IMMOBILIZATION OF A BACTERIAL INVERTASE IN SUGARCANE BAGASSE FOR INVERT SUGAR PRODUCTION

Pedro Henrique C. Moraes[1], Natanael, C. S. Gomes[1], Larissa, S. Saraiva[2], Rafaela R. Pinto[2], Catarina F. M. L Carvalinho[2], Gabriela E. Lacerda[1], Alex F. Almeida[2], Rafael F. Perna[3], Michelle C. A. Xavier[2], Sergio. A. V. Morales[2,3]*

[1]*Federal University of Tocantins, Food Engineering Department, Palmas, Tocantins, Brazil.*
[2]*Federal University of Tocantins, Graduate Program in Food Science and Technology, Palmas, Tocantins, Brazil.*
[3]*Federal University of Alfenas, Graduate Program in Chemical Engineering, Institute of Science and Technology, Poços de Caldas Campus, Minas Gerais, Brazil.*
** Corresponding author's email address: sergio.morales@mail.uft.edu.br*

ABSTRACT

Invertase is an enzyme of great industrial interest since it catalyzes the sucrose hydrolysis reaction to produce invert sugar. Furthermore, the immobilization of this enzyme improves its biotechnological applications as it can provide operational advantages such as greater operational and storage stability and enable its recovery and reuse. This work studied the immobilization by physical adsorption of invertase from *B. tequilensis*, isolated from the peach palm fruit (*Bactris gasipaes*), in alkaline treated sugarcane bagasse. After 8 hours of immobilization, 73.80% and 58.43% of immobilization yield and recovered activity, respectively, were obtained. The immobilized invertase showed stability for 9 days of storage under refrigeration whereas the soluble invertase activity decreased rapidly. Furthermore, the immobilized enzyme showed 31% of its initial activity in the second cycle of reuse. These results indicate that sugarcane bagasse has potential as support material for the immobilization of the bacterial invertase from *B. tequilensis* aiming the production of invert sugar via heterogeneous enzymatic systems.

Keywords: Invertase. Invert sugar. Immobilization. Sugarcane bagasse.

1 INTRODUCTION

Invert sugar has become an industrially advantageous product for food fabrication because it is highly soluble in water, do not crystallize and has a great sweetening capacity. This sugar can be produced by the hydrolysis of sucrose molecules catalyzed by enzymes invertase (β-fructofuranosidase, EC 3.2.1.26), which accelerate the reaction to obtain an equimolar mixture of glucose and fructose [1]. Furthermore, the use of the immobilized invertase provides operational advantages for the invert sugar production such as increasing its stability in relation to pH and temperature and avoiding the enzymatic denaturation. The enzyme immobilization also allows the recovery and reuse of the biocatalyst, which decreases the bioprocess cost [2]. However, the immobilized enzyme performance depends on the support material and technique used for immobilization. Sugarcane bagasse is an abundant and renewable residue from the sugar and alcohol industries and it is a potential enzymatic support material, as it has hydrophobic characteristics and its composition consists of cellulose, hemicellulose and lignin, which makes it an attractive material for enzyme immobilization, due to the wide possibility of reactive points [3]. In this sense, this work aimed to produce and evaluate a renewable biocatalyst for the hydrolysis reaction of sucrose into inverted sugar, through the immobilization of invertase from *Bacillus tequilensis* on sugarcane bagasse.

2 MATERIAL & METHODS

The cultivation was carried out inoculating the *B. tequilensis* strain (*bacillus sp* PP6) isolated from the peach palm fruit (*Bactris gasipaes*) in petri plate containing yeast extract, sodium chloride, tryptone and bacteriological agar. The following steps were the pre-inoculation, inoculation and fermentation stages [2]. The sugarcane bagasse fibers were pre-treated submerged in sodium hydroxide, washed with distilled water until pH neutralization and then dried at 70 °C for 24 h [4]. The enzyme was immobilized by physical adsorption, adding the support to the enzymatic extract. During the incubation period, at each hour, 1 mL of the enzymatic extract were removed for analyses. After the immobilization process, the immobilized enzymes were separated by vacuum filtration and stored for determination of enzymatic activity [5]. To enzymatic activity determination the reaction medium with the sample was added to DNS, and after procedures, received distilled water for spectrophotometer reading [5]. Immobilization yield and recovered activity were determined according to literature [6].

The storage stability was evaluated under refrigeration conditions (4 ºC) for 9 days and the operational stability was evaluated during sequential batch reaction cycles, where 1 g of the support containing the immobilized enzyme was added to the reaction medium. After each cycle, the biocatalyst was separated from the reaction medium through filtration to be used in a new cycle [7].

3 RESULTS & DISCUSSION

The immobilization of invertase on sugarcane bagasse by physical adsorption as a function of time is showed in Figure 1. It is observed that the enzymatic activity of the supernatant of the medium composed by the enzyme and support decreased along

the immobilization time, whereas the activity of the control sample (without support) remained practically constant. These results indicate that the enzyme was immobilized on the sugarcane bagasse.

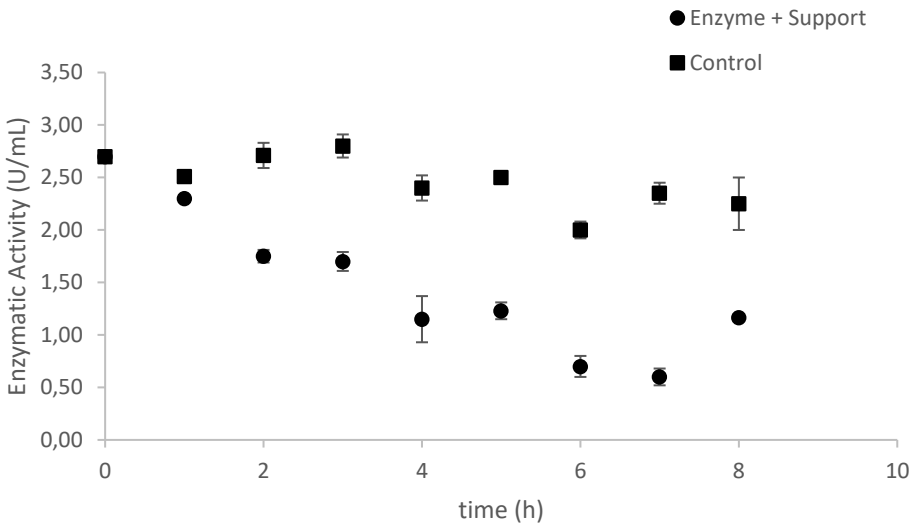

Figure 1 Kinetics profile of invertase immobilization on sugarcane bagasse.

The immobilization yield and recovered activity values were 73.80% ± 0.30 and 58.43% ± 5.4, respectively. The literature reports IY of 98% for invertase produced by the fungus *Cunninghamella echinulata* PA3S12MM and immobilized in calcium alginate [1]. However, this high value for immobilization yield can be explained by the fact that this enzyme was previously purified whereas in this work the invertase from *bacillus sp* PP6 was immobilized from a crude extract. Similarly, β-D-fructofuranosidase from *F. graminearum* was immobilized in sugarcane bagasse for 16 hours, aiming at the production of fructooligosaccharide [7]. It was reported that 66% of the initial activity of the enzyme was retained.

The storage stability of soluble and immobilized invertase are showed in Figure 2. After 9 days of storage under refrigeration (4 °C), the soluble enzyme lost approximately 80% of its activity, while the enzyme immobilized on sugarcane bagasse maintained its activity close to the initial one. This indicates that the immobilization of the enzyme on sugarcane bagasse increased significantly its storage stability. In other studies, invertase immobilized on silica nanoparticles and *Ocimum basilicum* seed showed 45 % and 78 % of its initial activity after 60 days of storage, respectively, while the soluble enzyme lost all activity in 25 days [9].

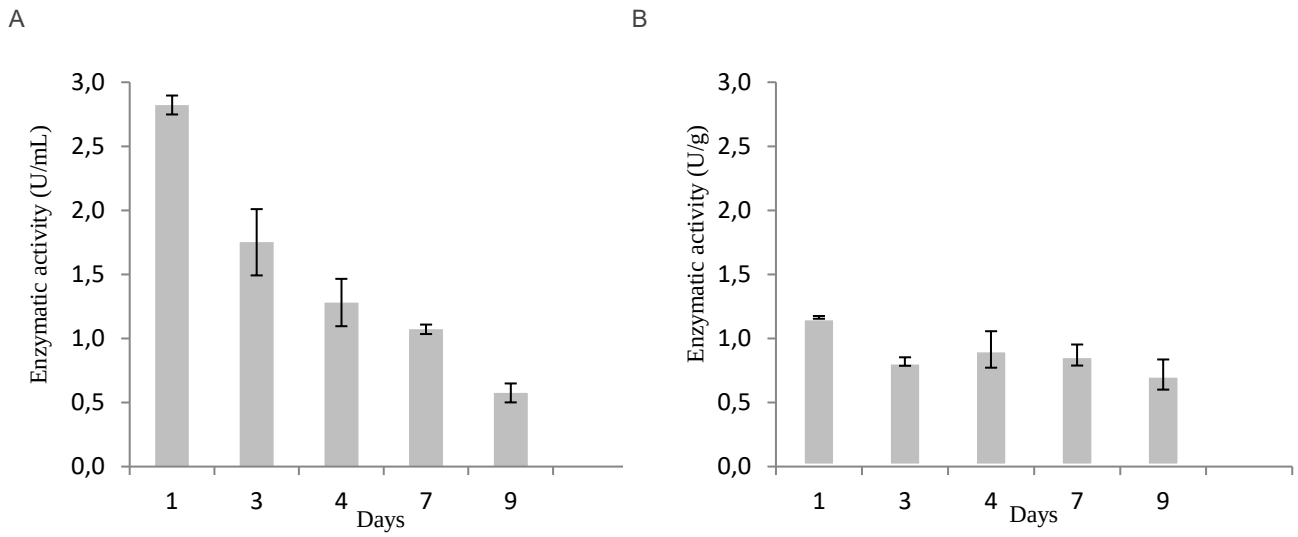

Figure 2 Storage stability of soluble enzyme (A) and immobilized enzyme (B).

The operational stability of the immobilized enzyme is showed in Figure 3. It is observed an activity reduction of approximately 69 % in the second reaction cycle. In the third cycle, the enzyme showed less than 15 % of its initial activity. The high decrease in activity suggests that enzymes weakly adsorbed on the outer surface of the support may have been removed during the reaction, while enzymes adsorbed more strongly in the porous of the support remained [8]. However, immobilization techniques such as covalent bonding could be applied in next studies aiming to improve the operational stability of the bacterial invertase.

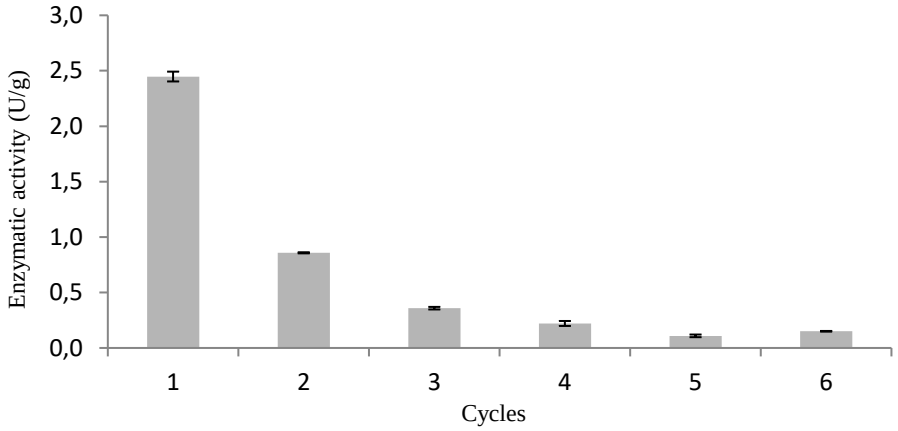

Figure 3 Operational stability of invertase immobilized in sugarcane bagasse.

L. brevis Mm-6 invertase was immobilized into alginate beads to preserve enzyme stability for continues industrial using [10,11]. Thermostability, loading capacity and reusability of the enzyme were enhanced by the immobilization process. Approximately 100% of the enzymatic activity was retained after 15 cycles of use, but it reduced to 80% after 20 cycles. Those results show the great influence of the immobilization technique and support on the reuse capacity of invertase.

4 CONCLUSION

The immobilization kinetics profile as well as the immobilization yield and recovered activity indicated that sugarcane bagasse pretreated with sodium hydroxide was capable of immobilizing invertase from *B. tequilensis* (*bacillus sp PP6*) isolated from peach palm fruit (*Bactris gasipaes*). The immobilized enzyme was stable under refrigerated conditions by 9 days whereas the soluble enzyme deactivated rapidly. Also, the immobilized enzyme kept 31% of its initial activity after the first reaction cycle. In this sense, the use of bacterial invertase immobilized on sugarcane bagasse, an abundant and renewable material, is an interesting strategy for the production of invert sugar in heterogeneous enzymatic reactors.

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