PRODUCTION AND CHARACTERIZATION OF PULLULAN PRODUCED FROM SUGARCANE BAGASSE ENZYMATIC HYDROLYSATE

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ABSTRACT

The strain *Aureobasidium pullulans* ATCC 42023 was employed to produce pullulan using xylose as the carbon source and $NaNO_3$ as the nitrogen source. Favorable results were achieved in terms of producing pullulan with low melanin content. Additionally, the production of exopolysaccharides (EPS) from enzymatic hydrolysate of sugarcane bagasse (SCB) was evaluated. The yield from the hydrolysate reached 16.02 ± 1.36 $g \cdot L^{-1}$ in 168 hours, resulting in pullulan with a white color. Further analysis using Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and thermogravimetric analysis (TGA) confirmed that the EPS obtained exhibit characteristics consistent with pullulan. These results collectively demonstrate the potential of using SCB enzymatic hydrolysate as an effective medium for the sustainable production of high-quality pullulan.

Keywords: *A. pullulanas* ATCC 42023. Pullulan. Sugarcane bagasse enzymatic hydrolysate.

1 INTRODUCTION

Pullulan is an exopolysaccharide (EPS) composed of α-(1–6) linked units of maltotriose (an α-(1–4) linked glucose trisaccharide) and is produced by strains of Aureobasidium pullulans. This biopolymer possesses intriguing intrinsic properties such as biodegradability, renewability, and biocompatibility. Specifically, pullulan, due to its non-toxic, hydrophilic, and biodegradable nature and its ability to avoid triggering an immunological response, finds widespread use in diverse industrial sectors including food and pharmaceuticals. In the pharmaceutical industry, its primary application lies in drug delivery systems where it serves as an encapsulant for controlled drug release.[1]

The increasing interest in pullulan production arises from its diverse applications. However, the production of pullulan still poses significant challenges, particularly in terms of concurrent melanin production, an undesirable biopigment at the industrial scale. Therefore, current research focuses on developing processes to produce melanin-free pullulan. Successful EPS production from fermentations hinges on both the intrinsic characteristics of the microbial species and specific fermentation conditions. Crucial factors include nitrogen and carbon sources, which are closely linked to melanin production during the process.

In this study, pullulan production was evaluated using SCB enzymatic hydrolyzate as a carbon source. Sugarcane bagasse was chosen due to its abundance as a by-product in Brazil. Initially, experiments were carried out using xylose, present in the enzymatic hydrolysate, as a substrate to evaluate the capacity of the microorganism to metabolize this pentose.

2 MATERIAL & METHODS

The strain A. pullulans ATCC 42023 was utilized for pullulan production. Initially, tests were conducted using NaNO3 as the nitrogen source and xylose as the carbon source at varying concentrations to achieve melanin-free pullulan production. This aimed to utilize xylose (and glucose) derived from the enzymatic hydrolysate of sugarcane bagasse. Subsequently, the enzymatic hydrolysate of sugarcane bagasse (SCB) was employed for EPS production. The hydrolysate, prepared at LBBSIM/EEL-USP Laboratory following the methodology of Prado *et al.*[2] contained 120 $g \cdot L^{-1}$ of glucose and 60 $g \cdot L^{-1}$ of xylose. Fermentation conditions were based on prior studies, starting with a total sugar concentration of 133.6 $g \cdot L^{-1}$ and 3 $g \cdot L^{-1}$ of $NaNO_3$, lasting 168 hours, under conditions of 200 rpm and 26 ºC. Finally, pullulan was recovered using the methodologies outlined by Terán-Hilares *et al.*[3] and characterized through FTIR, DR-X, and TGA analyses.

3 RESULTS & DISCUSSION

Table 1 presents the production of pullulan from xylose and $NaNO_3$ at various concentrations. Lower concentrations of the nitrogen source resulted in higher pullulan production, albeit with elevated melanin content. Conversely, increasing the nitrogen concentration to 3 $g \cdot L^{-1}$ ($NaNO_3$) reduced pullulan production but lowered melanin content. According to several authors, nitrogen acts as a stimulant for EPS production and affects the morphology of A. pullulans. Excessive nitrogen levels can increase biomass production instead of pullulan and intensify carbon source consumption,.[4,5] Other studies concluded that the carbon source influences UDP-glucose biosynthesis, the precursor enzyme for forming pullulan chains. Therefore, using xylose as a carbon source lengthens the biosynthetic pathway, ultimately reducing pullulan synthesis.[6]

Next, sugarcane bagasse enzymatic hydrolysate was used as a carbon source, which is rich in glucose and xylose. As shown in Table 2, pullulan with low melanin content was obtained, along with greater biomass production. Compared to the LB83 strain reported by Terán-Hilares et al.[7] the authors obtained 22 g·L^{-1} of pullulan from the sugarcane bagasse cellulosic hydrolysate, with glucose and xylose in a 2:1 ratio, under white light conditions over 168 hours. In comparison to the ATCC 42023 strain, the fermentation process was satisfactory, as the pullulan values with low melanin content are comparable to those obtained using hydrolysates from other strains.

Table 1. Production of biomass, pullulan, melanin and consumption of sugars in fermentation with xylose concentrations. Experiments carried ot with 0.38 and 3 g·L^{-1} of $NaNO_3$ and 0.2 g·L^{-1} of NaCl, in 168h; 1 g·L^{-1} of inoculum; 200rpm; temperature 26 ºC; pH 5.5.

Exp	Xilose (g·L^{-1})	$NaNO_3$ (g·L^{-1})	Biomassa (g·L^{-1})	Pululana (g·L^{-1})	Melanina ($U_{ABS}/g_{pululana}$)	Consumo de xilose (%)
1	90	0,38	11,00±1,33	31,77±2,40	0,434±0,067	45,01±0,66
		3,00	21,09±0,24	10,12±0,92	0,285±0,054	68,24±2,81
2	110	0,38	12,99±0,52	29,02±1,49	0,409±0,076	30,48±0,88
		3,00	23,18±1,67	12,91±1,18	0,227±0,008	45,87±5,74
3	130	0,38	10,97±0,68	29,39±0,33	0,424±0,016	32,95±1,57
		3,00	21,48±0,75	19,29±1,43	0,191±0,010	52,53±1,53

Figure 1. Pullulan production from different concentrations of xylose and $NaNO_3$, in 168h of cultivation. a) concentration of 0.38 g·L-1 of $NaNO_3$, with high melanin production; b) concentration of 0.38 g·L^{-1} of $NaNO_3$, with low melanin production.

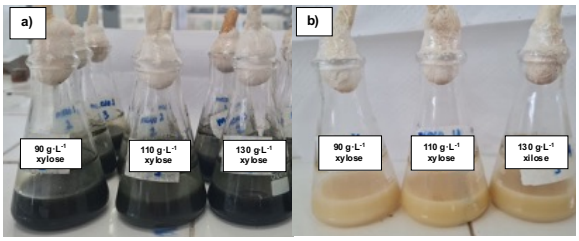
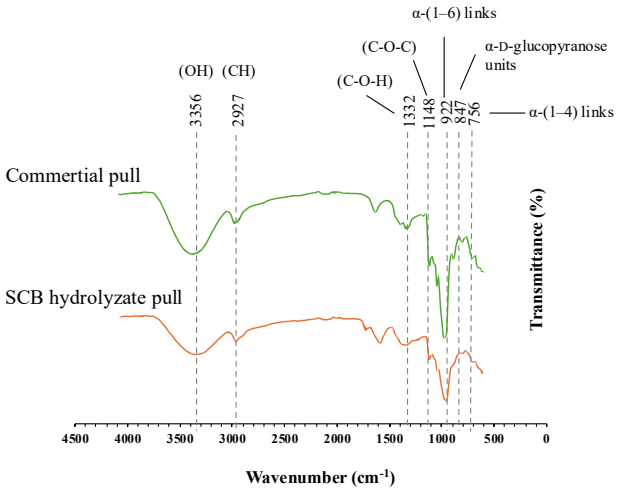


Table 2. Production of biomass, pullulan, melanin and sugar consumption in 168 h in a medium based on SCB enzymatic hydrolyzate. Experiments carried with 3 g·L^{-1} of $NaNO_3$ and 0.2 g·L^{-1} of NaCl, in 168h; 1 g·L^{-1} of inoculum; 200rpm; temperature 26 ºC; pH 5.5.

Biomass (g·L^{-1})	Pullulan (g·L^{-1})	Melanin ($U_{ABS}/g_{pululana}$)	Sugar consumption (%)
35,30±2,67	16,02±1,36	0,180±0,066	72,55±0,21

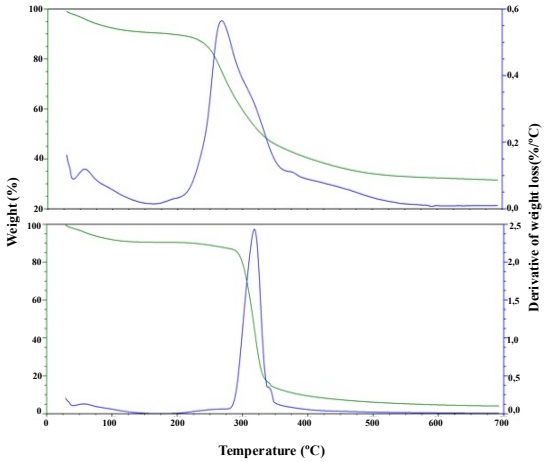
Subsequently, FTIR, XRD, and TGA analyses were conducted on the EPS obtained from sugarcane bagasse hydrolysate. Figure 2 shows the FTIR analysis, comparing commercial pullulan with the pullulan obtained in this study. Characteristic pullulan bonds were observed with absorption bands at 756 cm^{-1} and 922 cm^{-1}, corresponding to the α-(1–4) and α-(1–6)-D-glycosidic bonds, in addition to other functional groups. These results indicate that the EPS is primarily composed of pullulan.

Figure 2. FTIR analysis to identify functional groups in commercial pullulan and pullulan produced from a medium based on SCB enzymatic hydrolyzate.



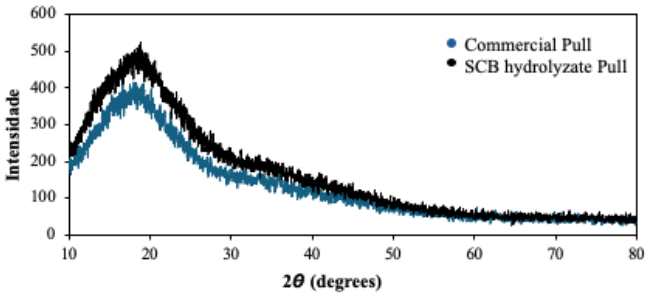
The TGA analysis (Figure 3) demonstrated that the initial thermal decomposition temperature (Td) of commercial pullulan occurred at approximately 200°C, while pullulan obtained from a sugarcane bagasse-based medium decomposed sharply starting at around 290°C. This Td was like the values reported by Kumar et al.[8] for pullulan produced from corn bran. This suggests that the molar mass and origin of pullulan production can result in differences in its structural properties and, therefore, alter the TGA curve.[9]

Figure 3. TGA analyzes for samples of a) commercial pullulan; b) pullulan produced from SCB enzymatic hydrolysate.



As shown in Figure 4, commercial pullulan and pullulan obtained from sugarcane bagasse enzymatic hydrolysate maintain the same amorphous structure, presenting a particular peak at 2θ = 19. According to Priyadarshi, Kim and Rhim[10], this peak corresponds to the accommodation of pullulan chains linked by H bonds.

Figure 4. X-ray diffraction patterns for samples of commercial pullulan and pullulan produced from SCB enzymatic hydrolysate.



4 CONCLUSION

The nitrogen source has a significant impact on the concomitant production of pullulan and melanin. In *Aureobasidium pullulans* ATCC 42023, the type and concentration of nitrogen source cause changes in the morphology of the microorganism and, consequently, influence the yield of low-melanin pullulan. This microorganism is capable of metabolizing xylose to produce pullulan, particularly when higher concentrations of $NaNO_3$ are used (3 $g \cdot L^{-1}$). Moreover, the SCB enzymatic hydrolysate can be utilized by *A. pullulans* ATCC 42023 as a carbon source for pullulan production, resulting in an exopolysaccharide with profiles like those of pullulan.

Further characterization of the pullulan produced from SCB enzymatic hydrolysate using advanced analytical techniques such as FTIR, XRD, and TGA confirmed that the structural and thermal properties are consistent with those of commercially available pullulan. These findings validate the effectiveness of using sugarcane bagasse hydrolysate in producing high-quality pullulan, thereby expanding the potential applications of this biopolymer in various industries.

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