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INSTITUTO DE PESQUISA EM BIOENERGIA

PH.D. PROGRAM IN BIOENERGY (UNESP, USP AND UNICAMP)

FRACTIONATION OF SPENT BREWER'S YEAST FOR HIGH VALUE-ADDED BIOMOLECULES PRODUCTION.

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Advisor: Prof. Dr. Pedro de Oliva Neto

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TÍTULO DA TESE: FRACTIONATION OF SPENT BREWER'S YEAST FOR HIGH VALUE-ADDED BIOMOLECULES PRODUCTION

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To my dear father, who always encouraged
me to overcome my own limits.

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ABSTRACT

Currently, the yeast biomass is an underutilized waste product of brewing industry. Moreover, the use of this biomass can be an economical source for the extraction of several compounds such as yeast extract, proteins, *β*-fructofuranosidase, RNA and 5'-ribonucleotides, which are by-products with wide applications in pharmaceutical and food industry. Faced with these issues, the objective of this work was to study and design an integrated bioprocess methodology using spent brewer's yeast biomass as feedstock for obtaining several high added value biomolecules through fractionation and downstream techniques. For this, the biotechnological potentials of yeast utilization are reviewed in the Chapter 1. The Chapter 2 presents the results of the initial stages of preparation and characterization of yeast from the brewing industry as well as the evaluation of autolysis parameters in order to maximize RNA extraction for subsequent 5'-ribonucleotides production. The Chapter 3 describes the use of acid shock at the beginning of autolysis in order to accelerate the autolytic process and the extraction of proteins. The results of enzymatic hydrolysis of RNA to produce 5'-ribonucleotides using 5'-phosphodiesterase from residual malted barley roots is presented in the Chapter 4. The recovery of extracellular β-fructofuranosidase enzyme as the first by-product before autolysis and studies on its immobilization in sodium alginate and activated charcoal beads were described in the Chapter 5. Finally, an economic analysis as well as a bioprocess plant design for yeast processing is proposed in the Chapter 6 in order to determine the profitability and financial viability of the methodology developed in this work. The proposed approach indicates an excellent strategy, since a better use of spent yeast from fermentation processes in the proposed biorefinery plant can open up a range of applications and add value to this product, making the industrial sectors more profitable.

Keywords: Biorefinery. Spent brewer's yeast. RNA. 5'-Ribonucleotides. 5'-Phosphodiesterase. β-Fructofuranosidase.

RESUMO

Atualmente, a biomassa de levedura é um resíduo subutilizado da indústria cervejeira. Além disso, o uso dessa biomassa pode ser uma fonte econômica para a extração de vários compostos, como extrato de levedura, proteínas, β-frutofuranosidase, RNA e 5'-ribonucleotídeos, subprodutos com amplas aplicações na indústria farmacêutica e de alimentos. Diante dessas questões, o objetivo deste trabalho foi estudar e propor uma metodologia integrada para o fracionamento de levedura residual cervejeira em diversas biomoléculas de alto valor agregado. Para isso, os potenciais biotecnológicos da utilização de leveduras são revisados no Capítulo 1. O Capítulo 2 apresenta os resultados dos estágios iniciais de preparação e caracterização da levedura da indústria cervejeira, bem como a avaliação de parâmetros de autólise para maximizar a extração de RNA para subsequente produção de 5'-ribonucleotídeos. O Capítulo 3 descreve o uso de choque ácido no início da autólise, a fim de acelerar o processo autolítico e a extração de proteínas. Os resultados da hidrólise enzimática do RNA para a produção de 5'-ribonucleotídeos usando 5'-fosfodiesterase proveniente de radícula de malte de cevada são apresentados no Capítulo 4. A recuperação da enzima extracelular da β-frutofuranosidase como o primeiro subproduto antes da autólise e estudos sobre sua imobilização em microesferas de alginato de sódio e carvão ativado são descritas no Capítulo 5. Por fim, é proposta no Capítulo 6 uma análise econômica, bem como um projeto de planta de bioprocesso para o processamento de leveduras, a fim de determinar a rentabilidade e viabilidade financeira da a metodologia desenvolvida neste trabalho. A abordagem proposta indica uma excelente estratégia, uma vez que um melhor uso de levedura residual de processos fermentativos pode abrir uma gama de aplicações e agregar valor a esse produto, tornando os setores industriais mais rentáveis.

Palavras-chave: Biorrefinaria. Levedura residual cervejeira. RNA. 5’-Ribonucleotídeos. 5'-Fosfodiesterase. β-Frutofuranosidase.

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1 GENERAL INTRODUCTION

Brazil occupies the third place in the world ranking of beer production, just behind China and the United States, and this sector contributes to generate development, jobs, income and prosperity. According to data from Brazilian Beer Industry Association (CERVBRASIL), which brings together the four largest brewers in the country and account for about 96% of the market, domestic production of beer has grown at an average rate of 5% per year over the last ten years (CERVBRASIL, 2016). The latest published bulletin shows that beer production was consolidated in 14.1 billion liters in 2018 (CERVBRASIL, 2018). Beer yields data for 2019-2020 has not yet been released but should remain around 14.1 billion liters beer per year. Due to this high production, the brewing industry is one of the most important of the Brazilian economy, which corresponds to 1.6% of the Brazilian Gross Domestic Product (GDP) and collects more than R$ 23 billion in taxes, contributing greatly to the development of the country (CERVBRASIL, 2016).

During brewing process, yeast biomass is an important by-product and is produced in large quantities. For each cubic meter of lager beer produced after wort fermentation, about 1.7 to 2.3 kg of spent yeast biomass are recovered on a dry basis (FERREIRA et al., 2010). Since Brazil annually produces approximately 14 billion liters of beer, the estimated yeast production is about 28 thousand tons just by the brewing sector. The bakery and alcohol sectors contribute to increase this number even more (RAMOS et al., 2011). Most of the dry yeast biomass produced, however, is sold at a low price by breweries and ethanol distilleries as a nutritional supplement in animal feed formulations (OLIVEIRA AND OLIVA NETO, 2011).

In many countries, on the other hand, yeasts are recognized as a high added value product, being the raw material for the extraction of several biomolecules. Yeast derivatives represent a rich source of nutrients such proteins, amino acids, minerals, vitamins, particularly those of the B complex, as well as carbohydrates, enzymes, lipids and nucleotides with important physiological properties (PÉREZ-TORRADO et al., 2015). Taking into account the abundance, the low cost and the richness in nutrient constituents, it becomes an excellent strategy the improvement of the technology of fractionation and purification of the yeast biomass.

Nowadays, the main challenges related to the beer and ethanol industry are the search for a more suitable destination for one of its main waste products: spent yeast.

If discarded incorrectly, such residues can cause environmental contamination besides representing a waste of material rich in nutrients from the biochemical point of view (RAKOWSKA et al., 2017). If used as a supplement in food products, the amount should be limited by the nucleic acid content, since in humans, RNA is metabolized to uric acid, which can cause gout (HUIGE, 2006). The change of this scenario, of spent yeast underutilization, began to be implemented in Brazil only in 1998 by the Cooperative of Sugar, Sugar and Alcohol Producers of the State of São Paulo (Copersucar) and by the Institute of Food Technology (Ital) in a project financed by FAPESP under the Partnership for Technological Innovation program (PITE). Preliminary results reported by Sgarbieri et al. (1999) indicated that yeast could be utilized more effectively in animal feed as well as it was possible to formulate four derived substances: autolysate, yeast extract, cell wall and protein concentrate.

Time passed and despite the residual yeast have gained more notoriety because of its great potential, the development of technologies geared to reuse this raw material keeps walking at a slow pace. South and southeast Brazil already have industries that develop other applications for yeast, however just last year, in 2018, Alagoas became the first state in the Brazilian northeast to own an alcohol distillery that dries yeast for sale as an animal supplement. Meanwhile, Japan use yeast as raw material for nucleic acids extraction and production of drugs with pharmaceutical applications since the 1980s. It is clear: for Brazil to become more competitive, innovation and research are needed. This path can be achieved more quickly by the partnership between research institutes that develop new technologies and bioprocess industries that invest in this market that has not been much explored in the Brazilian context.

The use of residual yeast from beer industry, for example, may be a promising approach to overcome the challenges related to RNA obtainment and hydrolysis in large scale for the production of 5'-ribonucleotides. The yeast biomass obtained by primary fermentation for the sole purpose of RNA extraction aiming to produce nucleic acid derivatives presents high costs and makes the product more expensive. The unique Brazilian company that produced RNA for 25 years, Omtek (Iracemápolis-SP), recently ended its activities due to the obsolete and expensive methodology used. Its process was based in primary fermentation to obtain yeast biomass and presented low yield of RNA extraction (only 55%), data not published. One of the alternatives to overcome this issue, object of the research in this work, would be to obtain RNA from

a more profitable starting point. Also, in the context of RNA extraction, another challenge to be overcome that deserves to be highlighted is the efficiency of its extraction once the biomass of yeast has been obtained. The current extraction techniques consist of the use of partial hydrolysis of the yeast cell wall at high temperatures, as well as the use of salts to osmotically force the RNA out into the extracellular environment (DIMOPOULOS et al., 2018). The use of acids such as H_2SO_4 and H_3PO_4 in addition to salts such as NaCl and KCl make the process expensive and not well seen by the food industry due to the high salt content in the final product (RAKOWSKA et al., 2017).

Yeast biomass is undoubtedly an underutilized waste from brewing and its use can be considered an economical source for the production of 5'-ribonucleotides and other compounds on a large scale in order to meet the growing demand for new inputs for food and pharmaceutical industries, as well as for the development of biorefineries, using simple and efficient strategies that guarantee economic viability. Therefore, it is necessary and justifiable the development of multidisciplinary and integrated research, aiming, in the medium and long term, to develop new products with more diversified functional and nutritional characteristics from this biomass. In view of the exposed characteristics, the present work appears as an attempt to answer the question: it is possible to establish a methodology capable of fractioning efficiently and with low cost the spent brewer's yeast into by-products of high added value? The yeast fractionation approach is promising because the byproducts have a higher value than whole yeast. The extract, for example, can be marketed in powder or paste to flavor meat in soups. The sale of the extract can yield up to R$ 8.00 per kilo, while the current price, with unprocessed yeast, reaches the value of R$ 0.80 per kilo. Yeast processing can at least double its marketing value or even reach 10 times more for other uses in food or pharmaceuticals.

In order to answer the posed question, the present work reviews the biotechnological potentials of yeast utilization in the Chapter 1. The Chapter 2 presents the results of the initial stages of preparation and characterization of yeast from the brewing industry, as well as the evaluation of yeast autolysis parameters in order to maximize RNA extraction for subsequent 5'-ribonucleotides production. The Chapter 3 describes the use of acid shock at the beginning of autolysis in order to accelerate the autolytic process and the extraction of proteins. The results of enzymatic hydrolysis of RNA to produce 5'-ribonucleotides using 5'-phosphodiesterase from residual malted

barley roots is presented in the Chapter 4. The recovery of extracellular β-fructofuranosidase enzyme as the first by-product before autolysis and studies on its immobilization in sodium alginate and activated charcoal beads were described in the Chapter 5. Finally, an economic analysis, as well as a bioprocess plant design for yeast processing were presented in the Chapter 6 in order to determine the profitability and financial viability of the methodology developed in this work. The proposed approach indicates an excellent strategy, since a better use of spent yeast from fermentation processes in the proposed biorefinery plant can open up a range of applications and add value to this product, making the industrial sectors more profitable.

2 GENERAL OBJECTIVE

The general objective of this work was to study and design an integrated bioprocess methodology using spent brewer's yeast biomass as feedstock for obtaining several biomolecules through fractionation and downstream techniques: yeast extract rich in proteins, *β*-fructofuranosidase, RNA and 5'-ribonucleotides.

2.1 SPECIFIC OBJECTIVES

- Reviewing the possible biotechnological applications of spent brewer's yeast;
- Evaluation of different cell disruption methods such autolysis and acid hydrolysis regarding the efficiency to obtain maximum RNA yields;
- Evaluation of the addition of acid at the beginning of the autolytic process aiming to accelerate the autolysis and high yields of yeast extract;
- Production of 5'-ribonucleotides by enzymatic hydrolysis of RNA using 5'-phosphodiesterase from spent malted barley roots.
- Recovering of extracellular β-fructofuranosidase enzyme before autolysis and evaluate its immobilization in sodium alginate and activated charcoal beads.
- Designing a bioprocess plant for spent brewer's yeast processsing and performing an economic analysis in order to determine the profitability and financial viability of the methodology developed under optimized conditions.

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