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Bed moisture estimation by monitoring of air stream temperature rise in packed-bed solid-state fermentation

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Abstract

We develop a method for predicting the moisture content of the fermenting solids in an intermittently mixed packed-bed bioreactor on the basis of measurements of the inlet and outlet gas stream temperatures and the assumption that both the gas streams are saturated with water vapor. This method can be used to estimate when an intermittently mixed packed bed should be mixed and how much water should be added during the mixing event. The predictions were experimentally verified for the growth of *Aspergillus niger* on wheat bran in a thin packed bed. We show that the application of a controller based on our estimation method would have led to adequate moisture control, with significant errors in moisture content prediction only occurring after the last mixing and water-addition event. Our method therefore represents a simple and practical method that can be incorporated into control schemes for intermittently mixed solid-state fermentation bioreactors with forced aeration.

Keywords: Solid-state fermentation; Packed bed; Moisture control; Wheat bran; *Aspergillus niger*

1. Introduction

Solid-state fermentation (SSF) of fast-growing fungi is associated with high rates of metabolic heat release. The removal of this metabolic heat at an adequate rate is a major challenge in the design of large-scale bioreactors for SSF processes, especially for those processes involving fungi, in which it is desirable for the bed to remain static during the majority of the process, since constant or frequent mixing typically causes excessive damage to the fungal hyphae or the substrate. Two bioreactor types are available that provide static, or largely static, operation: tray bioreactors and packed-bed bioreactors (Mitchell et al., 2000a,b). In tray bioreactors, in which air is not blown forcefully through the tray, heat removal is limited to the tray surfaces. In this case, heat removal can be promoted by circulating air around the tray or placing the tray in contact with cooling plates, but this does not prevent the development of large temperature and moisture gradients in the bed. As a result, bed heights within trays are limited to a few centimeters. Larger bed heights are possible with packed-bed bioreactors, in which air is forced through the bed and plays a significant role in heat removal. For example, packed-bed bioreactors with bed heights of 40 cm are used routinely in the *koji* industry and even higher bed heights are possible (Sato and Sudo, 1999).

Heat removal by evaporation can be a major mechanism for heat removal in packed beds and, as a result, in many cases water must be replenished intermittently during the process (Gutierrez-Rojas et al., 1996). The required frequency of water addition is dictated by the effect of the operational variables on the evaporation rate and on the moisture range that supports reasonable rates of fungal growth and product formation. Since it is not practical to mix added water uniformly into a static bed, the bed must be mixed thoroughly while water is sprayed on its surface. In other words, large-scale packed-bed bioreactors typically need to be operated in the intermittently mixed mode. The challenge is to maximize growth by maintaining

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appropriate water contents, while minimizing the number of mixing events in order to reduce damage to fungal hyphae. In order to do this it is necessary to identify the times at which water addition is necessary and the amount of water that needs to be added during each water-addition event.

Unfortunately, direct online measurement of bed moisture, although now becoming theoretically possible due to the development of sophisticated equipment involving microwave sensors and time domain reflectometry, is impractical due to cost constraints (Bellon-Maurel et al., 2003). Off-line measurement of bed moisture is also not practical, due to the difficulty of removing representative samples from the bed during the periods of static operation and the relatively long sample-processing times. The solution is to use indirect estimation methods, sometimes referred to as 'soft-sensors'. These methods are based on the measurement of gas stream variables that can be monitored online and the use of mathematical models to convert these measurements into estimates of bed moisture content (Pena y Lillo et al., 2001).

Indirect methods for water content estimation face their own challenges. Firstly, although sensors are available to monitor the relative humidity and temperature of the inlet and outlet gas streams, they typically do not perform well at a relative humidity near to saturation due to condensation of water on the sensor. Secondly, even if reliable values could be obtained for these variables throughout the fermentation, in order to estimate the moisture content of the bed it is also necessary to know the dry weight of the solids, and direct measurement of bed weight is costly and inaccurate for large bioreactors.

Several reviews have described mathematical models that simulate the performance of packed beds, and which might, therefore, potentially be used within soft sensors (Lenz et al., 2004; Mitchell et al., 2000a,b, 2003). Most of the early models have no practical significance as they either neglected evaporation or assumed continuous and uniform water addition. Only a few models have considered comprehensive balances (Pena y Lillo et al., 2001). Most of the models have been developed as tools for guiding bioreactor design; the prerequisite of such models is an accurate growth kinetic formulation, in which it is necessary to describe the dependence of growth on the bed temperature and moisture content. However, this is a difficult task since in many cases the kinetic behavior of the microorganism is history dependent (Dalsenter et al., 2005; Ikasari et al., 1999). This means that kinetic parameters determined on the basis of experiments performed under constant conditions will not give accurate predictions for real SSF processes, in which the key process conditions vary with time. For example, considerable deviation of kinetic model predictions from experimental data was reported for the growth of *Coniothyrium minitans* in a packed-bed bioreactor (Weber et al., 2002).

Given the difficulties in formulation of accurate kinetic models, a mathematical model designed for indirect estimation of bed moisture should not require a kinetic model but rather should rely on a measurable variable related to the rate of metabolism of the organism. In previous studies, the metabolic rate has been estimated from oxygen or carbon dioxide concentrations in the exit gas stream (Nagel et al., 2001a,b; Pena y Lillo et al., 2001; Weber et al., 2002), however, costly gas analyzers are needed. Given that the rate of heat release is directly related to metabolic activity, the metabolic rate can be estimated from the increase in temperature and humidity of the air stream. The need for online humidity measurements can be avoided if the humidity of the outlet air can be estimated from its temperature, which is the case if the air can be assumed to be in equilibrium with the solids.

The aim of this work was therefore to develop a method for estimating the moisture content of the fermenting solids that only requires measurement of the inlet and exit air temperatures. This was done by writing mass and energy balances and using them to derive equations to estimate water and dry matter loss of packed beds from the temperature difference between the air inlet and the air outlet. The predictions of the method were compared with experimental results. The error was within acceptable limits, which shows that this method is appropriate for estimating bed moisture content in intermittently mixed SSF bioreactors.

2. Materials and methods

Microorganism: *Aspergillus niger* ATCC 10864 was grown on PDA slants at 30 °C and stored at 4 °C until use. Spore suspensions were prepared by adding distilled water containing two drops of Tween 80 and scraping the mycelium with a glass rod.

Solid substrate: The solid substrate contained, per 100 g: 45.6 g wheat bran, 0.94 g corn flour, 0.94 g HCl (28%), 0.3 g $(NH_4)_2SO_4$, 0.01 g $FeSO_4{\cdot}7H_2O$, 0.01 g $ZnSO_4{\cdot}7H_2O$, 0.01 g $CuSO_4{\cdot}5H_2O$ and 52.2 g added H_2O. The particle-size distribution of the dry wheat bran was (w/w): 0% greater than 2 mm, 7.7% between 1 and 2 mm, 8.9% between 0.7 and 1 mm, 27.7% between 0.5 and 0.7 mm, 32.0% between 0.35 and 0.5 mm, 20.6% between 0.25 and 0.35 mm and 3.1% less than 0.35 mm. Solid substrate (3300 g) was autoclaved at 121 °C for 45 min and, after it had cooled to room temperature, was inoculated with 200 mL of spore suspension, which represented an inoculum density of approximately 1×10^8 spores per gram of wet substrate. The bioreactor was then loaded with 3500 g of inoculated substrate. The initial moisture content, taking into account the water in the wheat bran and inoculum suspension, was 55% (w/w), on a wet basis.

Bioreactor: The bioreactor was a fully insulated 100 cm long horizontal cylinder 28.5 cm internal diameter and tilted horizontally at an angle of 10° (Fig. 1). Inside the bioreactor, a longitudinal thin box (75 cm × 21 cm × 4 cm) acted as a base on top of which solid medium was spread at the desired height (5.5 cm), the bed being contained by a polystyrene foam frame mounted on top of the box. The upper surface of the box, which formed the lower surface of the bed, was perforated. To ensure uniform distribution of air, the underside of the perforated surface was covered with a finely meshed fabric. More details of the box are given in Fig. 1. A water ring blower supplied air. The saturated air was demisted in a surge tank and sterilized by passing through a 0.01-μm ceramic filter. It was then blown into the box at a rate controlled via a rotameter. The