INVERTASE IMMOBILIZATION ON POLYLACTIC ACID AND ACRYLONITRILE-BUTADIENE-STYRENE (ABS) POLYMER

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ABSTRACT

Invertases are enzymes that catalyze the hydrolysis of sucrose into D-glucose and D-fructose, known as invert sugar, which is widely applied in the food industry. Enzymes are one of the most expensive items in a bioprocess; then, their recovery and reuse is of utmost importance and immobilizing an enzyme makes the reaction more viable economically. Based on this, additive manufacturing is a new technology that is gaining more and more space in the industry, with enzyme immobilization on carriers obtained from a 3D printer being a promising area. The most commonly employed thermoplastics are polylactic acid (PLA) and acrylonitrile-butadiene-styrene (ABS) polymer, due to their mechanical strength, resistance and low cost. In this context, this work aimed to optimize the immobilization of invertase on PLA and ABS carriers by modifying several parameters in the immobilization methodology. As a result, it was possible to observe the enzymatic activities of 10.074 ± 1.894 U/g for PLA and 10.441 ± 0.456 U/g for ABS. However, PLA stood out as a promising polymer for the industrial application of immobilized enzymes, despite similar activity values of the immobilized derivative, the immobilization yield (31.397%) was almost eight times greater than that for ABS (4.114%).

Keywords: Invertase. Immobilization. Polylactic Acid. Acrylonitrile Butadiene Styrene Polymer.

1 INTRODUCTION

Invertases (E.C.3.2.1.26) are glycoproteins that catalyze the hydrolysis of sucrose, through the breaking of the alpha-1,4-glycosidic bond, forming the so-called inverted sugar, an equimolar mixture of monosaccharides D-glucose and D-fructose, widely used in food industry.[1,2]

Enzymes are one of the main variables that make the bioprocess costly, and for industrial application, economic viability is sought through enzyme recycling, ease of separation from the medium and high stability of the biocatalyst.[2] With this objective, the enzyme immobilization technique was developed, which denotes the confinement of the enzyme to a solid carrier, retaining its catalytic activity, facilitating its recovery and reuse.[1, 3, 4] Covalent bonding is one of the most applied methods when seeking irreversible immobilization, with stable bonding and high stability.[5]

An emerging technology with great potential for enzyme immobilization is additive manufacturing, or 3D printing, as it allows the construction of complex structures, in a simple, fast, accurate and low-cost way, without loss of material. The main materials used for 3D printing are polylactic acid (PLA) and acrylonitrile-butadiene-styrene (ABS) polymer, which have mechanical strength, resistance and low cost, but they require surface modifications for enzymatic immobilization.[6, 7, 8, 9] In this context, this project aimed to optimize the invertase immobilization on PLA and ABS carrier by modifying several parameters, such as chitosan and glutaraldehyde concentration, contact time of sodium hydroxide, chitosan and glutaraldehyde with the sample, ionic strength of the buffer and enzymatic loading in the immobilization methodology.

2 MATERIAL & METHODS

The immobilization of the commercial invertase isolated from *Saccharomyces cerevisiae* in crushed PLA and ABS followed the protocol described by Gkantzou *et. al*. (2022) with the variation of parameters for modifying the polymer surface. Some of the parameters analyzed were: contact time of sodium hydroxide with the sample (2, 24 and 48 hours), chitosan concentration (0.2%, 0.5% and 1.0% w/v), chitosan contact time with the sample (1 minute, 10 minutes, 1 hour and 2 hours), glutaraldehyde concentration (2.5%, 10.0% , 20.0% and 25.0% (v/v)), glutaraldehyde contact time with the sample (1, 2 and 4 hours), variation in the ionic strength of the phosphate buffer pH 7.0 used for the enzymatic solution (0.01M; 0.05M; 0.1M and 0.5M), and enzymatic loading (1.0 mg/mL, 2.5 mg/mL, 5.0 mg/mL, 10.0 mg/mL, 20.0 mg/ mL, 25.0 mg/mL and 30.0 mg/mL).[10]

To validate the best immobilization conditions, the enzymatic activity of the immobilized derivative was analyzed by measuring the hydrolysis of sucrose into reducing sugars using the Miller Method (1959).[11] In addition, the experiment was also carried out to determine the immobilization yield, quantifying the protein content, using the Bradford Method (1976), of the free enzyme and the supernatant after immobilization.[10,12] Finally, the results were analyzed statistically, using the Tukey test, with a level of 5% significance.[13]

3 RESULTS & DISCUSSION

PLA and ABS are the most used thermoplastics in additive manufacturing.[7,10, 14] However, for enzymatic immobilization, there is a need to modify these polymers, both in size and surface. For surface modification, the carriers need to have high affinity for the enzyme and availability of functional groups for binding.[5,15] One of the most used and reported techniques in the literature for immobilization by covalent bonding is the addition of amino groups to the surface of the support for binding to the enzyme through the cross-linking agent, glutaraldehyde.[9, 16, 17]

The chemical structure of PLA and ABS shows that they are hydrophobic plastics, therefore, a first strategy for surface functionalization is alkaline hydrolysis. The use of sodium hydroxide (NaOH) for this step is a simple, easy and inexpensive way to add reactive hydrophilic functional groups, such as carboxylic acids (-COOH) and hydroxyls (-OH), by breaking chemical bonds present in the PLA and ABS.[10,14,17]

The next step is the addition of reactive amino groups to the surface to make it biocompatible, which can be performed by various compounds. In the methodology presented in this work, the deposition of chitosan was used, a biopolymer from marine animal shells, which can be considered a bioadhesive for enzyme immobilization. With the presence of reactive amino groups, glutaraldehyde is responsible for the covalent bonds between enzyme and support through modification of the surface structure. Thus, as it is a bifunctional agent, it binds to both chitosan and the invertase, one on each side, creating a stable link between enzyme and support.[10]

After activation of the support, it needs to come into contact with the enzyme, so that immobilization can occur through covalent bonds.[10] In this regard, the ionic strength of the buffer used for the enzyme solution can change the immobilization rate and the region of the protein that will interact with the support.[18] Another factor is the enzyme loading provided for immobilization, as biocatalysts interact with the support until the point of saturation, where there are no more reactive groups on the support to interact with the enzyme.

Figure 1 schematizes the immobilization process by covalent bonding of the invertase enzyme on PLA and ABS carriers proposed in the project, with details of the chemical bonds.

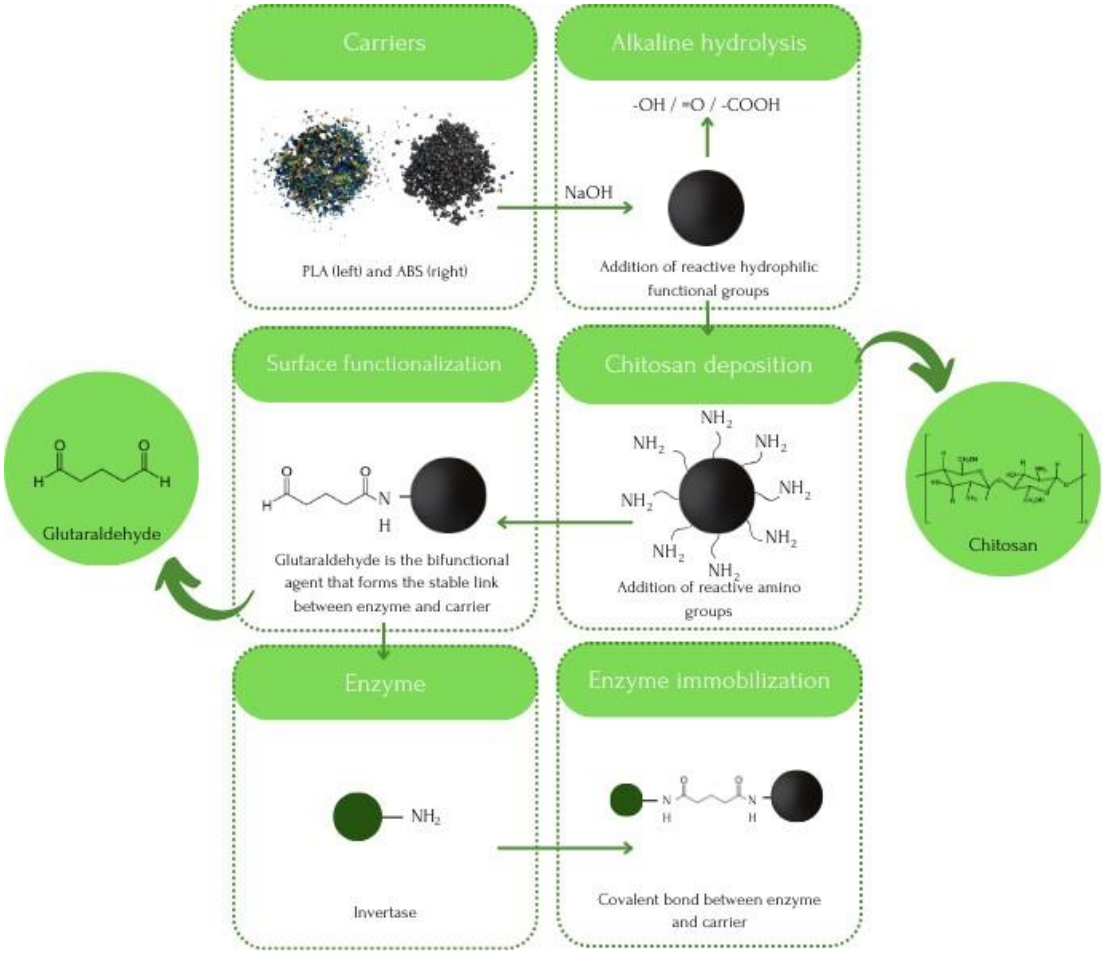

Figure 1 Scheme of the immobilization process by covalent bonding of the invertase enzyme on PLA and ABS carriers proposed in the project, with details of the chemical bonds.

Table 1 summarizes the best conditions measured in this project for the immobilization of the invertase in PLA and ABS, as well as the activity of the immobilized enzyme and the immobilization yield according to the protein content.

Table 1 Better conditions for immobilizing the invertase in PLA and ABS measured in the project , in accordance with the proposed methodology.

Parameters	Best immobilization conditions	
	PLA	ABS
Contact time of sodium hydroxide with the sample	2h	2h
Chitosan concentration	0.2% (w/v)	0.2% (w/v)
Chitosan contact time with the sample	10 minutes	1 minute
Glutaraldehyde concentration	25% (v/v)	2.5% (v/v)
Glutaraldehyde contact time with the sample	1h	1h
Ionic strength of the phosphate buffer pH 7,0 used for the enzymatic solution	0.05 M	0.05 M
Enzymatic loading	25 mg/mL	25 mg/mL
Enzymatic activity	10.074 ± 1.894 U/g	10.441 ± 0.456 U/g
Immobilization yield	31.397%	4.114%

As a result, it was possible to observe the enzymatic activities of 10.074 ± 1.894 U/g for PLA and 10.441 ± 0.456 U/g for ABS. However, PLA stood out as a promising polymer for the industrial application of immobilized enzymes, despite similar activity values of the immobilized derivative, the immobilization yield (31.397%) was almost eight times greater than that for ABS (4.114%).

These data may be due to the alkaline hydrolysis step. Although the literature reports the hydrolysis of ABS using sodium hydroxide, this does not occur effectively. Pokharna *et. al*. (2021) reported in their work the increase in the hydrophilicity of PLA and ABS with alkaline treatment, but just like the export here, the treatment was more effective for PLA, which achieved a greater bond between Poly-L-lysine and the surface, than for ABS.[14]

4 CONCLUSION

Additive manufacturing is an emerging technique, especially when applied to enzyme immobilization, with few studies reported in the literature. As it is a very advantageous technology, as it allows complex structures to be built in a simple, fast, precise and low-cost manner, exploratory studies are extremely important for the advancement of innovation.

The present work sought to report a methodology and its optimization for the enzymatic immobilization of invertase in two thermoplastics, PLA and ABS, used in 3D printing. Therefore, it was possible to observe that for both polymers, immobilization of the invertase enzyme occurred through covalent bonding. However, PLA stood out as a promising polymer for the industrial application of immobilized enzymes, despite similar activity values of the immobilized derivative, the immobilization yield was almost eight times greater than that for ABS.

Finally, although it is a new strategy for immobilization, little developed, it is possible to note the enormous technological potential involved, and it is only a matter of time before it becomes widely disseminated.

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