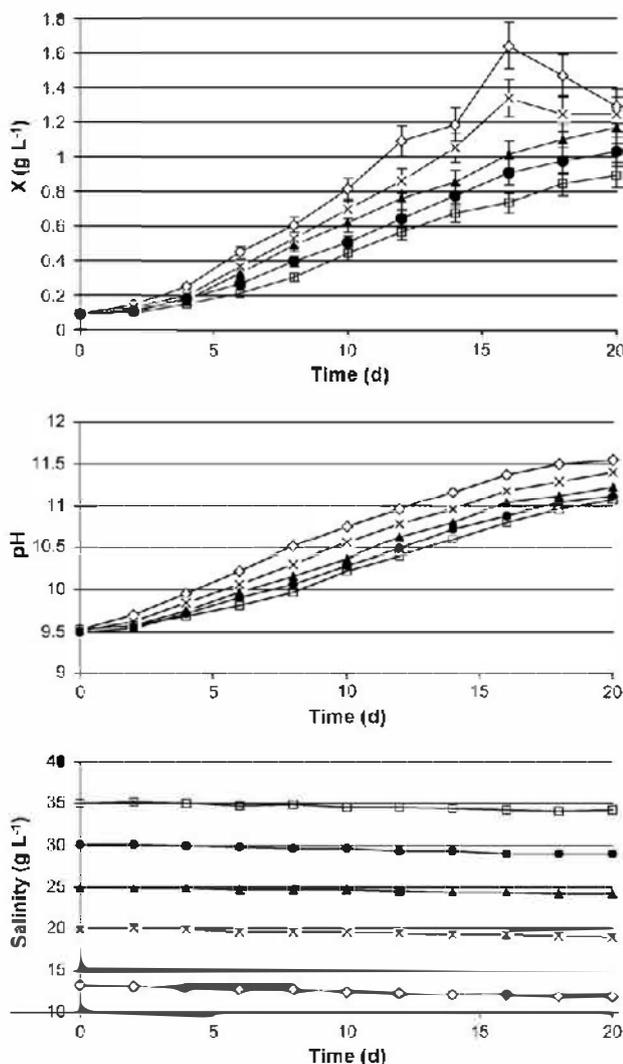


Fig. 5 – Biomass concentration X (g L⁻¹), pH and salinity (g L⁻¹) during cultivation of *Arthrospira platensis* from Toliara for different medium salinities: 13 g L⁻¹ (◇), 20 g L⁻¹ (×), 25 g L⁻¹ (▲), 30 g L⁻¹ (●) and 35 g L⁻¹ (□). Bars represent 95% confidence intervals.

Table 2 – Comparison of *Arthrospira platensis* cultivation for different percentages of CO₂ addition ($P_{X_{max}}$: productivity; μ_{max} : maximal growth rate; X_{max} : maximal biomass concentration).

	CO ₂ addition (%)			
	0	0.5	1	2
$P_{X_{max}}$ (g L ⁻¹ d ⁻¹)	0.10 ± 0.01	0.13 ± 0.02	0.16 ± 0.02	0.16 ± 0.02
Lag phase duration (day)	2	0	0	0
Exponential phase duration (day)	6	8	12	12
Linear growth phase duration (day)	12	12	8	8
μ_{max} (h ⁻¹)	0.020 ± 0.006	0.021 ± 0.002	0.024 ± 0.003	0.027 ± 0.005
X_{max} (g L ⁻¹)	2.4 ± 0.2	2.7 ± 0.2	3.3 ± 0.2	3.4 ± 0.2
Maximum number of whorls	20 ± 2	19 ± 2	22 ± 2	23 ± 2
Protein content (% of DW)	42 ± 1	44 ± 1	47 ± 1	46 ± 1
Chlorophyll content (% of DW)	0.22	0.22	0.24	0.23

Values are expressed as mean ± 95% confidence interval.

et al., 2005). As protein content is a very important parameter for nutritional uses of *Arthrospira*, the values have to be compared to the range of protein in *Arthrospira* products found on the market, which is 50–65%. The maximal value, near 50%, obtained for 13 g L⁻¹ is satisfactory for food applications.

3.4. Influence of CO₂ addition

To avoid limitation due to light transfer during growth, a green light intensity of 1200 lx was used for this part. Four experiments were carried out in discontinuous culture with different percentages of CO₂ in the sterile air flow: 0, 0.5, 1, and 2% (v/v). The results are illustrated in Fig. 6 and Table 2. First, a differ-

ence in the pH curves can be seen for the percentages of CO₂ tested. Addition of CO₂ allowed the pH to be regulated in a range of 9.5–10; the pH was lower when % CO₂ was higher. In contrast, without addition of CO₂, pH increased to 11.5 as explained above. Whatever the pH values, growth stopped after 16 h. Table 2 indicates an increase in growth parameters (X_{max} and $P_{X_{max}}$) with % CO₂ until 1%. No significant difference in growth parameters was observed between 1 and 2% of CO₂. Like all photosynthetic organisms, *A. platensis* from Toliara was able to use CO₂ as a carbon source for growth, which has been confirmed by several authors (Binaghi et al., 2003; Soletto et al., 2008). Most of these authors report an increase of growth with addition of CO₂ until an upper limit is reached which depends on light and pH conditions. However, Gordillo et al. (1999) reported a decrease of maximal biomass density for a CO₂ enrichment of 1%.

The increase in the duration of the exponential growth phase from 8 to 12 days, as indicated in Table 2, confirmed that carbon limited the growth in the previous experiment without CO₂ addition. In experiments with CO₂ addition, light could be responsible for the linear phase as culture was 1.5 times as concentrated as in previous experiments.

Table 2 indicates that protein content was significantly influenced by CO₂ addition. Supply of 1% of CO₂ allowed the highest protein content with a significant chlorophyll level. This value of 1% could constitute an economical compromise between productivity and the need for a high percentage of CO₂.

3.5. Semi-continuous experiment

A semi-continuous experiment was carried out with the best culture conditions found above: light 1200 lx, green light and 1% CO₂. When growth reached the exponential phase, 70% of the volume of the culture was harvested and fresh medium was added. During this experiment, four harvests were made, at 10(R1), 18(R2), 26(R3) and 34(R4) days of growth. Results are given in Fig. 7 in terms of biomass and pH. The results show a stability of the culture: productivity was maintained at 0.21–0.23 ± 0.03 g L⁻¹ d⁻¹ (Table 3). This productivity was quite high compared with values found in the literature. It varied from 0.02 to 0.05 g L⁻¹ d⁻¹ in open ponds (Radmann et al., 2007; Ravelo, 2001) and in a laboratory-scale aerated photobioreactor (Colla et al., 2007; Kim et al., 2007), and from 0.15 to 0.17 g L⁻¹ d⁻¹ for cultures in Erlenmeyer flasks (Pelizer et al., 2003; Wang et al., 2007). It could reach 0.43 g L⁻¹ d⁻¹ in more complex photobioreactors (Radway et al., 1999).

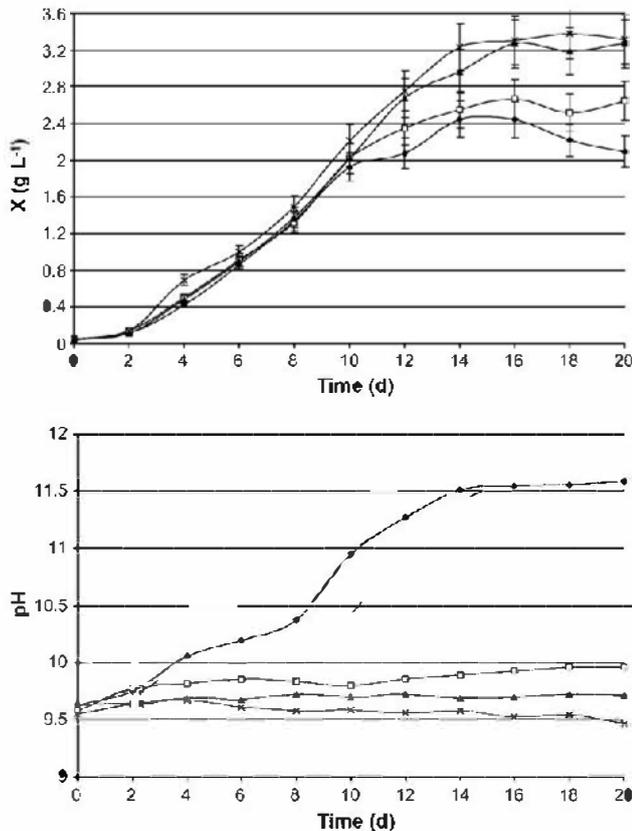


Fig. 6 – Biomass concentration X (g L⁻¹) and pH evolution during cultivation of *Arthrospira platensis* from Toliara at different percentages of CO₂ addition into the medium: 0% (◆), 0.5% (□), 1% (▲), 2% (×). Bars represent 95% confidence intervals.

Table 3 – Growth parameters for semi-continuous experiments ($P_{X_{max}}$: productivity; μ_{max} : maximal growth rate; X_{max} : maximal biomass concentration).

	Harvesting			
	R1 (day 10)	R2 (day 18)	R3 (day 26)	R4 (day 34)
X_{max} (g L ⁻¹)	2.1 ± 0.1	2.5 ± 0.2	2.4 ± 0.1	2.7 ± 0.2
Harvested volume (% of total volume)	69.4	72.3	64.0	72.0
$P_{X_{max}}$ (g L ⁻¹ d ⁻¹)	0.21 ± 0.03	0.23 ± 0.03	0.21 ± 0.03	0.22 ± 0.03
μ_{max} (h ⁻¹)	0.024 ± 0.003	0.011 ± 0.001	0.012 ± 0.002	0.014 ± 0.005
Protein content (% of DW)	–	–	–	53 ± 2

Values are expressed as mean ± 95% confidence interval.

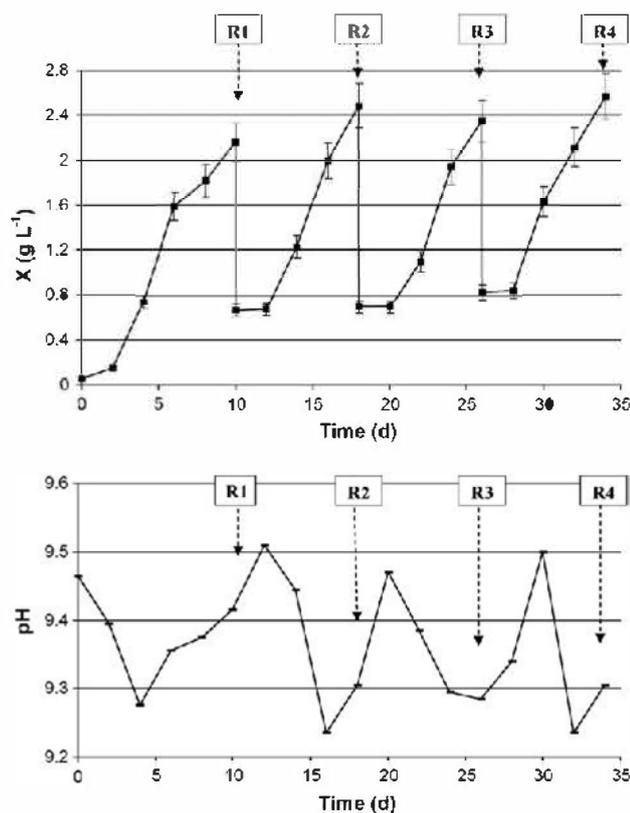


Fig. 7 – Biomass concentration X (g L⁻¹) and pH evolution during the cultivation of *Arthrospira platensis* from Toliara in semi-continuous mode. About 70% of the culture volume was taken and replaced by fresh medium at day 10 (R1), day 18 (R2), day 26 (R3) and day 34 (R4). Bars represent 95% confidence intervals.

The protein content after the 4th harvesting was satisfactory as it reached 53 ± 2%.

4. Conclusion

Growth of *A. platensis* from Toliara was studied in a bubble column, which was the most appropriate reactor. Significant growth was observed whatever the range of culture conditions tested (salinity varying between 13 and 35 g L⁻¹, CO₂ addition varying between 0 and 2% and light intensity varying between 600 and 1200 lx). However, optimal conditions in terms of productivity and protein content were obtained with a salinity of 13 g L⁻¹ and a percentage of CO₂ of 1%. The feasibility of semi-continuous culture with a high productivity (0.22 ± 0.03 g L⁻¹ d⁻¹) is a first step for the cultivation of *A. platensis* from Toliara in Madagascar. These results have to be confirmed in scaled-up culture and completed with economic

feasibility studies. In order to make this production profitable, further studies should be done to obtain a low-cost culture medium, for example from diluted sea water. Moreover, the use of CO₂ as a potential source of carbon for *Arthrospira* culture can help to attenuate the greenhouse effects of CO₂ produced, for example, by fermentation industries.

Acknowledgements

This work was performed under the management of URGPGE (Unité de Recherche en Génie de Procédés et Génie de l'Environnement) as a part of its photobioreactor research, and was supported by Fades (Fonds d'Appui au Développement de l'Enseignement Supérieur) of Madagascar.

References

- Anaga, A. and Gideon, O.A., 1996, A laboratory-scale cultivation of *Chlorella* and *Arthrospira* using waste effluent from a fertilizer company in Nigeria. *Bioresour. Technol.*, 58: 93–95.
- Bailey, J.E. and Ollis, D.F., (1986). *Biochemical Engineering Fundamentals* (2nd ed.). (McGraw-Hill, Singapore).
- Binaghi, L., Del Borghi, A., Lodi, A., Converti, A. and Del Borghi, M., 2003, Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis*. *Process Biochem.*, 38: 1341–1346.
- Borowitzka, M.A., 1999, Commercial production of microalgae: ponds, tanks, tubes and fermenters. *J. Biotechnol.*, 70: 313–321.
- Chojnacka, K. and Noworita, A., 2004, Evaluation of *Spirulina* sp growth in phototrophic, heterotrophic and mixotrophic cultures. *Enzyme Microb. Technol.*, 34: 461–465.
- Colla, L.C., Reinehr, C.O., Reichert, C. and Viera Costa, J.A., 2007, Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. *Bioresour. Technol.*, 98: 1489–1493.
- Dhiab, R.B., Ouada, H.B., Boussetta, H., Franck, F., Elabed, A. and Brouers, M., 2007, Growth, fluorescence, photosynthetic O₂ production and pigment content of salt adapted cultures of *Arthrospira (Spirulina) platensis*. *J. Appl. Phycol.*, 19: 293–301.
- Gordillo, F.J.L., Jimenez, C., Figueroa, F.L. and Niell, F.X., 1999, Effects of increased atmospheric CO₂ and N supply on photosynthesis, growth and cell composition on the cyanobacterium *Spirulina platensis (Arthrospira)*. *J. Appl. Phycol.*, 10: 461–469.
- Henrikson, R., (1989). *Earth Food Spirulina*. (Ronore Enterprises, USA).
- Jarisoa, T., Charpy, L., Vicente, N. and Langlade, M.J., (2003). *Culture de Spiruline en eau de mer à Toliara (Madagascar)*. (Colloque d'Ecologie Microbienne, Carry Le Rouet, France).
- Jeeji Bai, N., 1985, Competitive exclusion or morphological transformation? A case study with *Spirulina fusiformis*. *Arch. Hydrobiol. Suppl.* 71, *Algal. Stud.*, 191: 38–39.
- Kebede, E., 1997, Response of *Spirulina platensis (=Arthrospira fusiformis)* from Lake Chitu, Ethiopia, to salinity stress from sodium salts. *J. Appl. Phycol.*, 9: 551–558.

- Kim, C.-J., Jung, Y.-H. and Oh, H.-M., 2007, Factors indicating culture status during cultivation of *Spirulina (Arthrospira) platensis*. *J. Microbiol.*, 45(2): 122–127.
- Lewin, R.A., 1980, Uncoiled variants of *Spirulina platensis*. *Arch. Hydrobiol. Suppl.* 60, *Algal. Stud.*, 26: 48–52.
- Lu, C., Torzillo, G. and Vonshak, A., 1999, Kinetic response of photosystem II photochemistry in the cyanobacterium *Spirulina platensis* to high salinity is characterized by two distinct phases. *Aust. J. Plant Physiol.*, 26: 283–292.
- Lu, C. and Zhang, J., 2000, Role of light in the response of PSII photochemistry to salt stress in the cyanobacterium *Spirulina platensis*. *J. Exp. Bot.*, 51: 911–917.
- Mohanty, P., Srivastava, M. and Krishina, K.B., 1997, The photosynthetic apparatus of *Spirulina*: electron transport and energy transfer, in *Spirulina platensis (Arthrospira): Physiology, Cell-biology and Biotechnology*, Vonshak, A. (ed) (Taylor and Francis, London), pp. 17–42. (Taylor and Francis, London).
- Olaizola, M. and Duerr, E., 1990, Effects of light intensity and quality on the growth rate and photosynthetic pigment content of *Spirulina platensis*. *J. Appl. Phycol.*, 2: 97–104.
- Pelizer, L.H., Danesi, E.D., Rangel, C.O., Sansano, C.E.N., Carvalho, J.C.M., Sata, S. and Moraes, I.O., 2003, Influence of inoculum age and concentration in *Spirulina platensis* cultivation. *J. Food Eng.*, 56: 371–375.
- Radmann, E.M., Reinehr, C.O. and Costa, J.A.V., 2007, Optimization of the repeated batch cultivation of microalga *Spirulina platensis* in open raceway ponds. *Aquaculture*, 265: 118–126.
- Radway, J.C., Yoza, B.A., Benemann, J.R., Babcock, R.W., Chini Zintelli, G. and Tredici, M.R., 1999, Evaluation of a near-horizontal tubular photobioreactor system in Hawaii, In *Proceedings of the 8th International Conference on Applied Algology* Montecatini Terme, Italy, September 26–October 1,
- Ravelo, V., 2001, Bio-écologie, valorisation du gisement naturel de *Spiruline* de Belalanda (Toliara, sud-ouest de Madagascar) et technologie de la culture. PhD Thesis. University of Toliara, Madagascar.
- Ravelonandro, P.H., Ratianarivo, D.H., Joannis-Cassan, C., Isambert, A. and Rahehimandimby, M., 2008, Influence of light quality and intensity in the cultivation of *Spirulina platensis* from Toliara (Madagascar) in a closed system. *J. Chem. Technol. Biotechnol.*, 83: 842–848.
- Richmond, A., 1988, in Borowitzka, M.A. and Borowitzka, L.J., Borowitzka, L.J. (eds) (Cambridge University Press, Cambridge, UK), pp. 85–121. (Cambridge University Press, Cambridge, UK).
- Richmond, A., 1996, Efficient utilization of high irradiance for production of photoautotrophic cell mass: a survey. *J. Appl. Phycol.*, 8: 381–387.
- Rosales, N., Ortega, J., Mora, R. and Morales, E., 2005, Influence of salinity on the growth and biochemical composition of the cyanobacterium *Synechococcus* sp. *Cienc. Mar.*, 31: 349–355.
- Sarada, R., Pillai, M.G. and Ravishankar, G.A., 1999, Phycocyanin from *Spirulina* sp: influence of processing of biomass on phycocyanin yield, analysis of efficacy of extraction methods and stability studies on phycocyanin. *Process Biochem.*, 34: 795–801.
- Schlesinger, P., Belkin, S. and Boussiba, S., 1996, Sodium deprivation under alkaline conditions causes rapid death of filamentous cyanobacterium *Spirulina platensis*. *J. Phycol.*, 32: 608–613.
- Soletto, D., Binaghi, L., Ferrari, L., Lodi, A., Carvalho, J.C.M., Zilli, M. and Converti, A., 2008, Effects of carbon dioxide feeding rate and light intensity on the fed-batch pulse-feeding cultivation of *Spirulina platensis* in helical photobioreactor. *Biochem. Eng. J.*, 39: 369–375.
- Spolaore, P., Joannis-Cassan, C., Duran, E. and Isambert, A., 2006, Commercial applications of microalgae: a review. *J. Biosci. Bioeng.*, 101: 87–96.
- Thomas, W.H. and Gibson, C.H., 1990, Effects of small-scale turbulence on microalgae. *Appl. Phycol.*, 2: 71–77.
- Travesio, L., Hall, D.O., Rao, K.K., Benitez, F., Sanchez, E. and Borja, R., 2001, A helical tubular photobioreactor producing *Spirulina* in a semicontinuous mode. *Int. Biodeter. Biodegrad.*, 47: 151–155.
- Verma, K. and Mohanty, P., 2000, Changes of the photosynthetic apparatus in *Spirulina* cyanobacterium by sodium stress. *Z. Naturforsch.*, 55: 16–22.
- Vonshak, A., 1997, *Spirulina*: growth, physiology and biochemistry, in *Spirulina platensis (Arthrospira)*, Vonshak, A. (ed) (Taylor & Francis, London), pp. 43–65. (Taylor & Francis, London).
- Vonshak, A., Guy, R. and Guy, M., 1988, The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. *Arch. Microbiol.*, 150: 417–420.
- Wang, C.-Y., Fu, C.-C. and Liu, Y.-C., 2007, Effects of using light-emitting diodes on the cultivation of *Spirulina platensis*. *Biochem. Eng. J.*, 37: 21–25.
- Warr, S.R.C., Reed, R.H., Chudek, J.A., Foster, R. and Steward, W.D.P., 1985, Osmotic adjustment in *Spirulina platensis*. *Planta*, 163: 424–429.
- Weissman, J.C., Goebel, R.P. and Benemann, J.R., 1988, Photobioreactor design: mixing, carbon utilization, and oxygen accumulation. *Biotechnol. Bioeng.*, 31: 336–341.
- Wonnacott, T.H. and Wonnacott, R., (1990). *Introductory Statistics* (5th edition). (Wiley, New York, USA).
- Zarrouk, C., 1966. Contribution à l'étude d'une cyanophycée: influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de *Spirulina maxima* (Setch el Gardner) Geitler. PhD Thesis. University of Paris.
- Zeng, M.T. and Vonshak, A., 1998, Adaptation of *Spirulina platensis* to salinity-stress. *Comp. Biochem. Physiol. A*, 120: 113–118.