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Bioprocess development to add value to canola cake used as substrate for proteolytic enzyme production

A.C. Freitas[a], F.C.F. Baleeiro[b,c], R.F. Fonseca[b,c], V. Bertucci Neto[c], G.A.S. Pinto[d], C.S. Farinas[b,c,*]

[a] Departamento de Engenharia de Alimentos, Universidade Federal de Maranhão, Rua Urbano Santos S/N, Imperatriz, 65900-410, MA, Brazil
[b] Departamento de Engenharia Química, Universidade Federal de São Carlos, 13565-905, São Carlos, SP, Brazil
[c] Embrapa Instrumentação, Rua XV de Novembro 1452, 13560-970, São Carlos, SP, Brazil
[d] Embrapa Agroindústria Tropical, Rua Dra. Sara Mesquita 2270, 60511-110, Fortaleza, CE, Brazil

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ABSTRACT

Microbial proteases are one of the largest groups of industrial enzymes. This important market has led to a need for technically and economically efficient bioprocesses for protease production that could be exploited commercially. The aim here was to develop a complete bioprocess for protease production, from microbial fermentation up to dried product formulation. Evaluation was made of the effects of operational conditions on the production of protease by a selected strain of *Aspergillus oryzae*, cultivated under solid-state fermentation (SSF) with canola cake as a sole carbon source in an instrumented lab-scale bioreactor. Statistical experimental design revealed that the air flow rate, inlet air relative humidity, and initial substrate moisture content had significant effects on the efficiency of protease production. The highest protease production by *A. oryzae* was achieved at a fixed air flow rate of 12 mL/min, with inlet air relative humidity within the range 66–94% and initial substrate moisture content between 30% and 40%. The enzymatic extract produced under the selected conditions was spray dried using different concentrations of additives (glucose, maltodextrin, and CMC). The stability of the dried enzymatic powder during shelf storage was evaluated over a period of 90 days. There was a positive effect of CMC and a negative effect of glucose on protease activity and stability, while the influence of maltodextrin was negative in enzyme recovery at time zero, but it was positive on protease stability over a longer period. The spray dried proteolytic enzymatic formulation obtained from SSF of canola cake using *A. oryzae* has potential for applications in the industrial sector.

1. Introduction

Enzymes are increasingly used in many industrial sectors as an alternative to conventional chemical processes. The advantages of enzymes include efficiency at mild pH and temperature, high substrate specificity, and low toxicity (Sanchez and Demain, 2011). Proteases constitute one of the three largest groups of industrial enzymes and have a wide range of applications in the food, textile, and pharmaceutical industries (Rao et al., 1998). Microbial proteases account

* Corresponding author at: Embrapa Instrumentação, Rua XV de Novembro 1452, 13560-970, São Carlos, SP, Brazil. Tel.: +55 16 2107 2908; fax: +55 16 2107 2902.
E-mail address: cristiane.farinas@embrapa.br (C.S. Farinas).
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for approximately 40% of total worldwide sales of enzymes (Kumari et al., 2012). This important market has resulted in the need to develop technically and economically efficient bioprocesses for protease production. The search for ways of adding value to agro-industrial residues has driven research attention towards the development of solid-state fermentation (SSF) processes for the production of bio-products. The use of SSF for enzyme production has gained considerable attention over the past 20 years and the advantages of this cultivation system have attracted commercial interest (Barrios-Gonzalez, 2012; Chen and He, 2012; Singhania et al., 2010). The use of SSF is particularly advantageous for enzyme production by filamentous fungi, since it simulates the natural habitat of the microorganisms (Holker et al., 2004; Raimbault, 1998). However, certain operational limitations of SSF, such as difficulty in controlling the moisture level of the substrate and avoiding heat build-up, have held back its wider implementation. Recent studies have shown the importance of evaluating the influence of operational parameters on enzyme production by SSF in bioreactors under controlled conditions (Brijwani et al., 2011; Derakhti et al., 2012; Farinas et al., 2011; Pirota et al., 2013; Sukumprasertsri et al., 2013).

Protease production under SSF has been reported previously using different agro-industrial residues and fungal strains. For example, Macchione et al. (2008) and Sandhya et al. (2005) carried out comparative studies using different fungal strains to evaluate their performance in protease production. Thanapimmetha et al. (2012) investigated the production of protease by *Aspergillus oryzae* cultivated under SSF using deoiled *Jatropha curcas* seed cake as substrate. Vishwanatha et al. (2010) used wheat bran in an optimization study of SSF for protease production, while Belmessikh et al. (2013) evaluated tomato pomace as substrate. The use of agro-industrial residues as SSF substrates for enzyme production is a way to add value to these residues (Chen and He, 2012). In this respect, oil cakes have high nutritional value and are potentially valuable substrates for use in biotechnological SSF processes for the manufacture of high value products (Lomascolo et al., 2012; Ramachandran et al., 2007). The development of new canola varieties has enabled edible oil to be obtained from rapeseed, which is now the world's third largest source of vegetable oil. The by-product of oil extraction, canola cake, is not only a rich source of nitrogen, carbon, and minerals, but is also abundant and inexpensive (Lomascolo et al., 2012). However, despite the potential of *Aspergillus* strains and their recognized applications in industrial enzyme production, to the best of our knowledge there have been no studies concerning SSF bioprocess development for the production of proteolytic enzymes by *A. oryzae* using canola cake as solid substrate.

Developmental studies of bioprocesses for enzyme production usually focus on the optimization of cultivation conditions to increase productivity. Nevertheless, in order to obtain a final enzymatic product suitable for commercialization, the downstream operations such as product concentration and stabilization need to be addressed. In this context, dry solid formulations usually have a longer shelf life than liquid formulations. Among the various drying techniques, spray drying can efficiently dry heat-sensitive materials such as enzymes (Belghith et al., 2001; Namaldi et al., 2006). Spray drying is widely used industrially to obtain dry powder aerosols (Wang and Langrish, 2009). However, in order to increase the efficiency of product recovery during the drying process, it is often necessary to use additives to increase product stability. In the present context, it remains a challenge to identify the ideal conditions for both protease production and product stabilization, and to develop a complete bioprocess suitable for use in future commercial applications.

The aim of the present study was to develop a complete bioprocess for protease production, from microbial fermentation up to formulation of the dried product. Investigation was made of the effects of operational conditions on the production of protease by a selected strain of *A. oryzae*, cultivated under SSF of canola cake using an instrumented lab-scale bioreactor. Statistical experimental design with response surface analysis was used to study the influence of air flow rate, inlet air relative humidity, and initial substrate moisture content on the efficiency of protease production. The enzymatic extract produced under the selected conditions was concentrated and spray dried under different process conditions. The stability of the dried enzymatic powder during shelf storage was then evaluated over a period of 90 days.

2. Materials and methods

2.1. Instrumented SSF bioreactor

The SSF bioreactor used for all cultivations was a lab-scale system consisting of 16 columns (2.5 cm diameter, 20 cm length) placed in a water bath. The bioreactor was equipped with an on-line system to control the air flow rate and the inlet air relative humidity, as well as to monitor CO_2 at the outlets, as described previously (Farinas et al., 2011).

2.2. Raw material

Canola cake, a residue remaining after extraction of the oil, was kindly provided by Celena Alimentos (Eldorado do Sul, Rio Grande do Sul, Brazil). Canola cake composition was as follows (% of dry weight): protein $40.50 \pm 1.02\%$; total carbohydrates $35.60 \pm 0.80\%$; lipids $2.02 \pm 0.08\%$; ash $6.37 \pm 0.07\%$. A percentage of 80% of the cake particle sizes (described in terms of the characteristic diameter, d_p,) were in the range of $0.25 \leq d_p \leq 1.41$ mm and 20% of particle sizes had $d_p \leq 0.25$ mm. The cake was used as SSF substrate without any type of pretreatment.

2.3. Microorganism

A strain of *A. oryzae* (*A. oryzae* CCBP 001) from the collection maintained by Embrapa Tropical Agroindustry (Fortaleza, Brazil) was used for protease production. The strain was kept on dry sand at −18 °C and was activated in basic agar slants, as described by Couri and deFarias (1995). Conidia from the second activation step were suspended in Tween 80 solution (0.3%, v/v) that has been sterilized at 121 °C for 15 min.

2.4. Preparation of inoculum

One mililiter volume of the spore suspension was inoculated into a 250 mL Erlenmeyer flask containing ground corn cob and a nutrient medium (Couri and deFarias, 1995), and incubated for 5 days at 30 °C. The spores were then dispersed with 40 mL of 0.3% (w/v) Tween 80, using a sterile stick under aseptic conditions. The concentration of spores in the suspension was estimated by using a Neubauer chamber.