Biomass production by *Yarrowia lipolytica*: effect of C/N ratio and magnetic field intensity

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ABSTRACT

This study aimed to evaluate the effect of magnetic fields (MF) and carbon/nitrogen (C/N) ratio, on biomass production of *Yarrowia lipolytica* NRRL Y-1095, using Full Factorial Design (FFD). MF had no significant effect on biomass production. In addition, a descriptive model was able to approximate biomass concentration based on the C/N ratio. The experimental biomass concentration ranged from 8.43 (C/N ratio of 150) to 11.87 g L^{-1} (C/N ratio of 50), while the model-predicted biomass concentration varied from 8.94 (C/N ratio of 150) to 11.89 g L^{-1} (C/N ratio of 50). The lower the C/N ratio, the higher the biomass production was, highlighting the importance of nutrient balance in optimizing bioprocesses for higher biomass production.

Keywords: Bioprocess. Bioproduction. Full factorial design. Yeast.

1 INTRODUCTION

The yeast *Yarrowia lipolytica* is considered non-pathogenic and is approved by the FDA (Food and Drug Administration) and is GRAS (Generally Recognized as Safe) certified. Yeast biomass is rich in protein, is a source of B vitamins, metabolites with antimicrobial and anti-inflammatory activities, and lipids, which can be used as a supplement in foods[2]. Therefore, biomass of *Y. lipolytica* has potential for human[2] and animal[3] food applications.

The initial C/N ratio in the culture medium influences the amount of biomass produced. A high ratio, characterized by an abundance of carbon in relation to nitrogen, stimulates the accumulation of lipids in oleaginous yeasts. On the other hand, a low ratio indicates an abundance of nitrogen, which promotes cell growth[4]. Furthermore, magnetic field (MF) application in bioprocesses can increase microbial growth[5] and metabolic products, as carotenoids[6] and enzymes[7]. The effects of MF on different strains of microorganisms can be classified as positive, negative or null[8], depending on the intensity and time of application[9].

Therefore, the present study aimed to study cultivation medium conditions, MF and C/N ratio, to increase the biomass production of *Yarrowia lipolytica* NRRL Y-1095 using an experimental design.

2 MATERIAL & METHODS

Y. lipolytica NRRL Y-1095 was stored at 4°C on Yeast and Malt (YM) agar medium. To prepare the inoculum, the cells were transferred to a 500 mL Erlenmeyer flask with 200 mL of medium, according to Carsanba et al.[10].The flasks were kept on an orbital shaker at 180 rpm and 28 °C for 24 h.

A Full factorial design (FFD) 2^2, totaling 7 assays, was carried out to study the influence of MF (0 to 60 mT) and C/N ratio (50 to 150) on biomass concentration, with the medium adapted from Carsanba et al.[10] and composition in g L^{-1}: 60 glucose; 0.5 $(NH_4)_2SO_4$; $7KH_2PO_4$; 2.5 Na_2HPO_4; 1.5 $MgSO_4.7H_2O$; 0.15 $CaCl_2$; 0.15 $FeCl_3.6H_2O$; 0.02 $ZnSO_4.7H_2O$ and 0.06 $MnSO_4.H_2O$. The adaptation consisted of varying the nitrogen source (yeast extract) to obtain the different C/N ratio. Cultivations were carried out at 28 °C, initial pH 6.0, 10% (v v^{-1}) inoculum, 180 rpm, for 192 h. The experimental design was evaluated by Statistica version 5.0 (StatSoft, Tulsa, OK).

Biomass concentration was quantified at 192 h of cultivation using a spectrophotometer at 600 nm. The samples were centrifuged at 2034 x g for 20 min and biomass was resuspended in distilled water and centrifuged again. A standard curve relating biomass concentration and absorbance was constructed to obtain data in g L^{-1}. A linear equation was used to estimate the biomass concentration of *Y. lipolytica*.

3 RESULTS & DISCUSSION

MF did not affect the biomass concentration at 192 h. The biomass concentration predicted by Equation 1 was compared to experimental values using relative error calculations (Table 1).

Biomass concentration (g L^{-1}) = 10.41 - 1.48 C/N ratio (1)

The experimental biomass concentration ranged from 8.43 (C/N ratio of 50) to 11.87 g L^{-1} (C/N ratio of 50), while the model-predicted biomass concentration varied from 8.94 to 11.89 g L^{-1}, resulting in relative deviations between -4.68% and -0.20%. Thus, Equation 1 accurately predicts *Y. lipolytica* biomass concentration.

Table 1 - Experimental, predicted value and relative deviation for biomass concentration of yeast *Yarrowia lipolytica* NRRL Y-1095 at 192 h of cultivation.

Assays	C/N ratio	MF (mT)	Experimental - biomass concentration (g L^{-1})	Model - biomass concentration (g L^{-1})	Relative error (%)
1	-1 (50)	-1 (0)	11.87	11.89	-0.20
2	1 (150)	-1 (0)	8.43	8.94	-5.99
3	-1 (50)	1 (60)	11.01	11.89	-7.99
4	1 (150)	1 (60)	8.54	8.94	-4.68
5	0 (100)	0 (30)	10.97	10.41	5.05
6	0 (100)	0 (30)	11.10	10.41	6.14
7	0 (100)	0 (30)	10.99	10.41	5.22

Figure 1 shows the contour curves that relate the dependent variable (biomass concentration) with the independent variable (MF intensity and C/N ratio). It shows that, with the decrease in the C/N ratio from 150 to 50, there was an increase in biomass concentration. This is due to the greater relative availability of nitrogen, which stimulates microbial growth. Kuttiraja et al.[11] also studied the influence of C/N ratio in *Y. lipolytica* SKY7 cultures and observed an increase in biomass with a decrease in the C/N ratio from 150 (13.57 g L^{-1}) to 25 (22.62 g L^{-1}).

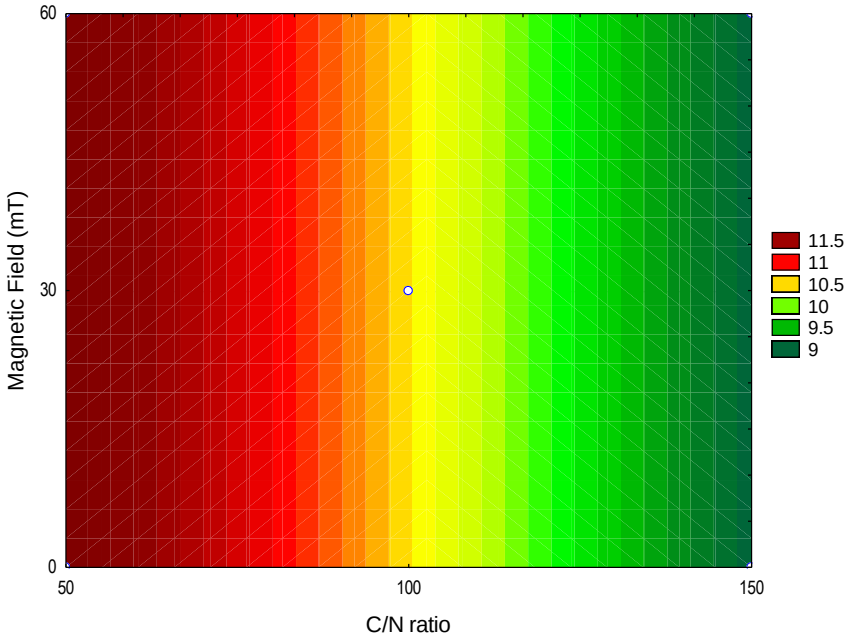

Figure 1- Contour curves obtained for biomass concentration of the yeast *Yarrowia lipolytica* NRRL Y-1095 as a function of MF intensity and C/N ratio.

These results provide a solid basis for designing cultivation strategies aimed at optimizing biomass production for this microorganism, highlighting the importance of carefully controlling the C/N ratio in industrial bioprocesses.

4 CONCLUSION

This study highlights the importance of the C/N ratio in *Y. lipolytica* NRRL Y-1095 biomass production, regardless of MF application. The use of FFD demonstrated accuracy in predicting biomass concentration, providing valuable information for bioprocess optimization.

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