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PRODUCTION OF HYBRID NANOFLOWERS WITH DIFFERENT LIPASE ENZYMES

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ABSTRACT

The synthesis of the hybrid nanoflowers is a simple, low cost and environmental friendly enzyme immobilization method. In this synthesis, the enzyme catalytic activity do not decrease due to unfavorable conformation of the enzyme or to mass transfer problems. In this context, it was produced the hybrid nanoflowers of lipases from Candida antarctica type B (CALB) and from Thermomyces lanuginosus (TLL) and, posteriorly, the crosslinking were realized by glutaraldehyde (GA) and divinylsulfone (DVS), changing some conditions like: the concentration of the GA and DVS, time of reticulation and temperature of reticulation. The activity of the hybrid nanoflowers was quantified by p-nitrophenyl butyrate (pNPB) hydrolysis. The best result was obtained producing TLL hybrid nanoflowers by $CuSO_4$ precipitation and DVS crosslinking (0.04 % v/v, at 25 ºC during 24 h) with retention of activity of 169.31 %. In addition, the CALB hybrid nanoflowers by $CuCl_2$ precipitation and GA crosslinking (0.8 % v/v, at 4 ºC during 24 h) retained 120.27 % of its activity.

1. INTRODUCTION

Lipases have been widely used in food industry, pharmaceutical processes, chemical and applications, because their high activity and high chemo-, regio- and enantio-selectivity (Altinkaynak et al., 2016; Cui et al., 2016). Therefore, the immobilization of enzymes technique has commonly used, since enable the easy separation of the reaction medium and the reuse of the biocatalyst, decreasing the effective cost of the process (Li et al., 2016). However, the activity of the most immobilized enzymes

by conventional methods of immobilization is decreased due to unfavorable conformation of the enzyme on the support and mass transfer problems (Altinkaynak et al., 2016; Cui et al., 2016). Thus, in recent years, it has been developed a simple, low cost and environmental friendly approach of enzyme immobilization, the synthesis of the hybrid nanoflowers (Li et al., 2016). In this context, it was produced the hybrid nanoflowers of lipases from *Candida antarctica* type B (CALB) and from *Thermomyces lanuginosus* (TLL) and, posteriorly, the crosslinking were realized by glutaraldehyde (GA) and divinylsulfone (DVS). The crosslinking of the nanoflowers were made in order to enhance the activity and the stability of the biocatalyst.

2. MATERIALS AND METHODS

2.1. Materials

The commercial TLL extract (24.9 mg of protein per mL) and CALB were obtained from Novozymes (Spain). The glutaraldehyde, divinylsulfone and *p*-nitrophenyl butyrate (*p*NPB) were purchased from Sigma Chemical Co (St. Louis, MO, USA). All others reagents were used of analytical grade.

2.2. Methods

2.2.1. Synthesis of the Hybrid Nanoflowers

Enzyme-inorganic hybrid nanoflower were synthesized as follow: 20 µL of aqueous $CuCl_2$ or $CuSO_4$ solutions (120 mM) was added to 3 mL of enzymatic solution (0.01 M, pH 7.4), containing 1 mg/mL of the lipase (CALB or TLL), then the mixture was incubated at 25 °C for 72 hours. Then the blue precipitates were settled, washed for three times by deionized water and collected after centrifugation (3500 rpm for 5 min) (Li et al., 2016). In order to optimize the reticulation procedure of the nanoflowers, the crosslinking was performed changing some conditions that are presented below, in the Table 1.

Table 1: Conditions of reticulation of the hybrid nanoflowers of CALB and TLL.

Conditions of reticulation	Glutaraldehyde (GA)	Divinylsulfone (DVS)
A	0.4 % v/v of GA at 4 ◦C, 24 h	0.02 % v/v of DVS at 4 ◦C, 24 h
B	0.8 % v/v of GA at 25 ◦C, 24 h	0.04 % v/v of DVS at 25 ◦C, 24 h
C	0.8 % v/v of GA at 4 ◦C, 24 h	0.04 % v/v of DVS at 4 ◦C, 24 h
D	0.4 % v/v of GA at 4 ◦C, 48 h	0.02 % v/v of DVS at 4 ◦C, 48 h
E	0.8 % v/v of GA at 4 ◦C, 48 h	0.04 % v/v of DVS at 4 ◦C, 48 h

F	0.4 % v/v of GA at 25 ◦C, 48 h	0.02 % v/v of DVS at 25 ◦C, 48 h
G	0.4 % v/v of GA at 25 ◦C, 24 h	0.02 % v/v of DVS at 25 ◦C, 24 h
H	0.8 % v/v of GA at 25 ◦C, 48 h	0.04 % v/v of DVS at 25 ◦C, 48 h

2.2.2. Determination of enzyme activity and protein concentration

The activities of the soluble and immobilized enzymes were determined according to the methodology described in the literature (Rios et al., 2016). In this procedure, enzyme activity was determined by the hydrolysis of *ρ*-nitrophenyl butyrate (*ρ*NPB) as a substrate, 50 mM in acetonitrile, at pH 7 and 25 °C. Protein concentration of the soluble enzymes and the supernatant was measured using Bradford method (Bradford, 1976) and bovine serum albumin was used as the reference.

2.2.3. Ethyl Hexanoate Hydrolysis

The ethyl hexanoate hydrolysis was performed according (dos Santos et al., 2015), with some modifications. The immobilized preparations were suspended in 0,5 mL of sodium phosphate buffer (100 mM, pH 7.4). Then, the reactions were conducted added 100 - 200 µL of the suspension of the immobilized preparations to 1 mL of 25 mM substrate at pH 5. The conversions were analyzed by HPLC (Thermo Scientific, Finnigan Surveyor with an UV detector PDA), using a Kromasil C18 (15cm×0.46cm) column.

3. RESULTS AND DISCUSSION

The activities before and after reticulation process of the nanoflowers were measured and the retention of activity were calculated. The results are shown in the Table 2.

Table 2: Data of rentention of activity of the nanoflowers after the reticulation process.

Conditions	Reticulation with Glutaraldehyde		Reticulation with Divinylsulfone	
	CALB	TLL	CALB	TLL
$CuCl_2$ (A)	98.57	47.99	59.82	79.57
$CuSO_4$ (A)	64.82	56.14	39.73	114.59
$CuCl_2$ (B)	97.52	58.36	59.37	107.43
$CuSO_4$ (B)	72.44	55.91	69.85	169.31
$CuCl_2$ (C)	120.26	39.52	83.37	78.96
$CuSO_4$ (C)	85.26	44.20	67.67	116.14
$CuCl_2$ (D)	51.39	23.45	51.62	53.26
$CuSO_4$ (D)	89.88	26.38	40.76	53.26

$CuCl_2$ (E)	71.27	34.98	58.96	65.29
$CuSO_4$ (E)	106.50	18.48	61.07	60.87
$CuCl_2$ (F)	42.53	9.88	55.78	68.10
$CuSO_4$ (F)	72.66	8.60	63.28	89.31
$CuCl_2$ (G)	39.86	65.07	88.24	115.85
$CuSO_4$ (G)	30.62	46.20	44.56	126.70
$CuCl_2$ (H)	43.05	51.07	79.59	59.00
$CuSO_4$ (H)	31.24	25.49	51.59	130.75

The results from the Table 2, show that the lipases were able to retain its activity or, even, enhance its activity, at some conditions of reticulation, in both salts studied. The best results of CALB nanoflowers reticulation was using glutaraldehyde at condition C (0.8 % v/v of GA at 4 ◦C, 24 h) and the best results of TLL nanoflowers reticulation was using divinylsulfone at condition B (0.04 % v/v of DVS at 25 ◦C, 24 h). In addition, the best retention of activity was obtained with time of reticulation of 24 h, in most cases.

4. REFERENCES

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