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Porosity changes during packed bed solid-state fermentation

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ABSTRACT

Porosity is one of the key parameters in a typical solid-state fermentation model that represents the intra and inter-particle void spaces in bioreactor bed. In this research, the particle density and moisture content of the bed were used to determine the porosity changes of the solid substrate bed during the fermentation of *Aspergillus niger* in a laboratory scale packed-bed bioreactor. Biomass growth was also monitored by means of measuring CO_2 and O_2 in the bioreactors outlet gas online. As such, an experimental relationship between humidity and porosity has been developed and can be used in solid-state fermentation modeling.

1. Introduction

Solid-state fermentation (SSF) is defined as a fermentation process in which microorganisms grow on solid materials in the absence of free water. The term arises from "solid-substrate fermentation" which was first proposed by Moo-Young et al. [1] to denote any type of the fermentation process that involves solids. In recent years, SSF has revealed great industrial potential and has found increased applications in bio-industries [2–4]. However, there continue to be a number of unresolved engineering problems in the industrial process that must be addressed. One key parameter helps resolve mentioned problem with the SSF process is the porosity. Since it appears in any typical solid sate modeling which has been used and is a promising tool that can help overcome the "scale-up" problems inherent to SSF systems [5–9]. Second because it would change during the process of fermentation and may affect other key variables accordingly. These variations may be due to different reasons such as, biomass grow, degradation of solid substrate, swelling or shrinking phenomena [10].

Porosity is one of the parameters of a typical SSF packed bed bioreactor model which represents the intra and inter-particle void spaces in a bioreactor bed. The body of any mathematical model consists of a series of variables (such as state, independent or operational variables) and parameters. Parameters may be constant or depend on the state of the fermentation system. In case a parameter is not constant it is critical to the mathematical model and the parameter to be modified by a function that reflects its variation during the process. In solid state fermentation modeling research has been conducted to determine the equation that describes the dependence of different parameters upon the state variables of the system. Among them, kinetic parameters and equations describing their dependence on temperature have largely been the focus [11–14]. It seems necessary to extract a function of porosity variation in order to use it as a mathematical model of solid state formation for a more accurate result.

Porosity of an SSF bed does not remain constant throughout SSF processes and this could be addressed to biomass grow, degradation of solid substrate, swelling or shrinking phenomena and water content variability in the packed bed. In the course of an SSF process biomass fill the inter particle spaces and this would directly affect porosity of the bed. Degradation of solid substrate happens when polymeric substrate provides the physical structure of the particles. During an SSF process, the support that gives the solid structure of the particles may be degraded by the microorganism. Such degradation leads to further variation in the shape and the volume of the solid substrate make the porosity of the whole bed change. Additionally, many of the solid substrate changes in terms of volume and shape with variation in the humidity. These changes have been referred to as swelling or shrinkage phenomena.

Previously, several reports have been published about investigating and modeling some of the variable characteristics of the bed in which porosity depends on. For example, Rajagopalan et al. [15] modeled the expansion of a bio-film for a single particle. Particle size reduction has also been modeled by Nandakumar et al. [16].

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Nomenclature

BD	bulk density (kg/m^3)
m_0	maintenance coefficient (kg oxygen/kg biomass/h)
MC	moisture content (kg H_2O/kg bed)
OUR	oxygen uptake rate (mole oxygen/h/kg initial substrate weight: IDW)
r_x	growth rate (kg biomass/h/kg IDW)
t	fermentation time (h)
V_g	volume of the bioreactor which is occupied by gases (m^3)
V_l	volume of the bioreactor which is occupied by liquids (m^3)
V_s	volume of the bioreactor which is occupied by solids (m^3)
x	biomass concentration (kg biomass/kg IDW)
x_m	maximum biomass concentration (kg biomass/kg IDW)
x_0	initial biomass concentration (kg biomass/kg IDW)
Y_{OX}	oxygen yield coefficient (kg oxygen/kg biomass)
ε	porosity (%)
ρ_w	water density (kg/m^3)
ρ_p	dried particle density (kg/m^3)
μ_m	maximum specific growth rate (h^{-1})

However, in their studies, the researchers assumed that the overall particle size is constant, but this is not necessarily true. Auria et al. [17] presented a parameter that indicates the fraction of initial free space filled by the microorganism during growth. They used an inert support as the solid structure to ensure that the solid particle structure remains constant during the process. Gutierrez Rojas et al. [18] also simulated porosity of the SSF on inert supports as a function of time. And Webber et al. [19] and Oostra et al. [20] investigated the influence of the humidity change on the bulk density.

Porosity variation during the SSF process have been measured and reported, but not modeled in the following papers: Richard et al. [21] investigated porosity change in the composting process both based on the theoretical equation presented by van Ginkel et al. [22] and experimental measurements computed by an air pycnometer [21]. They assumed the solid phase consists of organic material and ashes. Agnew et al. [23] reported porosity changes of a composting pile during the SSF process by using a simple theoretical equation and a modified air pycnometer.

Despite the relative importance of this consideration been mentioned in numerous texts, porosity changes have not been incorporated into any of the models describing the performance of the directors, [7,24]. Indeed, any changes in porosity affect heat and mass transfer within the bioreactor through effects on the size and shape of the inter-particle spaces. To consider the porosity changes, an equation should be developed to define porosity as a function of state variables such as growth, temperature and/or humidity.

Therefore, the goal of this research is to derive an equation capable of expressing porosity as a function of state variables to be used in SSF bioreactor modeling. Based on the theoretical equation of porosity [23] in the SSF bed, porosity of the bed is influenced by two state variables: humidity and biomass concentration. To effectively model the dependence of porosity on above mentioned state variables, the authors first investigated porosity variation in several laboratory scale bioreactors where the humidity remained constant. In assessing growth during the cultivation process, it became relevant to explore the influence of growth on the porosity during a typical SSF process in which other state variables stayed constant. Then different experiments were done to model the dependence of porosity on humidity by measuring porosity at various fermentation bed humidity levels. Particle density, bulk density and moisture content of the bed were being measured during the process so that the porosity of the bed can later be determined.

2. Materials and methods

2.1. Microorganism

Aspergillus niger PTCC 5010 was used for spore suspension preparation. Spores from a stock were cultured on potato dextrose agar (PDA) and incubated at 25°C for a week.

The spore content of PDA plates was suspended in 5 ml sterile distilled water. The suspension was passed through a filter to eliminate PDA particles and spore concentration was determined by Thoma chamber and modified to be in the order of 5×10^7 spores/ml by adding PFS-Tween solution (Peptone 1 g/l, NaCl 8.5 g/l, Tween 80 0.5 g/l). The spore suspension was maintained in −70°C for up to 6 months after adding 80% v glycerol.

2.2. Cultivation method

Wheat bran was used as a substrate. 250 g of milled wheat bran was mixed with enough water, HCl, salt solution and corn flour, to obtain 55% moisture content and a final pH of 5. Then the mixture was autoclaved at 121°C for 15 min, cooled to ambient temperature and inoculated with 27.5 ml of spore suspension to reach a concentration of 5.5×10^6 spores/g of initial dry substrate (IDW).

2.2.1. Porosity variation in constant humidity

In order to establish the porosity changes profile and develop a growth dependent equation on these changes, a small-scale experimental system was used so that other conditions of the bed could remain constant approximately. The system consists of seven identical columns (diameter 2.5 cm, height 20 cm) (Fig. 1a) and one modified column to measure pressure drop and the reactor temperature at various heights of the column during the process (Fig. 1b). Each column was aerated separately bottom to top. A polyethylene mesh was placed over the air inlet, at about 1 cm from the bottom, to support the solid substrate. Inlet air streams were saturated. The airflow through each column was set at 0.5 l/min. The columns were incubated in temperature-controlled water-circulation bath (30°C) (Fig. 2).

2.2.2. Porosity variation in variable humidity

A similar set of columns was used for experiments that sought to verify the equation developed for porosity. However, in these cases, columns were not incubated in a water bath. This allowed the operating condition to be increasingly similar to scaled-up packed bed bioreactors by reducing any heat transfer from the column's wall.

The temperature at various substrate bed heights was recorded using thermocouples inserted at the axis of the column. Growth was assessed by measuring oxygen consumption via an on-line gas analyzer. One column was removed every 4 h and then used as a sample to analyze porosity, moisture content and particle density of the bed.

Pressure drop was measured in a modified column with two connections to an on-line differential pressure indicator. One of the connections had been placed between the air inlet and the perforated polyethylene plate and the second connection was