THERMAL AND MORPHOLOGICAL EVALUATION OF LIPASE FROM *Bacillus* sp. IMMOBILIZED ON POLY (3-HYDROXYBUTYRATE-CO-HYDROXYVALERATE).

Rebeca Yndira Cabrera Padilla[1], Milena Chagas Lisboa[1], Marluce Santana dos Santos[1], Renan Tavares Figueiredo[1,2], Alini Tinoco Fricks[1,2], Elton Franceschi[1,2], Álvaro Silva Lima[1,2], Daniel Pereira Silva[1,2], Cleide Mara Faria Soares[1,2]

[1] Universidade Tiradentes, Aracaju-SE, Brazil, yndiracp@yahoo.com; milena.chagaslisboa@hotmail.com
[2] Instituto de Tecnologia e Pesquisa, Aracaju-SE, Brazil, Cleide_Mara@itp.org.br.

Resumo

A nova fonte de *Bacillus* sp. (ITP-001) foi imobilizada por adsorção física no polímero poli (3-hidroxibutirato-co-hidroxivalerato) (PHBV). O suporte e a biocatalizador imobilizado (BI) foram caracterizados em comparação com a lipase livre em relação à calorimetria exploratória diferencial, termogravimetria, área superficial específica, isotermas de adsorção-dessorção, volume de poro (*Vp*) e tamanho de poro (*dp*) por adsorção de nitrogênio. O perfil de perda de massa observado na análise térmica indicou que a redução de massa esteja relacionada à decomposição de compostos orgânicos e água.

Palavras-chave: lípase *Bacillus sp.*., PHBV, imobilização.

Abstract

A new source of lipase from *Bacillus sp*. ITP-001 was immobilized by physical adsorption on the polymer poly (3-hydroxybutyrate-co-hydroxyvalerate) (PHBV). The support and immobilized biocatalyst (IB) were characterised, compared to the free lipase, with regard differential scanning calorimetry, thermogravimetric analysis, the specific surface area, adsorption-desorption isotherms, pore volume (*Vp*) and size (*dp*) by nitrogen adsorption. The pattern of weight loss observed on thermal analysis indicated a reduction in mass is related to decomposition of organic compounds and water.

Key-words: lipase *Bacillus sp.*, PHBV, immobilization.

1. INTRODUCTION

Enzymes from microbial sources are currently receiving considerable attention due to their potential applications in industry such as in detergents, oleochemicals, organic synthesis, dairy, fat and oil modification, tanning, pharmaceuticals and sewage treatment [1,2]. Immobilization is a powerful tool for fine modifications to the catalytic properties of enzymes for industrial purposes. Immobilized lipases have the advantages of enhanced thermal and chemical stability, ease of handling, easy recovery and repeated use as compared with free forms [3-4]. Currently, eco-friendly supports are used for the immobilization of enzymes, but some have not yet been tested for *Bacillus* lipase, for example poly(3-hydroxybutyrate-co-hydroxyvalerate) (PHBV). Based on other applications for PHBV, our group recently immobilized *Candida rugosa* lipase (CRL) in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) and obtained an efficient immobilization yield, improved thermal stability and operational stability; this work is unique in the literature with lipase immobilized on PHBV [5]. Thermogravimetric analysis is an important tool for thermal stability studies of macromolecules and too is applied for free or immobilized enzymes. Consequently, PHBV has become a good alternative to be used as a support for the immobilization of

enzymes due to its biocompatibility, biodegradability, strength, easy absorption, as well as its eco-friendly and non-toxic properties.

2. OBJECTIVE

In this sense, the aim of this study was the thermal and morphological characterization of the immobilized lipase (*Bacillus* sp.) on PHBV by physical adsorption.

3. MATERIALS AND METHODS

3.1. Differential scanning calorimetry (DSC) and Thermogravimetric analysis (TG)

Samples of the free enzyme, PHBV and immobilized biocatalyst (PHBV-ITP) were obtained in according the literature [5,6], and were analysed using a differential scanning calorimeter apparatus (SHIMADZU-Model DSC 60) and DTA-TG apparatus (SHIMADZU-Thermogravimetric Analyser Model DTG-60 H), for DSC and TG analysis, respectively. For DSC analysis, about 4 to 6 mg of the sample were sealed in aluminium pans and submitted to a heating rate of 20°C/min from room temperature to 200°C; nitrogen was used as an inert gas at a flow rate of 30 mL/min. As for TG, in each analysis, approximately 4 mg of the sample were sealed in platinum pans and submitted to a heating rate of 10°C/min from room temperature to 600°C; nitrogen was used as an inert gas at a flow rate of 30 mL/min.

3.2. Nitrogen adsorption-desorption measurements

The surface area of the PHBV and immobilized lipase samples was calculated using the Brunauer-Emmett-Teller (BET) method [7]. Pore volume, pore size distribution and average pore diameter, based on BJH calculations [8], were evaluated by the BET apparatus software (Model NOVA 1200-Quantachrome Analyser) using N_2 adsorption at 77 K.

4. RESULTS

4.1. DSC and TG results

The behaviour of the free enzyme was slightly different compared to the support and the immobilized biocatalyst which showed similar behaviours (Fig. 1). It was possible to identify in all the samples a single peak corresponding to melting in the temperature range studied. For ITP-001, the melting temperature (T_m = 152.8°C) and enthalpy of fusion (ΔH_m = 2.54J/g) were determined. For PHBV, the melting temperature (T_m= 166.6°C) and enthalpy of fusion (ΔH_m= 78.35J/g) were also determined; these values were similar to those reported in the literature for pure PHBV [9] and immobilized *Candida rugosa* lipase (CRL) in poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [5]. For the immobilized biocatalyst (PHBV-ITP), T_m= 170.85°C and ΔH_m= 48.20 J/g were determined. Fig. 2 shows that in region I, the free enzyme (ITP-001) lost mass from the beginning, due to the weakly adsorbed water on the surface of the enzyme, while for the support (PHBV) and the immobilized biocatalyst (PHBV-ITP), the mass loss remained nearly constant. In region II, thermal decomposition started with considerable mass loss with increasing temperature for all three samples, although this was more pronounced for

the support and the immobilized biocatalyst. For PHBV, the maximum degradation occurred at 345°C, with onset ($T_{initial}$) at 275°C, consistent with previous reports in the literature stating that PHBV is thermally unstable above 250°C [10]. For PHBV-ITP, the maximum degradation occurred at 320°C, with onset at 260°C. The immobilized biocatalyst was more stable than the free ITP-001 lipase, as shown in region II in Fig. 2. In region III, it was observed that after the thermal decomposition of the support and the immobilized biocatalyst (PHBV-ITP), a slight variation of the mass occurred. For the free ITP-001 lipase, the mass loss was still substantial in region III. This was probably associated with the decomposition of organic compounds from the biocatalyst [11].

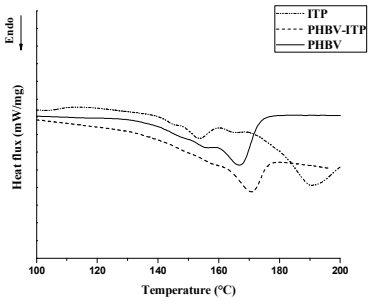

Fig. 1. DSC curves for the free enzyme (ITP-001), the support (PHBV) and PHBV-ITP

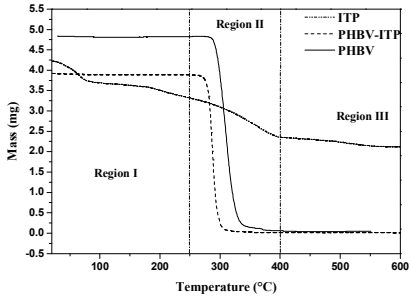

Fig. 2. The weight loss curves were divided into three regions: region I (25-250°C), region II (250-400°C) and region III (400-600°C).

4.2. Specific surface area and porous properties

The Brunauer-Emmett-Teller (BET) surface area of the pure PHBV and immobilized (PHBV-ITP) samples as well as their pore parameters are listed in Table 1. The nitrogen adsorption-desorption isotherms of PHBV before and after adsorption of ITP-001 can be classified as a type II isotherm which does not exhibit any type of hysteresis (Fig. 3). These are typical features of non-porous and macroporous materials [12].

Table 1. Textural properties of PHBV before and after adsorption of ITP-001.

Samples	Surface área (m^2/g)	Pore volume [cm^3/g ($x10^{-2}$)]	Mean pore diameter (Å)
Pure PHBV	**6.2**	**1.447**	**104**
PHBV-ITP	**5.7**	**0.994**	**61**

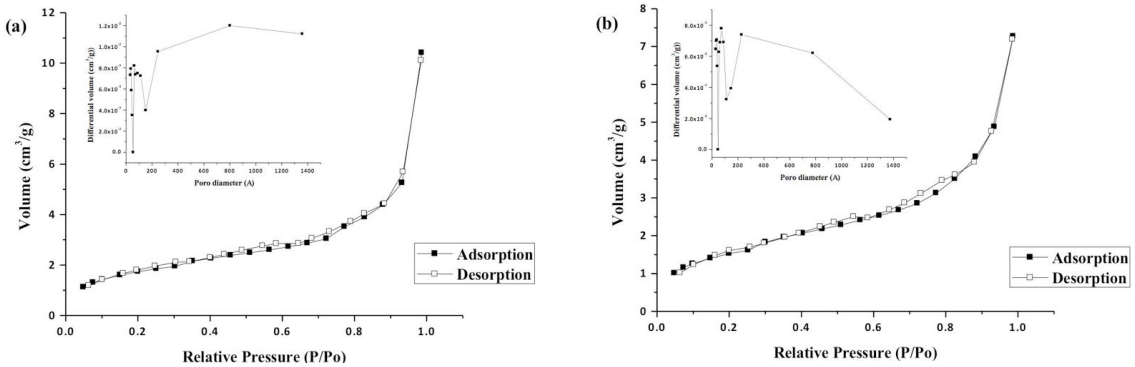

Fig. 3 Nitrogen adsorption-desorption isotherms of PHBV before (a) and after (b) Lipase from *Bacillus* sp. ITP-001 loading.

However, the slight decrease observed in the specific surface area (from 6.21 to 5.70 m^2/g) with the addition of the ITP-001 enzyme could be attributed to the blocking of some micropores, observed in the distribution of pore sizes present on the surface of the PHBV used as the support. A greater decrease was observed for pore volume, where the value for the support was $1.447x10^{-2}$ cm^3/g, while for the immobilized enzyme, this value was $0.994x10^{-2}$ cm^3/g (Table 1). On the other hand, the addition of the enzyme to the support exerted a strong

influence on the mean pore diameter. The marked decrease in the mean pore diameter and the decreased surface area and pore volume were not thought to be mediated by structural collapse caused by the immobilization of lipase. These results also suggest that the immobilization of ITP-001 lipase occurred in channels present in PHBV [13,14]. Despite this, the isotherms of both samples, according to the IUPAC classification, correspond to non-porous and macroporous materials; however, pure PHBV and PHBV-ITP are mostly mesoporous materials, as evidenced by the pore size distribution, although larger pore diameters were also observed.

5. CONCLUSIONS

The results obtained by N_2 adsorption-desorption isotherms clearly showed that the ITP-001 lipase was adsorbed into the channels of PHBV and the mesoporous and macroporous support structure was retained after the adsorption of ITP-001 lipase. The addition of the ITP-001 lipase onto pure PHBV did not significantly influence the values obtained for the surface area of the biocatalyst. The results obtained by DSC showed that the immobilization of the enzyme increased the enthalpy of fusion and thus provided greater thermal stability to the biocatalyst. Indeed, the TG profiles clearly showed that the immobilized biocatalyst was more stable than the free ITP-001 lipase. These results indicate that PHBV can be used as a support for the immobilization of lipase.

6. REFERENCES

1 SHARMA, R.; CHISTI, Y.; BANERJEE, U.C. Production, purification, characterization and applications of lipases. **Biotechnol. Adv., Oxford**, v.19, p.627–662, 2001.

2 GUPTA, N.; SAHAI, V.; GUPTA, R. Alkaline lipase from a novel strain *Burkholderia multivorans:* statistical medium optimization and production in a bioreactor. **Process Biochem.**, v.42, p.518–526, 2007.

3 KANWAR, S.S.; CHAUHAN, G.S.; CHIMNI, S.S.; KUMAR, Y.; RAWAT, G.S.; KAUSHAL, R.K. Methacrylic acid and dodecyl methyacrylate (MAc-DMA) hydrogel for enhanced catalytic activity of lipase of *Bacillus coagulans* MTCC-6375. **J. App. Polym. Sc.**, v.100, p.1420–1426, 2006.

4 GODOY, C.A.; DE LAS RIVAS, B.; FILICE, M.; FERNÁNDEZ-LORENTE, G.; GUISAN, J.M.; PALOMO, J.M. Enhanced activity of an immobilized lipase promoted by site-directed chemical modification with polymers. **Process Biochem.**, v.45, p.534–541, 2010.

5 CABRERA-PADILLA, R.Y.; LISBOA, M.C.; FRICKS, A.T.; FRANCESCHI, E.; LIMA, A.S.; SILVA, D.P.; SOARES, C.M.F. Immobilization of *Candida rugosa* lipase on poly (3-hydroxybutyrate-co-hydroxyvalerate): a new eco-friendly support. **J. Ind. Microbiol. Biotechnol.**, 2011 (Article impress).

6 BARBOSA, JM.P; SOUZA, R.L.; FRICKS, A.T.; ZANIN, G.M.; SOARES, C.M.F.; LIMA, A.S. Purification of lipase produced by a new source of Bacillus in submerged fermentation using an aqueous two-phase system. **J. Chrom. B.**, v. 879, p. 3853-3858, 2011.

7 BRUNAUER, S.; EMMETT, P.H.; TELLER, E. Adsorption of gases in multimolecular layers. **J. Am. Chem. Soc.**, v. 60, n. 2, p. 309-319, 1938.

8 SOARES, C.M.F.; DOS SANTOS, O.A.A.; OLIVO, J.E.; DE CASTRO, H.F.; DE MORAES, F.F.; ZANIN, G.M. Influence of alkyl -substituted silane precursors of silica gels on the enzymatic activity of immobilized microbial lipase from *Candida rugosa*. **J. Mol. Catal. B: Enzym.**, v.29, p. 69-79 2004b.

9 GONÇALVES, S.P.C; MARTINS-FRANCHETTI, S.M.; CHINAGLIA, D.L. Biodegradation of the films of PP, PHBV and its blend in soil. **J. Polym. Environ.**, v. 17, p. 280-285, 2009.

10 SINGH, S.; MOHANTY, A.K. Wood fiber reinforced bacterial bioplastic composites: Fabrication and performance evaluation. **Composites Sc. Technol.,** v. 67, p. 1753-1763, 2007.

11 SOARES, C.M.F.; SANTOS, O.A.; DE CASTRO, H.F.; MORAES, F.F.; ZANIN, G.M. Studies on lipase immobilization in hydrophobic sol-gel matrix. **Appl. Biochem. Biotechnol.**, v.113 p.307-319, 2004.

12 BROWN, M.E. Introduction to Thermal Analysis-Techniques and applications. **Kluwer Academic Publishers**, Second Ed., New York, 2004.

13 LI, Y.; ZHOU, G.; LI, C.; QIN D.; QIAO, W.; CHU, B. Adsorption and catalytic activity of *Porcine pancreatic* lipase on rod-like SBA-15 mesoporous material. **Colloids and Surfaces A: Physicochemical and Engineering Aspects.**, v.341, p. 79-85, 2009.

14 ZHOU, G.; CHEN, Y.; YANG, S. Comparative studies on catalytic properties of immobilized *Candida rugosa* lipase in ordered mesoporous rod-like silica and vesicle-like silica. **Micropor. Mesopor. Mater.**, v.119, p. 223–229, 2009.