



## Protein production by *Arthrospira (Spirulina) platensis* in solid state cultivation using sugarcane bagasse as support



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### ABSTRACT

The genus *Arthrospira* comprises a group of filamentous multicellular cyanobacteria and can be used for animal feed and human food. Solid state fermentation or cultivation (SSF) involves the use of a culture medium composed of solid material with given moisture content. No studies have been published about the cultivation of microalgae or cyanobacteria on solid medium. Furthermore, although sugar-cane bagasse is used as source of energy in alcohol distilleries in Brazil, the excess could be a support to photosynthetic microorganism growth. The experimental design methodology was used to evaluate the protein production by *Arthrospira platensis* under SSF using sugarcane bagasse as support, taking into account the moisture content of the medium, light intensity and inoculum concentration. Moisture was found to have a strong influence on the performance of the process. The best conditions were: moisture of 98.8%; inoculum concentration of 0.15 g biomass·kg wet culture medium<sup>-1</sup> and light intensity of 6.0 klx.

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### 1. Introduction

The genus *Arthrospira (Spirulina)* comprises a group of filamentous multicellular cyanobacteria (blue-green microalgae) [9]. Analyses of the chemical composition of *Arthrospira platensis* and *Arthrospira maxima* biomass indicate high protein content of about 70% (dry weight). So it can be used as protein source for both human food and animal feed [4].

Microalgae can be cultivated in open and closed systems with natural or artificial lighting [21,9]. The microorganism culturing techniques include submerged fermentation or cultivation (SmF) and solid state fermentation or cultivation (SSF). In SSF, the culture medium is composed of solid material with given moisture content. The necessary moisture exists in the absorbed or complexed form in the solid matrix. In this case, the nutrients are distributed heterogeneously [24].

Recently, there has been a resurgence of interest in SSF techniques due to the fact that SSF offers several advantages over SmF, such as lower energy requirements, smaller reactors, and the fact that the product is obtained in concentrated form. One of the greatest advantages of SSF methods is that they use little water, which drastically reduces the energy consumed in the recovery of products, in addition to producing low volumes of effluents [29,18,23,24,28,26,6].

Agroindustrial wastes used as raw materials for the generation of products of interest or energy are important objects of study for sustainable economic development [20,8,23,28,15]. In the 2012/2013 harvest, Brazil milled 588 million tons of sugarcane, with production of 23 billion liters of ethanol [25]. Although the bagasse can be used for obtaining energy in sugar-cane mills in Brazil, part of this raw material could be used as support to photosynthetic microorganism growth.

No study has been published about the variables involved in cultivation of photosynthetic microorganism on solid medium. The present study therefore examines the variables involved in the production of *A. platensis* on sugarcane bagasse as support, taking as independent variables the moisture of the culture medium (*M*), light intensity (*I*) and inoculum concentration (*C*). Since there are no studies in the literature about this type of process, the limits

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applied to the variables were based on solid state cultivation processes for other microorganisms and on a conventional *A. platensis* cultivation process.

## 2. Materials and methods

### 2.1. Microorganism and inoculum

The microorganism used was *A. platensis*. The inoculum was obtained by standard cultivation in liquid mineral medium [17]. The culture was carried out in 500 mL Erlenmeyer flasks with 200 mL of culture medium, kept in a shaker agitated at 160 rpm under a light intensity of 6.0 klx, in a room with controlled temperature (30 °C). The process time for production of the inoculum was 7 days, according to Pelizer et al. [19].

To obtain the desired concentration, the culture was filtered and diluted in standard mineral culture medium. The cell concentration was determined by spectrophotometry at 560 nm.

### 2.2. Experimental design

An experimental design was devised with three variables (2<sup>3</sup>), of the star type with orthogonal rotation [2]. The variables studied were: inoculum concentration (C), light intensity (I) and moisture content of culture medium (M). To obtain culture media with different moisture contents, the proportion of sugarcane bagasse and standard mineral liquid culture medium with agar was varied. So moisture content is related to the % of mineral liquid culture medium with agar that was added. X1, X2 and X3 are coded values for the chosen variables, whose combinations generated the experimental design under study (Table 1).

Based on the calculations performed to obtain the experimental design, the amount of inoculum concentration determined for X2 = -1.7 would be -0.02 g biomass·kg<sup>-1</sup> wet culture medium; however, this has no physical value for the experiments in question. The value used was therefore 0.02 g biomass·kg<sup>-1</sup> wet culture medium and the value of X2 was corrected to -1.3.

### 2.3. Culture conditions

The assays were conducted in 250 mL Erlenmeyer flasks with 50 g of culture medium, in temperature-controlled room (30 °C), maintained in static condition.

Illumination was provided by fluorescent lamps (40 W). The desired light intensity was obtained by changing the distance between the lamps and the culture and was measured using a light meter (Minolta TL-1) and expressed in klx (to be converted to photosynthetic photon flux density – PPF – the conversion for white fluorescent light: 12 μmol photons·m<sup>-2</sup>·s<sup>-1</sup>·klx<sup>-1</sup> can be used [14]). To keep the lighting uniform, the location where the cultures were performed was kept closed.

The culture medium was prepared by taking a given quantity of sugarcane bagasse ground in a Wiley mill with 0.5 mm mesh, according to each assay, which was transferred to the 200 mL Erlenmeyer flasks. Then, nutrient agar (8 g·L<sup>-1</sup>) was added to 500 mL of standard mineral culture medium [17] contained in a 1-L Erlenmeyer flask, which was placed in a microwave oven and left there for 3 min. The hot culture medium was distributed in the Erlenmeyer flasks containing sugarcane bagasse. Upon cooling, the medium gelled and then was homogenized for inoculation.

There is no need for sterilization since the initial pH of the medium is greater than 9.0.

The culture medium was inoculated with 2 mL of cellular suspension, whose concentration varied according to the assay.

In these assays, it was made an analysis of the protein content of the product obtained in 7 days of processing.

### 2.4. Analytical methods

#### 2.4.1. Determination of protein content

To determine the protein content in each process time, a sample of the cultured material was removed and homogenized. The homogenized material was washed with distilled water, filtered through a polyester screen and oven-dried at 55 °C until constant mass. The dried material was ground and the protein content was determined by the Kjeldahl method [10], using a conversion factor of 6.25 g protein·g<sup>-1</sup> nitrogen.

### 2.5. Analysis of the results

#### 2.5.1. Calculation of protein productivity

Protein productivity was determined in the assays in which cell growth occurred (Eq. (1)).

$$Pr_{\text{prot.}} = \frac{P_{\text{prod.}}}{T_{P_{\text{max}}}} \quad (1)$$

where  $Pr_{\text{prot.}}$  = protein productivity (g protein produced·100 g<sup>-1</sup> of dry culture medium·day<sup>-1</sup>);  $P_{\text{prod.}}$  = quantity of protein produced per 100 g of dry culture medium·day<sup>-1</sup> (g protein produced·100 g<sup>-1</sup> of dry culture medium·day<sup>-1</sup>) and  $T_{P_{\text{max.}}}$  = time taken to obtain the maximum amount of protein from the culture medium·day<sup>-1</sup>.

#### 2.5.2. Calculation of the nitrogen to protein yield ( $Y_{P/N}$ )

The nitrogen (KNO<sub>3</sub>) to protein yield ( $Y_{P/N}$ ) was calculated using Eq. (2) shown below. The amount of KNO<sub>3</sub> contained in each assay was calculated, since each culture medium was prepared with different quantities of liquid culture medium to obtain different moisture contents.

$$Y_{P/N} = \frac{P_{\text{prod.}}}{N} \quad (2)$$

where  $Y_{P/N}$  = nitrogen to protein yield (g protein·g KNO<sub>3</sub><sup>-1</sup>);  $P_{\text{prod.}}$  = quantity of protein produced per 100 g of dry culture

**Table 1**  
Variables, codes and levels of experimental design.

Variables	Codified variables	Levels				
		-1.7 (-1.3 for C)	-1	0	+1	+1.7
I = light intensity (klx)	X1	2.3	3.8	6.0	8.2	9.7
C = inoculum concentration (g biomass·kg <sup>-1</sup> wet culture medium)	X2	0.02	0.05	0.15	0.25	0.32
M = initial moisture of culture medium (%w/w)	X3	95.3	96.0	97.0	98.0	98.8

medium.day (g protein produced·100 g<sup>-1</sup> of dry culture medium·day<sup>-1</sup>) and  $N$ =quantity of KNO<sub>3</sub> in each culture medium (g KNO<sub>3</sub>·100 g<sup>-1</sup> dry culture medium).

## 2.6. Statistical analysis of the results

Multivariable regressions were performed for the results obtained in terms of protein productivity ( $Pr_{prot.}$ ) and the nitrogen to protein yield ( $Y_{P/N}$ ), using Statgraphics version 5.5.

## 3. Results and discussion

The literature lacks reports about *Arthrospira* production by solid state cultivation. Therefore, the choice of variables was based on processes that involve the use of other types of microorganisms and culture media. It is known that the microorganism requires minimal water content for its development, and that this requirement is lower in SSF processes. There is a minimum range of water content for bacteria, fungi and yeasts, but in the case of algae, nothing is reported in published studies.

One of the reported drawbacks of SSF is the high concentration of inoculum it requires. However, for *Arthrospira*, no information regarding this aspect was found in the literature. Pelizer et al. [19] conducted studies in liquid medium, observing this variable.

On the other hand, light intensity can be considered a widely studied aspect of utmost importance in the production of microalgae, as it is known that there is a light saturation point.

Therefore, these variables,  $M$ ,  $I$  and  $C$  were chosen based on the aforementioned considerations. Upon the conclusion of this work, the results obtained in the assays indicated that these three variables affect the process, and that the initial moisture content exerts the strongest influence (Table 2).

### 3.1. Moisture content

An analysis of the results of the assays conducted with  $I = 3.8$  klx (assays 01, 03, 05 and 07) clearly shows the influence of moisture content on the process. Assays 01 and 03, which were performed with lower initial moisture content (96.0%) than in assays 05 and 07 (98.0%), yielded unsatisfactory results. In assay 01, the microorganism showed no development and in assay 03, there was a negligible increase (1.6%), and it can be considered that cell

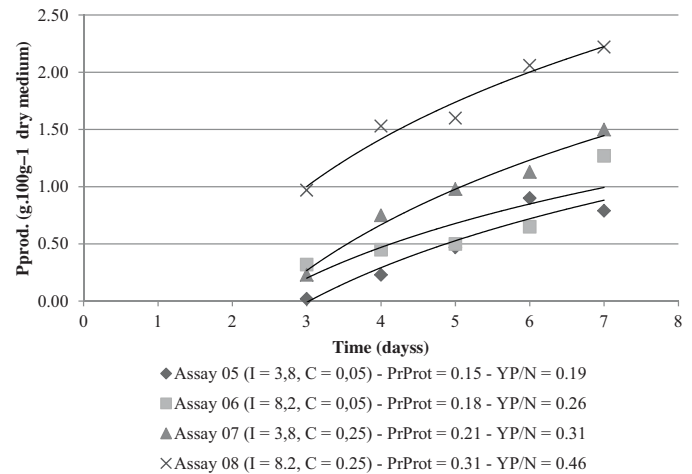


Fig. 1. Protein production ( $P_{prod.}$ ) in assays conducted with same initial moisture content (98.0%).

growth was virtually zero. The results obtained in assays performed with  $I = 8.2$  klx (02, 04, 06 and 08) were similar to those obtained in the assays performed with  $I = 3.8$  klx (Fig. 1).

Assays 09, 10, 13, 14, 15 and 16 were performed with the same concentration of inoculum, i.e., 0.15 g biomass·kg<sup>-1</sup> wet culture medium. Assay 13, performed with higher initial moisture content (98.8%), yielded the best results in terms of increased protein content (110.0%) and protein content of the product (5.18 and 5.19%, respectively), once again demonstrating the influence of the moisture content of the culture medium on the process. Assays performed with inoculum concentration of 0.25 g biomass·kg<sup>-1</sup> wet culture medium showed virtually no cell growth occurred due to the low moisture content of the initial culture medium in assays 03 and 04.

The results of assays 05, 06, 07 and 08, which were performed with an initial moisture content of 98.0%, showed higher increases in protein content than the assays conducted with an initial moisture content of 97.0%. The smallest increment was obtained in assay 01:43.0%, which was performed with lower illuminance (3.8 klx) and low inoculum concentration (0.05 g biomass·kg<sup>-1</sup> wet culture medium). In general, the results obtained in the assays carried out with the initial moisture content of 97% (09, 10, 11, 12, 15 and 16) did not show good results.

In the analysis of the significance of the initial moisture of the culture medium, this parameter was found to be a constraint of the process, which is in agreement with the literature on SSF. Very high moisture levels lead to diminished porosity, poor oxygen diffusion, a higher risk of contamination, decreased gas volume, and reduction in gas exchange. Low moisture levels lead to slower optimum growth, a low degree of substrate actually used, and an increase in water surface tension [16,7].

Castilho et al. [3] studied the influence of the substrate's initial moisture content on the production of pectinase by *Aspergillus niger* and noted that there was a limit of moisture for the synthesis of pectinases. A moisture content exceeding 40% reduced the production yield, while a moisture content of 25% induced virtually no cell growth.

dos Santos et al. [7] optimized solid state fermentation of potato peel for the production of cellulolytic enzymes. It was noted that approximately 50% moisture was ideal for obtaining the enzyme studied. In other studies the values ranged between 40% and 60%, with a decrease in fungal activity possibly related to inhibition of the fungus, marked by extrapolation of the ideal water level for the development of the line selected in the case of 60%, or low activity

Table 2  
Results of the experimental design.

Assay	X1	X2	X3	I	C	M	$Pr_{prot}$	$Y_{P/N}$
01	-1	-1	-1	3.8	0.05	96.0	-	-
02	1	-1	-1	8.2	0.05	96.0	-	-
03	-1	1	-1	3.8	0.25	96.0	0.01	0.01
04	1	1	-1	8.2	0.25	96.0	0.01	0.02
05	-1	-1	1	3.8	0.05	98.0	0.15	0.19
06	1	-1	1	8.2	0.05	98.0	0.18	0.26
07	-1	1	1	3.8	0.25	98.0	0.21	0.31
08	1	1	1	8.2	0.25	98.0	0.31	0.46
09	-1.7	0	0	2.3	0.15	97.0	0.03	0.04
10	1.7	0	0	9.7	0.15	97.0	0.09	0.16
11	0	-1.3	0	6.0	0.02	97.0	-	-
12	0	1.7	0	6.0	0.32	97.0	-	-
13	0	0	-1.7	6.0	0.15	95.3	-	-
14	0	0	1.7	6.0	0.15	98.8	0.45	0.45
15	0	0	0	6.0	0.15	97.0	0.14	0.17
16	0	0	0	6.0	0.15	97.0	0.14	0.18

X1, X2 and X3 = Codified variables,  $I$  = light intensity (klx),  $C$  = inoculum concentration (g biomass·kg<sup>-1</sup> wet culture medium),  $M$  = initial moisture of culture medium (%w/w),  $Pr_{prot}$  = protein productivity (g protein produced·100 g<sup>-1</sup> of dry culture medium·day<sup>-1</sup>),  $Y_{P/N}$  = nitrogen to protein yield (g protein·g<sup>-1</sup> KNO<sub>3</sub>).

of water needed for the fungus to develop as might have occurred in 40%.

Dhillon et al. [6] verified the potential of apple pomace as a solid substrate for fungal cellulase and hemicellulase bioproduction through SSF. The apple pomace used in this study was supplemented with 1% (w/w) rice husk. The moisture of the material was 71.28%. In this work, the initial moisture was adjusted to 75% with distilled water and the cultures were incubated in environmental chamber at 30 °C and 75% relative humidity for 7 days.

Abraham et al. [1] studied the potential of the solid-state fermentation of soy fiber residues for protease production. The water content of soy fiber in dry basis was about 82.5%. Wood chips were added to the materials in a 1:1 (w:w) ratio and acted as a bulking agent to provide the proper porosity.

Mahadik et al. [13] examined the production of acidic lipase by *A. niger* in SSF. To check the influence of moisture on lipase activity during the process, wheat bran was moistened with different amounts of liquid medium. Maximum enzyme yield was obtained when wheat bran was moistened with liquid medium in a 1:2.5 ratio. There was a decline in enzyme production above this ratio as the porosity of the medium is decreased.

Those studies consistently describe a minimum of moisture for growth, and in some cases, a maximum point. In the present study, only the beneficial result of increased moisture was observed, i.e., a maximum point for this variable was not reached. The use of sugar cane bagasse for *A. platensis* culture could help regarding porosity. The use of nutrient agar to maintain the moisture was of paramount importance, since sugarcane bagasse is not able to absorb the quantity of liquid medium necessary to reach the ideal moisture content for cultivation.

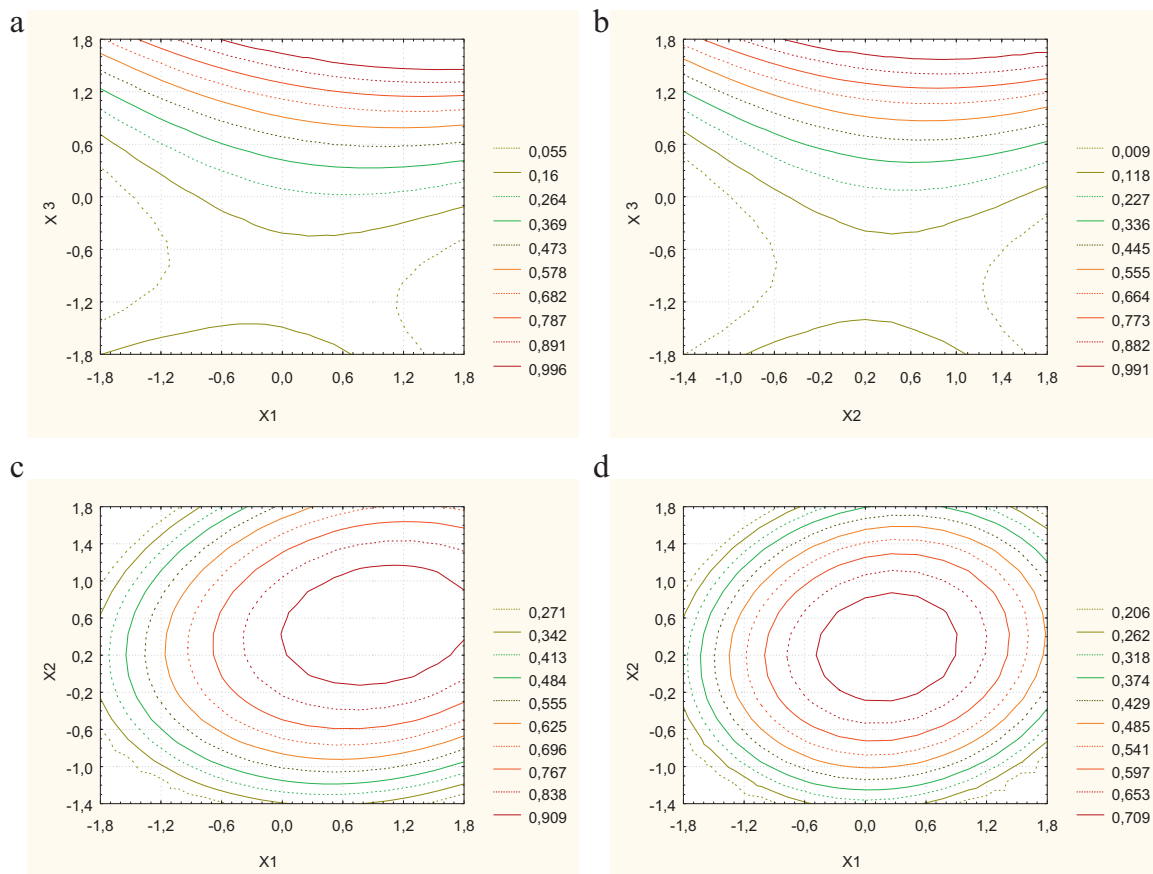
### 3.2. Light intensity

The results obtained in this work indicated that the optimal condition of illuminance was 6.0 klx. Zhang et al. [27] who studied variations in illuminance from 0.5 to 4.0 klx in a mixotrophic culture, found that the specific growth rate of the culture increased up to 2.0 klx, above which the growth rate no longer increased and suggested limit: light saturation which is in the order of 5–10 klx. Rodrigues et al. [22], studying fed-batch cultivation of *A. platensis* used minitanks at 13 klx and Matsudo et al. [14] at 9 klx. Jácome et al. [11] in fed-batch cultivation in a tubular photobioreactor used 10 klx. Leema et al. [12] used light intensity corresponding to 12 klx.

Danesi et al. [5] studied a two-step *A. platensis* cultivation process to observe the growth of *A. platensis* and the production of chlorophyll. Although the best growth result was observed at 5 klx, the biomasses from cultivations at 2 klx presented higher contents of chlorophyll.

The influence of illuminance on the process can be analyzed based on the results of assays 09, 10, 15 and 16. Increasing the illuminance from 2.3 klx in assay 09–6.0 klx in assays 15 and 16 was favorable, since the increments in protein content achieved in these assays were 7.3%, 35.6% and 37.7%, respectively. In assay 10, performed with illuminance of 9.7 klx, the increase in protein content was 33.5%, indicating that increasing the illuminance in this case is not recommended.

The results of assays 07 and 08, performed with the same initial moisture content (98.0%) and inoculum concentration (0.25 g biomass·kg<sup>-1</sup> wet culture medium), indicated that increasing the illuminance contributed to improve the process, with the protein content in assay 07 (3.8 klx) increasing by 64.4% and in



**Fig. 2.** Contour curves for protein productivity ( $\hat{Pr}_{\text{prot}}$ ): (a) as a function of X1 and X3, (b) as a function of X2 and X3, (c) as a function of X2 and X1, considering X3 = 0 (center point), and (d) X2 and X1, considering Vc = -1.7 (minimum point).



assay 08 (8.2 klx) by 95.3%. Assays 05 and 06, performed with an inoculum concentration of  $0.05 \text{ g biomass} \cdot \text{kg}^{-1}$  wet culture medium, also showed increments in protein content, i.e., assay 05 (3.8 klx) showed a 43.0% increase and assay 06 (8.2 klx) a 60.5% increase in protein content (Fig. 1).

### 3.3. Inoculum concentration

Using SSF process to cultivate fungi, some works refer to spores number in relation to solid medium. For example, [6], used spore suspension having  $1.0 \times 10^7 \text{ spores} \cdot \text{g}^{-1}$  substrate to produce fungal cellulose; Mahadik et al. [13] inoculated wheat bran with spore suspension of *A. niger* (1 mL) containing  $10^6$  spores from 7 days old culture grown to obtain lipase and dos Santos [7] added the quantity of  $10^7$  spores of *A. niger* per gram of dry basis substratum to the suspension to produce cellulolytic enzymes.

However, there are no studies about inoculum concentration to cultivate microalgae in SSF. Pelizer et al. [19] studied the influence of inoculum concentration to produce *A. platensis* in liquid medium. The inoculum concentrations tested were: 50, 100, 150 and  $200 \text{ mg L}^{-1}$ . For liquid medium, the  $50 \text{ mg L}^{-1}$  inoculum should be more appropriate to *A. platensis* cultivation because the higher inoculum concentrations did not present significant differences to justify their utilization.

In this work, inoculum concentrations of 0.02, 0.05, 0.15, 0.25 and  $0.32 \text{ g biomass} \cdot \text{kg}^{-1}$  wet culture medium were evaluated. Assays 12, 15 and 16 (assays 15 and 16 are repetitions) were performed with the same moisture content (97.0%) and illuminance (6.0 klx). It was found that the very high inoculum concentration used in assay 12 ( $0.32 \text{ g biomass} \cdot \text{kg}^{-1}$  wet culture

medium) did not contribute to improve the performance of the process, since this assay showed no cell growth. On the other hand, assays 15 and 16 ( $C=015 \text{ g biomass} \cdot \text{kg}^{-1}$  wet culture medium) showed an increase in cell growth of 35.6 and 37.7%, respectively.

Assays 06 and 08, with higher initial moisture content (98.0%), showed better results, clearly indicating the influence of the inoculum concentration. In assay 06, performed with  $C=0.05 \text{ g biomass} \cdot \text{kg}^{-1}$  wet culture medium, the increase in protein content was 60.5% while in assay 08, with  $C=0.25 \text{ g biomass} \cdot \text{kg}^{-1}$  wet culture medium, the increase was 95.3% (Fig. 1).

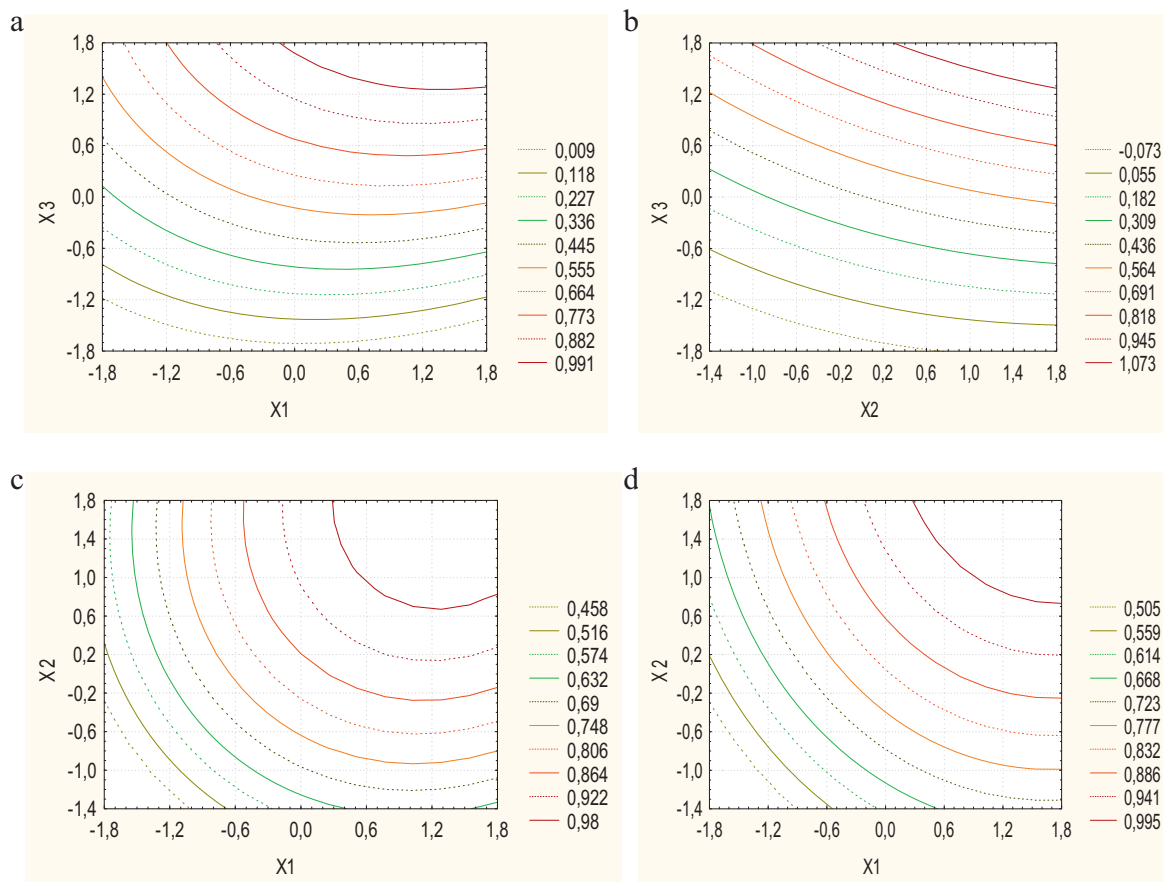
Assays 05 and 07 ( $M=98.0\%$ ) showed the influence of inoculum concentration. The increase in protein content in assay 05 was 43.0% and in assay 07, which was carried out with a higher concentration of inoculum ( $0.25 \text{ g biomass} \cdot \text{kg}^{-1}$  wet culture medium), the protein content increased by 64.5% (Fig. 1).

### 3.4. Statistical analysis of the results

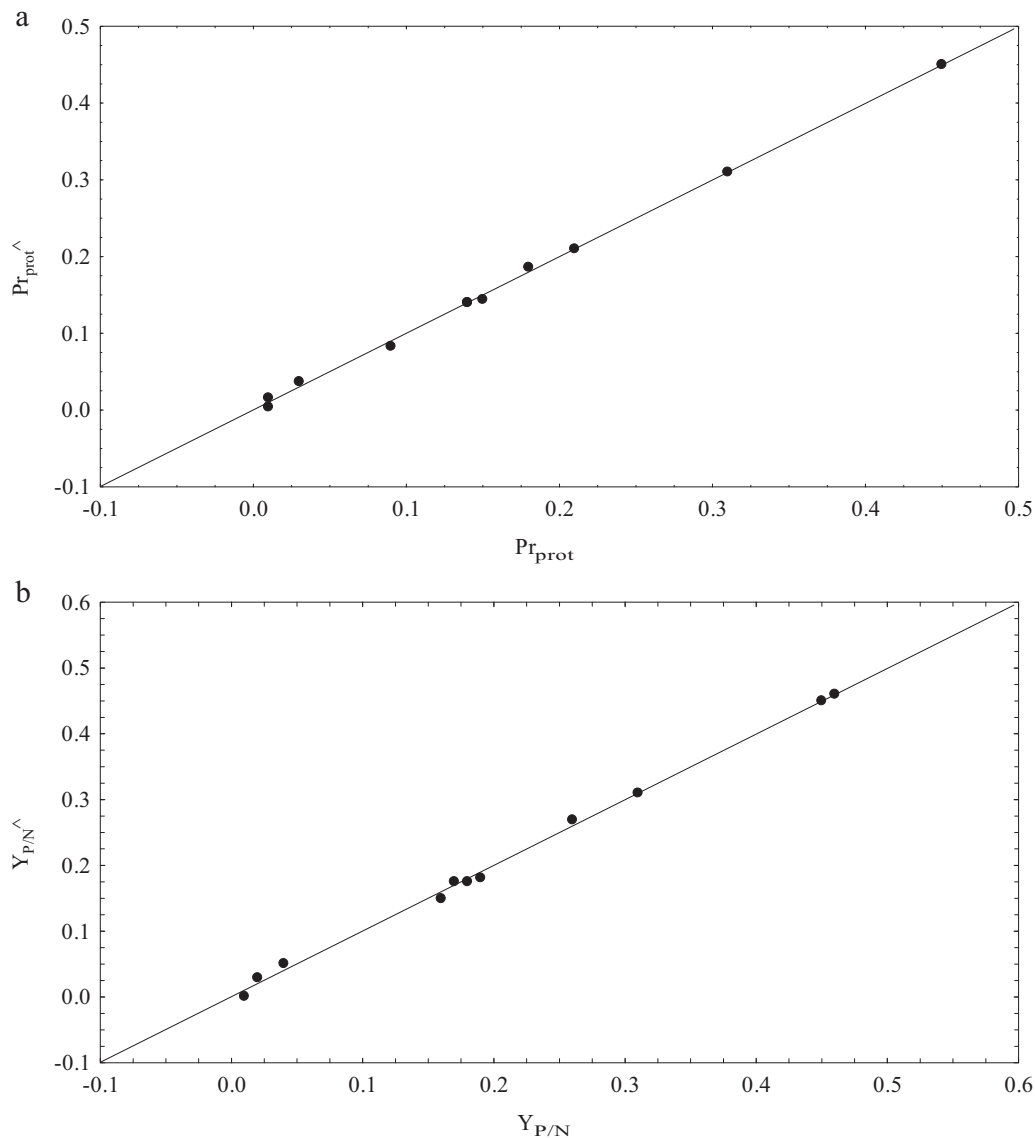
The results of protein productivity ( $Pr_{\text{prot}}$ ) and nitrogen to protein yield ( $Y_{P/N}$ ) were subjected to multivariable regressions, with a 10% limit of acceptance ( $p$ ) of each analysis.

Equations representative of the process were obtained for the variables under study. These equations are related with the encoded variables. To facilitate the discussion of the results, the values obtained in each assay are listed in Table 2.

Based on the equations obtained, graphical analyses were made by means of response surface plots and contour plots (Figs. 2–4, ,). Because the equation has three independent variables, the graphics obtained were constructed always considering two



**Fig. 3.** Contour curves of the nitrogen ( $\text{KNO}_3$ ) to protein yield ( $Y_{P/N}$ ): (a) as a function of  $X_1$  and  $X_3$ , (b) as a function of  $X_2$  and  $X_3$ , (c) as a function of  $X_2$  and  $X_1$ , considering  $X_3=0$  (center point), and (d) as a function of  $X_2$  and  $X_1$ , considering  $X_3=-1.7$  (minimum point).



**Fig. 4.** (a)  $\hat{Pr}_{\text{prot}}$  as a function of  $Pr_{\text{prot}}$ , considering an error range of 5%.  $r^2=0.9993$ .  $\hat{Pr}_{\text{prot}}=0.00021+0.99863 \cdot Pr_{\text{prot}}$ ; (b)  $\hat{Y}_{P/N}$  as a function of  $Y_{P/N}$  considering an error range of 5%.  $r^2=0.99876$ .  $\hat{Y}_{P/N}=0.00051+0.99752 \cdot Y_{P/N}$ .

variables so that, in the end, all the variables were combined with one another.

#### 3.4.1. Multivariable regression for protein productivity ( $Pr_{\text{prot}}$ )

The results of multivariate regression to assess the variables studied as a function of protein productivity ( $Pr_{\text{prot}}$ ) were:  $r^2=0.8657$ ,  $F=15.04$  and  $p=0.00196$ .

Eq. (3) was obtained by means of regression analysis:

$$\hat{Pr}_{\text{prot}} = 0.14 + 0.05X_2 + 0.13X_3 - 0.06X_2^2 + 0.03X_3^2 - 0.03X_1^2 + 0.02X_1X_2 \quad (3)$$

Based on Eq. (3), graphical analyses of the response surface plots and contour plots were performed, which are shown in Fig. 2.

#### 3.4.2. Multivariable regression for the nitrogen ( $KNO_3$ ) to protein yield ( $Y_{P/N}$ )

The results of the multivariable regression for the evaluation of the variables studied here as a function of the nitrogen ( $KNO_3$ ) to protein yield ( $Y_{P/N}$ ) were  $r^2=0.9531$ ,  $F=47.43$  and  $p=0.0000$ .

The following equation was obtained by means of regression analysis:

$$\hat{Y}_{P/N} = 0.18 + 0.03X_1 - 0.03X_1^2 + 0.08X_2 + 0.19X_3 \quad (4)$$

Based on Eq. (4), graphical analyses of the response surface plots and contour plots were performed and depicted in Fig. 3.

An analysis of the variables  $X_1$  and  $X_3$  as a function of estimated protein productivity  $\hat{Pr}_{\text{prot}}$  (Fig. 2a) reveals that augmented productivity requires a high moisture content and light intensity close to the center point (6.0 klx). From the contour plots, it can be seen that for high productivity, the value of  $X_3$  must be close to the maximum point and light intensity ( $X_1$ ) close to the center point, as was found for  $\hat{Pr}_{\text{prot}}$ .

An analysis of Fig. 2b with respect to  $X_2$  and  $X_3$  as a function of  $\hat{Pr}_{\text{prot}}$  also reveals the same response profile.

For the variables  $X_1$  and  $X_2$  evaluated at the center point of moisture,  $X_3=0$  (Fig. 2c), note that there is an optimal point close to the values of the center point. However, the estimated productivity values were also very low. An analysis of the same variables for  $X_3=-1.7$  (Fig. 2d) indicates that high productivity

values are obtained with a high inoculum concentration and values of light intensity close to the center point.

In the analysis of the variables under study, X1, X2 and X3 as a function of the nitrogen to protein yield ( $Y_{P/N}$ ) (Fig. 3), the observations were similar to those corresponding to  $Pr_{\text{prot}}$ .

Based on Figs. 3 and 4, which compare the estimated and experimental values for protein productivity ( $Pr_{\text{prot}}^{\wedge}$  and  $Pr_{\text{prot}}$ ) and for the nitrogen ( $\text{KNO}_3$ ) to protein yield ( $Y_{P/N}^{\wedge}$  and  $Y_{P/N}$ ), one can verify that there is a good fit data.

#### 4. Conclusions

In conclusion, it was found that moisture strongly influences the performance of the process. The best conditions were: moisture of 98.8%; inoculum concentration of 0.15 g biomass/kg wet culture medium<sup>-1</sup> and light intensity of 6.0 klx.

The values of  $r^2$ ,  $p$ , and  $F$  show a good fit of the statistical model found for the studied process.

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