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Comparative study of the multiplication and fermentation yields by ussing different *Saccharomyces* yeast strains to ethanol production

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Abstract

The use of five *Saccharomyces* yeasts inoculums and yeasts multiplication kinetics were studied during the fermentation process of sugar cane molasses. Yeasts behavior in aerobic cultivation conditions was given by yeast multiplication dynamics, kinetic multiplication parameters, cells autolysis degree and weight of dried biomass. Yeasts Ethanol RedTM and Safdistil C-70 had the best fermentation kinetic parameters.

Keywords: *Saccharomyces* yeasts, ethanol, multiplication kinetics

1. Introduction

The gradual depletion of crude oil and the environment deterioration resulted from over consumption of petroleum derived fuels have generated the idea to urgent develop alternatives that are both renewable and environmentally friendly [1].

Ethanol is one of the most important fuels from renewable sources that are currently produced at competitive prices [2]. Bioethanol is an attractive, sustainable energy source to fuel. Based on the premise that fuel bioethanol can contribute to a cleaner environment, the demand for this fuel is increasing [3].

For the production of ethanol as an alcoholic beverage several raw materials have been exploited over the centuries, including molasses resulted from sugar production. Molasses represents a ready and renewable source for alcoholic fermentation because of its high availability and low cost [4,5]. 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 production of baker's yeast, in 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 [6,7].

The fermentative yeasts Saccharomyces cerevisiae is largely used in ethanol production using renewable biomass as sugar cane or sugar beet molasses as the main carbon source [8,9]. Accumulation of ethanol in yeast cultures leads to decreased viability, growth and ethanol yields. Yeast cells grown in the presence of ethanol showed changes in the lipid composition of the plasma membrane and tolerance to high levels of external ethanol improved [10]. The lack of robustness of the fermentation in the presence of raw materials quality fluctuations, which leads to changes in the kinetic behavior with impact on yield, productivity and conversion, is among the main problems related to the alcoholic fermentation process [3].

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Estimation of kinetic parameters of differential models is usually complex, mainly due to non-linearities, great number of parameters and interactions among them. In biochemical engineering the most classical method involves the mathematical estimation of model parameters based on the minimization of some cost function built-up with the parameters to be estimated. Several kinetic models have been proposed for the alcoholic fermentation [3,11,12,13].

The main objective of this work was to study the multiplication kinetics for five different yeast strains used to ethanol production.

Yeasts behavior in aerobic cultivation conditions was given by yeast multiplication dynamics, kinetic multiplication parameters, cells autolysis degree and weight of dried biomass.

2. Materials and Method

Yeast strains. The yeasts used for fermentation process are various types of active dried yeasts *Saccharomyces cerevisiae*: Safdistil C-70 and Ethanol Red^{TM} from SC Enzymes & Derivates SA, Trockenhefe and Fali^{R} from SC Protect Consult SRL and Pakmaya from SC Pakmaya SA.

The yeasts were coded as it is shown in table 1.

Table 1. The codes used for yeast strains in the experiment

No. crt.	Yeast strain	Code used
1	Safdistil C-70	D1
2	Ethanol Red^{TM}	D2
3	Fali^{R}	D3
4	Trockenhefe	D4
5	Pakmaya	D5

The dried yeasts have the following characteristics: dry matter 94-96%, nitrogen content 4-8% from dry matter, P_2O_5 1-4% from dry matter, living cells $6x10^8$ cells/g, NTG < 10^5/g, coliform bacteria < 10^2/g, lactic bacteria < 10^3/g.

Lyophilized yeasts were reactivated, directly in the molasses medium. Inoculum for fermentation assays were incubated in shaker at 200 rpm, at room temperature for 24 h. Volumes transferred to the fermentation media were calculated so that initial biomass concentration was 1 x 10^8 viable cells/mL. The multiplication kinetics was studied in *S.C. Euroavipo S.A. Ploiesti, Romania* Laboratory.

Yeast culture conditions. The study focused on the yeast multiplication was realized by cells cultivation in submerged conditions in a liquid medium based on 100 mL of sterile diluted molasses (12^o Bllg), acidified to pH ≈ 4.5 with sulphuric acid and enriched with 0.5% ammonium sulphate.

Cultivation was performed on a rotary shaker at 200 rpm, observing the yeast behavior during 48 hours cultivation.

Similar, to evaluate yeast fermentative capacity in a volume of 250 mL of sterile diluted molasses (15^{o}Bx - percentage w/v of soluble solids), acidified to pH ≈ 4.5 with sulphuric acid and enriched with 0.5% ammonium sulphate were added the equivalent quantities of inoculum. Fermentation was done in stationary cultivation conditions, during 48 hours at room temperature, by using the same concentrations of vegetative inoculum.

Multiplication kinetics. Yeast growth was monitored by using direct cytometry. Colony forming units per ml (CFU/mL) were calculated, and microbial population vs. time was modeled according to the reparametized Gompertz equation proposed by Zwietering (1990) [14] by using the following model:

$$y = a * \exp\{-\exp[((\mu_{max} * e)/A) * (\lambda + t)) + 1]\}$$

Where: $y = \ln(N/N_0)$, N_0 is the initial microbial population; N the microbial population at time t, $A = \ln(N\infty / N_0)$ is the maximum value reached with $N\infty$ as the asymptotic maximum population, μ_{max} is the maximum specific growth rate, and λ the lag phase period.

After 12, 24, 36 and 48 h, biomass concentration, number of viable cells, number of generations, rate of multiplication, generation time and mother cells 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).

Biomass concentration in g/L was determined by gravimetric analysis after drying to constant weight.

*Fermentation yields and kinetic parameters.*The maximum theoretical ethanol yield from sugar was calculated according to the stoichiometric relation represented by Eq. (1), i.e., 100 g of hexoses produce 51.1 g of ethanol and 48.9 g of CO_2.

Ethanol yields over total initial sugars (Y_1) and consumed sugars (Y_2) were calculated according to Eqs. (2) and (3).

$$C_6H_{12}O_6 = 2CH_3CH_2OH + 2CO_2 \quad (1)$$

$$Y_1\ (\%) = [(P_f-P_0)*100]/(S_0*0.511) \quad (2)$$

$$Y_2\ (\%) = [(P_f-P_0)*100]/[(S_0-S_f)*0.511)] \quad (3)$$

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 and 48 h, the total CO_2 loss, 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.

Sugars and ethanol concentration determination

Sugar content was approximated by using soluble solids content. The percentage (w/v) of soluble solids (^{o}Brix) was determined with a refractometer Abbe.

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.

3. Results and Discussion

Yeast cells multiplication and stability were quantified by studying yeast multiplication dynamics in asynchrony conditions, determining multiplication kinetic parameters such as generation number, multiplication rate, generation time, cells autolysis grade and weight of dried biomass.

The yeast multiplication curve is presented in figure 1 for 48 hours monitoring.

The yeast multiplication curve is in accordance with fermentation dynamics, expressed as grams of CO_2 loss from 250 ml of medium, presented in figure 2.

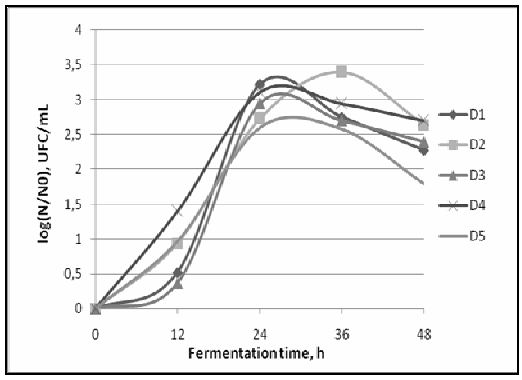

Figure 1. Yeast multiplication dynamics in submerse cultivation conditions

As it can be observed, yeast strains noted D_2 and D_1 have emitted the highest CO_2 amount.

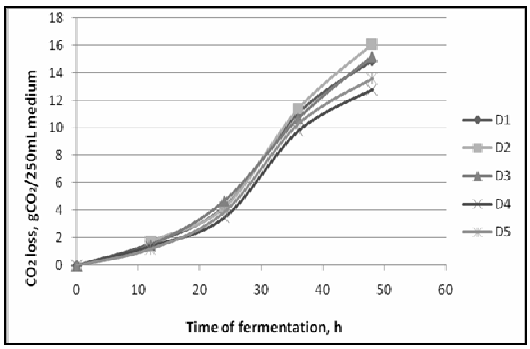

Figure 2. Fermentation dynamics for the yeasts studied for 48 hours

For these yeast strains (D_1 and D_2), the sugar consumption rate and the ethanol formation rate were determined during the 48 hours of fermentation, and the results are presented in figures 3 and 4.

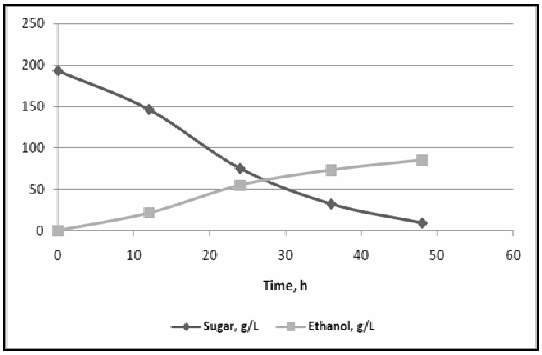

Figure 3. Sugar consumption rate and ethanol formation rate during 48 hours of fermentation for yeast D_1

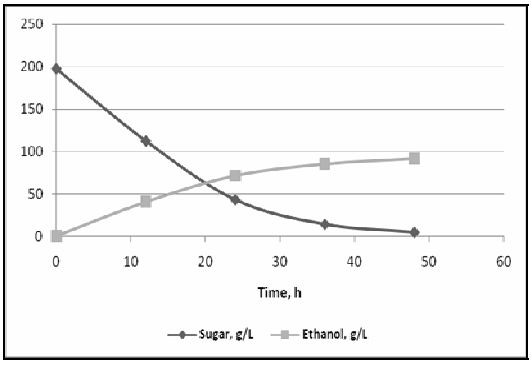

Figure 4. Sugar consumption rate and ethanol formation rate during 48 hours of fermentation for yeast D_2

As figures 3 and 4 show, yeast D_2 has a higher ethanol formation rate than yeast noted D_1.

In table 2 the kinetic parameters for the five yeast strains used in the experiment are presented.

Table 2. The kinetic parameters for the yeasts used in experiment

Yeast strain	Generation number, n	Multiplicatio n rate (v), n/h	Generation time (t), 1/v
D1	*8,982025*	*0,748502*	*1,336002*
D2	9,179909	0,764992	1,307203
D3	6,685078	0,55709	1,795043
D4	5,683091	0,473591	2,111527
D5	6,032349	0,502696	1,989275

As it can be observed from table 2 and figures 5, 6 and 7, the best kinetic parameters were obtained for Ethanol Red^{TM} - D_2 yeast strain.

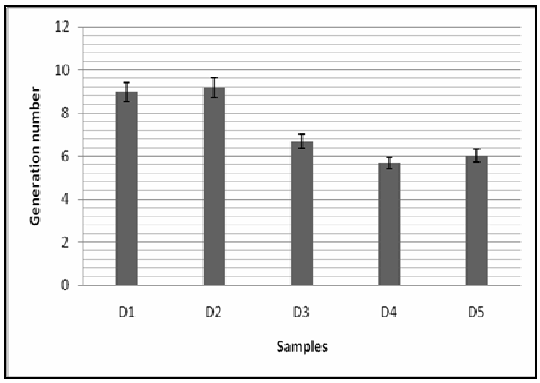

Figure 5. The generation number of the yeast strains studied

For this yeast strain, generation number was 9.179909, multiplication rate was 0.764992 cells/hour, and generation time was 1.307203.

Large ethanol plants produce high amounts of residual yeast cells, which is partly reused as inoculum and the remaining cells are used for yeast extract production or for animal feeding.

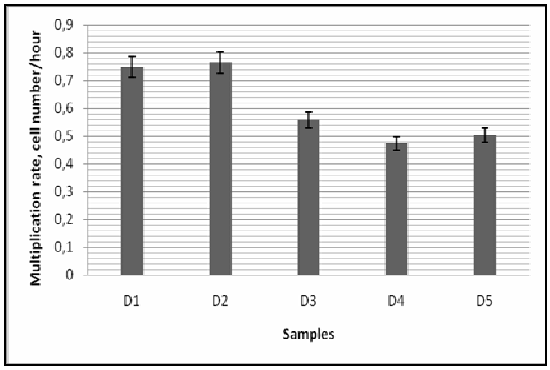

Figure 6. The multiplication rate for the yeasts studied

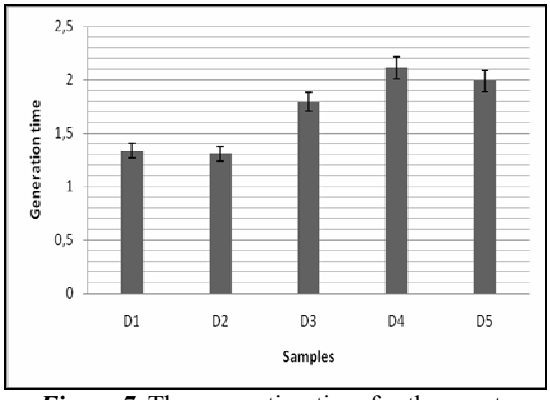

Figure 7. The generation time for the yeasts

In industry, it is important to be able to determine the growth rate of yeasts and understand the factors which affect it in order to generate maximum product by the most economic means.

The generation time refers to the average time it takes between two cell divisions. The generation time for the yeast strains used in experiment is presented in figure 7. It can be observed that D_2 yeast has the lowest generation time and D_4 the highest generation time.

In fact, all kinetic parameters were lower for D_4 Trockenhefe yeast and higher for D_2 and D_1 yeasts, as figures 5, 6 and 7 shows.

By studying yeasts metabolic stability, it can be observed (figure 8) that after 48 hours of fermentation, D_2 (Ethanol Red^{TM}) yeast has the lowest autolysis grade – 70.37% and is followed by D_1 (Safdistil C-70) yeast, the differences between these yeasts are very small. When the dry biomass yield was calculated, grams of dried biomass/100 mL medium, after 60 hours of cultivation, the results previously obtained were confirmed.

The highest multiplication yield was obtained for D_1 and D_2 (figure 9).

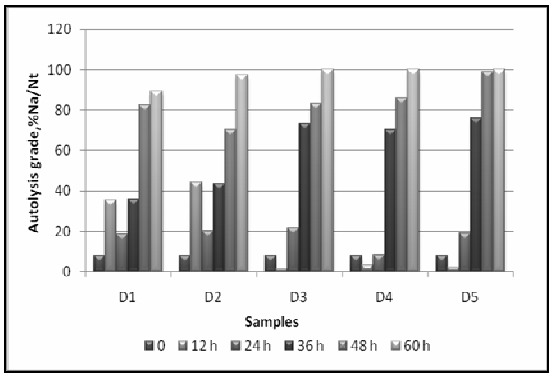

Figure 8. Yeasts metabolic stability

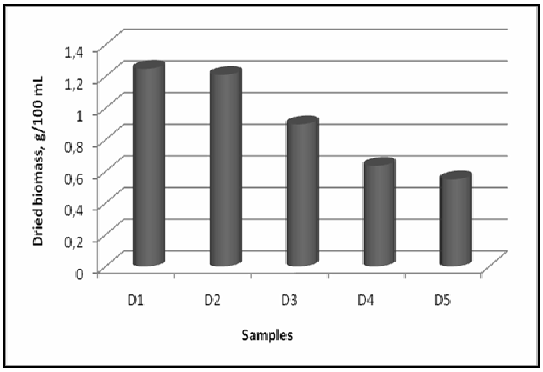

Figure 9. Dried biomass amount of the yeast strains studied

4. Conclusion

The multiplication kinetic parameters were studied for five yeast strains used in industrial alcoholic fermentation. The best kinetic parameters were obtained for Ethanol Red™ and Safdistil C-70 yeast strains, and the differences between two yeasts were small. The lowest kinetic parameters were obtained for Trockenhefe yeast strain.

When dry biomass yield was evaluated, the highest multiplication yield was obtained for Ethanol Red™ and Safdistil C-70 yeast strains too.

The kinetic parameters were correlated with fermentation dynamics, sugar consumption rate and ethanol formation rate. Yeast strains Ethanol Red™ and Safdistil C-70 had the best fermentation rate and multiplication parameters.

Yeast strain used for alcoholic fermentation directly influences the ethanol yield and also ethanol quality.

Acknowledgements

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