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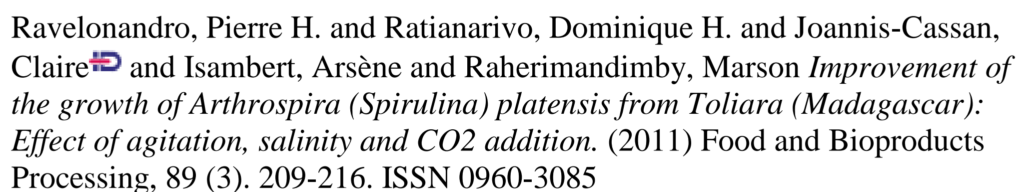

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Improvement of the growth of *Arthrospira* (*Spirulina*) *platensis* from Toliara (Madagascar): Effect of agitation, salinity and CO_2 addition

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ABSTRACT

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 $g\,L^{-1}$) and CO_2 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 CO_2 improved the productivity by near 60%. The feasability of semi-continuous culture was demonstrated and optimal culture conditions led to a mean productivity of $0.22 \pm 0.03\,g\,L^{-1}\,d^{-1}$, a mean specific growth rate of $0.015 \pm 0.002\,h^{-1}$ and a protein content of $53 \pm 2\%$ of total dry weight.

Keywords: *Arthrospira* (*Spirulina*) *platensis*; Salinity; CO_2 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 environment 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, CO_2 addition, nutrient addition, inoculation size, stirring, pH, etc. (Zarrouk, 1966; Richmond, 1988; Chojnacka and Noworita,

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2004). However, there is a lack of agreement on their influence for *A. platensis*. An understanding of the biotechnological caracteristics 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 phtosynthetic 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 g L^{-1} than in sea water with an average salinity of 44 g L^{-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 CO_2 addition to the medium. In most *Arthrospira* cultures in closed reactors, CO_2 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, CO_2 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 CO_2 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 temparature. The composition of the medium was per liter of distilled water: 10 g $NaHCO_3$, 0.5 g K_2HPO_4, 2.5 g $NaNO_3$, 1.0 g K_2SO_4, 1.0 g NaCl, 0.20 g $MgSO_4{\cdot}7H_2O$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.08 g EDTA and 0.04 g $CaCl_2$ (Ravelonandro et al., 2008). The medium was autoclaved for 20 min at 121 °C. The salinity of the medium was 13 g L^{-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.

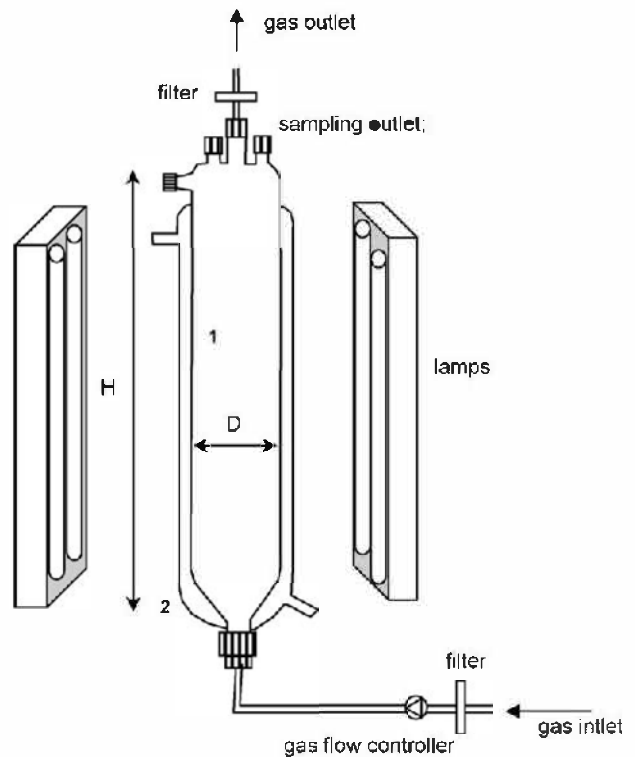

Fig. 1 – Photobioreactor made of cylindrical glass column (1) and a double-jacket (2); inlet diameter D = 9 cm; height H = 41 cm, surface/volume ratio: 44 m^{-1}.

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/CO_2 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 mg L^{-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 g L^{-1}).

2.5. Effect of CO_2 addition

The variation of CO_2 percentage in the inlet feed was obtained by addition of pure CO_2 (SOAM, Madagascar). Four percentages of CO_2 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: H_2; 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 (Iin) and outgoing light (Iout) 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, chlorphyll content and number of spires were made in duplicate so the results were expressed as mean ± 95% confidence interval, ie: $m \pm ts/\sqrt{n}$ 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 distibution 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

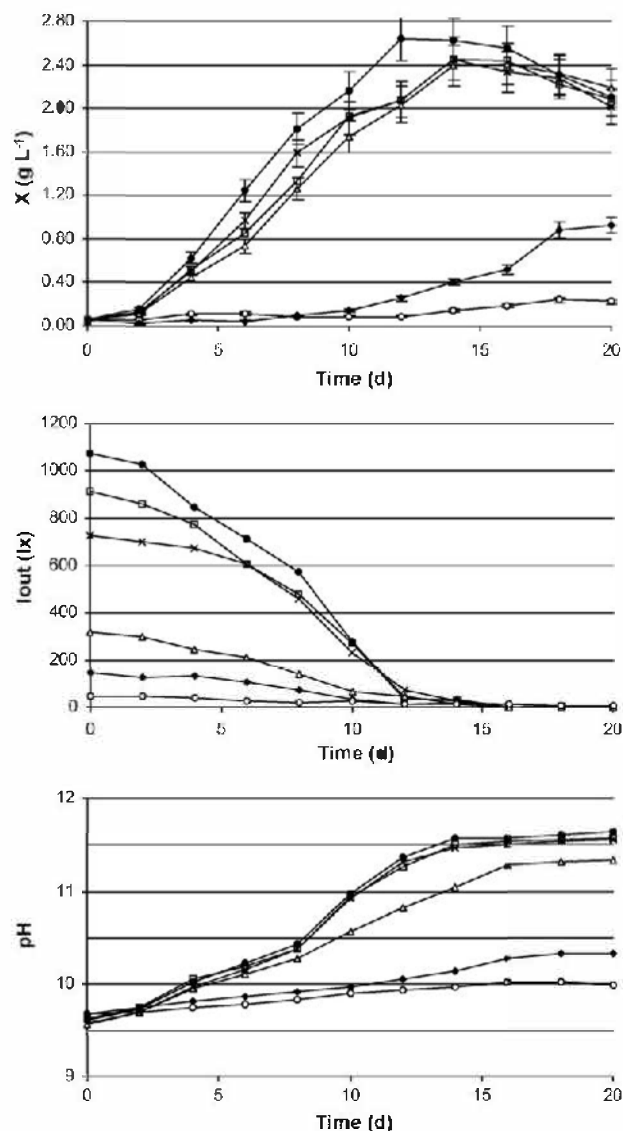

Fig. 2 – Biomass concentration X (g L^{-1}), outgoing light and pH during cultivation of *Arthrospira platensis* from Toliara for different green light intensities: 100 lx (○), 200 lx (♦), 400 lx (Δ), 800 lx (×), 1000 lx (□) and 1200 lx (●). Bars represent 95% confidence intervals.

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 μ_{max}opt, was obtained for 1200 lx (μ_{max}opt = 0.023 ± 0.003 h^{-1}). Fig. 3a presents the variation of μ_{max} with light intensity in comparison with μ_{max}opt. For values above 400 lx, values of μ_{max} were near 90% of μ_{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 ± 2% of dry weight (DW) (Fig. 3b). The same result was observed for X_{max} (X_{max}opt for 1200 lx was 2.6 ± 0.1 g L^{-1}). Thus, for large scale

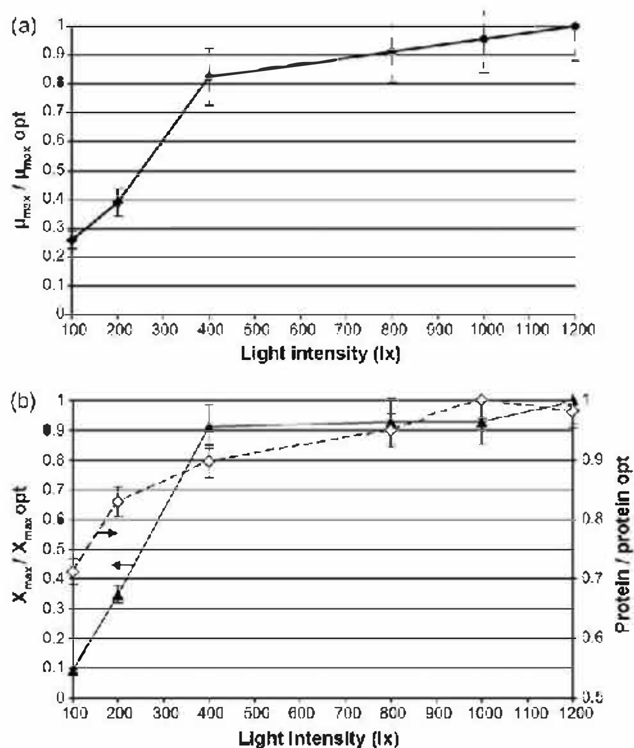

Fig. 3 – Influence of light intensity on: (a) specific growth rate compared to optimal specific growth rate (μ_{max}/μ_{max}opt), and (b) ratio of protein content to optimal protein content (◇) and ratio of X_{max} to optimal X_{max} (▲) for *Spirulina platensis* from Toliara. Bars represent 95% confidence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

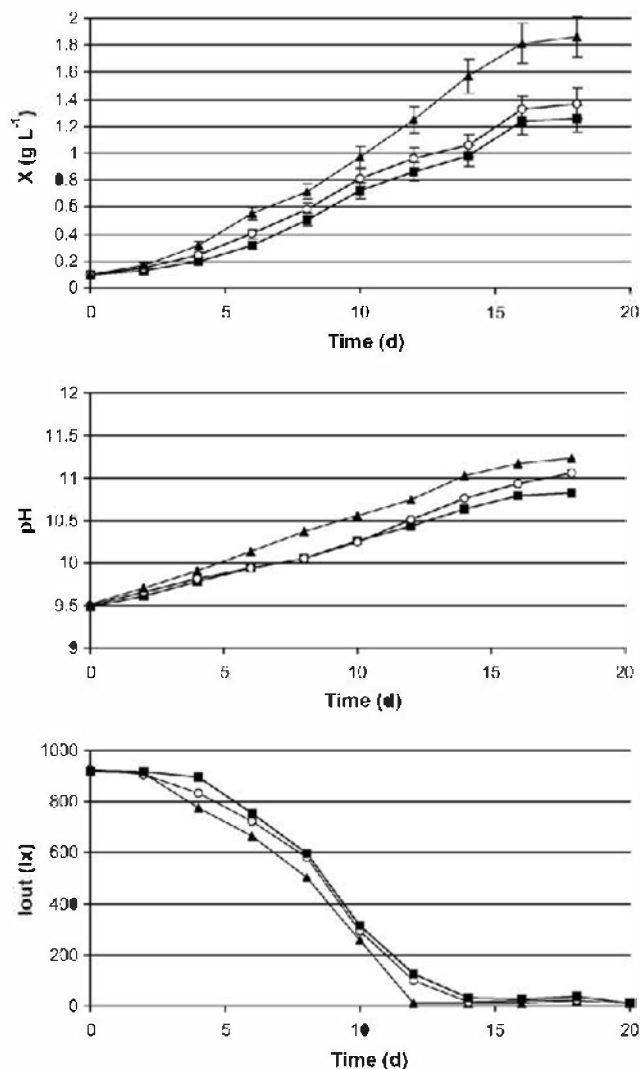

Fig. 4 – Biomass concentration X ($g\,L^{-1}$), pH and outgoing light during cultivation of *Arthrospira platensis* from Toliara for different types of agitation: magnetic agitator (■), recirculation (◇), and bubbling (▲). Bars represent 95% confidence intervals.

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 $0.0122 \pm 0.0006\,h^{-1}$. Stirring and pumping gave similar values of 0.009 ± 0.001 and $0.010 \pm 0.001\,h^{-1}$ respectively. The maximal biomass was achieved with a bubble column ($1.8 \pm 0.1\,g\,L^{-1}$).

The experiments did not distinguish among the influence of hydrodynamics, light transmission in the reactor and CO_2 transfer. However, the difference in growth appeared from 4 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 counterproductive 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 ($0.0076 \pm 0.0003\,h^{-1}$). 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).

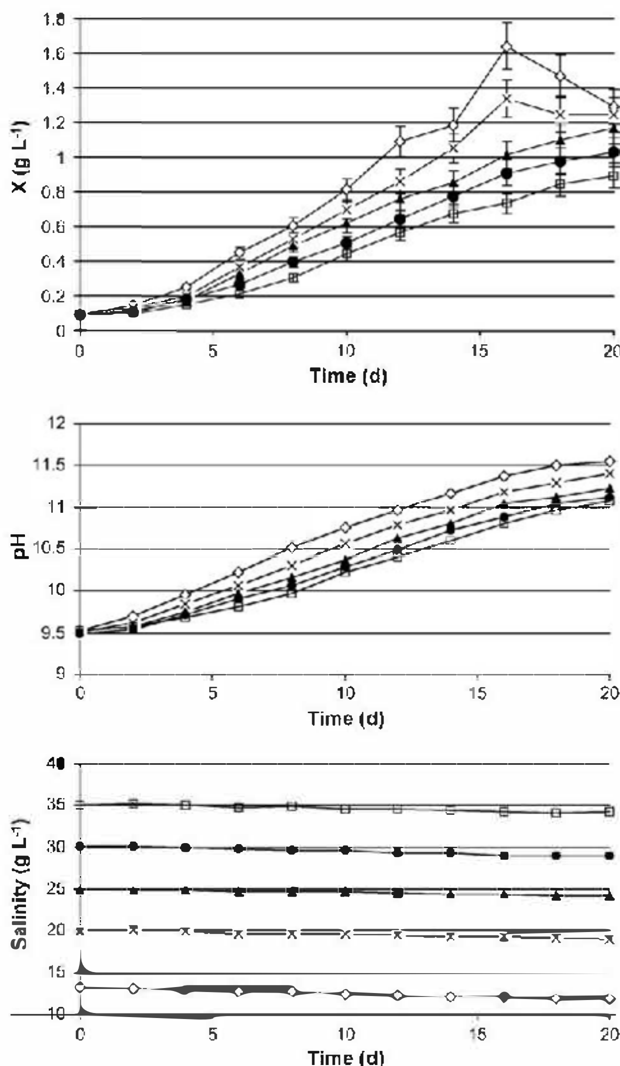

Fig. 5 – Biomass concentration *X* ($g\,L^{-1}$), pH and salinity ($g\,L^{-1}$) during cultivation of *Arthrospira platensis* from Toliara for different medium salinities: 13 $g\,L^{-1}$ (◊), 20 $g\,L^{-1}$ (×), 25 $g\,L^{-1}$ (▲), 30 $g\,L^{-1}$ (●) and 35 $g\,L^{-1}$ (□). Bars represent 95% confidence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

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 $50 \pm 2\%$ for a salinity of 13 $g\,L^{-1}$ to $38 \pm 1\%$ 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

Table 2 – Comparison of *Arthrospira platensis* cultivation for different percentages of CO_2 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 CO_2 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 CO_2 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 difference in the pH curves can be seen for the percentages of CO_2 tested. Addition of CO_2 allowed the pH to be regulated in a range of 9.5–10; the pH was lower when % CO_2 was higher. In contrast, without addition of CO_2, 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 % CO_2 until 1%. No significant difference in growth parameters was observed between 1 and 2% of CO_2. Like all photosynthetic organisms, *A. platensis* from Toliara was able to use CO_2 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 CO_2 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 CO_2 enrichment of 1%.

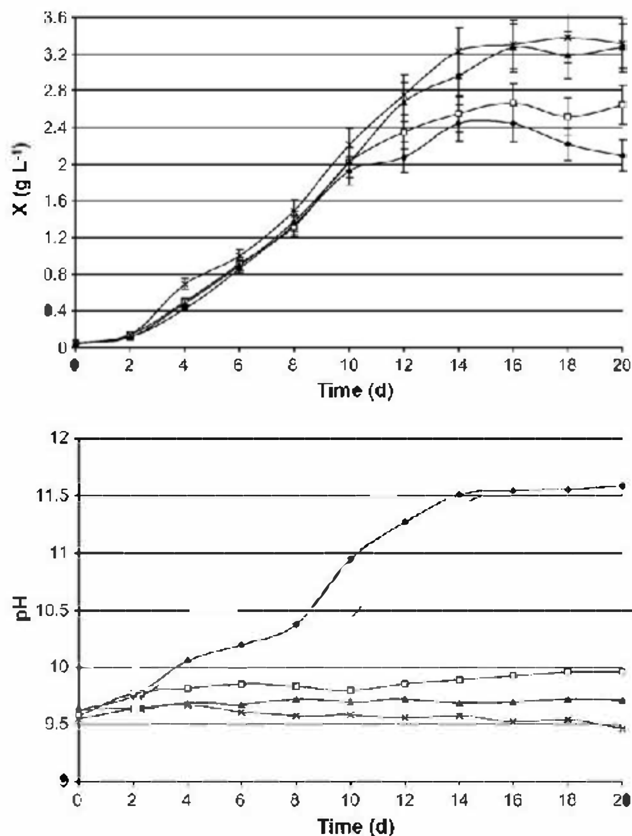

Fig. 6 – Biomass concentration X ($g L^{-1}$) and pH evolution during cultivation of *Arthrospira platensis* from Toliara at different percentages of CO_2 addition into the medium: 0% (♦), 0.5% (□), 1% (▲), 2% (×). Bars represent 95% confidence intervals.

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 CO_2 addition. In experiments with CO_2 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 CO_2 addition. Supply of 1% of CO_2 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 CO_2.

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% CO_2. 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).

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.

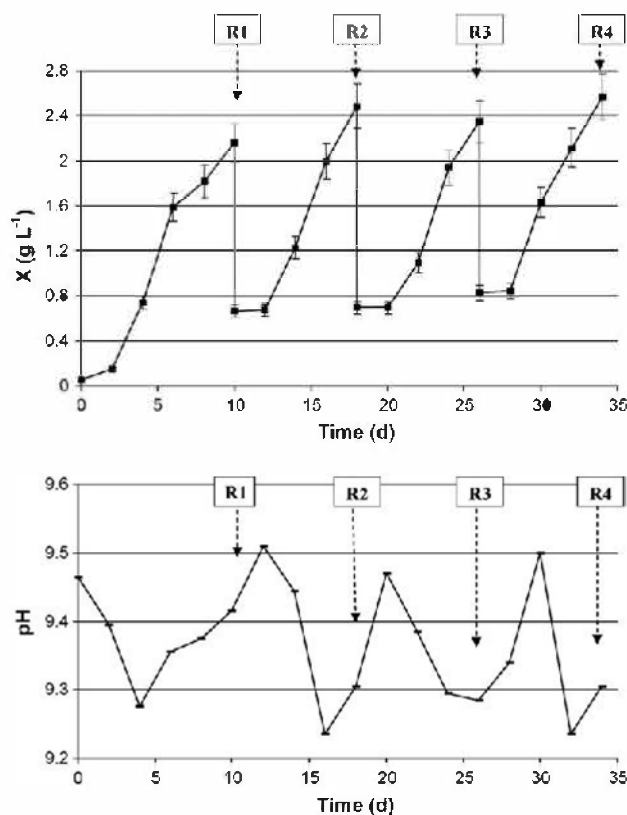

Fig. 7 – Biomass concentration X ($g L^{-1}$) and pH evolution during the cultivation of *Arthrospira platensis* from Toliara in semi-continuous mode. About 70% of the culture volume was taken and replaced by fresh medium at day 10 (R1), day 18 (R2), day 26 (R3) and day 34 (R4). Bars represent 95% confidence 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 $35 g L^{-1}$, CO_2 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 CO_2 of 1%. The feasibility of semi-continuous culture with a high productivity ($0.22 \pm 0.03 g L^{-1} d^{-1}$) 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 CO_2 as a potential source of carbon for *Arthrospira* culture can help to attenuate the greenhouse effects of CO_2 produced, for example, by fermentation industries.

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