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Optimization of the repeated batch cultivation of microalga *Spirulina platensis* in open raceway ponds

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Abstract

The cyanobacterium *Spirulina platensis* is extensively used in human nutrition because it is a source of beneficial phenolics, proteins, unsaturated lipids and vitamins. Repeated batch cultivation of photosynthetic microorganisms is widely used industrially because it allows actively growing cultures to be maintained for long periods of time. Despite this, there have been few detailed studies involving the repeated batch cultivation of *S. platensis*. We used a Box–Behnken factorial design to optimize the repeated batch cultivation of *S. platensis* in open raceway ponds at 30 °C under a light intensity of 3000 lx and a 12 h photoperiod for a total cultivation time of 1500 h (60 days) where the variables were blend concentration (0.40, 0.60 and 0.80 g l^{-1}), renewal rate (20, 40 and 60%) and culture medium, the culture media being Zarrouk medium in three different dilutions. We found that *S. platensis* productivity was 0.028 to 0.046 g l^{-1} day^{-1} and the maximum specific growth rate (μ_{max}) 0.038 to 0.138 day^{-1}, all the process variables being statistically significant at $p<0.05$. Our results show that semicontinuous cultivation of *S. platensis* can be optimized using a medium consisting of 20% (v/v) Zarrouk Medium, a *S. platensis* blend concentration of 0.40 g l^{-1} and a renewal rate of between 40 and 60%.

Keywords: Open raceway ponds; Repeated batch cultivation; *Spirulina platensis*

1. Introduction

The commercial cultivation of microalgae and cyanobacteria on an industrial scale began with the culture of *Chlorella* in Japan in the 1960s followed by the cultivation of *Spirulina* in Mexico, the USA and China in the 1970s. In the last 30 years the biotechnological industry of photosynthetic microorganisms has grown and become very diversified. The most important commercially produced photosynthetic microorganisms are *Spirulina*, *Chlorella* and *Dunaliella*. A common characteristic of them is that because they grow in highly selective media they can be cultivated in open systems but remain relatively free from contamination by other microorganisms (Borowitzka, 1999).

The commercial production of *Spirulina* biomass is based almost exclusively on open reactors, especially open raceway ponds (Richmond, 1990). Although some

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problems in production plants are frequently observed, the open cultivation of *Spirulina* is easily accomplished. The tanks are relatively simple to build and to operate, and can have scale increase up to 5000 m^2 or even more (Tredici et al., 1993).

In both open and closed (batch) cultivations, the composition of *S. platensis* is considerably influenced by the cultivation conditions, including pH, light intensity and the presence of contaminants (Marek et al., 1987; Vonshak et al., 1982), temperature (Vonshak et al., 1982), the presence of bicarbonate ions (Costa et al., 2002; Vonshak et al., 1982), nitrogen source (Danesi et al., 2002; Costa et al., 2000), bioreactor type (Mirón et al., 1999), initial biomass concentration (Pelizer et al., 2003; Costa et al., 2000) and population density (Gitelson et al., 1996; Qiang et al., 1996; Vonshak et al., 1982). However, these factors have only been studied for batch cultivation although industrial scale batch cultivation of photosynthetic microorganisms is generally economically unviable because of the time and expense involved in loading, discharging and cleaning the bioreactor.

The repeated batch cultivation is an alternative form of operation for microalgae production. In repeated batch cultivation the reactor is initially filled with the cultivation medium and incubated under ideal conditions. After certain period a specific cultivation volume is removed and replaced with an equal amount of fresh medium. Consequently a part of cultivation medium is kept in reactor as starting inoculum (Otero et al., 1998). Repeated batch mode of operation provides an excellent means of regulating the nutrients feed rate to optimize the productivity while at the same time preventing the over and underfeeding of nutrients (Giridhar and Srivastava, 2001). Repeated batch cultivation presents several operational advantages, the most important of which are the maintenance of a constant inoculum and high growth rates (Fábregas et al., 1996, 1995a). It is curious that although repeated batch cultivation is very often used for growing microalgae and cyanobacteria relatively few papers have been published on the dynamics of this type of system involving variables such as blend concentration and renewal rate (Kaewpintong et al., 2007; Reinehr and Costa, 2006; Reichert et al., 2006; Hata et al., 2001; Travieso et al, 2001; Otero et al., 1998; Fábregas et al., 1996, 1995a,b).

Response surface methods are often used when curvature in the response surface is suspected. As with central composite designs, Box–Behnken designs are response surface methods used to examine the relationship between one or more response variables and a set of experimental parameters. These designs also ensure that all factors are never set at their high levels, simultaneously. Furthermore, Box–Behnken designs have fewer design points. Also, each factor requires only three levels, which may be experimentally more convenient and less expensive to run than central composite designs with the same number of factors (Box and Behnken, 1960).

The objective of our study was to optimize the repeated batch cultivation of *S. platensis* in open raceway ponds, evaluating the influence of *S. platensis* biomass concentration, medium composition and renewal rate on specific growth rate and productivity.

2. Material and methods

2.1. Microorganism and cultivation media

In this study we used *Spirulina platensis* strain LEB-52 (Costa et al., 2000) maintained and grown in undiluted Zarrouk medium (Zarrouk, 1966).

For the experiments Zarrouk medium was used in three different dilutions: undiluted, 50% Zarrouk medium and 50% distilled water, 20% Zarrouk medium and 80% distilled water. Zarrouk medium was first defined in 1966, and previous works have showed that this medium can be diluted because the concentration of nutrients is high enough ((Reichert et al., 2006, Reinehr and Costa, 2006). All reagents were of analytical grade and purchased from Merck KGaA. (Darmstadt, Germany), Vetec (São Paulo, Brazil) or Synth Chemical Co. (São Paulo, Brazil).

2.2. Cultivation conditions and analytical determinations

Cultivations were carried out in 6 l acrylic open raceway ponds (Fig. 1) containing 5 l of culture medium and an initial *S. platensis* biomass concentration of 0.15 g l^{-1} (Costa et al., 2003). Raceway ponds are normally used in open commercial cultivation of microalgae. A geometric scale-down was done in order to obtain the dimensions of our reactor. The raceway ponds were maintained in a non-sterile chamber at 30 °C and the cultures agitate using a paddle-wheel rotating at 18 revs min^{-1}, illumination being provided by Osram 40 W (3000 lx) daylight-type fluorescent lamps under a 12 h photoperiod (Reichert et al., 2006; Tanticharoen et al., 1994; Vonshak et al., 1982).

Samples were collected daily and the *Spirulina* biomass concentration monitored by optical density (o.d.) at 670 nm using a Varian Cary 100 spectrophotometer and a calibration curve of o.d. against dry weight (g l^{-1}) of *Spirulina* biomass (Muliterno et al., 2005).

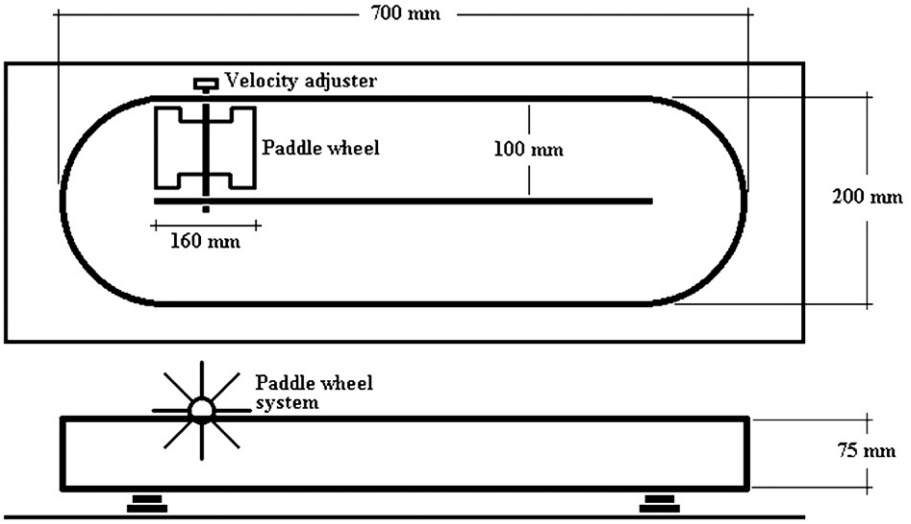

Fig. 1. Diagram of the open raceway pond used in the cultivations.

2.3. Experimental design

A Box–Behnken experimental design was used (Prvan and Street, 2002) with three factors varying each one in three levels. Table 1 presents the matrix of the factorial design with the variables and their respective levels.

When the *S. platensis* biomass concentration in the culture reached a predetermined level (0.40, 0.60 or 0.80 g l^{-1}, named as the 'blend concentration', X_1) a portion of the medium (20, 40 or 60% (v/v), the 'renewal rate', X_2) was withdrawn and the same amount of fresh medium added. Each run lasted about 60 days (1500 h).

The specific growth rate (μ_x) in the exponential phase was obtained by exponential regression and the biomass doubling time (t_d) calculated using $t_d=(\ln 2)/\mu_x$. The *Spirulina* biomass productivity (P_x) was expressed as g l^{-1} day^{-1}. All these values were calculated for each growth cycle (i.e. after the addition of each batch of fresh medium when new exponential growth occurred) along with the averages and standard deviations for each run.

2.4. Statistical analysis

The influence of the variables studied on μ_x and P_x was adjusted using a second degree polynomial function (Eq. (1)):

$$Y = b_0 + \sum_{i=1}^{3} b_i X_i + \sum_{i=1}^{3} b_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} b_{ij} X_i X_j \quad (1)$$

where b_0 is the independent term representing the average value of the experimental result, b_i are the linear regression coefficients explaining the influence of the variables, b_{ii} are the quadratic regression coefficients explaining the influence of the variables, and b_{ij} are the regression coefficients defining the interactions between the variables. The significance levels for each variable obtained by the analysis of variance (ANOVA) are presented in the tables along with their effects.

A lack-of-fit test was also applied to compare the variation of the residues of the model with the variation of the observed results. Statistical significance higher than 0.05 in the lack-of-fit test implies that the model is suitable for the observed data with a confidence interval

Table 1
Box–Behnken matrix showing the variables and their respective levels for the *Spirulina platensis* growth experiments

Run	Blend concentration (g l^{-1}, coded as X_1)	Renewal rate (%, coded as X_2)	Zarrouk medium (%, coded as X_3)
1	0.40	20	100
2	0.80	20	100
3	0.40	60	100
4	0.80	60	100
5	0.40	40	50
6	0.80	40	50
7	0.40	40	20
8	0.80	40	20
9	0.60	20	50
10	0.60	60	50
11	0.60	20	20
12	0.60	60	20
13	0.60	40	100
14	0.60	40	100
15	0.60	40	100

of 95% and that there are no significant effects outside the model.

3. Results and discussion

The average μ_x, t_d and P_x values and their standard deviations are shown in Table 2, from which it can be seen that runs 3, 5 and 7 with low biomass and high and medium renewal rates presented the highest μ_x values with an increase of up to 260% as compared to the lowest μ_x values obtained in the low renewal-rate runs 9 (high blend concentration) and 2 (medium blend concentration) indicating that low *S. platensis* blend concentration and higher renewal rates result in higher μ_x values. The highest productivity was obtained in run 7 in which the blend concentration was low and the renewal rate high, the P_x value for this run being 70% higher than the lowest P_x values obtained in the low renewal-rate runs 1 (low blend concentration), 2 (medium blend concentration) and 9 (high blend concentration) (Tables 1 and 2).

Vonshak et al. (1982) verified that *Spirulina* biomass concentrations of 0.40 to 1.00 g l^{-1} resulted in a decrease in the photosynthetic potential of *Spirulina*, because of the absence of incident light on most of the cells caused by the shadowing effect of the cells themselves. Even at concentrations of about 0.50 g l^{-1} (considered ideal for maximal photosynthetic efficiency) about 80% of *Spirulina* filaments spent some time in complete darkness (Vonshak et al., 1982). Batch culture studies on *Spirulina* biomass production carried out by Gitelson et al. (1996) and Qiang et al. (1996) showed that at higher *Spirulina* biomass concentrations μ_x tends to decrease due to the shadowing effect. These authors concluded that when other cultivation conditions are ideal high μ_x values can be expected in systems with low biomass concentrations. These observations are similar to our results in which we found that the runs with the highest μ_x values were those in which the *Spirulina* biomass was never higher than 0.40 g l^{-1}, probably allowing optimal, or semi-optimal, photosynthetic efficiency. The three growth curves (runs 3, 5 and 7) which presented the highest μ_x values (0.134, 0.116 and 0.138 day^{-1}, respectively) are shown in Fig. 2.

Table 2
Growth parameters for *Spirulina platensis* undergoing repeated batch cultivation in open raceway ponds

Run	Growth cycles (N)	Specific growth rate (μ_x, day^{-1})	Doubling time (t_d, day)	Biomass productivity (P_x, g l^{-1} day^{-1})
1	17	0.0716±0.0076	9.7±1.0	0.0278±0.0032
2	8	0.0389±0.0045	17.8±2.2	0.0285±0.0026
3	9	0.1339±0.0115	5.2±0.5	0.0377±0.0035
4	4	0.0549±0.0050	12.7±1.2	0.0317±0.0026
5	12	0.1156±0.0107	6.0±0.5	0.0396±0.0038
6	6	0.0659±0.0058	10.6±0.9	0.0396±0.0038
7	14	0.1377±0.0189	5.1±0.7	0.0462±0.0051
8	5	0.0570±0.0047	12.2±1.1	0.0364±0.0031
9	7	0.0382±0.0035	18.2±1.6	0.0276±0.0010
10	5	0.0799±0.0052	8.7±0.6	0.0335±0.0025
11	13	0.0602±0.0099	11.8±1.8	0.0355±0.0059
12	5	0.0944±0.0053	7.4±0.4	0.0375±0.0010
13	9	0.0787±0.0103	8.9±1.2	0.0360±0.0039
14	9	0.0807±0.0079	8.7±0.9	0.0372±0.0037
15	8	0.0759±0.0103	9.3±1.2	0.0353±0.0051

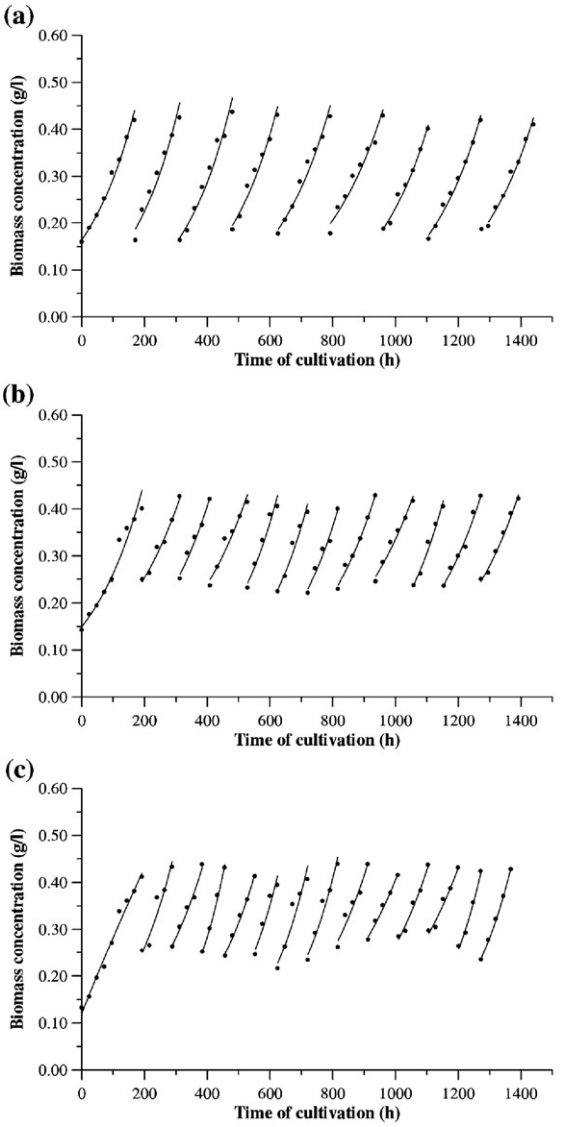

Fig. 2. *Spirulina platensis* biomass as a function of cultivation time for runs 3 (a), 5 (b) and 7 (c).

The behavior of μ_x and P_x as a function cultivation time for runs 3, 5 and 7 are shown in Fig. 3 from which it

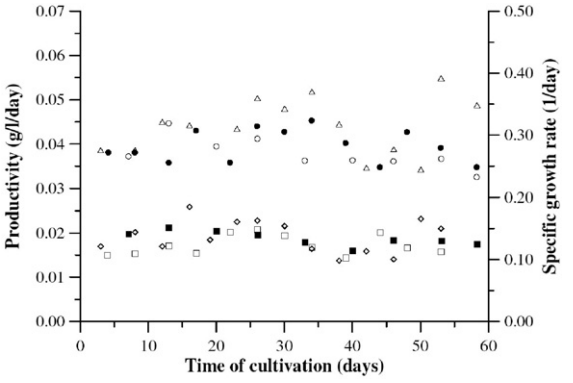

Fig. 3. Productivity (P_x) and specific growth rate (μ_x) as a function of cultivation time. (○) P_x and (□) μ_x for run 3; (●) P_x and (■) μ_x for run 5; (△) P_x and (◇) μ_x for run 7.

can be seen that both μ_x and P_x remained approximately constant over time, indicating that repeated batch cultivation is suitable for the cultivation of *S. platensis*. In all the runs μ_x and P_x remained high for more than 40 days (1000 h), contrasting with batch cultivation studies in which *S. platensis* reaches the end of the exponential phase after about 20 days (500 h) (Costa et al., 2003).

The significance levels and the resulting effects from the ANOVA of the factorial design are presented in Table 3. The three factors studied (*S. platensis* blend concentration (X_1), medium dilution rate (X_2) and cultivation medium (X_3)) significantly ($p<0.05$) influenced

Table 3
Statistical significances (p) and effects obtained from analysis of the factorial design used in the repeated batch cultivation of *Spirulina platensis*

Factors	Specific growth rate (μ_x)		Biomass productivity (P_x)	
	p	Effect	p	Effect
Blend concentration (X_1)				
X_1(L)	<0.0001 *	−0.0624±0.0035	0.0007 *	−4.3±1.2
X_1(Q)	0.0001 *	−0.0104±0.0025	0.1992	−1.1±0.9
Renewal rate (X_2)				
X_2(L)	<0.0001 *	0.0346±0.0037	0.0004 *	4.8±1.3
X_2(Q)	<0.0001 *	0.0153±0.0024	<0.0001 *	5.5±0.8
Cultivation medium (X_3)				
X_3(L)	0.0029 *	0.0111±0.0036	0.0043 *	3.6±1.2
X_3(Q)	0.0004 *	−0.0087±0.0024	<0.0001 *	−4.0±0.8
Interactions				
$X_1 \cdot X_2$	<0.0001 *	−0.0280±0.0048	0.0131 *	−4.3±1.7
$X_1 \cdot X_3$	0.0903	−0.0080±0.0047	0.0096 *	−4.1±1.5
$X_2 \cdot X_3$	0.1148	−0.0088±0.0055	0.2682	−2.2±1.9

L: linear effect, Q: quadratic effect.
* Statistically significant at the 95% confidence interval.

the μ_x both linearly and quadratically. Since the blend concentration quadratic effect was significant it follows that the behavior of μ_x as a function of blend concentration was not linear, with an increase in μ_x occurring at the lowest blend concentration (0.40 g l^{-1}). The quadratic effect of the renewal rate was also significant, with the maximum point occurring at a renewal rate of between 40 and 60%. Regarding cultivation medium, we found that μ_x was lowest with undiluted Zarrouk medium and highest with 20% Zarrouk medium. The interaction between blend concentration and renewal rate ($X_1 \cdot X_2$) was also significant (Fig. 4). At all medium renewal rates μ_x increased when the blend concentration decreased ($p<0.0001$) and μ_x also increased as the renewal rate increased from 20 to 40% ($p<0.0001$), although between renewal rates of 40 and 60% the μ_x values were statistically equal and for blend concentrations of 0.60 ($p=0.4991$) and 0.80 g l^{-1} ($p=0.6948$).

With regard to biomass productivity (P_x), the linear effect of the blend concentration was significant and negative, i.e. P_x was highest at a blend concentration of 0.40 g l^{-1}. The linear effect of the renewal rate showed a significant positive influence although the quadratic effect was also significant with a maximum between the 40 and 60% renewal rates, P_x being lowest sides with undiluted Zarrouk medium and highest for 20% Zarrouk medium. The interaction effects between blend concentration and the renewal rate ($X_1 \cdot X_2$) and between blend concentration and cultivation medium ($X_1 \cdot X_3$) on P_x were also significant ($p<0.05$), with P_x being lowest when the renewal rate was 20% ($p<0.0001$) independent of the blend concentration. However, when the renewal rate was 60% P_x increased significantly ($p=0.0314$) with decreasing blend concentration from 0.80 to 0.40 g l^{-1}.

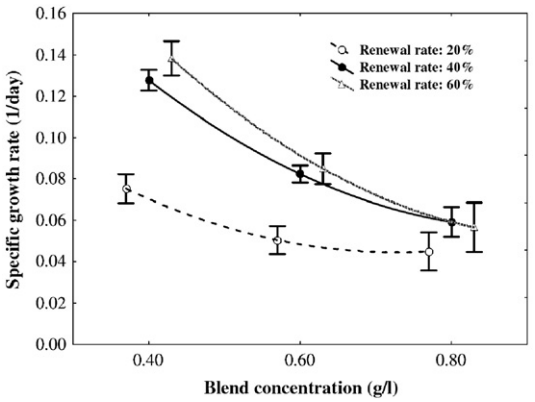

Fig. 4. Influence of *Spirulina platensis* blend concentration and medium renewal rate on specific growth rate (μ_x). Renewal rates: (○) 20%, (●) 40% and (Δ) 60%.

Travieso et al. (2001) studied different renewal rates (from 5 to 25%) during the repeated batch cultivation of *S. platensis* in a tubular photobioreactor with daily renewal of the culture medium (Zarrouk medium). They found that there was significant increase in productivity as the renewal rate increased from 5 to 20%, although productivity began to decline when the dilution rate reached 25%. The results of Travieso partially support our results although we found an increase in productivity even with a renewal rate of 60%, probably due to the fact that Travieso renewed the medium each day (with consequent nutrient dilution) which caused decreased biomass productivity because t_d for *S. platensis* exceeds three days.

The models presented in Eqs. (2) and (3) were obtained from the statistical analysis of the factorial design and they predict μ_x and P_x as a function of blend concentration, renewal rate and cultivation medium for the repeated batch production of *S. platensis*. These models are useful for the optimization of *S. platensis* production because the levels of the variables can be chosen so that maximum values are obtained.

$$\mu_x = 0.0779 - 0.0311 X_1 + 0.0100 X_1^2 + 0.0169 X_2 - 0.0150 X_2^2 + 0.0066 X_3 + 0.0093 X_3^2 - 0.0141 X_1 X_2 \quad (2)$$

$$P_x = 0.0358 - 0.0023 X_1 + 0.0022 X_2 - 0.0053 X_2^2 + 0.0017 X_3 + 0.0043 X_3^2 - 0.0023 X_1 X_2 - 0.0021 X_1 X_3 \quad (3)$$

The residues obtained from the observed and predicted values of these models are shown in Table 4. The correlation coefficient of the μ_x model was 0.9853 and that of the P_x model was 0.9633 while the lack-of-fit tests was showed a significance of 0.1374 for the μ_x model and 0.1638 for the P_x model, indicating that these models faithfully represent the observed data which show no significant deviation from the models.

The contour plots (projected response surface diagrams) based on the statistical models for P_x are presented in Fig. 5(a) for undiluted Zarrouk medium, Fig. 5(b) for 50% Zarrouk medium and Fig. 5(c) for 20% Zarrouk medium as a function of blend concentration and renewal rate. The maximum productivity of the model occurred at a blend concentration of 0.40 g l^{-1} and a renewal rate of 50% using 20% Zarrouk medium. The productivity obtained with diluted cultivation medium and a blend concentration of 0.40 g l^{-1} was significantly higher ($p=0.0005$) than that with undiluted Zarrouk medium.

Our results are supported by those of Fábregas et al. (1995a) who studied the marine microalga *Dunaliella tertiolecta* and found that the highest productivities occurred at a nitrate concentration of 0.25 g l^{-1}, which is seven times lower than the 1.80 g l^{-1} occurring in Zarrouk medium. Fábregas et al. (1996) evaluated the semicontinuous cultivation of the microalga *Phaeodactylum tricornutum* by varying the renewal rate and the nitrogen concentration from saturation (0.99 g l^{-1}) to limiting levels (0.03 g l^{-1}) and found that the optimum productivity occurred when the renewal rate was high and the nitrogen concentration was 0.25 g l^{-1}. A study of the diatom *Skeletonema costanum* growing under different levels of illumination and inorganic nitrogen showed that when the nitrogen concentration

Table 4
Residues obtained for the specific growth rate (μ_x) and productivity (P_x) models

Run	μ_x (day^{-1})			P_x (g l^{-1} day^{-1})		
	Observed	Predicted	Residues	Observed	Predicted	Residues
1	0.0716	0.0730	−0.0014	0.0278	0.0283	−0.0005
2	0.0389	0.0390	−0.0001	0.0285	0.0283	0.0002
3	0.1339	0.1350	−0.0011	0.0377	0.0373	0.0004
4	0.0549	0.0446	0.0103	0.0317	0.0281	0.0036
5	0.1156	0.1217	−0.0061	0.0396	0.0386	0.0010
6	0.0659	0.0595	0.0064	0.0396	0.0382	0.0014
7	0.1377	0.1349	0.0028	0.0462	0.0462	0.0000
8	0.0570	0.0727	−0.0157	0.0364	0.0374	−0.0010
9	0.0382	0.0487	−0.0105	0.0276	0.0309	−0.0033
10	0.0799	0.0825	−0.0026	0.0335	0.0353	−0.0018
11	0.0602	0.0619	−0.0017	0.0355	0.0343	0.0012
12	0.0944	0.0957	−0.0013	0.0375	0.0387	−0.0012
13	0.0787	0.0779	0.0008	0.0360	0.0358	0.0002
14	0.0807	0.0779	0.0028	0.0372	0.0358	0.0014
15	0.0759	0.0779	−0.0020	0.0353	0.0358	−0.0005

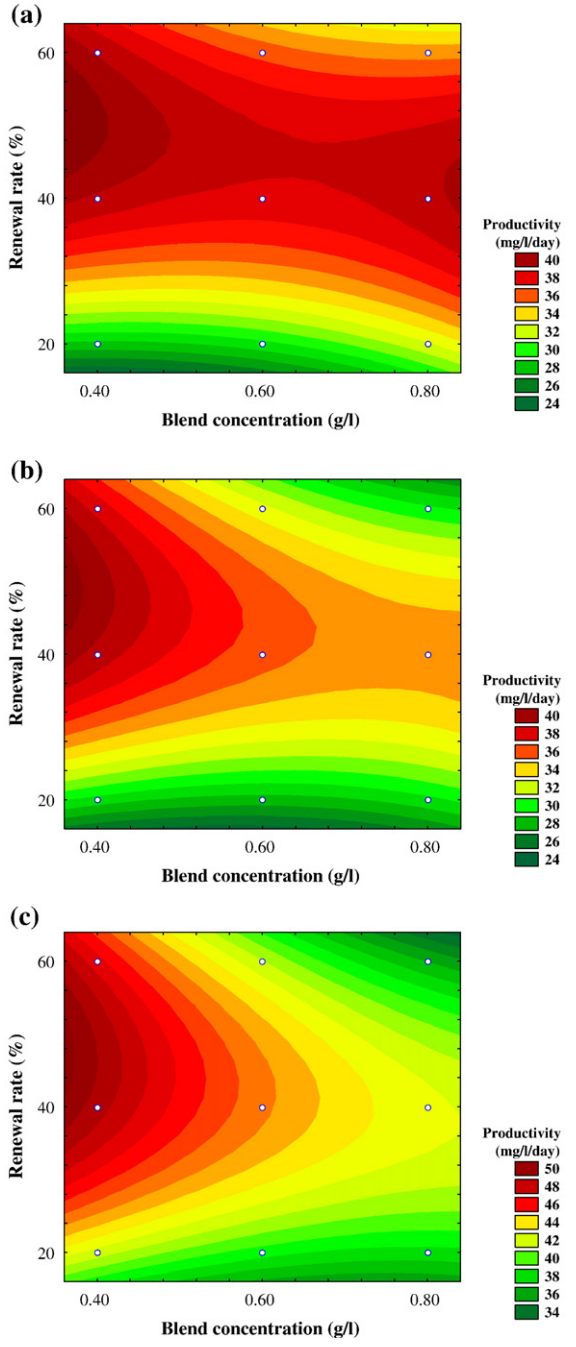

Fig. 5. Contour plots (projected response surfaces) for productivity as a function of blend concentration and renewal rate using: (a) undiluted Zarrouk medium; (b) 50% (v/v) Zarrouk medium plus 50% (v/v) distilled water; (c) 20% (v/v) Zarrouk medium plus 80% (v/v) distilled water.

was very high *S. costanum* needed more energy for the incorporation and reduction of nitrate and that this reduced its photosynthetic efficiency (Smith et al., 1992). Similar observations have been reported during the repeated batch cultivation of the marine microalga *Tetraselmis suecica*, where high nutrient concentration decreased productivity and inhibited the activity of enzymes related to photosynthesis (Fábregas et al., 1995b). Taken together, these observations indicate that the nutrient concentration of undiluted Zarrouk medium is unsuitably high for the repeated batch cultivation of *Spirulina*.

During the batch cultivation of *S. platensis* in undiluted Zarrouk medium in open raceway ponds for 25 days the maximum productivities were 0.026 g l^{-1} day^{-1} (Duarte Filho et al., 2002a) and 0.024 g l^{-1} day^{-1} (Duarte Filho et al., 2002b), significantly ($p<0.05$) lower than most of the repeated batch cultivation P_x values shown in Table 2. Previous works with the same strain (LEB-52) of *S. platensis* showed a productivity of 0.023 g l^{-1} day^{-1} (for batch cultivation) and between 0.019 and 0.042 g l^{-1} day^{-1} (for repeated batch cultivation in closed photobioreactor) (Reichert et al., 2006, Reinehr and Costa, 2006).

Under batch cultivation in open raceway ponds containing lagoon water supplemented with bicarbonate and urea μ_{max} for *S. platensis* was 0.157 day^{-1} but exponential growth continued for only 15 days (Costa et al., 2003), whereas in our experiments although the highest μ_x value was only 0.138 day^{-1} (Table 2) the exponential phase lasted for almost 60 days (1500 h) resulting in higher overall productivity. Reinehr and Costa (2006) studied the repeated batch cultivation of *S. platensis* (LEB-52 strain) in closed photobioreactor and reported μ_x between 0.035 and 0.111 day^{-1}, most of them lower than the values obtained in our study.

An extrapolation of the results obtained in this study to the real application must be carefully evaluated, because the conditions of illuminance and photoperiod are not constant in the environment. Further studies involving the repeated batch cultivation of *Spirulina platensis* under open-air conditions should be developed in order to apply a better adjustment of the variables involved in this type of cultivation.

4. Conclusions

The repeated batch cultivation of *S. platensis* was influenced by both *S. platensis* blend concentration and media renewal rate, highest specific growth rates (μ_x) and biomass productivities (P_x) being obtained at a blend concentration of 0.40 g l^{-1} and a media renewal rate of between 40 to 60%. With undiluted Zarrouk medium μ_x was 0.134 day^{-1} and P_x 0.038 g l^{-1} day^{-1} whereas with 20% Zarrouk medium μ_x was 0.138 day^{-1} and P_x 0.046 g l^{-1} day^{-1}, respectively.

The data presented in this paper demonstrate that the repeated batch cultivation of *S. platensis* in open raceway ponds can be optimized by using diluted Zarrouk medium and selecting suitable medium renewal rate and blend concentration. The implementation of such a system on an industrial scale could lead to a significant decrease in *S. platensis* production costs and may also be applicable to the production of other photosynthetic microorganisms.

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