

**INFLUENCE OF THE TEMPERATURE ON BATCH CULTIVATION OF
Candida utilis IZ-1840 ON A SYNTHETIC MEDIUM CONTAINING
GLYCEROL AS THE MAIN CARBON SOURCE**

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SUMMARY. The highest values of the specific growth rate at the exponential phase (0.144 h^{-1}) and of the yeast cells productivity ($0.80 \text{ g.L}^{-1}.\text{h}^{-1}$) were obtained at 34°C and 30°C , respectively. The cells yield factor decreased from 0.495 to 0.275 when the temperature was increased from 26°C to 42°C .

INTRODUCTION

A great deal of experimental work, programmed and supported in Brazil by the former Secretaria de Tecnologia Industrial (MIC/STI, 1985a,b), led to the following main conclusions: 1. mixtures of ethyl esters, obtained by transesterification of different vegetable oils, gave very good performance when used as the sole fuel in Diesel engines; 2. 1,000 kg of vegetable oil led to 100 kg of glycerol as a by-product of the transesterification process. Consequently if, for instance, only 10% of the Diesel oil consumed in Brazil were replaced by the ethyl esters cited above, about 200,000 metric tons of glycerol could be produced. The use of such glycerol as a raw material must obviously be studied. Several microorganisms can use glycerol as a carbon source leading to the production of organic acids, AMP, antibiotics, enzymes, vitamins, sugars, steroids derivatives, enzyme inhibitors and polysaccharides. In spite of the fact that almost 150 different microorganisms can grow on media containing glycerol, no information was found regarding the use of glycerol as a raw material for the production of single cell protein, certainly due to its present relatively high cost. This situation, however, will change if a National Program were to be established with the purpose to replace Diesel oil by mixtures of ethyl esters. Based on the above facts, a research program was defined with the main intention to study the influence of experimental conditions on the production of *Candida* yeasts using glycerol as carbon source. The purpose of this paper is to show the influence of the temperature on batch cultivation of *Candida utilis* IZ-1840 on a synthetic medium containing glycerol as the main carbon source.

MATERIALS AND METHODS

The microorganism was inoculated in slant tubes containing sterilized (121°C; 15 min) culture medium (g.L⁻¹: glucose, 10.0; peptone, 5.0; yeast extract, 3.0; malt extract, 3.0; agar, 20.0). The inoculated tubes were incubated at 30°C for 72 h (or at 24°C for 120 h) and then maintained at 4-7°C for no more than 3 months. The inoculum was prepared as follows. A 125-mL Erlenmeyer flask, containing 20 mL of sterilized culture medium (g.L⁻¹: glycerol, 10.0; peptone, 5.0; yeast extract, 3.0; malt extract, 3.0) was inoculated from a recently developed culture of the yeast and was then incubated at 30°C for 22-26 h in a rotary shaker (300 min⁻¹). The obtained suspension was used to inoculate a 500-mL Erlenmeyer flask containing 80 mL of sterilized medium (g.L⁻¹: glycerol, 10.0; K₂HPO₄, 0.49; KH₂PO₄, 1.74; NH₄Cl, 4.0; MgCl₂.6H₂O, 2.54; Na₂SO₄, 1.67; citric acid, 0.21; ZnSO₄.7H₂O, 7.0×10⁻³; biotin, 4.0×10⁻⁶. Medium pH = 4.5) that was then incubated at 30°C for 16-20 h in a rotary shaker (300 min⁻¹). The final suspension was used to inoculate the fermenter. The tests were carried out in 15-L Biolafitte fermenters under the following conditions: volume of inoculum = 1.0 L; volume of medium (g.L⁻¹: glycerol, 72.0; K₂HPO₄, 3.53; KH₂PO₄, 12.53; NH₄Cl, 28.8; MgCl₂.6H₂O, 18.29; Na₂SO₄, 12.0; citric acid, 1.51; ZnSO₄.7H₂O, 0.050; biotin, 2.9×10⁻⁵) added to the reactor = 5.0 L; pH = 4.5 (automatically controlled by addition of 4.0 N solution of NaOH); temperatures = 26, 30, 34, 38 and 42°C; impeller speed, 500 min⁻¹; air rate = 1.0 L.L⁻¹.min⁻¹; anti foam: 5% aqueous suspension of Silcolapse (ICI). The culture medium composition was based on information published by Hong et al. (1987). The yeast cell concentrations were measured as follows: 10.0 mL of the fermenting medium was centrifuged (1,500×g; 10 min); the sediment was suspended in about 5 mL of distilled water, filtered through a Millipore membrane (pore diameter = 1.2 μ_m) and vacuum dried at 70-80°C until constant weight (about 4 h). The glycerol concentrations were determined in the centrifuged medium by means of a Merckotest kit (Fossati et al., 1982; McGowan et al., 1983). The yeast cells yields were calculated by the method proposed by Stouthamer (1969) and the productivities were evaluated by equations (1) and (2).

$$P = \frac{(X_f - X_0)}{t_f} \quad (1)$$

$$P' = \frac{10^3 \times Y_{x/s}}{t_f} \quad (2)$$

The value of t_f was the time elapsed from the beginning of the test until the moment correspondent to $S = 0$. The number of tests carried out at 26, 30, 34, 38 and 42°C were, respectively, 3,5,3,3 and 2.

RESULTS AND DISCUSSION

Figure 1 shows the results obtained in a typical experiment. In almost all the tests carried out at 26, 30, 34, and 38°C, the exponential growth phase begun just after the fermenter inoculation. At 42°C the yeast growth was very slow, reaching a cell concentration of 6.3 g.L⁻¹ (about 21% of the expected final concentration) after 122 h. Figure 2 presents the influence of the temperature on the average values of μ_m (maximum value = 0.144 h⁻¹ at 34°C), P (maximum value = 0.80 g.L⁻¹.h⁻¹ at 30°C), P' (maximum value = 13 g.kg⁻¹.h⁻¹ at 30°C), $Y_{x/s}$ and $\Delta t/t_f$. Equation (3) may be proposed to correlate $Y_{x/s}$ and T .

$$Y_{x/s} = 0.600 - \frac{2.232}{48.95 - T} \quad (3)$$

Small concentrations of ethanol (average = 0.6 g.L⁻¹) and acetic acid (average = 0.3 g.L⁻¹) were detected in all the centrifuged fermented media by gas chromatography, but the obtained results did not permit to explain the influence of T on Y_{x/s}. Equation (3) permits to conclude that when T = 45.23°C the value of Y_{x/s} will be zero. In other words, 45°C is probably the approximate value of T that completely inhibits the cells growth. Figure 2 also shows that Δt/t_f decreased when μ_m increased. Equation (4) may be used to correlate Δt/t_f and μ_m.

$$\Delta t / t_f = 1.645 - 7.670 \mu_m \quad (r = -0.997) \quad (4)$$

NOMENCLATURE

P	: yeast cells productivity
P'	: yeast cells productivity
r	: correlation coefficient
S	: glycerol concentration
t	: time
t _f	: duration of the test
T	: temperature
X	: yeast cells concentration, dry matter
X ₀	: initial value of X
X _f	: final value of X
Y _{x/s}	: yeast cells yield
Δt	: duration of the exponential phase
μ _m	: specific growth rate at the exponential phase

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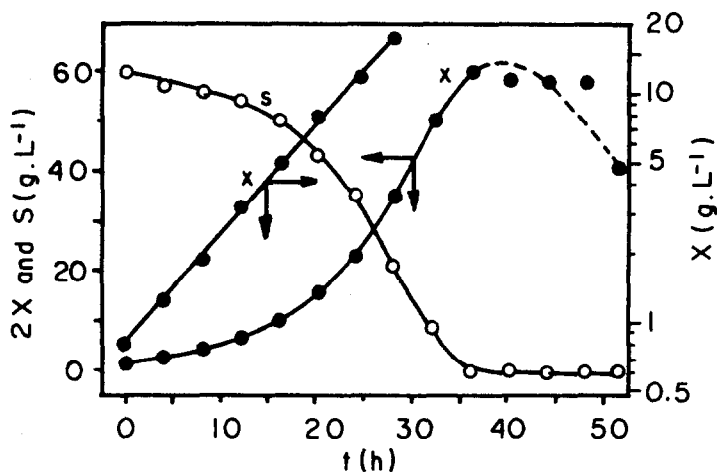


Fig. 1 - Variation of the yeast cells concentration (X) and of the glycerol concentration (S) during a test carried out at 30°C .

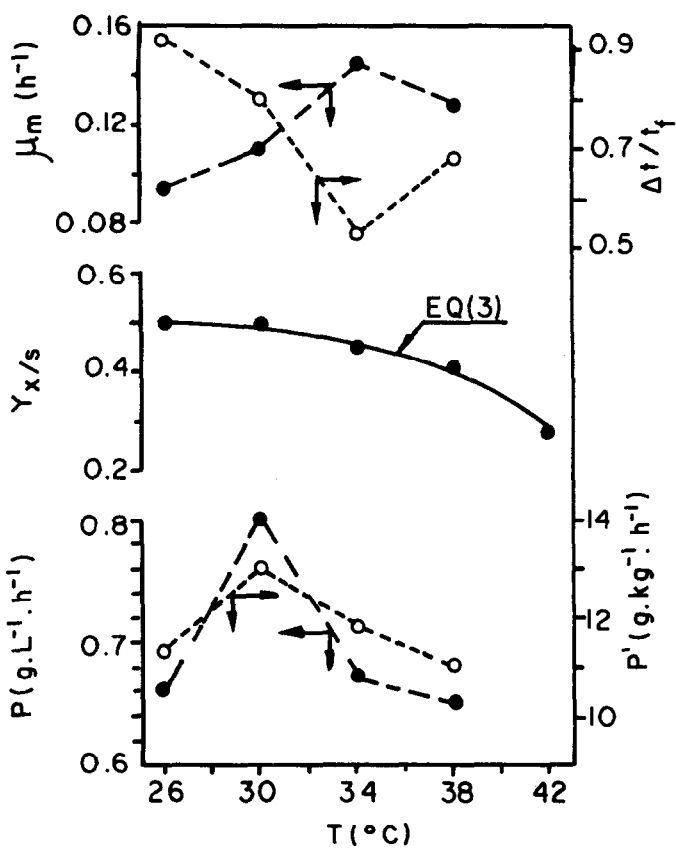


Fig. 2 - Influence of the temperature (T) on the following parameters: specific growth rate at the exponential phase (μ_m), yeast cells yield ($Y_{x/s}$), yeast cells productivities (P and P') and $\Delta t / t_f$.