COMBINED ALCOHOLIC FERMENTATION OF GRAPE MUST

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Abstract: this article presents results about combined alcoholic fermentation of grape must.
Key words: cell, biomass, yeasts, fermentation, biotechnology.

Introduction

In the last years, an emphasis on agro-food industry especially in wine, it is using immobilized microorganisms. These organisms have the advantage that they can be easily inserted and removed from wine or wine after total or partial biotransformation of substrates [1, 2, 3, 4, and 5].

One of the techniques currently used for immobilization of microorganisms is the inclusion or encapsulation, this method is the introduction of microorganisms in a rigid polymer matrix without flow cell holder [6, 7, and 8].

Fermentation process stops or slow fermentation processes are among the key issues that may meet during winemaking, and are often caused by a total or partial block consumption of sugar by yeasts to produce ethanol [9, 10, 11, 12, and 13].

From the beginning, since it is used in wine, it was selected yeasts of the genus Saccharomyces preferred ellipsoideus. Operation for elliptic yeasts was and is justified by the fact that they compared with wild yeasts have enough alcohol power high concentrations of sulfur dioxide supports relatively high, giving a high yield in alcohol, possession quickly put environment and lead always to achieve a wine fair and healthy [14, 15, 16, 17, and 18].

Yeast responsible for alcoholic fermentation, in addition to sugar conversion, production and partial decomposition of malic acid in non acid products. This degradation, independent of malo-lactic fermentation, 15-25% of the initial amount of malic acid is converted into alcohol and carbon dioxide [19, 20].

The research was carried out alcoholic fermentation with immobilized yeast; obtaining pure yeast is a biomass that can be used in various industries: food, cosmetic, medicine, pharmaceutics [21].

The aim of this research is to achieve a pure yeast biomass in polyethylene tube after completion of the alcoholic fermentation.

Materials and Methods

As research objects were accepted Sauvignon musts with different selected yeast from import, and of course membrane filter of 0,6 μm pore diameter and made tubes on which they were immobilized yeasts.

Alcoholic fermentation went with and without oxygen dosage. Dosed oxygen which had a positive influence on biomass accumulation. Fermentation was monitored daily, as determined by refract meter thus УРЛ-1 sugars remaining in the environment that were to be converted into alcohol. After, completion of alcoholic fermentation with yeast immobilized tubes were then dried and subjected to dialysis prompting and measuring, it

was determinate exact t formed pure yeast biomass. Finally, the stage described above was established scheme then we get pure yeast biomass.

For determination of physical and chemical indexes of obtained wine were used analytical methods appropriate standard and recommended by the OIV.

Results

Following research found yeast biomass accumulation in polyethylene tube after the process of alcoholic fermentation. As samples were taken from the Sauvignon grape. Fermentation took place in three bottles of 5 liters each temperature for 17 ... 18 ^{0}C.

For fermentation in each bottle (figure 1) was introduced each polyethylene tube 1.25 g each selected yeast imported (Oenoferm Freddo, Sihai Activhefe 10 and LittoLevure Sauvignon). Before being introduced, tubes were activated yeast in warm temperature of 37C mash for 15 to 20 min.

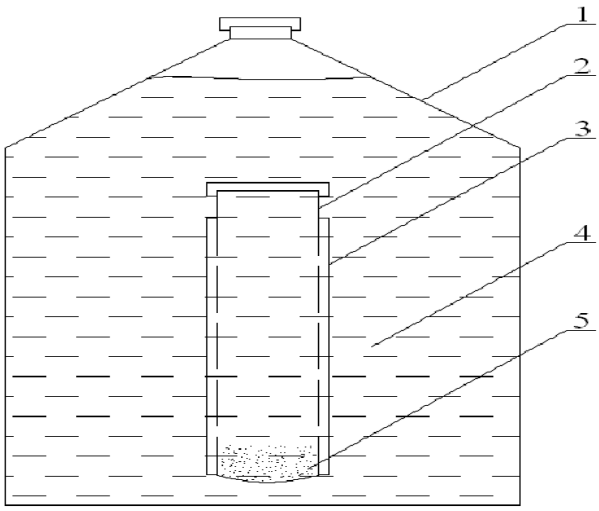
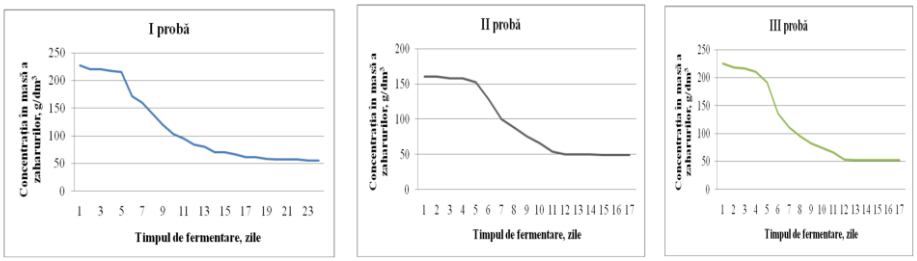


Fig. 1. Scheme of fermentation installation using immobilized yeast: 1 - fermentation tank, 2 - perforated polyethylene tube, 3 - diameter membrane filter pores of 0,6 μm, 4 - must; 5 - selected yeasts.

Daily was conducted of monitoring fermentation process, determines the amount of sugar that were to be fermented. The fermentation process is represented graphically in figure 2 (a, b, c).



a) b) c)

Fig. 2. Fermentation chart of sample 1 (a), sample 2 (b) and sample 3 (c)

We can see, from the figure 2, that the alcoholic fermentation is in normal regime. It sees a slight decrease in sugar in the first 3 to 4 days, because occurs yeast multiplication. Starting with day 4-5, yeasts were most intense activity and transforms large quantities of sugars into alcohol and CO_2. Towards, the end, because alcohol is formed in the environment, the power decreases gradually fermenting yeasts metabolize quantities increasingly smaller sugars. Yeasts are growing increasingly more difficult and some cells die.

To obtain pure yeast biomass were several stages: packing, weighing yeast, activate yeast, weighing tube, alcoholic fermentation, dialysis, drying and scales.

Following the general scheme for obtaining pure yeast biomass following results was shown in table 1.

Table 1. Biomass accumulation in process of alcoholic fermentation

Nr.	Weight of tube before fermentation, g	Yeast mass, g	Weight of tube after fermentation, dialysis and drying, g	Accumulated biomass, g
1.	41,61	1,25 Oenoferm Freddo	43,30	0,44
2.	42,51	1,25 Siha Activhefe 10	45,10	1,34
3.	42,49	1,25 LittoLevure Sauv.	46,12	2,38

From the table 1, it is observed that the use of yeasts in the fermentation process LittoLevure Sauvignon accumulated biomass is highest, so the experience of the alcoholic fermentation was carried out by dosing O_2 have used the same yeast.

In the obtained wine samples were determined physical-chemical indices (alcohol concentration and titratable acidity). The results are presented in the table 2.

Table 2. Physical-chemical indices of obtained wines

Nr.	Physical-chemical indices	
	Alcohol, % vol.	Titrable acidity, g/L
1.	13,8	9,3
2.	9,5	6,5
3.	13,4	6,6

We can conclude, from the table that alcoholic fermentation went all the way through adding selected yeasts environment. We can deduce from this an alcohol concentration measured.

The second experience consisted of dosing fermentation with yeast oxygen tube (figure 3).

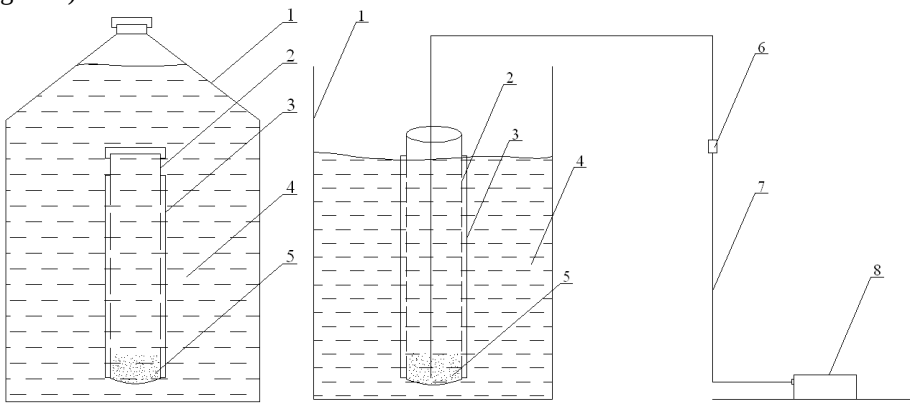


Fig. 3. Diagram of fermentation with O_2 dosing: 1 - fermentation tank, 2 - perforated polyethylene tube, 3 - membrane filter with a pore diameter of 0.6 μm, 4 - must; 5 - selected yeasts, 6 - sterile; 7 - polyethylene hose, 8 - pump oxygen.

It was determined daily average remaining sugars as with in the previous experiment. Fermentation process is represented graphically in figure 4.

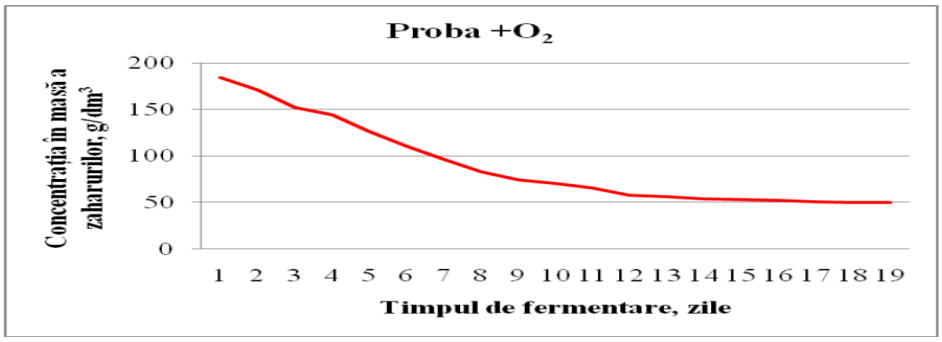


Fig. 4. Graph of fermentation sample with oxygen dosing (initial sugar = 184 g / dm^3)

We can see from the chart 4 a sudden drop in sugar, this debt is rapid multiplication of yeasts from the early days. An important part of their multiplication which had been dosed oxygen inside the tube with yeast. As in table grape sugars are consumed, multiplying yeast fermentation ceases and so well.

In the table 3 is the accumulated biomass for alcoholic fermentation of a dose.

Table 3. Biomass accumulation

Nr.	Weight of tube before fermentation, g	Yeast mass, g	Weight of tube after fermentation, dialysis and drying, g	Accumulated biomass, g
1.	34,94	0,25 LittoLevure Sauv.	36,77	1,58

Presence of oxygen in the alcoholic fermentation had a big influence on biomass accumulated.

Yeasts need oxygen to multiply and turn, so fermentation took place in the presence of more intense and accumulated biomass is much higher than in its absence.

Table 4. Physical-chemical indices

Nr.	**Physical-chemical indices**	
	Alcohol, % vol.	**Titrable acidity, g/L**
1.	7,7	8,6

Alcoholic strength obtained is lower than for non-use of oxygen dispenser.

This is because when oxygen while fermentation assay was carried out and the breathing, and some of the sugars present in wine was consumed in breathing.

Conclusions

- Cell immobilization has become an important practice in biotechnology recent years leading to increased performance and economy fermentative processes.
- Using fixed and fixed in wine microorganisms are highly appreciated worldwide and therefore to increase performance wine industry in Moldova have been researching for pure yeast biomass growth.
- The process of alcoholic fermentation with yeast immobilized allows pure yeast biomass accumulation.
- A stately yeast biomass was obtained when carrying out alcoholic fermentation oxygen dosing.
- This study has allowed the development of a scheme for obtaining pure yeast biomass.
- Yeast biomass has gained wide use: food, cosmetic, medicine, pharmaceuticals.

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