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Immobilization of enzymatic extract in polyester fiber emulsified with polyurethane resin

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ABSTRACT
The objective of this work was to evaluate the immobilization of lipolytic and pectinolytic enzymatic extracts in a Polyester Fiber Emulsified with Polyurethane Resin (FPERP) support without and with functionalization with glutaraldehyde. In the first method, the support was emulsified with the enzymatic extract and then the shape of the immobilized was determined before the completion of the polymerization, and could be flat, coiled or double-layered. In the second method, the functionalization of PFRP with glutaraldehyde was performed before immobilization. The esterification activity and operational stability of lipolytic and pectinolytic immobilizes were determined by the synthesis of ethyl oleate and hydrolysis of citrus pectin, respectively. The immobilization of the lipolytic extract in support of PFRP functionalized with 50 and 25% glutaraldehyde resulted in immobilized patients with activity of 28.05 and 15.52 U/g, respectively. On the other hand, the immobilized without functionalization presented higher activity in the form of a double layer (132.17 U/g). The immobilization of the pectinolytic extract resulted in immobilizations with exo-polygalacturonase (exo-PG) and pectinmethylesterase (PME) activity of 1.78 and 12.34 U/g, respectively, for the double-layer immobilized. Regarding operational stability, the immobilized did not present satisfactory values, possibly due to the inefficiency of the polyurethane resin as a support for enzymatic immobilization on the fiber.

Keywords: Lipases, Pectinases, Glutaraldehyde, Reuse, Esterification.

1 INTRODUCTION

Lipases (triacylglycerol acylhydrolases; E.C. 3.1.1.3) are enzymes that catalyze hydrolysis of carboxylic ester bonds of triacylglycerols, releasing organic acids and glycerol. In industry, enzymes can be applied in oleochemicals, detergents, polymers, food processing, pharmaceuticals, waste, cosmetics and biodiesel (GUERRAND, 2017).

In turn, pectinases constitute a group of enzymes responsible for the degradation of pectin, a polysaccharide present in the middle lamella and primary cell wall of higher plants (SUDEEP et al.,

2020; HOSSEINI et al., 2021). Enzymes belonging to this class find applications in the fruit and vegetable juice industry, production of alcoholic beverages, in the textile industry, in the extraction of vegetable oils, in the fermentation of teas and processing of coffee beans, among others (SOUZA and KAWAGUTI, 2021).

Although enzymes have a wide range of industrial applications, most commercial enzymes are used only once, due to the fact that they are free and soluble in the reaction medium (RIGO et al., 2021). This aspect can raise the costs of biocatalysts, making their application less attractive for some industries. In this context, enzymatic immobilization emerges as an alternative to facilitate recovery and reuse in several reaction cycles, in addition to contributing, in many cases, to the improvement of enzymatic stability.

The proper choice of supports for immobilization plays a fundamental role in the success of the process, resulting in immobilized with good activity, resistance and stability. It should be noted that some immobilization methods can compromise the activity of the enzyme, due to the use of unfavorable conditions, including the use of multiple reagents, high temperatures and shear forces, among other factors (KUJAWA et al., 2021). Therefore, immobilization by simple and direct methods can ensure the maintenance of enzyme activity and stability.

The use of polyurethane monomers for enzyme immobilization has been investigated by several authors, including in works of this research group (NYARI et al., 2016; FERNANDES et al., 2018; NYARI et al., 2018; SBARDELOTTO et al., 2018; CUI, LI and LI, 2019; BRESOLIN et al., 2019; BUSTAMANTE-VARGAS et al., 2015; BUSTAMANTE-VARGAS et al., 2019; MOENTAMARIA et al., 2020). However, this study sought to develop a simple immobilization method with mild reaction conditions to avoid the denaturation of enzymes, using a commercial material known as Polyester Fiber Emulsified with Polyurethane Resin (FPERP). It is worth mentioning that polyurethane resin is responsible for the rigidity of synthetic gypsum when in contact with water and can act as an immobilizing agent.

Thus, this study aims to contribute to the advancement of knowledge in the field of enzyme immobilization, by investigating the use of PFRP as a promising support, and evaluating the operational stability of immobilized lipolytic and pectinolytic enzyme extracts. The results obtained may have relevance both for the industry, by providing more efficient and sustainable biocatalyst alternatives, and for academic research, by expanding knowledge about the immobilization of enzymes in different supports.

2 MATERIALS AND METHODS

2.1 MATERIALS

The reagents and solvents used were: oleic acid (99.7%; Synth); ethyl alcohol (99.3%; Modern Chemistry); acetone (99.5%; Neon); sodium hydroxide (97%; Modern Chemistry), glutaraldehyde (50%; Vetec); glutaraldehyde (25%; Scientific Exodus); α-D-galacturonic acid (97%; Fluka); citrus pectin (Vetec); sodium acetate (Synth); acetic acid (99.7%; Vetec); 3,5 dinitrosalicylic acid (99%; Vetec); Sodium and Potassium Tartrate (99.35%; Neon). All reagents used in the present study were of analytical grade and used without previous treatment.

2.2 BIOCATALYSTS

Immobilization was performed using the commercial lipolytic enzymatic extract Eversa® Transform 2.0 (Novozymes Latin America Ltda, Araucária - PR), obtained from the fungus *Aspergillus oryzae* and kindly provided by an oil company located in the state of Rio Grande do Sul. The enzymatic extract presents in its composition 50% of glycerol, 43% of water, 5% of lipase and 2% of stabilizers.

The pectinolytic enzyme extract Pectinex® Ultra SP-L has been kindly provided by Novozymes Latin. America Ltda, Araucária - PR. This enzymatic extract is composed of polygalacturonase obtained from *Aspergillus aculeatus and* other pectinases, hemicellulases and beta-glucanases. The extract has a density of 1.16 g/mL and is composed of 45% glycerol, 45% water, 5% polygalacturonase and 5% potassium chloride. The storage temperature recommended by the manufacturer is from 0 to 10 ºC.

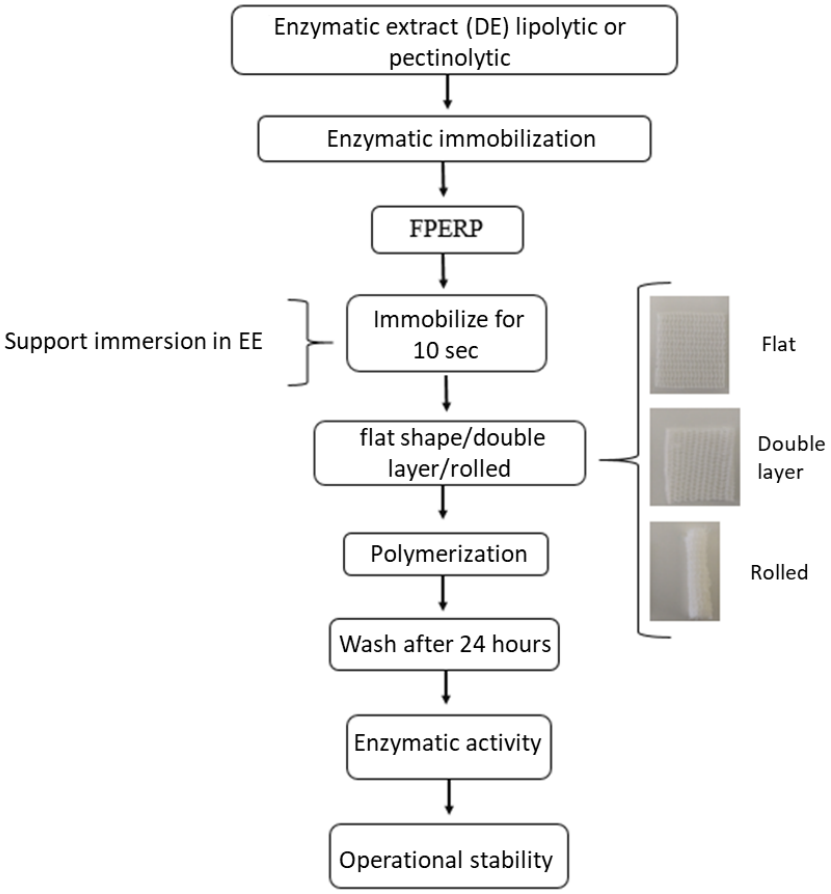
2.3 METHODS OF ENZYMATIC IMMOBILIZATION

2.3.1 Incorporation of enzymatic extracts in FPERP

FPERP or synthetic gypsum (Hygia Cast, South Korea) has a layer of polyurethane. When exposed to water, the polyurethane polymerizes, resulting in the fiber hardening in approximately 5 to 10 seconds of contact, according to the manufacturer.

For immobilization of the enzymatic extracts, PFRP strips with dimensions of approximately 3 cm x 4 cm were used. These strips were arranged in flat, coiled or double-layered shapes. In the immobilization process, the aqueous enzymatic extract was used instead of water, as illustrated in the flowchart shown in Figure 1.

Figure 1 – Enzymatic immobilization process in polyester fiber emulsified with polyurethane resin (FPERP).



The immobilization of the enzymatic extracts in the flat form was performed by submerging the support in 3 mL of enzymatic extract, enough to cover the support, for about 10 s (maximum time stipulated by the manufacturer). Then, the support was removed and the end of the polymerization was waited, which occurs after 5 minutes, resulting in the hardening of the material.

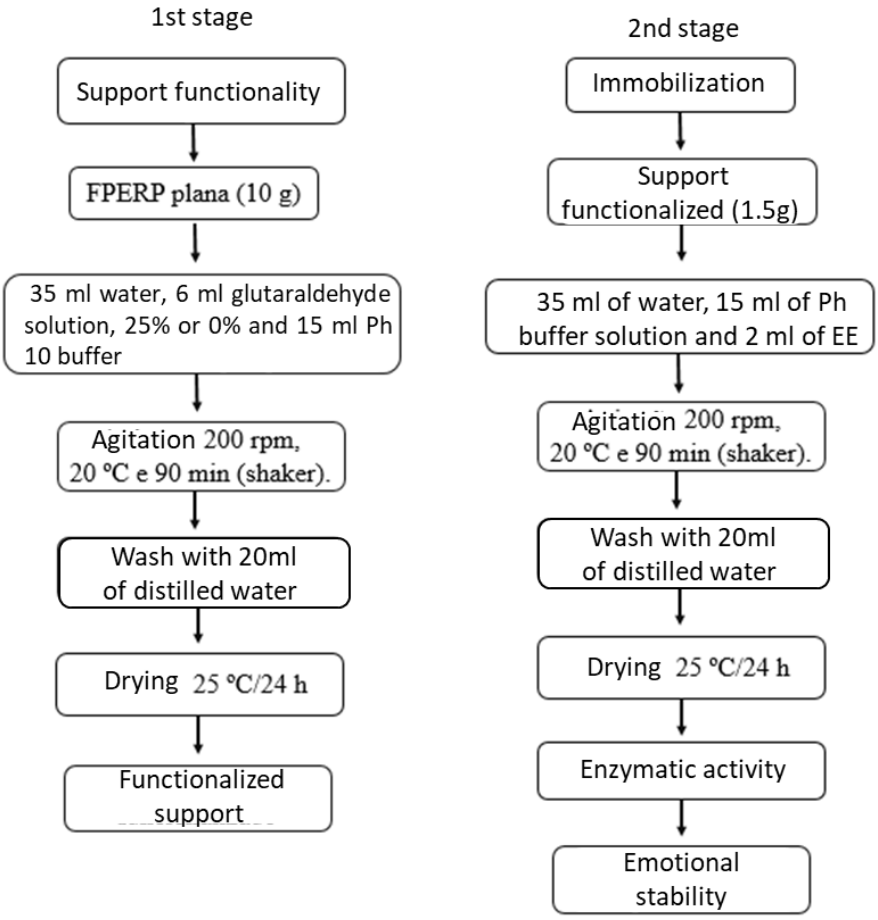
In the case of double-layered and coiled supports, the same procedure was performed, with the difference that before the end of polymerization, the support was manually rolled with the aid of tweezers, or superimposed on two layers, which were pressed until the end of polymerization to finish the shape of the immobilized. After a period of 24 hours, all immobilized individuals were washed with distilled water to remove excess enzyme extract. A control was also performed using only distilled water, with the objective of evaluating the support without the presence of the enzymatic extract.

2.3.2 Functionalization of PFRP with glutaraldehyde and enzymatic immobilization

The functionalization of PFRP was performed based on the study by Dariff et al. (2021). The procedure consisted of preparing a solution of glutaraldehyde 50% or 25%, in buffer pH 10 (Figure 2), in order to obtain a conjugate base of Schiff, formed by a covalent bond between the functional groups

aldehyde of glutaraldehyde and the deprotonated amine groups of the enzyme, resulting in the formation of an imin.

Figure 2 - Scheme of the steps of immobilization of enzymatic extract in support of PFERP functionalized with glutaraldehyde.



For each assay, 10 g of support was added to a flask containing 15 mL of buffer solution (pH 10), 35 mL of distilled water and 6 mL of glutaraldehyde (50% or 25%). The solution and support were submitted to 200 rpm agitation in an orbital agitator (New Brunswich, Excella E25R) at 20 °C for 90 min. Then, the support was separated from the solution and washed with 20 mL of distilled water to remove the excess reagents. The same procedure was performed for the control support, without the subsequent addition of enzymatic extract.

After drying the support at room temperature for 24 h, about 1.5 g of the functionalized support was added to an erlenmeyer containing 15 mL of buffer solution (pH 10), 35 mL of distilled water and 2 mL of enzymatic extract. The system was placed in an orbital agitator at 20 °C with agitation at 200 rpm for 90 min. Then, the support was separated and washed with 20 mL of distilled water to remove

the excess solution and enzyme extract. The immobilized was left at room temperature (25 ºC) until the enzymatic activity tests of the immobilized and the washing water were performed.

2.4 CHARACTERIZATION OF FPERP SUPPORTS AND FIXED ASSETS

2.4.1 Density of media

The bulk density of the materials was determined according to ASTM D3574-02 (ASTM, 2011). 5 support samples were cut out and height, length and width measurements were performed using a caliper. Then, the samples were weighed on a precision scale and the values were expressed in g/cm³. All trials were performed in triplicate.

2.4.2 Surface area

The characterization of the surface area of the flexible polyurethane and FPERP samples was performed using a Quantachrome Autosorb-1 (Nova-2200e). The specific surface areas of the materials were determined using the BET method (BRUNAUER, EMMETT and TELLER, 1938). The volume and mean diameter of the pores were calculated by the method of BJH (BARRET, JOYNER and HALENDA, 1951). For the analysis of the surface area, 0.1 g of samples were previously subjected to a heat treatment at 40 °C and reduced pressure, for 12 h. The analysis was performed at a constant temperature of -196 °C (77 K).

2.5 DETERMINATION OF ESTERIFICATION ACTIVITY

The esterification activity of the enzymatic extract, the support and the immobilized was quantified through the synthesis reaction of ethyl oleate from ethyl alcohol and oleic acid (molar ratio 1:1 (m/m)). The methodology was based on Ferraz et al. (2015), with modification in the molar ratio. The flasks with the substrates were placed in an orbital agitator (New Brunswich, Excella E25R) at 160 rpm, 40 ºC and 10 min. After that, 5 mL of the substrate mixture was removed and approximately 0.1 g of enzyme was added to the reaction medium. A blank assay (without enzyme) was performed to determine the acidity of the reaction medium. The reaction, as well as the white one, was conducted in closed glass flasks and kept in an orbital agitator under the same agitation and temperature conditions for 40 minutes. Then, aliquots of 0.5 mL were removed from the reaction medium and added to 15 mL of an acetone-ethyl alcohol solution (ratio 1:1; v/v) to stop the reaction. All trials were done in triplicate.

The amount of acid consumed was determined by titration with sodium hydroxide (NaOH) 0.05 M up to pH 11, with the aid of a pH meter (Tecnal, TEC-7). Enzymatic activity was expressed in units per gram of substrate (RIGO et al., 2010), being defined as the amount of oleic acid (μmol) converted

per gram of enzyme per minute, under the conditions of the assay. The enzymatic activity was calculated using Equation 1.

$$AE = \frac{(V_b - V_a) * M * 1000 * V_f}{t * M_{EE} * V_c} \quad (1)$$

Where:

AE: esterification activity (U/g);

Va: volume of NaOH spent on titration of the sample taken after 40 min (mL);

Vb: volume of NaOH spent on titration of the sample without enzyme (mL);

M: molarity of NaOH solution;

Vf: final volume of reaction medium (mL);

t: time (min);

MEE: mass of the enzymatic extract or fixed assets (g);

Vc: aliquot volume of the reaction medium removed for titration (mL).

2.6 ENZYMATIC ACTIVITY OF EXO-POLYGALACTURONASE (EXO-PG)

The exo-PG activity was determined by the 3,5-dinitrosalicylic acid (DNS) method, described by Miller (1959), to evaluate the activity of the extract, the support and the pectinolytic immobilization. In this method, galacturonic acid production resulting from the hydrolysis of citric pectin is quantified using a calibration curve established with α-D-galacturonic acid (molecular weight of 212.15 g/mol) as the reducing sugar and the DNS reagent.

For this assay, a volume of 1 mL of substrate (0.5% (m/v) of citrus pectin in sodium acetate buffer 100 mM; pH 5.0) was used. The substrate was incubated at 40 °C for 15 min for temperature stabilization. Then, 0.1 g of the free or immobilized pectinolytic enzymatic extract was added to the substrate and the reaction was incubated at 40 °C for 6 min. After this period, the biocatalyst was removed (when possible) and 1 mL of the DNS reagent was added. For the DNS staining reaction, the samples were heated in a water bath at 100 °C for 8 min, cooled in an ice bath and added 8 mL of double sodium and potassium tartrate solution (50 mM) for color stabilization. Finally, the absorbances of the samples were measured in a spectrophotometer (Logem, LS-7052-BIV) at 540 nm using a control prepared under the same conditions, but replacing the enzyme with distilled water.

A unit of pectinolytic activity was defined as the amount of galacturonic acid generated per min (U = μmol/min), the value can be divided by the mass of immobilized or enzymatic extract used to obtain the value in U/g.

2.7 DETERMINATION OF THE ENZYMATIC ACTIVITY OF PECTINMETHYLESTERASE (PME)

The SME activity was based on the methodology described by Hultin, Sun and Bulger (1966), with changes in temperature. The procedure consisted of adding 30 mL of a 1% citrus pectin solution in 0.2 M NaCl to an erlenmeyer, adjusting the pH to 4.5. Then the immobilized or enzymatic extract was added. The enzymatic reaction was performed in an orbital agitator (New Brunswich, Excella E25R) at 55 ºC for 30 min. After the reaction, the pH of the solution was readjusted to 4.5 with a 0.005 M NaOH solution and the volume spent was used to calculate the activity of the SME from Equation 2, where an SME unit was defined as the amount of enzyme capable of catalyzing the demethylation of pectin corresponding to the consumption of 1 μmol of NaOH/min.mL, under the test conditions.

$$\boldsymbol{AE} = \frac{\boldsymbol{V_{NaOH} * M * 1000}}{\boldsymbol{t * MEE}} \qquad (2)$$

Where:

AE: enzymatic activity (U/g);

V: volume of NaOH spent in mL;

M: molarity of NaOH (0.005 M);

t: reaction time (30 min);

ESM: mass of enzymatic or immobilized extract (g).

2.8 ASSESSMENT OF THE OPERATIONAL STABILITY OF LIPOLYTIC FIXED ASSETS

The operational stability of the fixed assets was determined following the method described by Ficanha et al. (2015), with some modifications in the separation of the fixed assets from the environment. In this case, the immobilized was only removed from the reaction medium, with the aid of tweezers, and added in a new synthesis reaction of ethyl oleate. Then, the residual activity was calculated.

This process was repeated until the residual activity of the fixed assets reached approximately 50%. The residual activity (RA) of each cycle was calculated with the aid of Equation 3 (FICANHA et al., 2020).

$$\boldsymbol{AR}(\%) = \frac{\boldsymbol{AE_i}}{\boldsymbol{AE_0}} * 100 \qquad (3)$$

Where:

RA: residual activity;

AEi: esterification activity at time "i";

AE0: esterification activity at initial time.

2.9 EVALUATION OF THE OPERATIONAL STABILITY OF PECTINOLYTIC FIXED ASSETS

To evaluate the operational stability of pectinolytic enzymatic fixed assets, a specific analysis was performed for exo-PG (item 2.6) and SME (item 2.7). This evaluation was conducted through successive reactions of hydrolysis of citrus pectin, following the methodologies proposed by Miller (1959) and Hultin, Sun and Bulger (1966), respectively. After each reaction batch, the PFRP-derived immobilization was removed and added in a new reaction medium. Based on the values of enzymatic activity, the residual activity was calculated through Equation 3.

3 RESULTS AND DISCUSSION

3.1 STUDY OF THE IMMOBILIZATION OF THE LIPOLYTIC ENZYME EXTRACT IN PFERP

The immobilization of the lipolytic enzyme extract in PEPERP was performed with and without previous treatment with glutaraldehyde 50 and 25% (v/v), and the results are presented in Table 1.

Table ERRO! NENHUM TEXTO COM O ESTILO ESPECIFICADO FOI ENCONTRADO NO DOCUMENTO. - Initial enzymatic activity (AE), operational stability (N. from recycles) and residual activity (RA) of the lipolytic enzyme extract immobilized in FPERP with and without the functionalization of the support with glutaraldehyde (GA).

Biocatalysts	Initial AE* (U/g)	No. of recycling	AR (%)
Free enzyme extract	151,41±26,62	-	-
Support	0	0	0
Functionalized support-GA 50%	109,09±11,46	0	0
Functionalized support-GA 25%	37,14±1,75	0	0
Functionalized Fixed Assets-GA 50%	28,05±6,33	1	50,40
Functionalized fixed assets-GA 25%	15,52±1,94	0	0
Fixed assets flat	87,27±6,67	1	77,08
Fixed assets in double layer	132,17±4,62	1	61,41
Immobilized rolled up	81.39±9.89	1	50,31

*Activity of the immobilized discounting the activity of the functionalized support.

It was verified that the FPERP support did not show activity when immobilized with the lipolytic enzymatic extract. However, when the support was functionalized with glutaraldehyde, it began to present activity, increasing proportionally (from 37 to 109 U/g) with the concentration of glutaraldehyde (from 25 to 50%). The functionalized support probably presents acidic characteristics that consume the titrating agent, requiring an evaluation of the acidity of the support.

The enzymatic immobilized units using the functionalized support, presented activities (discounted the support activity) of 15.52 U/g, for 25% and 28.05 U/g, for 50% of glutaraldehyde. This trend of increased activity with glutaraldehyde concentration in the functionalization step can be associated with a greater number of active aldehyde sites on the support surface when activated with a 50% solution of glutaraldehyde. The higher concentration of the same tends to favor the incorporation

of aldehyde groups to the support and, consequently, increase the probability of binding between the enzyme and the aldehyde group.

Among those immobilized with the support without functionalization, the immobilized produced by submersion of two layers in the enzymatic extract followed by adhesion of the same stands out, generating a double layer of immobilized, with activity of 132.17 U/g. In this case, the configuration of the support may have favored the incorporation of the enzymatic extract, in relation to the other forms (flat and coiled).

Although some immobilization assays have shown enzymatic activity, in the first batch of ethyl oleate synthesis, when submitted to the second batch (1st cycle), only the support functionalized with 50% glutaraldehyde and the supports that were submerged in the enzymatic extract showed enzymatic activity higher than 50%. However, in the 2nd recycling these fixed assets presented activity of less than 50%. This tendency can be attributed to the leaching of the support enzyme suggesting that the immobilization processes evaluated do not provide an effective incorporation of the enzyme to it, probably by adsorption.

Thus, according to the tests performed in this study, PFRP did not prove to be an efficient support in enzymatic immobilization, both in terms of activities obtained and operational stability, contradicting the hypothesis that the support could be a good enzyme immobilizing agent.

Other studies were successful in immobilization using polyurethane, however, the immobilization method was in *situ*. Nyari et al. (2016) immobilized *in situ* the lipase of *Candida antarctica* B (CALB) in polyurethane foam by the confinement method and found enzymatic activity values of 302.9 U/g and an operational stability of 36 continuous cycles with 51% activity.

Nyari et al. (2018) used *Candida antarctica* B lipase (CALB) immobilized *in situ* in polyurethane foam in the synthesis of isoamyl acetate by mechanical and ultrasonic agitations and found the operational stability of eight cycles for mechanical agitation and 27 cycles for ultrasonic agitation, both with residual activity greater than 60%.

Thus, it can be assumed that enzymatic immobilization is a process that depends on several factors, such as the proportions of polyols and isocyanates and other additives necessary for polymerization, polymerization temperature, surface area, material density, among others.

According to Correia, Fonseca and Ferreira-Dias (2011), enzymatic immobilization in polyurethane is usually performed with precursor proportions that result in a flexible foam, with porous structure, where lipase can be fixed by covalent bonds with the support or bonds between the monomers in the polymerization step. It is also possible that the enzyme acts with a binding link in the polymer structure (ANTUNES, 2015).

The polyurethane present in PFERP is responsible for the stiffness of the plaster after polymerization, a characteristic necessary for an effective immobilization of the fracture, and thus, the polyurethane must be presented in a compact form, with high density, unlike the flexible foam. Probably, the characteristics of the material are interfering in the immobilization process, either in the incorporation of the enzyme to the support or due to the diffusional limitation of the substrate to the enzyme, due to its low surface area (Table 2).

In order to verify the density of the material, the apparent density of flexible polyurethane was determined, which was 0.088±0.001 g/cm3, a value approximate to that found by Facin (2017) of 0.094 g/cm 3, and the density of FPERP was 0.705±0.002 g/cm3. The difference in density between the materials may be a factor that hindered the enzymatic immobilization in the polyurethane resin present in the polyester fiber.

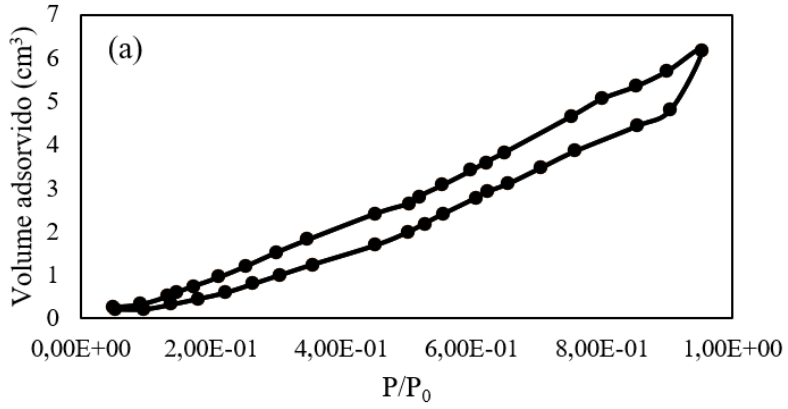
The values of surface area and the adsorption and desorption isotherms for flexible polyurethane foam, polyester fiber and polyurethane resin are presented in Table 2 and Figure 3, respectively.

Table 2 – Surface area of the materials flexible polyurethane foam, polyurethane resin and polyester fiber.

Material	**Surface area (m^2/g)**
Flexible polyurethane foam	6,05
Polyurethane resin	1,71
Polyester fiber	3,78

The surface area found for the flexible polyurethane foam was about 3 times larger than the surface area of the polyurethane resin. Thus, the lower surface area and higher density of polyurethane resin compared to polyurethane foam may have contributed to the low efficiency of PFPERP as an enzymatic support.

Figure 3 – Adsorption and desorption isotherms of flexible foam (a), polyurethane resin (b) and polyester fiber (c).



The profiles of the nitrogen adsorption and desorption isotherms presented by the three materials (Figure 3) do not correspond to any standard defined by the International Union of Pure and Applied Chemistry (IUPAC). The disordered syneresis of the isotherms suggests that the structure is disorganized and there is low affinity between the gas and the sample.

3.2 STUDY OF THE IMMOBILIZATION OF PECTINOLYTIC ENZYME EXTRACT IN PFERP

Despite the low operational stability of the lipolytic immobilization, the immobilization of pectinolytic enzymatic extract (exo-PG and PME) in PFERP was evaluated, using or not the functionalization of the support with 50 and 25% glutaraldehyde, and the results are presented in Table 3.

Table 3 – Enzymatic activity (AE), operational stability (N. of recycles) and residual activity (RA) of pectinolytic extract (exo-PG and PME) and fixed assets in PFRP with and without functionalization with glutaraldehyde (GA).

Materials	AE initial exo-PG (U/g)	No. of recycling (RA%)	Initial AE SME (U/g)	N. of reciclos (AR%)
Free enzyme extract	2,51±0,28	-	142,58±1,36	-
Support	0	-	0	-
Functionalized support-GA 50%	0	-	0	-
Functionalized support-GA 25%	0	-	0	-
Functionalized Fixed Assets-GA 50%	1,64±0,02	1 (51,04)	0,36±0,01	0 (41,59)
Functionalized fixed assets-GA 25%	0,83±0,21	1 (50,97)	0	-
Fixed assets flat	1,54±0,02	1 (52,24)	4,99±0,22	0 (11,94)
Fixed assets in double layer	1,78±0,48	1 (53,46)	12,34±0,98	0 (11,26)
Immobilized rolled up	1,55±0,03	1 (54,06)	11.72±0.13	0 (10,31)

Unlike what was observed for lipase, the functionalized supports for the pectinase activity reaction did not present titrating agent consumption. This indicates the specificity of glutaraldehyde with NaOH in terms of reaction interference. Similar to the results found for lipase, the best result obtained for exo-PG was for the immobilized in double layer of FPERP (1.78 U/g), followed by the functionalized immobilization with glutaraldehyde 50% (1.64 U/g).

The immobilization of the pectinolytic extract and the activity of exo-PG followed a behavior similar to the enzymatic activity of lipase, presenting activity in the first reaction batch, but the activity decreased throughout the process, and after the first recycling the residual activity of the immobilized was less than 50%.

The determination of free SME pectinase was 142.48 U/g, however, the immobilization of pectinase in FPERP reduced the enzymatic activity. The highest value was found for the double-layer support (12.34 U/g), but the immobilized ones did not present operational stability. In situ immobilization of pectinolytic enzyme extracts in rigid polyurethane foam was studied by Bustamante-Vargas et al. (2015). The authors observed that the operational stability of the

immobilized, under the conditions of pH 4.5 and 55 ºC with reaction time of 6 min, was 6 cycles with residual activity of 35%.

As in the immobilization assays of the lipolytic enzyme extract, the FPERP support was able to immobilize part of the pectinolytic enzymatic extract, however it was not efficient in maintaining the operational stability of the immobilized. The decrease in activity along the recycling may be related to the inactivation of the enzyme and/or leaching of the enzyme from the support to the reaction medium. This is because the enzyme extract was likely adsorbed on the surface by weak bonds, which can be easily broken.

4 CONCLUSION

The objective of this work was to immobilize aqueous enzymatic extracts in an innovative polymeric support, FPERP, which is a commercial material capable of hardening when in contact with water, due to the presence of polyurethane. The purpose of enzymatic immobilization is to facilitate recovery and enable the reuse of immobilization, and operational stability is a crucial parameter to determine the success of an immobilization process.

However, the evaluation of the operational stability (reuse) of the fixed assets contradicted the expectation that the polyurethane layer could act as an immobilizing agent under the conditions employed. Polyurethane has different densities, and can be rigid or soft, and this characteristic seems to influence the ability of the material to immobilize the enzyme. Thus, this study may contribute to expand the information regarding the immobilization of enzymes in polyurethane.

However, it is important to note that each enzyme-support system is unique and requires careful evaluation of the specific conditions. Therefore, although PFRP has not been shown to be an effective support in this study, it is possible that, with adjustments in the immobilization conditions or with the use of other polymeric supports, more promising results can be achieved.

In summary, the results obtained in this study indicate that the enzymatic immobilization in polyurethane, using FPERP as a support, was not successful under the tested conditions. However, this study contributes to the understanding of the challenges and limitations associated with the immobilization of enzymes in polyurethane and may direct future research in the search for more efficient methods of enzymatic immobilization.

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