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Utilisation of grape seeds for laccase production in solid-state fermentors

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Abstract

The aim of the present paper was to investigate the feasibility of grape seeds as a support-substrate for laccase production by the white-rot fungus *Trametes hirsuta* under solid-state conditions, operating in laboratory-scale bioreactors. Two bioreactor configurations were considered in order to determine the most suitable one: immersion and tray. As regards bioreactor design, the tray configuration led to the highest laccase activities. In addition, the nature of the support employed (inert or non-inert) on laccase production was also evaluated. The results obtained clearly showed the superiority of grape seeds for laccase production over nylon sponge, since they produced much higher activities (around threefold).

On the other hand, decolourization of structurally different dyes by the extracellular liquid obtained in the tray configuration operating with grape seeds as a support was assayed. The results showed that the individual dye structures influenced the decolourization extent. However, in all cases a decolourization kinetic of first order with respect to dye concentration was found.

Keywords: Bioreactors; Food processing industry; Grape seeds; Laccase; Solid-state fermentation

1. Introduction

Laccase (benzenediol: oxygen oxidoreductase, E.C. 1.10.3.2) is an enzyme of considerable biotechnological interest, since it can be employed in numerous environmental applications. Although their specific physiological functions are not completely understood, there are several indications that laccases are involved in the formation and/or degradation of complex organic substances (Thurston, 1994).

SSF is defined as any fermentation process carried out on a solid material (employing either a natural support or an inert support) in absence of free flowing liquid (Pandey, 1992). In recent years, SSF has received more and more interest from researchers, since several studies have demonstrated superior product yields and simplified downstream processing (Robinson & Nigam, 2003). The utilisation of food-industrial wastes in different bioprocesses provides alternative substrates and also helps solving pollution problems (Pandey, Selvakumar, Soccol, & Nigam, 1999). In addition, costs are much lower due to the efficient utilisation and value-addition of food lignocellulosic wastes (cassava, pomace, bagasse,...). However, their utilisation presents several problems such as support degradation and/or support accretion may occur during the fermentation process. This would affect mass transfer and oxygen supply into the reactor bed hampering the proper performance of the bioreactor. Moreover, it was found that the different non-inert support employed lost about 40% of their weight at the end of cultivation with modification of their physical integrity (Rodríguez Couto, Rivela, Muñoz, & Sanromán, 2000). Therefore, it would be of

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great interest to find a system support-substrate/bioreactor configuration that allows operating in solid-state conditions for prolonged times. This configuration should be applicable to continuous operation as well as permit the scale-up of the process.

From the different available food-industrial wastes, grape seeds could be an adequate support-substrate to perform fermentation processes due to the following reasons: they have the physical integrity to serve as a supporting material (necessary to operate at bioreactor scale for prolonged times), they can function as a carbon source and their availability and low cost, since they are wastes from the wine industry. Therefore, their reutilisation would also contribute to solve pollution problems.

The selection of an adequate bioreactor is crucial to carry out solid-state processes, since the success of the process depends on it. The most important factors to take into account to design a bioreactor operating in solid-state conditions are the parameters of the process and the nature of the support employed (Durand et al., 1993; Pandey, 1991). In addition, the cost and simplicity also deserve great attention. It is necessary to find an adequate combination support/bioreactor, which allows obtaining high productivity at low cost.

The aim of this paper is to evaluate the potential of a lignocellulosic material such as grape seeds as a support-substrate for laccase production by the white-rot fungus *Trametes hirsuta* in solid-state bioreactors. In order to develop a bioprocess with high profitability, two bioreactor configurations (immersion and tray) were studied. In addition, the influence of the nature of the support employed on the efficiency of the process was evaluated. The ability of the laccase complex secreted in such conditions to decolourize several structurally different dyes was investigated.

2. Materials and methods

2.1. Microorganism and growth medium

T. hirsuta (BT 2566), obtained from Dr. G.M. Gübitz (Institute for Environmental Biotechnology, Graz University of Technology, Graz, Austria) was maintained on potato dextrose agar (PDA) plates at 4 °C and subcultured every three months.

The growth medium contained per litre: 4 g glucose, 15 g yeast extract, 2 g thiamine, 0.75 g NH_4Cl, 2 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2 \cdot 2H_2O$, 0.5 g KCl and 20 mM acetate buffer (pH 4.5).

2.2. Supports

Grape seeds were isolated from wine bagasse and employed as a non-inert support. The chemical composition of grape seeds is 44% (w/w) lignin, 7% (w/w) cellulose and 31% (w/w) hemicellulose (Moldes, Gallego, Rodríguez Couto, & Sanromán, 2003).

Cubes ($0.5 \times 0.5 \times 0.7$ cm^3) of fibrous nylon sponge (Scotch Brite^{TB}, 3M Company, Spain) were used as inert support. Prior to use, the cubes of nylon sponge were pre-treated by boiling for 10 min and washing thoroughly three times with distilled water. After that, the cubes were dried overnight at room temperature.

Prior to use, both supports were autoclaved at 121 °C for 20 min.

2.3. Bioreactor cultivation

2.3.1. Tray bioreactor

The static tray bioreactor is the generally used bioreactor for SSF. The configuration employed in this work consisted of a glass culture flask Fernbach type, conical shape (1.8 L), where the support were placed forming a layer of about 1 cm of thickness (90 g grape seeds or 15 g nylon sponge/200 mL medium). Inoculation was carried out directly in the bioreactor with 10 agar disks (diameter, 3 mm), from actively growing fungus on PDA plates. The bioreactor was kept in a chamber at 30 °C, 90% humidity, in complete darkness.

2.3.2. Immersion bioreactor

This bioreactor configuration was designed by our research group (Rivela, Rodríguez Couto, & Sanromán, 2000). It consisted of a jacketed cylindrical glass vessel with a round bottom (working volume of 0.5 L). A wire mesh basket was filled with grape seeds colonised by the fungus and placed into the bioreactor vessel as indicated in a previous work (Rivela et al., 2000). It was moved upwards and downwards by means of a pneumatic system, remaining 90 s outside and 10 s inside the medium, which gave an immersion frequency of 0.1 s^{-1}. The bioreactor was kept at 30 °C by means of temperature-controlled water and humidified air was supplied in a continuous way at 0.5 vvm.

Both bioreactors were carried out in batch and samples were collected once a day, centrifuged at $8000 \times g$ for 10 min and analysed. Duplicate experiments were run for comparison and samples were analysed twice. The values in the figures correspond to mean values with a standard deviation lower than 15%.

2.4. Analytical determinations

2.4.1. Reducing sugars

They were measured by the dinitrosalicylic acid method using D-glucose as a standard according to Ghose (1987).

2.4.2. Laccase activity

This was determined spectrophotometrically as described by Niku-Paavola, Raaska, and Itävaara (1990)