Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1806-2563
ISSN on-line: 1807-8664
Doi: 10.4025/actascitechnol.v39i4.30770

Optimization of olive oil hydrolysis process using immobilized Lipase from *Burkholderia cepacia* sp. in Polyurethane

Nádia Ligianara Dewes Nyari*, Alessandro Rogério Paulazi, Clarice Steffens, Marcelo Mignoni, Jamile Zeni and Rogério Marcos Dallago

*Departamento de Engenharia de Alimentos, Universidade Regional Integrada do Alto Uruguai e Missões, Avenida Sete de Setembro, 1621, 99700-000, Erechim, Rio Grande do Sul, Brazil. *Author for correspondence. E-mail: nadialigianara@hotmail.com*

ABSTRACT. The aim of this study was to achieve the best conditions for the olive oil hydrolysis process at optimal pH and temperature using *Burkholderia cepacia* lipase immobilized *in situ* in rigid polyurethane support. The influences of the temperature (13.85 to 56.5°C) and pH (4.18 to 9.82) were evaluated by a central composite rotational experimental design 2^2. The operational stability and storage conditions were also studied. The olive oil hydrolysis process was optimized in pH 7.0, at 40°C and 15 min of reaction, with 66 and 93 U g^{-1} of hydrolysis activity in free and immobilized lipase, respectively, with > 700% yield. The immobilized remained stable for up to 40 days of storage at temperatures of 60°C, and for 100 days from 4 to 25°C. The operational stability of the immobilized was 6 continuous cycles. In this way, immobilization showed to be a promising alternative for its application in olive oil hydrolysis, having storage stability and reuse capability.

Keywords: biocatalysis, lipase, *in situ* immobilization, polyurethane, hydrolysis.

Otimização do processo de hidrólise do azeite de oliva utilizando lipase *Burkholderia cepacia* sp. imobilizada em poliuretano

RESUMO. As lipases são enzimas que, naturalmente, atuam na hidrólise de óleos e gorduras, porém, em meios com quantidade baixa de água, são capazes de atuar em reações como a esterificação, alcoólise, acidólise, aminólise, entre outras. Sendo utilizadas na redução da concentração dos lipídios contidos nos efluentes, promovendo a hidrólise dos óleos e gorduras presentes. Sendo assim, o objetivo do presente estudo foi obter as melhores condições do processo de hidrólise do óleo de oliva em termos de pH e temperatura, utilizando lipase de *Burkholderia cepacia* imobilizada *in situ* em suporte rígido de poliuretano. O processo de hidrólise do óleo de oliva foi obtido utilizando as condições de pH de 7,0, temperatura de 40°C e 15 min de reação com atividade hidrolítica para a enzima livre e imobilizada de 66 e 93 U g^{-1} respectivamente, com rendimento de > 700%. O imobilizado apresentou estabilidade de 40 dias de estocagem nas temperaturas de 60°C e 100 dias de armazenamento nas temperaturas entre -4 a 25°C e a estabilidade operacional de 6 ciclos contínuos. O imobilizado se mostrou uma alternativa promissora para aplicação no processo de hidrólise frente à enzima livre, tendo estabilidade de estocagem e possibilidade de reutilização operacional.

Palavras-chaves: biocatálise, lipase, imobilização *in situ*, poliuretano, hidrólise.

Introduction

The increasing demand for manufactured products has created a great load of waste, especially with lipid characteristics such as oils and fats, mainly from industrial processing such as slaughterhouses, dairy farms, oil extraction factories, domestic sewage, drugs, petroleum, hospitals, trade, among others (Cammarota & Freire, 2006, Jeganathan, Nakhla, & Bassi, 2007, Battimelli, Loisel, Garcia□Bernet, Carrere, & Delgenes, 2010, Salum et al., 2010, Valadão & Benedet, 2011, Zhang, Sathitsuksanoh, Zhu, & Zhang, 2011, Mannarino, Moreira, Ferreira, & Arias, 2013, Rezende et al., 2013, Barros, Maia, Souza, & Di, 2014, Buss & Henkes, 2014, Santos, Guimarães, Rosa, & Carvalho, 2014, Frinhani & Moreira, 2014, Santos, Henrique, Shhlindwein, Ferreira, & Stachiw, 2015).

In order to overcome these difficulties, industries have invested in efficient and environmentally sustainable waste treatment systems. In this context, we highlight the enzymatic treatments that correspond to the most recent technology for the biological treatment of effluents (Buss & Henkes, 2014, Santos et al., 2015, Silva, Silva, Gois, Almeida, & Abud, 2015). The lipolytic enzymes (lipases) can,

by hydrolytic method reduce the concentration and degrade a large number of toxic and persistent substances (Liu et al., 2011, Baldo et al., 2013, Chen, Zhang, Xu, & Yan, 2013, Zadinelo et al., 2013, Costa et al., 2014).

The use of immobilized lipase on a suitable support increases the process financial viability, mainly due to its reuse (reaction medium separation), it improves the thermal and mechanical properties (stability at different temperatures), operational stability, storage and the enzymatic activity (Cadena et al. 2010, Hu et al., 2012, Palomino-Romero et al., 2012, Colla et al., 2014, Silva et al., 2014, Manoel et al., 2015, Nyari, Zeni, Steffens, Dallago, & Rigo, 2015, Shao, Jing, Tian, Zheng, & Shang, 2015, Nyari et al., 2016).

Immobilization processes of different materials are described in the literature, especially the entrapment (with or without binding) during support synthesis. Within this context, the polyurethane (PU) support is highlighted, demonstrating the possibility of lipase *in situ* during the polymerization stage, presenting excellent activity and yield results in terms of esterification (Nyari et al., 2016). In addition, it has presented chemical compatibility with lipase, resistance to pH, temperature and organic solvents (Simões, Mori, Faria, Castro, & Mendes, 2011, Cui, Tao, Li, Chen, & Tan, 2013, Cipolatti et al., 2015, Nyari et al., 2015). In this context, this study aimed at evaluating the hydrolytic capacity of *Burkholderia cepacia* sp. lipase immobilized in PU support, as well as the pH and temperature effects on its activity. The immobilization process, stability, storage, and reuse have also been evaluated The chosen reaction model was the olive oil hydrolysis.

Material and methods

Materials

The *Burkholderia cepacia* sp. lipase used in this study was obtained from Amano Pharmaceuticals Co., Ltd. (Nagoya, Japan). The commercial polyol and isocyanate monomers used in this work were produced for a specific formulation for mattresses and foam injected by the Flexible Polyurethanes – Mannes Company (Erechim, State Rio Grande do Sul, Brazil). The solvents used were acetone (FMaia), ethanol (Merck), olive oil, arabic gum, sodium phosphate (monohydrate and heptahydrate - Nuclear) and sodium hydroxide (Nuclear).

Methods

Determination of protein profiles of commercial *Burkholderia cepacia* sp. lipase

The lipase protein content was determined by Bradford (1976), using bovine serum albumin as standard. The specific activity (U mg^{-1}) was determined by the enzyme activity quotient (U mL^{-1}) and total protein content (mg mL^{-1}).

Immobilization of *Burkholderia cepacia* sp. lipase in polyurethane (PU)

The lipase from *Burkholderia cepacia* sp. immobilization on PU was performed using 6 mL of polyol and 4 mL of isocyanate (60-40%, v v^{-1}), with 2 mL of the enzymatic solution, corresponding to 0.2 g of the enzyme (1 g of the enzyme in 10 mL of distilled water) (Nyari et al., 2015). The enzymatic solution was added to the polyol and homogenized (5 s) and after that, isocyanate was added at constant stirring (10 s). The *in situ* polymerization was conducted at 20°C, until the growing of PU foam (5 min), remaining stationary for 3 hours and then crushed, producing a homogeneous product.

Morphology characterization using scanning electron microscope (SEM)

The morphology of PU support and *Burkholderia cepacia* sp. lipase in polyurethane (PU) were evaluated by scanning electron microscope (SEM) (DSM960 Zeiss). The samples were mounted on double-sided conductive carbon tape adhered to an aluminum sample port and sputter coated with gold in a sputtering unit (SCD050/ LEICA) at 40 mA for 90 s. Next, they were characterized with SEM operating at 10kV.

Reaction time study

The reaction time to evaluate the hydrolytic activity was monitored for 40 min. The reaction was conducted at 35°C, 0.2 g enzyme, pH 7 and 160 rpm. An independent test was conducted for each time.

Optimization of hydrolysis process

The hydrolytic activity of the immobilized and free lipase was conducted using an experimental design composite (RCCD) 2^2, with triplicate at a central point, evaluating the influence of the pH and temperature variables. The reaction time was set at 15 min for the optimization test.

Enzymatic hydrolytic activity

The reaction medium was composed of olive oil (10%, w v^{-1}) and arabic gum (5%, w v^{-1}) in 100 mM sodium phosphate buffer at pH 7.0. 19.8 mL of this emulsion and 0.2 g lipase (immobilized or free) were added into a flask. The flasks were incubated at different temperatures (13.8, 20; 35; 50 and 56.5°C) and pHs (4.2; 5; 7; 9 and 9.8). After the incubation, the reaction was interrupted adding 15 mL of acetone-ethanol (1:1, v v^{-1}). The fatty acids content was determined by titration, until pH 11, with NaOH 0.05 M (Alonso-Morales et al., 2008, Liu & Chang, 2008).

Parallel to this, blank tests have been done using only the emulsion and the acetone-ethanol solution, without the addition of lipase. The definition of hydrolytic activity of the lipase (free and immobilized) has been done according to Equation 1.

$$AH = \frac{(Va - Vb).M.1000}{t.X} \quad (1)$$

where:
AH = hydrolytic activity (U mL^{-1} or U g^{-1});
M = NaOH molarity;
Va = NaOH volume used in the sample titration after the reaction (mL);
Vb = NaOH volume used in the black titration (mL);
X = enzymatic extract volume (mL) or mass of immobilized (g) used in the reaction;
t = reaction time (min).

One lipase activity (hydrolytic) unit was determined as the amount of enzyme that released 1 μmol of fatty acids per minute.

Yield immobilization

The immobilized yield was calculated considering the total activity of the free lipase in solution given on the immobilization process (that considers the volume of the enzymatic extract employed on the immobilization test and its activity [U mL^{-1}]) and the immobilized total activity (that considers the total mass of the produced immobilized and its activity (U g^{-1}) according to Equation 2.

$$RI\ (\%) = \frac{UT_{immobilize\ d}}{UT_O} \times 100 \quad (2)$$

where:
$RI\ (\%)$ = Immobilization yield;
$UT_{immobilized}$ = total activity on synthesized immobilized;
UT_o = total activity of the enzymatic solution offered for immobilization.

The hydrolysis reaction was conducted with 0.2 g of biocatalyst, at 35°C, pH 7, 15 min reaction time and 160 rpm.

Stability

Storage and operational

The immobilized stability during the storage was evaluated in temperatures ranging from -4 to 40°C. The hydrolytic activity was evaluated until it presented residual activity lower than 50%. The immobilized operational stability was studied by consecutive batches, with reuse.

Residual activity (%)

The residual activity was used to evaluate the storage activity (free and immobilized thermal stability) and operational/reuse (immobilized), calculated using the initial activity for each system as a reference, according to Equation 3.

$$RA(\%) = \frac{U_X}{U_{initial}}\ x\ 100 \quad (3)$$

where:
$RA\ (\%)$ = Residual activity;
U_X = Enzymatic activity after reuse and storage;
$U_{initial}$ = Initial enzymatic activity.

Statistical analysis

Each experiment was done in triplicate. Data were expressed as means ± standard deviation, and subjected to one-way analysis of variance (Tukey) using Statistic 8.0 (StatSoft) software. A significance level of 95% ($p < 0.05$) was used.

Results and discussion

Characterization of immobilized *Burkholderia cepacia* sp. lipase

The support morphology with the presence of the *Burkholderia cepacia* sp. lipase is shown in Figure 1.

It is possible to notice that the pores formed during synthesis are heterogeneous and randomly arranged.

Protein content of *Burkholderia cepacia* sp. lipase

The enzymatic solution used in this work was characterized in terms of quantification of protein

content. The results obtained in this stage from *Burkholderia cepacia* sp. lipase are 0.19 mg mL^1 with specific activity of 306.4 U mg^{-1}.

Study of the reaction time

The results of hydrolytic activity for the free and immobilized lipases, in terms of reaction time, are found in Figure 2.

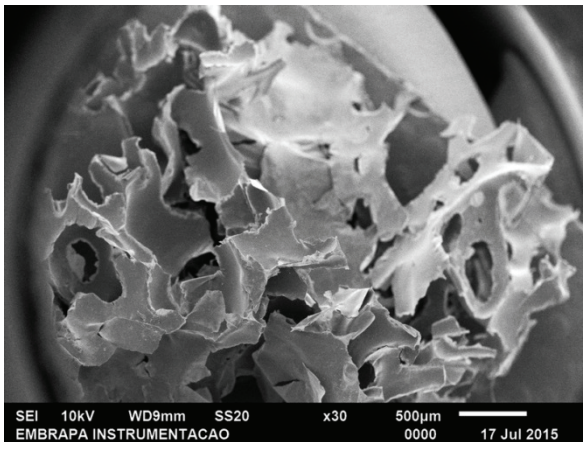

Figure 1. SEM microscopy of polyurethane foam used as support without *Burkholderia cepacia* sp. lipase *in situ* in polyurethane (PU).

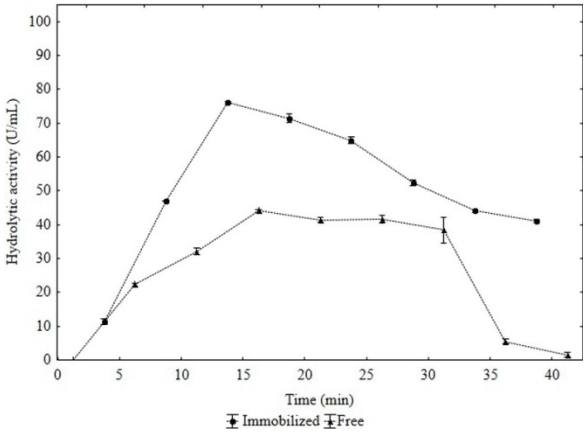

Figure 2. Hydrolytic activity of free and immobilized lipase from *Burkholderia cepacia* in terms of reaction time.

The free and immobilized lipase forms presented the same behavior, with an increase of the hydrolytic activity on the first 15 min of reaction time, followed by a decrease in subsequent times. It demonstrated, at 40 min of reaction time, activities of 2 and 20 U g^{-1} for the free and immobilized forms, respectively. This trend suggests the presence of two reaction mechanisms (hydrolyses and esterification), both linked to lipases (Liu & Chang, 2008, Liu et al., 2011). Initially, there is a predominance of hydrolyses reaction on the first reaction minutes, which generates alcohol and carboxylic acid as products. The esterification reaction occurs with the concentration increase of alcohol and acid on the reactive system, consuming both the acid and the alcohol, causing a decrease in the hydrolytic activity.

The greatest hydrolytic activities with 75.5 and 43.7 U g^{-1} for the immobilized and free forms have been observed at 15 min of reaction time and such time was chosen for the subsequent tests.

It is noted that on all periods the immobilized lipase demonstrated greater activity than the free one. Such behavior suggests a positive effect of the immobilization over the enzyme's activity in terms of the hydrolysis reaction. It is worth mentioning that both tests have been done with the same catalyst mass (free and immobilized). In this context, this effect is sharper when considering that the immobilized mass (0.2 g) employed on the test has, theoretically, 0.0037 g of the enzyme. In other words, 5.4 times less enzyme mass (0.02 g) was present on the test with free enzyme, which was conducted with 0.2 mL of the enzymatic extract prepared with solubilisation of 1 g enzyme lyophilized in 10 mL of water.

Optimization of hydrolytic process: effect of pH and temperature

The hydrolyze results obtained by the experimental design 2^2 showed the best activities for free and immobilized forms with 65.1 and 92.2 U g^{-1}, respectively, at the optimal range (optimal condition) (pH 7 and 40°C) (Table 1).

Table 1. The matrix of experimental design 2^2, the influence of temperature and pH on the hydrolytic activity (U g^{-1}) of *Burkholderia cepacia* lipase immobilized and free.

Test	pH	Temperature (°C)	Enzyme Free (U g^{-1})	Enzyme Immobilized (U g^{-1})	Yield (%)
1	-1 (pH 5)	-1 (20°C)	18.4 ± 1.8	17.7 ± 8.7	521.9
2	1 (pH 9)	-1 (20°C)	34.2 ± 1.4	35.5 ± 4.1	561.6
3	-1 (pH 5)	1 (50°C)	29.2 ± 1.0	28.8 ± 7.8	535.3
4	1 (pH 9)	1 (50°C)	18.7 ± 0.7	17.7 ± 6.8	514.1
5	-1.41 (pH 4.18)	0 (35°C)	15.0 ± 0.6	12.2 ± 1.9	439.2
6	1.41 (pH 9.82)	0 (35°C)	20.4 ± 0.5	23.3 ± 2.7	618.8
7	0 (pH 7)	-1.41 (13.85°C)	38.9 ± 0.9	26.6 ± 4.7	370.7
8	0 (pH 7)	1.41 (56.50°C)	6.2 ± 0.4	6.6 ± 7.7	577.0
9	0 (pH 7)	0 (40°C)	65.2 ± 8.3	92.2 ± 8.7	765.0
10	0 (pH 7)	0 (40°C)	65.1 ± 8.3	93.3 ± 2.7	776.2
11	0 (pH 7)	0 (40°C)	66.4 ± 8.3	91.1 ± 4.1	742.5

*Variables fixed: 160 rpm, 0.1 g enzyme, and 15 min reaction.

According to the data, it can be noted that the process optimization condition had been achieved. Equation 4 and 5 show the second order coded model for hydrolytic activity of free and immobilized lipase in terms of temperature and pH, within the ranges studied.

$$\text{Hydrolytic activity (U g}^{-1}) = 65.6 + 1.6.\text{pH} - 22.7\ \text{pH}^2 - 6.4.\text{T} - 20.3.\text{T}^2 - 6.6\ \text{pH.T} \quad (4)$$

$$\text{Hydrolytic activity (U g}^{-1}) = 92.2 + 2.8.\text{pH} - 35.4\ \text{pH}^2 - 4.4.\text{T} - 35.9.\text{T}^2 - 7.2\ .\text{pH.T} \quad (5)$$

where:
T = temperature (°C).

The model was validated by variance analysis and the non-significant parameters were added to the lack of fit for the analysis of variance (ANOVA) test. The correlation coefficient was 0.94 and 0.98, the F calculated value was 16.7 and 55.5 times higher than the F tabulated value for the free and immobilized lipase, allowing the contour curve construction in Figure 3, the free lipase (a) and (b) and immobilized lipase (c) and (d) .

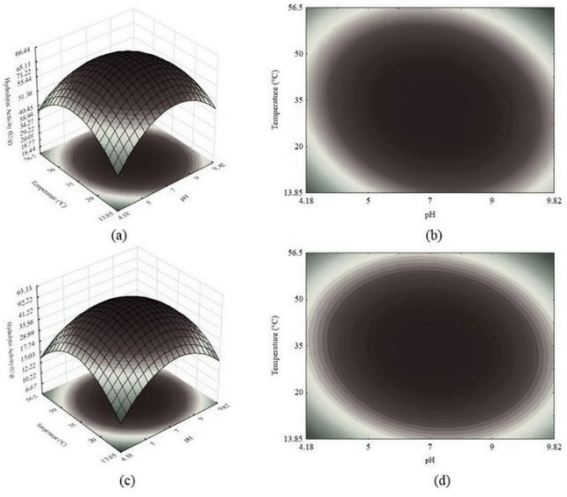

Figure 3. Response surface and contour curve in accordance with the predictive model for the hydrolytic activity of free (a) and (b) immobilized (Figure 4c and d) lipase in terms of pH and temperature.

The optimal range (optimal condition) for the hydrolytic reaction was at pH 7 and at 40°C. The pH adaptability should be associated with the carriers charge. The presence of cations in polyurethane hydroxyl radicals might have reduced the low pH sensitivity. Furthermore, the structure screen function would play an important role on the adaptability enhancement (Hu et al., 2012).

The results obtained in the current work are similar to those from previous reports in the literature by Souza et al. (2014), using *Burkholderia cepacia* lipase encapsulated with polyethylene glycol (1% of PEG1500) was immobilized in sol-gel with 89.91 U g^{-1} activity and 91.4% yield. For Padilha, Ferreira, Castiglioni, Alegre, and Tambourgi (2011), using *Burkholderia cepacia* lipase the activity showed maximum value (1.85 U mL^{-1}) to a specific activity of 29.30 U mg^{-1} and the others Jegannathan, Chan, and Ravindra (2009), Liu and Chang (2008) and Hu et al. (2012), on different supports and consequent industrial processes.

The pH and temperature optimization minimize the enzymes' activity loss since they avoid conditions that would favor their inactivation (Soares, Santana, Zanin, & Castro, 2003, Padilha et al., 2011). The immobilization yield calculations that evaluate the process efficiency were conducted at an optimized condition.

Efficiency of immobilization

The immobilization process' efficiency was evaluated in relation to yield. The free enzyme on the enzymatic extract demonstrates 65.1 U mL^{-1} of hydrolytic activity that corresponds to the total activity given on the 130.2 U immobilization, where 2.0 mL of enzymatic extract were applied at the immobilization stage. The immobilized (10.8 g) showed 90.2 U g^{-1} of hydrolytic activity, corresponding to 974.2 U of total activity, in other words, an immobilization yield of 748.2%. Yields greater than 100%, for this same support, on esterification and hydrolysis activities were reported by Nyari et al. (2016) and Bustamante-Vargas et al. (2015) for *Candida antarctica* lipase and (Rohapect™ DA6L) pectinase enzymes from *Aspergillus niger*, respectively, both *in situ* immobilized in polyurethane. These results suggest a beneficial effect of immobilization on the enzyme activity. This trend may be associated with several factors, such as easy accessibility of new active sites.

On the proposed immobilization process, the support's isocyanate groups (-SCN) might be linking covalently with lipase hydroxyl groups (-OH) during the support's polymerization stage, which occurs with volume expansion. When expanded, the support with the covalently linked lipase cause structural modifications on the lipase, similar to the unwind of a thread ball (a form assigned to lipases that best describes its conformational structure) (Uppenberg, Hansen, Patkar, & Jones, 1994, Uppenberg et al., 1995), displaying new active sites facilitating the substrate dispersion access to it, therefore increasing the activity.

This yield is greater than on reported studies in the literature for other supports. For the *Candida rugosa* immobilized in silica (CPS) Soares et al. (2003) obtained an immobilization yield of 59.6%. For Liu and Chang (2008), the yield was 42.6% in K-carrageenan. For Hu et al. (2012), the yield was 84.3% in NKA macroporous resin. For

Palomino-Romero et al. (2012) the yield was 87% in the SBA-15 mesoporous material. For Shao et al. (2015) the yield was 100% in Accurel MP.

Stability

Thermal stability

The lipase stability is a determining factor for its application in many biotechnological processes and thermal stability is important to determine practical conditions for its use in biotechnology and food processing. However, the lipase characteristics such as its activity and stability in organic solvents must be taken into account for each single process.

The results relating to the thermal stability of immobilized *Burkholderia cepacia*, conducted at different temperatures (from -4 to 40ºC) are found in Figure 4.

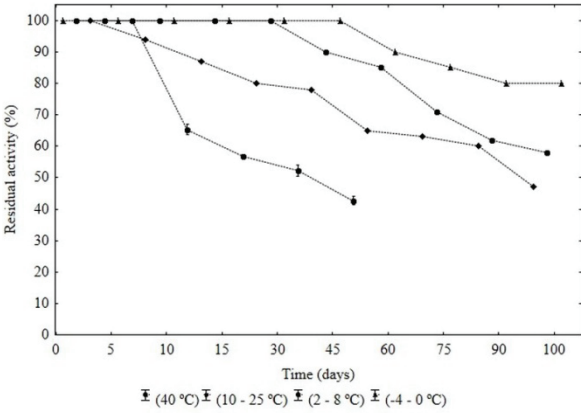

Figure 4. Stability of the immobilized lipase storage at different temperatures: (a) freezer (-4 to 0°C), refrigerator (2 to 8°C), ambient (10 to 25°C) and 40ºC.

Based on the results, a negative effect of temperature can be observed on the thermal stability of the immobilized lipase from *Burkholderia cepacia*, at 40ºC temperature, producing only 30 days of residual activity greater than 50% when compared to the initial one. In other words, it is approximately 3 times less than the 100 days observed for storage temperatures from -4 to 25ºC.

This trend is coherent with the literature, in which it describes that the Lipase inactivation is proportional to temperature increase (Liu & Chang, 2008, Jegannathan et al., 2009). In this respect, the smaller the denaturation the greater the time of denaturation and therefore, it was stable at the temperature analysis.

The thermal stability results of the immobilized lipase from *Burkholderia cepacia*, stored at ambient (10 to 25ºC), refrigerator (2 to 8ºC) and freezer (-4 to 0ºC) temperatures have shown days 5, 30 and 45 with 100% of residual activity in relation to the initial one (93.3 U g^{-1}). The residual activities noted at 100 days were 47.6, 62.0 and 80%, respectively.

The results obtained from the current study are similar to those from previous reports in the literature by Chiou and Wu (2004), Liu and Chang (2008), Jegannathan et al. (2009) and Hu et al. (2012) (Table 2).

Results indicated that polyurethane provides better microenvironment to endure high temperatures and it might protect enzyme conformations from destruction by high temperatures.

Operational stability

One of the most important properties of the immobilized is its recovery and reuse abilities, in financial and environmental terms; it may be a limiting factor for industrial applications. In this way, the immobilized's operational stability was evaluated in multiple operating cycles on hydrolytic reactions. The immobilized lipase showed a residual activity of 32%, relative to 93.33 U g^{-1} initial activity (Figure 5) during 6 continuous cycles.

The results obtained from the current study are similar to those from previous reports in the literature by Jegannathan et al. (2009) and Liu et al. (2011). The immobilization efficiency can be determined by the possibility of recovery, use, and reuse in continuous biocatalytic processes. Among these factors, it is possible to mention the support type used, binding, the reaction medium and processes that will be submitted (Cadena et al, 2010).

Table 2. Examples of *Burkholderia cepacia* lipase immobilized in different supports.

Support	Yield (%)	Opt. (ºC)	Opt. pH	Ther stab. (ºC)	T (hour min^{-1})	Ope. Stab. (%)	References
Accurel MP 1000	100	40	-	-	24 hours	8 cycles	Shao et al. (2015)
macroporous resin NKA	84.3	37	-	-	1 hour	5 cycles	Hu et al. (2012)
andrographolide	99	50	-	-	4 hours	8 cycles	Liu et al. (2011)
mesoporous material SBA-15	87	60	6.0 – 8.5	70°C - 24%	6 hours	-	Palomino-Romero et al. (2012)
K-carrageenan	42.6	45	6.0 – 9.0	room - 50°C	10 days	6 cycles	Liu and Chang (2008)
Poly-hydroxybutyrate particles (PHB)	95,8	50	8.5	-	2 hours	6 cycles	Hara, Hanefeld, and Kanerva (2011)
mesoporous magnetic	96	-	7.5	30°C - 83% - 56 days	4 hours	10 cycles	Cadena et al. (2010)
in situ in polyurethane	700	40	7.0	- 4 the 25ºC - 100 days 40 the 80ºC - 40 days	15 min	6 cycles	This work

*Where: Optimal temperature (ºC) = Opt. (ºC); Optimal pH = Opt pH; Thermal stability (ºC) = Ther. Stab. (ºC); Time (hours min^{-1}) = T and Operational Stability (%) = Op. Stab. (%). *Produced by the author.

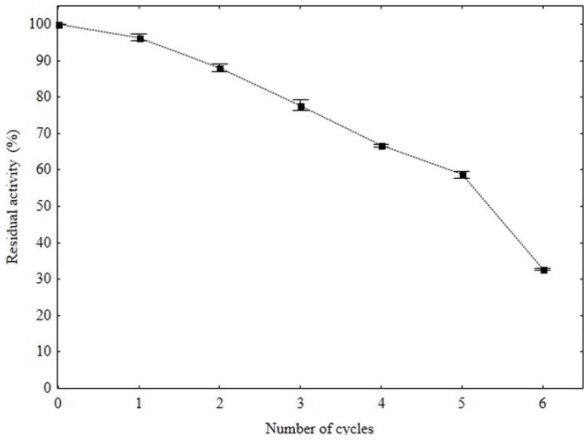

Figure 5. Operational stability of immobilized lipase in continuous cycles of use.

Conclusion

According to the results, it was possible to achieve the best conditions for the olive oil hydrolysis process in pH and temperature terms using *Burkholderia cepacia* lipase *in situ* immobilized in a polyurethane support. The immobilized achieved a hydrolytic activity of 93 U g^{-1} in reaction conditions of pH 7, at 40°C for 15 min. The immobilized remained stable for up to 40 days of storage, at temperatures between 40 and 80°C, and 100 days at 4 to 25°C. It also showed the operational stability of 6 continuous cycles. Thus, the immobilized has proven to be a promising alternative for the implementation of olive oil in the hydrolysis process.

Acknowledgements

The authors would like to thank URI University - Erechim Campus, CNPq, Capes and Fapergs for their infrastructure and financial support for this research.

References

Alonso-Morales, N., Lopez-Gallego, F., Betancor, L., Hidalgo, A., Mateo, C., Fernandez-Lafuente, R., & Guisan, J. M. (2008). Reversible immobilization of glutaryl acylase on sepabeads coated with polyethyleneimine. *Biotechnology Progress, 20*(1), 533-536.

Baldo, C., Baggio, L. M., Moreno, T. G., Magri, A., Melo, M. R., Gasparin, F. G. M., & Celligoi, M. A. P. C. (2013). Estudo da produção de lipase por bactérias isoladas de efluente de abatedouro avícola. *Biochemistry Biotechnology Reports, 2*(1), 347-341.

Barros, H. S., Maia, C. E. M., Souza, L., & Di, A. (2014). Extração do óleo presente na escuma de esgoto para uso na produção de biodiesel. *Revista Sociedade de Ciência Química e Tecnologia, 2*(1), 42-50.

Battimelli, A., Loisel, D., Garcia□Bernet, D., Carrere, H., & Delgenes, J. P. (2010). Combined ozone pretreatment, and biological processes for removal of colored and biorefractory compounds in wastewater from molasses fermentation industries. *Journal Chemistry Technology Biotechnology, 85*(1), 968-975.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1-2), 248-254.

Buss, D. A., & Henkes, J. A. (2014). Estudo dos impactos ambientais causados por laticínios com foco no reaproveitamento dos resíduos gerados. *Revista Gestão Sustentabilidade Ambiental, 3*(1), 392-401.

Bustamante-Vargas, C. E., Oliveira, D., Nyari, N. L., Valduga, E., Soares, M. B. A., Backes, G. T., & Dallago, R. M. (2015). *In situ* immobilization of commercial pectinase in rigid polyurethane foam and application in the hydrolysis of pectic oligosaccharides. *Journal of Molecular Catalysis B: Enzymatic, 122*(1), 35-43.

Cadena, P. G., Jeronimo, R. A. S., Melo, J. M., Silva, R. A., Lima Filho, J. L., & Pimentel, M. C. B. (2010). Covalent immobilization of invertase on polyurethane, plast-film and ferromagnetic Dacron. *Bioresource Technology, 101*(1), 1595-1602.

Cammarota, M. C., & Freire, D. M. G. (2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology, 97*(1), 2195-2201.

Chen, D., Zhang, H., Xu, J., & Yan, Y. (2013). Effect of sub-and supercritical CO_2 treatment on the properties of *Pseudomonas cepacia* lipase. *Enzyme Microbiology Technology, 53*(1), 110-117.

Chiou, S. H., & Wu, W. T. (2004). Immobilization of *Candida rugosa* lipase on chitosan with activation of the hydroxyl groups. *Biomaterials, 25*(1), 197-204.

Cipolatti, E. P., Moreno-Pérez, S., Souza, L. T. A., Valério, A., Guisán, J. M., Araújo, P. H., & Pessela, B. C. (2015). Synthesis and modification of polyurethane for immobilization of *Thermomyces lanuginosus* (TLL) lipase for ethanolysis of fish oil in solvent free system. *Journal Molecul Catalysis B: Enzymatic, 122*(1), 163-169.

Colla, L. M., Ficanha, A. M., Rizzardi, J., Bertolin, T. E., Reinehr, C. O., & Costa, J. A. V. (2014) Production and characterization of lipases by two new isolates of *Aspergillus* through solid-state and submerged fermentation. *Biomed Medical Research International, 7*(1), 77-73.

Costa, D. M., Castro, R. S. S., Padilla, R. Y. C., Silva, D. P., Ruzene, D. S., Lima, Á. S., & Soares, C. M. F. (2014). Uso de sabugo de milho pré-tratado para a imobilização de lipases de *Burkholderia cepacia*. *Sempesq, 16*(1), 24-25.

Cui, C., Tao, Y., Li, L., Chen, B., & Tan, T. (2013). Improving the activity and stability of *Yarrowia lipolytica* lipase Lip2 by immobilization on polyethyleneimine-coated polyurethane foam. *Journal Molecular Catalysis B: Enzymatic, 91*(1), 59-66.

Frinhani, E. M. D., & Moreira, M. C. G. (2014). Otimização da oxigenação de efluentes domésticos por meio da variação das configurações de operação de aeradores de fluxo descendente. *Unoesc Ciência-ACET, 5*(1), 73-82.

Hara, P., Hanefeld, U., & Kanerva, L. T. (2011). Immobilised *Burkholderia cepacia* lipase in dry organic solvents and ionic liquids: a comparison. *Green Chemistry, 11*(1), 250-256.

Hu, Y., Tang, S., Jiang, L., Zou, B., Yang, J., & Huang, H. (2012). Immobilization of *Burkholderia cepacia* lipase on functionalized ionic liquids modified mesoporous silica SBA-15. *Process Biochemistry, 47*(1), 2291-2299.

Jeganathan, J., Nakhla, G., & Bassi, A. (2007). Oily wastewater treatment using a novel hybrid PBR–UASB system. *Chemosphere, 67*(1), 1492-1500.

Jegannathan, K. R., Chan, E. S., & Ravindra, P. (2009). Physical and stability characteristics of *Burkholderia cepacia* lipase encapsulated in κ-carrageenan. *Journal Molecul Catalysis B: Enzymatic, 58*(1), 78-83.

Liu, C. H., & Chang, J. S. (2008). Lipolytic activity of suspended and membrane immobilized lipase originating from indigenous *Burkholderia* sp. C20. *Bioresource Technology, 99*(1), 1616-1622.

Liu, T., Liu, Y., Wang, X., Li, Q., Wang, J., & Yan, Y. (2011). Improving catalytic performance of *Burkholderia cepacia* lipase immobilized on macroporous resin NKA. *Journal Molecul Catalysis B: Enzymatic, 71*(1), 45-50.

Mannarino, C. F., Moreira, J. C., Ferreira, J. A., & Arias, A. R. L. (2013). Avaliação de impactos do efluente do tratamento combinado de lixiviado de aterro de resíduos sólidos urbanos e esgoto doméstico sobre a biota aquática. *Ciência Saúde Coletiva, 18*(1), 3235-3241.

Manoel, E. A., Ribeiro, M. F., Santos, J. C., Coelho, M. A. Z., Simas, A. B., Fernandez-Lafuente, R., & Freire, D. M. (2015). Accurel MP 1000 as a support for the immobilization of lipase from *Burkholderia cepacia*: Application to the kinetic resolution of myo-inositol derivatives. *Process Biochemistry, 50*(1), 1557-1564.

Nyari, N. L. D., Fernandes, I. A., Bustamante-Vargas, C. E., Steffens, C., Oliveira, D., Zeni, J., … Dallago, R. M. (2016). *In situ* immobilization of *Candida antarctica* B lipase in polyurethanefoam support. *Journal of Molecular Catalysis B: Enzymatic, 124*(1), 52-61.

Nyari, N. L. D., Zeni, J., Steffens, C., Dallago, M. R., & Rigo, E. (2015). Easy and fast method of *Candida antarctica* B lipase immobilization in polyurethane foam. *Indian Journal Advance Chemical Science, 4*(1), 315-322.

Padilha, G. S., Ferreira, J. F., Castiglioni, G. L., Alegre, R. M., & Tambourgi, E. B. (2011). Avaliação da lipase extracelular de *Pseudomonas cepacia* para purificação em sistema bifásico aquoso. *Ciências Tecnologia de Alimentos, 31*(1), 16-22.

Palomino-Romero, J. A., Leite, O. M., Eguiluz, K. I. B., Salazar-Banda, G. R., Silva, D. P., & Cavalcanti, E. B. (2012). Tratamentos dos efluentes gerados na produção de biodiesel. *Química Nova, 35*(1), 367-345.

Rezende, J. H., Carboni, M., Murgel, M. A. D. T., Capps, A. L. A. P., Teixeira, H. L., Simões, G. T. C., & Oliveira, C. D. A. (2013). Composição gravimétrica e peso específico dos resíduos sólidos urbanos em Jaú (SP). *Revista Engenharia Sanitária Ambiental, 18*(1), 1-8.

Salum, T. F. C., Villeneuve, P., Barea, B., Yamamoto, C. I., Côcco, L. C., Mitchell, D. A., & Krieger, N. (2010). Synthesis of biodiesel in column fixed-bed bioreactor using the fermented solid produced by *Burkholderia cepacia* LTEB11. *Process Biochemistry, 45*(1), 1348-1354.

Santos, A. D. E., Guimarães, J. F., Rosa, P. E. C., & Carvalho, B. J. J. (2014). Redução da turbidez e *Escherichia coli* ATCC 25922 em efluente sintético de laticínio pós-tratamento com *Moringa oleifera* Lam. *Blucher Food Science Proceedings, 1*(1), 637-640.

Santos, A. T. L., Henrique, N. S., Shhlindwein, J. A., Ferreira, E., & Stachiw, R. (2015). Aproveitamento da fração orgânica dos resíduos sólidos urbanos para produção de composto orgânico. *Revista Brasil Ciência Amazônia, 3*(1), 15-21.

Shao, Y. B., Jing, T., Tian, J. Z., Zheng, Y. J., & Shang, M. H. (2015). Characterization and optimization of mesoporous magnetic nanoparticles for immobilization and enhanced performance of porcine pancreatic lipase. *Chemical Papers, 69*(1), 298-1311.

Silva, C., Silva, I., Gois, G., Almeida, R., & Abud, A. (2015). Avaliação das condições de pré-tratamento e hidrólise enzimática do resíduo do processamento de graviola visando a obtenção de etanol 2G. *Blucher Chemistry Engineering Proceedings, 1*(1) 5698-5704.

Silva, N. C., Miranda, J. S., Bolina, I. C., Silva, W. C., Hirata, D. B., Castro, H. F., & Mendes, A. A. (2014). Immobilization of porcine pancreatic lipase on poly-hydroxybutyrate particles for the production of ethyl esters from macaw palm oils and pineapple flavor. *Biochemistry Engineering Journal, 82*(1), 139-149.

Simões, A. S., Mori, R. Y., Faria, R., Castro, H. F. D., & Mendes, A. A. (2011). Desempenho da matriz híbrida SiO2-quitosana na imobilização da lipase microbiana de *Candida rugosa. Química Nova, 34*(1), 33-38.

Soares, C. M. F., Santana, M. H. A., Zanin, G. M., & Castro, H. F. (2003). Efeito do polietilenoglicol e da albumina na imobilização de lipase microbiana e na catálise em meio orgânico. *Química Nova, 26*(6), 832-838.

Souza, R. L., Faria, E. L., Figueiredo, R. T., Fricks, A. T., Zanin, G. M., Santos, O. A., & Soares, C. M. (2014). Use of polyethylene glycol in the process of sol–gel encapsulation of *Burkholderia cepacia* lipase. *Journal of Thermal Analysis and Calorimetry, 117*(1), 301-306.

Uppenberg, J., Hansen, M. T., Patkar, S., & Jones, T. A. (1994). The sequence, crystal structure determination, and refinement of two crystal forms of lipase B from *Candida antarctica. Structure, 2*(1), 293-308.

Uppenberg, J., Oehrner, N., Norin, M., Hult, K., Kleywegt, G. J., Patkar, S., & Jones, T. (1995). Crystallographic and molecular-modeling studies of lipase B from *Candida antarctica* reveal an estereospecificity pocket for secondary alcohols. *Biochemistry, 34*(1), 16838-16851.

Valadão, F. C. A., & Benedet, K. D. (2011). Variação nos atributos do solo em sistemas de manejo com adição de cama de frango. *Revista Brasileira Ciência Solo, 35*(1), 2073-2082.

Zadinelo, I. V., Sereniski, R. M., Borin, R., Fagnani, K. C., Stremel, D. P., & Gomes, L. F. S. (2013). Potencial da produção de biogás a partir de efluente pré-tratado de abatedouro de aves da região do Oeste do Paraná. *Revista Brasileira Energias Renováveis, 2*(1), 1-11.

Zhang, X. Z., Sathitsuksanoh, N., Zhu, Z., & Zhang, Y. H. P. (2011). One-step production of lactate from cellulose as the sole carbon source without any other organic nutrient by recombinant cellulolytic *Bacillus subtilis*. *Metabolic Engineering, 11*(1), 364-372.

Received on January 27, 2016.
Accepted on May 25, 2016.