DETERMINATION OF LEVURIEN BIOMASS IN BIOREACTOR

DETERMINAREA BIOMASEI LEVURIENE ÎN BIOREACTOR

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Abstract. *In the bioreactor, optimal growth and multiplication conditions were created by applying growth and aeration factors, reaching a multiplication rate of 32x106 cells/mL in the bioreactor versus 12x106 cells/mL at the control. The amount of yeast biomass obtained in the bioreactor was 78,6% higher than in the control by the aerobic stimulation effect of the synthesis of cellular precursors of biomass multiplication. In the bioreactor, the conditions of respiratory multiplication of the yeast have been established, as evidenced by the reduced alcohol content of 7.5% alcohol and the large amount of biosynthesis obtained by biosynthesis compared to the control where the fermentative processes are at the expense of the respiratory.*
Key words: biomasă, yeast, bioreactor

Rezumat. *În bioreactor s-a creat conditii optime de creştere şi multiplicare levuriană prin aplicarea factorilor de creştere şi aerare, ajungându-se la o rată de multiplicare de $32x10^6$ celule/mL în bioreactor comparativ cu $12x10^6$ celulle/mL la martor. Cantitatea de biomasă levuriană obtinută în bioreactor a fost cu 78,6% mai mare decăt la martor prin efectul de stimulare aerobică a sintezei precursorilor celulari ai multiplicării biomasei. În bioreactor s-au creat condiţiile de multiplicare levuriană pe cale respiratorie dovadă sta concentraţia redusă in alcool de 7,5% vol alcool şi cantitatea mare de biomasă obţinută prin biosinteză comparativ cu martorul unde domină procesele fermentative în detrimentul celor respiratorii.*
Cuvinte cheie: biomasă, drojdii, bioreactor

INTRODUCTION

The use of bioreactors in the production of protein biomass with a high nutritional value is common practice in the industrialised countries for a long period of time.

In our country there are experimental trials for monitoring and overseeing of bioreactors (Cascaval and Ungureanu, 2000; Selişteanu, 2001), but we need a new scientific aproach in the sense that we need to move pass the experimental stage to the pilot stage and finaly to the industrial production stage if we want to put these biotechnologies to work in development acording to A. Sasson (Sasson, 1993).

V. Magearu and S. Jurcoane worked on the analytical control of biotechnological processes and foundations in the bioreactors (Magearu, 1988; Jurcoane, 1999-2000).

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In our experiment we wanted to increase the return of levurian biomass in the bioreactor versus a control of traditional fermentation without shaking, more nutrients or a change in pH value, the factors which have been used in the bioreactor.

The fermentation process was shorter in the biorector (9 days until dry) due to the above mentioned factors compared to the control (14 days) when the fermentation process had to be stopped as proven by the rezidual amount of sugar of 26 g/L.

Aeration is known as a physical factor of levurian multiplication as well as the separated influence of some B complex vitamins on the growth of yeasts.

MATERIAL AND PROCEDURE

The grape must is introduced in the bioreactor-5. lt (236g/L sugars; 6.6g/L tartric acid; pH-3.35) and after that 60mg/L of SO_2 must (sterilized at 105°C for 15 min.) was added to provide antioxidant protection together with the pH sensors, DO, foaming sensor; cooling, air and temperature. All the entrances and exits in the fermentation tank were covered with cotton-wool and aluminium foil to avoid contamination.

At the same time 1lt of must was introduced in a UV sterilized plastic bottle to obtain a control sample without the imposed parameters from the bioreactor.

The transfer of the must in the control sample and in the reactor was followed by sterilization at 105°C for 15 min.

Sacharomyes cerevisiae -Killer strand 10701 USAMV Bucharest was added in 500 mL starter must and thermostated 3 days at 28°C/72 h.

Parameters settings in the bioreactor:
-stirring speed was set to 50 rot./min.;
-Nutrient dosage, in a dose of 31mL/day;
-pH-4.06;
-fermentation temperature 22°C;
-level of aeration-10;
-Basic Pump; (NaOH 1 N pH-13)

The pH, the metabolism of sugars in the must, numerical evaluation of yeasts in fermentation, foaming and the colour of the medium , quantitative evaluation of yeast biomass at the end of fermentation and physical-chemical analysis of the resulting wine were monitored.

Peristaltic pumping additives in Bioreactor
Nutrients:-B complex vitamins forte (5 tablets la %);
1. Essential nutrients for the yeasts:
-Pantothenic acid: 45 mg (9 mg/tablet)-ideal for the yeasts -250 µg;
-Vitamin B1: 8,250mg (1,65 mg/tablet)-ideal for the yeasts -250 µg;
-Biotin: 375 µg (75 µg/tablet)-ideal for yeasts 250 µg:
2. Glutation Activator Nutrient:
-30g/hL (cell walls, sdteroli vitamins, growth factors)
Basic Pump Na OH 1N, pH 13

RESULTS AND DISCUSSIONS

The fermatation process was stopped through exposure to cold air (12°C) and sulfiting at 100mg/L SO_2.

The bioreactor was disconnected and the fermented liquid, 5 L, was taken from the bioreactor and was left to decant in the cold so the the yeast sets and the crude wine decants; the control sample continued to ferment until 17.07.2017 when it was put in the cold to obtain the yeast and the crude wine;

During the multiplication of yeasts and the showing of alcohol fermentation we proceded to the monitorisation of the alcoholic fermentation through the daily metabolism of sugars, numerical evaluation of the yeasts in fermentation, and the pH, the quantitative assesment of the levurian biomass at the end of fermentation according to table 1.

Table 1

The multiplication of yeasts

Data	Procedures/ obs.	Yeast no. Bioreactor /Control	Residual sugars g/L Bioreact./ Control	pH Bioreact. / Control
5.07.	The yeast dose is innoculated /Lag stage	$5x10^6/5x\ 10^6$	236/236	4.06/3.24
6.07.	Multiplication/Exponential Stage	$10x\ 10^6/6x\ 10^6$	205/220	4.03/3.25
7.07.	Exponential Stage	$22x10^6/8x10^6$	175/190	4.00/3.26
8.07.	Exponential Stage	$25x10^6/10x10^6$	150/170	4.00/3.26
9.07.	Exponential Stage	$28x10^6/11x10^6$	100/135	3.99/3.27
10.07.	Exponențial Stage/Staționary	$32x10^6/12x10^6$	60/105	3.98/3.27
11.07	Final Stage	$16x10^6/8x10^6$	30/70	4.03/3.37
12.07.	Final Stage	$10x10^6/8x10^6$	15/50	4.03/3.37
13.07.	Decanting and racking (Bioreactor)	$8x10^6/8x10^6$	7/40	4.02/3/39

The rate of multiplication of yeasts in the bioreactor reached a maximum of $32x10^6$/ml after six days compared to the control sample where the multiplication rate was of only $12x10^6$/mL for the same period of time. The metabolism of sugars happens at the same speed in the bioreactor when after 8 days all the sugars are metabolised while in the control sample there are still 26g/L sugars even after 13 days. The appearance of the fermentation process represents the Gaussian bell.

Physico-chemical analysis of the resulting raw wines 236 g sugar=13.8% vol. alcohol

Why is there more biomass in the bioreactor?

Why is the wine from the bioreactor of lower alcoholic concentration (7.5% vol. alcohol) while in the control sample the concentration is higher (13.7%vol. alcohol)? (tab. 2)

Table 2

The kinetics of biosynthesis processes

No	Sample	Alcohol % vol.	Total acidity g/L ac. tartric	Acidity vol. g/L ac. acetic	SO2 total mg/L	SO2 free mg/L	Sugars red. g/L	Non-reductive extract. g/L	pH	Polyphenols g/L	Turbidit. NTU
1	Wine control	13.7	7.4	1.11	151	10	26	26	3.25	0.380	0.64
2	Wine bioreactor	7.5	7.4	0.87	130	6	4	30	3.88	0.409	1.52

The answer comes from the the repression and enzymatic stimulation of some biochemical processes of glycolysis and breathing. Through `the Pasteur effect` the fermentation is inhibited by aeration and the aerobic (respiratory) pathway of sugar degradation is intensified. The modification of the Pasteur effect is called `the Crabtree` and represents the repression of breathing and the stimulation of fermentation through which the yeast strand *Saccharomyces cerevisiae* produces ethanol (alcohol) in anaerobic conditions and high concentrations of glucose while the pyruvate is transformed in ethanol and carbon dioxide amd the energy production is lowered to 2 moles ATP/mole of glucose.

Of course in the bioreactor only a small amount of sugars were used in glycolysis to obtaine ethyl alcohol (7.5 % vol. alcool), the rest was used in the respiratory processes and oxidative phosphorylation of yeast mitochondria with the production of a large amount of energy required in the aerobic biosynthesis of levurian mass and nucleotide and nucleic acids via the hexose monophosphate pathway for the synthesis of biomass-forming cellular precursors according to figure 1.

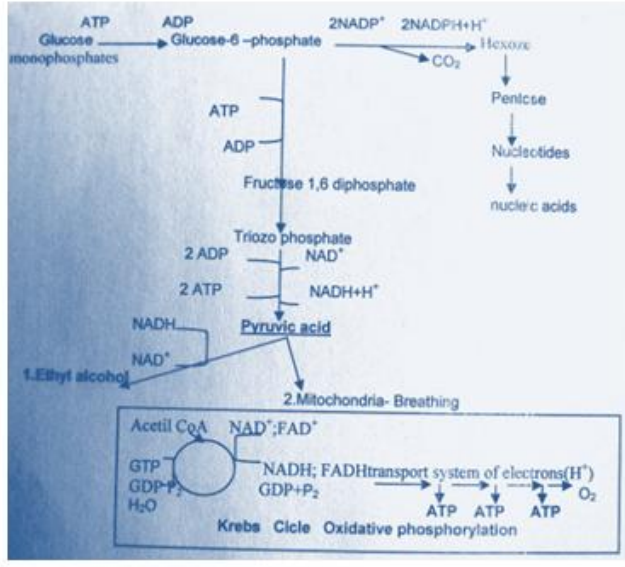

Fig.1 Sugar metabolism in the yeast cell

Wet yeasts mass:

In the control sample through the must fermentation of 1200 mL raw wine 16g of wet yeast were obtained.

In the bioreactor through the must fermentation of 5000 mL raw wine 119g of wet yeast were obtained.

The calculation of yield in the bioreactor:

If the control sample we have 1200 ml raw wine……16.0 yeast

Related to the bioreactor `s 5000 ml raw wine, we have…… 66.6 g yeast;

66,6 g drojdie.....100%

119 g drojdie.....x**;** **X =78,6 %**

The yield of levurian biomass in the bioreactor is 78.6% higher than in the control sample (fig.2, fig.3).

The factors that have led to increased yields in biomass

1. Aeration by air dosing at a dose of 10;
1. Rhythmic addition of nutritional factors of 31 mL / day for 5 days;
2. The change of pH in the bioreactor to 4.06;
3. Temperature: 22°C. Stirring to 50 rot/min.

Fig. 2 The Bioreactor working

Fig. 3 Sampling of the product

CONCLUSIONS

In the bioreactor optimal conditions of growth and levurian multiplication were created by applying aeration and growth factors, reaching a multiplication rate of $32x10^6$ cells/mL versus $12x\ 10^6$ cells/mL in the control sample.

The obtained amount of levurian biomass in the bioreactor was with 78.6% higher than the amount obtained in the control sample due to the effect of aerobic stimulation of the synthesis of cellular precursors of biomass multiplication.

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