Journal of Molecular Catalysis B: Enzymatic 5 (1998) 75–78

Lipase production by solid state fermentation of olive cake and sugar cane bagasse

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Received 10 October 1997; accepted 27 November 1997

Abstract

Olive oil cake (OOC) from Morocco and sugar cane bagasse (SCB) were used for lipase production using thermostable fungal cultures of *Rhizomucor pusillus* and *Rhizopus rhizopodiformis*. The maximum production of lipase by *Rhizomucor pusillus* and *Rhizopus rhizopodiformis* in solid state fermentation (SSF) using SCB, was 4.99 U/g DM equivalent to 1.73 U/ml and 2.67 U/g DM equivalent to 0.97 U/ml, respectively. However, the mixture of OOC and SCB, 50% each, increased the lipase activity as high as 79.6 U/g DM equivalent to 43.04 U/ml and 20.24 U/g DM equivalent to 10.83 U/ml obtained by *Rhizopus rhizopodiformis* and *Rhizomucor pusillus*, respectively. These data compare favourably with most of the activities reported for other lipase hyperproducing microorganisms.

Keywords: *Rhizomucor pusillus*; *Rhizopus rhizopodiformis*; Optimisation

1. Introduction

As an agricultural country, Morocco produces various agricultural products and part of these are being transformed on the industrial scale each year. By-products such as about 300,000 tons of sugar cane bagasse and 180,000 tons of olive oil cake (by-products generated by olive oil extraction plants) are being produced annually [1]. Almost 80 to 85% of bagasse are being used as fuel. While olive oil cake is sometimes used as fuel, it is also mostly discarded as waste in the environment. Thus, very few of the large quantity of the olive oil cakes are being valorized. The disposal of most untreated olive oil cake is a threat to the environment.

Solid state fermentation of olive oil cake might have a strong scientific, industrial, and environmental impact. For instance, the production of enzymes, such as thermostable lipases, by thermophilic filamentous fungi, fermenting

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1381-1177/98/$19.00
PII: S1381-1177(98)00067-8

Table 1
List of filamentous fungal strains

Number	Strain	Source
A13	*Rhizopus rhizopodiformis*	ORSTOM culture collection
A16	*Rhizomucor pusillus*	ORSTOM culture collection

olive oil cake and bagasse as solid matrix, might be a way of valorizing these by-products.

2. Materials and methods

2.1. Substrates

The bagasse was given by the sugar refinery Emiliano Zapata (Zapatepec, Morelos, Mexico) and by Dar El Gadari (Morocco). Raw olive oil cake was given by various traditional olive oil extraction plants (maâsra in Morocco).

2.2. Microbial strains

Table 1 shows the list of filamentous fungal strains.

2.3. Assay techniques

The relative humidity and dry matter were determined by drying samples of substrates at 105°C for 24 h. The pH value of the sample of substrates was assayed following dilution of 1 g in 10 ml of distilled water, and by using a digital pH meter Knick. Protein content was assayed according to Bradford [2]. Reducing sugars were measured according to Miller [5]. Lipase was assayed according to Kwon and Rhee [4].

2.4. Apparatus used to monitor the solid state fermentation

The apparatus used to monitor the solid state fermentation is shown in Fig. 1

2.5. Culture medium

Table 2 shows the components used in the preparation of the culture medium.

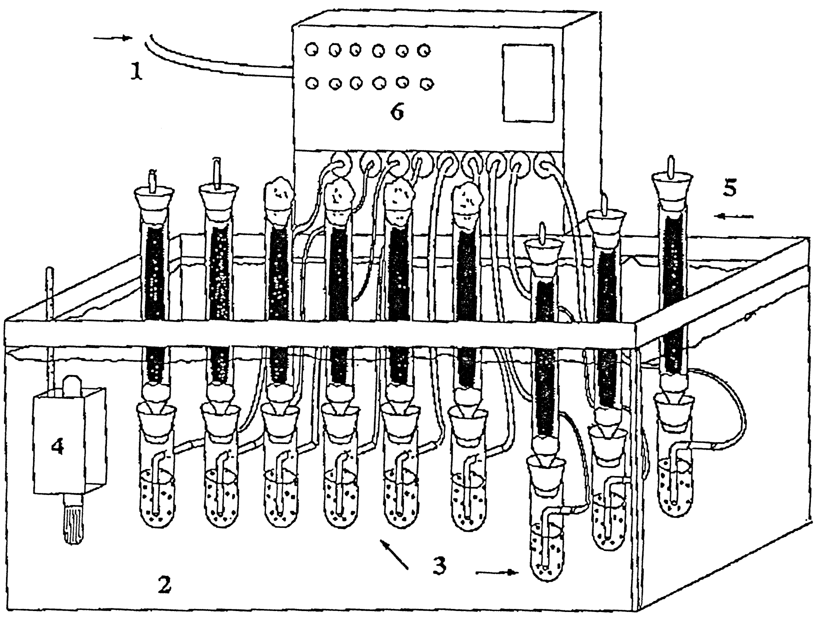

Fig. 1. Apparatus used to monitor the solid state fermentation. 1. Air inlet; 2. Thermoregulated waterbath; 3. Humidifier; 4. Heating element; 5. Fermentation column; 6. Aeration manifolds.

Table 2
Culture medium used for lipase production [3]

Bactopeptone	50 g
Glucose	20 g
KH_2PO_4	1 g
$NaNO_3$	1 g
$MgSO_4$	0.5 g
Distilled water	1000 ml

2.6. Physico-chemical characterization of bagasse and olive oil cake substrates

Each sample of substrate was finely ground and screened in order to obtain particles with a diameter smaller than 1 mm, and was assayed for mineral matter (crude ashes), fat [6], total nitrogen (Kjeldahl), and cell wall components [7] such as total fibres (NDF), lignocellulose (ADF), and lignin (ADL).

3. Results and discussion

3.1. Characterization of substrates

The chemical composition of the bagasse and olive oil cake substrates is shown in Table 3.

3.1.1. Bagasse

Bagasse has a low protein and fat content, and a high level of cell wall components, more particularly cellulose (Table 3).

Table 3
Chemical composition of bagasse and olive oil cake substrates

Components	% Dry matter	
	Bagasse	Olive oil cake
Organic matter	97.21	91.9
Protein	1.98	5.25
Cellulose	41.13	10.58
Hemicellulose	35.84	15.74
Lignin	19.76	25.48
Fat	2.55	18.4
Ash	2.79	8.1

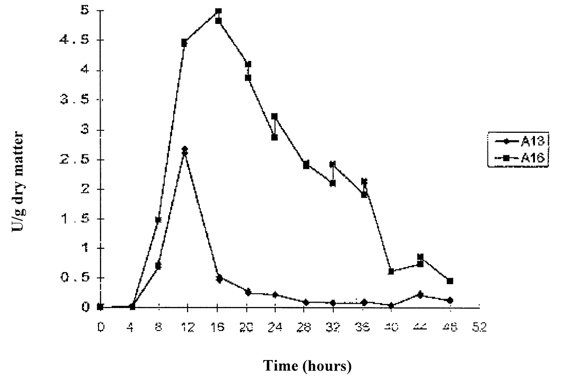

Fig. 2. Time course of lipase activity from *Rhizopus rhizopodiformis* strain A13 and *Rhizomucor pusillus* strain A16 during solid state fermentation of sugar cane bagasse.

3.1.2. Olive oil cake

Olive oil cake from maâsra has a relatively high protein and fat content, and a high lignin content (Table 3). This indicates that olive oil cake might be a suitable substrate for solid state fermentation.

3.2. Enzyme production by solid state fermentation

Screening of strains of filamentous fungi producing thermostable lipases has been performed on bagasse alone, and on a mixture of olive oil cake and bagasse (50:50).

The maximal production of lipase was reached, on bagasse alone, by *Rhizomucor*

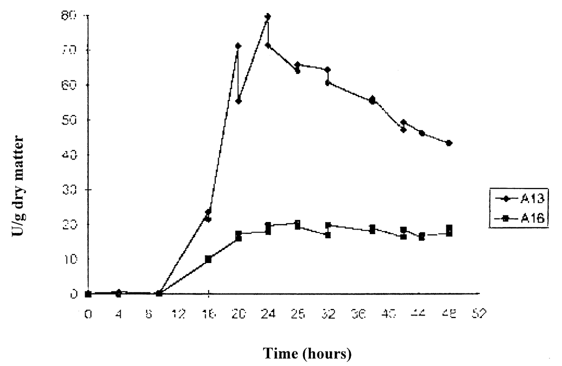

Fig. 3. Time course of lipase activity from *Rhizopus rhizopodiformis* strain A13 and *Rhizomucor pusillus* strain A16 during solid state fermentation of sugar cane bagasse and olive oil cake.

pusillus with 4.99 U/g dry matter (equivalent to 1.73 U/ml) and by *Rhizopus rhizopodiformis* with 2.67 U/g dry matter (equivalent to 0.97 U/ml) (Fig. 2).

When cultivated on bagasse and olive oil cake as solid substrates, *Rhizopus rhizopodiformis* produced 79.60 U of lipase per g of dry matter (equivalent to 43.04 U/ml). Under the same conditions, *Rhizomucor pusillus* produced 20.24 U/g dry matter (equivalent to 10.83 U/ml). This suggests that the addition of olive oil cake had a synergistic effect with bagasse on enzyme production, and that olive oil cake might bring some precursors necessary for lipase production (Fig. 3).

4. Conclusion

The use of solid state fermentation for the production of thermostable lipases is an interesting alternative to the valorization of bagasse and olive oil cake. The synergistic effect of olive oil cake added to bagasse has been confirmed. Lipase production could be optimized by adding the appropriate precursors found in olive oil cake.

Acknowledgements

This work was supported by the International Development Research Centre (Canada).

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