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DIAUXIC GROWTH OF *SACCHAROMYCES CEREVISIAE*

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ABSTRACT

Glucose is preferred source of carbon and energy for yeast cells. Cultivation of *Saccharomyces cerevisiae* in medium with high glucose concentration cells consume at first the glucose and when the glucose is depleted, yeast starts consuming the by-product of their own metabolism, ethanol. This change from growth on glucose to growth on ethanol is known as the diauxic shift. The aim of this work was to observed diauxic behaviour of two yeast strains *Saccharomyces cerevisiae*. In this study were used measuring of optical density of yeast suspension at 600 nm and counting procedure using Burker chamber. The total number of cells was recorded without determining viability. It was found that diauxic shift of strain 612 occured after 12 hours of incubation (1.7 x 10^7 cells.ml^{-1}) and for strain Kolín after 8 hours of incubation (2.12 x 10^7 cells.ml^{-1}).

Keywords: Diauxic shift, *Saccharomyces cerevisiae*, stationary phase, carbon source

INTRODUCTION

The yeast *Saccharomyces cerevisiae* is the most commonly used species in biotechnological applications and is the most dominating species in yeast research. *Saccharomyces cerevisiae* is well adapted to life in a wide range of chemical and nutritional conditions (**Šillerová *et al.*, 2011**), so one of their exploitation is to prepare supplemented cells rich of microelement (**Poláková *et al.*, 2011**) or they can be a source of important antioxidant substances (**Lavová *et al.*, 2013**). Yeast has evolved a particularly effective mechanism for adapting its physiology to the changing concentration and composition of the carbon-containing nutrients that provide both the carbon and energy required for growth **(Ambroziak, 2008)**.
Growth and proliferation of microorganisms such as the yeast *Saccharomyces cerevisiae* are controlled in part by the availability of nutrients. *Saccharomyces cerevisiae* has the ability to grow with either anaerobic or aerobic metabolism, depending on the carbon source. The preferred sources of carbon and energy for yeast cells are fermentable sugars, such as glucose. When yeast cells are grown in liquid cultures in rich media containing glucose, they metabolize glucose predominantly by glycolysis, releasing ethanol in the medium (**Brauer *et al.*, 2005)**. When glucose becomes limiting, the cells enter a diauxic shift characterized by decreased growth rate and by switching metabolism from glycolysis to aerobic utilization of ethanol (**Albers *et al.*, 2002**). When ethanol is depleted from the medium and no other carbon source is available because proliferating yeast cells exhaust available nutrients, they enter a stationary phase (**Werner-Washburne *et al.*, 1993)**. This phase is characterized by cell cycle arrest and specific physiological, biochemical, and morphological changes (**Maris *et al.*, 2001)**. These changes include thickening of the cell wall, accumulation of reserve carbohydrates, and acquisition of thermotolerance **(Galdieri *et al.*, 2010; Zampar *et al.*, 2013)**.
The aim of this study was to observe changes in growth process of two yeast strains *Saccharomyces cerevisiae* cultivated in YPD medium. This study is focused on a) the monitoring of the diauxic behaviour of yeast *Saccharomyces cerevisiae* and b) determining when yeast enter into the stress-resistant stage known as the stationary phase.

MATERIAL AND METHODS

Microorganisms

Experiments were carried out with yeasts *Saccharomycescerevisiae* Meyen ex E.C. Hansen strains 612 and Kolín. The yeast strains were obtained from distillery Slovenské liehovary a likérky, a.s. Leopoldov, Slovakia. The yeast were individually maintained at 4 °C on malt extract agar plates and subcultured at monthly intervals.

Inoculum preparation and cultivation conditions

For inoculum preparation 5 loops of cells from an isolated colony on malt extract agar plate were aseptically transferred to 100 mL of liquid YPD (Yeast Peptone Dextrose) medium containing 10 g.L^{-1}yeast extract, 20 g.L^{-1} peptone and 35 g.L^{-1} glucose. The culture was incubated at 30 °C with shaking at 280 rpm. Then 20 mL of this over-night culture was transferred into 100 mL fresh YPD with the same composition and initial cell density was 0.5 x 10^6 cells.ml^{-1}. Yeast cells were grown under shaking (280 rpm) at 30 °C in dark. Yeast cell abundance was determined by a hemocytometer (Burker counting chamber). Optical density (OD) at 600 nm was the criterion of yeast cells growth. Growth curves of *Saccharomyces cerevisiae* were plotted by drawing absorption and number of yeast cells against process time.

RESULTS AND DISCUSSION

In present study we monitored changes in growth process of two yeast strains *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, strains 612 and Kolín, cultivated in YPD medium. The indicator of cells growth was optical density of medium, which was measured at 600 nm.
Herman (2002) characterized that during the initial logarithmic phase of growth, the budding yeast grows by fermentation of the available glucose. When glucose becomes limiting, the cells transiently arrest growth and switch to a respiratory mode of energy production. This period of transition is known as the “diauxic shift” (Figure 1).

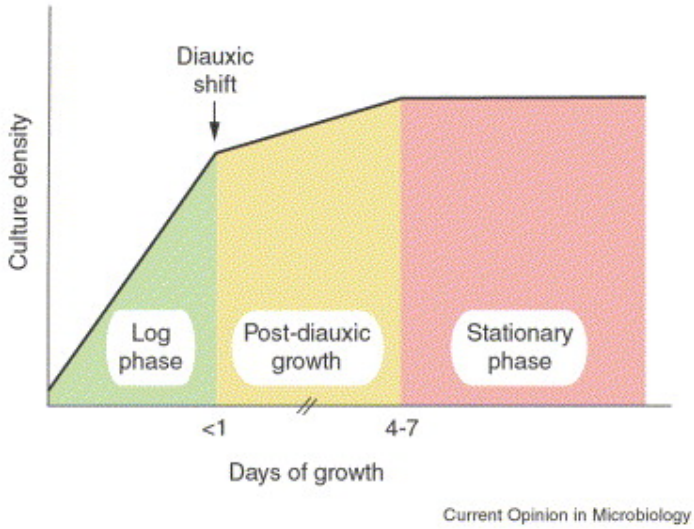

Figure 1 Growth phases exhibited by *Saccharomyces cerevisiae* cultures grown on glucose-based media **(Herman, 2002)**

From our results of *Saccharomyces cerevisiae* cultivation (Figure 2,3) was found that there was diauxic behaviour of cells what is present by typical growth curve. The finding was confirmed for both analysed strains. Based on our results it was found that the growth of strain 612 (Figure 2) was exponential with increasing value of absorbance from 0.06 to 0.75 during the 8 hours process time. **Fabrizio and Longo (2003)** described exponential growth of *Saccharomyces cerevisiae* cultivated in medium containig 20 g.L^{-1} glucose. They found that after 10 hours of yeast growth occurred diauxic shift.
After 8 hours of cultivation the rate of growth was slower but exponential too. The diauxic shift of strain 612 was observed after 12 hours of cultivation with increasing value of absorbance to 0.85. This results confirmed finding of **Werner-Washburne *et al.* (1993)** who reported that diauxic shift occurs in first 24 hours process time.In the next hours of cultivation yeast cells enteredinto the post-diauxic growth.

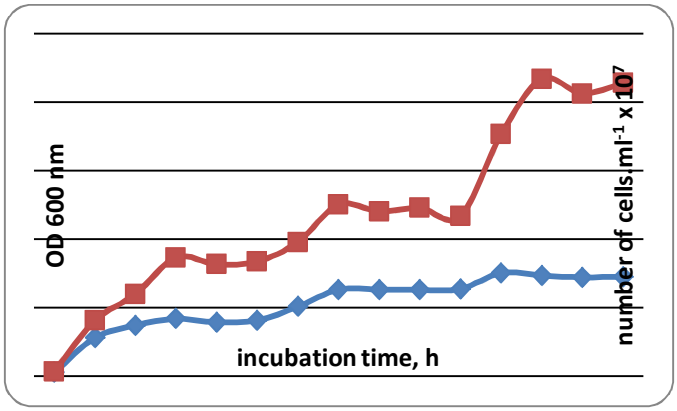

Figure 2 Diauxic growth of *Saccharomyces cerevisiae* strain 612 on YPD medium with addition of 35 g.L^{-1} glucose ; OD 600 nm (♦), number of cells.ml^{-1} x 10^{7}(■)

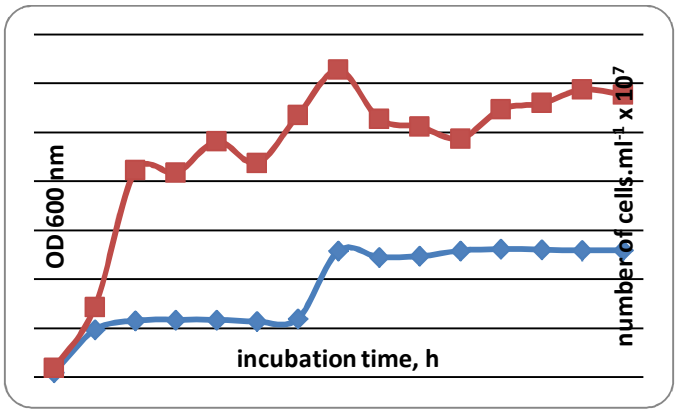

Figure 3 Diauxic growth of *Saccharomyces cerevisiae* strain Kolín on YPD medium with addition of 35 g.L^{-1} glucose ; OD 600 nm (♦), number of cells.ml^{-1} x 10^{7}(■)

Exponential growth phase of strain Kolín (Figure 3) was observed during the 4 hours process time (absorption value increased from 0.05 to 0.49). This statement agree with detection of **Al-mhanna (2010),** who described exponential growth of *Saccharomyces cerevisiae* during first 4 hours. Then the absorbance value increased from 0.49 to 0.59. It means that the rate of growth was still exponential but slower than during first 4 hours. The diauxic shift was found after 8 hours of cultivation when value of absorbance raised to 0.59. With prolongation of incubation time, yeast entered into post-diauxic phase. After 36 hours of cultivation followed a true stationary phase, when the cell number is no longer increasing.
During the subsequent post-diauxic growth period, the cells grow rather slowly and utilize the ethanol that was produced during the previous period of fermentation. When this ethanol is finally exhausted, the cells enter into the true stationary phase, the growth period when the cell number is no longer increasing **(Herman, 2002)**.
Diauxic-shift is very important way of cells growth on mixture of carbon sources. Our result shows that *Saccharomyces cerevisiae* Meyen ex E.C. Hansen, strains 612 and Kolín, cultivated in batch aerobic conditions in YPD medium instead simultaneously utilization of sources exploit them separately which caused two growth phases. During the first phase their main metabolic pathway is fermentation despite aeration and low energy efficiency.

CONCLUSION

This study dealt about diauxic behaviour of yeast *Saccharomyces cerevisiae*. The results indicated that cultivation of *Saccharomyces cerevisiae* in glucose limiting substrate has diauxic behaviour for high glucose concentration. It was found that diauxic shift of strain 612 occurs after 12 hours of incubation and for strain Kolín after 8 hours of incubation.

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