COBEQ 2018 XXII CONGRESSO BRASILEIRO DE ENGENHARIA QUÍMICA
23 a 26 de Setembro de 2018
Hotel Maksoud Plaza
São Paulo – SP

ENBEQ 2018 XVII ENCONTRO BRASILEIRO SOBRE O ENSINO DE ENGENHARIA QUÍMICA
27 a 28 de Setembro de 2018
USP
São Paulo – SP

EFFECT OF ENZYME LOADING ON THE IMMOBILIZATION PARAMETERS OF A LIPASE ADSORBED ON FUNCTIONALIZED RICE HUSK SILICA

MIGUEZ JP, MACHADO NB, MENDES AA

Federal University of Alfenas, Institute of Chemistry
E-mail: adriano.mendes@unifal-mg.edu.br

ABSTRACT – *Commercial lipase from Thermomyces lanuginosus (TLL) was adsorbed on hydrophobic Octyl–SiO_2 via interfacial activation and anion exchanger Amino–SiO_2 via ionic interaction. In this study, rice husk silica was used as matrix to prepare such supports. The effect of enzyme loading on the immobilization parameters has been evaluated. Maximum immobilized protein loading of 12.3 and 21.9 mg/g for Amino–SiO_2 and Octyl–SiO_2 was observed, respectively. Experimental data on Octyl–SiO_2 and Amino–SiO_2 adsorption were well-fitted to Langmuir isotherm model. Similar hydrolytic activity values (between 630 and 645 IU/g of biocatalyst) were observed. However, strong diffusional limitation in hydrolysis reaction has been observed for immobilized TLL on hydrophobic support (Octyl–SiO_2). These results show the potential application of the supports prepared in lipase immobilization for further use in biotransformation reactions.*

1. INTRODUCTION

Lipases (triacylglycerol ester acylhydrolases, EC 3.1.1.3) are hydrolases that cleavage carboxylic ester bonds in tri-, di-, and monoacylglycerols to glycerol and free fatty acids at the water-lipid interface. In environments with low water content, these enzymes also catalyze other biotransformation reactions such as esterification, interesterification and transesterification (Adlercreutz, 2013). The use of free lipases in industrial processes has some limitations such as difficult reusability and poor solvent tolerance capability (Alves *et al.*, 2017). These problems can be overcome by immobilizing lipases using different techniques. Among them, physical adsorption has attracted significant commercial attention in the recent years because it is simpler and less expensive than other techniques and high catalytic activity and stability may be retained (Vescovi *et al.*, 2016; Alves *et al.*, 2017). This method allows the reusability of supports after inactivation of immobilized enzyme using several chemicals, including surfactants, urea and guanidine (Adlercreutz, 2013).

In this study, TLL was immobilized by physical adsorption on functionalized supports prepared via functionalization of rice husk silica with octyl (Octyl–SiO_2) aminopropyl (Amino–SiO_2) groups. The effect of initial protein loading on the immobilization parameters was evaluated in the hydrolysis of olive oil emulsion and adsorbed TLL concentration. Isotherm studies were also performed to elucidate the adsorption process.

2. MATERIALS AND METHODS

2.1. Materials

TLL was purchased from Sigma-Aldrich (St. Louis, MO, USA). Rice husk silica was prepared via a hydrothermal process (Zhang *et al.*, 2017). Octyl–SiO_2 and Amino–SiO_2 were prepared via functionalization of rice husk silica according methodology described in a study performed in our group (Vescovi *et al.*, 2016).

2.2. Physical adsorption of TLL on silica-based supports – Isotherm studies

1 g of support was incubated in 19 mL of 5 mM sodium acetate containing different protein loadings ranging from 5 to 40 mg protein/g of support. The suspensions were kept under agitation (200 rpm) in an orbital shaker at room temperature within 15 h. The heterogeneous biocatalysts were filtered under vacuum, washed with distilled water (volume ratio 1:5) and stored at 4 °C for 24 h prior to use. In this study, non-linear Langmuir isotherm model (Eq. (1)) was fitted to the experimental data (Alves *et al.*, 2017).

$$q_e = \frac{q_{\max} \times C_e}{K_L + C_e} \quad (1)$$

where q_e is the adsorption capacity at equilibrium (mg protein/g of support), C_e is defined as the residual mass of protein in unit mass of lipase solution (mg protein/g of solution), q_{max} is the maximum adsorption capacity (mg protein/g of support) and K_L is the Langmuir constant related to the energy of adsorption (mL/mg of protein).

2.3. Determination of immobilization parameters

The hydrolytic activity (HA) was determined in the hydrolysis of olive oil emulsion at pH 8.0 (buffer sodium phosphate 100 mM) and 37 °C under agitation in an orbital shaker (200 rpm) (Alves *et al.*, 2017). One international unit (IU) of activity was defined as the mass of enzyme required to release 1 µmol of free fatty acids per minute of reaction. Protein was determined by the Bradford's method (Bradford, 1976). Immobilized protein (IP) was calculated after determining the amount of disappeared protein in the supernatant and comparing to the initial concentration (mg/g support). Specific activity (SA) was calculated as the hydrolytic activity of the biocatalyst per milligram of immobilized protein (IU/mg_{IP}).

3. RESULTS AND DISCUSSION

The effect of initial protein loading on the immobilization parameters has been studied in order to determine TLL maximum adsorption capacity on both functionalized supports. The immobilization procedure was performed under optimal experimental conditions: buffer sodium acetate pH 4.0 for Amino–SiO_2 and pH 5.0 for Octyl–SiO_2 at 25 °C and 15 h of incubation. From the data summarized in Table 1, it is possible to observe an increase in immobilized enzyme concentration as the initial protein loading rose to 20 and 30 mg/g for Amino–SiO_2 and Octyl–SiO_2, respectively. Further increase in protein loading did not improve TLL adsorption on both support surfaces due to support saturation. These results indicate that both supports have a limited number of adsorption sites on their surfaces that can

interact with TLL. Maximum capacity of Amino–SiO_2 and Octyl–SiO_2 to adsorb TLL was 12.3 ± 0.1 mg/g and 21.9 ± 0.1 mg/g of support, respectively.

Table 1 – Influence of enzyme loading on immobilization parameters of TLL adsorbed on Octyl–SiO_2 and Amino–SiO_2.

Enzyme loading (mg/g)	Octyl–SiO_2			Amino–SiO_2		
	IP (mg/g)	HA (IU/g)	SA (IU/mg_{IP})	IP (mg/g)	HA (IU/g)	SA (IU/mg_{IP})
5	5.0	192.7 ± 5.5	38.1 ± 2.4	4.8 ± 0.1	443.7 ± 9.8	92.5 ± 3.3
10	9.9 ± 0.1	429.9 ± 9.2	43.2 ± 3.1	9.1 ± 0.2	570.9 ± 6.2	62.7 ± 1.8
15	14.8 ± 0.1	511.0 ± 3.3	34.3 ± 1.3	11.5 ± 0.1	645.8 ± 6.9	56.2 ± 0.9
20	19.7 ± 0.3	554.8 ± 1.0	28.2 ± 2.1	12.3 ± 0.1	617.4 ± 15.2	50.2 ± 1.5
25	20.4 ± 0.1	557.2 ± 4.3	27.3 ± 0.9	11.9 ± 1.0	618.6 ± 12.4	51.7 ± 6.1
30	21.9 ± 0.1	561.4 ± 6.9	25.6 ± 2.4	11.6 ± 0.7	616.7 ± 20.4	53.4 ± 4.2
35	21.7 ± 0.5	630.3 ± 5.8	29.0 ± 1.7			
40	21.3 ± 0.4	618.9 ± 4.9	26.5 ± 0.8			

The experimental data of adsorption of TLL on both functionalized supports were adequately explained by the Langmuir isotherm model ($R^2 \geq 0.974$) – Fig. 1A,B. Moreover, values of theoretical maximum adsorption capacity obtained for Octyl–SiO_2 (q_{max}=21.8 mg/g) and Amino–SiO_2 (q_e=12.4 mg/g of support) for initial protein loading of 20 mg/g) were very similar to experimental data – 21.9 mg/g for Octyl–SiO_2 and 12.3 mg/g for Amino–SiO_2. Langmuir isotherm is the most widely used model for lipase adsorption on several supports. This model assumes monolayer adsorption of adsorbate molecules on an adsorbent surface containing a limited number of adsorption sites (Alves *et al.*, 2017).

As it can be seen in Table 1, there was greater hydrolytic activity by increasing initial protein loading. The hydrolytic activity using Octyl–SiO_2 varied from 192.7 ± 5.5 (5 mg/g) to 630.3 ± 5.8 IU/g (35 mg/g) while this increase was around 1.5 times higher – from 443.7 ± 9.8 (5 mg/g) to 645.8 ± 6.9 IU/g (15 mg/g) for Amino–SiO_2. Further increase of initial protein loading over 15 mg/g of support and 35 mg/g of support for Amino–SiO_2 and Octyl–SiO_2, respectively, has not promoted increase in hydrolytic activity. On the other hand, specific activity values decreased as initial protein loading increased. These results could be attributed to a larger number of protein–protein interactions (e.g. intermolecular steric interactions) on the biocatalyst surface, thus limiting the access of substrate molecules to the active sites of immobilized TLL (Alves *et al.*, 2017). Specific activity values of the biocatalysts prepared by using Amino–SiO_2 were 2-3 times higher than Octyl–SiO_2 due to orientation of TLL on the support surface towards the reaction medium, thus allowing better accessibility of droplets of

oil to the active sites of immobilized TLL molecules (Vescovi *et al.*, 2016; Alves *et al.*, 2017).

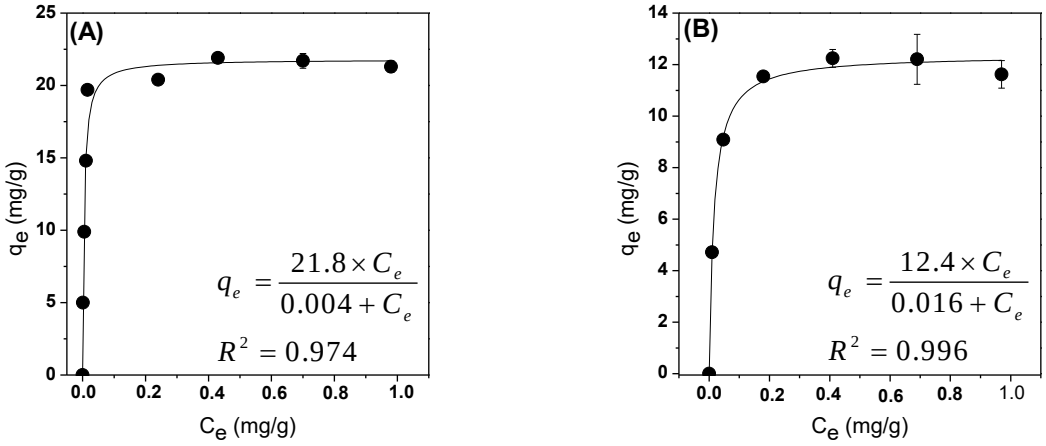

Figure 1 – Plot of Langmuir isotherm model for equilibrium data of TLL adsorption on Octyl–SiO_2 (A) and Amino–SiO_2 (B) at 25 °C.

The present study showed that TLL was successfully immobilized on both functionalized rice husk silica. Octyl–SiO_2 provided biocatalysts with the highest immobilized enzyme concentration. Thus, strong diffusional limitations were observed for these biocatalysts in olive oil hydrolysis. These results show the modulation of the catalytic properties of TLL due to its different orientation on the support surfaces.

4. ACKNOWLEDGEMENTS

This study was financially supported by FAPEMIG (Process APQ–02196–15), CNPq (Process 404929/2016–8) and CAPES (Brazil).

5. REFERÊNCIAS

ADLERCREUTZ P, Immobilisation and application of lipases in organic media. *Chem. Soc. Rev.*, v. 42, p. 6406-6436, 2013.

ALVES MD, ARACRI FM, CREN EC, MENDES AA, Isotherm, kinetic, mechanism and thermodynamic studies of adsorption of a microbial lipase on a mesoporous and hydrophobic resin. *Chem. Eng. J.*, v. 311, p. 1-12, 2017.

BRADFORD MM, Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye. *Anal. Biochem.*, v. 72, p. 248-254, 1976.

VESCOVI V, KOPP W, GUISAN JM, GIORDANO RLC, MENDES AA, TARDIOLI PW, Improved catalytic properties of *Candida antarctica* lipase Bmulti-attached on tailor-made hydrophobic silica containing octyland multifunctional amino-glutaraldehyde spacer arms. *Process Biochem.*, v. 51, p. 2055-2066, 2016.

ZHANG S, GAO H, LI J, HUANG Y, ALSAEDI A, HAYAT T, XU X, WANG J, Rice husks as a sustainable silica source for hierarchical flower-like metal silicate architectures assembled into ultrathin nanosheets for adsorption and catalysis. *J. Hazard. Mater.*, v. 321, p. 92-102, 2017.