EXPLORING THE PRODUCTION OF BIOSURFACTANT BY YEAST IN NON-DETOXIFIED HEMICELLULOSIC HYDROLYSATE OF SUGAR CANE BAGASSE: A Proposal for Technological Innovation.

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ABSTRACT

Biosurfactants are amphiphilic microbial metabolites, meaning they are molecules that have both hydrophilic and hydrophobic groups. Due to their structure, biosurfactants exhibit excellent surfactant, emulsifying, and antimicrobial properties. Their main industrial applications include use as emulsifiers, foaming agents, detergents, dispersants, and antimicrobials. Additionally, biosurfactants have advantages over synthetic surfactants due to their eco-friendly characteristics, as they are biodegradable, non-toxic, biocompatible, and can be produced through fermentation processes using agro-industrial by-products as raw materials, such as the hemicellulosic hydrolysate of sugarcane bagasse. However, biosurfactant production from hydrolysate still faces limitations since the non-detoxified hydrolysate obtained from acid pretreatment contains compounds such as furans and phenolics at concentrations that can impair the performance of fermentation processes. Thus, the present work aims to evaluate the production of biosurfactants in non-detoxified hemicellulosic hydrolysate of sugarcane bagasse by the yeast *Scheffersomyces shehatae*.

Keywords: Biosurfactants, non-detoxified hemicellulosic hydrolysate, sugarcane bagasse, inhibitory compounds, *Scheffersomyces shehatae*

1 INTRODUCTION

Due to the specific physical and chemical characteristics they possess, surfactant compounds are widely used in industry, households, and various other areas. This implies that these compounds will inevitably be dispersed in various environments, which have negative impacts on wildlife, especially aquatic organisms.[1] Due to these toxic and polluting characteristics, in recent years there has been interest in the development and production of environmentally friendly processes and compounds, such as microbial biosurfactants (BS).

BS are considered sustainable products because they can be obtained from various types of microorganisms, such as bacteria, filamentous fungi, and yeasts, in fermentation processes that use agro-industrial by-products as raw materials [2]. Different microorganisms can produce BS, but the use of yeasts offers the great advantage of not presenting a risk of toxicity or pathogenicity [3].

Currently, the industry is still in the early stages of processing biosurfactants from hemicellulosic hydrolysates. A major obstacle remains the high cost of production and recovery, with raw materials typically representing 30% of these costs. Consequently, research has focused on producing biosurfactants and improving yields from low-cost substrates, such as lignocellulosic biomass [4]. However, utilizing lignocellulosic by-products requires a pretreatment step to degrade the lignocellulosic structure. Various pretreatments exist for lignocellulosic biomass, with acid pretreatment demonstrating high yields in sugar content, resulting in acidic hydrolysates [5]. However, these hydrolysates also contain inhibitory compounds, such as phenolic compounds and furans, which can interfere with the fermentation process. Although detoxification improves the fermentability of hydrolysates, it is economically desirable to minimize detoxification requirements [6] [7].

Therefore, considering the premises presented about the potential use of hemicellulosic hydrolysates and the need to seek more economical alternatives, the present work aims to evaluate the production of BS in non-detoxified hemicellulosic hydrolysate from sugarcane bagasse by the yeast *Scheffersomyces shehatae*, aiming to reduce process costs, as well as to gain a better understanding of the biotransformation mechanisms involved in the metabolism of furanic and phenolic compounds.

2 MATERIAL & METHODS

The fermentations were conducted in 50 mL Erlenmeyer flasks containing 10 mL of the culture medium proposed by Kitamoto *et al*. (1990) (g/L: monopotassium phosphate, 2; yeast extract, 1; ammonium nitrate, 20; magnesium sulfate heptahydrate, 2) supplemented with non-detoxified hemicellulosic hydrolysate with an initial xylose concentration of 40 g/L. Approximately 1 g/L of cells were inoculated into the fermentation flasks, The flasks were incubated in a rotary shaker at 200 rpm and 30 ºC for a period of 144 hours [8]. During this period, samples were collected at time intervals of 4, 8, 12, 24, 36, 48, 72, 96, 120, and 144 hours, with each sampling point being performed in triplicate. At the end of each sampling time, the fermentations were interrupted by centrifugation at 3500 rpm for 15 minutes to separate the cell biomass and the supernatant.

The quantification of specific monosaccharides (xylose, glucose, and arabinose) was performed using an Agilent Technologies 1200 series HPLC system equipped with a refractive index detector and a Bio-Rad Aminex HPX-87H column. To determine the levels of furans (furfural and 5-hydroxymethylfurfural) and phenolic compounds (4-hydroxybenzoic acid, gallic acid, ferulic acid, vanillic acid, p-coumaric acid, syringaldehyde, vanillin, and pyrocatechol), the same HPLC apparatus was used. This system was configured with a quaternary pump and a UV detector set to 276 nm, employing a Zorbax Eclipse C18 column (4.6 × 250 mm) at 30 °C [9].

Consequently, to demonstrate biosurfactant production, surface tension tests were conducted using a bench tensiometer. Additionally, emulsification tests were performed by mixing 1 ml of kerosene with 1 ml of the sample and vortexing for 2 minutes. The resulting emulsion was measured in centimeters [10] [11]. All the described procedures are illustrated in Figure 1.

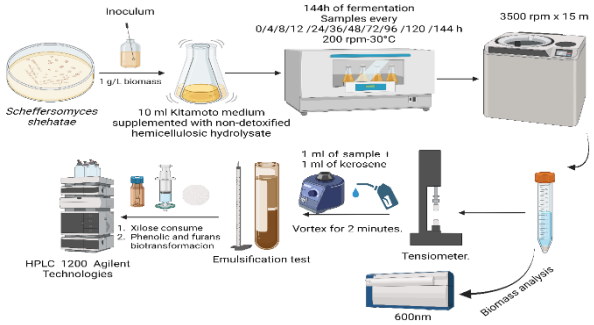

Figure 1 Fermentation process of non-detoxified hemicellulosic hydrolysate from sugarcane bagasse.

3 RESULTS & DISCUSSION

Fermentation was carried out in non-detoxified hemicellulosic hydrolysate from sugarcane bagasse, evaluating the kinetic growth profile and the production of BS (IE24 and surface tension). Regarding microbial growth. As shown in Figure 2a, after 72 hours, the amount of xylose present in the medium was almost completely consumed, demonstrating that the yeast has the ability to consume xylose even in the presence of inhibitory compounds in higher concentrations [12].

Regarding the surfactant and emulsifying properties of the BS produced in cultures using non-detoxified hemicellulosic hydrolysate from sugarcane bagasse, a slight alteration in the surface tension value was also observed. At the beginning of fermentation, the surface tension was 72.8 mN/m, but from 96 hours of cultivation, it was reduced to 61.3 mN/m. As for the emulsifying action of the BS, an IE24 of 6% was obtained from 36 hours of fermentation, and throughout the process, the values increased to approximately 70 - 80% (Figure 2b).

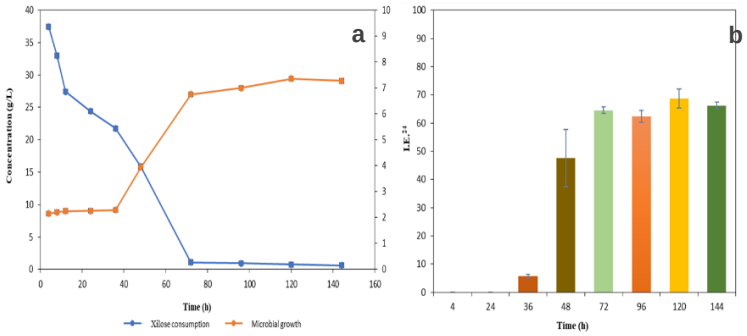

Figure 2 Analysis of the kinetic profile by the yeast *S. shehatae* in non-detoxified hemicellulosic hydrolysate from sugarcane bagasse-based medium. Evaluation of microbial growth, xylose consumption (a), and emulsification index (b).

As observed in Figure 3, the different inhibitory compounds showed reductions in their concentrations throughout the fermentation process. Regarding gallic acid, it is observed that after 144 hours, there was a reduction of almost 40% compared to its initial concentration, p-catechol 54%, vanillic acid 67%, and p-coumaric acid 63%. It should be noted that 5-HMF was completely metabolized and/or bio transformed during the fermentation process. These results may indicate the yeast's need to produce BS in advance to metabolize and/or biotransform the insoluble inhibitory compounds present in larger quantities in non-detoxified hemicellulosic hydrolysate from sugarcane bagasse [13].

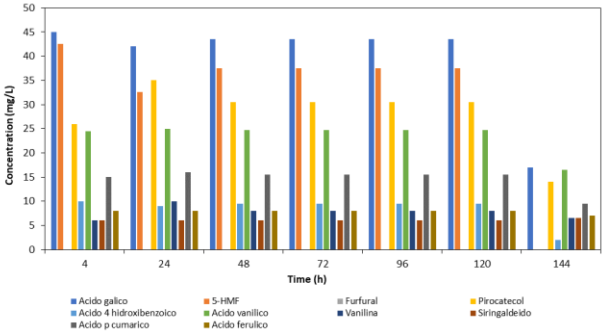

Figure 3 HPLC analysis of various phenolic compounds and furans during the fermentation process by the yeast *Scheffersomyces shehatae* in non-detoxified hemicellulosic hydrolysate from sugarcane bagasse.

4 CONCLUSION

Based on the results obtained so far, the present study showed that the yeast *S. shehatae* can produce BS in a culture medium supplemented with non-detoxified hemicellulosic hydrolysate from sugarcane bagasse. On the other hand, an increase in BS production was also noted, demonstrating the yeasts' ability to function under conditions with higher concentrations of phenolic compounds and furans. The results obtained contribute to the sustainable production of BS according to the biorefinery concept and indicate that it is possible to eliminate the detoxification step, thereby reducing process costs.

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