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Hygroscopic properties of solid agro-industrial by-products used in solid-state fermentation

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ABSTRACT

One of the main drawbacks in operating solid-state fermentation (SSF) bioreactors is the reduction in moisture content (MC) of the solid phase due to microbiological and physicochemical issues. Therefore, heat and mass transfer models require hygroscopic information concerning the solid substrate. This contribution addresses the study of the sorption isotherms of sugarcane bagasse (SCB) and wheat bran (WB) obtained using the static gravimetric method, with temperature and water activity as the controlled variables. The Oswin model was selected to construct the sorption isotherm for SCB, and the Halsey model, modified in the present study, for WB. The Bates and Watts curvature measurements and the Box parameter bias were used to analyze the nonlinear behavior and assess the goodness of the fitting process. Fermentative assays were carried out in glass flasks for the production of endoglucanase employing the fungus *Myceliophthora thermophila* I-1D3b, controlling the environmental water activity, in order to assess the influence of the loss of moisture from the solid phase on fungal metabolic activity.

1. Introduction

Solid-state fermentation (SSF) is a general term used to designate a biotechnological process involving cultivation of microorganisms on moist solid substrates, without the dripping in of water and with a continuous gas phase in the inter-particle space (Rahardjo et al., 2006). Although there may be thin films of water at the particle surface, a gas phase fills most of the inter-particle space and the majority of the water in the system is absorbed within the moist solid particles (Mitchell et al., 2006).

One of the main advantages of SSF is the possibility of using agro-industrial by-products as substrates to generate high added-value products such as enzymes. Wheat bran (WB) is considered to be an ideal substrate for SSF, due to the correct balance between the carbon, nitrogen and phosphorous sources (Couri et al., 2000; Pandey et al., 1999; Brijwani et al., 2010). This substrate is rich in carbohydrates (58–74%, dry basis, d.b.), protein (12–17%), fat (0.5–1.6%), lignin (4–7%), minerals (1.5–6%), bioactive compounds and vitamins (Theander and Westerlund, 1986; Maes and Delcour, 2002; Shenoy and Prakash, 2002; Slavin, 2003), this being the reason why WB is widely used in several SSF processes. Sugarcane bagasse (SCB) is composed of cellulose (37–42%, d.b.), hemicelluloses (21–32%), lignin (20–25%) and ash (3%) (Trickett and Neytzell-de Wilde, 1982; Aguilar et al., 2002; Kim and Day, 2011), this being the reason why SCB has been used to produce cellulases (Da Silva et al., 2005; Sukumaran et al., 2008; Gao et al., 2008; Zanelato et al., 2012) and hemicellulases (Milagres et al., 2004; Badhan et al., 2007; Dhillon et al., 2011).

One of the main drawbacks in operating SSF bioreactors is the reduction in moisture content (MC) of the solid phase during the process due to microbiological and physicochemical issues. Water has many functions in SSF as the nutrient and metabolite solvent; stabilizer of biopolymer structures, e.g. proteins, nucleic acids and carbohydrates; stabilizer of the lamellar plasmatic membrane; and maintainer of cell volume due to linkages to polyols, carbohydrates and enzymes (Schwan, 1965; Kuntz, 1971; Crowe et al., 1982; Quinn, 1985). Hence, microorganisms are quite sensitive to a reduction in water concentration and many studies have been published in the literature about the dependence of enzyme production on the water content of the porous media (Lonsane et al., 1992; Grajek and Gervais, 1987; Sato and Sudo, 1999; Gervais and Molin, 2003; Singhania et al., 2009). If the MC is insufficient, gas and solute diffusions decrease, and the cell metabolism is affected due to a lack of nutrients or the accumulation of toxic compounds in the cell vicinity. Water also solubilizes enzymes, which are fundamental for the metabolic system of the cell as a whole (Todd, 1972; De

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Loecker et al., 1978; Wolfe and Steponkus, 1983). In order to fulfill the needs of the microorganisms during SSF, the water content of the solid material must be well above its equilibrium moisture content (EMC) with the surrounding air at the local temperature. The EMC of a solid material is defined as the thermodynamic state in which the water vapor pressure in the solid phase is equal to the vapor pressure in the surrounding air.

Even if saturated air enters a SSF bioreactor, its relative humidity (RH) will decrease due to the metabolic heat generated, producing a driving force transferring water from the solid to the fluid phase. In some bioreactors, mainly in rotating drum apparatuses, air may enter the equipment with moderate humidity in order to remove evaporative heat from the solid and reduce the temperature of the solid matrix, providing an effective alternative temperature control for the fermentation process. In this class of fermenters, the water can be replenished during fermentation, restoring the ideal MC of the solid phase. However, in packed bed bioreactors, water replenishing is not possible and therefore the MC cannot be controlled during fermentation. Thus drying is a serious drawback for the use of this type of bioreactor.

Modeling of heat and mass transfer in SSF bioreactors requires knowledge of the sorption isotherms of the solid constituents. However, due to the low cost of the agro-industrial by-products, few articles are available in the literature on biomass materials. Some studies have been published on wheat straw (Duggal and Muir, 1981); biomass briquettes (composed of saw dust and de-oiled castor cake); cotton stalk and saw mill waste (Singh, 2004); corn stover (Igathinathane et al., 2005); flax straw, hemp stalks and reed canary grass (Nilsson et al., 2005); pellets made from sorghum stalks, corn stover, wheat straw and big bluestem (Theerarattananoon et al., 2011); sunflower stems (Sun et al., 2013); orange pulp and peel (Casciatori et al., 2013a,b); and on switchgrass and prairie cord grass (Karunanithy et al., 2013a).

All the models available in the literature to represent EMC are non-linear with respect to the parameters, and all the articles published on the determination of biomass EMC, with the exception of that by Casciatori et al. (2013a,b), did not take this characteristic into account when estimating the parameters. Even though several commercial softwares are available for non-linear estimations, little care was taken with respect to the validity of the estimated parameters or to the goodness of fit. The authors usually assume that the statistical analysis performed for parameters estimated from linear model is also valid for non-linear ones.

The Battes and Watts curvature measurements, Box parameter bias and residual analysis are powerful techniques to provide reliable estimations of the parameters. Several textbooks and articles are available in the literature about non-linear estimation and therefore it is not the intent of the present authors to dig deeply into this subject in the present paper (see for instance Draper and Smith, 1985; Gallant, 1987; Ratkowsky, 1990). Basically, the Bates and Watts (1980) curvature measurements are divided into the Intrinsic Curvature (cur IN) and the Parameter Effect Curvature (cur PE). Excessive cur IN means the model is inadequate to represent the experimental data set, while excessive cur PE implies that the mathematical model is acceptable, although all or some of the estimated parameters are biased and the model must be reparametrized. The statistical significances of cur IN and cur PE are assessed by comparing their calculated values with the radius of curvature ρ, given by:

$$\rho = \frac{1}{\sqrt{F_{(p,N-p,1-\alpha)}}}$$

where F is the Fisher statistics, p is the number of parameters being estimated, N is the number of experimental data, and α is the significance level. Excessive cur PE indicates that one or more parameters are not well defined, but the calculations are unable to identify which parameters are responsible for this effect. Therefore, the Box (1971) bias measurements can be applied and a parameter is said to be inadequate when its bias is greater than 2%. When the Bates and Watts (1980) curvatures or the Box (1971) bias are excessive, the estimated parameters are only valid for the experimental data set used for the estimation, and extrapolation is not recommended.

Another important statistical tool to assure the quality of the fit is the graphical residual analysis. This analysis is important not only for non-linear estimations but also for linear ones, since a random residual distribution is expected within the whole domain of the solution, which is an indication of homoscedasticity. Some authors have used the residual analysis to represent experimental data from biomass when discriminating models (Igathinathane et al., 2005; Nilsson et al., 2005; Cordeiro et al., 2006; Acharjee et al., 2011; Karunanithy et al., 2013a).

Therefore, this article addresses the sorption isotherms of sugarcane bagasse and wheat bran, and the kinetics of water adsorption–desorption of these materials for several air relative humidity values. The deleterious effect of the loss of water from the solids on the production of endoglucanase by the thermophilic mold *Myceliophthora thermophila* I-1D3b, cultivated in sugarcane bagasse and wheat bran at 45 °C, was also evaluated and discussed. Special care was taken in estimating the parameters, since all the models applied were non-linear with respect to the parameters.

2. Materials and methods

2.1. Solid materials

Sugarcane bagasse (SCB) and wheat bran (WB) were the solid materials tested. They were washed with an abundance of tap water to remove filth and oven dried at 105 °C to constant weight, a common procedure for SSF substrates. Water was then sprayed on to the solids to produce samples with several MC values, and the moist samples maintained at 5 °C in thick-walled plastic bags for 15 days, being turned over daily to homogenize the moisture. The SCB was kindly provided by the *Usina Colombo*, Ariranha-SP, Brazil, an industrial ethanol and sugarcane producer. Only fibers that passed through a 3 mm sieve and were retained by a 1.44 mm sieve were used. The WB was bought at a local retailer and was not sieved.

2.2. Sorption isotherm experiments

The standard static gravimetric method was used as described by Spiess and Wolf (1983). This method is widely used for biomass EMC experiments (Igathinathane et al., 2005; Zanoelo, 2005; Cordeiro et al., 2006; Acharjee et al., 2011; Karunanithy et al., 2013a). Samples of 1 g of each material were individually placed in recipients whose air relative humidity (RH) was controlled by saturated salt solutions. The recipients were placed in climatic chambers at the temperatures shown in Table 1. The samples were weighed to constant weight on analytical balances with an accuracy of ±0.1 mg, and all experiments were carried out in triplicate. Special care was taken to avoid fungal infestation that could alter the experimental results. The substrates and salt solutions were sterilized in an autoclave at 121 °C for 25 min, and thermally sensitive materials were rinsed in an ethyl alcohol solution at 70% volume.

The models fitted to the experimental data are shown in Table 2 and the Least Square Method (LSM) was applied to estimate the parameters (Ratkowsky, 1983, 1990). The commercial software *Origin 6.0* (Microcal Software Inc., Massachusetts, USA) was used to estimate the parameters, using the Levemberg–Marquardt algorithm to optimize the search for the minimum of the objective function. Several sets of initial values for the parameters were used,