IMPROVING BIOMASS PRODUCTION OF *Rhodotorula glutinis* BY NUTRIENTS MODIFICATION OF THE CULTURE MEDIUM

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Introduction. Oleaginous microorganisms are defined as microbial with lipid content more than 20% (1). Lipid accumulation in oleaginous yeast occurs in two cultural steps. In the first step, the microorganism grows in a balanced culture medium. Then, in a second step, a medium with an excess of carbon source and limited in at least one nutrient, is supplied to achieve lipid accumulation (2).
Microbial oils compared to vegetables oils have many advantages, such as: short time production, not affected by season or climate and facility to scale-up.
Yeasts have higher growth rates and lipid contents; and they consume cheap nutrients compared to other microorganisms (3, 4).
The aim of this research was to improve *R. glutinis* biomass concentration by modification of nitrogen source and glucose concentration in the culture medium.

Methods. *Rhodotorula glutinis* was purchased to ATCC (204091) and maintained on YPD agar slants. Inoculum was prepared in YPD medium, reaching a yeast concentration between 2.3 and $2.7*10^7$ cells/mL. Culture medium was (g/L): glucose, 100; $NaNO_3$, 26.7 or $(NH_4)_2SO_4$, 21.0; KH_2PO_4, 1.40; $MgSO_4$, 0.20; $CaCl_2$, 0.11. The pH was adjusted at 5.5. Cultures were performed in 125 mL flasks containing 25 mL of medium with 10% of inoculum, incubated at 30°C and 150 rpm. Glucose concentrations were varied from 20 to 300 g/L (Fig. 1). Samples were taken every 24h for further assays (cell growth by dry weight and glucose by the dinitrosalicylic acid method).

Results. Ammonium sulfate is the most common inorganic nitrogen source used for *R. glutinis* cultures; however, in this work, the use of $NaNO_3$ increased greatly biomass production. Maximal biomass concentrations of 5.1 and 46.1 g/L were obtained using $(NH_4)_2SO_4$ and $NaNO_3$, respectively. These results could be explained by the decreasing effect of pH during the cultures. Using $(NH_4)_2SO_4$, pH quickly decreased until 3.0, while by using $NaNO_3$, pH remained almost constant. Biomass production was gradually augmented as glucose concentration was increased (from 20 to 150 g/L), and decreased for higher concentrations (Fig.1).

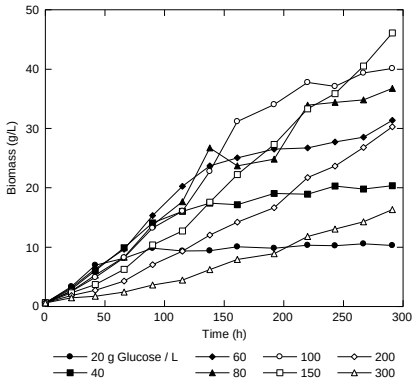

Fig.1 Kinetics of growth of *R. glutinis* using different glucose concentrations and $NaNO_3$ as nitrogen source.

Specific growth rate (μ) decreased as glucose concentration augmented. For glucose concentrations ≥ 150 g/L, μ remained constant (Table 1). Yields of biomass ($Y_{x/s}$) values had no tendency, oscillating between 0.387 and 0.525 g/g.

Table 1. Specific growth rate of *R. glutinis* at different glucose concentrations.

Glucose(g/L)	μ (h^{-1})	Glucose(g/L)	μ (h^{-1})
20	0.065	100	0.025
40	0.035	150	0.016
60	0.030	200	0.014
80	0.018	300	0.014

Conclusions. The highest biomass concentration (46.1 g/L) of *R. glutinis* was obtained using $NaNO_3$ as nitrogen source and a glucose concentration of 150 g/L. The highest values of μ (0.065 h^{-1}) and $Y_{x/s}$ (0.525 g/g) were found at 20 and 60 g/L of glucose, respectively. Work is ongoing to stimulate lipid accumulation in *R. glutinis.*

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References.

1. Meng X., Yang J., Xu X., Zhang L., Nie Q., Xian M. (2009). *Renewable Energy*. vol (34): 1-5.
2. Ratledge C. (2004). *Biochimie.* vol (86): 807-815.
3. Lopes da Silva T., Feijão D., Roseiro J., Reis A. (2011). *Bioresource Technology.* vol (102): 2998-3006
4. Li Q., Du W., Liu D. (2008). *Appl Microbiol Biotechnol.* vol (80): 749-756.