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MICROBIAL LIPASE PRODUCTION ON A POLYMERIC RESIN

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ABSTRACT

Rhizopus delemar was grown in a submerged culture and produced a lipase at 14 U/ml. It was compared with solid state fermentation with a polymeric resin (Amberlite). The lipase was produced and simultaneously adsorbed on the support: 96 U/g initial dry matter were obtained when dextrin was used as the carbon source against only 68 and 58 U/g for maltose and glucose, respectively.

INTRODUCTION

Lipases are widely used enzymes that can be obtained from animals, plants and microorganisms. Microbial lipases are used in the food industry, mainly in dairy products, and are also important in detergent, pharmaceutical, cosmetics and leather processing (Seitz, 1973). New trends are directed toward the use of immobilized lipases in organic solvent for ester synthesis, triglycerides hydrolysis or flavouring compound synthesis (Christen & López-Munguía, 1994).

Solid state fermentation (SSF) has been shown to be a suitable method for producing enzymes such as pectinases, amylases, or cellulases (Lonsane & Ghildyal, 1992), but few papers have dealt with the production of lipases. Recently, Rivera-Muñoz *et al.* (1991), using *Penicillium candidum* grown on wheat bran, found that solid state fermentation (SSF) could be appropriate for lipase production. Moreover, anionic supports can be suitable for immobilization as exemplified by Rizzi *et al.* (1992) who studied the synthesis of isoamyl acetate with a commercial immobilized *Mucor miehei* lipase.

It is known that the lipase of *Rhizopus delemar* is excreted to the production medium (Iwai & Tsujisaka, 1984). The aim of this work was to investigate if it is possible to produce and immobilize simultaneously the *Rhizopus delemar* lipase on an ion exchange resin. Lipase production in submerged culture and SSF was also compared.

EXPERIMENTAL AND ANALYTICAL PROCEDURES

Microorganisms and culture media. *Rhizopus delemar* CDBB H313 (CINVESTAV-México) was grown in the nutritive medium previously optimized by Martinez Cruz *et al.* (1993) and was used both in submerged culture and SSF. Submerged cultures were made in 250 ml Erlenmeyer flasks placed on a shaker at 29°C with an inoculum size of 1×10^7 spores/ml. The pH was adjusted to 6 and the agitation speed set at 180 rpm. At the end of the fermentation, the biomass was allowed to settle and the supernatant decanted. In SSF, an anionic resin (Amberlite IRA-900; Rohm & Haas, USA) was used as support

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and prepared according to Auria *et al.* (1990). The nutritive medium was added to the dried support to complete 58% (v/w) final water content, the maximum absorption capacity of the resin. Three different carbon sources at 20 g/l in the submerged were studied: glucose, maltose and dextrin. The cultures were carried out in small columns placed in a temperature controlled bath. Initial conditions were identical to those used for submerged cultures with an inoculum of 1.10^7 spores/g initial dry matter (IDM) and an aeration rate of 0.5 l/h.g IDM. At the end of the fermentation, the Amberlite spheres were separated manually from the biomass.

Analytical procedures. In submerged cultures, growth was followed by the dry weight method and in SSF respirometry was used, which allowed to calculate CO_2 production rate as previously described (Christen *et al.*, 1993). Water activity, moisture content and pH were also determined at the end of the fermentation.

Lipolytic activity was assayed with the method used by Nahas (1988), with some modifications. The substrate was a 5 % tributyrin emulsion prepared in a 1 % Tween solution in 2.5 M Tris-maleate buffer (pH = 6) by homogenizing with an Ultraturrax apparatus (8000 rpm during 2 mn). The reaction mixture contained 18 ml substrate solution and 12 ml of supernatant culture medium. In SSF, the lipase activity was assayed from one gram of Amberlite. The determination was performed with a pH stat, at 37°C and pH adjusted to 6. The butyric acid released was titrated with 50 mM NaOH solution during 5 min. One unit (U) was defined as the amount of enzyme releasing one µmol of free fatty acid per minute.

RESULTS and DISCUSSION

Growth and lipase production in submerged culture

Results are given in Figure 1. It can be seen that maximum activity (14 U/ml) was obtained when biomass attained its maximum concentration (12.3 mg/ml). These values were reached within 2 days and are similar to those obtained by Martinez Cruz *et al.* (1993). The decrease in activity after 2 days is frequently observed in these systems and may be due to the action of proteases excreted by the fungus (Iwai & Tsujisaka, 1984) or changes in the environment of the enzyme.

Growth and lipase production in SSF

As previously reported for *Aspergillus niger* (Auria *et al.*, 1990) and for *Candida utilis* (Christen *et al.*, 1994), growth on Amberlite was found to occur only on the surface and between the particles forming a mesh. Growth was monitored by CO_2 production rate and did not display significative differences between the three substrates (see Figure 2). Maximum growth rates were reached between 15 and 20 hours and attained values of 3.5 ml CO_2 /h.g IDM. Maximum lipase production was found after 20 hours, just after the maximum of CO_2 production rate (see Figure 3). The best activity was found for dextrin (96 U/g IDM) against 68 U/g IDM for maltose and 58 U/g IDM for glucose. Similar glucose repression on lipase production was observed in submerged culture by Martinez Cruz *et al.* (1993) and Haas & Bailey (1993). As in the submerged culture, the activity decreased once the maxima had been achieved. The pH evolution was similar in both submerged culture and in SSF: first a decrease, and then an acid reduction to attain final values near neutrality (see Figure 4). The pH decrease was lower in SSF probably due to the buffering capacity of the support.

The most relevant data concerning the submerged cultures and SSF for lipase pro-

Figure 1 : Rhizopus delemar lipase production kinetic in submerged culture

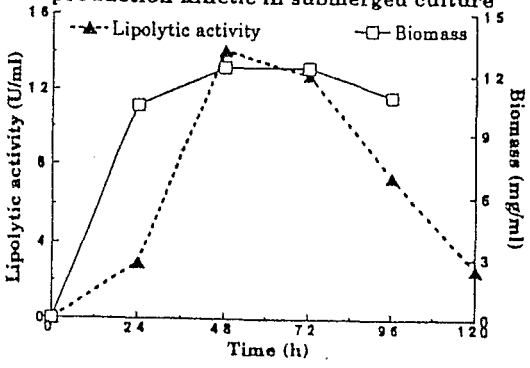


Figure 2 : Respirometric activity of R. delemar grown in SSF with 3 different carbon sources

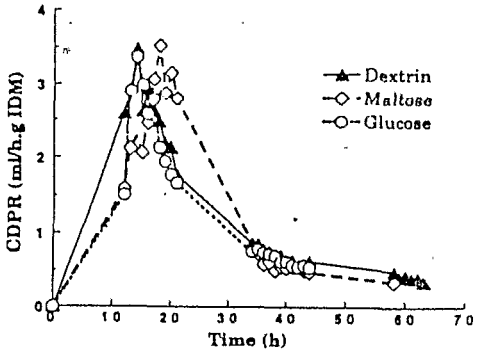


Figure 3 : Rhizopus delemar lipase activity in SSF with 3 different carbon sources

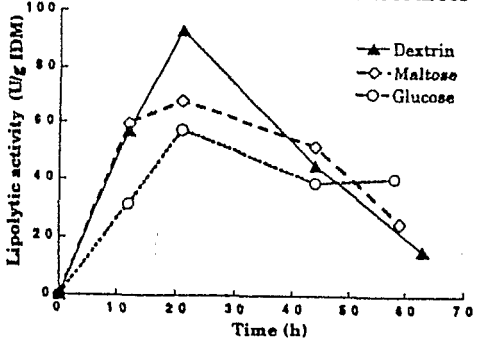
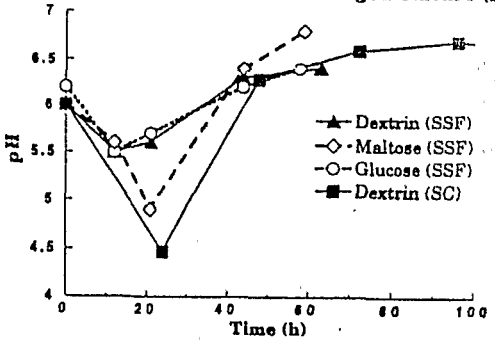


Figure 4 : pH evolution in solid state fermentation (SSF) and submerged culture (SC)



duction by *R. delemar* are summarized in Table 1, where it is shown that SSF gave a five fold increase in productivity with the same substrate (dextrin) compared with submerged culture. Amberlite proved to be an adequate support for this purpose: it provided good stability for pH, moisture content and Aw, all of them being key parameters in SSF. This support was also able to adsorb the lipase excreted by the fungus given that the activities reported in Table 1 were determined directly from the support once the biomass was removed. The best carbon source was dextrin as has already been observed in submerged culture (Martinez Cruz *et al.*, 1993). The catabolite repression due to glucose was at the same levels as those obseved in submerged culture (a decrease of 40% against dextrin). The respiratory quotients (R.Q.) observed are typical of the oxidative use of the carbon sources.

Fermentation	Submerged	SSF		
Carbon source	Dextrin	Glucose	Maltose	Dextrin
Time max. production (h)	48	18	18	15
CDPR# max (ml/h.g IDM)	-	2.8	3.5	3.48
R.Q. (Range)	-	1-1.3	1-1.3	1-1.4
pH max. production	-	5.7	4.9	5.6
Aw (final)	-	0.998	0.999	0.994
Lipolytic Act. (U/g IDM)	-	58	68	96
Lipolytic Act. (U/ml)	14	14.5*	17*	24*
Productivity (U/ml.h)	0.29	0.81	0.94	1.6

Table 1: Comparative results for lipase production. # CDPR: Carbon dioxide production rate. * These values refer to the lipolytic activity per volume of packed bed with an apparent packing density of 0.25 g IDM/ml reactor (Auria *et al.*, 1990).

CONCLUSION

Rhizopus delemar showed a good capacity to grow on Amberlite with various carbon sources (dextrin, maltose and glucose). The evolution of the fermentation was similar in submerged culture and in SSF except for the fact that in the case of SSF, the enzyme was produced in shorter times and was adsorbed onto the support. While many variables may still be optimized, this work demonstrated that it was possible to simultaneously produce the lipase and to separate it from the biomass by adsorption onto a resin allowing obtention of a ready-to-use immobilized lipase.

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