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Optimization of the immobilization of a microbial lipase via physical adsorption on mesoporous hydrophobic resin

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ABSTRACT

Microbial lipase from Thermomyces lanuginosus (TLL) was immobilized via physical adsorption (interfacial activation) on poly-(styrene-divinylbenzene) (PSty-DVB) resin. The effect of pH, ionic strength and temperature was evaluated. The immobilization parameters were determined in the hydrolysis of olive oil emulsion and immobilized protein concentration. In this set of experiments, the initial protein lading used was 10 mg/g of support. Maximum immobilized protein concentration and hydrolytic activity was observed at pH 5.0 and ionic strength of 5 mM (≈10 mg/g of support and hydrolytic activity of 235 IU/g of support). The temperature of immobilization ranging from 10 to 25°C did not display strong influence on the immobilized protein concentration. High affinity of TLL with the hydrophobic support was verified due to its high surface area and porous size.

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

INTRODUCTION

The application of lipases (triacylglycerol ester acylhydrolases, EC 3.1.1.3) in a large-scale process is often limited due to their high cost, and sensitivity to high temperature and organic solvents. Moreover, it is difficult to separate them from the reaction system, which limits its recovery and may lead to contamination of the final product (Adlercreutz, 2013). In order to overcome these problems, lipases have been immobilized by several protocols (Fernández-Lafuente, 2010; Adlercreutz, 2013). Physical adsorption on hydrophobic supports is an attractive protocol from the industrial point of view because it allows the reuse of the support by desorption of inactive enzyme molecules from the biocatalyst surface (Fernández-Lafuente, 2010; Hernández et al., 2011). This protocol promotes the stabilization of the lipases in open conformation (interfacial activation), thus making it especially suitable for lipase immobilization (Salis et al., 2005; Fernández-Lafuente, 2010; Hernández et al., 2011; Adlercreutz, 2013; Miranda et al., 2014; Lage et al., 2016). In this study, microbial lipase from *Thermomyces lanuginosus* (TLL) was immobilized via physical adsorption on poly-(styrene-divinylbenzene) resin, a highly hydrophobic and porous support. The effect of certain parameters, including pH, ionic strength and temperature, was evaluated on the catalytic properties of the prepared biocatalysts.

MATERIALS AND METHODS

Materials

Lipase from *Thermomyces lanuginosus* (TLL) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further treatment. It is a liquid enzymatic preparation with a specific activity of 1100.3 IU/mg of protein and 17.7 mg protein/mL of enzyme solution. Mesoporous PSty-DVB resin (Diaion® HP-20) was purchased from Supelco (Bellefonte, PA, USA). Olive oil (low acidity) 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 protein concentration

The catalytic activity of soluble and immobilized lipase was determined in the hydrolysis of olive oil emulsion at pH 8.0 (buffer sodium phosphate 100 mM), 37 °C and 5 min of reaction under continuous agitation (200 rpm) in an orbital shaker (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 according to the Bradford (1976). Bovine serum albumin was used as standard protein. In this study, all solutions were prepared using Milli-Q water.

Preparation of biocatalysts via physical adsorption and characterization

The wet support (10 g) was then incubated in 190 mL of enzymatic solution containing 10 mg protein/g of support (Miranda et al., 2014; Lage et al., 2016). The suspensions were kept under agitation (200 rpm) in an orbital shaker for 12 h of incubation. In this study, the effect of pH (4.0 to 9.0), ionic strength (0 to 200 mM) and temperature (10 to 25 °C) on the catalytic properties of the biocatalysts was evaluated. The biocatalysts were then filtered (Whatman filter paper 41) under vacuum, washed with distilled water (volume ratio 1:5) and stored at 4 °C for 24 h prior to use. Immobilization yield (IY) was calculated by measuring the units of hydrolytic activity in the supernatant before and after immobilization procedure. 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}).

RESULTS AND DISCUSSION

Initially, the effect of pH on the catalytic properties of immobilized TLL was evaluated. In this set of experiments, the ionic strength used was fixed at 5 mM. As it can be observed in Table 1, the pH varying from 4.0 to 9.0 did not influence the values of immobilized protein concentration (IP) and immobilization yield (IY) which shows that the immobilization of TLL on PSty-DVB was clearly governed by hydrophobic interactions. However, at pH 5.0 was reached the highest hydrolytic activity and specific activity (SA) values. These results clearly show that maximum catalytic activity was observed very near to the isoelectric point of TLL (p*I* ≈4.4) (Fernández-Lafuente, 2010). Under this condition, a better orientation of the enzyme with the support surface due to its higher hydrophobicity near to its p*I* value is expected. These results are in agreement with those ones previously reported

for the physical adsorption of lipase from *Mucor javanicus* on silica particles (Salis et al., 2005), and lipase B from *Candida antarctica* (CALB) on activated carbon (Rodrigues et al., 2008). Thus, subsequent tests were conducted at pH 5.0 (buffer sodium acetate).

Table 1. Influence of pH of immobilization on the catalytic properties of immobilized TLL on STY-DVB particles

pH	IY[a] (%)	IP[b] (mg/g of support)	HA[c] (IU/g of support)	SA[d] (IU/mgIP)
4.0	99.4 ± 0.1	9.9 ± 0.1	155.7 ± 16.3	15.6 ± 1.6
5.0	99.6 ± 0.1	9.9 ± 0.1	235.0 ± 5.1	23.5 ± 0.5
6.0	98.9 ± 0.2	9.9 ± 0.1	211.2 ± 12.8	21.2 ± 1.3
7.0	98.4 ± 1.1	9.8 ± 0.3	121.6 ± 3.2	12.2 ± 0.4
8.0	98.1 ± 1.0	9.7 ± 0.1	89.3 ± 11.0	8.9 ± 1.1
9.0	96.4 ± 1.2	9.6 ± 0.2	82.8 ± 1.8	8.3 ± 0.1

a – Immobilization yield; b – Immobilized protein concentration; c – Hydrolytic activity; d – Specific activity

The effect of ionic strength on the catalytic properties of the biocatalysts was evaluated (Table 2). High immobilization yield and immobilized protein concentration for ionic strength varying from 0 to 200 mM was also observed. However, maximum hydrolytic activity and specific activity values were verified up to 100 mM and a slight decrease of the activity at 200 mM may be observed. This result could be attributed to possible distortion of the three dimensional structure of some enzyme molecules by influence of high ions concentration. Further studies were then performed at 5 mM buffer sodium acetate pH 5.0.

Table 2. Influence of the ionic strength on the catalytic properties of immobilized TLL on STY-DVB particles.

Ionic strength (mM)	IY[a] (%)	IP[b] (mg/g of support)	HA[c] (IU/g of support)	SA[d] (IU/mgIP)
0[e]	99.7 ± 0.1	9.9 ± 0.1	221.3 ± 9.3	22.3 ± 0.9
5	99.6 ± 0.1	9.9 ± 0.1	235.0 ± 5.1	23.7 ± 0.5
20	99.7 ± 0.2	9.8 ± 0.1	237.6 ± 8.8	24.2 ± 9.0
50	99.6 ± 0.2	9.8 ± 0.1	229.3 ± 5.0	23.6 ± 5.8
100	99.5 ± 0.4	9.7 ± 0.2	237.8 ± 1.3	24.5 ± 0.2
200	99.6 ± 0.2	9.8 ± 0.1	195.4 ± 5.4	19.9 ± 0.5

a – Immobilization yield; b – Immobilized protein concentration; c – Hydrolytic activity; d – Specific activity; e – distilled water adjusted with HCl solution (1 M)

The effect of temperature ranging from 10 to 25 °C was also studied. The enzyme was highly adsorbed on the support surface and immobilization yield above 99.5% was observed. Similar hydrolytic activity values were also observed (data not shown). This indicates that the effect of temperature was not significant. These results may be credited to high affinity of the

lipase with this support due to its high hydrophobicity and porous size. PSty-DVB resin has an average particle diameter of 250–850 μm, specific surface area of ≈500 m^2/g and average porous size of 260 Å (Supelco technical information). The porous size of this support are 5-fold higher that the molecular diameter of TLL – around 53.2 Å (Lage et al., 2016). Thus, high immobilization yield is expected because these porous are large enough to accommodate lipase molecules in its internal surface.

CONCLUSION

The results show that the immobilization of TLL on PSty-DVB was governed by hydrophobic interactions due to high affinity of this lipase with highly hydrophobic supports (mechanism of interfacial activation of lipases with hydrophobic surfaces). The immobilization procedure conducted near the isoelectric point (pH 5.0) and low ionic strength (5 mM buffer sodium acetate) allowed a better conformation of the enzyme with the support surface, thus exhibiting higher hydrolytic activity and specific activity.

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