**Xylitol Production From Rice Straw Hemicellulosic Hydrolysate Using Cells of *Candida Guilliermondii* Permeabilized With Triton X-100.**

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**Highlights**

* The cellular permeabilization medium affects xylitol production in hydrolysate.
* Permeabilized cells increases xylitol productivity by 40 % and yield by 32 %.
* High xylitol productivity (2.0 g/L.h) was obtained with permeabilized cells.
* Permeabilized cells can be reused for at least 4 cycles with preserved viability.

**1. Introduction**

Xylitol is a five-carbon sugar alcohol with notable applications in the food, medical and pharmaceutical areas. The production of xylitol by biotechnological route is based on the use of whole cells of microorganisms (fermentation process) or by its isolated enzymes (enzymatic technology). The fermentation process has shown significant advances but finds commercial constraints due to low productivities. On the other hand, costs of isolation/purification of enzymes has been pointed as the major barriers to the enzymatic technology. Therefore, it is important to develop an alternative method (biotransformation process) to make the biotechnology route more attractive. In our previous study [1] was demonstrated that under selected process conditions, resting cells of *Candida guilliermondii* permeabilized with Triton X-100 is able to efficiently produce xylitol from a medium composed only by D-xylose, potassium phosphate buffer and MgCl2.6H2O. In the present study, the potential use of the rice straw hemicellulosic hydrolysate (RSHH) as xylose source to produce xylitol from *C. guilliermondii* permeabilized with Triton X-100 was evaluated. The effects of permeabilization medium (semi-defined medium or hemicellulosic hydrolysate), as well as the addition of potassium phosphate buffer and/or MgCl2.6H2O on the RSHH biotransformation was evaluated as an attempt to improve the results of this biotransformation process. The reuse of the biocatalyst in repeated batch was also assessed.

**2. Methods**

The rice straw hemicellulosic hydrolysate (RSHH) was obtained under conditions previously optimized by our research group. Initially, rice straw was treated with 80 mg NaOH/g of biomass, at 70 °C for 45 min. Then, the solids recovered were hydrolyzed with 85 mgH2SO4/g solid, at 150 °C for 10 min. Both treatments were performed in a batch reactor with liquid to solid ratio of 10:1. Cells of *C. guilliermondii* ATCC 201935 were permeabilized by adding Triton X-100 directly to the growth medium at cell to surfactant ratio of 1:0.9 g/g [1]. In this step, two different growth media were assessed: (1) semi-defined medium (SDM), containing in g/L: 30.0 xylose, 1.0 MgCl2·6H2O, 3.0 (NH4)2SO4, 0.1 CaCl2.2H2O, 20 % (v/v) rice bran extract and 0.1 M potassium phosphate buffer (pH 6.5); and (2) rice straw hydrolysate hemicellulosic (RSHH) containing 30 g/L xylose without any supplementation. After defining the best permeabilization medium, assays of biotransformation were carried out. For this, hemicellulosic hydrolysate (56.0 g/L D-xylose, pH 6.8) and permeabilized cells in MSD (10-12 g/L) were added to a 50-mL Erlenmeyer flask to a final volume of 25 mL. The flasks were incubated in a rotary shaker at 200 rpm, 35°C for 21 h. In this step, the effect of potassium phosphate buffer and MgCl2 .6H2O on biotransformation parameter, as well as the reusability of the permeabilized cells were also assessed. The reusability of the permeabilized cells was evaluated in RSHH without any salt addition. At each 15 h, cells were collected by centrifugation (5600g, 10 °C, 20min), washed twice with 0.1 M potassium phosphate buffer pH 6.5, and then recycled for new reaction medium. Xylose, xylitol and sub-products concentrations were measured using HPLC.

**3. Results and discussion**

As shown in Table 2, the growth medium used for cellular permeabilization signiﬁcantly affects the biotransformation parameters of rice straw hemicellulosic hydrolysate (RSHH). The highest QP values (2.10 g/L.h) was achieved with permeabilized cells in semi-defined medium (SDM), corresponding to increse of 34.8% in relation to permeabilized cells in RSHH. It can be noted also that the biocatalysts efficiency was not influenced by permeabilization medium showing similar YP/S values (about 0.57 g/g. These results suggest that affinity of the detergent to the cell wall/membrane was reduced in hemicellulosic hydrolysate.

**Table1.** Effect of permeabilization medium of *C. guilliermondii* on biotransformation parameters of RSHH, after 15 h.

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| --- | --- | --- |
| Biotransformation Parameters | Permeabilization medium | |
| **MSD** | **RSHH** |
| Xylitol production (g/L) | 30.8±2.05 (21.94±0.60) | 23.7±0.11 |
| Xylose utilized (%) | 93±1.7 (85±0.5) | 75 |
| YP/S (g/g) | 0.57±0.03 (0.43±0.01) | 0.56±0.01 |
| QP (g.L.h) | 2.10±0.13 (1.47±0.04) | 1.38±0.01 |

Values between parentheses correspond to data from non-permeabilized cells.

In this study, was also verified that the addition of potassium phosphate buffer and MgCl2.6H2O to the RSHH did not affect the biotransformation parameters, being observed a average production of xylitol of 29 g/L and 92 % of xylose utilized. As compared to the non-permeabilized cells, the impact of using permeabilized cells on xylitol production from RSHH was an increase of 40 and 32 % on QP and YP/S, respectively. Besides, permeabilized cells remained viable during 4 cycles of 15 h in repeated batch maintaining its catalytic activity (QP~1.93 g/L.h and YP/S~0.59 g/g).

**4. Conclusions**

The yeast *C. guilliermondii* permeabilized with Triton X-100 proved to be an effective biocatalyst for xylitol production from rice straw hemicellulosic hydrolysate. Moreover, the permeabilized cells may be reused up to 4 consecutive reaction cycles without reducing production rates and yields. This approach may allow the future development of xylitol production from xylose present in lignocellulosic biomass, with additional potential for implementation in biorefinery.

**References**

1. D.V. Cortez, S.I. Mussatto, I.C. Roberto, Appl. Biochem. Biotechnol. 180 (2016) 969-979.

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