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Structural properties of beds packed with agro-industrial solid by-products applicable for solid-state fermentation: Experimental data and effects on process performance

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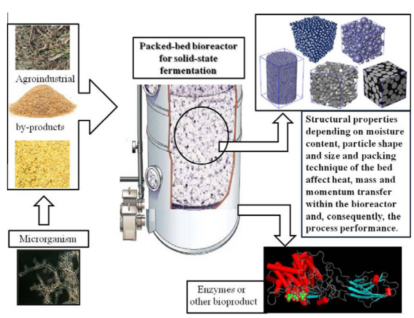
HIGHLIGHTS

- Paper addressed structural properties of beds packed with agro-industrial wastes.
- Moisture content and packing technique markedly affected bed density and porosity.
- Sugar cane bagasse increases porosity of beds packed with milled material and bran.
- It was discussed how bed porosities influence on packed-bed bioreactor performance.
- Results are useful for heat and mass transfer modeling and simulation in reactors.

GRAPHICAL ABSTRACT



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ABSTRACT

The knowledge of structural properties of beds packed with particles from agro-industrial solid wastes is important for modeling and simulation of heat and mass transfer in solid-state fermentation (SSF) in packed-bed bioreactors. This paper addresses the experimental determination of particle and bulk densities (ρ_{part} and ρ_{bulk}) and porosities (ε) of beds packed with sugar cane bagasse (SCB), wheat bran (WB) and orange pulp and peel (OPP) and with mixtures of them. The effects of moisture content (MC) and packing technique on structural properties were evaluated. Microscopic analysis of cell-size was performed and the porosity along the fermentation was determined, as well as the endoglucanase yields for different proportions of a medium composed by SCB/WB. Results showed that MC affects significantly ρ_{part}, ρ_{bulk} and ε. For OPP and WB, ε ranged from 0.4 to 0.7, depending on MC, and for SCB from 0.7 to 0.9, depending on MC and packing technique. For the composed media SCB:OPP:WB (1:2:2, weight) and SCB:WB (7:3), ε values were similar to the ones obtained for SCB. The growth of the fungi *Myceliophthora thermophila* I-1D3b and *Trichoderma reesei* QM9414 did not

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affect ε of the medium composed by SCB:WB. A comprehensive discussion on how the structural properties of solid matrices affect the performance of SSF processes was done, considering both operational aspects and bioproducts yields.

1. Introduction

Solid-state fermentation (SSF) is a sustainable alternative for bio-based processes in which agro-industrial by-products may be used as culture media or substrates, leading to the achievement of high-added value bio-products, such as several kinds of enzymes, useful in food and chemical industries. In SSF, the continuous phase is gaseous and water is either impregnated within the particles or forms a thin film over them [1].

Several agro-industrial by-products have been used as substrates or inert supports on SSF processes, e.g. grasses, bagasses, brans, straws, hulls, sawdust and pulp and peel [2,3]. Due to its high pectin content, orange pulp and peel (OPP) has been successfully applied as substrate to synthesize pectinolytic enzymes, an important group of enzymes in juice processing [4,5]. Sugar cane bagasse (SCB) has been applied for cellulolytic enzymes production, a key-group of enzymes for the second-generation ethanol route [2,6]; furthermore, this by-product has also been used in the composition of several SSF matrices as an inert support [4,7]. Wheat bran (WB) has been widely employed in SSF processes for the synthesis of a variety of bio-products, since it is considered an ideal substrate, providing well-balanced sources of carbon, nitrogen and phosphorous [8].

It is well known that the porosity (ε) of a packed-bed directly affects the fluid dynamics and the effective heat and mass transfer properties of the porous media, such as the bed permeability, the effective thermal conductivity and the effective mass diffusivity [9]. Therefore, an ideal porous matrix for SSF bioreactors should fulfill the nutritional microbial needs and provide adequate physical structure for air percolation, in order to guarantee efficient transfer of the respiratory gases and facilitate metabolic heat removal. However, typical papers on SSF usually bring few information about bed structure, while some information can be found in composting literature, showing that ε of composting piles decrease linearly with the moisture content (MC) of the materials [10,11].

Kumar et al. [12] used *Aspergillus niger* to individually ferment SCB and WB, enriched by molasses or sucrose, and observed agglomeration in WB beds at MC as low as 65%, while SCB beds kept adequate structure up to 85% MC. Asha Poorna and Prema [13] studied the production of endoxylanase from *Bascillus pumilus* cultivated in WB and found best result for 71.4% MC. According to the authors, low MC reduces swelling capacity of the substrate, increasing water surface tension and consequently reducing the water activity for the microbial metabolic requirement. In opposition, very high MC reduces inter particle spaces, leading to a decrease in the bed voidage and resulting in less space for microbial growth and deficient gaseous exchange. The particle density (ρ_{part}) regulates the utilization of the substrate by the microorganism at a molecular level, as well as the gas–liquid interfacial area and the thickness of the wet fungal layer. Small particle size interferes with microbial respiration, leading to deficient microbial growth, while large particle size limits the available surface for the microbial attack [14]. Bulk density (ρ_{bulk}) affects SSF yields, especially due to microbial growth. Dorta and Arcas [15] studied the effect of packing density on spores yield of *Metarhizium anisopliae* cultivated in a mixture of rice bran and rice husk, and observed that for low ρ_{bulk} (from 0.270 to 0.357 g/cm^3), no significant differences took place on both the total biomass production and the spore yield. However, a significant reduction on the total accumulated biomass was observed when ρ_{bulk} was increased up to 0.496 g/cm^3.

The current paper addresses the structural properties bulk density, particle density, and porosity of beds packed with SCB, WB and OPP and with mixtures among them, correlating these properties with the moisture content and the packing technique employed. The swelling of SCB with MC increasing was microscopically observed, and a correlation of the cell-size with MC was established. Fermentation experiments were carried out on beds composed by SCB and WB, fermented by the thermophilic mold *Myceliophtora thermophila* I-1D3b and by the mesophilic *Trichoderma reesei* QM9414, and the effect of fungal growth on the selected properties was measured. Cellulase (filter paper activity) has been synthesized in this fermenting system for several SCB/WB proportions and MCs, and the influence of the bed structure on the enzyme yield was discussed. The effect of the bed structure on the airflow head-loss was assessed and discussed. The results here presented certainly will be useful for heat and mass transfer modeling in SSF processes in packed bed bioreactors.

2. Materials and methods

2.1. Materials

SCB, OPP, WB, and mixtures of them were tested. The raw materials were abundantly washed with tap water to remove filth and oven dried at 65 °C until constant weight. SCB was kindly provided by Usina Vale, from Onda Verde-SP, Brazil, an industrial ethanol and sugar producer. Only fibers restrained between 3 and 1.44 mm opening sieves were used. OPP was kindly provided by Citrovita, from Catanduva-SP, Brazil, an industrial orange juice producer. The material was ground in knife mill and only the particles restrained between 2 and 1.44 mm opening sieves were used. WB was bought in a local retailer and was not sieved.

Water was sprayed over the solids in order to provide samples at several MCs, and 1 kg of each dried material was used for each MC. Moist samples were stored at 5 °C in thick-walled plastic bags for 15 days and daily revolved to homogenize the MC. For SCB, the MC ranged from 4.0% to 75.0% (w.b.); for WB, from 4.5% to 65.0% and for OPP, from 6.0% to 75.0%.

Mixtures of SCB and WB (7:3 w/w) and SCB, OPP and WB (1:2:2 w/w) were also tested in the MC ranging from 20% to 80%. These mixtures were tested since they have been previously used in literature to produce cellulases and xylanases (SCB + WB) and of pectinases (SCB + OPP + WB) [4,6].

2.2. Packing techniques

For SCB, the major volumetric component studied here, three packing techniques were evaluated, as follows:

(LP) Loose Packing: with the aid of tweezers, portions of fibers of approximately 5 g were gently accommodated in layers in a cylindrical recipient made of stainless steel with known volume (10 cm diameter, 12.7 cm height, 1 L approximate volume) up to the maximum height;

(CP) Compressed Packing: after the addition of a portion of fibers, as described for the loose packing, a weight of 0.545 kg was applied