JAP&T TPA-Timişoara

Available online at http://journal-of-agroalimentary.ro

Journal of Agroalimentary Processes and Technologies 2022, *28* (1), 20-26

Journal of Agroalimentary Processes and Technologies

Active wine yeast biomass obtained through biotechnological process

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Abstract

The aim of this paper was to study the influence of some parameters of the fermentation process at laboratory level, using an orbital incubator, with the aim to obtain a high yield of wine active yeast biomass. In order to start the process, previously we isolated the yeasts strain, identified as *S. cerevisiae*, from the Fetească regală grapes from Pietroasa Viticulture and Winemaking Research and Development Station. The study considered the influence of 3 parameters on the biomass yeast development: the ratio working volume / total volume (Vu/Vt) (100 mL/500 mL; 150 mL/500 mL; 200 mL/500 mL; 300 mL/500 mL); the inoculate rate (5%, 10%, 15%, 20%); and the stirring rate (150 rpm, 170rpm, 200rpm, 240 rpm). After the fermentation process, the medium was centrifuged and washed with sterile distilled water, and the resulting purified wet yeast biomass was dried by lyophilization. The answers of this study were the weight wet (WCW g/100 mL) and the dry biomass (DCW/100 mL), the cellular viability (UFC/ mL), the bioconversion yield regarding the transformation of substrate (sugar) in biomass; pH. The best experiment was for the following cultivation conditions: Vu/Vt ratio 200 mL/500 mL; inoculation rate: 20%; stirring rate 240 rpm; for a WCW of 4.96 g/100 mL. The active yeast wine biomass will be further investigated for a scale-up winemaking process, in order to assess the terroir behavior.

Keywords: grapevine; Pietroasa vineyard; Feteasca regala; yeast biomass

1. Introduction

Saccharomyces cerevisiae is the dominant microflora during fermentation, used worldwide in the industry. *Saccharomyces cerevisiae* is the main strain for baker's or brewer's biomass production, widely used in the production of beverages and fermented foods [1]. The yeast genome is composed of 45%–60% of structural proteins, peptide hormones and enzymes, totaling 5858 proteins [2]. *S. cerevisiae*, as a unicellular eukaryotic microorganism, can provide important compounds and nutrients necessary for living cells and, for this reason, can play an important role in wine fermentation. Oligopeptides, amino acids, polysaccharides, especially β-glucans and mannans, lipids, B-complex vitamins (except vitamin B12) can be obtained from *S. cerevisiae cells.*

Today, many yeasts are available in a number of different formats – all with good viability and utility. Large-scale wineries generally use specifically selected starter cultures of *S. cerevisiae* in preference to relying on the fermentative activities of naturally occurring yeasts [3]. These cultures are available as commercial active dry yeast (ADY) and inactive dry yeast (IDY). Michał Wójcicki et al (2022) isolated microorganisms from three grape varieties: Seyval blanc, Regent and Solaris [4]. They studied the effect of newly isolated native vine varieties and *S. cerevisiae* yeast strains

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on the content of selected metabolites of wine fermentation.

For many years, yeast starters have been selected, *Saccharomyces* yeasts being preferred when it comes to natural grape microflora, due to their suitable characteristics, especially high alcohol-tolerance. Genus *Saccharomyces* appears to be perfect for carrying out alcoholic fermentations, being the result of the adaptation to high sugar concentrations in must and subsequent conversion into ethanol, carbon dioxide and organoleptic compounds [5]. The aim of our study was to determine the best conditions of aeration, agitation and inoculation ratio in order to increase biomass production of isolated *Saccharomyces cerevisiae* yeast.

2. Materials and Method

The grape samples (Fetească regală variety) came from Pietroasa Viticulture and Winemaking Research and Development Station during the 2021 harvest (Figure 1).

Figure 1. 2021 grape sample harvest from Pietroasa Viticulture and Winemaking Research and Development Station

Decimal dilutions were made on the collected samples in order to isolate new yeast strains. The isolated pure culture was identified using PCR ITS-RFLP technique [6]. The PCR products of isolated yeast were sequenced in both directions, using ITS1/ITS4 primers. The sequenced sequence analysis was performed using the BLAST program to determine the percentage of identity with the sequences available in the NCBI database, applying the program http://blast.ncbi.nlm.nih.gov, these being aligned using the Clustal Omega program [7,8].

For *maintenance culture* was used the selected yeast culture cultivated at slant tube with YMSP agarised medium (yeast extract, sucrose, malt extract and peptone hy-soy) [9].

Preinoculum preparation was obtained using previously obtained pure cultures which were inoculated into tubes with YMSP agarised and incubated at 30 °C for 48 hours (Figure 2).

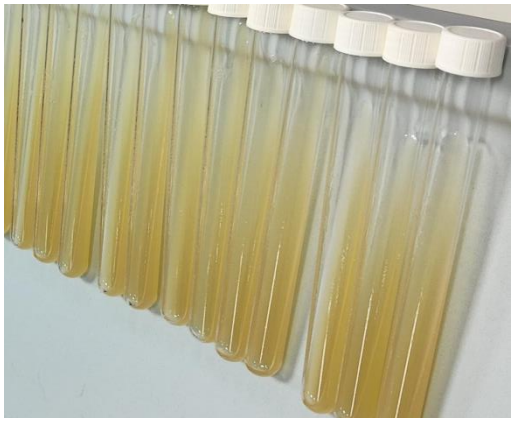

Figure 2. Preinoculum medium

Inoculum preparation – the liquid culture medium based on yeast extract, hy-soy peptone and sucrose was dissolved in 100 mL distilled water and distributed in Erlenmeyer flasks by 500 mL and sterilized at 121 °C for 15 min (Figure 3).

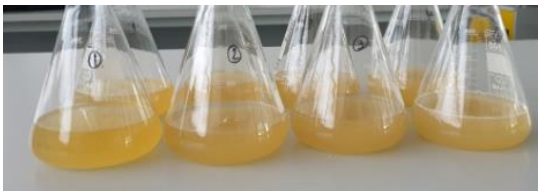

Figure 3. Inoculum medium, ready for seeding with pre-inoculum yeast culture

After the culture medium for the inoculum reached room temperature, it was seeded with the pre-inoculum culture and incubated at 30 °C for 17-19 h at Orbital Shaker (Figure 4).

In order to achieve the study aim, the following steps were followed:

For experimental fermentations, the influence of 3 parameters on the biomass yeast development was studied: the ratio with culture media working volume / total volume (Vu/Vt) (100 mL/500 mL; 150 mL/500 mL; 200 mL/500 mL; 300 mL/500 mL); the inoculate rate (5%; 10%; 15%; 20%); and the stirring rate (150 rpm; 170 rpm; 200 rpm; 240 rpm). This was used to determine the influence of

cultivation parameters on different types of biomasses (WCW and DCW).

The nutrients used for the fermentation process were the following: yeast extract, sugar as carbon source, $NH_4H_2PO_4$, $MgSO_4 \times 7H_2O$, KCl.

Figure 4. Inoculum medium, freshly seeded with pre-inoculum yeast culture

Upstream -process

The batch fermentation was:

1. Influence of ratio working volume / total volume (Vu/Vt) towards yeast biomass development

Parameters of fermentation process:

- the ratio working volume / total volume (Vu/Vt): 100 mL/500 mL; 150 mL/500 mL; 200 mL/500 mL; 300 mL/500 mL);

- temperature: 30 °C;

- stirring rate: 240 rpm;

- cultivation time: 19 h;

- inoculation ratio: 15%.

2. The influence of the inoculation ratio on the development of yeast biomass

- the ratio working volume / total volume (Vu/Vt): 200 mL/500 mL

- temperature: 30 °C;

- stirring rate: 240 rpm;

- cultivation time: 19 h;

- inoculation ratio: 5%, 10%, 15% and 20%.

3. The influence of agitation speed on the growth of yeast biomass

- the ratio working volume / total volume (Vu/Vt): 200 mL/500 mL

- temperature: 30 °C;

- stirring rate: 150 rpm, 170 rpm, 200 rpm, 240 rpm;

- cultivation time: 19 h;

- inoculation ratio: 20%.

Downstream process

- *Separation of yeast biomass*: after completing each batch (Figure 5) the yeast cream biomass was separated from the culture fermented media by centrifugation.
- *Purification of yeast cream biomass*: the yeast biomass was purified with sterilized distillated water followed by centrifugation at 4000 rpm for 5-10 min (Figure 6).
- Determination of the WCW was performed according to [9].

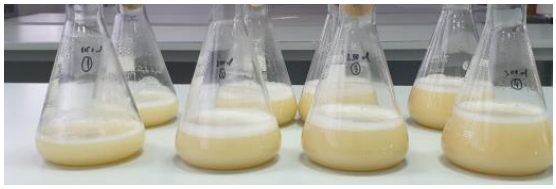

Figure 5. Fermented culture media at the end of the fermentation

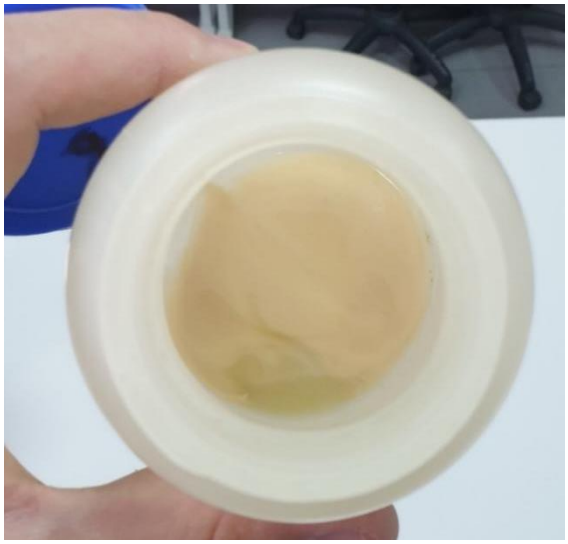

Figure 6. Yeast cream biomass after the centrifugation and purification

To freeze-dried the samples obtained after centrifugation, the working protocol described by Stan et al. was used [10].

For the determination of DCW, the resulting biomass was dried in an oven at 105 °C for 4 h; this was continued until the biomass reached a constant weight, and then, it was weighed.

The yield of transforming the carbon source into yeast biomass was determined

It is calculated according to the formula:

$\Delta X / \Delta S = y$, where:

X = the amount of yeast biomass

S = substrate concentration (carbon source)

y = yield of transforming the substrate into biomass

3. Results and Discussion

The selected yeast strain was identified as *S. cerevisiae* yeast using PCR method. The *S. cerevisiae* after cultivation on malt extract for 3 days is in the form of spherical, ellipsoidal, cylindrical, elongated cells, arranged alone or in pairs and occasionally forms chains and agglomerations [11,12]. *S. cerevisiae* is the most common yeast used for alcoholic fermentations in wine production. *S. cerevisiae* wine strains showing low acetic acid production under aerobic conditions [13].

We studied the influence of 3 parameters on the biomass yeast development: the ratio working volume / total volume (Vu/Vt) (100 mL/500 mL; 150 mL/500 mL; 200 mL/500 mL; 300 mL/500 mL); the inoculation rate (5%, 10%, 15%, 20%); and the stirring regime (150 rpm, 170 rpm, 200 rpm, 240 rpm. We followed the experiments described above.

From the data presented in Table 1 it is observed that the highest amount of wet and dry biomass was obtained when working on a volumetric ratio 200 mL / 500 mL.

For the next experiment the best result, 200 mL / 500 mL, in terms of wet biomass content was carried out.

The higher inoculation rate was shown to have a positive effect towards yeast biomass development. It has been shown that there is a small difference between variants 3 and 4; and a more pronounced difference between variant 4 and variants 1 and 2.

Table 1. Influence of ratio working volume / total volume (Vu/Vt) towards yeast biomass development

Cultivation time (h)	Samples	pH	D.M (dry matter) (%)	WCW g	WCW g/100 mL	DCW g/100 mL	Consumed sugar g/100 mL	Y (%)
0	1 - 150 mL	5	7.8					
	2 - 200 mL	5	7.9					
	3 - 250 mL	5	7.8					
	4 - 300 mL	5	7.9					
17	1 - 150 mL	4	1.5					
	2 - 200 mL	4	1.6					
	3 - 250 mL	4	1.6					
	4 - 300 mL	4	1.5					
19	1 - 150 mL	4.5	1.4	11.22	3.74	0.81	6.4	58
	2 - 200 mL	4.5	1.5	15.84	3.96	0.99	6.4	61.8
	3 - 250 mL	4.5	1.6	19.19	3.84	0.86	6.2	61.9
	4 - 300 mL	4.5	1.5	22.92	3.82	0.85	6.4	59.6

The experiment with a 20% inoculation rate had a higher carbon consumption, which also led to a higher amount of wet yeast biomass (the conversion rate of the carbon source in g WCW / 100 mL average was higher compared to the other 3 variants).

The data presented in the Table 2 shows that at an inoculation ratio of 20% a higher dry final biomass concentration (1.04g /100 mL) has been obtained than in the case of using an inoculation ratio of 5 % batch 1 (0.77 g / 100 mL), the rest of the experimental conditions being the same.

It was highlighted that the variant 4 showed an average carbon source bioconversion rate of 0.98 g WCW / 100 mL, compared to 0.8 g for variants 2 and 3 and 0.68 g for variant 1 (Table 3).

Table 2. The effect of the inoculation ratio on the accumulation of yeast biomass

Cultivation time (h)	Samples	pH	D.M (dry matter) (%)	WCW g	WCW g/100 mL	DCW g/100 mL	Consumed sugar g/100 mL	Y (%)
0	1 - 5%	6	8.2					
	2 - 10%	6	8.1					
	3 - 15%	6	7.8					
	4 - 20%	6	7.6					
17	1 - 5%	~5	2.2					
	2 - 10%	~5	1.7					
	3 - 15%	~5	1.7					
	4 - 20%	~5	1.6					
19	1 - 5%	4.5	2.1	16.07	4.02	0.77	6.1	65.9
	2 - 10%	4.5	1.6	16.91	4.23	0.87	6.5	65.07
	3 - 15%	4.5	1.7	19.15	4.79	0.95	6.1	78.5
	4 - 20%	4.5	1.6	19.84	4.96	1.04	6	82.6

Table 3. The influence of stirring rate on the growth of yeast biomass

Cultivation time (h)	Samples	pH	D.M (dry matter) (%)	WCW g	WCW g/100 mL	DCW g/100 mL	Consumed sugar g/100 mL	Y (%)
0	1 - 150 rpm	5.5	7.8					
	2 - 170 rpm	5.5	7.8					
	3 - 200 rpm	5.5	7.7					
	4 - 240 rpm	5.5	7.7					
17	1 - 150 rpm	4	2.1					
	2 - 170 rpm	4	1.7					
	3 - 200 rpm	4	1.6					
	4 - 240 rpm	4	1.5					
19	1 - 150 rpm	4	1.6	15.42	3.86	0.68	6.2	63.8
	2 - 170 rpm	4	1.7	15.37	3.84	0.8	6.1	62.9
	3 - 200 rpm	4	1.6	15.32	3.83	70	6.1	62.78
	4 - 240 rpm	4	1.6	19.24	4.81	0.98	6.1	78.8

From the data presented in the tables (final values) it is observed that a better development of the yeast biomass was achieved at a stirring of 240 rpm. Also, the accumulation of a higher amount of yeast biomass is correlated with a higher rate of consumption of the substrate (sugar), namely Y= 6.1-6.4%.

The viability was 2×10^{10} UFC/ mL for the variant 4, at the ratio working volume / total volume (Vu/Vt): 200 mL/500 mL; (culture media: yeast extract, sugar as carbon source, $NH_4H_2PO_4$, $MgSO_4 \times 7H_2O$, KCl); temperature: 30 °C; stirring regime: 240 rpm; cultivation time: 19 h; inoculation ratio: 20%.

4. Conclusions

In order to optimize the bioprocess, the following technological parameters were studied: stirring rate, average volume/total V ratio (aeration), inoculation ratio.

The optimal values of these biotechnological parameters were established experimentally.

A better development of the yeast biomass was obtained by using a stirring rate of 240 rpm.

Studying the influence of the inoculation ratio on the biomass concentration, the optimal values regarding the specific growth rate of biomass were obtained at an inoculation ratio of 20%.

After the processing phase of the culture medium resulting from the bioprocess, a final biomass concentration > 4.96 g / L was obtained.

The best variant was when the ratio working volume/total volume (Vu/Vt): 200 mL/500 mL.

Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards. Authors declare that they present their own literature survey and results/discussion/conclusion in the article.

Acknowledgements: This work was supported by a grant of the Ministry of Research, Innovation and Digitization, CNCS/CCCDI - UEFISCDI, project number PN-III-P3-3.5-EUK-2019-0213, within PNCDI III.

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