The Annals of the University *Dunarea de Jos* of Galati
Fascicle VI – Food Technology, New Series Year III (XXXIII)
Paper presented at the International Symposium *Euro - aliment 2009*, 9th – 10th of October 2009, Galati – ROMANIA

BIOETHANOL PRODUCTION FROM MOLASSES BY DIFFERENT STRAINS OF *SACCHAROMYCES CEREVISIAE*

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Received 11 June 2009
Revised 31 August 2009

In commercial ethanol production producers often use sugar cane molasses as raw material due to their abundance and low costs. The most employed microorganisms used for fermentation is *Saccharomyces cerevisiae* yeasts due to their ability to hydrolyze sucrose from cane molasses into glucose and fructose, two easily assimilable hexoses. The aim of this study was to evaluate the application of different strains of *Saccharomyces cerevisiae* for sugar cane molasses in order to produce bioethanol. According to the obtained results the strain D1 (Safdistil C-70) achieved higher values of the specific growth rate in comparison with other strains used. The maximum ethanol productivity of 2.33 g/L·h was achieved around 36 hours of fermentation by using the yeast D1. Therefore, the optimal duration of the fermentation process in technical and economic terms should be considered.

Keywords: yeast, bioethanol, fermentation, ethanol yield

1. Introduction

The ethanol production is among the oldest technology and is produced commercially by fermentation of cereal grains, molasses or other materials with high starch and/or sugar contents. The fermentation process involves conversion of sugars to alcohol and carbon dioxide by the yeast *Saccharomyces cerevisiae.* Ethanol is produced through the fermentation of agricultural by-products such as sugarcane, corn and wheat, sugar beet and cassava, among others.

Molasses is commonly used as a feedstock for bioethanol production. Molasses, the noncrystallizable residue remaining after sucrose purification, has some advantages: it is a relatively inexpensive raw material, readily available, it does not require starch hydrolysis and already used for ethanol production

The molasses obtained after sugar beet processing contains about 60% sucrose and 40% other components. The nonsucrose substances include inorganic salts, raffinose, kestose, organic acids and nitrogen containing compounds.

Molasses is used in the baker's yeast production, in the fermentation technology for ethanol, citric, lactic and gluconic acids production, as well as glycerol, butanol and acetone production, as an ingredient of mixed feeds or in the production of amino acids (Belitz et al., 2009, Satyanarayana et al., 2009).

The fermentative yeast *Saccharomyces cerevisiae* is largely used in ethanol production using such renewable biomass as sugar cane or sugar beet molasses as the main carbon source. (Echegaray et al., 2000, Sanchez and Cardona, 2008). These types of *S. cerevisiae* were selected as production microorganisms on account of their commercial availability and an extensive application in food industry.

The main objective of this work was to develop an economical fermentation process to produce bio-ethanol from sugar cane molasses by a selected strain of the yeast *Saccharomyces cerevisiae*.

2. Materials and methods

2.1. Materials

Molasses were obtained from a sugar factory and were used as a fermentation medium.

Table 1 shows the compositions of sugar cane molasses used as a substrate fermentation.

Table 1. Composition of the sugar cane molasses used for experiment

Molasses type	*water content, %*	*soluble solids content, %*	*total sugar, %*	*total nitrogen content, %*	*mineral substances content, %*	*pH*
Sugar cane molasses	18.2	81.8	54.6	0.5	6.2	7.6

Sugar cane molasses were diluted with water to a resultant sugar content of 180-200 g/l. pH value of culture media was adjusted with 10 % v/v sulphuric acid to the value of 4.50.

Yeast strains. The yeasts used for the fermentation process were various types of active dried yeasts *Saccharomyces cerevisiae*: Safdistil C-70 and Ethanol Red™ purchased from SC Enzymes & Derivates SA –yeasts for strong alcoholic drinks production, Trockenhefe-yeast for wine production and Fali®-bakery yeast purchased from SC Protect Consult SRL and Pakmaya purchased from SC Pakmaya SA-bakery yeast. The yeasts were coded as it is shown in Table 2.

Table 2. The yeast strains characteristics and codes used in the experiment

No. crt.	*Yeast strain*	*Characteristics*	*Code used*
1	Safdistil C-70	% dry weight: 94.0 – 96.5 Living cells at packaging: > 14 x 10^9/g Total bacteria: < 1 x 10^4/g Acetic acid bacteria: < 1 x 10^3/g Lactic bacteria: < 1 x 10^3/g	D1
2	Ethanol Red™	% dry weight: 94.0 – 96.5 Living cells at packaging: > 20 x 10^9/g Total bacteria: < 1 x 10^4/g Acetic acid bacteria: < 1 x 10^3/g Lactic bacteria: < 1 x 10^3/g	D2
3	Fali®	% dry weight: 94.0 – 96.0 Living cells at packaging: > 6 x 10^8/g NTG < 10^5/g Acetic acid bacteria: < 1 x 10^3/g Lactic bacteria < 10^3/g	D3
4	Trockenhefe	% dry weight: 94.0 – 96.0 Living cells at packaging: > 10 x 10^8/g NTG < 10^5/g Acetic acid bacteria: < 1 x 10^3/g Lactic bacteria < 10^3/g	D4
5	Pakmaya	% dry weight: 94.0 – 96.0 Living cells at packaging: > 10 x 10^8/g NTG < 10^5/g Acetic acid bacteria: < 1 x 10^3/g Lactic bacteria < 10^3/g	D5

Before utilisation all dried yeasts were stored at 4°C.

Yeasts were reactivated, directly in the molasses medium. Inoculum for fermentation assays was incubated in shaker at 200 rpm, at room temperature (25 °C), for 24 h. Volumes transferred to the fermentation media were calculated so that the initial biomass concentration was 1 x 10^8 viable cells/mL.

2.2. Yeast culture conditions

The study focused on the yeast multiplication and fermentation was realized by cells cultivation in submerged conditions in a liquid medium based on 300 mL of sterile diluted molasses (20-21%), acidified to pH ≈ 4.5 with sulphuric acid and enriched with 0.5% ammonium sulphate.

Fermentation was done in stationary cultivation conditions, during 60 hours at room temperature, by using an initial biomass concentration of 1x10^8 viable cells/mL.

After 12, 24, 36, 48 and 60 h, the number of viable cells and the rate of multiplication were evaluated. Yeast viability assay was examined by microscopy in the presence of the blue methylene indicator, based on the viable capacity of reducing the redox indicator from the blue oxidized form (blue) to the reduced form a leuco-derivative (colorless).

2.3. Fermentation yields and kinetic parameters

The yeast specific growth rate, μ (h^{-1}), was calculated from the slope of the linear dependence of the yeast cell number logarithm (log N) on the fermentation time of culture media (h) during an exponential phase of the growth using the equation:

$$\text{slope} = \mu/2.303 \quad (1)$$

The maximum theoretical ethanol yield from sugar was calculated according to the stoichiometric relation represented by Eq. (2), i.e., 100 g of hexoses produce 51.1 g of ethanol and 48.9 g of CO_2.

Ethanol production yields over total initial sugars (Y_1) and consumed sugars (Y_2) were calculated according to Eqs. (3) and (4).

$$C_6H_{12}O_6 = 2CH_3CH_2OH + 2CO_2 \quad (2)$$

$$Y_1\ (\%) = [(P_f\text{-}P_0)*100]/(S_0*0.511) \quad (3)$$

$$Y_2\ (\%) = [(P_f\text{-}P_0)*100]/[(S_0\text{-}S_f)*0.511)] \quad (4)$$

Where: S_0 initial sugar concentration (g/L), S_f final sugar concentration (g/L), P_0 initial ethanol concentration (g/L), P_f final ethanol concentration (g/L), r_S sugar consumption rate - dS/dt (g/L h), r_P product formation rate - dP/dt (g/L h), $Y_{P/S}$ ethanol yield from sugar, r_P/r_S (g/g).

In order to evaluate the yeasts fermentative capacity after 12, 24, 36, 48 and 60 h, the sugar consumption rate (g/L · h), ethanol formation rate (g/L · h), ethanol yield over total initial sugars (Y_1), ethanol yield over consumed sugars (Y_2) and ethanol yield from sugar ($Y_{P/S}$) were calculated.

2.4. Sugars and ethanol concentration determination

Sugar content was approximated by using the 3,5-dinitrosalicylic acid method (Miller, 1959).

Ethanol was separated and measured using a TRACE GC having the following characteristics: SPLIT injector, FID detector, fused silica capillary 30 m long, inner diameter 0,25 mm, stationary phase CARBOWAX 20M.

For each experiment three independent fermentations were carried out and the results shown in this paper represent average values.

3. Results and discussions

Determination of cells number during the fermentation indicated that there was an intensive and almost linear increase of yeast cell counts during the first 24 h of fermentation in the case of all the applied yeast strains.

Figure 1 shows the dependence of the yeast specific growth rate, μ, on the yeast strains (according to Eq. (1)). Average values of the specific growth rate of five applied yeast strains amounted to 0.74 h^{-1} (D1), 0.76 h^{-1}(D2), 0.55 h^{-1} (D3), 0.47 h^{-1}(D4) and 0,50 h^{-1}(D5).

It is evident that strains D1 and D2 attained higher values of the specific growth rate in comparison with yeast strains D3, D4 and D5.

The exponential phase of the yeast cell growth was underway, due to the remaining oxygen content of the fermenting media. The total number of yeast cells was almost constant during further fermentation under anaerobic conditions.

During the exponential phase of growth, yeast cells incorporated sugar assimilated from the culture media into biomass inducing the intensive cell growth, at the same time, being produced ethanol and CO_2. In second, anaerobic phase, yeast cells used assimilated sugar mostly for ethanol synthesis.

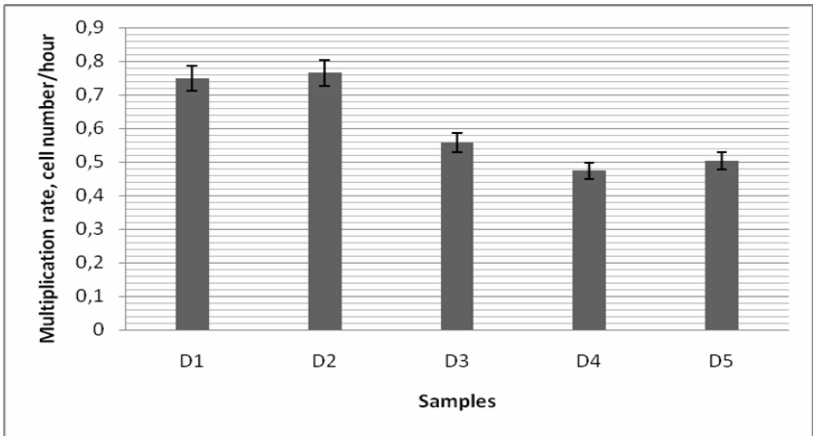

Figure 1. The multiplication rate for the yeast strains

The growth rates are very small in comparison to product (ethanol) yields. These small values were expected since there is no oxygen supply and the soluble solids concentration is high (20%) and, consequently, dissolved oxygen concentration is low.

Echegaray et al. (2000) reported values ranging from 0.24 to 0.019 for the growth rate in the case of the anaerobic fermentation of sugarcane molasses by *Saccharomyces cerevisiae* containing around 170 g/L of sugars (for this substrate, equivalent to 17% soluble solids). The decrease in μ along time was possible due to the product inhibition.

Figure 3 illustrates the dependence of the ethanol yield over total initial sugars (Y_1) on the time and yeast strains (according to Eq. (1)).

The value of the ethanol yield over total initial sugars (Y_1) in applied experimental conditions ranged from 4.92 % to 72.60 %.

Average values of the ethanol yield over total initial sugars (Y_1) were maximum for D1 and D2 (21.93% and 15.03%) and 4.92%, 6.35% and 5.81% for D3, D4 and D5 respectively. Although sugar utilization after 60 h of fermentation was almost total, more significant variations of ethanol yield for different strains were evident and average values amounted to 72.06, 72.60, 64.99, 67.85 and 64.72 % for strains D1, D2 D3, D4 and D5.

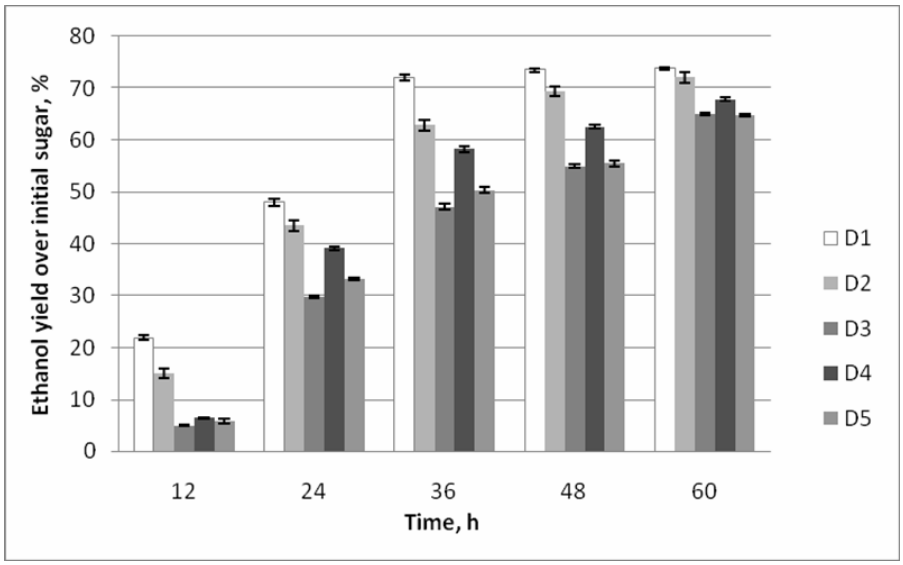

Figure 2. The dependence of the ethanol yield over total initial sugars (Y_1) on the time and yeast strains

The dependence of ethanol yield from sugar ($Y_{P/S}$) on the time of fermentation and yeast strains is depicted in Figure 3.

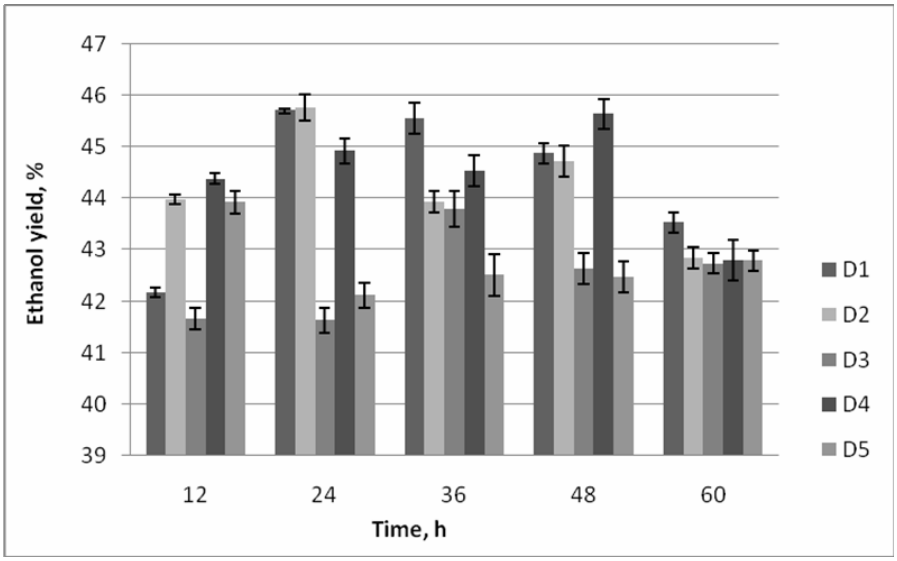

Figure 3. The dependence of the ethanol yield ($Y_{P/S}$) on the time and yeast strains

The value of the ethanol yield in experimental conditions ranged in the interval of 41.6 and 45.8 %, which is close to the theoretical yield of 51.1 g of ethanol per 1 g of glucose (Roehr, 2001).

Generally, the strain D3 had lower ethanol yields in comparison with the other strains used. It is also evident that strains D4 and D5 had lower ethanol yields in comparison with yeast strains (D1 and D2). By using yeast strain D1 a product yield ($Y_{P/S}$) of 45.5% from consumed substrate represents 89.1% of the theoretical maximum.

The dependence of the sugar consumption rate (g/L·h) on the time of fermentation and strain yeasts is shown in Figure 4.

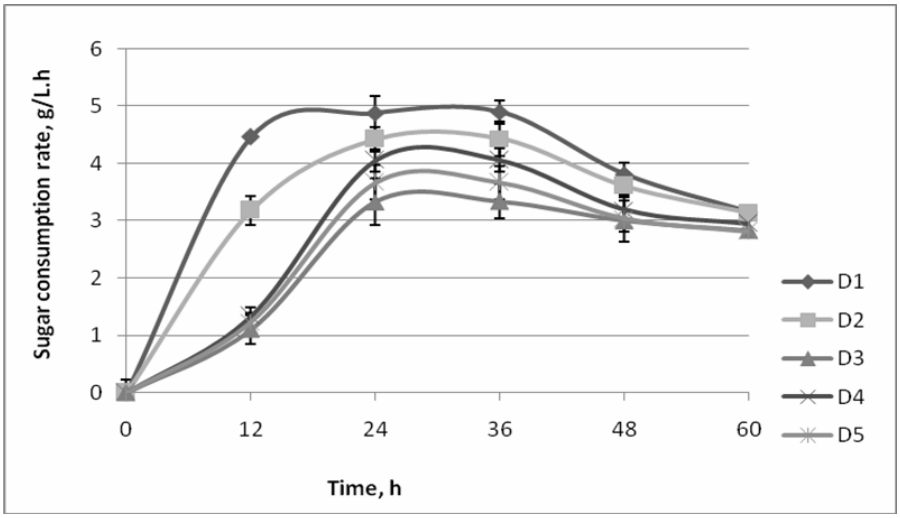

Figure 4. The dependence of the sugar consumption rate on the time and yeast strains

It can be observed that average values of the sugar consumption rate after 12 h of fermentation ranged from 1.1 to 4.84 g/L·h depending on the yeast strains used. The maximum value of the sugar consumption rate was achieved for D1 after 36 h of fermentation amounted of 4.90 g/L·h.

In Figure 5, it is presented the dependence of average values on the ethanol productivity (product formation rate) on the fermentation time and applied yeast strains. According to the obtained results, the maximum productivity was achieved at around 36 h of fermentation and amounted to 2.33 g/L·h for D1 strain.

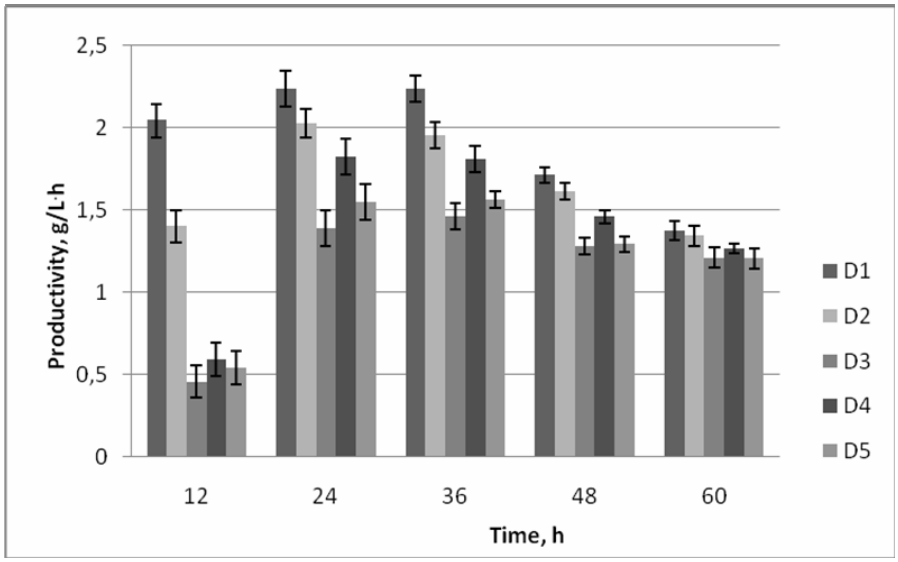

Figure 5. Ethanol productivity dependence on the time and yeast strains

Roukas (1996) reported a maximum productivity value of 3.8 g/L h for the fed-batch fermentation of beet molasses with initially 250 g/L sugars and 3.7 x 10^8 cells/mL. These conditions are similar to those of Siqueira et al., 2008, when a productivity of 6.916 g/L h was achieved from soybean molasses. However, it is important to remark that the sugar beet molasses is composed mainly of sucrose (Vicik et al., 1990), while soybean molasses contains almost 50% of non-fermentable sugars, consequently, ethanol concentration is lower and product inhibition may be less expressive.

The ethanol yield over consumed sugars related to the theoretical yield (Y_2) was plotted depending on the time of fermentation and yeast strains (Figure 6).

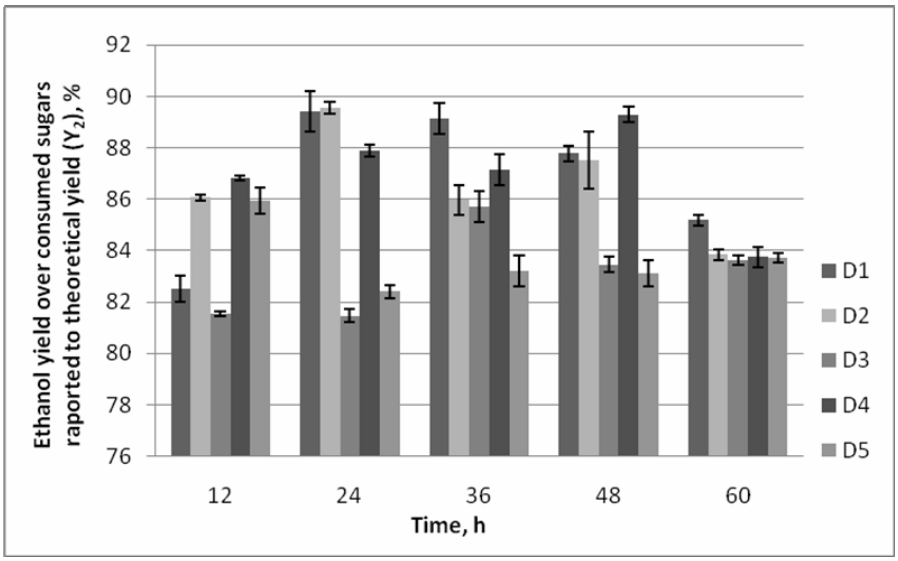

Figure 6. Ethanol yield over consumed sugars related to the theoretical yield (Y_2) on the time and yeast strains

The average values of the yield over consumed sugars related to the theoretical yield (Y_2) after 60 h of fermentation were situated from 83.62 to 83.83%.

4. Conclusions

The presented results demonstrated that the efficient ethanol production molasses is possible with all examined strains of yeast *Saccharomyces cerevisiae* (D1, D2, D3, D4 and D5).

Maximum ethanol productivity 2.33 g/L·h was achieved by using the yeast D1, after 36 h of fermentation followed by D2 (2.1 g/L·h), D4 (1.8 g/L·h), D5 (1.56 g/L·h) and D3 (1.46 g/L·h). A further prolongation of the fermentation time induces a gradual decrease of the ethanol productivity, suggesting that the optimum duration of the process should be considered both technically and economically.

Acknowledgments

The authors acknowledge funding support from S.C. Euroavipo S.A. Ploiesti, Romania and BIOALIMENT Platform (http://www.bioaliment.ugal.ro/).

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