Lipid production and cell growth by *Lipomyces starkeyi* using experiment design in shake flasks

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Introduction

Studies attempting the establishment of a microbial lipid production process from renewable resources, mainly xylose, will be developed. This pentose, obtained from sugar cane bagasse hydrolysis, is expected to be available in large quantities and is going to be used as a substrate in fermentation processes in the near future.

The ability of some microorganisms to produce significant lipid quantities, called as single-cell oil (SCO), is already known and in the eighties received great attention from the scientific community. Most oleaginous yeasts can accumulate lipids at levels of more than 40% of their dry weight and as much as 70% under nutrient-limiting conditions (Beopoulos et al. 2009). However, the lipid content and fatty acid profile differ between species (Beopoulos et al. 2009, Li et al. 2008b, Meng et al. 2009).

Some of the yeasts with high oil content are *Rhodotorula glutinis, Cryptococcus albidus, Lipomyces starkeyi,* and *Candida curvata* (Meng et al. 2009). The main requirement for high lipid production is a medium with an excess of carbon source and other limiting nutrients, mostly nitrogen. Hence, production of lipids is strongly influenced by the C/N ratio, aeration, inorganic salts, pH, and temperature (Papanikolaou et al. 2006).

Results and Conclusions

A 2^2 full factorial design was used to evaluate the effect of two variables: carbohydrate concentration and C/N ratio, on cell growth and lipid accumulation in flask culture conditions (250 mL Erlenmeyer flasks containing 50 mL of medium). The Statistica (version 7.0) software was used for regression and graphical analysis of the obtained data.

Significant variation on cell mass and lipid content exist within the 12 runs and the highest values were obtained in different runs: a maximum biomass concentration (around 30 g/l) was observed in run 2 (90 g/L xylose, C/N 15). Highest lipid content around 44% was found for the center points (60 g/ xylose and C/N 30), and the highest cellular productivity, 0.1574 g/L.h was found for the run 7 (60 g/L and C/N 8.9). Run 8 was done with 60 g/L of glucose plus xylose and C/N ratio of 51.2, resulting in 19.5 g/L cell mass concentration. Run 5 contained the lowest carbohydrate concentration (17.7), which was depleted at 72h, justifying the lower cell growth and productivities. The number of cells for the experiments varied from 1.5×10^8 to 3.7×10^8cells/mL.

The results for cell mass were analyzed for regression analysis and variance (ANOVA). The model coefficients were calculated by regression analysis for each variable. The ANOVA indicated that the model was significant and adequate to represent the actual relationship between the response and the significant variables with very small p-value (0.05).

The pure error was very low, indicating good reproducibility from the biomass data obtained. Fisher's *F*-test also demonstrates a very high significance for the regression model since the computed *F*-value (97.03) is much greater than the tabular *F*-value (4.12) at 5% level for the cell mass concentration. Through the Analysis of Variance, the correlation coefficient obtained for the cell mass concentration and the result of F-test (23.55 times higher than F_{tab}) were good indicators for a model (coded equation) representative of the actual relationship among the selected reaction parameters (Eq. 1).

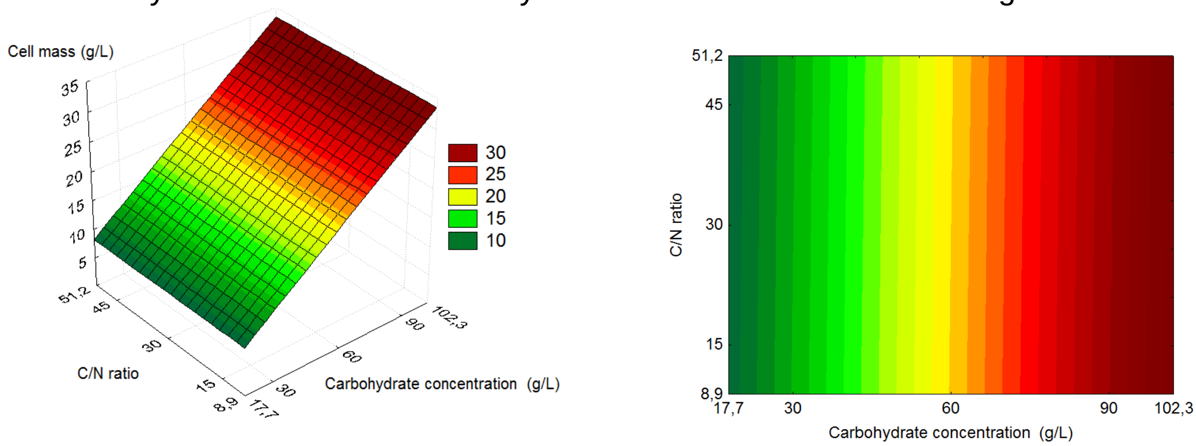
A direct correlation between the C/N ratio and cell mass and lipid content was obviously found. Clearly, higher carbohydrate concentration leads to higher cell mass. Comparing the cell mass concentration at the low level of C/N ratio with the cell mass concentration at the high level indicates that the cell mass was somewhat negatively affected by C/N ratio (22.7 vs. 19.5 g/l) since there would be less available nitrogen for biosynthesis of yeast cells.

The R^2 value for Equations (1) is 0.9822, indicating that about 98% of the variations in cell mass can be explained by the quadratic polynomial. This means that Equation (1) is adequate for correlating the experimental results. The factors x_1 and x_2 are specified in their coded units.

$$\text{Cell mass} = 20{,}98 + 8{,}92x_1 - 0{,}09x_1^2 - 0{,}01\, x_1x_2 \qquad (1)$$

According to the *t* and *p* value, the term of x_2 and x_2^2, did not have the statistical significance. The analysis indicated that independent variable x_2 (C/N ratio) in investigated range, did not have significant effect on the response variable.

The regression model was used to construct the response surface and contour plot. It can be seen (Figure 1) that there is no clear optimum within the experimental area investigated because the best cell mass concentration lies at the upper bound of the carbohydrate concentration range. Nevertheless, the contour plot indicates that the best cell mass concentration occurs at a higher carbohydrate concentration at any C/N ratios within the studied range.



(a) (b)

Figure 1. Response surface (a) and contour plot (b) of the experimental design.

Angerbauer et al. (2008) studied the influence of the C/N ratio on lipid accumulation by *L. starkeyi* comparing C/N ratios of 150, 60, 30, 20 and 15. The highest lipid content was measured at a C/N ration of 150, with 68% lipids of the dry matter. The dry matter was 9.5 g/L, giving a lipid concentration of 6.4 g/L.

Nutrient imbalance in the culture medium has long been known to trigger lipid accumulation by oleaginous microorganisms. When cells run out of key nutrients, usually nitrogen, excess substrate continues to be assimilated by the cells and converted into fat for storage. However, under nitrogen-limited conditions, cell propagation is drastically depressed, which in many cases restricts cell density and cell productivities (Li, Zhao et al. 2007).

It was reported that when C/N ratio was increased from 25 to 70, oil content increased from 18% to 46% (Hassan et al. 1996). Different nitrogen sources also affected the oil production. Both inorganic nitrogen sources and organic nitrogen sources can be used for yeast cultivation with differences in oil accumulation(Li, Du et al. 2008).

Results of the study illustrated the importance of these factors on cell mass concentration. The experimental results clearly showed that the cell mass directly depends on carbohydrate concentration and secondary on C/N ratio. The optimum conditions of fermentation promoted biomass concentration of 29 g/L.

Keywords: oleaginous yeast, biodiesel, fermentation, shake flasks, experimental design

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