POTENTIAL OF *Yarrowia lipolytica* FOR BIOSURFACTANT AND XYLITOL PRODUCTION IN BIOREFINERIES

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ABSTRACT

Synthetic surfactants are petroleum derivatives, amphipathic molecules, with high toxicity and exhibit a difficult decomposition. These molecules present tensioactive and/or emulsifier properties, with a variety of applications in many industrial sectors. However, because of the environmental damage caused by these compounds, the search for ecologically sustainable molecules has been intensified. That's the case of biosurfactants, which are biologically originated molecules with biodegradable characteristics, high biocompatibility, with low or null toxicity and presenting the same or better physical-chemical properties than synthetic surfactants. These biomolecules can be synthesized in fermentation processes, using as nutritional source agricultural and industrial byproducts. Therefore, the present project was made using lignocellulosic biomass combined with hydrophobic substrate or vinasse as feedstock to the sustainable production of biosurfactants in the context of biorefineries, using *Yarrowia lipolytica*, as the fermentative agent.

Keywords: Biosurfactants. Biorefineries. Sugarcane. Vinasse.

1 INTRODUCTION

Surfactants are amphipathic molecules, formed by hydrophobic and hydrophilic parts. Because of this dual structure, these molecules can reduce the superficial and interfacial tension, besides acting as an emulsifier agent[1]. However, synthetic surfactants, produced by the organic synthesis of petroleum derivatives, are harmful to the environment, presenting low biodegradability, low biocompatibility, and high toxicity, causing problems to the environment[2]. That's why there was an increase in the interest of natural surfactants, once they present high biodegradability, low toxicity, and better properties in extreme conditions regarding temperature, pH and saline concentration and, because of that, biosurfactants are known as sustainable and ecofriendly products[3].

Generally, biosurfactants produced by yeasts may present *GRAS* status (Generally Recognized as Safe), meaning that these molecules are classified as non-toxic or non-pathogenic, allowing an unrestricted application of these compounds, including food and pharmaceutical industry[4]. Literature describes the unconventional yeast *Yarrowia lipolytica*, which has *GRAS* status, as a versatile organism because of the variability of high-value compounds it can produce, including organic acids and lipases, indicating that it may be the standard for biosurfactant production in biorefineries[5,6].

In the last decade, the Bioprocesses and Sustainable Products Laboratory (*LBios)* has been researching the utilization of lignocellulosic biomass, such as sugarcane bagasse, and vinasse, a byproduct of ethanol industry, to the development of processes and products of economic and ecological interests, in addition to the concretization of biorefineries in the national scene. Thus, this project was made to further comprehend sustainable biosurfactant production, using *Yarrowia lipolytica* as the fermentative agent in cultures containing sugarcane bagasse hemicellulosic hydrolysate, hydrophobic substrate, and vinasse as nutritional source.

2 MATERIAL & METHODS

The sugarcane bagasse used in this work was characterized[7]. The characterized sugarcane bagasse was pre-treated using an acid hydrolysis process[7] by adding a sulfuric acid solution in a stainless-steel reactor at 121 ºC, 50 rpm, for 20 minutes obtaining the hemicellulosic hydrolysate (SBHH). The solid fraction (cellulignin) obtained was treated with sodium hydroxide (0.12 g/g)[7], holocellulose and the liquid fraction (SBHH) resulting were reserved for concentration and detoxification. The SBHH was concentrated and detoxicated using sodium hydroxide, concentrated phosphoric acid, and activated carbon to remove compounds that inhibit cell growth.

The physical-chemical characterization of vinasse was made analyzing conductivity, pH, fraction of insoluble solids, concentration of total sugars[8], concentration of reducing sugars[9], concentration of proteins[10], and concentration of lipids[11].

Yarrowia lipolytica cells were cultivated in medium[12] containing sugarcane bagasse hemicellulosic hydrolysate, vinasse, soybean oil and a mixture of residual oils, at 150 rpm, 27 ºC, for 168 hours. The interruption time of the fermentative process was different for each medium. The flasks were centrifuged, and the fermentation broth was removed. The cellular concentration was determined by gravimetry methods[13], and the emulsification index was determined on the supernatant by an emulsion with kerosene.

Monosaccharides sugars (xylose, glucose and arabinose), phenolic compounds (gallic acid, pyrocathecol, 4-hidroxybenzoic acid, vanillic acid, vanillin, p-coumaric acid, ferulic acid and syringaldehyde) and furans (furfural and 5-hidroxy-methyl-furfural), that were present in the hydrolysate and fermentation broth supernatant, were quantified using HPLC[7].

3 RESULTS & DISCUSSION

It is shown in Table 1 the physical-chemical characterization of the SBHH before and after detoxification of furans and phenolic compounds.

Table 1 Physical-chemical characterization of SBHH before and after detoxification

Components	Sugarcane bagasse hemicellulosic hydrolysate	
	Concentrated and non-detoxified (g/L)	Concentrated and detoxified (g/L)
Gallic acid	0,0695 ± 0,0334	0,0457 ± 0,0012
5-HMF	0,0105 ± 0,0115	0,0092 ± 0,0008
Furfural	0,1268 ± 0,0622	0,0578 ± 0,0086
Pyrocatechol	0,04 ± 0,0219	0,0148 ± 0,0031
4-hidroxybenzoic acid	0,0288 ± 0,0013	0,012 ± 0,0
Vanillic acid	0,0743 ± 0,0281	0,0263 ± 0,0005
Vanillin	0,029 ± 0,0230	0,006 ± 0,0
Syringaldehyde	0,0278 ± 0,0242	0,0033 ± 0,0026
P-coumaric acid	0,017 ± 0,0142	0,0025 ± 0,0020
Ferulic acid	0,0285 ± 0,0235	0,005 ± 0,0
Glucose	8,71	6,44
Xylose	67,8	61,29
Arabinose	9,81	7,39

In cultures of *Yarrowia lipolytica* containing hydrophobic carbon sources, there was the formation of oil droplets surrounded by cellular aggregates. This fact may explain a change in the hydrophobicity of the cell surface, proven after centrifugation of the medium containing SBHH and vegetable oils, due to the formation of a layer of biomass in the middle of the aqueous and oily layers. The phenomenon of hydrophobization is related to the presence of protrusions on the surface of cells present in culture media with hydrophobic substrates. These protrusions can be characterized as channels that connect the cell wall to its interior, probably participating in the consumption of oils[14]. It should be noted that the change in hydrophobicity in some cultures prevented the determination of cell biomass, as it made it difficult to separate cells and the supernatant. After the end of fermentation, a variation in pH was observed in relation to the cultivation medium (Figure 1). *Y. lipolytica* can produce citric acid during its growth[15], explaining the decrease in pH in medium supplemented with cellulosic hydrolysate containing soybean oil and oil mixture. The increase in pH in some crops can be explained by some nitrogenous metabolites produced by Y. lipolytica during fermentation, such as amino acids[16].

In Figure 1, the consumption of sugars by *Y. lipolytica* during the production of biosurfactants can also be seen. The sugars consumed in greatest quantities were xylose and glucose. It is noteworthy that in the cultivation medium containing the mixture of Vinasse + SBHH, 38% of the xylose was consumed after 168 h of cultivation and it was noted, in addition to the production of biosurfactant with an emulsification index of 60%, the presence of 5.84 g/L of xylitol. These results demonstrate the potential of using vinasse and SBHH to produce biosurfactant and xylitol. Due to the highlighted emulsifying characteristics, the biosurfactant produced by *Y. lipolytica* under the studied conditions can be considered a good emulsifier.

The results obtained so far demonstrate the potential for using *Y. lipolytica* in lignocellulosic biorefineries to obtain biosurfactants and other value-added products, such as xylitol.

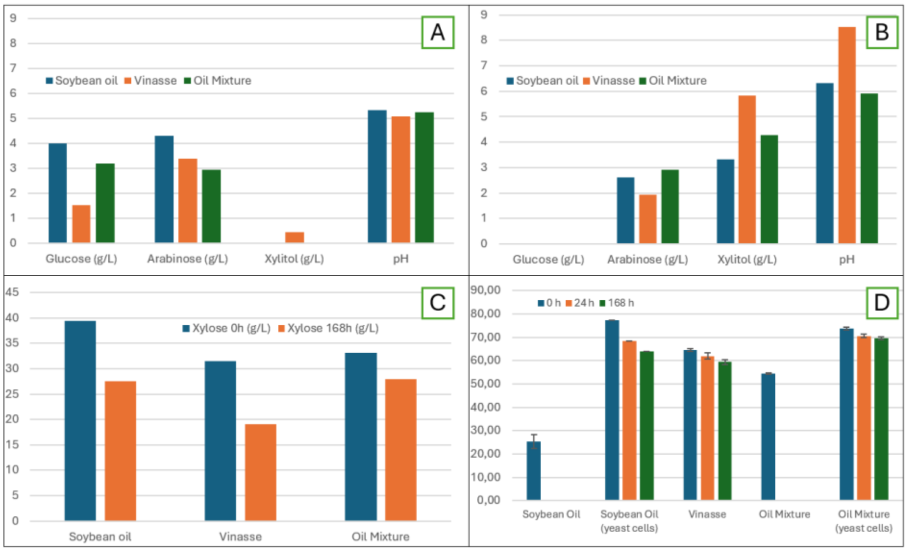

Figure 1 : (A) Initial concentration of sugars and pH present in the cultivation medium ;(B) Final concentration of sugar and pH present in the cultivation medium; (C) Evaluation of the xylose concentration in the beginning and ending of the fermentation process; (D) Emulsification indexes of the biosurfactant present in the fermentation medium

4 CONCLUSION

Among the results presented, the oleaginous yeast *Yarrowia lipolytica* revealed to be a promising alternative for biorefineries, as it can use vinasse, a by-product of ethanol, in addition to sugarcane bagasse hemicellulosic hydrolysate, for the biosurfactant production, a high-added value product. Furthermore, the change in the cellular hydrophobicity in media containing hydrophobic sources and the formation of cellular aggregates are prime examples of the possibility in using *Yarrowia lipolytica* for the ecologic and sustainable treatment in marine ecosystems in cases of oil spills.

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