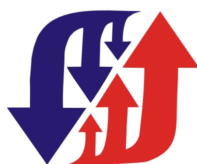

XII Seminário Brasileiro de Tecnologia Enzimática
ENZITEC 2016

Influence of protein loading on the catalytic properties of immobilized lipase from *Thermomyces lanuginosus* on poly-(styrene-divinylbenzene): Determination of thermodynamic and isotherm parameters

Michelle D. Alves[1], Fernanda M. Aracri[2], Jaquelinne J. Bassi[2], Érika C. Cren[1] and Adriano A. Mendes[2]

[1]Universidade Federal de Minas Gerais – Depto. de Engenharia Química
31270-901 Belo Horizonte – MG
[2]Universidade Federal de Alfenas – Instituto de Química
37130-000 Alfenas – MG - E-mail: adriano.mendes@unifal-mg.edu.br

ABSTRACT

In the present study, Thermomyces lanuginosus lipase (TLL) was physically adsorbed on poly-(styrene-divinylbenzene) (PSty-DVB) resin in order to prepare highly active biocatalysts. The effect of initial protein loading on the catalytic properties of the biocatalysts was evaluated. The adsorption capacity at 25 °C and pH 5.0 at low ionic strength (5 mM buffer sodium acetate) was around 134 mg of protein/g of support using initial protein loading of 150 mg/g of support. Maximum hydrolytic activity varied from 235 to 470 IU/g of support. The equilibrium adsorption data fitted to the Langmuir isotherm model (R^2=0.9776). Thermodynamic analysis showed that the adsorption was a spontaneous process ($-18.7 \leq \Delta G \leq -9.0$ kJ/mol).

Keywords: Physical adsorption, Lipase, Poly-(styrene-divinylbenzene) resin, Catalytic properties.

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 (Lage et al., 2016).

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 (Fernández-Lafuente, 2010; Adlercreutz, 2013). This method allows the reusability of supports after inactivation of immobilized enzyme using several chemicals, including surfactants, urea and guanidine (Fernández-Lafuente, 2010). In this study, TLL was immobilized on PSty-DVB resin via physical adsorption at low ionic strength. The effect of initial protein loading on the catalytic properties was evaluated in hydrolysis reaction and immobilized protein concentration. Thermodynamic and isotherm studies were also performed.

MATERIALS AND METHODS

Materials

Thermomyces lanuginosus lipase (TLL) was purchased from Sigma-Aldrich (St. Louis, MO, USA). PSty-DVB resin (average particle diameter of 250–850 µm, surface area of ≈500 m^2/g and average porous size of 260 Å) was purchased from Supelco (Bellefonte, PA, USA). Olive oil from Carbonell (Córdoba, Spain) was purchased at a local market (Alfenas, MG, Brazil). Arabic Gum was acquired from Synth® (São Paulo, SP, Brazil). All other chemical reagents were of analytical grade acquired from Vetec Química Ltd. and Synth®.

Determination of the hydrolytic activity and immobilized protein concentration

The hydrolytic activity (HA) of the biocatalysts was determined in the hydrolysis of olive oil emulsion at pH 8.0 (buffer sodium phosphate 100 mM), 37 °C and 5 min under agitation in an orbital shaker (200 rpm) (Lage et al., 2016). One international unit (IU) of hydrolytic activity was defined as the mass of enzyme required to release 1 µmol of free fatty acids per minute of reaction under the conditions above described. Protein was determined by the Bradford's method (Bradford, 1976), using bovine serum albumin as standard protein.

Physical adsorption of TLL – Isotherm and thermodynamic studies

10 g of wet PSty-DVB resin was incubated in 190 mL of 5 mM sodium acetate pH 5.0 containing different protein loadings to vary the support loading from 10 to 200 mg protein/g of support (Lage et al., 2016). The suspensions were kept under agitation (200 rpm) in an orbital shaker at room temperature for 15 h. The biocatalysts were filtered under vacuum, washed with distilled water (volume ratio 1:5) and stored at 4 °C for 24 h prior to use. Immobilized protein (IP) was calculated after determining the amount of protein disappeared in the supernatant and comparing to the initial protein concentration offered (mg/g support). Specific activity (SA) was calculated as the hydrolytic activity of the biocatalyst per milligram of immobilized protein (IU/mg_{IP}). Equilibrium constant (K_c) was determined as the ratio between immobilized protein concentration (mg/g) and residual protein (RP) in solution (RP – mg/mL), respectively. Free energy Gibbs (ΔG) was determined according to Eq. 1:

$$\Delta G\left(kJ/mol\right) = -RT\ln K_c \quad \therefore \quad K_c = \frac{IP}{RP} \tag{1}$$

where R is the gas universal constant (8.314×10^{-3} kJ/mol.K), T is the experimental temperature (298.15 K), and K_c is the equilibrium constant.

In this study, Langmuir isotherm model (Eq. 2) was used to fit the experimental data from TLL adsorption on PSty-DVB (Lage et al., 2016).

$$q_e = \frac{q_{\max} \times C_e}{K_L + C_e} \tag{2}$$

where q_e is the adsorption capacity at equilibrium (mg protein/g of support), C_e is the residual protein concentration after immobilization (mg protein/mL), q_{max} is the maximum adsorption capacity (mg protein/g support), K_L is the Langmuir constant (mL/mg protein).

RESULTS AND DISCUSSION

The influence of protein loading on the catalytic properties of the prepared biocatalysts by immobilizing TLL on PSty-DVB resin via physical adsorption is shown in Table 1.

Table 1. Influence of initial protein loading on the catalytic properties of immobilized TLL on STY-DVB particles

Protein loading (mg/g)	IP (mg/g)	RP (mg/mL)	HA (IU/g)	SA (IU/mg_{IP})	Kc	ΔG (kJ/mol)
10	9.9 ± 0.1	0.005	235.0 ± 5.1	23.7 ± 0.5	1881.1	–18.7
20	19.2 ± 0.5	0.042	432.7 ± 10.1	23.8 ± 1.1	432.1	–15.0
30	29.0 ± 0.4	0.053	467.9 ± 22.9	16.1 ± 0.6	551.0	–15.0
70	69.3 ± 0.6	0.037	445.2 ± 49.4	6.5 ± 0.6	1454.5	–18.1
115	108.7 ± 3.1	0.331	450.7 ± 30.7	4.2 ± 0.3	327.8	–14.4
125	117.0 ± 2.3	0.416	469.9 ± 60.2	4.0 ± 0.6	277.9	–14.0
150	133.9 ± 1.3	0.847	443.0 ± 25.2	3.3 ± 0.1	158.0	–12.5
175	133.9 ± 4.4	2.163	431.8 ± 50.6	3.2 ± 0.5	61.9	–10.2
200	133.5 ± 3.1	3.501	412.6 ± 19.3	3.1 ± 0.1	38.1	–9.0

The immobilized lipase concentration (IP) increased greatly with the offered initial protein concentration up to 150 mg/g of support as expected. Maximum immobilized protein concentration was around 134 mg/g of PMA particles. This support exhibited higher immobilized protein loading when compared to poly-methacrylate particles – ≈100 mg of TLL/g of support (Lage et al., 2016). After, no significant effect was verified due to possible support saturation. Hydrolytic activity of the prepared biocatalysts varied from 235 to 470 IU/g of support. The biocatalysts previously prepared using initial protein loading from 20 to 200 mg/g presented similar hydrolytic activity (HA). These results indicate strong diffusional limitation of oil molecules to the internal microenvironment of the biocatalysts. On the other hand, specific activity (SA) values were influenced by the protein loading, varying from 23.7 ± 0.5 to 3.1 ± 0.1 IU/mgIP. The reduction of SA values may be attributed to strong mass transfer effects because the substrate molecules (droplets of oil) are large, thus reducing their accessibility to the internal biocatalyst surface (possible reduction of porous diameter after immobilization) or steric hindrances because the active center of the enzyme is oriented towards the support surface.

Thermodynamic considerations of an adsorption process are necessary to conclude whether the process is spontaneous or not. Adsorption processes occur spontaneously at a given temperature whether ΔG is a negative value. K_c values (ratio between IP and RP) varied from 1881.1 to 38.1 for lowest and highest protein loading, as shown in Table 1. This parameter was then used to determine ΔG values by using Eq. (1). ΔG values varied from −18.7 to –9.0 kJ/mol, thus indicating that the adsorption of TLL on PSty-DVB was a spontaneous process.

The physical adsorption results were analyzed by applying the Langmuir isotherm model (Fig. 1). The experimental data from the adsorption of TLL on PMA particles were adequately explained by the Langmuir isotherm model (R^2=0.9776). From these observations,

it is possible to verify that the immobilization of TLL on PMA particles occurred via monolayer adsorption (Lage et al., 2016). The theoretical value of maximum adsorbed protein amount on PMA particles (q_{max}) was 148.1 mg/g of support. The difference between maximum observed loading (experimental – 133.9 mg/g of PMA particles) and theoretical q_{max} value could be attributed to compounds (e.g. salts, polyols and sugars) present in crude TLL extract that could also adsorb on the support surface (Fernández-Lafuente, 2010).

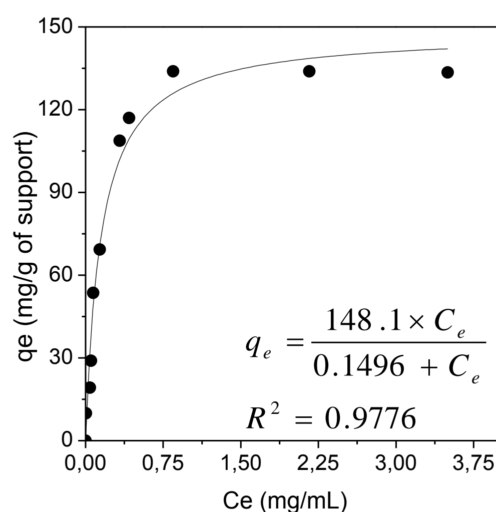

Figure 1. Langmuir isotherm model for TLL immobilization on PSty-DVB resin.

CONCLUSION

The present study showed that TLL was successfully immobilized on PSty-DVB resin (maximum protein loading around 134 mg/g of support). The adsorption was a spontaneous process (ΔG<0). Hydrolytic activity and specific activity values indicated preferential immobilization of the enzyme in the internal support surface. PSty-DVB resin showed to be highly promising to prepare robust biocatalysts due to its large surface area and porous size.

ACKNOWLEDGEMENTS

This paper was financially supported by FAPEMIG (APQ–02196–15) and CNPq.

REFERENCES

Adlercreutz P. 2013. Immobilisation and application of lipases in organic media. Chem Soc Rev 42:6406-6436.

Bradford MM. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248-254.

Fernandez-Lafuente R. 2010. Lipase from *Thermomyces lanuginosus*: Uses and prospects as an industrial biocatalyst. J Mol Catal B Enzym 62:197-212.

Lage FAP, Bassi JJ, Corradini MCC, Todero LM, Luiz JHH, Mendes AA. 2016. Preparation of a biocatalyst via physical adsorption of lipase from *Thermomyces lanuginosus* on hydrophobic support to catalyze biolubricant synthesis by esterification reaction in a solvent-free system. Enzyme Microb Technol 84:56-67.