J Appl Phycol (2010) 22:745–752
DOI 10.1007/s10811-010-9515-9

Modeling growth and photosynthetic response in *Arthrospira platensis* as function of light intensity and glucose concentration using factorial design

Ben Dhiab Rym · Ghenim Nejeh · Trabelsi Lamia · Yahia Ali · Challouf Rafika · Ghozzi Khemissa · Ammar Jihene · Omrane Hela · Ben Ouada Hatem

Received: 17 August 2009 / Revised and accepted: 24 February 2010 / Published online: 24 March 2010

Abstract Combined effect of light intensity and glucose concentration on *Arthrospira platensis* growth and photosynthetic response was evaluated using a 3^2 factorial design. This design was carried out with light levels of 50, 100, and 150 μmol photons $m^{-2}s^{-1}$ and glucose concentrations of 0.5, 1.5, and 2.5 gL^{-1}. Results from the response surface methodology were that the highest level of light intensity and glucose concentration improved biomass (1.33 gL^{-1}), maximum specific growth rate (0.49 day^{-1}), and net photosynthetic rate (139.89 μmol O_2 mg $Chl^{-1}h^{-1}$). Furthermore, the interaction of both factors showed that at low light, glucose had a low effect on maximum biomass and maximal net photosynthetic rate. However, at the highest light levels, the effect of glucose was more sensitive and the increase of glucose concentration increased the levels of all responses. The rates of the instantaneous relative growth, net photosynthesis, and dark respiration of growth cultures showed two different phases in mixotrophic condition. The first was distinguished by the preponderance of the photoautotrophic mode; the second was based mainly on photoheterotrophy.

Keywords *Arthrospira platensis* · Growth · Mixotrophy · Net photosynthetic rate · Response surface methodology · *Spirulina*

B. D. Rym (✉) · G. Nejeh · T. Lamia · Y. Ali · C. Rafika · G. Khemissa · A. Jihene · O. Hela · B. O. Hatem
Laboratoire de Biodiversité et de Biotechnologie Marine, Institut National des Sciences et Technologies de la Mer, BP 59, Route de Khniss Monastir 5000, Tunisia
e-mail: bendhiab_r@yahoo.fr

Introduction

Arthrospira (Spirulina) platensis is a photosynthetic cyanobacterium which is able to convert the energy of sunlight into chemical compounds usable by the cell to fix carbon dioxide and release oxygen. This cyanobacterium was also shown to be capable of using organic carbon sources in heterotrophic and mixotrophic culture conditions. (Marquez et al. 1993; Chen et al. 1996; Zhang et al. 1999; Vonshak et al. 2000; Chojnacka and Noworyta 2004; Lodi et al. 2005; Andrade and Costa 2007). Industrial and commercial biomass cultivation of this microalga was exclusively performed in autotrophic mode. Mixotrophic growth offers a possibility of greatly increasing microalgal cell concentration in batch culture (Richmond 1988; Marquez et al. 1993, 1995; Zhang et al. 1999).

In mixotrophic growth, there are two distinctive processes within the cell, photosynthesis and aerobic respiration. The former is influenced by light intensity and the latter is related to the organic substrate concentration.

The interaction of light and glucose on specific growth rate was found to follow multiplicative growth kinetics (Chojnacka and Noworyta 2004). The level of light intensity and glucose concentration and their interaction may influence both autotrophic (photosynthesis) and heterotrophic (oxidative metabolism of glucose) processes and therefore influence cell growth.

Several scientists have modeled mixotrophic cultures to determine optimal growth conditions using empirical mathematical models. The most widely used models are those of Monod and Haldmane (Zhang et al. 1999; Chojnacka and Noworyta 2004). Most of the results were obtained by taking into account only one variable (light or glucose) at a time, which does not allow the possible impact of interactions between the different factors to be established. These

traditional optimization methods are not only time-consuming (Box and Draper 1987) but also do not express the possible interactions between factors. The application of an experimental design using response surface methodology (RSM), on the other hand, can greatly reduce the number, time, and cost of experiments. In addition, the effect of a single or multiple interacting variables can be quantified.

In the present study, the optimal conditions for the effective mixotrophic culture of *A. platensis* were partially established using RSM.

This study was primarily aimed at evaluating the combined effects of light intensity and glucose concentration on maximal biomass concentration, maximum specific growth rate, maximum net photosynthetic rate, and dark respiration rate.

Growth and photosynthetic responses measured during 11 days of culture in photoautotrophic conditions were compared with those in mixotrophic conditions.

Methods

Microorganism and growth conditions

The strain of *Arthrospira platensis* used was Compere no. 1968/3786, isolated from Lake Chad. The culture media was Zarrouk's medium (Zarrouk 1966). Each component of this medium was sterilized separately by autoclaving at 120°C for 20 min, after which the cooled solutions were mixed and the medium supplemented with 0.5, 1.5, or 2.5 g L^{-1} glucose.

Cultivation was carried out in photobioreactors consisting of 5-L sterilized flasks (diameter of bottom, 14 cm; height, 36 cm) containing 3.5 L medium and equipped with a device for aseptic removal of samples. The cultures were incubated at 32°C and sparged continuously with air at constant flow rate. Illumination was provided by fluorescent tubes to give constant light intensities of 50, 100, or 150 μmol photons $m^{-2}s^{-1}$. Each culture was inoculated with an initial biomass concentration of 0.15 gL^{-1} (Andrade and Costa 2007).

Biomass concentration determination

Biomass concentration was determined daily by measuring the optical density of samples at 560 nm and comparing these values with previously prepared calibration curves of optical density versus biomass dry weight as described by Leduy and Therien (1977) and applied by Vonshak et al. (1988), Marquez et al. (1993), Chojnacka and Noworyta (2004), and Danesi et al. (2004).

The instantaneous relative growth rate (IRGR, day^{-1}) was calculated using a fitting program applied to the growth curve (calculated from the daily growth data) using the following formula: $IRGR = 1/x\ (dx/dt)$, where (x) is the biomass concentration and (t) is the time; unit of (IRGR) is day^{-1}.

The maximum specific growth rate (μ_{max}, day^{-1}) was determined from the linear region of the slope of the growth curve: $\mu = (\ln x_2 - \ln x_1)/(t_2 - t_1)$, where x_2 is the cell concentration at experimental time t_2 and x_1 the cell concentration at time t_1.

Chlorophyll content determination

Chlorophyll content was determined by centrifuging 10 mL of culture at 4,000 rpm for 20 min. The pellet was resuspended in 10 mL absolute methanol. The whole mixture was maintained for 2 h at 4°C in the dark under agitation before being filtered. Absorption spectrum was measured with a scanning spectrophotometer (DU-530, Beckman Coulter, USA). Chlorophyll-a concentration was calculated according to Lichtenthaler (1987).

Measurement of photosynthetic and dark respiration rates

Samples of the culture suspension were diluted with fresh Zarrouk's medium and then dark-adapted for 30 min as described by Vonshak et al. (1988). Dark respiration and photosynthetic rates were measured as O_2 exchange rates using a Clark-type oxygen electrode at 25°C (Chlorolab 2, Hansatech Instruments). Actinic light was provided by light emitting diodes with a maximum emission around 650 nm. For photosynthesis, light saturation curves were obtained by measuring O_2 exchange rates during successive 1.5-min illumination periods with a stepwise increase from 0 to 1,500 μmol photons $m^{-2}s^{-1}$. For dark respiration, O_2 consumption rates were measured in darkness over 5 min. Each value is based on three repetitions, and the corresponding means are presented.

Glucose content determination

Glucose was analyzed with dinitrosalicylic acid method (Miller 1959).

Experimental design

The two factors (light intensity and glucose concentration) were examined at three levels as listed in Table 1. The levels of each factor were chosen based on the available literature.

To evaluate the effect of the two factors on growth and photosynthetic responses, a quadratic full factorial design was used. A total of 11 experiments (nine points of the factorial design and two center points to establish the experimental errors) were carried out in randomized run order.

Using this design, both factors were tested at three different levels: light intensity at 50 μmol photons $m^{-2}s^{-1}$ (level −1),

Table 1 Maximal specific growth rate (μ_{max}), maximal biomass concentration (X_{max}), net photosynthetic rate (P_n), and dark respiration rate (R_d) for various culture conditions of light intensity and glucose concentration

Experiment	Factors		Responses			
	Light (μmol photons $m^{-2}s^{-1}$)	Glucose ($g\ L^{-1}$)	Maximal biomass, X_{max} ($g\ L^{-1}$)	Maximum specific growth rate, μ (day^{-1})	Net photosynthesis, P_n (μmol O_2mg $Chl^{-1}h^{-1}$	Dark respiration, R_d (μmol uptake O_2mg $Chl^{-1}h^{-1}$)
1	50	0.5	0.51	0.28	43.77	45.85
2	100	0.5	0.44	0.24	48.56	41.60
3	150	0.5	0.73	0.43	84.32	46.76
4	50	1.5	0.73	0.33	46.91	45.85
5	100	1.5	0.80	0.19	47.61	50.87
6	150	1.5	0.83	0.49	135.79	46.16
7	50	2.5	0.85	0.35	49.21	50.74
8	100	2.5	0.91	0.37	67.78	63.27
9	150	2.5	1.33	0.49	132.7	65.00
10	**100**	**1.5**	**0.86**	**0.23**	**67.83**	**52.93**
11	**100**	**1.5**	**0.84**	**0.23**	**65.29**	**39.94**

Bold values correspond to the central point repeated two times

100 μmol photons $m^{-2}s^{-1}$ (level 0), and 150 μmol photons $m^{-2}s^{-1}$ (level 1) and glucose concentration 0.5 gL^{-1} (level −1), 1.5 gL^{-1} (level 0), and 2.5 gL^{-1} (level 1). The response variables selected were the maximum specific growth rate of *A. platensis* (μ_{max}, day^{-1}), maximum biomass concentration (X_{max}, $g\ L^{-1}$), maximum net photosynthetic rate (P_n, μmol O_2mg $Chl^{-1}h^{-1}$), and dark respiration rate (R_d, μmol uptake O_2mg $Chl^{-1}h^{-1}$).

The parameters of the model were estimated by multiple linear regression using program MODDE 7.0, a software for experimental design and optimization (Umetrics AB, Umeå, Sweden).

The accuracy of the model fit was evaluated by the explained variation (R^2 adjusted) and the model validity (lack of fit).

Results

Growth was evaluated by following the increase of the biomass concentration of the culture over 11 days under the same light intensity (150 μmol photons $m^{-2}s^{-1}$) but different trophic conditions: photoautotrophic, without addition of glucose, and mixotrophic with the addition of 2.5 gL^{-1} glucose concentration (Fig. 1).

As shown in Fig. 1, during the whole time course of growth, the mixotrophic culture had the highest biomass concentration at any given time when compared to photoautotrophic culture. Maximum biomass concentration produced mixotrophically over 11 days cultivation was 2.3 times greater than that achieved in photoautotrophic condition.

Photosynthesis, dark respiration, and instantaneous relative growth rate

Photosynthesis and oxidative glucose metabolism existed simultaneously in *A. platensis* cells grown under mixotrophic conditions, and indeed, the two processes may affect each other. Oxygen levels in photoautotrophic and mixotrophic conditions were measured daily at the same time for 11 days. Photosynthetic parameters obtained from *P–I* curves are net photosynthetic rate (P_n) and dark respiration rate (R_d). IRGR was also followed every day during 11 days of culture.

The changes in the instantaneous relative growth rates, net photosynthetic rates, and dark respiration rates with cultivation time under photoautotrophic and mixotrophic conditions are plotted, respectively, in Fig. 2a, b.

For both cultures, two phases are revealed. The first phase, during the first 3 days of growth, was characterized by an increase of the instantaneous relative growth rates and the highest rates of net photosynthesis and dark respiration. The second phase, beyond 3 days of culture, is recognizable by a fast decrease of the rates of the instantaneous relative growth and photosynthesis. However, dark respiration rates are maintained at high values.

During the first phase, mixotrophic cultures are characterized by the highest rates of instantaneous relative growth, net photosynthesis, and dark respiration, which are about 1.5 times higher than those observed in autotrophic culture.

During the second phase, mixotrophic cultures show a rapid decrease of net photosynthetic rates to negative values (−31 μmol O_2mg $Chl^{-1}h^{-1}$), indicating the enhancement of

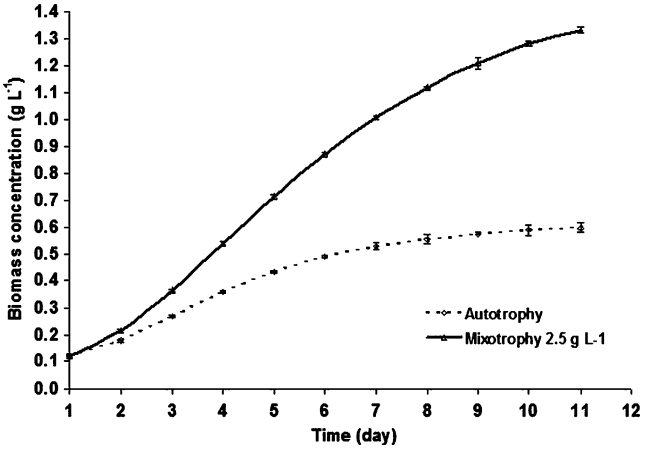

Fig. 1 Growth curve of *A. platensis* cells under photoautotrophic and mixotrophic conditions at light intensity of 150 μmol photons $m^{-2}s^{-1}$

photoheterotrophy. However, dark respiration rates are maintained at higher values and even increase from the sixth day (78.61 μmol uptake O_2mg Chl^{-1} h^{-1}).

For photoautotrophic cultures, in the second phase, net photosynthetic rates are maintained approximately stable at 70 μmol O_2mg $Chl^{-1}h^{-1}$; however, dark respiration rates reduced from 43.99 to 18.28 μmol uptake O_2mg Chl^{-1}.

Specific rates of glucose consumption (Fig. 3) showed that all quantity of available glucose was totally consumed during the first 3 to 4 days of growth.

Experimental design

The influence of light intensity and glucose concentration on growth and photosynthesis was investigated using a RSM. The results are summarized in Table 1. Analysis of the results was performed by MODDE. 7.0. The effect of each factor and their interactions was obtained by ANOVA with confidence interval of 90%.

Growth was characterized by two responses: maximum biomass concentration (X_{max}) and maximum specific growth rate (μ_{max}), whereas photosynthesis was evaluated by maximum net photosynthetic rate (P_n) and dark respiration rate (R_d).

The results of the ANOVA are reported in Table 2 and indicate that the models developed for maximum biomass concentration and maximum specific growth rate have a good R^2 ($R \geq 0.91$) without any lack of fit. These models appeared therefore adequate to accurately fit the experimental data. As indicated by the P value, linear and quadratic effects of light and glucose and their interaction were significant ($P<0.1$) for maximum biomass concentration. For the maximum specific growth rate, the P value indicated that glucose has only a significant linear effect; however, light has both significant linear and quadratic effects ($P<0.1$).

For photosynthetic responses, ANOVA results (Table 3) indicate that the polynomial model describing dark respiration rate was not good ($R<0.8$). However, for maximum net photosynthetic rate, the model appeared adequate without any lack of fit and has a good R^2 ($R=0.95$). The P value indicated that linear effects of light and glucose and the quadratic effect of light were significant on maximum net photosynthetic rate ($P<0.1$). The interaction effect of light and glucose can be considered significant ($P \leq 0.1$).

Analysis of the predicted data by response surface plots (Fig. 4a) revealed that the maximum biomass concentration (1.33 gL^{-1}) was achieved at the highest level of light intensity (150 μmol photons $m^{-2}s^{-1}$) and glucose concentration (2.5 g L^{-1}).

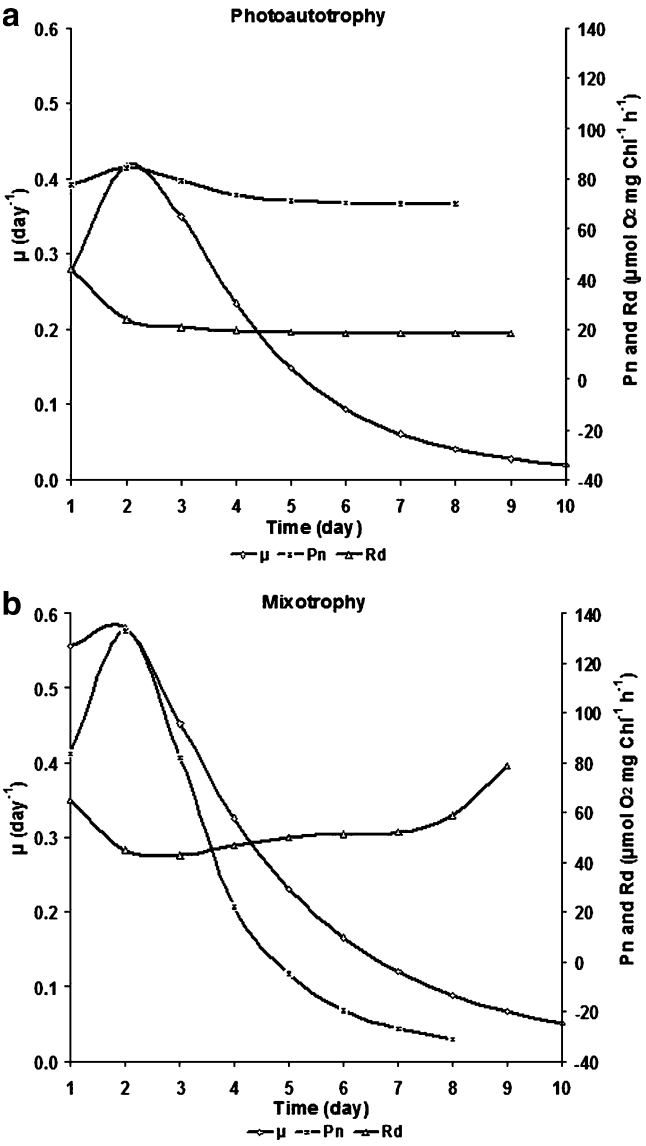

Fig. 2 Rates of the instantaneous relative growth, net photosynthesis, and dark respiration under photoautotrophic (**a**) and mixotrophic (**b**) conditions at a light intensity of 150 μmol photons $m^{-2}s^{-1}$

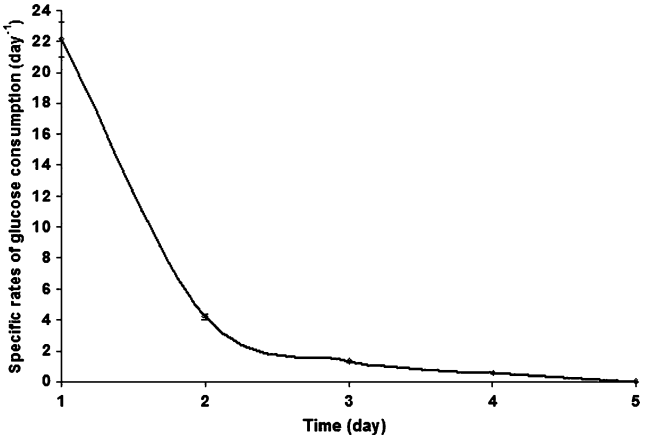

Fig. 3 Specific rates of glucose consumption at a light intensity of 150 µmol photons $m^{-2}s^{-1}$ and initial glucose concentration of 2.5 g L^{-1}

As shown in Fig. 4b, the increase of light intensity from 50 to 100 µmol photons $m^{-2}s^{-1}$ induced a slight decrease of the maximum specific growth rate. Over 100 µmol photons $m^{-2}s^{-1}$, the maximum specific growth rate increased with the increase of light intensity from 100 to 150 µmol photons $m^{-2}s^{-1}$.

The highest maximum specific growth rate (0.49 day^{-1}) was set at the highest level of light intensity (150 µmol photons $m^{-2}s^{-1}$) and glucose concentration (2.5 g L^{-1}).

The maximum net photosynthetic rate (130 µmol O_2 mg $Chl^{-1}h^{-1}$) was observed at the highest level of light intensity and glucose concentration (Fig. 4c). The interaction effect of glucose concentration and light intensity on maximal net photosynthetic rate was more sensitive when increasing light from 100 to 150 µmol photons $m^{-2}s^{-1}$ and essentially at high glucose concentrations (more than 1.4 g L^{-1}).

Discussion

The results showed that *A. platensis* grows in the presence of organic substrate (glucose) in the light. Independently of light intensity and glucose concentration, rates of the instantaneous relative growth, net photosynthesis, and dark respiration demonstrated two different phases.

The first phase occurred during the first 3 to 4 days. It was characterized by the highest rates of instantaneous relative growth and net photosynthesis, as well as the preponderance of photosynthetic activity even in mixotrophic cultures, as seen by the increase in pH until the third day (data not shown). This result might be supported by the data reported by Yang et al. (2000) who found that light was the major source for ATP production in the early phase of mixotrophic cultivation.

The second phase occurred from the fourth day onwards. It was characterized by the decrease of the instantaneous relative growth rates. In photoautotrophic cultures, rates of maximal net photosynthesis and dark respiration were maintained at constant values which are lower than those observed during the first phase. However, in mixotrophic cultures, net photosynthetic rate was reduced to negative values and dark respiration rate increased. Thus, we suggest that metabolic activity was based essentially on photoheterotrophy. This suggestion is supported by the decrease of pH during this phase, due to the release of carbon dioxide, caused by the heterotrophic component of mixotrophic metabolism as reported by Hase et al. (2000).

These results are in contrast with those observed by Chen and Zhang (1997) who showed that heterotrophic metabolism dominated in the first phase, then decreased subsequently as the glucose was consumed, and later

Table 2 R^2 and lack of fit of the polynomial models and coefficients (scaled and centered) and *P* values of both linear and quadratic effects of light intensity and glucose concentration and their interaction for growth responses: X_{max}, maximum biomass concentration, and μ_{max}, maximum specific growth rate

	X_{max} (g L^{-1})		μ_{max} (day^{-1})	
	R^2	Lack of fit	R^2	Lack of fit
	0.99	0.27	0.91	0.17
	Coefficient SC	*P* value	Coefficient SC	*P* value
Constant	0.818	3.23821e−006	0.243	0.000137
Light	0.187	0.000682	0.076	0.009223
Glucose	0.235	0.000157	0.045	0.060531
Light × Light	0.145	0.008037	0.136	0.004918
Gluc × Gluc	−0.120	0.017006	0.026	0.402707
Light × Gluc	0.065	0.035157	−0.001	0.957984

X_{max}=0.5987–0.0098 Light + 0.4639 Glucose + 5.81.e^{-005} Light × Light −0.1196 Glucose × Glucose + 0.0013 Light × Glucose

μ_{max}=0.6059–0.0099 Light + 0.0445Glucose + 5.71.10^{-5} Light × Light

Table 3 R^2 and lack of fit of the polynomial models and coefficients (scaled and centered) and P values of both linear and quadratic effects of light intensity and glucose concentration and their interaction for photosynthetic responses: P_n, net photosynthetic rate, and R_d, dark respiration rate

	P_n (μmol O_2 mg $Chl^{-1}h^{-1}$)		R_d (μmol uptake O_2 mg $Chl^{-1}h^{-1}$)	
	R^2	Lack of fit	R^2	Lack of fit
	0.95	0.55	0.77	0.79
	Coefficient SC	P value	Coefficient SC	P value
Constant	62.722	$9.68569e^{-005}$	47.585	$1.328e^{-005}$
Light	35.487	0.000493	2.580	0.301735
Glucose	12.173	0.040597	7.467	0.020747
Light × Light	24.908	0.014774	−1.087	0.765416
Gluc × Gluc	−8.272	0.279815	5.343	0.182049
Light × Gluc	10.735	0.105145	3.338	0.278310

P_n=93.1963–1.4285 Light − 9.2967 Glucose + 0.0091 Light × Light + 0.2147 Light × Glucose

photosynthesis became predominant. The same comment was signaled by Andrade and Costa (2007). Indeed, these suggestions were based only on the glucose consumption and phycocyanin content.

Our results showed effectively that the total glucose in the medium was consumed during the first 3 days, considered as the photoautotrophic phase and characterized by the highest net photosynthetic rate. Thus, we hypothesise that glucose was stored as reserve carbohydrate (glycogen) to be metabolized in the second phase considered as heterotrophic. Indeed, Pelroy et al. (1972) showed in *Synechocystis* sp. that exogenous glucose is mainly stored as glycogen under illumination before being metabolized for the maintenance of cells (Yang et al. 2000).

Our hypothesis is supported by results of Martinez and Orus (1991) who noted that respiratory rate was noticeably enhanced in mixotrophic cultures, reflecting the increasing rate of glucose metabolism after the induction of glucose uptake ability.

Moreover, in the second phase and additional to the decrease of the net photosynthetic rate, we noted a reduction of the photochemical efficiency of photosystem II (data not shown) accompanied by lower electron transport rate. Therefore, organic carbon sources reduced the photosynthetic efficiency in this phase, and the enhancement of biomass of *A. platensis* implied that organic sources had more pronounced effects on respiration than on photosynthesis. These conclusions are in agreement with results obtained with *Phaeodactylum tricornutum* under mixotrophic culture (Liu et al. 2009).

All the above results confirmed that, in the second phase, *A. platensis* might use and metabolize glucose and then shift to the heterotrophic nutrition mode.

Comparison between mixotrophic and autotrophic cultures showed that the former were characterized by the highest values of the instantaneous relative growth rates and maximal biomass concentration. These results are consistent with those obtained by Marquez et al. (1993,

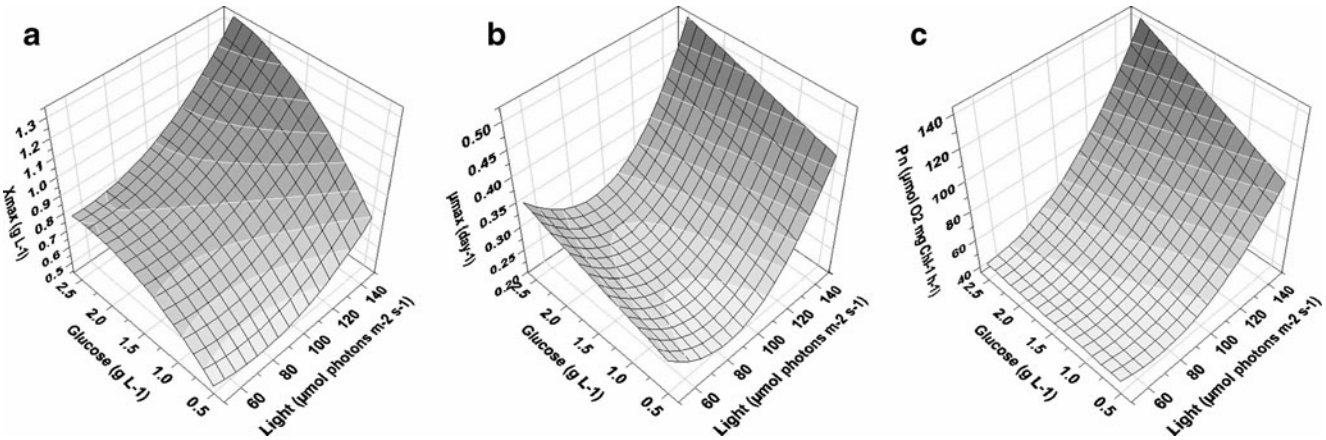

Fig. 4 Response surface plot vs light intensity and glucose concentration for maximum biomass concentration (**a**), maximum specific growth rate (**b**), and net photosynthesis (**c**)

1995), Vonshak et al. (2000), Zhang et al. (1999), and Andrade and Costa (2007).

Marquez et al. (1993) and Hata et al. (2000) suggested that in mixotrophic culture, a simultaneous uptake of organic compounds and CO_2 takes place as carbon sources for cell synthesis, and it is then expected that CO_2 will be released via respiration and will be rapidly trapped and reused under sufficient light intensity. Thus, mixotrophic cells acquire the energy by catabolizing organic compounds via respiration and converting light energy into chemical energy via photosynthesis (Hata et al. 2000).

Effectively, both photosynthetic and dark respiration rates were the highest in mixotrophic cultures, as observed by Vonshak et al. (2000) in *A. platensis*, Kang et al. (2004) in *Synechococcus* sp., Yu et al. (2009) in *Nostoc flagelliforme*, and Orus et al. (1991) in *Chlorella vulgaris*.

The biomass gain recorded in mixotrophic cultures was achieved in the second phase characterized by the nutritive salt reduction and the decrease of the available photons for cells as a consequence of shading caused by the increase in cell density. Such conditions seem to stimulate heterotrophic growth which gives the possibility to increase biomass concentration (Chen et al. 1996; Chen and Zhang 1997).

Martinez et al. (1997) indicated in *Chlorella pyrenoidosa* that light contributes the energy needed for growth and cell maintenance, while glucose is used for the formation of biomass.

Furthermore, the results of Yu et al. (2009) demonstrated that, during the first 4 days, the cell concentration in mixotrophic culture of *N. flagelliforme* was lower than the sum of those in photoautotrophic and in heterotrophic culture. However, from the fifth day, the cell concentration in mixotrophic culture surpassed the sum of those obtained from the other two trophic modes. The reason for this phenomenon requires further investigation.

Growth and photosynthetic activity of mixotrophic cultures were evaluated with respect to light intensity and glucose concentration using response surface methodology. The experimental design showed that polynomial models dependent on light intensity and glucose concentration could describe relatively accurately the maximal biomass concentration, maximum specific growth rate, and maximum net photosynthetic rate.

The results showed clearly that all these responses were influenced by both factors. Furthermore, the interaction of both factors showed that at low light intensity, glucose had a low effect on maximum biomass concentration and maximum net photosynthetic rate. However, at the highest light intensity (150 μmol photons $m^{-2}s^{-1}$), the effect of glucose was positive and the responses were more sensitive to it. The effect of this organic carbon substrate might be attributed to the protective role of glucose or to the shift in light intensity at which photo-inhibition occurs, as explained by Chojnacka and Marquez-Rocha (2004). Thus, cells growing heterotrophically might use part of the O_2 produced by cells growing photoautotrophically, decreasing dissolved oxygen concentration; this can help reduce photo-oxidative damage.

As has been observed in the response surface plot, the maximum biomass concentration (1.33 gL^{-1}) was obtained at the highest light intensity (150 μmol photons $m^{-2}s^{-1}$) and glucose concentration (2.5 gL^{-1}). The same conditions improved maximum specific growth rate (0.49 day^{-1}) and maximum net photosynthetic rate (139.89 μmol O_2mg Chl^{-1} h^{-1}). Conditions favoring high biomass production and maximum net photosynthetic rate were, however, not optimal. Indeed, the optimal conditions of light intensity and glucose concentration are not achieved in the experimental range used in this study. Therefore, further studies that extend the experimental range of light intensity and glucose concentration might be required to reveal optimal conditions that maximize growth and photosynthesis of mixotrophic cultures.

For the same species, Zhang et al. (1999), using a number of mathematical models, found that the optimal initial glucose concentration and light intensity were 2.5 gL^{-1} and 48 μmol photons $m^{-2}s^{-1}$ (4 klx), respectively. However, Chojnacka (2003) determined optimal growth parameters to be 2.5 gL^{-1} glucose concentration and 126 μmol photons $m^{-2}s^{-1}$ (10.5 klx) light intensity. This difference in the optimal light intensity, as commented by Chojnacka and Marquez-Rocha (2004), could be due to the different methods of light intensity measurement and distribution of cells inside the culture vessels. Considering all the findings drawn from the experimental design, it is also recommended that data from batch cultures should be further examined to develop much more accurate models.

In conclusion, the present paper reports that both maximum net photosynthetic and dark respiration rates of mixotrophic cells were significantly higher than these rates in photoautotrophic cells. This suggests that the increased growth rate of *A. platensis* is due to the synergistic effect of photosynthesis and glucose oxidation. The first phase of mixotrophic cultures was identified as mainly photoautotrophic, while the second was photoheterotrophic.

The experimental design showed that polynomial models dependent on light intensity and glucose concentration described relatively accurately maximal biomass concentration, maximal specific growth rate, and maximal net photosynthetic rate. Therefore, these models can be useful for the optimization of *A. platensis* productivity and can be employed to predict biomass production in mixotrophic cultures.

Acknowledgments We are grateful to Dr. Kristina Raab, researcher in Wageningen IMARES, for her considerable comments in improving the language of this manuscript.

References

Andrade MR, Costa JAV (2007) Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture 264:130–134

Box GEP, Draper NR (1987) Empirical model building and response surfaces. Wiley, New York

Chen F, Zhang Y (1997) High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. Enzyme Microb Technol 20:221–224

Chen F, Zhang Y, Guo S (1996) Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnol Lett 18:603–608

Chojnacka K (2003) Heavy metal ions removal by microalgae *Spirulina sp*. in the processes of biosorption and bioaccumulation. PhD dissertation, Wroclaw University of Technology, Poland

Chojnacka K, Marquez-Rocha FJ (2004) Kinetic and stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. Biotechnology 3:21–34

Chojnacka K, Noworyta A (2004) Evaluation of *Spirulina* sp. Growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme Microb Technol 34:461–465

Danesi EDG, Rangel-Yangui CO, Carvalho JCM, Sato S (2004) Effect of reducing the light intensity on the growth and production of chlorophyll by *Spirulina platensis*. Biomass Bioenerg 26:329–335

Hase R, Oikawa O, Sasao C, Morita M, Watanabe Y (2000) Photosynthetic production of microalgal biomass in a raceway system under greenhouse conditions in Sendai city. J Biosci Bioeng 89:157–163

Hata JI, Hua Q, Yang C, Shimizu K, Taya M (2000) Characterization of energy conversion based on metabolic flux analysis in mixotrophic liverwort cells, *Marchantia polymorpha*. Biochem Eng J 6:65–74

Kang R, Wang J, Shi D, Cong W, Cai Z, Ouyang F (2004) Interaction between organic and inorganic carbon sources during mixotrophic cultivation of *Synechococcus* sp. Biotechnol Lett 26:1429–1432

Leduy A, Therien N (1977) An improved method for optical density measurement of the semimicroscopic blue green algae *Spirulina maxima*. Biotechnol Bioeng 19:1219–1224

Lichtenthaler HK (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In: Packer L, Douce R (eds) Methods in enzymology, Vol 148. Academic Press, New York, pp 350–382

Liu X, Duan S, Li A, Xu N, Cai Z, Hu Z (2009) Effect of organic carbon sources on growth, photosynthesis, and respiration of *Phaeodactylum tricornutum*. J Appl Phycol 21:239–246

Lodi A, Binaghi L, De Faveri D, Carvalho JCM, Converti A, Del Borghi M (2005) Fed-batch mixotrophic cultivation of *Arthrospira (Spirulina) platensis* (Cyanophyceae) with carbon source pulse feeding. Ann Microbiol 55(3):181–185

Marquez FJ, Sasaki K, Kakizono T, Nishio N, Nagai S (1993) Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. J Ferment Bioeng 76:408–410

Marquez FJ, Nishio N, Nagai S, Sasaki K (1995) Enhancement of biomass and pigment production during growth of *Spirulina platensis* mixotrophic culture. J Chem Tech Biotech 62:159–164

Martinez F, Orus MI (1991) Interactions between glucose and inorganic carbon metabolism in *Chlorella vulgaris* strain UAM 101. Plant Physiol 95:1150–1155

Martinez F, Camacho F, Jiménez JM, Espinola JB (1997) Influence of light intensity on the kinetic and yield parameters of *Chlorella pyrenoidosa* mixotrophic growth. Process Biochem 32:93–98

Miller GL (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 31:426–428

Orus MI, Marco E, Martinez F (1991) Suitability of *Chlorella vulgaris* UAM 101 for heterotrophic biomass production. Bioresour Technol 38:179–184

Pelroy RA, Rippka R, Stanier RY (1972) Metabolism of glucose by unicellular blue-green algae. Archiv für Mikrobiologie 87:303–322

Richmond A (1988) *Spirulina*. In: Borowitzka MA, Borowitzka LJ (eds) Micro-Algal biotechnology. Cambrige University Press, Cambridge, pp 85–121

Vonshak A, Guy R, Guy M (1988) The response of the filamentous cyanobacterium *Spirulina platensis* to salt stress. Arch Microbiol 150:417–420

Vonshak A, Cheung SM, Chen F (2000) Mixotrophic growth modifies the response of *Spirulina (Arthrospira) platensis* (cyanobacteria) cells to light. J Phycol 36:675–679

Yang C, Hua Q, Shimizu K (2000) Energetics and carbon metabolism during growth of microalgal cells under photoautotrophic, mixotrophic and cyclic light–autotrophic/dark–heterotrophic conditions. Biochem Eng J 6:87–102

Yu H, Jia S, Dai Y (2009) Growth characteristics of the cyanobacterium *Nostoc flagelliforme* in photoautotrophic, mixotrophic and heterotrophic cultivation. J Appl Phycol 21:127–133

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* Geitler. PhD thesis, University of Paris

Zhang XW, Zhang YM, Chen F (1999) Application of mathematical models to the determination optimal glucose concentration and light intensity for mixotrophic culture of *Spirulina platensis*. Process Biochem 34:477–481