Plant Archives Vol. 22, No. 2, 2022 pp.491-502 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2022.v22.no2.089

RESPONSE OF ORCHID CUT FLOWERS AS AFFECTED BY FLORAL PRESERVATIVES ON THE POSTHARVEST QUALITY

Ajish Muraleedharan[1]*, K. Sha[1], S. Kumar[1], G. Usha[1], P.K. Karthikeyan[2], C. Praveen Sampath Kumar[3] and J.L. Joshi[3]

[1]Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai nagar, Tamil nadu, India - 608002.

[2] Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University, Annamalai nagar, Tamil nadu, India - 608002.

[3] Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai nagar, Tamil nadu, India - 608002.

*Author for correspondence : ajishm1000@gmail.com

(Date of Receiving : 03-09-2020; Date of Acceptance : 25-11-2020)

ABSTRACT

In this experimental study, a novel cultivation mode in a conical annular photobioreactor (*CAPBR*) was proposed to enhance microalgae biomass production of *Arthrospira platensis*. Three operating factors under varying conditions of initial inoculum concentration (0.25–1.25 $g.L^{-1}$), light intensity (6–180 $\mu mols^{-1}m^{-2}$) and surface/volume (S/V) ratios (94–112 m^{-1}) were optimized. Box-Behnken design combining with response surface methodology was used to evaluate the effect of operating parameters on response variables (Volumetric productivity, Growth rate). Optimal parameters of the chosen variable were found by considering the quadratic model, as well as by evaluating the desirability function. The obtained results showed that the light intensity and S/V ratio were the most significant factors conditioning volumetric productivity and growth rate. Moreover, an important interaction between light intensity and S/V ratio ($P < 0.05$) on volumetric productivity and growth rate was reported.

The maximum volumetric productivity and growth rate of 542 $mgL^{-1}day^{-1}$, 0.785 day^{-1} respectively was obtained at 180 $\mu mols^{-1}m^{-2}$, with inoculum concentration of 0.351 $g.L^{-1}$ and S/V ratio of 112 m^{-1}. According to ANOVA analysis, this model reasonably predicts match the experimental data.

Keywords: Photobioreactor**,** Biomass productivity, *Arthrospira platensis*, optimization, Box-Behnken design.

Introduction

The industrial production of phototrophic microorganisms is gaining momentum in different fields particularly in as food and pharmaceutical industries. Photosynthetic microorganisms present a most attractive energy source because it is renewable and without greenhouse effect. Among the photosynthetic microorganisms of commercial interest, *Arthrospira platensis* is one of the most interesting species. It is unbranched spiral filamentous with 200 to 300 µm in length and 5 to 10 µm in width (Benelhadj *et al*., 2016). They are filamentous cyanobacteria that are commonly found in the marine environment under highly alkaline and brackish living conditions, their commercial applications are prospective, widely used for the production of organic foods, cosmetics, food additives, pigments with important antioxidant molecules and anti-inflammatory properties (Pedrosa *et al.,* 2011; Larrosa *et al*., 2018; Hadizadeh *et al*., 2019; Manirafasha *et al*., 2018; and Chaiklahané *et al.,* 2022). *Chlorella* and *Arthrospira* are the most prominent species in the microalgae market due to their primary uses as human nutrition (Morocho-Jacome *et al*., 2016). In 1974, the word Food Conference of United Nations declared *Arthrospira* as "the best for tomorrow" (UNWFC, 1974).

Arthrospira platensis is a blue-green alga rich in balanced proteins and great source of various nutraceuticals, has a particular chemical composition with 55 to 70% of proteins, 15 to 25% of carbohydrates and 4 to 7% of lipids (Ljubic *et al.,* 2018). It has been successfully produced in open raceway ponds for commercial use (Lu *et al.* 2011).Therefore, special strategies have to be introduced to increase *A. platensis* productivity as different factors could impact biomass yield including, the mode of the operation, light intensity, cell concentration and reactor design (Chaiklahan *et al*., 2022; da Silva *et al*., 2016; Li *et al*., 2021; Bezerra *et al*., 2011).

According to previous studies, *Arthrospira platensis* can be cultivated in open ponds and closed photobioreactor, mainly with tubular configuration (Ferreira *et al*., 2012; Morocho-Jácome *et al*., 2012; Xie *et al*., 2015). In open and closed cultures, various parameters greatly influence the growth of *Spirulina platensis* including pH, light intensity, inoculum level and culture contamination (Xie *et al*., 2015; Vonshak *et al*., 1982; Richmond, 2004; Danesi *et al*., 2011).

The optimal bioreactors have to present a high specific surface with high Surface/volume (S/V) ratio for efficient transfer of light energy. The current culture systems include batch operated shallow open ponds (15-30 cm of depth) (Belay. 1997), tubular photobioreactors (Carlozzi *et al*., 2000; Molina *et al*., 2001) and flat plat photobioreactors (Sforzal *et al*., 2014).

In the study of process optimization, generally, the "one parameter at time" approach is widely used, where only one parameter varied while the others are held constant. This method can be used as a rough assess of optimal levels (Norfarina *et al*., 2017).

This strategy necessitates a long time and costs a lot of money because there could be numerous experiments required (Adinarayana *et al*., 2002; Androga *et al*., 2014). In this respect, statistical experimental designs such as factorial design and response surface method are interesting alternatives. Response surface methodology (RSM) is a series of statistical methods for the experiment design, the creation of models, the estimation of the effect of variables and the search for optimal conditions desirable response factors (Bas *et al*., 2007; Haddar *et al*., 2010; Sathendra *et al*., 2019; Montgomery *et al*., 2012). The main advantage of RSM is the limited required experimental studies to test different parameters and to take into account their interactions (Ojha and Das, 2018). Several research works on the use of experimental design to optimize variables for biomass production are reported in literature including: biomass production by open raceway pond (Radmann *et al*. 2007; Junique *et al*., 2021), close photobioreactors (Carlozzi *et al*., 2003; Ho *et al.,* 2012), phototrophic and mixotrophic cultures growth (Chojnacka *et al*., 2003; Verma *et al*., 2020).

In phototrophic growth through batch culture always ends with a stationary phase when environmental factors become unfavorable such as depletion of nutritional source (C/N), proton potential (pH), and viscosity. Another factor is added to the photobioreactor system which is the availability of light by the effect of light attenuation at the depth of the culture tank particularly for high cell density (Richmond, 2004).

In this context, we are looking for the combined effect of the specific surface ratio (S/V) with culture parameters that have positive interactions to minimize the limiting effect of attenuation and improve system performance with the goal of increasing productivity and maximizing final biomass concentration. Relatively, few data are published in this respect, and it remains interesting to investigate the interaction of surface area with other production parameters including light strength and inoculum concentrations.

The contribution of this work is the use of a new conical-annular PBR design with a high S/V ratio and the coupling of the specific surface area ratio (S/V) of the PBR with the light intensity and the inoculum concentration. This study was conducted using the design experiment approach. Regression models of biomass productivity and growth rate to the independent variables (initial biomass concentration, light intensity and ratio S/V of PBR) and their interaction were developed and optimized to get the best results.

Material and Methods

Bacteria and culture media

Arthrospira platensis used in this research was collected from the Laboratory of Biotechnology and Process Engineering, ENP Elharrach, Algerie. For the production of spirulina culture, the microalgae were seeded on synthetic Zarrouk medium (Zarrouk, 1966).

Experimental procedure

As shown in the figure.1, the photobioreactor used in this research consisted of a conical glass vessel and annular structure with 60° inclination. This tilt allows the light to be projected perpendicularly on the PBR surface and to reduce radiation losses by bending. In addition, this configuration provides a 360° exposure surface and a short light path, allowing for light homogenization in the culture's volume.

This reactor significantly improved the production of H_2 by *S. platensis* compared to conical and cylindrical PBR (Ainas *et al*., 2017). The CAPBR is characterized by an external cone (Ec), an internal cone (Ic) and an annular chamber of small thickness (17-25 mm) with a high surface/volume(S/V) ratio (greater than 90 m^{-1}). The top opening of the annular chamber is capped with an outlet to limit the entry of air borne contaminants, which creates a low positive pressure inside the reactor that further reduces contamination risks and agitation culture. It is provided by air sparging filtered using an air pump. The CAPBR was kept in a temperature-controlled room (to maintain the temperature at 30°C±2, illumination was provided by Osram 40 W (36 µmol $m^{-2}s^{-1}$) daylight type fluorescent lamp under 12h photoperiod.

In order to evaluate the influence of initial inoculum concentration, light intensity and S/V ratio on volumetric productivity (P_V) and growth rate (μ_{max}), three PBRs of different capacities (Table 1) were tested.

Measurements and analytical methods

A. Platensis concentration was calculated by measuring the optical density (OD) at 618 nm by spectrophotometer (model Shimadzu UV -1201, Tokyo, Japan) and expressed in gram of dried biomass per liter of medium ($g.L^{-1}$). At 618 nm a DO 618 nm of 1.0 corresponded to 0.799 g DW/L.

The biomass production performance was quantified by growth rates and volumetric productivity. The growth rate μ_{max} (estimated from the linear phase during exponential bacterial growth) is calculated by equation (1).

$$\mu_{max} = \frac{\ln X_2 - \ln X_1}{(t_2 - t_1).\ln 2} = \frac{1}{(t_2 - t_1).\ln 2} \ln\left(\frac{X_2}{X_1}\right) \quad ...(1)$$

Where X_1 and X_2 correspond to the biomass concentration ($g.L^{-1}$) at time t_1 and t_2 (days), respectively.

The volumetric productivity (P_V) was defined by the equation (2).

$$P_V = \frac{C_b - C_{b_0}}{t - t_0} \quad ...(2)$$

Where C_b and C_{b0} are the biomass concentration at time t and the starting time t_0 respectively.

The chlorophyll-a content in microalgae cells was estimated using a spectrophotometric method at 665, 645 and

630 nm. Chlorophyll contents in mg/g become calculated the usage of the following equation (Ruiz *et al*. 2015).

Chlorophyll-a (mg/g) = 11.6 (A_{665})-1.31(A_{645})–0.14(A_{630}) (3)

Experimental design

Response surface methodology is an important technique for optimizing a multivariate system. It is an empirical statistical modeling used for multiple regression using quantitative data derived from properly designed experiment (Rao *et al*., 2000).

The RSM was used in combination with Box-Behnken design through Design Expert software trial version 13 to examine the effect of three independent variables on response functions and to evaluate the optimum conditions for maximizing biomass production by *Arthrospira platensis* batch cultures. The optimization method includes observing the response of the statistically modeled combination, evaluation the coefficients by fitting the experimental data to the response function. Predicting the response of the fitted model and testing the model's suitability (Wang *et al*., 2016).

The three independent factors in this research were the initial inoculum concentration (X_1), light intensity (X_2) and S/V ratio (X_3). The response functions were the volumetric productivity (P_V) and growth rate (μ_{max}). The range and the levels of the factors are given in Table 2. In the development of the statistical model, the relation between the coded values and actual values is described by Eq (4).

$$x_i = \frac{X_i - X_i^0}{\Delta X_i} \quad ...(4)$$

Where x_i and X_i are coded and uncoded values of the ith independent variable: X_i^0 is the uncoded values of the ih independent variable at the center point, and ΔX_i is the step change value between low level (-1) and high level (+1).

The *quadratic* model was also used to correlate the relation between independent variables and dependent variables, estimated with the following Eq (5)

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j + \varepsilon \quad ...(5)$$

Where Y is the predicted response, β_0 intercept the term, β_i the coefficient of linear effect, β_{ii} the coefficient of squared effect, β_{ij} the coefficient of interaction effect and ε the random error.

X_i and X_j are the input n variables which influence the response of variable Y.

The Analysis of variance (ANOVA) was used to determine the relationship between the factors and the response variable. The quality of fit of the predictive model was expressed by R^2 and its statistical significance was tested with the F- test. Plots of the contour (2D) and response surface (3D) were used to investigate the influence of variable interaction over response (Behera *et al*., 2020).

Results and Discussion

The application of RSM provides an analytical relationship between the response function and the independent factors. Model terms were evaluated by the P-value (productivity) with confidence level of 95%. The contours plots were obtained according to the effects of the three factors (initial inoculum concentration, light intensity and surface/volume ratio) at three levels.

Table 3 present the experimental biomass production results from a three-factor and three-level factorial design of experiment.

Fitting of quadratic mathematical model and analysis of variance

The results of the study were fitted by an empirical relation represented by a 2nd polynomial equation with interaction terms. Based on findings of the experimental design (Table 3), the regression models with codes factors for describing volumetric productivity (P_V) and growth rate (μ_{max}) are seen in Eqs. (6) and (7) respectively.

$$P_V = 0.2253 - 0.0175X_1 + 0.1094X_2 - 0.0919X_3 - 0.0140X_1X_2 - 0.0080X_1X_3 + 0.0663X_2X_3 - 0.0428X_1^2 - 0.0240X_2^2 + 0.0445X_3^2 \quad (6)$$

$$\mu_{max} = 0.2403 - 0.0243X_1 + 0.1695X_2 0.0890X_3 - 0.0195X_1X_2 - 0.0075X_1X_3 + 0.1105X_2X_3 + 0.0333X_1^2 + 0.0738X_2^2 - 0.0052X_3^2 \quad (7)$$

An analysis of variance was used to assess the suitability of the fitting of the second-order mathematical function to the experimental results. Table 4, displays the ANOVA analyses for volumetric productivity and growth rate. This analysis indicated that the P_V model was significant, as shown by the F-value of 20.89, which had a p-value of 0.19 percent. The P-values quantify the statistical significance of each variable, low P-values indicated that the variables in the model are statistically significant (Liu *et al*, 2003).Moreover, the ANOVA result for growth rate model shows *F*-value of 14.53 expressing a significant effect on the response with p = 0.44% (table 4). In addition, quadratic model statistics show high reliability in the estimation of volumetric productivity and growth rate (R^2= 0.974 and R^2= 0.963, respectively). A high correlation coefficient ensures a satisfactory adaptation of the quadratic model to the experimental results. The P-vales (as seen in Table 5) were used to evaluate the significant and effective terms of the established models. The lower P value (below 0.05 at 95 % significance) for a model term. The P value greater than 5% is negligible as it indicates that the corresponding coefficient in the model is insignificant.

Diagnostic plots such as predicted versus actual value plots and standard probability plots of the studentized residual confirm the suitability of the chosen model as a reliable approximation of the real system. To evaluate a model's suitability, these plots are used.

Fig. 2 (a and b) confirms that the predicted response values from the models are well aligned with the observed values; the data points are distributed relatively close to the single direction (y = x). These graphs show an acceptable agreement between the actual data and the data derived from the models. Data were also examined to confirm the residues' normality. Figure 3(a-b). presents the normal probability

graph for these residues. This plot's data points are quite closely to a straight line, may be inferred that the data are uniformly distributed.

Evaluation of variables effect on the volumetric productivity and growth rate

Using RSM by the combined effect of independent variables offers prediction more than the conventional methods. In order to investigate the effect of each factor and their interactions on the responses, 3D surface response plots and their corresponding contour plots for the predicted response were developed. This plot, which is based on the model mathematical equation to analyze the change response surface, can facilitate the truthful examination of the effects of the experimental factors on the responses (Yetilmezsoya *et al*., 2009). In a 3D surface plot, a graphical representation of the quadratic model showing the impact of each variable on the obtained response. These graphs assist in explaining the effect of two unrelated factor on the response while retaining the third factor constant.

The contour plots offer the opportunity to determine the type of interactions that exist between variables. The spherical shape of the response surface suggests that there is negligible interaction. On the other hand, the elliptical shape of the contour plots indicates that the interaction between two variables is important (Muralidhar *et al*., 2001).

The surface plots for the volumetric productivity (Pv) and growth rate (μ_{max}) as a function of inoculums concentration, light intensity and S/V ratio, as formulated by equation 7 and 8 is presented in Figs. 4 and 5.

The combined effect of inoculums concentration and light intensity on volumetric productivity and growth rate is shown in figs 4(a) and 5(a).The effect of the light intensity was investigated in the range of 6-180 µmol m^{-2} s^{-1}. According to the results shown in table 5, the effect of light intensity on the volumetric productivity and growth rate was significant (P value < 0.05).

While keeping the S/V ratio constant, it was deduced from the P-values of 0.4266 and 0.4975 there was no significant interaction between inoculum concentration and light intensity (Table 5).

The monitoring of both productivity and growth rate under the effect of the two tested variables reveals a higher volumetric productivity and growth rate of 0.317 $gL^{-1}Day^{-1}$ and 0.558 day^{-1} respectively with a lower inoculum and a hither light intensity and growth rate when the light intensity decreases from 180 to 6 µmol m^{-2} s^{-1}. Therefore, it appearing that, in order to stabilize volumetric productivity at these ideal levels, a light intensity of 180 µmol m^{-2} s^{-1} qnd an initial inoculums concentration of 0.25 gL^{-1} must be introduced.

The combined effect of the two variables, light intensity and specific surface of PBR (S/V) on volumetric productivity and growth rate is illustrated in figs. 4b and 5b an increase of both volumetric productivity and growth rate are observed with the increase of the two variables (I, S/V) at alight intensity of 6 µmol m^{-2} s^{-1}, the volumetric productivity are generally less than 0.157$g^{-1}L^{-1}d^{-1}$; however, the maximum volumetric productivity (0.51$g^{-1}L^{-1}d^{-1}$) was observed at 180 µmol m^{-2} s^{-1} and S/V 112 m^{-1} (Fig. 4b).

The 3D plot (Fig. 4b, 5b) also reveals that the volumetric productivity and growth rate was more sensitive to the change of the two variables, it presents a steeper slope between the two axes (axis of light intensity and axis of the S/V ratio). As shown in the Fig. 4(b), the contour plots are elliptical shape for volumetric productivity response, showing that interactive effect are no negligible. This was verified by the results of ANOVA in table 5, in which the P-value is less than 0.05 for X_2X_3. On the other hand, Fig. 5(b) also shows a significant interaction (P value < 0.05) between light intensity and S/V ration on the growth rate.

The presence of such a linear response can be interpreted by the independence of the volumetric productivity and growth rate responses to the simultaneous variations of two studied parameters. The increase in light intensity and S/V ratio leads to increased biomass productivity which is essentially conditioned by the efficient conversion of light energy (Vonshak *et al*., 2014).

Figures 4(c) and 5(c) shows the effect of initial inoculum concentration and the specific surface area (S/V ratio) on volumetric productivity and growth rate at constant light intensity of 93 µmol $m^{-2}s^{-1}$. The results showed the best value in terms of Pv and μ_{max} (0.367 $gL^{-1}day^{-1}$, 0.387 day^{-1} respectively) are obtained at a high S/V ratio and a less culture density.

According to table 5, the P-value (0.6420 and 0.7898) imply that the interactive (X_1*X_3) is insignificant terms for volumetric productivity and growth rate.

Application of photobioreactor with reduced light path (higher S/V ratio) allows more effective use of light and achieves the highest productivity (Gupta *et al*. 2015).

A low productivity of culture can be explained by the effect of self-shading by microalgae filaments when the biomass concentration in the broth becomes too dense leading to poor growth (Grima *et al*., 1996; Richmond, 2004).

Under these conditions, Ogbonna and Tanaka (2000) proposed that this is related to the direction of the phototrophic cell metabolism towards heterotrophy when the illumination is limited.

However, the effect of surface/volume ratio and light intensity on maximum biomass production was investigated. As shown in Fig. 6, a maximum biomass concentration of *A. Platensis* was reached at 4,548g/L after 7 days and then stopping growth. Bezerra *et al.* (2011) reported that as the capacity of light to penetrate the culture determines the cell concentration in the saturation region. This behavior can be attributed to photo-saturation or autchading of the culture (Bezerra *et al*. 2016). In addition, it is widely recognized that illuminance is attenuated exponentially upon entry into the culture, trying to impose strict limits on cell density culture (Ferreira, 2016).

In order to investigate the influence of light intensity and S/V ratio on chlorophyll content during *A. platensis* cultivation, the two vital parameters were maintained 6, 93 and 180 µmol $m^{-2}s^{-1}$ and 94, 103 and 112 m^{-1}.

Figure 7, show chlorophyll-a content measurements performed on *A. platensis* cells grown under different light intensity and S/V ratio.

The result in figure 7, show a proportionality between the chlorophyll-a concentration obtained and the light intensity applied with a maximum chlorophyll concentration of 5.792 mg/g for the CAPBR with à low S/V ration of 94 m^{-1} and an intensity of 180 µmol m^{-2} s^{-1}. However, in shallow CAPBR of 103; 112 m^{-1}, the chlorophyll content increased linearly with light intensity ranging from 6 to 93 µmol m^{-2} s^{-1} with maximum chlorophyll-a values of 4.28; 3.218 mg/g for two dimensions 103 and 112 m^{-1} respectively (Fig.6). On the other hand, at high intensity in the order of 180 µmol m^{-2} s^{-1}, the chlorophyll-a concentration obtained from the two dimensions 103 and 112 m^{-1} were 2.98 and 2.704 mg/g, respectively. These last results showed that there is a reduction of 16 and 30% respectively compared to the maximum values obtained at the intensity of 93 µmol m^{-2} s^{-1} (Fig. 7).

For algae, modification in their photosynthesis process to light have an adaptive significance with the aid of maximizing their capability increase. At light limitation, microalgae operate less photosynthesis and produce less energy. To compensate for this lack their chlorophyll content increase a two to ten times, these adjustments known as light-shade adaptations (Candioti *et al*., 2014).

However, at high level of light intensity (180 µmol $m^{-2}s^{-1}$), a reduction in concentration of chlorophyll likely due to photo inhibition effects in this irradiance or may be due to an acclimatization response by reducing Chlorophyll-a content to decrease light harvesting ability and an accumulation of other pigments like phycocyanin, carotenoids which have antioxidant activity in these conditions (Sforza *et al*., 2014).

The outcomes of this work were compared with other research published in the literature on the optimization of biomass productivity and growth rate. Table 7, summarizes some experimental studies, combining various environmental parameters such as light intensity and S/V ratio have an important impact on biomass production. In addition, the Pv and μ_{max} obtained from this study is consistent with past research.

Optimization by response surface methodology

In order to find the best process condition, numerical optimization was conducted using the RSM. The desirability function (D) is used in Design-Expert software to select the ideal parameters of predicted volumetric productivity and growth rate. Desirability typically ranges from 0 to 1. A greater value signifies that the response is more desirable, while a zero value signifies that the response is completely undesirable (El-Mekkaoui *et al*., 2020).

The operational factors (inoculums concentration, light intensity and S/V ratio) were kept in range while the volumetric productivity and growth rate were set at maximum. Table 6 shows the optimal conditions with the estimated and experimental of the volumetric productivity and Growth rate. These optimal variables were chosen according to the highest desirability of 0.98.

At the suggested operating conditions of 0.35 gL^{-1}, 180 $m^{-2}s^{-1}$ and 112 m^{-1} initial inoculums concentration, light intensity and S/V ration respectively, there was no discernible difference between the experimental and predicted data of the two models in terms of productivity and growth rate. Therefore, the predictive model was found to be suitable for predicting and optimizing the microalgae cultivation mode.

Conclusion

In this research, an annular conical photobioreactor was used to enhance biomass production from cyanobacterium *A. platensis* by the Box-Behnken (*BBD*) design. The impact of inoculum concentration, light intensity and S/V ratio have been studied by RSM in attempt to optimize growth conditions. The correlation between the independents variables and the biomass production was obtained by a 2nd polynomial function. According to the results, light intensity and S/V ratio were the most important factors affecting volumetric productivity and growth rate, while inoculums concentration had minor effect on growth rate. However, significant interaction was observed between light intensity and S/V Ratio (P value < 0.05) on the volumetric productivity. Nevertheless, a significant interaction between light intensity and S/V ratio was found to affect volumetric productivity (P value < 0.05. This study shows that *A. platensis* can be cultivated in CAPBRs with the maximum volumetric productivity, growth rate and chlorophyll content (0.542 g L^{-1} day^{-1}, 0.785 day^{-1}, 2.704 mgg^{-1}) respectively, when the light intensity and S/V ratio are suitable.

The predictive model of RSM is found to be able to better predict the maximum biomass production under the imposed conditions.

Table 1 : Characteristic of conical photobioreactor of the *Spirulina platensis* culture

Type	Working volume (mL)	Culture depth (cm)	S/V ratio (m^{-1})
CAPBR1	200	1.25	112
CAPBR2	500	1.75	103
CAPBR3	800	2.25	94

Table 2 : Range and levels of experimental variables tested in the BBD design

Variables	Unit	Factors	Levels of variables		
			(-1)	(0)	(+1)
Inoculum concentration	$g.L^{-1}$	X_1	0.25	0.75	1.25
Light intensity	µmol m^{-2} s^{-1}	X_2	6	93	180
Surface/volume (S/V) ratio	m^{-1}	X_3	94	103	112

Table 3 : Experimental design table for optimization of biomass production and growth rate

No. of run	X_1 : Inoculum concentration ($g\ L^{-1}$)	X_2: Light intensity ($\mu mol\ m^{-2}\ s^{-1}$)	X_3 : S/V ratio (m^{-1})	Volumetric productivity ($g\ L^{-1}\ day^{-1}$)	Growth rate (day^{-1})
1	1.25	6	103	0.083	0.13
2	0.25	180	103	0.298	0.604
3	0.75	180	94	0.206	0.223
4	0.25	93	94	0.173	0.209
5	0.25	6	103	0.095	0.163
6	1.25	180	103	0.23	0.493
7	0.25	93	112	0.347	0.353
8	0.75	93	103	0.23	0.256
9	0.75	93	103	0.224	0.23
10	1.25	93	112	0.301	0.313
11	0.75	6	94	0.076	0.168
12	0.75	93	103	0.222	0.235
13	0.75	180	112	0.548	0.671
14	1.25	93	94	0.159	0.199
15	0.75	6	112	0.153	0.174

Table 4 : Analysis of variance (ANOVA) for the volumetric productivity and growth rate model.

Source	DF	Sum of squares	Mean squares	F-value	P-value
Volumetric productivity					
Model	9	0.1969	0.0219	20.89	0.0019
Residual	5	0.0052	0.0010		
Lack of fit	3	0.0052	0.0017	100.08	0.0099
Pure error	2	0.0000	0.0000		
Total	14	0.2022			
R^2		0.974			
Adj-R^2		0.9274			
Growth rate					
Model	6	0.3485	0.0581	12.29	0.0012
Residual	8	0.0378	0.0047		
Lack of fit	6	0.0374	0.0062	32.77	0.0299
Pure error	2	0.0004	0.0002		
Total	14	0.3863			
R^2		0.9632			
Adj-R^2		0.8969			

Table 5: Coefficients from the RSM optimization for the volumetric productivity and Growth rate model.

Term	Volumetric productivity, $g.L^{-1}\ day^{-1}$				Growth rate, day^{-1}			
	Coefficient Estimate	DF	F-Value	P-Value	Coefficient Estimate	DF	F-Value	P-Value
Intercept	0.2253	1	20.89	0,0019*	0.2403	1	14.53	0.0044*
X_1	-0.0175	1	2.34	0,1868	-0.0243	1	1.65	0.2548
X_2	0.1094	1	91.34	0.0002*	0.1695	1	80.79	0.0003*
X_3	0.0919	1	64.45	0,0005*	0.0890	1	22.27	0,0052*
$X_1.X_2$	-0.0140	1	0.7482	0.4266	-0.0195	1	0.5346	0.4975
$X_1.X_3$	-0.0080	1	0.2443	0.6420	-0.0075	1	0.0791	0.7898
$X_2.X_3$	0.0663	1	16.76	0.0094*	0.1105	1	17.17	0.0090*
$X_1.X_1$	-0.0248	1	2.17	0.2011	0.0333	1	1.44	0.2836
$X_2.X_2$	-0.0240	1	2.04	0.2129	0.0738	1	7.07	0.0449*
$X_3.X_3$	0.0445	1	6.97	0.0460*	-0.0052	1	0.0346	0.8597

Table 6 : Results of RSM optimization

Inoculum concentration $g\ L^{-1}$	Light intensity $\mu mol\ m^{-2}\ s^{-1}$	S/V ratio, m^{-1}	Volumetric productivity, $g\ L^{-1}\ day^{-1}$		Growth rate, day^{-1}	
			Pred	Exp	Pred	Exp
0.351	180	112	0.529	0.542	0.739	0.785

Table 7: Comparison of biomass productivity and growth rate of *Arthrospira platensis* obtained from this study with others research's

Algae type	Type of photobioreactor	S/V ratio m^{-1}	Volumetric productivity, P_V (g $L^{-1}day^{-1}$)	Growth rate, μ_{max} (day^{-1})	References
A. platensis	HoP HeP	194 388	0.86 1.12	0.988 0.750	da Silva *et al.* 2016
M. aeruginosa	ABCPBR	90	0.163	-	El-Mekkaoui *et al.* 2020
Chlorella pyrenoidosa	- MTA PBR - H-T PBR	78 125	0.107 0.147	- -	Sukacová *et al.* 2021
A. platensis	CAPBR	112	0.542	0.785	Present study

CAPBR: Conical annular Photobioreactor; HoP: Horizontal Photobioreactor;
HeP: Helicoidal Photobioreactor; ABC PBR: airlift bubble column photobioreactor
MTA PBR: MultiTubular Airlift photobioreactor; H-T PBR: Helical-Tubular photobioreactor

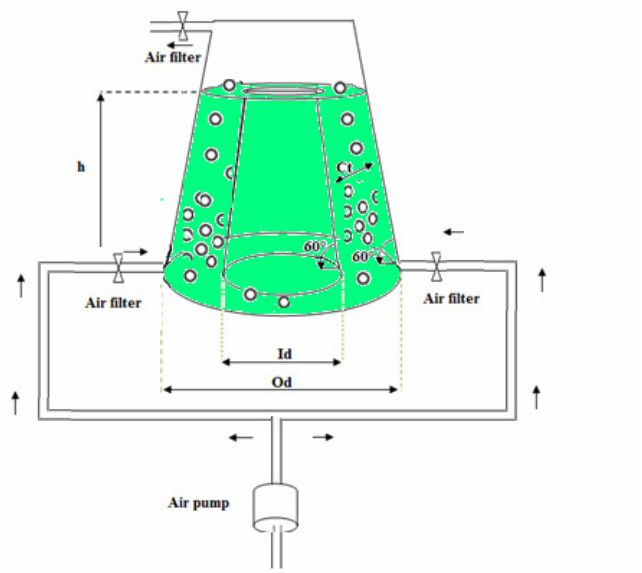

Fig. 1 : Schematic of conical annular photobioreactor (CAPBR)

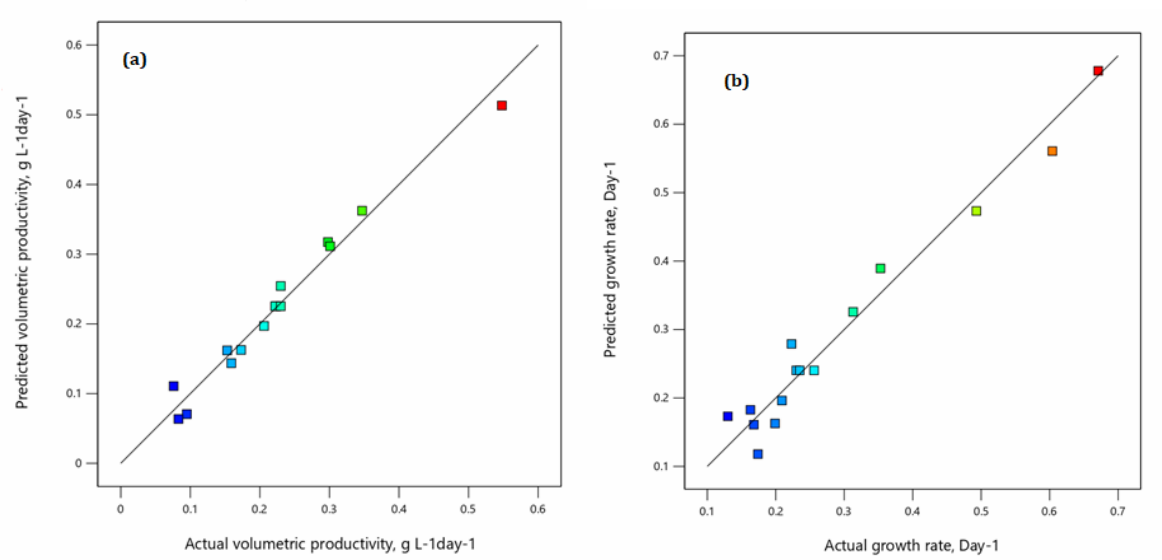

Fig. 2 : Plot of predicted values versus to actual values for (a) volumetric productivity and (b) growth rate

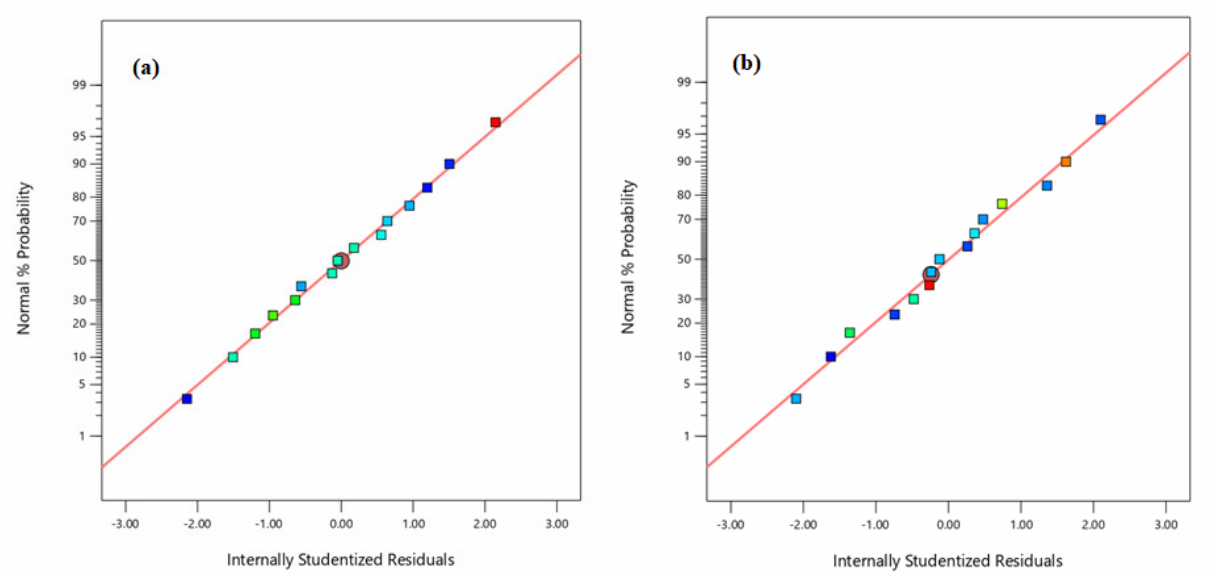

Fig. 3 : Normal plot of residual for (a) volumetric Productivity and (b) growth rate

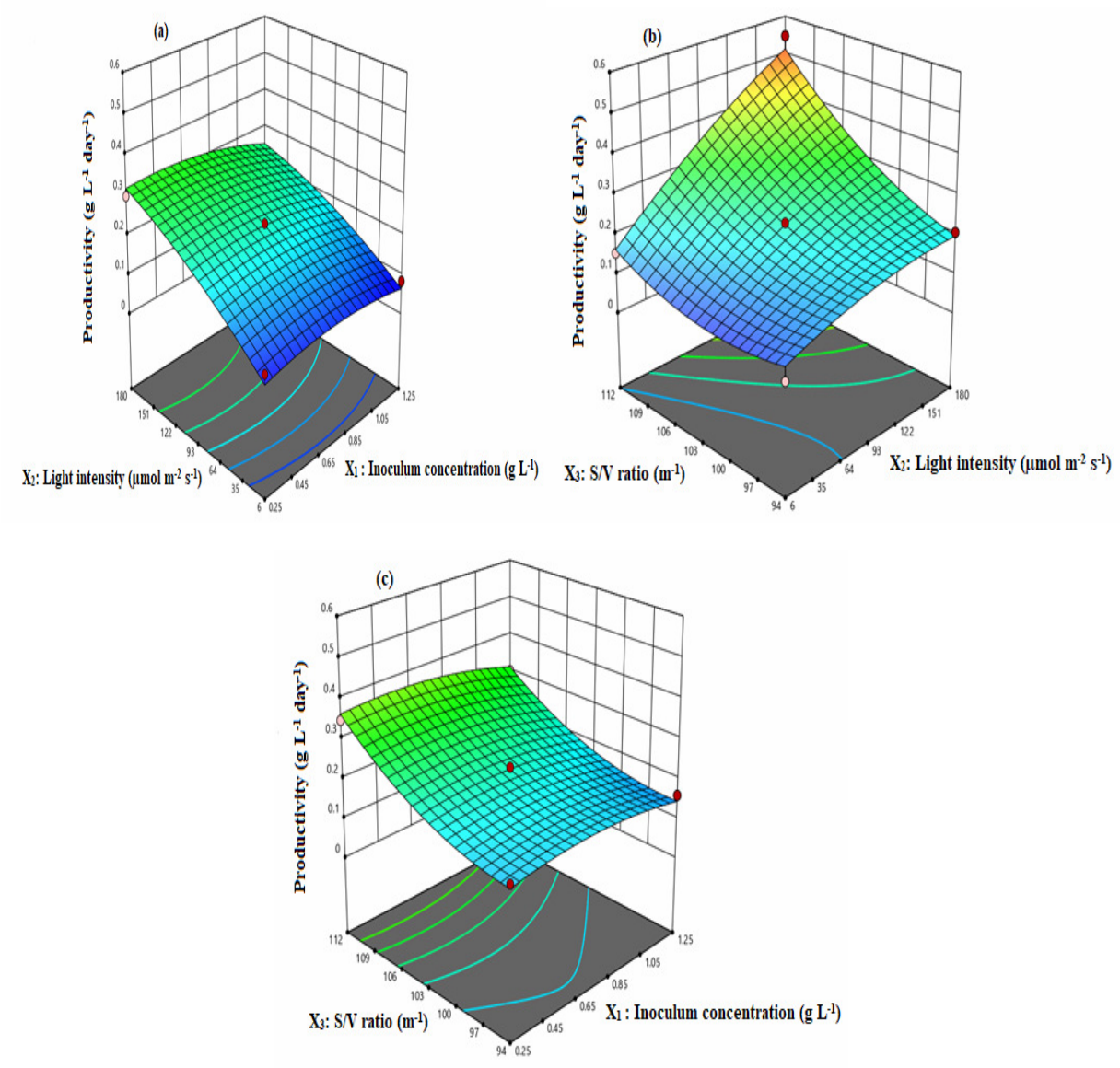

Fig. 4 : 3D surface plots of the volumetric productivity at (a) light intensity and inoculum concentration; (b) light intensity and S/V ratio; (c) inoculum concentration and S/V ratio

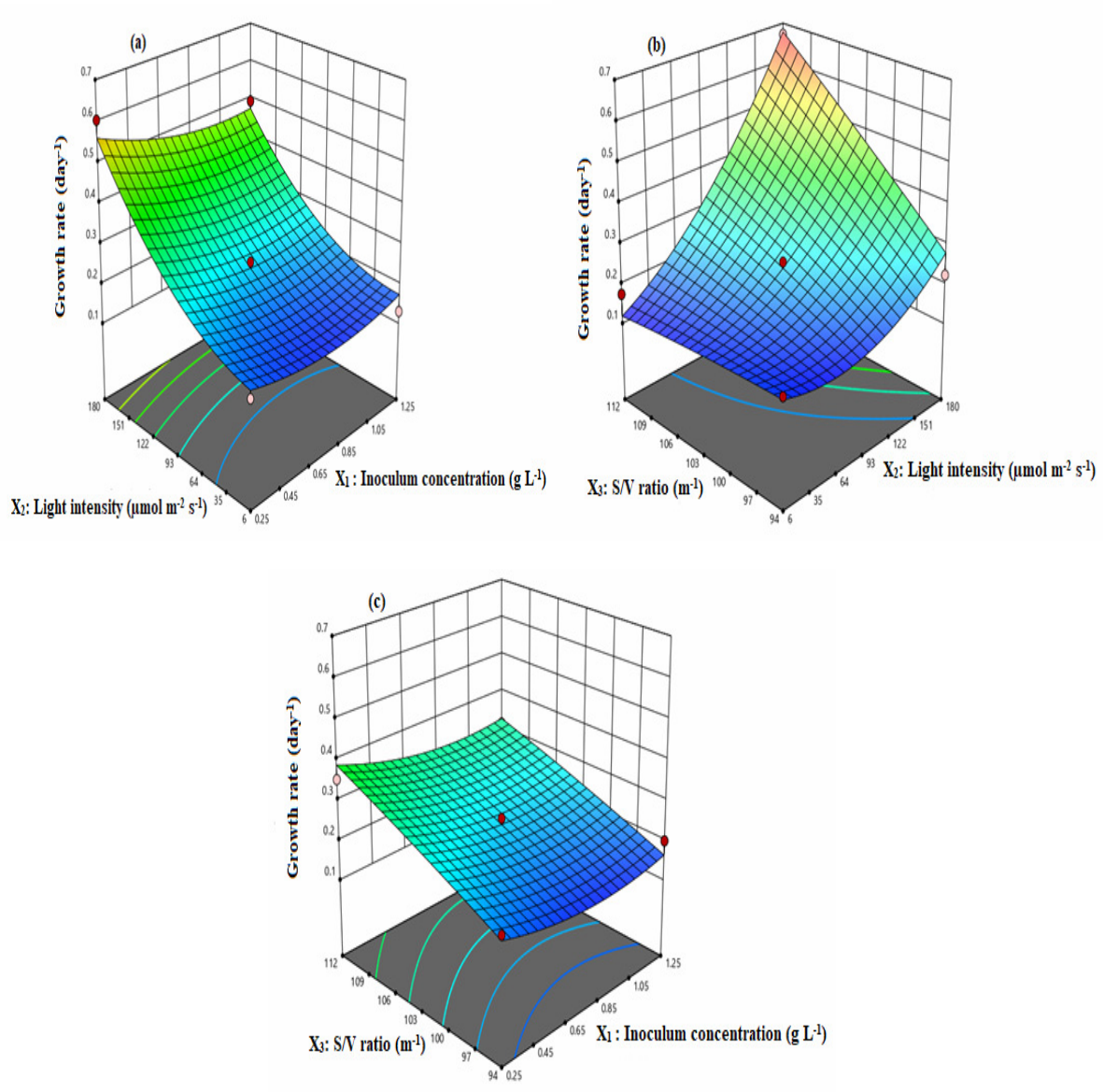

Fig. 5 : 3D surface plots of the Growth rate at (a) light intensity and inoculum concentration; (b) light intensity and S/V ratio; (c) inoculum concentration and S/V ratio

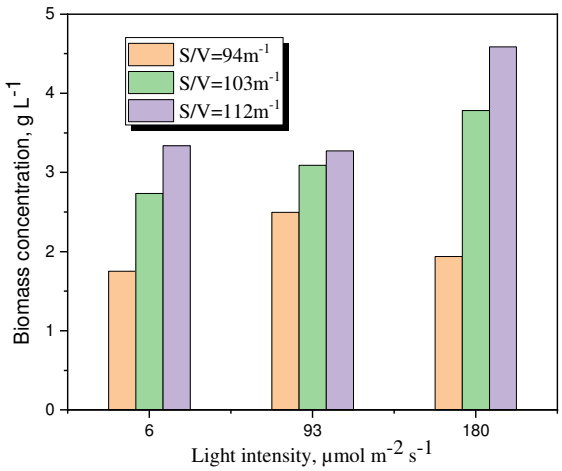

Fig. 6 : Effect of light intensity and S/V ratio on biomass concentration

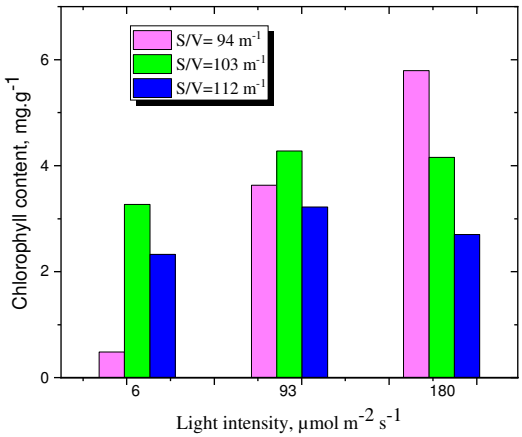

Fig. 7 : Effect of light intensity and S/V ratio on chlorophyll content

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Acknowledgements

The authors thank the Laboratory of Biotechnology and Process Engineering; ENP Elharrach, Algeria and the Department of Nature and Life of Medea University for offering equipment and assistance for this research.

Reference

Adinarayana, K. and Ellaiah, P. (2002). Response surface optimization of the critical medium components for the production of alkaline protease by a newly isolated *Bacillus* sp. *J. Pharm. Pharm. Sci*., 5: 272–278. file:///C:/Users/intel/Downloads/Response_Surface_Optimization_of_the_Critical_Medi%20(1).pdf

Ainas, M.; Hasnaoui, S.; Bouarab, R.; Abdi, N.; Drouiche, N. and Mameri, N. (2017). Hydrogen production with the cyanobacterium *Spirulina platensis*. *Int J Hydrogen Energy*. 42: 4202-4207.https://doi.org/10.1016/j.ijhydene. 2016.12.056.

Androga, D.D.; Sevinc, P.; Koku, H.; Yücel, M.; Guduz, U. and Eroglu, I. (2014). Optimization of temperature and light intensity for improved photofermentative hydrogen production sing *Rhodobacter capsulatus* DSM 1710. *Internat. J. Hydrog Energ*, (39) 6: 2472-248. https://doi.org/10.1016/j.ijhydene.2013.11.114.

Bas, D. and Boyaci, I.H. (2007). Modeling and optimization I: Usability of response surface methodology. *J of Food Engin*. 78: 836-845. https://doi.org/10.1016/j.jfoodeng. 2005.11.024.

Behera, B.; Patra, S. and Balasubramanian, P. (2020). Biological nutrient recovery from human urine by enriching mixed microalgal consortium for biodiesel production. *J. Environ. Manage*. 260: 110111. https://doi.org/10.1016/j.jenvman.2020.110111.

Belay, A. (1997). Mass culture of Spirulin aoutdoors – the earthrise farms experience. In:Vonshak, A. (Ed.), *Spirulin platensis* (*Arthrospira*): Physiology, Cell-Biology and Biotechnology. Taylor and Francis, London, 131–158. https://doi.org/10.1201/ 9781482-272970.

Benelhadj, S.; Gharsallaoui, A.; Degraeve, P. and Attia, H. (2016). Effect of pH on the functional properties of *Arthrospira* (*Spirulina*) *platensis* protein isolate. *Food Chem.,* 194: 1056-1063. http://dx.doi.org/10.1016/j.foodchem. 2015.08.133.

Bezerra, R.P.; Ortiz Montoya, E.Y.; Sato, S.; Perego, P.; Carvalho, J.C.M. and Converti, A. (2011). Effects of light intensity and dilution rate on the semicontinuous cultivation of *Arthrospira* (Spirulina) platensis. A kinetic Monod-type approach. *Bioresour. Technol*. 102: 3215-3219. https://doi.org/10.1016/j.biortech. 2010.11. 009.

Candioti, L.V.; De Zan, M.M.; Camara, M.S. and Goicoechea, H.C. (2014). Experimental design and multiple response optimization. Using the desirability function in analytical methods development. *Talanta* 124: 123-138. https://doi.org/10.1016/j.talanta.2014. 01. 034.

Carlozzi, P. (2000). Hydrodynamic aspects and Arthrospiragrowth in two outdoor tubular undulating row photobioreactors. *Appl. Microbiol. Biotechnol*. 54: 14–22. https://doi.org/10.1007/s002530000355.

Carlozzi, P. (2003). Dilution of solar radiation through ‘‘culture’’ lamination in photobioreactor rows facing South–North: a way to improve the efficiency of light utilisation of cyanobacteria (*Arthrospira platensis*). *Biotechnol. Bioeng*. 81: 305–315. https://doi.org/10.1002/ bit.10478.

Chaiklahan, R.; Chirasuwan, N.; Srinorasing, T.; Attasat, S.; Nopharatana, A. and Bunnag, B. (2022). Enhanced biomass and phycocyanin production of *Arthrospira* (Spirulina) platensis by a cultivation management strategy: Light intensity and cell concentration. *Bioresour. Technol.,* 343: 126077. https://doi.org/10.1016/j.biortech.2021.126077.

Chojnacka, K. and Noworyta, A. (2004). Evaluation of Spirulina sp. Growth in photoautotrophic, heterotrophic and mixotrophic cultures. *Enzyme Microbial. Technology*, 34: 461-465. https://doi.org/10.1016/j.enzmictec.2003.12.002.

Danesi, E.D.G.; Rangel-Yagui, C.O.; Sato, S.; de Carvalho, J.C.M. (2011). Growth and content of *Spirulina platensis* biomass chlorophyll cultivated at different values of light intensity and temperature using different nitrogen sources, *Brazilian Journal of Microbiology*.,

42: 362-373. https://doi.org/10.1590/S1517-83822011000100046.

da Silva, M.F.; Casazza, A.A.; Ferrari, P.F.; Perego, P.; Bezerra, R.P.; Converti, A. and Porto, A.L. (2016). A New Bioenergetic and Thermodynamic Approach to Batch Photoautotrophic Growth of *Arthrospira* (*Spirulina*) Platensis in Different Photobioreactors and Under Different Light, *Bioresour. Technol*., 207: 220–228. https://doi.org/10.1016/j.biortech.2016.01.128.

El-Mekkaoui, S.A.; El-Ibiari, N.N.; El-Ardy, O.A.; Abdelmonem, N.M.; Elahwany, A.H.; Abadir, M.F. and Ismail, I.M. (2020). Optmizatin of cultivation for *Microcystis aeruginosa* for biodiesel production using response surface methodology. *Bulletin of National Research Centre,* 44: 6. https://doi.org/10.1186/s42269-019-0265-9.

Ferreira, L.S.; Rodrigues, M.S.; Converti, A.; Sato, S. and Carvalho, J.C.M. (2012). *Arthrospira (Spirulina) platensis*) cultivation in tubular photobioreactor: use of no-cost CO_2 from ethanol fermentation. *Appl. Energy.* 92: 379–385. https://doi.org/10.1016/j. apenergy. 2011. 11.019.

Ferreira, V.S.; Pinto, R.F. and Sant'Anna, C. (2016). Low light intensity and nitrogen starvation modulate the chlorophyll content of *Scenedesmus dimorphus*. *Journal Applied Microbiology,* 120: 661-670. DOI: 10.1111/jam.13007.

Gupta, P.L.; Lee, S.M. and Choi, H.J. (2015). A mini review: photobioreactors for large scale algal cultivation. *World J Microbiol Biotechnol*., 31: 1409–1417.https://doi.org/ 10.1007/s11274-015-1892-4.

Grima Molina, E.; Fernandez, Sevilla J.M.; Sanchez Pérez, J.A. and Camacho, G.F. (1996). A study on simultaneous photolimitation and photoinhibition in dense microalgal cultures taking into account incident and averaged irradiances. *Journal of Biotechnology*, 45: 59-69. https://doi.org/10.1016/0168-1656(95)00144-1.

Haddar, A.; Fakhfakh-Zouari, N.; Hmidet, N.; Frikha, F.; Nasri, M. and Sellami, K.A. (2010). Low-cost fermentation medium for alkaline protease production bt *Bacillus mojavenis* A21 using hulled grain of wheat and sardinella peptone. *Journal of Bioscience and Bioengineering*, 110: 288-294. https://doi.org/10.1016/ j.jbiosc.2010.03.015.

Hadizadeh, M.; Ofoghi, H.; Kianirad, M. and Amidi, Z. (2019). Elicitation of pharmaceutical alkaloids biosynthesis by salicylic acid in marine microalgae *Arthrospira platensis*. *Algal Res*., 42: 101597. https://doi.org/10.1016/j.algal.2019.101597.

Ho, S.H.; Chen, Y. and Chang, C.J.S. (2012). Effect of light intensity and nitrogen starvation on CO_2fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresour. Technol.* 113: 244–252. https://doi.org/10.1016/ j. biortech. 2011.11.133.

Junique, L.; Watier, L.; Lejeune, H.; Viudes, F.; Deblieck, M. and Watier, D. (2021). Determination by response surface methodology of optimal protein and phycocyanin productivity conditions in *Arthrospira* (Spirulina) platensis under different combinations of photoperiod variation and lighting intensity. *Bioresour. Technol. Reports,* 15: 100763. https://doi.org/10.1016/ j.biteb.2021.100763.

Larrosa, A.P.Q.; Camara, A.S.; Pohndorf, R.S.; Rocha, S.F.D. and LADA, P. (2018). Physicochemical, biochemical, and thermal properties of *Arthrospira* (*Spirulina*) biomass dried in spouted bed at different conditions. *J Appl Phycol* 30: 1019–1029. http://dx.doi. org/ 10.1007/s10811-017-1265-5.

Li, L.; Zhong, X.; Zheng, Y.; Guo, S.; Shi, X. and Huang, J. (2021). Enhancement of biomass production and productivity of *Arthrospira platensis* GMPA7 using response surface monitoring methodology and turbidostatic cultivation strategy. *J. Appl. Phycol*. 33: 755–763. https://doi.org/10.1007/s10811-020-02360-5.

Liu, J.Z.; Weng, L.P. and Zhang, Q.L. (2003). Optimization of glucose oxidase production by *Aspergillus niger* in a benchtop bioreactor using response surface methodology. *World, J. Microbiol. Biotechnol*., 19: 317-323. https://doi.org/10.1023/A:1023622925933.

Ljubic, A.; Safafar, H.; Holdt, S.L. and Acobsen, C. (2018). Biomass composition of *Arthrospira platensis* during cultivation on industrial process water and harvesting. *J. Appl. Phycol*. 30: 943–954. https://doi.org/10.1007/ s10811-017-1332-y.

Lu, Y.M.; Xiang, W.Z. and Wen, Y.H. (2011). *Spirulina* (*Arthrospira*) industry in Inner Mongolia of China: current status and prospects. *J. Appl. Phycol*., 23: 265–269. http://dx.doi.org/10.1007/s10811-010-9552-4.

Manirafasha, E.; Murwanashyaka, T.; Ndikubwimana, T.; Ahmed, N.R.; Liu, J.; Lu, Y.; Zeng, X.; Ling, X. and Jing, K. (2018). Enhancement of cell growth and phycocyanin production in *Arthrospira (Spirulina) platensis* by metabolic stress and nitrate fed-batch, *Bioresour. Technol*. 255: 293–301. https://doi.org/10. 1016/ j.biortech.2017.12.068.

Molina, E.; Fernandez, J.; Acien, F.G. and Chisti, Y. (2001). Tubular photobioreactor design for algal cultures. *J Biotechnol*., 92: 113–131.https://doi.org/10.1016/ S0168-1656 (01)00353-4.

Montgomery, D.C.; Design and Analysis of Experiments (2012) 8th Edition, John Wiley & Sons, New York. P752.

Morocho-Jácome, A.L.; Converti, A.; Sato, S. and Carvalho, J.C.M. (2012). Kinetic and thermodynamic investigation on *Arthrospira* (*Spirulina*) *platensis* fed-batch cultivation in tubular photobioreactor using urea as nitrogen source. *J. Chem. Technol. Biotechnol*., 87: 1574–1583.https://doi.org/10.1002/jctb.3795.

Morocho-Jacome, A.L.; Mascioli, G.F.; Sato, S. and Carvalho, J.C.M. (2016). Evaluation of physicochemical treatment conditions for the reuse of a spent growth medium in *Arthrospira platensis* cultivation. *Algal. Res*., 13: 159-166. https://doi.org/ 10.1016/ j.algal.2015.11.022.

Muralidhar, R.V.; Chirumamila, R.R.; Marchant, R. and Nigam, P.A. (2001). A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources. *Biochem. Eng. J.* 9: 17-23. https://doi.org/ 10.1016/ S1369-703X(01)00117-6.

Norfarina, M.N.; MohdShamzi, M.; TeckChwen, L.; Hooi, L.F.; Raha, A.; Joo, S.T.; Rosfarizan, M. (2017). Comparative analyses on medium optimization using one-factor-at-a-time, response surface methodology and artificial neural network for lysine–methionine biosynthesis by *Pediococcus pentosaceus RF-1*.

Biotechnol & Biotechnolog Equip. 31: 935–947.https://doi.org/10.1080/13102818.2017.1335177.

Ogbonna, J.C. and Tanaka, H. (2000). Light requirement and photosynthetic cell cultivation–Development of processes for efficient light utilization in photobioreactors. *J. Appl. Phycol.,* 12: 207–218. https://doi.org/ 10.1023/A:1008194627239.

Ojha, N. and Das, N. (2018). A Statistical approach to optimize the production of Polyhydroxy alkanoates from *Wickerhamomyces anomalus* VIT-NN01 using Response Surface Methodology. *Int. J. Biol. Macromol.* 107: 2157-2170. https://doi.org/10.1016/ j.ijbiomac. 2017.10.089.

Pedrosa, B.R.; Montoya Ortiz, E.Y.; Sato, S.; Perego, P.; Carvalho, J.S.M. and Converti, A. (2011). Effects of light intensity and dilution rate on the semicontinuous cultivation of *Arthrospira* (*Spirulina*) platensis. A kinetic Monod-type approach, *Bioresour. Technol.,* 102: 3215–3219. http://dx.doi.org/10.1016/j.biortech.2010.11.009.

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. https://doi.org/10.1016/ j.aquaculture.2007.02.001.

Rao, K.J.; Kim, C.H. and Rhee, S.K. (2000). Statistical optimization of medium for the production of recombinant hirudin from *Saccharomyces cerevisiae* using response surface methodology. Process *Biochem.* 35: 639–647. https://doi.org/10.1016/S0032-9592(99) 00129-6.

Richmond, A. (2004). Handbook of Microalgal Culture: Biotechnology and Applied Phycology. Edition: 9600 Garsington Road, Oxford OX4 2DQ, UK, pp 577.

Ruiz, J.C.R.; Ordonez, Y.B.M.; Basto, A.M. and Campos, M.R.S. (2015). Antioxidant capacity of leaf extracts from two *Stevia rebaudiana* Bertoni varieties adapted to cultivation in Mexico. *Nutricion Hspitalaria* 31: 1163-1170. DOI : 10.3305/nh.2015.31.3.8043.

Sathendra, E.R.; Baskar, G.; Kumar, R.P. and Gnansounou, E. (2019). Biomass production from palm wood using *Trichoderma reesei* and *Kluveromyces marxianus*, *Bioresour. Technol*. 271: 345–352. https://doi.org/ 10.1016/j.biortech.2018.09.134.

Sforza, E.; Gris, B.; De Farias Silva, C.E.; Morosinotto, T. and Bertucco, A. (2014). Effects of Light on Cultivation of *Scenedesmus obliquus* in Batch and Continuous Flat Plate Photobioreactor. *Chem Engin Transact.* 38: 211–216.https://doi.10.3303/CET1438036.

Sukacová, K.; Lošák, P.; Brummer, V.; Máša, V.; Vícha, D. and Zavrel, T. (2021). Perspective Design of Algae Photobioreactor for Greenhouses. A Comparative Study. *Energies,* 14: 1338. https://doi.org/ 10.3390/ en 14051338

UNWFC (United Nations World Food Conference), Report of the World Food Conference, Rome, 5-16 November 1974. University of Michigan, United Nations. file:///C:/Users/intel/Downloads/E_CONF.65_20-EN. pdf.

Verma, R.; Kumari, K.V.L.K.; Srivastava, A. and Kumar, A. (2020). Photoautotrophic, mixotrophic, and heterotrophic culture media optimization for enhanced microalgae production. *Journal of Environmental Chemical Engineering*. 8: 104149. https://doi.org/ 10.1016/j.jece.2020.104149.

Vonshak, A.; Abeliovich, A.; Boussiba, S.; Arad, S. and Richmond, A. (1982). Production of *Spirulina* biomass: Effects of environmental factors and population density. *Biomass.* 2: 175–185. https://doi.org/10.1016/0144-4565(82)90028-2.

Vonshak, A.; Laorawat, S.; Bunnag, B. and Tanticharoen, M. (2014). The effect of light availability on the photosynthetic activity and productivity of outdoor cultures of *Arthrospira platens is (Spirulina). J. Appl. Phycol*. 26: 1309–1315. https://doi.org/10.1007/ s10811-013-0133-1.

Wang, L.; Li, M.; Feng, C.; Hu, W.; Ding, G.; Chen, N. and Liu, X. (2016). Ti nano electrode fabrication for electrochemical denitrification using Box-Behnken design. *Journal of electroanalytical chemistry*. 773: 13-21. http://dx.doi.org/10.1016/j.jelechem.2016.04.025.

Xie, Y.; Jin, Y.; Zeng, X.; Chen, J.; Lu, Y. and Jing, K. (2015). Fed-batch strategy for enhancing cell growth and C-phycocyanin production of *Arthrospira (Spirulina) platensis* under phototrophic cultivation. *Bioresour Technol*. 180: 281–287. https://doi.org/ 10.1016/j.biortech.2014.12.073.

Yetilmezsoya, K.; Demirel, S. and Vanderbei, R.J. (2009). Response surface modeling of Pb (II) removal from aqueous solution by *Pistacia vera* L: Box-Behnken experimental design. *Journal of Hazardous Materials,* 171: 551-562. doi: 10.1016/j.jhazmat.2009.06.035.

Zarrouk, C. (1966). Contribution to the study of a Cyanophycea: influence of various physical and chemical factors on the growth and photosynthesis of *Spirulina platensis* (Thesis). University of Paris.