Insights into physical characterization of solid state fermentation: from preliminary knowledge to practical application

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Solid state fermentation (SSF) involves the growth of microorganisms especially filamentous fungi on moist solid substrates in the absence of free-flowing water. SSF offers a more favorable environment for fungal growth, yielding higher productivity in a relatively low-cost process by using nutrient-rich agro-industrial residues as substrates. With the increasing interest in SSF nowadays, researchers are keen to discover as many new ways to explore the usage of this technology as possible to develop new added-value materials from by-products. In addition to nutritional composition of solid substrate used for SSF medium, physical properties of solid substrate also represent another important idea to be focused on during SSF that will influence the behavior and the productivity of microorganisms involved for substrate utilization and product formation. The main objective of this reported article was to study solid substrate particles and their physical characteristics in relation to SSF, analyzing how of these characteristics vary with different solid substrate particles and moisture contents. The importance of physical properties, such as bulk density, particle density, specific volume, porosity, particle size, surface area, volumetric specific surface area, water retention value (WRV) and tortuosity, was identified in this study. This primary information can be advantage in the microbial SSF process to be carried out.

Keywords: Solid state fermentation; wheat bran; soybean hulls; rapeseed meal.

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Introduction

Solid state fermentation (SSF) refers to the microbial fermentation, which take place in the absence or near absence of free water, thus being close to the natural environment to which the selected microorganisms, especially fungi, are naturally adapted. Future biorefineries based on modern SSF aim to exploit the vast complexity of the technology to modify biomass produced by agriculture and food industry for valuable by-products through microbial bioconversion [1, 2].

Nowadays, SSF is an economically viable, practically acceptable technology for large-scale bioconversion and biodegradation processes. Development of sustainable SSF and bioprocess technology is an emerging, multidisciplinary field with possible application to the production of enzymes, chemicals, bioethanol, and pharmaceuticals. SSF offers many advantages over conventional liquid fermentation such as simple and inexpensive substrates, elimination of the need for solubilization of nutrient from within solid substrates, elimination of the need for rigorous control of many parameters during

fermentation, product yields are mostly higher, lower energy requirements, produce less waste water, no foam generation, and relatively easy recovery of end products. SSF provides flexibility in terms of the raw materials to be used and their capability to produce various value-added products. Thus, SSF hold the highest potential for biorefinery.

The solid substrate is a major element in SSF. The particle size properties of solid substrates will lead to the shape, accessible area, surface area, and porosity of the solid substrates [3]. Processes like chopping, grinding, and cutting create a condition for microorganisms to be active at the initial stages of growth and increase the degradation and hydrolysis rate since the solid substrate is insoluble [4]. The most important physical factor is the particle size that affects the surface area to volume ratio of the solid substrate [5]. Smaller particle size would provide a larger surface area per volume and allow full contact of microorganisms with the nutrients, but the diffusion of oxygen would be affected [6]. Larger particle size provides small area per volume ratio and gives excellent diffusion of oxygen but contact with nutrients is affected [7]. A suitable particle size should satisfy both mycelial growth and the demand for oxygen and nutrients [8]. Particle size also affects the size of inter-particle voids and porosity [9]. Any change in porosity of the solid substrate bed changes the apparent density of solid substrate and diffusion of gases into the bed. A large pore size is suitable for an adequate oxygen supply [10]. If porosity is limited, the effective diffusivity of gases is less. Particle size and properties may change during fermentation. These do not only affect the growth of microorganisms, but also affect the monitoring of heat conductivity, substrate consumption, products concentration and water content [11]. In addition to providing nutrients such as carbon and nitrogen, the solid substrate also performs the role of the physical structure that supports the growth of microorganisms [12]. Another important factor in the selection of substrate is the water holding capacity that maintains moisture content of the fermented substrate [6].

The objective of this study is to analyze the viability of wheat bran, soybean hulls, and rapeseed meal for solid state fermentation (SSF) processes by exploring their physical properties. Wheat bran, soybean hulls, and rapeseed meal, the low-cost residues of the milling industry, are interesting solid substrates for SSF. These materials may be the model of cheap and abundant agricultural waste and they have potential in making the entire SSF process feasible. Such properties include bulk density, particle density, porosity, water retention value (WRV) and tortuosity, and the percentage of pores filled with water. Indeed, in SSF, the availability of surface area plays a critical role for microbial attachment and mass transfer [13] and is dependent upon the particle size of the support. Particle size affects the rigidity and porosity of the solid substrate, which further influences mass transference and heat transference, as well as microbial growth and metabolite production [7, 8, 14].

Materials and Methods

Solid substrate

Wheat bran was obtained from Cargill Wheat Processing Plant (Manchester, UK). Soybean hulls and rapeseed meal were obtained from Brocklebank Oilseed Processing Division, Cargill Wheat Processing Plant (Liverpool, UK). All the substrates were kept in airtight container and stored in cold room for future use.

The composition of wheat bran [15], soybean hulls [16], and rapeseed meal [17] used in this study are reported in Table 1. Rapeseed meal is composed of a large proportion of protein (38.9%) followed by wheat bran (15.4%) and soybean hulls (14.2%). However, wheat bran is particularly rich in starch (23.4%). Soybean hulls are considered to have a much less nutritional and can be classified as lignocellulosic material. Carbohydrates are a major component of

soybean hulls (50.7%) represents a structural component constituent in cell walls, including cellulose (36.4%) and hemicellulose (12.5%).

Table 1. Composition of solid substrate.

Component	Wheat bran [15] (%)	Soybean hulls [16] (%)	Rapeseed meal [17] (%)
Moisture	10.3	10.1	10.6
Ash	5.9	4.2	7.5
Crude fibre	8.7	32.3	14.97
Cellulose	10.6	36.4	-
Hemicellulose	29.7	12.5	-
Protein	15.1	14.2	38.9
Total nitrogen	2.4	2.3	6.2
Lignin	2.42	0.75	8.9
Starch	23.3	1.8	4.9
Oil	-	-	2.5
Phosphorus	1.0	0.20	0.8

Evaluating particle size

The method proposed by Baker and Herrman [18] was used to evaluate particle size of wheat bran, soybean hulls, and rapeseed meal. Samples were sieved using a SATAKE PLSB-Series 2000 (Simon Laboratory Sifter, UK). A series of known test sieves values of 45, 53, 120, 180, 212, 500, 850, 1180, 1400, 1700, and 2000 µm aperture size were used in order to evaluate particle size distribution in the original solid substrate. Each sieve separates solid substrate particles according to size. In this method, the first step in particle size analysis is to obtain a representative sample. A 150.0 g sample was measured by using a full stack of sieves to avoid accumulation of more than 20.0 g over any one sieve. After the 150.0 g sample had been weighed, the following stages in the separating process were followed: The sieve stack was arranged with the following order: the greatest size at the top and the finest at the bottom. The sample was placed onto the top sieve and the sieve stack was placed onto the shaker. The shaker was allowed to run for about 5 min on laboratory sifter to ensure complete separation. The sides of each sieve were gently tapped with a brush before removing the sieve from the stack. The sieve stack was removed from the shaker. Each sieve was placed with the retained solid particles on a balance to weigh the sieve and retained solid particles together. Solid particles were removed, and the sieves were thoroughly cleaned. The empty sieves were weighed, and the weights recorded. The difference between the weight of the sieve with and without material was calculated to determine the weight of material. The weight values were entered in the appropriate columns of a spreadsheet. The average particle size of material retained on each sieve is calculated as the geometric mean of the diameter openings in the two adjacent sieves in the stack. Equation 1 [18] shows this calculation.

$$d_i = (d_u \times d_0)^{0.5} \quad (1)$$

As it is not practical to count each particle individually and calculate an average, the average particle size can be calculated on the basis of weight. This can be done with Equation 2 [18]:

$$d_{gw} = log^{-1}\left(\frac{\sum(W_i \times \log d_i)}{\sum W_i}\right) \quad (2)$$

The standard deviation can be calculated as follows [18]:

$$S_{gw} = log^{-1}\left(\frac{\sum W_i(log\, d_i - log\, d_{gw})^2}{\sum W_i}\right)^{0.5} \quad (3)$$

Where:

d_{gw} = the average particle size

S_{gw} = standard deviation (dimensionless)

d_i = diameter of ith sieve in the stack

d_u = diameter opening through which particles will pass (sieve preceding the ith)

d_0 = diameter opening through which particles will not pass (ith sieve)

W_i = weight of retained solid particles in every sieve (ith sieve) (g)

The number of particles per gram and amount of surface area can be calculated from d_{gw} and S_{gw} obtained from Equations 2 and 3, respectively.

From this value, the particles per gram and surface area can be calculated as follows [18]:

$$Particles/g = \left(\frac{1}{\rho_p \beta_v}\right) \exp\left(4.5\ ln^2 S_{gw} - 3 \ln d_{gw}\right) \quad (4)$$

$$SA = \left(\frac{\beta_s}{\rho_p \beta_v}\right) \exp\left(0.5\ ln^2 S_{gw} - \ln d_{gw}\right) \quad (5)$$

Where:
SA = surface area (cm^2/g)
β_s = shape factor for calculating surface area of particles = 6
β_v = shape factor for calculating volume of particles = 1
ρ_p = particle density of solid substrate (g/cm^3)

For these calculations, the shape factors β_s and β_v are assumed to be 6 and 1, respectively. The particle density (ρ_p) can be obtained within the next section. Since the specific weight is expressed in g/cm^3, it is necessary to convert the average particle size (d_{gw}) to cm. This could be done by multiplying the value by 0.0001.

Volumetric specific surface area (VSA) (cm^{-1}) can then be obtained from Equation 6 [18] by multiplying it by particle density (ρ_p) of solid substrate (g/cm^3), that is:

$$VSA = SA \times \rho_p \quad (6)$$

Properties of solid substrate

Density is measured as mass per unit volume (mass divided by volume). Solid substrate density depends on the chemical composition and structure of the minerals in the solid substrate. Density of any materials (solid substrate) can be divided into two categories: (1) bulk density and (2) particle density. Bulk density refers to the volume of the solid portion of the solid substrate particles along with the spaces where the air and water exist. Bulk density differs from particle density as particle density is only concerned with solid substrate particles and the pore spaces occupied within the solid substrate. Bulk density is used along with particle density to calculate porosity. Porosity (expressed in percentage) refers to pore space occupied by air and water within a solid substrate. This knowledge about the properties of a solid substrate makes it possible to have a better understanding of how the solid substrate functions within specific conditions. It also allows for a more accurate interpretation of solid substrate measurements to be carried out. Percentage of pores filled with water and bed tortuosity can be defined as a function of moisture content.

Procedures

(1) Bulk density

Solid substrates (wheat bran, rapeseed meal, and soybean hulls) with different moisture contents were poured into a measuring cylinder of known volume (30.0 mL) and weighed to determine the bulk density [19]. Bulk density (P_b) of solid particles was calculated using Equation 7.

$$\rho_b = \frac{mass\ of\ dry\ solid\ substrate\ (g)}{total\ volume\ of\ solid\ substrate\ and\ air\ (mL)} \quad (7)$$

(2) Particle density

Particle density (ρ_p) at various moisture contents was determined using a standard soil particle density protocol [20]. The weight of an empty 100.0 mL volumetric flask without a cap was measured. Approximately 12.0 g of solid substrate was weighed and mixed homogenously with a suitable amount of water to obtain an initial moisture content of 0, 11, 35, 50, 60, 65, 70, 75, and 80%. The solid substrate was placed in the volumetric flask using a funnel. The weight of the volumetric flask containing the solid substrate was measured at different moisture contents. About 50 mL distilled water was added to the solid substrate in the volumetric flask. The solid substrate/water mixture was brought to a gentle boil by placing the volumetric flask on a hot plate. The flask was gently swirled for 10 seconds once every minute to keep the solid substrate/water mixture from foaming over. The boiling process was continued for 10 min to remove air bubbles. The volumetric flask was removed from the heating plate and the mixture was allowed to cool. Once the volumetric flask has cooled, the flask was capped and let to sit for

24 h. After 24 h, the cap was removed and the flask filled with distilled water, so that the bottom of the meniscus is at the 100 mL line. The 100 mL solid substrate/water mixture was weighed in the volumetric flask. The weight values were recorded in the appropriate columns of the spreadsheet for further data analysis.

$$\rho_p = \frac{mass\ of\ dry\ solid\ substrate\ (g)}{Volume\ of\ solid\ substrate\ particles\ only\ (mL)} \quad (8)$$

(3) Porosity

Porosity can also be expressed as percentage of pores filled with air. The amount of pore space, or porosity, is expected to decrease by increasing the moisture content of the solid substrate. Porosity (ε) can be calculated with the following equation:

$$\varepsilon = 1 - \left(\frac{\rho_b}{\rho_p}\right) \times 100 \quad (9)$$

(4) Tortuosity

Bed tortuosity (τ), which accounts for elongation of the diffusion path due to the presence of solid substrate particles, is expected to increase with bulk density [21]. Bed tortuosity (dimensionless) can be calculated using the following equation:

$$\tau = \frac{1}{(0.2+\varepsilon)^2} \quad (10)$$

(5) Percentage of pores filled with water

By knowing the bulk density, porosity, water density and the ratio of water mass to solid sample mass, the percentage of pores filled with water (ε_w) can be measured as followed:

$$\varepsilon_w = \left(\frac{g\ water/g\ substrate}{\varepsilon}\right) \times \left(\frac{\rho_b}{density\ of\ water}\right) \times 100 \quad (11)$$

(6) Water retention value

Water retention value (*WRV*) is an empirical measurement of the capacity of a test solid substrate to hold water. *WRV* was calculated as the ratio of weight of water retained by wet solid particles after centrifugation under specified conditions to the weight of the same solid sample after oven drying. The procedures were carried out by using a specially modified centrifuge-holding tube, as illustrated in Figure 1. The method used to determine *WRV* has been taken from Scandinavian pulp, paper, and board [22] with slight modifications appropriate for the apparatus available. In addition to measuring *WRV* of solid substrates, the same procedure was also performed to measure *WRV* of fungus cell material. Details operating conditions are described details as reported elsewhere [23].

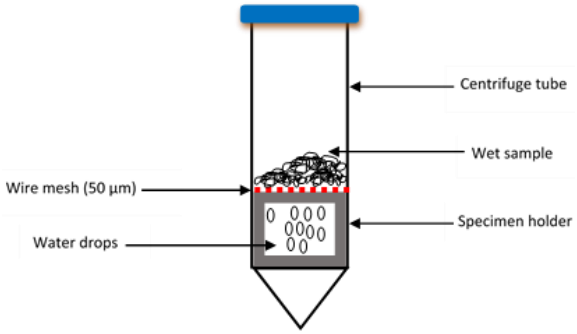
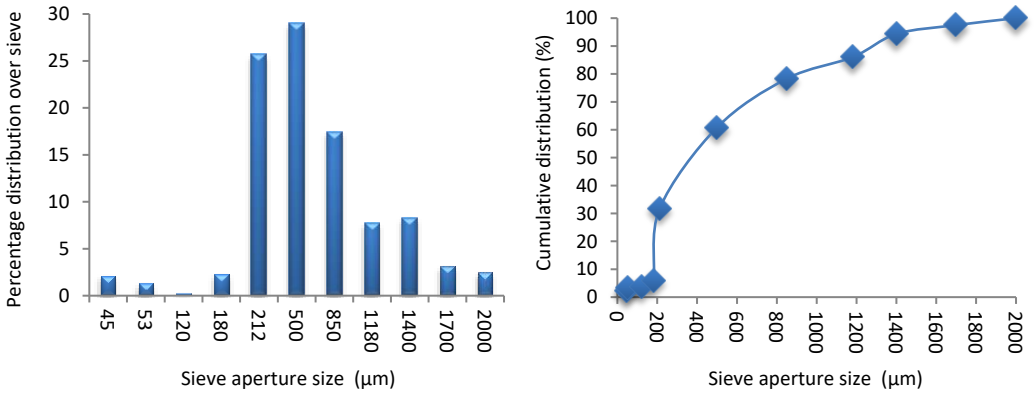


Figure 1. Modified centrifuged-holding tube to hold the rim of the centrifuge rotor [23].

Results and discussion

Particle size is one of the most important physical parameters in SSF. Particle size distribution will affect the surface area to volume ratio of the particles that are initially accessible to the microorganism and the packing density within the surface mass [24]. The space between particles is occupied by a continuous gas phase [25, 26] and the size of the substrate particles determines the pore space that is occupied by air. This space will aid gaseous exchange as well as heat and mass transfer between particles. As the rate of O_2 transfer into the pore space affects growth, the substrate should contain particles of suitable size to enhance mass transfer [27, 28]. In addition, the chemical composition of solid substrate will determine its ability to retain sufficient water supplies to support growth. The results presented here provide a basic idea of the limitations and difficulties that are faced in the development of SSF. Furthermore, understanding some of these physical properties will help in

[**2a**]



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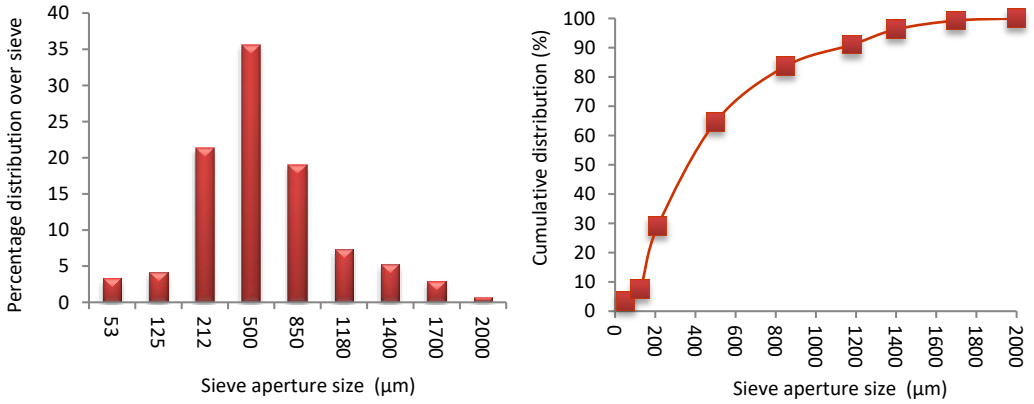

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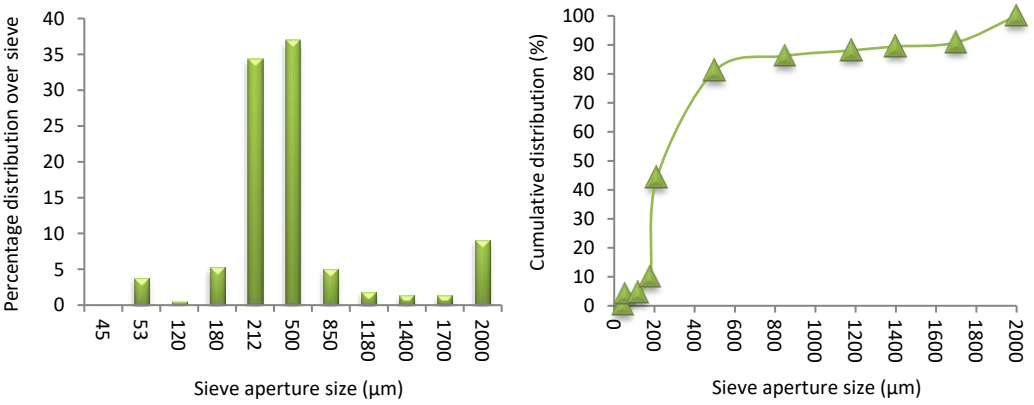

Figure 2. Left: Bar chart of percentage solid particle distribution over sieve. Right: Cumulative size distribution of solid particles. [**2a**]: wheat bran; [**2b**]: soybean hulls; [**2c**]: rapeseed meal.

developing the design of research strategies and experiments and in defining experimental parameters such as setting a suitable initial moisture content prior to the start of the SSF process.

Evaluating particle size

Figure 2 shows percentage distribution of solid particles over the sieve and cumulative data from particle size distribution. In summary, particle size analysis of wheat bran revealed cumulative undersize of 29.05%, 25.75% and 17.49% at

diameter of 500, 212, and 850 µm, respectively (Figure 2a). For soybean hulls, about 35.65% of particles are 500 µm in size followed by 21.43% and 19.06% the particles 212 and 850 µm in size, respectively (Figure 2b). For rapeseed meal, about 36.95% of particles are 500 µm in size followed by 34.35% and 9.16% of particles 212 and 200 µm in size (Figure 2c).

Particle analysis provided volumetric specific surface area measurements, for wheat bran (301.99/cm) > rapeseed meal (214.0/cm) > soybean hulls (173.40/cm). The higher volumetric specific surface area in wheat bran can be correlated with its starch content (23.3%) [15]. However, by considering the particles per gram value of wheat bran compared to rapeseed meal and soybean hulls, which contained only husk, wheat bran presents as a promising substrate in SSF. Soybean hulls might be useful as an inert carrier. Mixed with other substrates, soybean hulls can be used to create inter-particle spaces, thus increasing the surface area for better air circulation and nutrient diffusion. However, this is not the only factor, which will determine its suitability because factors such as the type of microorganism and moisture content will also have an important impact on SSF. According to Ishizawa *et al*., volumetric specific surface area is attributed to the creation of surface openings or internal pore spaces and by the removal of cell wall components, which enhances the direct physical contact between the enzymes and the substrate [29].

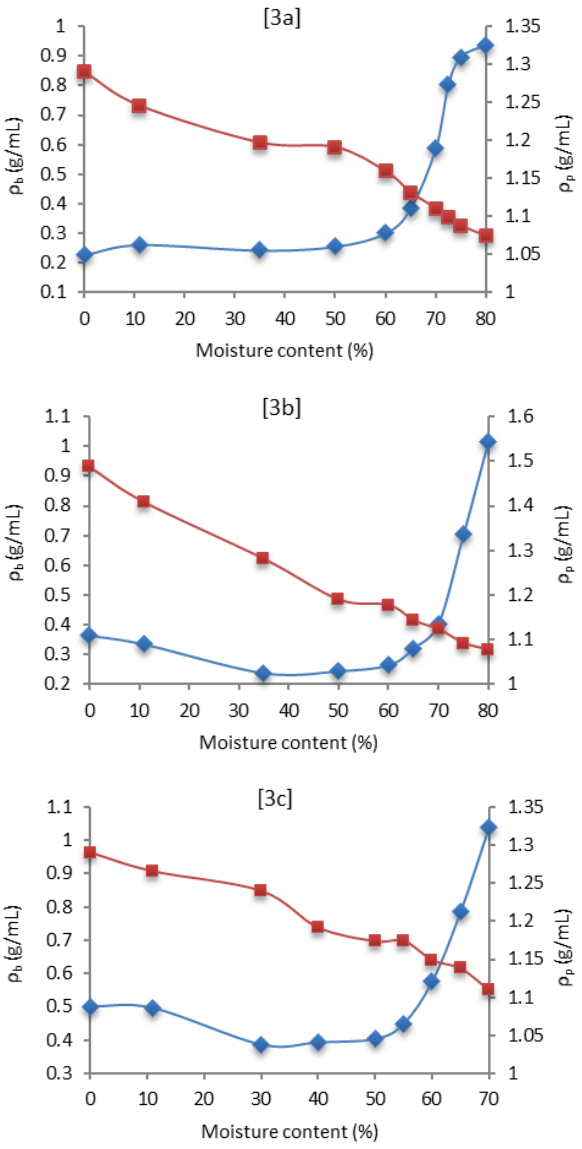

Figure 3. Bulk density (◆) and particle density (■) at different moisture contents. [**3a**]: wheat bran; [**3b**]: soybean hulls; [**3c**]: rapeseed meal.

Properties of solid substrate

In this study, bulk density, particle density, porosity, tortuosity, and percentage of pores filled with water for wheat bran, soybean hulls, and rapeseed meal were experimentally measured at different moisture contents. According to Figure 3a, bulk density decreased from 0.26 to 0.24 g/mL when moisture content increased from 11 to 35% in wheat bran. Then, it increased from 0.24 to 0.94 g/mL when moisture content further increased from 35 to 80%. The same trend was observed in soybean hulls where the bulk density decreased from 0.33 to 0.23 g/mL when moisture content increased from 11 to 35%, then increased from 0.23 to 1.01 g/mL when moisture content changed from 35 to 80% (Figure 3b). Figure 3c also shows that the bulk density of rapeseed meal decreased from 0.5 to 0.39 g/mL when moisture content changed from 11 to 30%. The bulk density then started to increase from 0.39 to 1.04 g/mL when moisture content increased from 30 to 70%. Different trends in bulk density due to increase in moisture content can contribute to different responses of

solid materials to moisture content. Particle density decreased from 1.29 to 1.07, 1.49 to 1.08, and 1.29 to 1.11 g/mL for wheat bran, soybean hulls, and rapeseed meal, respectively, showing a linear relationship, when moisture content changed from 0 to 80 % (Figure 3). If the increase in moisture content results in greater decrease in solids volume than mass, particle density will have a negative relationship with the moisture content.

By gaining information on both the bulk density and particle density of the solid substrate, it is possible to measure the pore space (porosity) that is occupied by air (oxygen) (Equation 9). Furthermore, by knowing the solid bulk density, particle density and the density of water, the ratio of the volume of water to the volume of solid substrate may be calculated along with the percentage of the pore space filled with water (Equation 11). Figure 4 shows the correlation between porosity (pores filled with air/O_2) and the percentage of pores filled with water. In all three solid substrates tested, porosity decreased from 82.45 to 6.26%, showing a non-linear relationship when moisture content increased from 0 to 80%. By contrast, the opposite trend was observed for the percentage of pores filled with water, which increased exponentially when moisture content increased from 0 to 80%.

By using the information in Figure 4, a preliminary decision about the most suitable moisture content for initial SSF experiments can be made. For example, with wheat bran and soybean hulls, suitable moisture content might be set at 65% (Figure 4a and 4b) while this would be 55% for rapeseed meal (Figure 4c). For wheat bran and soybean hulls, the percentage of pores filled with air and the percentage of pores filled with water were 65.95% and 91.64%, and 79.97% and 74.11%, respectively, at moisture content of 65%. For rapeseed meal, the percentage of pores filled with air and the percentage pores filled with water were 61.69% and 72.22%, respectively, at moisture content of 55%. The percentage of pores filled with water for wheat bran was slightly higher due the high starch component, which has a high ability to absorb water.

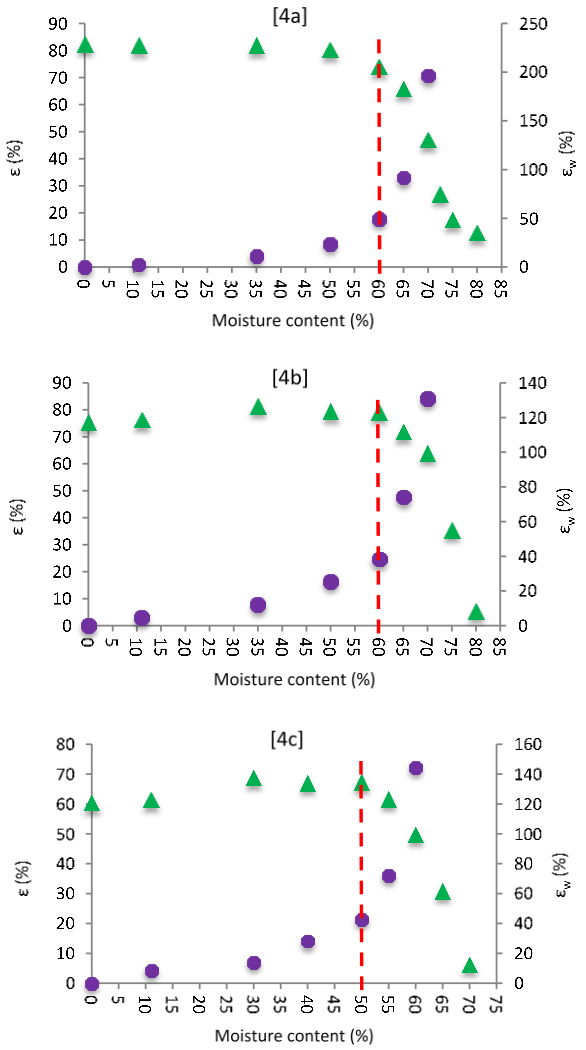

Figure 4. Relationship between the percentage of pores filled air (porosity - ε) (▲) and those filled with water (ε_w) (●) as a function of moisture content. [**4a**]: wheat bran; [**4b**]: soybean hulls; [**4c**]: rapeseed meal.

Bed porosity ensures O_2 availability between the moist solid substrate particles. At high moisture content, water flooded the system at one point, not allowing air in or out and leading the bulk density to increase, particle density to decrease and specific volume to decrease. High moisture content also resulted in porosity decreasing to almost zero. This could provide a reason for the fungal growth being restricted to the surface of

the solid particle as difficulty with penetration results in poor mycelium growth. At low moisture content (below 30%), even with high O_2 content, conditions are not favorable to support a greater fungal growth due to lack of water. According to Valera *et al*., there are two effects of particle size on SSF at any given moisture content [30]. The first effect is the increase in surface area for the growth of the microorganism with decreased particle size. The second is the reduction in pore space and hence gas phase O_2 transfer with decreased particle size. Arasaratnam *et al*. found that paddy husk mixed with substrates, such as rice bran, corn flakes, soya flour, and soy meal powder, during SSF increased glucoamylase production [31]. This has been attributed to the efficient air circulation and nutrient diffusion caused by the inter-particle space created by paddy husk, along with the increase in the surface area for the spores to germinate and mycelia to grow, providing them with easy access to nutrients. This phenomenon was also observed by Rahardjo *et al*. when using various model solid substrates with different porosities for the production of α-amylase in SSF of *A. oryzae* [11, 32]. They reported that model substrates with high porosity exhibited better enzyme production compared to those with less porosity.

The pore spaces between particles are occupied with a continuous gas phase. Gas phase in SSF is strongly affected by the size of particles, the shape of particles, and the tortuosity of a network of gas-filled pores (porosity) [33, 34, 35]. According to Moldrup *et al*., a tortuosity phenomenon of pore spaces influences the transport of water solutes and gases within the solid substrate [35]. Figure 5 shows the relationship between porosity and tortuosity for wheat bran, soybean hulls and rapeseed meal at different moisture contents. At high porosity (low moisture content), the low value of tortuosity indicates that the transport of water solutes and gases is facilitated. In addition, within this study, tortuosity and porosity were considered together because they were simultaneously altered when solid substrate particles were subjected to a particular moisture content (specified in this study).

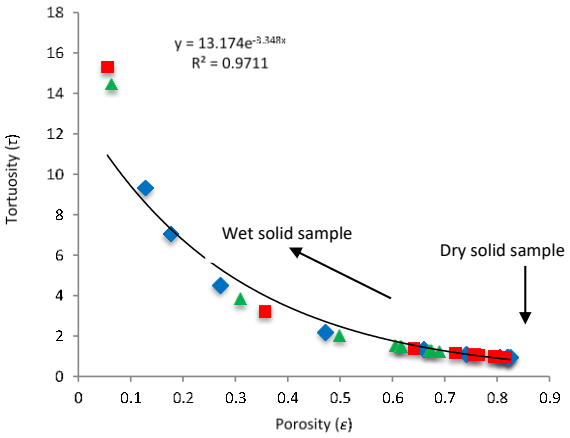

Figure 5. Correlation between porosity (ε) and tortuosity (τ) for three different solid substrates measured at different moisture contents. (◆): wheat bran; (■): soybean hulls and (▲): rapeseed meal.

Furthermore, at low porosity (high moisture content), the value of tortuosity was higher indicating that the transport of water and gases were difficult. This is due to the decrease of inter-particle spaces and substrate agglomeration in most of the cases, which may interfere with microbial respiration and mass transfer. By contrast, high water content provides less respiration efficiency due to decreased inter-particle space. As a result, this condition will create limited surface area for microbial penetration and attachment. From this study, the values of tortuosity (dimensionless) for wheat bran, soybean hulls, and rapeseed meal are 1.35, 1.18, and 1.50, respectively. These values are based on moisture content of 65% for wheat bran and soybean hulls and 55% for rapeseed meal.

Water retention value

WRV is used to determine the amount of water absorbed under specified conditions. It is also often used to quantify fiber swelling behavior and it represents the water absorbency that is a key quality in solid substrate particles [22]. The ability of solid substrate to retain water was addressed details previously [23]. This is highly

relevant to the study as it provides a basis to understand other important solid particle properties for SSF, such as how much water can be stored in the solid substrate, how fast water and heat will be transferred through the solid particle, how easily the mycelium of fungus can penetrate through the solid particle, and the potential of total water needed to be supplied both at the beginning and throughout the entire process of SSF to support the growth of microorganisms [36, 37, 38].

The pore characteristics, such as volume and size distribution, are key parameters for water capacity and absorbency. Values of *WRV* measured for wheat bran was 263.55% followed by soybean (164.56%) and rapeseed meal (132.36%) (Table 2). Details information and results from this study are discussed details as reported elsewhere [23]. The ability of wheat bran to retain water in the solid substrate is 56% higher than that of soybean hulls and rapeseed meal. This may be explained by the high starch content in wheat bran (23.3%) [15] as starch absorbs more water compared to the other two solid substrates. In addition, starch content can be related to the available surface area of the wetted solid particles. Nevertheless, these polysaccharide components are an important factor in terms of the physicochemical properties of solid substrates in the SSF process as they contribute a nutritional value.

Table 2. *WRV* for wheat bran, soybean hulls, rapeseed meals, and for fungal mycelium *A. awamori* and *A. oryzae*.

Solid substrates	*WRV* (%)
Wheat bran	263.55
Soybean hulls	164.56
Rapeseed meal	132.36
A. awamori cells	167.20
A. oryzae cells	289.77

In addition, experiments were carried out in order to determine the ability of the fungus itself to retain water within its own cells. The average *WRV* for *A. awamori* was 167.20%, while *A. oryzae* showed an average of 289.77%. Briefly, these results showed that the ability of *A. oryzae* to retain water in the cells was higher (73%) than that of *A. awamori*. Different fungi have different cell morphologies, which results in differing abilities to retain water [39]. The ability of fungus *A. awamori* and *A. oryzae* cells to retain water was reported previously [23]. This factor will influence the ability of a fungus to fully utilize the water content provided in the system and also its ability to maintain the moisture content at an optimum level during the SSF process.

In summary, it can be concluded that, in addition to nutritional value, there are many important characteristics that a good solid substrate should have. Many factors are involved in a successful fermentation process. These include physical factors discussed and listed in Table 3. In addition to the solid substrate used, the fungus itself has physical properties that influence the SSF process. For example, the ability of fungus cells to absorb and retain water was explored and found that *Aspergillus oryzae* is able to retain water content about 5 times higher than *Aspergillus awamori* [23]. In addition, moisture content loss from *A. awamori* is 46% higher than that from *A. oryzae*. This property will also determine the moisture content during SSF and show how cells of the fungus itself play an important role in maintaining moisture content.

Conclusion

Many factors are involved in a successful SSF process. The study deals with the physical and aspects of the system, which may vary from process to process depending on solid substrates and microorganisms. In addition to nutritional composition of solid substrate used for SSF medium, physical properties of solid substrate also represent another important idea to be focused on during SSF. Physical properties measured in this study include bulk density, particle density, porosity, tortuosity, water retention value, which will influence the behavior and the productivity of microorganisms involved

Table 3. Physical properties of wheat bran, soybean hulls, and rapeseed meal particles.

Parameter	Values		
	Wheat bran	**Soybean hulls**	**Rapeseed meal**
ρ_b of sample at moisture content 0%	0.22 g/mL	0.36 g/mL	0.50 g/mL
ρ_b of sample at moisture content 11%	0.26g/mL	0.33 g/mL	0.50 g/mL
ρ_b of sample at moisture content 65%	0.39 g/mL	0.32 g/mL	0.79 g/mL
ρ_p of sample at moisture content 0%	1.29 g/mL	1.49 g/mL	1.29 g/mL
ρ_p of sample at moisture content 11%	1.25 g/mL	1.41 g/mL	1.27 g/mL
ρ_p of sample at moisture content 65%	1.13 g/mL	1.14 g/mL	1.13 g/mL
ε of sample at moisture content 0%	82.45 %	75.57 %	60.42 %
ε of sample at moisture content 11%	81.96 %	76.32 %	61.51 %
d_{gw} at moisture content 11%	0.0494 cm	0.0474 cm	0.0391 cm
S_{gw} at moisture content 11%	1.04	2.21	2.31
Particles/gram at moisture content 11%	6.95×10^3 particles/gram	1.12×10^5 particles/gram	3.01×10^5 particles/gram
SA at moisture content 11%	241.51 cm^2/gram	122.98 cm^2/gram	168.54 cm^2/gram
VSA at moisture content 11%	301.99/cm	173.40/cm	214.0/cm
WRV	263.55 %	164.56 %	132.36 %
τ (dimensionless)	1.35 (MC: 65% ε: 65.95%)	1.18 (MC: 65% ε: 79.97%)	1.50 (MC: 65% ε: 61.69%)

for substrate utilization and product formation. Primary physical knowledge and information about the solid substrates that going to be used in the microbial SSF is important to make sure the successful of the process.

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