Innovative Romanian Food Biotechnology *Vol. 2, Issue of June 30, 2008*

Received March 24, 2008 / Accepted April 25, 2008

RESEARCH ARTICLE

CHARACTERIZATION OF TWO *SACCHAROMYCES CEREVISIAE* STRAINS OBTAINED BY UV MUTAGENESIS

Leontina PETREA *

Department of Bioengineering in Food Industry, Microbiology, Faculty of Food Science and Engineering , Dunarea de Jos University, Domneasca Street, No. 111, 800 008, Galati, ROMANIA

Abstract

The UV radiation was used in this experiment in order to obtain *Saccharomyces cerevisiae* strains hearing improved biotechnological properties. Two *Saccharomyces cerevisiae* mutants were obtained in a suspension containing $1.8x10^2$ cells / cm^3, under irradiation for 50 seconds.

The capacity of the *Saccharomyces cerevisiae* mutants to ferment glucose, galactose, sucrose, maltose and raffinose was tested as well as their capacity to produce ethanol.

The results indicate that only *Saccharomyces cerevisiae t1* mutant is capable to ferment all this glucides and it is also able to produce a higher quantity of ethanol than parental strain.

Keywords: UV radiations, *Saccharomyces cerevisiae*, ethanol

Financial support and Acknowledgements : This work was supported by the CNCSIS Project, PN-II- RU – MD – 1- 28, No.17/1.X. 2007

Introduction

S. cerevisiae is a very attractive organism to work with since it is nonpathogenic, and due to its long history of application in the ethanol production. It is used extensively in batch fermentations to convert sugars to ethanol for the production of beverages (Dequin, 2001).

Despite the obvious importance of this process, the physiological constraints which limit the rate of glycolysis and ethanol production are not fully understood. Identification of these constraints represents an important step toward the development of improved organisms and process conditions for more rapid ethanol production (Kielland-Brandt, 1994).

Although the improved design fermentative engineering and optimal cultural conditions can quantitatively enhance the microbial products, this

* Corresponding author: lili_petrea2006@yahoo.com

will only be up to a limit. Genetic improvement of the organism is fundamental to the success of fermentation technology.

The improvement of the yeast strains was traditionally based on random mutagenesis or classical breeding and genetic crossing of two strains followed by screening for mutants exhibiting enhanced properties of interest.

Microorganisms are genetically endowed with a mechanism that adjunts the production of metabolic cells to a level that should meet their own needs (Quain, 1998).

A certain amount of mutational changes in the genome occurs as a natural process, though the probability is low. Exposing a culture of a micro-organism to UV light or chemicals enhances the mutations occurrence rate.

But it is a tremendous task for the industrial genetics engineer to screen the very large number of randomly produced mutants and to select the ones with the desired characteristic features.

This study was made in order to obtain mutants capable to ferment the same glucides as parental strain do but bearing the ability to produce a higher ethanol quantity.

Two mutants of *Saccharomyces cerevisiae* strain were isolated and tested for their fermentative properties.

Materials and methods

Obtaining the mutant strains by UV mutagenesis

Saccharomyces cerevisiae strain was maintained on YPG medium (containing 4% glucose, 2% peptone and 1% yeast extract) for 18 hours.

Yeast cells during the stationary phase of growth were harvested by centrifugation (4000rpm, 10 min) and washed twice by means of distilled water.

Suitable dilutions were performed and $1.8x10^2$ cells / cm^3 were plated on YPGA medium (YPG medium solidified by adding 4% agar).

Each plate was exposed to ultraviolet radiation (λ=254nm) for different periods of time: 10, 20, 30, 40 and 50 seconds. The dishes were incubated at 30°C in the dark for three days (Vassu, 2001), treated with ultraviolet radiation for 50 seconds, two strains of mutant yeasts were isolated from the Petri dish under 50 minutes ultraviolet radiation. The mutant strains: *S. cerevisiae t1* and *S. cerevisiae t2* were replicated in test-tubes with wort agar and kept in the dark.

Spectrum of fermented glucides

Sterile solutions of galactose, glucose, sucrose, maltose and raffinose were added separately in five test tubes under Durham tube and Wickerham medium, everything being placed in the reversed order into the fermentation medium.

Wickerham medium containing 40g of glucose, 6g of $(NH_4)_2SO_4$, 5g of bacto-peptone, 2.5g of yeast extract, 2g of KH_2PO_4, 0.25g of $CaCl_2.2H_2O$, and 0.25g of $MgSO_4.7H_2O$ (per liter).

The concentration of the raffinose solution was 40% and for other glucides 20%.

Parental and mutant yeast cells were inoculated into liquid fermentation media and then were incubated at 28ºC for 10 days. CO_2 gas trapped in the Durham tube was visually monitored (Tofan *et al*., 2002).

Testing the raffinose potential of fermentation

For the qualitative determination of the remaining glucides in the medium after raffinose fermentation, in the case of the parental strain, the gas chromatography analysis was carried out.

A sample of 100µl of fermented medium, 0.5ml of hexamethyldisilazane, and 1ml of freshly distilled dimethylformamide was retained overnight.

The gas chromatograph (GC - Varian CP 3800) was equipped with a DB -1 capillary column (30 m × 0.25 mm × 0.2 µm, Perkin Elmer) and a flame-ionization detector. Helium was used as a carrier gas bearing a linear velocity of 35 cm s^{-1}. The column was operated carrying an initial temperature of 140°C, adjusted to 160°C at 3°C min^{-1}, was held at 160°C for 3 min, and adjusted to 300°C at 3°C min^{-1}.The final temperature was held for 20 min.

The injector port was operated in the split mode (1:100) at 260°C, and the detector was maintained at 300°C.

The study of alcoholic fermentation

Inoculum preparation. The mutant yeast cells from each test-tube with pure culture, were carried into 25cm^3 balloons bearing wort and incubated at 30ºC for 24 hr.

From this inoculum 4% is transferred into three Erlenmeyer flasks fitted with fermentation valves, each containing 150 cm^3 of wort. After fixing the fermentation valves, 2 cm^3 concentrated sulfuric acid was added through the outer opening. Each recipient was weighed and then was incubated at 30°C for 3 days.

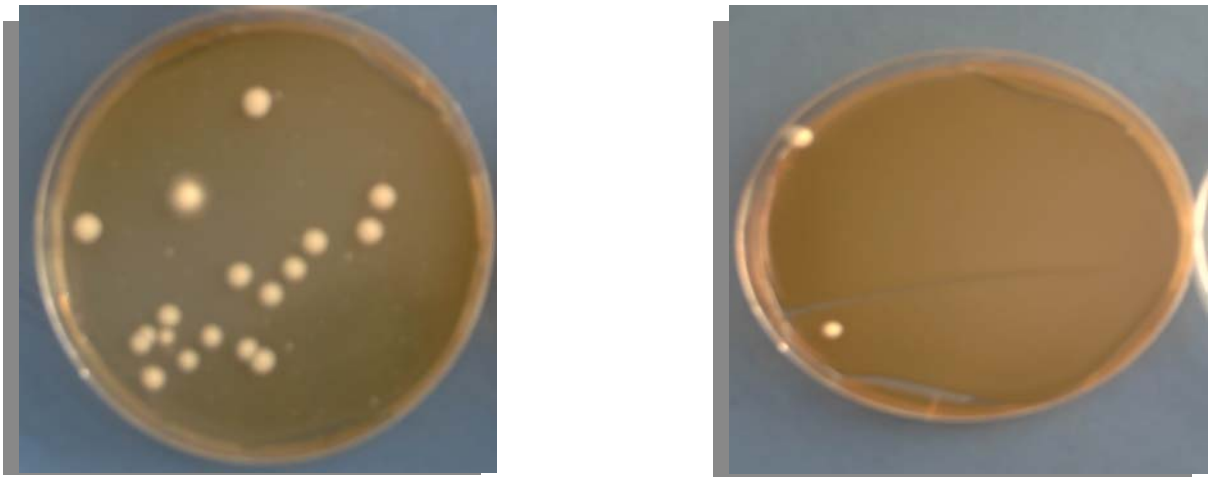
After 6, 24, 48 and 72 hours the average quantity of CO_2 released was calculated. Ethanol concentration was established by the end of fermentation after using pyknometer method for distilation.

Results and discussions

Morphological characteristics (the color, form, profile and diameter) of the colonies grown after irradiation are analyzed because all these have an important role in alcoholic fermentation. (Table 1 and Figure 1).

Table 1 *Morphological characterization of the irradiated yeast colonies*

Media	Colonies morphology	
	Parental colonies (10^{-5} dilution)	Irradiated colonies (50 seconds of irradiation , 10^{-5} dilution)
WLN (Wallerstein Laboratorius Nutrient)	Greenish colonies, circular perimeter, slippery and with 1.5 -2mm in diameter;	Greenish colonies, circular perimeter, mat colonies and with 1.5mm in diameter;
YPGA (Yeast-Peptone-Glucose-Agar)	Cream-colored colonies , convex profile with circular perimeter, slippery and with 1.5-2mm in diameter;	Cream – colored colonies, raised profile with circular perimeter, matte and with 1.5 mm in diameter;



A ***B***

Fig. 1. *Colonies of Saccharomyces cerevisiae (A – parental colonies; B – colonies formed by t1 mutant strain)*

Received March 24, 2008 / Accepted April 25, 2008

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After mutagenesis, these qualities were almost the same as in the case of cells undergoing no treatment.

Ultraviolet radiation damages cells in a number of ways. The lethal effect of the UV radiation is due to many causes, for instance-mutations of the genes or chromosomes, destruction of the cell-membrane (Figure 2) or of some structures or cell organelles. The presence of the steady diploid phase offers the possibility to study the lethal, either dominant or recessive mutants.

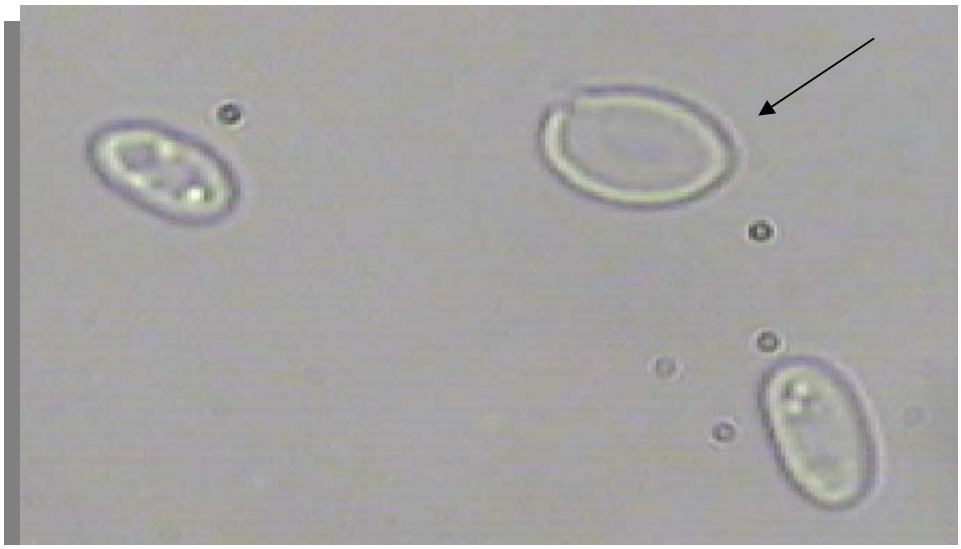

Fig. 2. *Saccharomyces cerevisiae cells after 50 seconds of irradiation*

Owen and collaborators (1990) put forward that the frequency of the lethal dominant mutant is equal to the degree of ploidy of the irradiated cell.

Some of these mutants can only reproduce in accept of their growing on wort agars.

As it is mentioned in the literature, the main glucides fermented by Saccharomyces *cerevisiae* yeast, are: glucose, galactose, sucrose, maltose and raffinose.

From that reason, the potential of *S. cerevisiae* yeast potential to ferment the glucides was tested.

Each strain's ability to ferment the glucides previously mentioned was tested relying on the mutant strains having the samephysiological characteristics as the parental strain.

The first stage was testing the fermentation potential of glucose both for the parental strain and for the mutant ones.

The experiment proved that the irradiated *S. cervisiae t1* strain and the wild - type *S. cerevisiae* strain were able of fermentig the glucides. It was also proved that the *S. cerevisiae t2* was not able of fermenting the maltose as compared to the wild-type, *S. cerevisiae* strain and to mutant strain *S. cerevisiae t1*.

Maltose is taken up via maltose permease and then hydrolyzed intracellular by maltase into two units of glucose. Maltose permease is encoded by MALT. MAL genes cannot be induced if maltose cannot be transported into the cell (Goffeau, 2000).

It is possible that the maltose transport to have been affected by irradiation. That can explain why the strain, *Saccharomyces cerevisiae t2*, obtained by mutagenesis under UV radiation, cannot fermented maltose.

During the process of wort fermentation, glucose is consumed first, followed by fructose and sucrose. (Anghel *et al*., 1989). The disaccharide sucrose and trisacharide raffinose are hydrolyzed outside the cell membrane into monosaccharide, which are then taken up by the cell (Ostergaard *et al.,* 2000).

The experiment also proved that *S. cerevisiae t1* brings about sucrose fermentation faster than parental strain does. That proof was also supported by the formation of a higher quantity of gas in the Durham tub in first five days in case of *S. cerevisiae t1*compared to parental *S. cerevisiae* strain, as shown in figure 3.

The presence of glucides in the medium of fermentation was analyzed by means of gas chromatography after raffinose fermentation and it was discovered that:

Both in the case of parental and mutant strains, the pick obtained through chromatograms show the presence of α-glucose and the β-glucose in the medium. The pick of α-glucose was identified as the position with a retention time (RT) of 18.212 min. and the pick of β-glucose was identified as the position with an RT of 21.271 min. Also at the RT of 44.273 min. one pick which corresponds to melibiose (Figure 4) was obtained..

The absence of fructose and the presence of glucose in the medium prove a partial degree of raffinose fermentation. In the case of parental strain the fermentation degree represents 0.33% (Gasent-Ramires *et al*., 1995).

Out of the 2 mutant strains whose fermentation capacity were tested, only the *Saccharomyces cervisiae t1* proves an increase in the CO_2 amount released on the 72h period compared to the parental *Saccharomyces cerevisiae* strain.

As for the other mutant strain, the amount of CO_2 released after 72 h doesn't significantly differ from the parental strain (Figure 5).

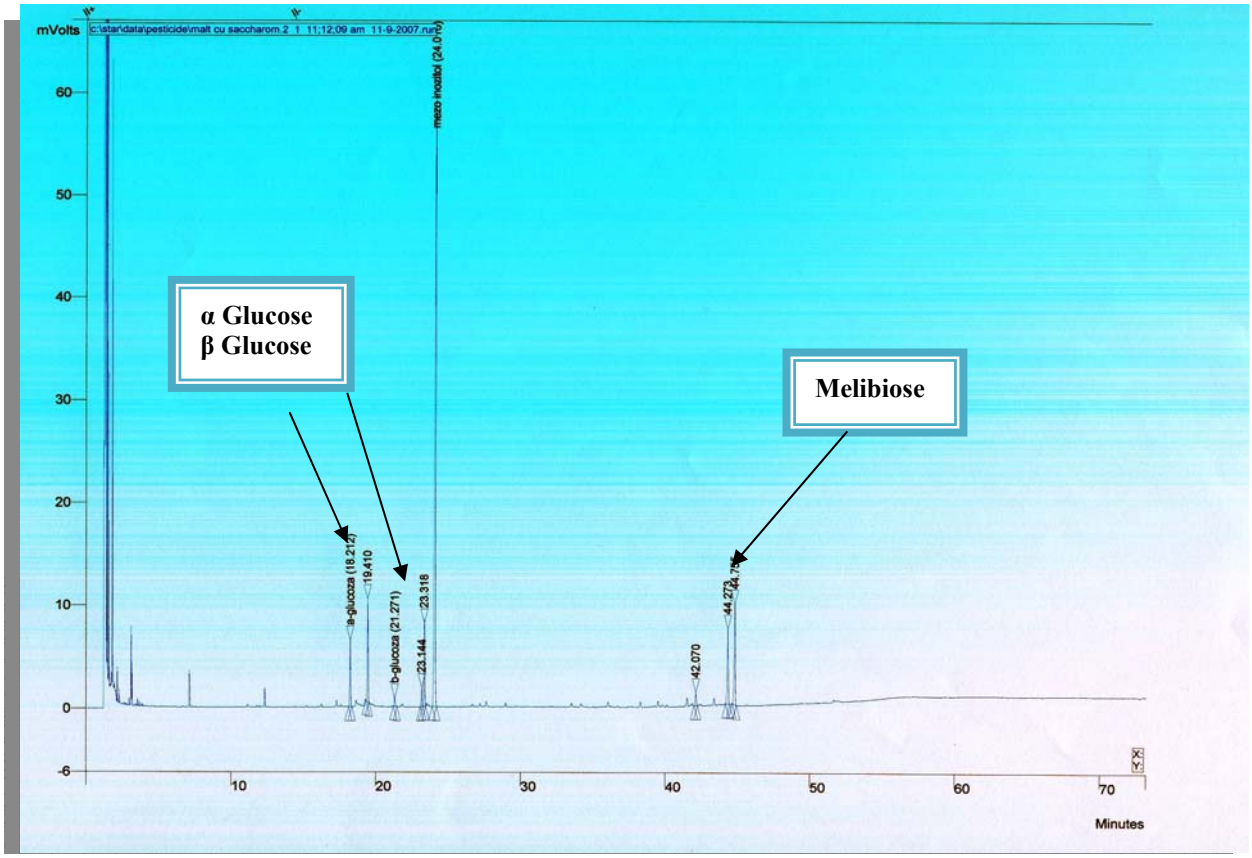

Fig. 3 *Chromatogram showing the residual glucides after sucrose fermentation by the Saccharomyces cerevisiae strain*

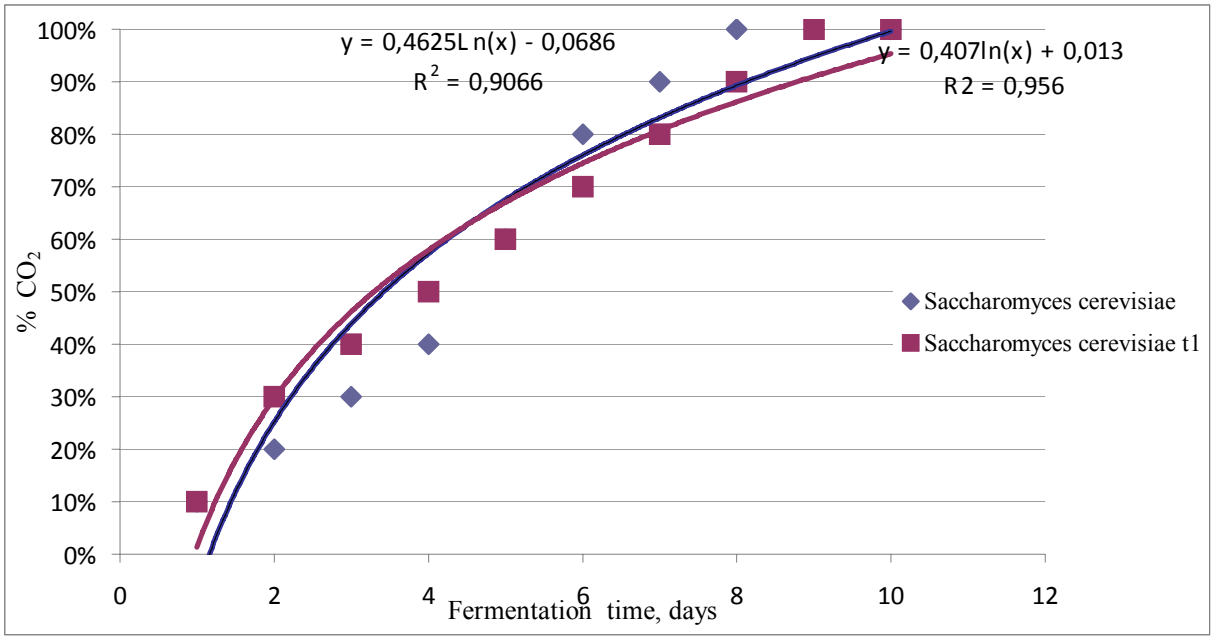

Fig. 4. *Raffinose fermentation dynamics*

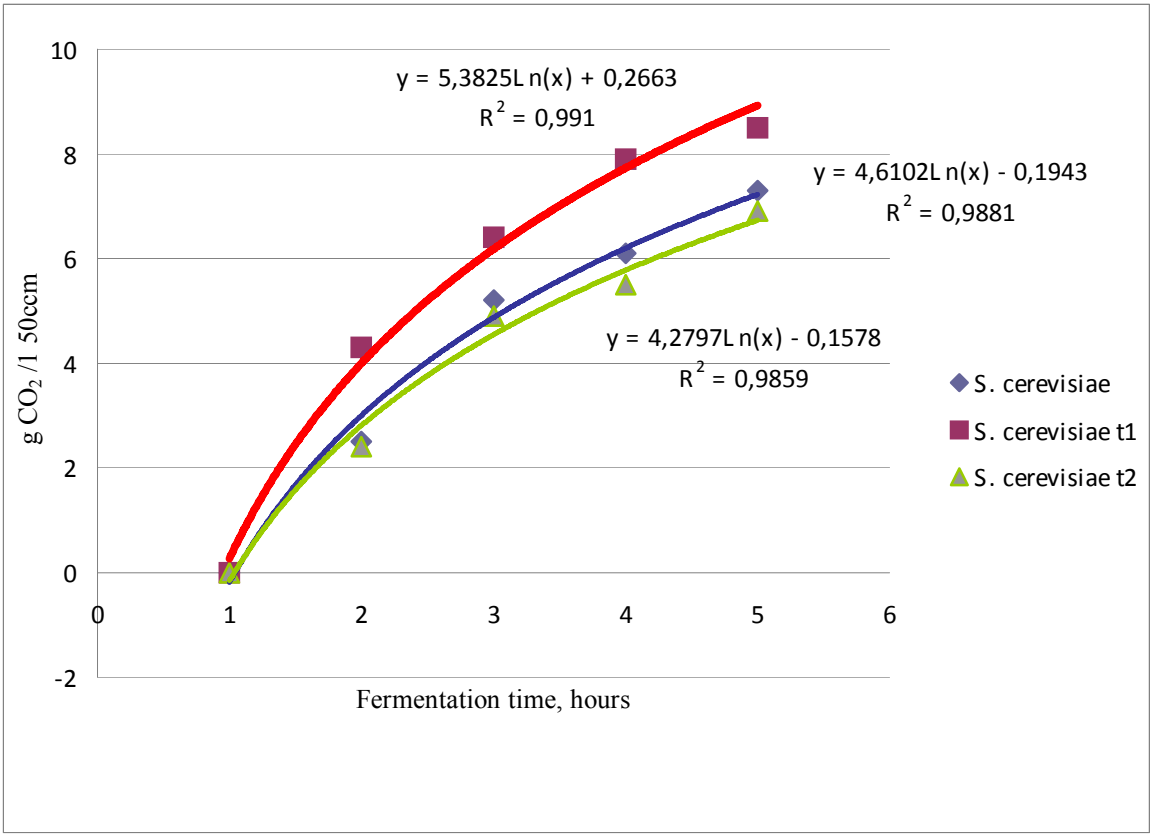

Fig. 5. *Wort fermentation dynamics*

The transformation of glucides into alcohol leads to a lower must density (Walker, 1998).

The fermentation dynamics can be observed by measuring the malt extract concentration. At the end of fermentation the malt extract had a concentration of 3.0ºBx (for *S. cerevisiae t1 strain*) and of 5ºBx (for *S. cerevisiae t2 strain*) as compared to 4ºBx in case of parental strain.

Saccharomyces cerevisiae yeast produces ethanol from 4.5 to 18% v/v by means of alcoholic fermentation. (Dan *et al.*, 1999)

After 72 hours of fermentation, parental *Saccharomyces cerevisiae* strain produces 7.3% v/v ethanol.

Figure 6 shows the rate of ethanol production comparing the yeast parental strain and the *Saccharomyces cerevisiae t1* strain as a result, by means o fermentation, the mutant strain obtained in the process of UV mutagenesis stands o a proof of a higher rate of ethanol production, 1.15% v/v.

This research shall be continued for grounds of ethanol production improvement.

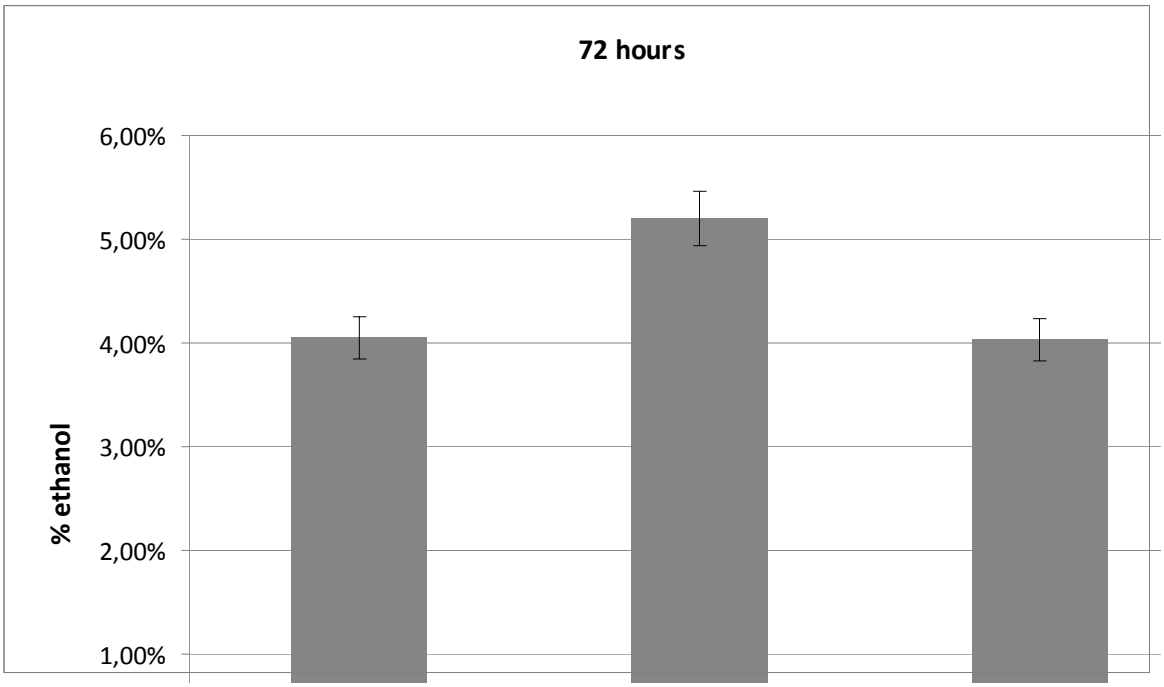

Fig. 6 *The dynamics of ethanol production*

Conclusions

Ultraviolet (254nm) radiation effects the ethanol productivity growth of *Saccharomyces cerevisiae* strain. The glucides tested were fermented under *S. cerevisiae* and *S. cerevisiae t1*strainas follows: glucose first, sucrose, maltose, galactose and raffinose last.

Different rates of fermentation of individual sugars were observed.

In the case of *S. cerevisiae t1* mutant strain sucrose underwent fermentation faster than glucose did during the first five days.

Galactose underwent fermentation more slowly than glucose and sucrose for each strain tested.

The *S. cerevisiae t2* strain does not bring about maltose fermentation.

Also, the *Saccharomyces cerevisiae t1*strain mutant ethanol production proved higher than the wild type strain by a rate of 1.15%.

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