

Yeast from Ethanol Production – Source of SCP (Single Cell Protein)

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Abstract— Dry yeast, one of sugarcane ethanol manufacturing byproducts, is largely marketed as a source of protein for preparation of animal feed. We have analyzed the behavior of four different *Saccharomyces cerevisiae* strains isolated from ethanol production industrial process (CAT, PE, SMBP1 and NARBP2) in a cultivation medium formulated from sugarcane molasses in laboratory conditions. The assessed parameters were: Cell yield ($Y_{x/s}$) and protein concentration in the mass. The protein analysis was carried out with the use of the TruSpecN (LECO) equipment. The results suggest that the amount of cell yield and the percentage of protein in the mass are different for each strain tested. It was also possible to clearly elucidate that there is a correlation between the two parameters studied. The larger the amount of cell mass produced, the smaller the amount of protein.

Index Terms – Alcoholic fermentation, dry yeast, *Saccharomyces cerevisiae*, unicellular protein

I. INTRODUCTION

According to UNICA [1], Brazil produced about 4,832.7 million liters of ethanol in 2014. This ethanol is a product of metabolic activity by *Saccharomyces* genus yeast, capable of efficiently transforming sugar in feedstock (sugarcane juice, molasses or both) into ethanol. However, it is known that part of this sugar is compromised with the maintenance of yeast cell metabolism, and other non-ethanol products are ultimately generated. The amount of sugar diverted, and the nature of the compounds produced may vary depending on the operation conditions of each industrial unit. Menezes [2] reports that 5% of sugar of the fermentation process is diverted to ethanol production. Yeast cell is one of the various products formed in alcoholic fermentation. It is estimated that for every liter of ethanol produced, yeast produces nearly 51g of cells on dry basis. From this total, nearly 22g [3] may be removed without impairing the process.

The use of this strategy guarantees continuous young cell population and constant cell mass volume in the process. Once removed from the process, this yeast is usually dried and sold as a source of protein for feed manufacturing [4]. As reported by Santos [5] the dry-yeast market is thriving, with

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Europe as the leading buyer of this protein. The European demand is explained by the ban on adding animal origin protein to ruminant feed in that continent. This ban aims to control mad cow disease.

The exact amount of dry yeast production in Brazil is unknown, but it is estimated at about 50 thousand tonnes/year [6], with the most part exported

Even though dry yeast protein is classified as SCP (single-cell-protein), referring to protein originated from bacteria, yeasts, filamentous fungi or algae [7], it differs from other products of this category, since it is not produced for this purpose. Dry yeast is actually a byproduct of ethanol production, which may be commercialized as SCP. Considering the particularity of this SCP, it is unlikely that ethanol production units carry out their processes aiming at obtaining yeast with high protein content. The strategy used by units is to carry out endogenous fermentation before drying the mass to increase protein content in their yeast. This fermentation consists of keeping yeast at high temperatures in anaerobiosis for extended periods of time, thus causing a drop in carbohydrate reserves consumed for ethanol production [8]. According to Pulzatto [9], yeast submitted to endogenous fermentation process guarantees factory remuneration about 40% higher than dry yeast obtained directly from alcoholic fermentation, which covers production costs of this byproduct, as explained by this author.

Steckelberg [10] notes the varying amount of protein found in different yeast strains isolated at distinct production units. Therefore, working with yeast naturally able to accumulate higher levels of protein seems to be an interesting strategy for this industry. Within this context, this work evaluated the ability of four different yeast strains isolated from ethanol production processes to produce cell mass and accumulate protein in this mass.

II. SUBJECTS AND METHODS

A. Strains: We evaluated four different *Saccharomyces cerevisiae* strains isolated from industrial processes. They are known as CAT, PE, SMBP1 and NARBP2. These strains belong to the industrial yeast collection of the Bioprocesses Division of CPQBA/UNICAMP.

B. Cultivation conditions: The tests were carried out under sterile conditions in 250 ml Erlenmeyer flasks containing 100 ml of medium prepared with molasses and incubated as follows: 24 hours/30°C/150 rpm. Molasses was used at concentration of 347 g/liter to reach concentration of fermentescible sugars (sucrose, fructose and glucose) of approximately 150 g/liter. Fermentation inoculation was

made by adding 10 ml yeast grown in synthetic medium (15% glucose, 0.5% KH_2PO_4 , 0.5% KH_2PO_4 , 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% KCl and 0.6% yeast extract) for 24 hours/30°C/150 rpm. Parameters assessed were cell yield ($Y_{x/s}$ = cell mass produced in relation to sugar available) and protein percentage in this mass.

C. *Analytical methods:* Cell mass (MS) produced was obtained through gravimetric quantification. Samples were centrifuged twice to wash cells. Cells were resuspended in distilled water and transferred to glass plates previously weighed. Plates were dried in oven with air circulation at 60°C until constant weight. Protein concentration was obtained with TruSpecN (LECO).

D. *Result analysis:* All samples were made in triplicate and results were assessed statistically using STATISTICA® 8.0.

III. RESULTS AND DISCUSSION

Table 1 shows the results for the four strains evaluated for cell mass produced, mass protein concentration and cell yield ($Y_{x/s}$).

Even though the results of this work were obtained in laboratory conditions, they coincide with the results obtained under industrial conditions. Researchers analyzed yeast from ethanol production and protein concentration in industrial yeast was of 39.6% [11].

Among the four strains studied, the one with the highest yield was CAT (0.024013 gMS/g TRS) followed by PE (0.021941gMS/g TRS), NARBP2 (0.020142gMS/g TRS). The strain producing the lowest number of cells was SMBP1 (0.020727gMS/g TRS). Strains with the highest yield in cells also presented the lowest amounts of protein in their masses. SMB1 presented 42.02% of total protein in cell mass produced. NARBP2 presented 40.70%, PE, 39.38% and CAT, 37.99%.

The analysis of this phenomenon has allowed us to confirm that there is a linear relation between cell yield $Y_{p/s}$ and the protein amount found in this mass. With $R^2=0.9445$, it is possible to state that the higher the cell yield, the lower the protein concentration in this mass (Fig.1). Even though the findings point to the CAT strain as the most indicated when the goal is to obtain protein, it is important to highlight that in ethanol production, the commercialization of this protein as a strategy used to add value to an ethanol production byproduct is not the priority. Ethanol production units are not conceived to produce SCP (Single Cell Protein), that is, they have not been designed for massive microorganism growth for animal or human consumption [7]. The removal of part of the yeast from the process ("bleeding") é a highly recommended practice to guarantee constant yeast population concentration in the process. Another positive factor in removing part of the cells from the process is the possibility of renewing yeast cells in process, a renewal that guarantees young cells, which in turn promote healthy fermentation.

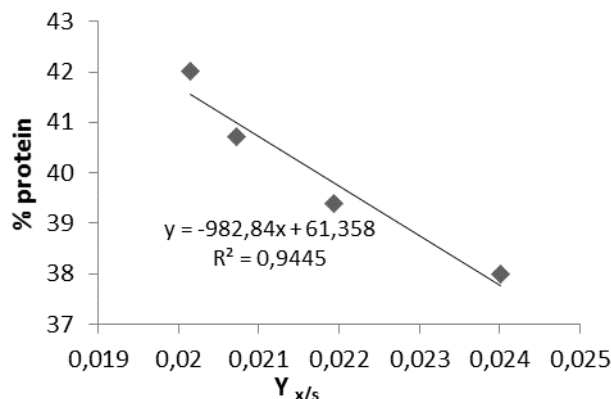


Figure 1 – Regression curve for $Y_{x/s}$ and % protein parameters for the four strains studied.

Drying cell mass removed from the process is an interesting aspect from the economical point of view, since this dry material is commercialized based on its protein concentration. Some strategies are used by units that dry yeast to increase protein concentration before the drying stage. One of them, and maybe the most widely used by industrial units, is endogenous fermentation, which consists of keeping cell mass under high temperatures (40°C) for enough time to consume carbohydrate reserve of the cell, which consequently increases cell protein concentration. Ferreira et al., [4] show that with application of endogenous fermentation in cell mass removed from the process, it is possible not only to increase mass protein levels, but also transform cell reserve into ethanol. According to these authors, the gain achieved with endogenous fermentation was 40 and 68 liters per tonne of dry yeast with 25 and 27% protein increment for PE-2 and VR-1 *S. cerevisiae* commercial strains, respectively.

From the data presented in this work, it was possible to clearly verify that protein concentration obtained in yeast mass removed from fermentation for ethanol production varies depending on the strain used. In addition, it was possible to notice a correlation between yield in cells and the concentration of protein in this cell mass. The higher the cell yield, the lower the protein concentration in this mass.

Caution is required when using the capacity to accumulate protein as parameter for the choice of yeast to start up the season, since the cell composition of this yeast will be influenced by the unique characteristics of each unit, that is, cultivation medium composition, operation temperature, plant type (batch or continuous). Moreover, recent research has shown that replacing commercial yeasts for those inhabiting feedstock is a common practice [12]. Units producing SCP exclusively may benefit from the unique properties found in yeast strains isolated from ethanol production units, and may start cultivating these strains in that type of industry.

Table I – Cell mass produced x protein concentration x cell yield ratio

Strain	MS (g/kg medium)	Protein (%)	MP (g/kg medium)	Y _{x/s}
CAT	7.087 ± 0.071	37.996 ± 0.787	2.692 ± 0.036	0.024013±0.000179
PE	6.481 ± 0.032	39.381 ±0.187	2.552 ± 0.006	0.021941±0.000074
SMBP1	5.954 ± 0.138	42.021 ± 0.387	2.502 ± 0.038	0.020142±0,000322
NARBP2	6.116 ± 0.106	40.720 ± 1.010	2.491 ± 0.094	0.020727±0.000105

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