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Lipase Production in Discontinuous Operation System Using a *Candida lipolytica* Strain

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Abstract

The optimal growth conditions for a Candida lipolytica strain and an increased lipase production in a batch culture system have been determined, by gradually increasing the impeller speed (400 – 600 – 800 – 900 rpm) and the aeration (2.0 – 2.5 – 3.0 – 4.0 L/min) during the bioprocess time .

The lipolytic activities into the broth reached maximum levels within 20 - 22 h after inoculation, when the microorganisms were at the beginning of the stationary growth phase.

Key words: *Candida lipolytica*, lipase, discontinuous cultivation

Introduction

Lipases or triacylglyceral acyl ester hydrolases (E.C.3.1.1.3) are carboxylesterases that catalyse both the hidrolysis and the synthesis of esters formed from glycerol and long - chain fatty acids.

Enzymatic hydrolysis of triglycerides has the advantage of product quality and less energetic demand.

The inverse processes of glyceride synthesis and transesterification are also of great importance, especially in food and cosmetic industry [1, 2].

The lipases for industrial use are mainly produced by microorganisms: bacteria, yeast and fungal strains.

In recent years, new applications of the microbial lipases such as: hydrolysis of fats, transesterification, stereospecific hydrolysis of racemic esters, organic syntheses, and their use in the detergent and paper industry have been developed [3].

This triggered a growing interest in the microbial lipases research concerning the improvement of producing strains, of culture conditions as well as of the biochemical properties of these enzymes [4, 5].

Yeast strains of *Candida, Yarrowia* and *Hansenula* genera can produce cell-bound or excret lipase into the medium by submerged cultivation under optimum growing conditions [6, 7, 8, 9].

This work presents the influence of some operational parameters in lipase batch culture production, using the *Candida lipolytica* ICCF – 214 strain.

Materials and Methods

Microorganism, culture media and cultivation conditions

Candida lipolytica ICCF – 214 strain selected previously by screening was used [10].

Liquid medium containing (w/v): glucose (2.0 %), corn steep liquor (1.0 %), yeast extract (0.5 %), Tween 80 (1.0 %), mineral salts ($K^+$and Mg^{2+}) and antifoam agent (mineral oil) have been used for the submerged cultivation [11].

The initial pH of the media was 4.5 – 5.0.

The inoculum was prepared on modified GY·P medium in Erlenmeyer flasks, incubated at 27 ^oC for 24 hours.

The fermenter was inoculated with 10 % (v/v) of inoculum.

The submerged cultivation was performed in a 14 l glass fermenter type MF-114 (New Brunswick), containing 6 l medium, at 27 ^oC for 20 – 24 h.

The culture was variously stirred (agitation speed 400; 500; 600; 700; 800; 900 r.p.m.) and aerated (2.0; 2.5; 3.0; 3,5; 4.0; 5.0 L/min) during fermentation corresponding to the growth phase of the microorganism.

Enzyme assay

The activity of the extracellular lipase was assayed in the cell-free liquid, after separation of biomass by centrifugation at 3500 rpm for 20 min (Janetski centrifuge).

The lipolytic activity was determined according to a modified Willstatter method, on olive oil as substrate [12].

One activity unit was defined as the amount of enzyme which hydrolises the vegetable oil, and thus causes the release of 1 μ equivalent of carboxyl groups per minute, under the reaction conditions (30 ^oC, pH = 7.2).

Other analytical methods

Sugar concentration of the culture was determined using the orto – toluidine method [13].

Biomass concentration was determined measuring the optical density (OD) of the broth by visible spectrophotometry (SPEKOL - C. Zeiss - Jena) at 570 nm.

Results and Discussions

The effect of broth agitation and aeration, related to oxigen level into the culture, on the lipase batch production using *Candida lipolytica* ICCF 214 strain was studied.

The optimal conditions for high lipolytic activities were obtained when the impeller speed was gradually increased (400 – 600 – 800 – 900 r.p.m.), and so was the air flow (2.0 – 2.5 – 3.0 – 4.0 L/min), during the growth phases of the microorganism (**Table 1**).

Table 1. The effect of some biotechnological parameters on lipase batch production by *C. lipolitica* ICCF -214 strain.

No of Exp	Time of cultivation (h)	Agitation rate (r.p.m.)	Air flow (L/min)	Lipolytic activity (U/mL)
1	**2**	**3**	**4**	**5**
1.	0	500	2.0	-
	4	700	3.0	-
	8	800	3.5	7.8
	12	800	4.0	13.7
	16	900	4.0	19.6
	20	900	4.0	36.8
	22	900	4.0	34.5
	24	900	4.0	32.6
2.	0	400	2.0	-
	4	600	2.5	-
	8	600	2.5	9.7
	12	800	3.0	17.4
	16	900	4.0	22.6
	20	900	4.0	34.8
	22	900	4.0	38.8
	24	900	4.0	33.7
3.	0	500	2.0	-
	4	500	2.5	-
	8	800	3.0	10.6
	12	800	3.0	16.9
	16	800	4.0	21.3
	20	800	5.0	35.0
	22	800	5.0	28.6
4.	0	600	2.0	-
	4	700	2.0	-
	8	800	2.5	6.9
	12	850	3.0	17.1
	16	850	3.0	23.3
	20	850	3.0	27.7
	22	850	3.0	25.7
5.	0	500	2.0	-
	4	600	2.0	-
	8	600	2.0	8.4
	12	800	3.0	12.03
	16	800	4.0	17.5
	20	800	4.0	26.7
	22	800	4.0	29.6
	24	800	4.0	24.8

During the first growing stage of the culture (4 – 6 hours from inoculation) the agitation rate was kept low (400 – 500 r.p.m.), then it was gradually increased to 800 – 900 r.p.m., and the air flow at the beginning of the bioprocess was 2.0 – 2.5 L/min, then it was increased up to 4.0 l/min during the next 8 – 10 hours of cultivation, when the microbial growth reached the maximum value.

The results are comparable with the ones obtained by BARTH and al. [14] for lipase production in a *Yarrowia lipolytica* submerged culture.

The time course of lipase biosynthesis in a *Candida lipolytica* ICCF 214 submerged liquid culture is shown in **Figure 1**.

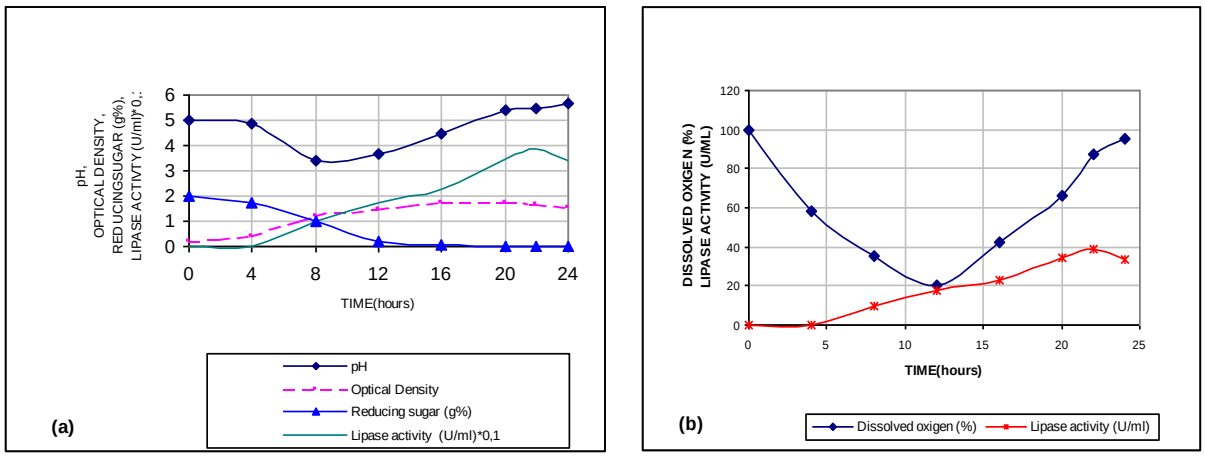

Figure 1: Time course analysis of lipase production in a *Candida lipolytica ICCF- 214* submerged liquid culture:
(a) - pH - optical density - reducing sugar - lipase activity;
(b) - dissolved oxigen - lipase activity.

The lipase production was initiated at the end of the exponential phase, as the growth of the microorganism attained a plateau, and reached maximum levels within 20–22 hours of cultivation (the beginning of the stationary phase).

The carbon source (glucose) was exhausted after 16 hours of cultivation, corresponding to the maximum growth rate value of the microorganism.

The pH value decreased during the first 8–10 hours of cultivation from initial value of 4.5 – 5.0 to around 3.5, then it increased to 5.2–5.4 as the lipase biosinthesis rate was high.

The rate of enzyme synthesis decreased when the product started to accumulate, suggesting that the bioprocess was regulated by a feed-back repression.

The results correspond to the data that have been reported by VALERO et al. [15], and HADEBALL et al. [16].

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