Brazilian Journal
of Chemical
Engineering

Vol. 36, No. 03, pp. 1131 - 1141, July - September, 2019
dx.doi.org/10.1590/0104-6632.20190363s20180245

WATER HOLDING CAPACITY AND HEAT TRANSFER ASPECTS OF A MIXTURE OF SUGAR CANE BAGASSE AND WHEAT BRAN IN A PARTIALLY FILLED ROTATING DRUM

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(Submitted: June 6, 2018 ; Revised: March 7, 2019 ; Accepted: April 12, 2019)

Abstract - Maximum water holding capacity of a mixture of sugarcane bagasse and wheat bran was determined in static conditions and in a drum rotated at 1 rpm. The variables filling degree, water flow rate, volume of added water and number of sprinklers were tested to control the moisture content of the solid and the number of rotations to achieve homogeneous moisture content. None of the selected variables was significant, giving the apparatus high flexibility to control moisture content. For the heat transfer experiments, the tested variables were introduction of air through an inner tube amidst the particles, water sprinkling over the bed and drum rotation. The selected variables represented limited mechanisms of heat removal, although efficient when coupled with the drum rotation. The results are of value to control the temperature and the moisture content in solid-state cultivation bioreactors.

Keywords: Rotating drum; Solid-state cultivation; Sugar cane bagasse; Water holding capacity; Heat transfer.

INTRODUCTION

Solid-state cultivation (SSC) is a term commonly used to characterize the growth of microorganisms in a wet solid substrate in the absence of dripping water (Pandey, 2003). This water restriction favors the use of filamentous fungi, since the imposed conditions are similar to their natural habitat and hamper the possibility of exogenous contamination by bacteria (Hölker and Lenz, 2005). Besides, the cost-benefit ratio of the SSC is positive as residues from agroindustry can be used as substrates to produce high added-value compounds.

In SSC, the gas phase is continuous and the presence of a particulate solid phase characterizes a heterogeneous system in which the water is incorporated into both phases, not always in equilibrium. This non-equilibrium affects the water activity (α_w), which is defined as the ratio between the partial pressure of water vapor in the solid material and the partial pressure of pure water at a given temperature. In practical terms, α_w corresponds to the fraction of water available for chemical and biochemical reactions and the microbial growth. The literature has reported several studies correlating this property to both the growth of microorganisms and the synthesis of compounds of interest (Gervais et al., 1988; Parra et al., 2004; Corona et al., 2005; Pardo et al., 2005; Nuñez-Gaona et al., 2010; Giorni et al., 2012; Rivas et al., 2014; Zhang et al., 2015; Casciatori et al., 2015). According to Gervais and Molin (2003), the water is adsorbed on the solid phase and the growth of microorganisms occurs preferentially in a superficial liquid film in which nutrients, metabolites and respiration gases are dissolved. Nevertheless, an excess of water tends to block the interstitial spaces of the porous media, restricting the offer of oxygen.

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Therefore, keeping the moisture content at adequate levels is fundamental for the best performance of the process.

The moisture content of the porous media is strongly related to the cultivation temperature, which is variable along the process due the heat metabolically generated. In SSC, heat dissipation is hampered by the poor effective thermal conductivity of the porous media, normally made of organic materials (Casciatori et al., 2013). Hence, undesirable gradients of temperature are noted during the cultivation, promoting harmful loss of water from the substrate to the continuous gas phase.

The temperature increase can be minimized by mean of convection and of water evaporation, both related to the air flow rate and its relative humidity. However, the air flow rate has a limited capacity of heat removal, since low rates must be used in SSC to avoid injuries to the fungal mycelium due to the shear stress. On the other hand, while water evaporation is a strong alternative for heat removal, the reduction of α_w negatively affects fungal metabolism. Such a trend has been observed in packed-bed SSC bioreactors (Grajek, 1998; Saucedo-Castañeda et al., 1990; Sangsurasak and Mitchell, 1995), since the simple project of these bioreactors does not contemplate water replenishing.

Effects of the cooling associated with evaporation have been addressed in the literature. Barstow, Dale and Tengerdy (1988) presented an investigation on temperature control by flowing air in a rotating drum for the cultivation of *Rhizopus oligosporus* on granular yellow corn. Two kind of experiments were performed: with integrated control of temperature based on the variation of the unsaturated airflow rate and with constant air flow rate. The authors concluded that the evaporative cooling had an important effect on the control of the temperature, since variations of temperature as high as 17ºC were observed in the uncontrolled experiments, while the increase in temperature was around 5 ºC under monitored and controlled fermentative assays. Moreover, the final dry weight of the samples and the biomass generated were very close for both experiments.

Ryoo et al. (1991) presented a study on temperature and moisture control in a rotating drum using the same substrate and microorganism of Barstow, Dale and Tengerdy (1988), in which the relative moisture of the air was varied. The results showed the importance of the maintenance of temperature and moisture content on the fungal protein production along time, since a reduction of 33% of the yield of proteins was reported between controlled and uncontrolled experiments.

Schutyser et al. (2003a) presented a model to predict the moisture profiles in a rotating drum after some seconds of continuous water sprinkling over a bed of wheat grains. The authors recommended water sprinkling followed by immediate mixing to remove moisture gradients in the bed. Indeed, mixing and motion of particles intensifies the contact between solid and gas phases, increasing the mass and heat transfer rates (Schutyser et al., 2003b; Kalogeris et al., 2003; Poletto et al., 2017; Frinkler et al., 2017). However, alternatives for temperature and moisture control, such as water sprinkling and mixing of the bed, should be employed taking into account the operational conditions offered by the rotating drum and its capacity to achieve the thermal equilibrium under different mechanisms of heat and mass transfer.

In this context, this work is the initial part of a broader investigation, and presents an experimental study of water sprinkling on a RD partially filled with a mixture of sugar cane bagasse and wheat bran (SCB/WB) and the effects of sprinkling on the thermal equilibrium of the bed are addressed. Initially, the water holding capacity of the bed was determined under static and moving conditions. The homogenization of the water sprinkled on the bed surface was assessed as a function of the water flow rate, volume of water added, number of sprinkles and the bed filling degree, while the bed was rotated at a constant angular velocity. The heat transfer aspects were qualitatively analyzed by imposing an initial temperature gradient in the bed and controlling the drum wall temperature, the air flow rate and the parameters related to the water being sprinkled. The results obtained here will be useful for the temperature and moisture content control of solid-state cultivation rotating drum bioreactors.

MATERIAL AND METHODS

Particles

Sugar cane bagasse (SCB) was kindly provided by Usina Colombo, Ariranha- SP, Brazil, at 48.3% moisture content (MC on a wet basis, as all MCs in this text). The bagasse was sieved and the fibers restrained between the sieves with openings of 3 mm and 1.41 mm were used in the experiments, in which the average diameter and length were 0.81 ± 0.21 mm and 13.8 ± 2.4 mm, respectively. Part of the bagasse was used at the moisture content as it was received and part was dried in a convective oven at 60 ºC until constant weight, reaching 7.6% MC. The bagasse was kept in thick wall polyethylene plastic packages and stored at 5 ºC until its use. Wheat bran (WB) was purchased in local retailers at 11.0% MC and it was dried to 7.6% or moisturized up to 48.3%. The bran was sieved using sieves of 1mm and 0.84 mm and the fractions retained between these two sieves were used in the experiments.

The weight proportion of the mixture SCB/WB was fixed at 7:3 (dry basis), following the recommendation of Zanelato et al. (2012) for the production of endoglucanases with the thermophilic fungus *Myceliophtora thermophila* I-1D3b by SSC. The materials were individually moisturized at the desired moisture content and mixed at the indicated proportion in plastic bags, and the final homogenization was visually observed.

Maximum water holding capacity under static conditions

The mixture SCM/WB was gently accommodated in a stainless-steel cylinder 7.5 cm in diameter and 10 cm in length and was supported by a metal screen with 1 mm opening, fixed at the basis of the cylinder, which was suspended over a Plexiglas box with a paper tissue placed in its bottom, as presented in Figure 1. The initial MC of the mixture was 7.6 % or 48.30%, with 23 g or 15g of particles being added in the tube, respectively. The MC were determined using a MB45 halogen analyzer (Ohaus, Parsipanny, USA).

Tap water at room temperature was pumped using a Micropump gear pump (Cole-Parmer, Vernon Hill, USA) to a full cone agricultural sprinkler and from it to the free surface of the bed of particles. The clearance between the sprinkler and the bed surface was 1 cm and the diameter of the circular wet area was coincident with the stainless-steel cylinder diameter, provided that water drops did not reach the tube wall. Volumes of 5 mL of water were intermittently sprinkled at 1 min intervals up to the moment when the first water drop was observed at the paper tissue. The total volume of water added to the porous medium was defined as the maximum water holding capacity (WHC), expressed as MC. The experiment was replicated five times.

The adopted methodology does not correspond to classical methodologies reported in the literature for determination of the maximum water holding capacity of the solid material, since the conception of the experiment was aimed at the real application of a SSC system.

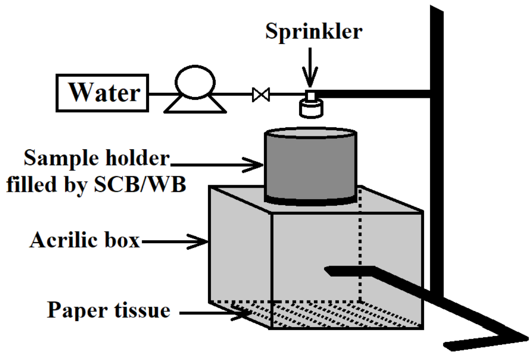

Figure 1. Experimental set up for the determination of the maximum water holding capacity of the SCB/WB porous matrix.

Water retention in a rotating drum

The ideal MC of SSC processes depends on the substrate and microorganism. In this article, the MC suggested by Zanelato et al. (2012) for the cultivation of *M. thermophila* I-1D3b in a SCB/WB mixture will be adopted. The initial MC of the SCB/WB mixture was 48.3 %, and an aqueous solution of Rhodamine B at 0.02% (w/w) was sprinkled over the bed up to 75% MC, following Zanelato et al. (2012).

The experiments were carried out in a drum similar to the one used by Tada et al. (2017a) for the experiments of mixture and motion of SCB during the drum rotation. Briefly, the drum was made of Plexiglas 31 cm in diameter and 74 cm in length with four longitudinal straight baffles 6 cm wide each. Full cone sprinklers were connected to a gear pump and were positioned over the bed surface. Preliminary experiments were carried out sprinkling the Rhodamine solution continually at a flow rate of 1.5 L min^{-1} over the bed surface and it was observed that the solution quickly reached the opposite drum wall. Therefore, intermittence was introduced between consecutive additions of solution, and the intermittence proved to be a function of the drum filling degree (f) and was equal to 13 min for f = 0.4, 15 min for f = 0.5 and 17 min for f = 0.6. The volume of Rhodamine solution sprinkled over the bed at each individual addition was tested and it was observed that volumes lower than 100 mL suffered severe effects of evaporation and volumes higher than 400 mL promoted leak of the solution. The number of sprinkler and their clearance to the bed surface were carefully studied to avoid water drops reaching the drum wall and the intersection of wetted areas from consecutive sprinklers, while promoting wetted areas as large as possible. For more details on the sprinklers positioning see Grajales-Agudelo (2014).

In order to test the homogeneity of the MC after the total volume of Rhodamine solution was sprinkled, the controlled variables were the drum filling degree, the number of sprinkles, the flow rate of solution and the volume of solution at each individual intermittent interval. Table 1 presents the levels of the controlled variables. The ranges chosen for the variables load of particles and rhodamine flow rate were based on the results available in literature for the same rotating drum used in this work (Grajales-Agudelo, 2010; Grajales-Agudelo, 2014; Tada et al., 2017a). Note that the load of particles is greater than those usually reported in the literature for SSC in rotating drums (Barstow, Dale and Tendergy, 1988; Ryoo et al., 1991; Oostra, Tramper and Rinzema, 2000; Stuart and Mitchell, 2003). The number of sprinklers was adopted considering the area covered by each individual jet, in order to avoid overlapping of wetted areas or non-wetted areas. In preliminary tests, the volume of rhodamine solution

Table 1. Levels of the controlled variables adopted for the experiments of Rhodamine solution sprinkling over the SCB/WB mixture surface in the drum rotated at 1 rpm.

Variable	Levels		
Rhodamine solution flow rate (L min^{-1})	1.5		2.5
Number of sprinklers	3		5
Load (kg) (filling degree, f)	3 (f = 0.4)	4.5 (f = 0.5)	6 (f = 0.6)
Volume of rhodamine solution in each intermittent interval for each f (mL)	100 200	150 300	200 400

in each intermittent interval was determined, it being observed that volumes under 100 mL required long experiments, resulting in partial evaporation of the added water, while volumes over 400 mL resulted in leak of the solution from the bottom of the drum. Based on these preliminary tests, a range of volume of rhodamine solution in each intermittent application has been adopted for each load of particles.

The drum was rotated at 1 rpm, following Tada et al. (2017a), while the Rhodamine solution was sprinkled over the bed surface. When the final volume of solution was added to the bed, the drum was halted and the bed surface was photographed, using a DCR-DVD408 digital camera (Sony, Tokyo, Japan), in the five longitudinal positions indicated in Figure 2. The digital images were analyzed using the LensEye image analysis software (Engineering & Cyber Solutions, Gainesville, USA), following methodology described by Grajales-Agudelo et al. (2012) and the results were considered satisfactory when a homogeneous color distribution was noticed. The mixture of particles was sampled from the bed surface in the longitudinal positions indicated in Figure 2, besides one extra sample from a position close to the drum wall for the analysis of moisture content.

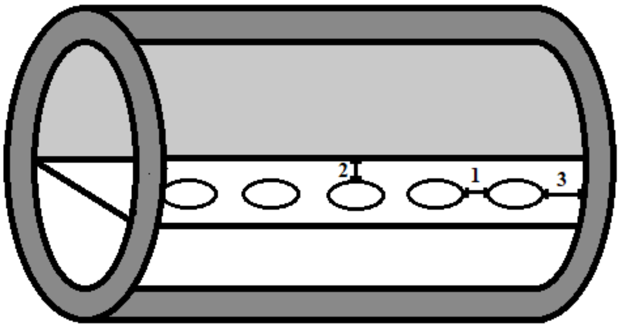

Figure 2. Longitudinal positions selected for the image analysis and the moisture content determination. The distances corresponding to the numbering in the figure indicate: “1”= 3 cm; “2”= 3 cm; “3”= 6 cm.

Heat transfer experiments

The heat transfer experiments were carried out in the drum presented in Figure 3, in order to assess the effectiveness of different heat transfer mechanisms on the bed temperature variation. The mechanisms evaluated were predominantly convective, promoted by the air flowing amidst the particles, by the water sprinkled over the porous medium and by the contact between the drum wall and the bed. Besides, the effect of mixing the particles by means of drum rotation was evaluated in some experiments. The effects of heat removal by evaporation was neglected due to the high relative humidity of the air (RH ≥ 91.53 %).

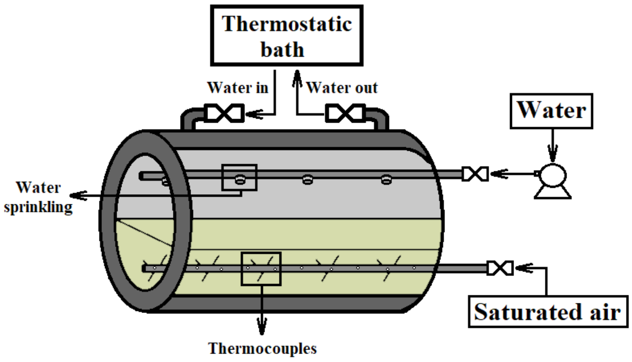

Figure 3. Diagram of the stainless-steel rotating drum, presenting the inner tube to introduce air in the system, the thermocouples and the sprinklers. A system of gears and chains rotate the lateral wall of the drum at a constant frequency, while the front and end lids are kept still.

The jacketed stainless-steel drum was 31 cm in diameter and 74 cm long, in which water flowed throughout the jacket and air at 91.53% relative humidity (RH) was introduced through a perforated tube longitudinally placed amidst the particles. The air RH was measured using a Humicap HMP7 probe (Vaisala, Helsink, Finland). Ten type T thermocouples were fixed to the wall of the aeration tube, whose tips were positioned near to the bed free surface and between the inner tube and the drum wall. The longitudinal locations of the thermocouples were 10, 20, 30, 40 and 50 cm from the front lid, and they were connected to a data acquisition system COMPAQ-DAQ (National Instruments, Austin, USA), managed by a LabView v. 8 routine. The length of the heat transfer experiments was usually three hours, except when stated otherwise.

For the experiments, the empty drum was let to reach the thermal equilibrium with the drum wall and the air flowing at the desired flow rate, both at 45°C, before the particles (f = 0.4, corresponding to a load of 3 kg) were introduced at 25°C. Three experiments were carried out, as presented in Table 2, in which the

Table 2. Operational conditions to evaluate the temperature variation of a SCB /WB bed in a rotating drum (f = 0.4; air inlet and drum wall temperatures 45°C; water sprinkling temperature 65°C; air flow rate 5 L min^{-1}; drum rotation at 1 rpm) (*).

Run	Water sprinkling	Drum rotation	Bed initial moisture content (%, w.b.)
1	○	○	75
2	●	○	50
3	●	●	50

(*) Note: ●: Yes, ○: No.

air inlet temperature and flow rate were 45°C and 5 L min^{-1}, respectively, while the drum wall was kept at 45°C. When pertinent, the water flow rate and temperature were 2.5 L min^{-1} and 65°C, respectively, and the drum rotation frequency was 1 rpm.

Statistical analysis

The mean values of the final moisture contents of the beds of SCB/WB after the water holding capacity experiments were compared by a Tukey test at 95% confidence interval. The water sprinkling experiments in the rotating drum followed a full general experimental design with analysis of variance at 95% confidence interval. The statistical analysis was done using the software Minitab 18 (Minitab Inc., State College, USA)

RESULTS AND DISCUSSION

Maximum water holding capacity

Experiments were carried out to determine the maximum water holding capacity (WHC) of a mixture of sugar cane bagasse and wheat bran (proportion 7:3) at two initial moisture contents, 7.61 and 48.30%, corresponding to the MC obtained after drying the bagasse and the MC of the bagasse as it was provided by the sugar cane mill, respectively. Table 3 presents the final MC of the mixture after the first drop of water leaked from the bottom of the bed, where one may notice that no statistical difference was observed between the means, according to the Tukey test at 95% confidence level.

According to Casciatori et al. (2015), the absorption of water by SCB and WB is very slow. These authors wetted dry samples (5% MC) of SCB and WB, separately and under static conditions, using enough water to reach 80% MC. Both materials took about 12 days to reach the maximal absorption capacity, which were 80% for WB and 75% for SCB. Therefore, the values found in this work indicate that the contact time was too short for the water to be absorbed by the solid phase in large concentrations; therefore, the liquid was mainly present on the surface of the particles, as a liquid film, and trapped within the void spaces, by capillarity. This hypothesis is reinforced by the fact that the initial moisture content of the samples did not influence the final MC, pointing out that the liquid was not absorbed by the solid phase. As previously stated, in SSC an excess of water must be avoided in order to keep the air as a continuous phase. This way, the actual MC of the solid material used in SSC must be under its WHC. Grigelmo-Miguel and Martín-Belloso (1999) determined the WHC of cellulosic fibers and Ramaswamy et al. (2013) of potato pulp and reported values of 90.0 % and 88.1%, respectively, agreeing with the values obtained in this work.

Table 3. Final moisture contents corresponding to the maximum water holding capacity of a SCB/WB mixture.

	Moisture content (%, w.b.)	
Initial	7.63	48.30
Final*	87.30^{A} ± 1.38	88.18^{A} ± 2.04

(*) Means followed by the same letters do not differ statistically, according to the Tukey test at 95% confidence interval.

Martínez and co-workers (2017) evaluated the production of aromatic compounds by *Kluyveromyces marxianus* cultivated in SCB enriched with sugar beet molasses, with moisture content ranging from 58 to 77 %, and the highest concentration of aromatic compounds was observed from 66 to 68 % MC. The highest used MC (77%) was claimed to be the maximum absorption capacity of the substrate, even though the authors did not present the experimental method to determine it and did not specify whether substrate refers to the solid phase or to the porous medium. This maximum absorption capacity is in between the value reported by Casciatori et al. (2015) for individual fibers of SCB and the value presented here for the SCB/WB mixture.

Kumar et al. (2003) cultivated *Aspergilus niger* in SCB enriched with sucrose or sugar cane molasses to produce citric acid, varying the MC from 55 to 85%. The results showed that the highest concentration of citric acid took place at 75% MC, although the sugar consumption was similar at 75 and 85%. The authors attributed the lower citric acid concentration at 85% to the reduction of the bed porosity, which hampers heat and mass transfer processes. Casciatori et al. (2014) determined the bed porosity of SCB and WB, individually and of mixtures of both, at several MC and with different techniques of packing, and proposed equations to forecast the porosity. Appling the equation of Casciatori et al. (2014) for loose packings, as seems to be the pack used by Kumar et al. (2003), the porosity of SCB beds for the moisture contents 75 and 85% results in 0.71 and 0.64, respectively. Such slight porosity reduction does not seem to be able to interfere significantly in the heat and mass transfer processes. Hence, the presence of liquid water blocking the void spaces is more likely to have happened.

In the forecited work of Zanelato et al. (2012), *M. thermophila* I-1D3b was cultivated in a mixture of SCB/WB in plastic bags for MC equal to 75, 80 and 85%, and the best results were observed at 80%. However, when this condition was applied to a vertical packed bed bioreactor, a leak of water was noticed from the bottom of the column and the authors proceeded with other experiments in the bioreactor at 75%.

The classical technique for the determination of the water retention capacity of porous media was not used in this work since it is not compatible with the needs of SSC. In the classical method (see e.g. Shaw, 1927; Kauffman et al., 1986; Naeth et al., 1991), a porous medium is flooded with water for a period, followed by drainage of water in excess. Hence, after drainage, some pores could be blocked by water and for SSC, the presence of air in the voids is fundamental for the microbial activity. Besides, water will be sprinkled over the bed when it is needed, i.e., when the temperature increases above an acceptable limit or when the water evaporated from the porous matrix must be replenished. Therefore, the method applied here was developed to fulfill the specific needs of a SSC system.

From the above, experiments to determine the optimum moisture content for a specific SSC process must be done carefully, since the water added to the porous medium might be blocking the void spaces.

Water retention in the rotating drum

The moisture content of the bed surface after Rhodamine solution sprinkling is presented in Table 4, where the mean value of six samples, and respective standard deviation (SD), is shown for each bed filling degree, solution flow rate, number of sprinklers and volume of water sprinkled at each individual interval. The initial MC of the SCB/WB mixture was 48.3% and the volume of solution added should be enough for the mixture to reach 75%. The analysis of variance for this experiment is presented in Table 5. It must be pointed out that the number of rotations required for the mixture to reach a homogeneous MC was observed by image analysis using the software *LensEye*. The gravimetric analysis of MC was made only after the image analysis indicated that the bed achieved homogeneity. The experiments in the rotating drum lasted from 2.4 to 3.2 hours, depending on the adopted operational conditions (solution flowrate, volume of solution, and filling degree).

Only three conditions resulted in a MC under 70% and all of them were observed for low flow rate, low volume of solution and three sprinklers, as can be seen in Table 4. Since the volume of solution added was enough for the mixture to reach 75%, these MC under 75% indicate that part of the solution was evaporated, due to the long duration of the experiments and the favorable environmental conditions (temperature higher than 30^{o}C and relative humidity under 50%). Only three values of MC were higher than 76% and all of them took place at the higher flow rate, representing that the mixing process should last a little longer. The standard deviations reported in Table 4 were of the same order of magnitude, the variation

Table 4. Mean values and standard deviations of moisture content of samples of the mixture SCB/WB after sprinkling the bed with a solution of Rhodamine B in a rotating drum.

Filling degree (f) Load	Flow of solution (L min^{-1})	Sprinkled volume (mL)	Number of sprinklers	Mean MC (% w.b.)	VC (%)
f = 0.4 3.0 kg	1.5	100	3	58.48 ± 2.99	5.1
			5	73.63 ± 3.81	5.2
		200	3	72.73 ± 3.84	5.3
			5	75.04 ± 1.88	2.5
	2.5	100	3	73.71 ± 4.19	5.7
			5	70.91 ± 1.78	2.5
		200	3	70.00 ± 1.00	1.4
			5	76.66 ± 1.75	2.3
f = 0.5 4.5 kg	1.5	150	3	66.45 ± 3.84	5.8
			5	71.85 ± 6.46	9.0
		300	3	75.78 ± 4.72	6.2
			5	70.29 ± 5.29	7.5
	2.5	150	3	78.47 ± 4.48	5.7
			5	76.24 ± 1.72	2.3
		300	3	75.89 ± 2.43	3.2
			5	71.30 ± 3.54	5.0
f = 0.6 6.0 kg	1.5	200	3	68.29 ± 4.22	6.2
			5	71.23 ± 2.46	3.5
		400	3	73.95 ± 5.32	7.2
			5	72.34 ± 4.80	6.6
	2.5	200	3	70.35 ± 4.38	6.2
			5	73.28 ± 2.33	3.2
		400	3	74.88 ± 3.00	4.0
			5	71.66 ± 3.85	5.4

(*) VC - variation coefficient (VC = SD/Mean * 100).

Table 5. ANOVA for the final moisture contents of the SCB/WB mixture after Rhodamine solution sprinkling in the rotating drum (*).

Source	DF	Adj. SS	Adj. MS	F	p-value
Filling degree	2	9.366	9.366	3.36	0.057
Solution flow rate	1	10.124	10.124	7.27	0.015
Solution volume	1	0.029	0.029	0.02	0.886
Number of sprinklers	1	0.776	0.776	0.56	0.465
Error	18	25.071	1.393		
Total	23	45.366			
R^2 = 44.74%		R^2 (adjusted) = 29.39%			

(*) Note: DF - degree of freedom; Adj. SS - adjusted sum of squares; Adj. MS - adjusted mean square; F - calculated Fisher's statistics; R^2 - regression coefficient.

coefficients (VC) were under 10% and the Levene's test for equal variance showed that they were equal at the 95% confidence interval. These statistical results demonstrate that the moisture content is quite homogeneous, independent of the experimental condition.

The ANOVA indicated that the model is not predictive, since the sum of the squares of the stochastic part is greater than that of the deterministic part. Therefore, it is meaningless to observe the p-values presented in Table 5, which could indicate the solution flow rate as significant, since the non-explained part of the model (stochastic) surpasses the explained part (Adjusted determination coefficient = 0.29). Under this consideration, any experimental condition could be used to moisten the bed in this system; however, it is advisable to avoid the combination of low flow rate and low volume of water in order to prevent low MC in the SSC process. Therefore, for the heat transfer experiments the highest flow rate (2.5 L min^{-1}) and the highest volume of sprinkling for each specific filling degree were used. The configuration of three sprinklers was chosen due to its simplicity.

Heat transfer results

The heat transfer experiments were carried out using the filling degree 0.4 and initial temperature of the particles at 25°C: flowing air at 45°C and keeping the drum still; flowing air and sprinkling water and keeping the drum still; similar to the previous one, but rotating the drum at 1rpm. Therefore, the experiments here reported the bed temperature increase, while in a real SSC system one must decrease the bed temperature due to heat generated by microbial activity. In fact, due to the limitations of the equipment, experiments of temperature decrease could not be performed. Nevertheless, the general trend of the system towards thermal equilibrium probably does not change whether the temperature increases or decreases.

Figure 4 presents the first set of experiments, where the data points referred as *inner* correspond to the temperatures measured between the inner tube and the drum wall and the data points identified as *surface* were collected close to the bed free surface. The longitudinal positions where the temperatures were measured are identified from 1 to 5, corresponding to the distances from 10 to 50 cm measured from the drum right lid represented in Figure 3.

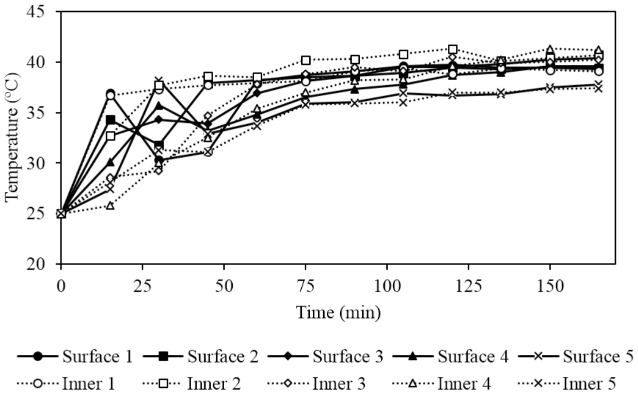

Figure 4. Temporal temperature profiles in a bed of a SCB/WB mixture, keeping the drum still and without water sprinkling (T_{in} = 45 °C; RH = 91,53%; Q = 5 L min^{-1}; $T_{b,0}$ = 25 °C; $U_{b,0}$ = 75%; T_w = 45 °C).

In Figure 4 one must note that while the temperature in the positions *inner* increased steadily along time, the temperature in the positions *surface* showed an erratic trend. Tada et al. (2017b) carried out experiments in a still drum partially filled with glass beads, with air flowing in parallel to the free surface, and noted that the wall-to-bed convective mechanism dominates the heat transfer process, justifying the steady trend observed for the *inner* temperatures here presented. The analysis of the irregular trend at the *surface* position requires the understanding that the low flow rate used in the experiment, as normally applied in SSC, is distributed along the bioreactor length; consequently, the superficial air velocities are very low and so is the convective heat transfer. Besides, the air relative humidity is below saturation (91.53%) and evaporative heat is removed, while at the bed free surface heat transfer takes place at almost natural convection. Such a combination of mechanisms is complex and the consequences for the temperature variation are difficult to forecast. To represent such complex phenomena, two-phase models, in which independent heat balances are proposed for the solid and for the gas phase, are more adequate. This class of model should include many heat transfer mechanisms when applied to this system, such as: fluid-to-particle forced convection caused by the air introduced through the inner tube, moving in the radial and angular directions; fluid-to-particle natural convection, due to movement of the air from hot (near to the wall) to cold (surface) regions; convection from the drum wall to the air present in the voids of the bed; solid-solid contact between drum wall and the bed solid phase; heat removal by evaporation, due to the unsaturation of the air; water condensation from the air, which flows from hot to cold regions; natural convection between particles in the bed free surface and the air in the headspace.

Regarding the longitudinal temperature profile, one may note that the temperatures measured at the positions *inner 1* and *inner 2* were always higher than the others; nevertheless, at the positions *surface* no specific trend was realized. Such trend at the *inner* temperatures indicates that some end effects took place through the drum lid, even though Tada et al. (2017b) showed that the axial heat dispersion is negligible in a drum similar to the one used here. As previously stated, in Tada et al. (2017) air was introduced parallel to the bed surface, while in the present contribution air was inserted into the drum throughout a perforated tube. It seems that fin effects took place by means of the inner tube, influencing the bed temperatures at the positions *inner* 1 and 2.

According to Figure 4, approximately 75 minutes were required to reduce the heating rate and the steady state was not reached up to 165 minutes of experiment. At the end of the test differences of temperature up to 5°C were observed between the positions *inner* and *surface*. Therefore, one may conclude that the introduction of the air at low flow rates is not efficient to reduce the thermal heterogeneity in the bed.

Figure 5 presents the experiments carried out sprinkling water on the bed surface and keeping the drum still, where one may notice that the temperatures measured at the positions *inner* and *surface* increased steadily along the experiment. For the positions *inner*, the trend of the temperatures did not differ significantly from the ones presented in Figure 4, showing that sprinkling water promoted some effect only in the top layers of the bed. Nevertheless, the heat transfer mechanisms associated with the drum wall were still more effective than the ones associated with the air flow and the water sprinkling, since the average final temperature of the positions *inner* are 3°C higher than the ones of the positions *surface.* By the end of the 135 min of experiment, the bed did not reach the desired temperature of 45°C, indicating that some heat loss to the environment took place.

The effect of the rotation of the drum on the temperature distribution is shown in Figure 6, where one may realize that the rotation was positive for the thermal homogenization from the initial moments of experiment. The time required to reduce the heating rate was also reduced when rotation was introduced, since this time was nearly 75 min without rotation (Figure 5) and 45 min with rotation (Figure 6). However, the thermal equilibrium at 45°C was not observed, confirming that a significant heat loss to the external environment occurred.

The temperature increase in SSC is slow and is related to the metabolic heat generated by the microorganism. In experiments in packed beds, Zanelato et al. (2012) cultivated *M. thermophila* I-1D3b in a SCB/WB mixture and observed a maximum temperature of 51°C (ideal temperature 45°C) after 18 h of cultivation; Castro et al. (2015) cultivated *Aspergillus awamori* IOC-3914 in babassu cake and observed an increase of 20 ºC upon the ideal temperature of 20 ºC after 24 hours of the process. Even if the rate of temperature increase is slow, the options to avoid an excess of temperature are limited in packed beds, since the

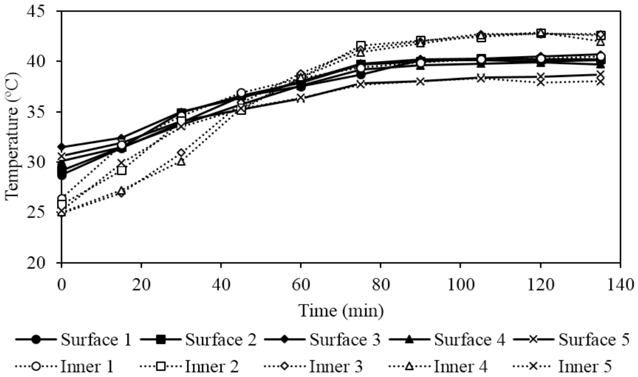

Figure 5. Temporal temperature profiles in a bed of a SCB/WB mixture, keeping the drum still, with water sprinkling at 65°C and 2.5 L min-1 (T_{in} = 45 ºC; RH = 91,53%; Q = 5 L min^{-1}; $T_{b,0}$ = 25 ºC; $U_{b,0}$ = 50%; T_w = 45 ºC).

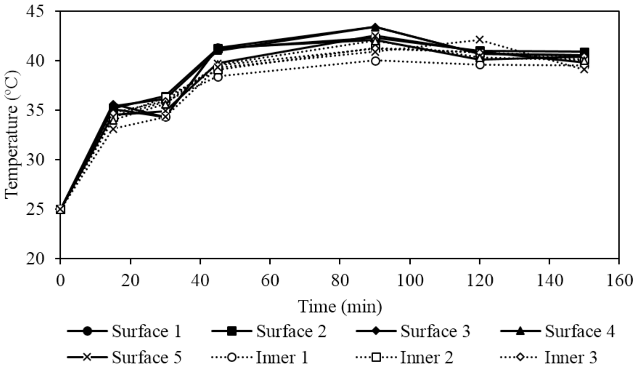

Figure 6. Temporal temperature profiles in a bed of a SCB/WB mixture, with water sprinkling at 65°C and 2.5 L min-1 and drum rotation at 1 rpm (T_{in} = 45 ºC; RH = 91,53%; Q = 5 L min^{-1}; $T_{b,0}$ = 25 ºC; $U_{b,0}$ = 50%; T_w = 45 ºC).

effective thermal properties of agricultural wastes are very low (Casciatori et al., 2013; Casciatori and Thoméo, 2018). The only operational alternatives in packed beds are the increase of the air flow rate and the reduction of the bed wall temperature, alternatives that would cause serious concerns to the cultivation process.

Rotating drum bioreactors usually are kept still during long periods, when air is inserted only to fulfill the respiratory needs of the microorganism, when the drums behave similarly to packed beds. During the first stages of microbial growth, when conidia germinate and the hyphae are polarized, rotation must be avoided. When the fungal biomass achieves its maximal concentration, the heat generated is also maximum. However, at this condition, the solid is already colonized and the drum might be rotated, provided that the microorganism tolerates some shear stress. From the heat transfer results obtained here, it was noted that the temperature changes caused by the flowing air and the sprinkling water are not enough to provide thermal homogenization, which was observed only when the drum was rotated.

Further steps

From the results presented here, the next step would be the cultivation of the porous medium with the fungus *Myceliophtora thermophila* I-1D3b, or any other microorganism of interest, in order to identify the modifications of the porous medium structure due to both the substrate consumption and the mycelial growth. The first aspect does not seem to be very significant, since this fungus attacks more intently the wheat bran than the sugar cane bagasse, due to the high recalcitrance of the bagasse (see Gomes, 2015). Therefore, considering that WB answers for only 30% of the mixture, little modification of the results presented here can be expected. On the other hand, the mycelial growth can promote deep modifications in the structure of the pores, since this microbe has long aerial hyphaes, resulting in a dense mycelium that would reduce the pore size or even block it, modifying the free path for the flows of water and air.

As for the heat transfer experiments, they have been carried out in order to observe how long the system requires to reach the thermal equilibrium from a non-equilibrium condition, considering the alternatives of temperature control usually found in the SSC literature for rotating drums. For cultivation experiments, the heat generation is not constant and depends on the microbial biomass concentration, which is related to several microbiological aspects, such as: the resistance of fungus to the shear stress, which determines the rotation regime of the drum; the kinetic parameters for the fungal growth, which determines the amount and rate of heat released; the density and distribution of the fungal mycelium, which is responsible for the restriction to the flow of air and water in the void spaces.

CONCLUSIONS

Studies on water sprinkling over a bed of a mixture of sugar cane bagasse and wheat bran have been carried out aiming at its application in rotating drums bioreactors for solid-state cultivation. Operational conditions for water sprinkling in a rotating drum partially filled with sugar cane bagasse and wheat bran were presented, taking into account the filling degree, water sprinkling flow rate, water volume at each individual intermittence and number of sprinklers, as well as the maximum holding capacity of the porous media. From these results, heat transfer experiments were carried out in order to identify the alternative that promoted thermal homogenization. The heat transfer experiments showed that the heat transfer mechanisms provided by the flowing air, the drum wall and the sprinkled water are limited to promote thermal homogenization and that drum rotation is needed to achieve it. The reported results will be valuable for the operation of bioreactors for SSC in partially filled drums that will be kept still during long periods of cultivation, but could be rotated to avoid reaching high cultivation temperatures.

NOMENCLATURE

DG	Degree of freedom
f	Filling degree
F	Calculated Fisher's statistics
MC	Moisture content, % w.b.
Q	Air flow rate, L min^{-1}
RD	Rotating drum
RH	Relative humidity, %
R^2	Regression coefficient
SCB	Sugar cane bagasse
SD	Standard deviation, % w.b.
SSC	Solid state cultivation
$T_{b,0}$	Initial temperature of the bed, % w.b.
T_{in}	Temperature of the air inlet, ºC
T_w	Temperature of the drum wall, ºC
$U_{b,0}$	Initial moisture content of the bed, % w.b.
VC	Variation coefficient, %
WB	Wheat bran
WHC	Water holding capacity, % w.b.
α_w	Water activity

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