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Lovastatin production by *Aspergillus terreus* using lignocellulose biomass in large scale packed bed reactor

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A B S T R A C T

The effect of superficial air velocity on lovastatin production by *Aspergillus terreus* PL10 using wheat bran and wheat straw was investigated in a 7 L and a 1200 L packed bed reactor. Mass transfer and reaction limitations on bioconversion in the 1200 L reactor was studied based on a central composite design of experiments constructed using the superficial air velocity and solid substrate composition as variables and lovastatin production as response. The surface response prediction showed a maximum lovastatin production of 1.86 mg g^{-1} dry substrate on day 5 of the bioconversion process when the reactor was operated using 0.19 vvm airflow rate (23.37 cm min^{-1} superficial air velocity) and 54% substrate composition (w_C). Lovastatin production did not increase significantly with superficial air velocity in the 7 L reactor. Variation in temperature and exit CO_2 composition was recorded, and the Damköhler number was calculated for lovastatin production at these two scales. The results showed that in larger reactors mass transfer limitation controlled bioconversion while in smaller reactors bioconversion was controlled by reaction rate limitations. In addition, mass transfer limitations in larger reactors reduced the rate of metabolic heat removal, resulting in hot spots within the substrate bed.

Keywords: Superficial air velocity; Solid-state bioconversion; Lovastatin production; Packed bed bioreactor; Damköhler number; *Aspergillus terreus*

1. Introduction

Lovastatin is a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase and widely used in the treatment of hypercholesterolemia. Lovastatin, a natural statin, also serves as a precursor for many synthetic and semi-synthetic statins. Statins have benefits beyond their cholesterol-reducing effects. These pleiotropic effects include improvement of endothelial function, stabilization of atherosclerotic plaques, and reduction of the effects of oxidative stress (Haramaki and Ikeda, 2003; Kumar et al., 2011). Thus, lovastatin continues to be a drug of interest and importance.

Enzymes, secondary metabolites and animal feed have been produced by solid-state bioconversion process from a variety of substrates that includes apple pomace, soybean cake, sugarcane bagasse and wheat straw, rice straw and oil palm frond (Soccol and Vandenberghe, 2003; Jahromi

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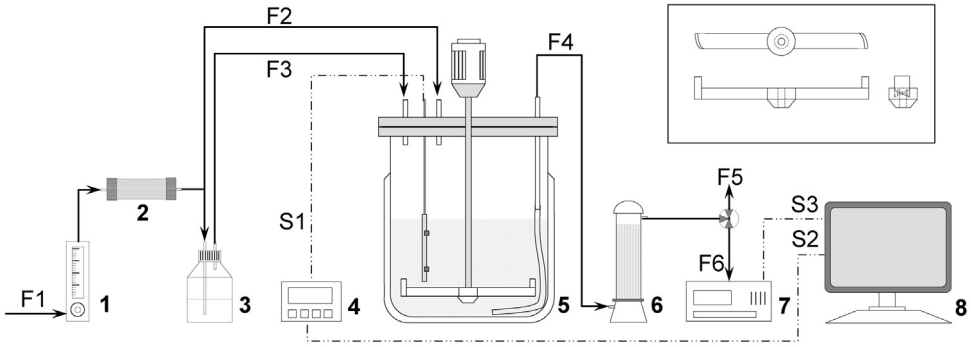

Fig. 1 – Flow diagram showing the equipment used for carrying out bioconversion of solid substrate to lovastatin. A seven liter bioreactor normally used for submerged culture was adapted for solid-state bioconversion. The different units of the laboratory setup for solid state bioconversion are (1) Flow meter, (2) Inlet air ceramic filter, (3) Bottle containing sterile water for moisturizing air, (4) Temperature recorder, (5) Solid-state reactor, (6) Desiccator, (7) CO_2 analyzer, (8) Computer for recording data. The inset figure shows the schematic diagram (not to scale) of the impeller used for mixing. The flow streams are described as F1: Air inlet stream, F2: Dry air stream, F3: Moisturized air stream, F4: Air exit stream, F5: Vent air, F6: Air stream for CO_2 analysis. The signals are described as S1: Temperature measurement signal, S2: Temperature recording signal, S3: CO_2 concentration recording signal.

et al., 2012). Solid-state bioconversion can be an economically attractive alternative to submerged fermentation because product yields can be higher in the natural environment provided by the solid medium, operational costs lower and the requirement effluent treatment minimal (Singhania et al., 2009). But the large amount of metabolic heat generated within the reactor is difficult to remove because the substrate is a poor conductor of heat. Aeration, which provides the primary means of heat removal through convective heat transfer, also affects the humidity, and local O_2 and CO_2 gradients within the reactor (Raghavarao et al., 2003). Hence, controlling reactor condition, and operating it to achieve the process goals, does not translate linearly from one scale to another. This study was carried out to understand the problems arising in different scales of operation for the production of lovastatin in a 7 L and 1200 L solid-state packed bioreactor under different conditions.

The performance of a solid-state bioconversion process is affected by both preparatory as well as operating conditions (Basu et al., 2002; Bhatnagar et al., 2008). The variables associated with the preparatory conditions include pre-treatment of substrate, initial pH, the age, morphology and the amount of inoculum per unit weight of substrate. For most processes, these conditions are standardized to ensure repeatability of results. However, the more important variables affecting productivity, such as, mixing, temperature, humidity and airflow rate vary from one process to another. Despite its prospects and the continuing interest in solid-state fermentation, the variation in production at different scales has not been adequately studied.

In this paper, results of lovastatin production by solid-state bioconversion in packed bed reactors with forced aeration and intermittent mixing has been reported. The time profiles of temperature and CO_2 have been presented. In addition, the variation in superficial air velocity and Damköhler number (*Da*) were determined for the 7 L and 1200 L reactor, to understand the effect of mass transfer and reaction rate limitations on bioconversion of substrate. We also report for the first time, about large-scale production of lovastatin by *Aspergillus terreus* PL 10 in a 1200 L packed bed reactor by solid-state bioconversion. The effect of solid substrate composition and aeration rate on lovastatin production was optimized using the central composite experimental design.

2. Materials and methods

2.1. Micro-organism and spore suspension

Aspergillus terreus PL-10 was used for lovastatin production. For preparing a spore suspension, 10 mL of sterilized 0.1% Tween-80 solution was added to a well-sporulated slant. The surface of the slant was scraped with an inoculation loop, and agitated thoroughly using a cyclomixer to suspend the spores. The concentration of the spore-suspension was measured using a haemocytometer and adjusted to 1×10^8 spores mL^{-1} by diluting it suitably. A spore suspension of this concentration was used as inoculum throughout the study (Valera et al., 2005).

2.1.1. Preparation of seed culture

Preparation of seed culture for 7 L reactor. The seed culture medium contained dextrose 20 gL^{-1}, lactose 20 gL^{-1}, Na-glutamate 12.5 gL^{-1}, KH_2PO_4 5 gL^{-1}, K_2HPO_4 5 gL^{-1}, $MgSO_4{\cdot}7H_2O$ 0.1 gL^{-1}, $CaCl_2{\cdot}2H_2O$ 20 mgL^{-1}, $CuCl_2{\cdot}2H_2O$ 5 mgL^{-1}, H_3BO_3 11 mgL^{-1}, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 5 mgL^{-1} and adjusted to pH 6.5 (Hajjaj et al., 2001). For bioconversion in 7 L reactor the required volume of seed culture was prepared in Erlenmeyer flasks of volume 250 mL in duplicate. Each flask was inoculated with 1×10^8 spores mL^{-1} and incubated in a shaken incubator for 24 h at 30 °C and 150 rpm agitation speed and the pellets formed were used as seed culture.

Preparation of seed culture for 1200 L reactor. For solid state bioconversion in 1200 L reactor, the seed culture was grown in two stages – the pre-seed culture and the seed culture. The composition of the pre-seed and seed culture medium was the same as that used for preparation of seed culture for the 7 L reactor. The pre-seed culture was grown in five 2 L flasks containing 1 L of medium. The culture grown in the flasks was used to inoculate the 150 L reactor (Bioengineering AG, Switzerland) for preparing the seed culture. 50 L of medium was prepared for each bioconversion experiment carried out in the 1200 L reactor. The 150 L reactor was operated for 45 h at a temperature of 30 °C, pH of 6.5, agitation speed of 100 rpm and aeration rate of 1.0 vvm. The average size of the pellets obtained was 1.45 mm. After harvesting, the required volume of the seed culture was pumped aseptically to inoculate the 1200 L reactor.