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Water Retention Value: A Study Model-based by *Asperglillus awamori* and *Aspergillus oryzae* Embrace Three Models of Solid Substrate

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Abstract: The goal of this study was to evaluate the water retention value (*WRV*) of a test solid substrate and a fungal cell in solid state fermentation (SSF). *WRV* is the ratio of the weight of water retained after centrifugation under specific conditions by a wet sample to the oven dry weight of the same sample. SSF refers to the microbial fermentation, which takes place in the absence or near absence of free water, thus being close to the natural environment. Many factors are involved in a successful SSF process. In addition to biological parameters, the SSF process is also dependent on physical factors such as *WRV*. A centrifugal technique has been modified and applied to the evaluation of *WRV*. Wheat bran, soybean hulls and rapeseed meal were used as model substrate. *Aspergillus awamori* and *Aspergillus oryzae* were used as model microorganism. Results revealed that the ability of wheat bran to retain water in the solid substrate is 56% higher than that of soybean hulls and rapeseed meal. In the term of fungal cell, the ability of *A. oryzae* to retain water in the cells was higher (73% higher) than that of *A. awamori*. In addition, through oven method moisture content loss from *A. awamori* is 46% higher than that from *A. oryzae* during drying process. Nevertheless, it can be seen that *A. oryzae* is able to retain water content about 5 times higher than *A. awamori*. Through this results, we found that WRV varies depending on solid substrates and microorganisms. This initial information can be beneficial in the SSF process to be carried out.

Key words: Water retention value (*WRV*), solid state fermentation, solid substrates, *Aspergillus awamori*, *Aspergillus oryzae*.

1. Introduction

Solid state fermentation (SSF) has been defined in many ways. In the latest definition, Mitchell *et al*. [1] defined SSF as a process that involves the growth of microorganisms on moist particles of solid materials in beds in which the spaces between the particles are filled with a continuous gas phase. Whatever the definition, we can understand that SSF is referring to the microbial fermentation, which takes 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. SSF processes are clearly different from submerged fermentation (SmF). In most cases, it is soluble substrate supported on a solid insoluble matrix in an environment of low moisture content. The advantage of SSF comes from its simplicity and its closeness to the natural habitat of many microorganisms. The solid substrate is a major element in SSF. In addition to providing nutrients such as carbon and nitrogen, the solid substrate also performs the role of the physical properties that supports the growth of microorganisms [2]. The importance of physical properties in SSF, experimental studies have focused on bulk density, particle density, specific volume, porosity, particle size, surface area, volumetric specific surface area and tortuosity. They were analyzing how of these

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characteristics vary with different solid substrate particles and moisture contents. Another important factor in the selection of substrate is the water holding capacity, which refers to water retention value that maintains moisture content of the fermented substrate [3]. However, this factor has not been investigated or reported yet in open literature regards to factors influence of performance SSF.

The water requirements of microorganisms for microbial activity can be expressed quantitatively in the form of water activity (a_w) of the environment or substrate. The a_w gives an indication of the amount of free water in the substrate and determines the type of microorganisms that can grow in SSF. The required a_w value for SSF varies depending on the microorganism, but it is usually recommended that the a_w be enough to permit growth of mycelium through the solid substrate particles without disintegrating the particles. According to Nigam and Singh [4], microorganisms capable of carrying out their microbial activities at lower a_w values are suitable for SSF process. Bacteria mainly grow at higher a_w values of about 0.9, while yeasts grow at values of 0.8 and filamentous fungi are adaptable to lower a_w values ranging between 0.6 and 0.7 [4, 5]. However, according to Ruijiter *et al*. [6], the fungus *A. oryzae* accumulates high concentrations of polyols at water activities between 0.96 and 0.97 during SSF, which seems to be unusual for this type of processing. As the fermentation starts at low moisture content, the culture dries out. Consequently, the fungus grows poorly and growth does not occur before the fermentation is completed. To overcome this problem, an appropriate amount of water is occasionally added throughout the fermentation period [7].

The ability of solid substrate and selected fungi to retain water is addressed in this report. 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 fungi. In addition, experiments were carried out in order to determine the ability of the fungus itself to retain water within its own cells. Filamentous fungi continue to dominate as an important microorganism in SSF due to their mycelia mode of growth as well as their neutral physiological capabilities [1]. Different fungi have different cell morphologies, which results in differing abilities to retain water. 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.

2. Materials and Methods

2.1 Microorganisms

Aspergillus awamori and *Aspergillus oryzae* obtained from the School of Chemical Engineering and Analytical Science, Faculty of Engineering and Physical Science, University of Manchester are used throughout this study. Fungal spores in universal bottle were stored at 4 °C in agar slopes of solid sporulation medium containing 5% (w/v) whole wheat flour and 2% (w/v) agar (Sigma-Aldrich) as a stock culture and they were sub-cultured in the time interval of every two months. This strain produces compact colonies with huge spores and accumulates large quantities of black and green pigments during growth in the form of mycelium for *A. awamori* and *A. oryzae*, respectively.

2.2 The Substrates

The solid substrates: wheat bran, soybean hulls and rapeseed meal, were used. 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 three selected substrates were used without any treatment.

2.3 Glucosamine

The method was adapted from Swift [8] with slight modification to suit the sample study. The method is based on the fact that glucosamine is a monomer component of chitin, present as acetylglucosamine. Chitin is an insoluble polymer present in the mycelium [9]. The process consists of the polymerisation of chitin; followed by the liberated glucosamine.

2.4 Water Retention Value

Water retention value (*WRV*) is an empirical measurement of the capacity of a test solid substrate to retain 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.

This was 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 SCAN-C 62: 00 [10] with slight modifications appropriate for the apparatus available.

Procedures:

5.0 g of solid substrate at moisture content 0% after drying at 80 °C overnight was immersed in distilled water for about 1 h at room temperature. Sample was removed from the water and allowed to drain for a few minutes. Some amount of the wet sample was then transferred into the specially modified centrifuge tube (Figure 1) and centrifuged at 7,500 rpm for 15 min. During centrifugation, the unit hangs on the rim of the centrifuge rotor, leaving enough space underneath to accommodate drained water. After centrifugation, samples were weighed (W_{ww}) and next dried in an oven at 80 °C for overnight. The samples were weighed as quickly as possible after centrifugation in order to obtain a wet weight prior to any evaporation occurring. After drying, samples were put into a desiccator to cool and then weighed (W_{dw}). The *WRV* was expressed in grams per gram, according to the following equation:

$$WRV = \frac{W_{ww} - W_{dw}}{W_{dw}}$$

Where;

W_{ww} = wet weight of sample after centrifugation (g)

W_{dw} = dry weight of sample after drying (g)

In addition to measuring *WRV* of solid substrates, the same procedure was also performed to measure WRV of fungus cell material. The procedure for WRV for fungi was carried out according to the following steps: First, culture fungus (7 days old) was autoclaved at 121 °C for 5 min to kill the fungus and after that melted agar was removed. Fungal mycelium

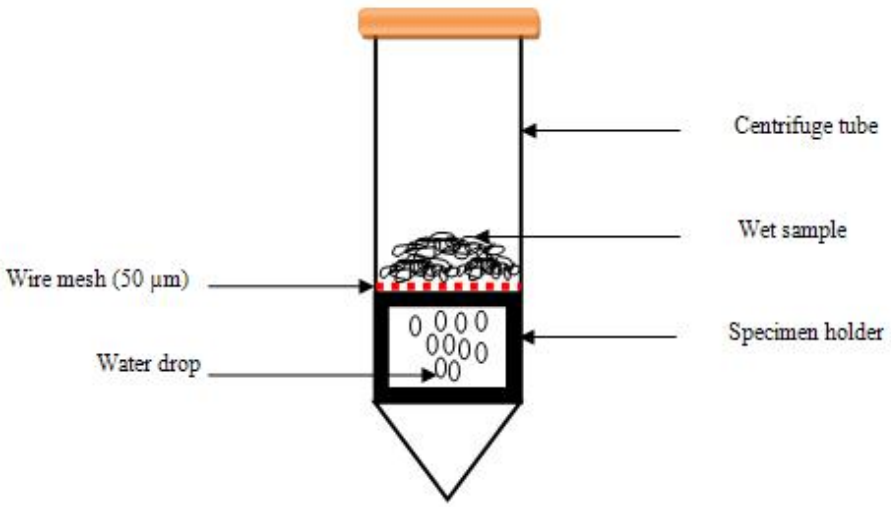

Fig. 1 Modified centrifuge-holding tube to the rim of the centrifuge rotor.

mat was obtained and washed under running tap water. Sample (fungal mycelium mat) was then dried in an oven at 80 °C for 24 h until the moisture content was almost zero. Dried fungal mycelium mat was crushed using a mortar to obtain pieces of fungal mycelium. About 2.0 g of fungal pieces was weighed and placed in 250 mL flask. 40.0 mL of distilled water was added and the flask was placed on a shaker at 250 rpm and 30 °C for about 24 h. The sample was then transferred into a normal centrifuge tube and centrifuged at 10,000 rpm for 10 min. The supernatant was discarded. The wet sample was transferred into a new centrifuge tube equipped with a wire mesh (50 μm) on top of the specimen holder (Figure 1) and centrifugation was performed according the procedure explained above. *WRV* was calculated, in units of gram per gram, according to equation above.

3. Results and Discussion

WRV is a measure of water retained by a material after centrifuging under specified conditions [11]. WRV is a useful reference to evaluate the performance of solid substrates and microorganisms relative to moisture behavior during SSF. 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 (solid substrates as well as fungus itself) will help in 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.

Figure 2 shows values of *WRV* measured for wheat bran, soybean hulls and rapeseed meal. An average *WRV* for wheat bran was 2.63 g/g (range between 2.53 and 1.78 g/g), with an average coefficient of variation between replicates of 1.12%. Soybean hulls had an average *WRV* of 1.69 g/g (range between 1.60 and 1.79 g/g) with an average coefficient of variation between replicates of 0.82%. Rapeseed meal had an average *WRV* of 1.36 g/g (range between 1.30 and 1.43 g/g) with an average coefficient of variation between replicates of 0.63%. 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%) [12] as starch absorbs more water compared to the other two solid substrates. Meanwhile, it is worthy of note that the solid substrates have different hydrophilic properties leads to vary *WRV* measurement.

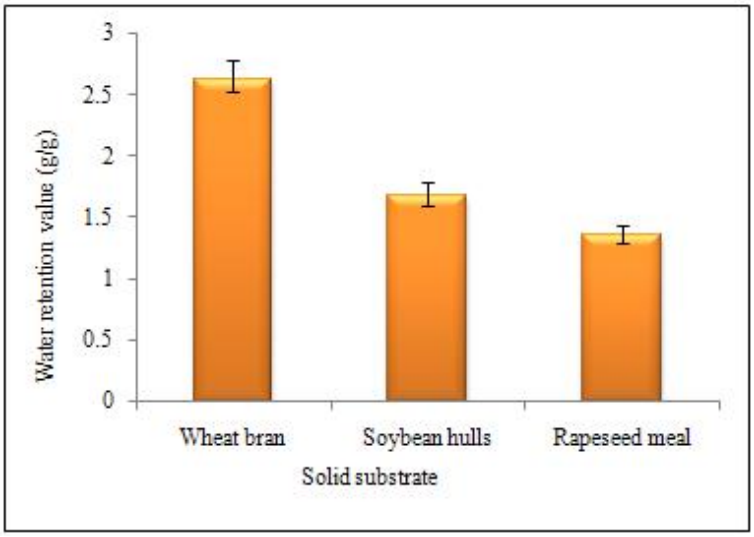

Fig. 2 Water retention values for wheat bran, soybean hulls and rapeseed meal.

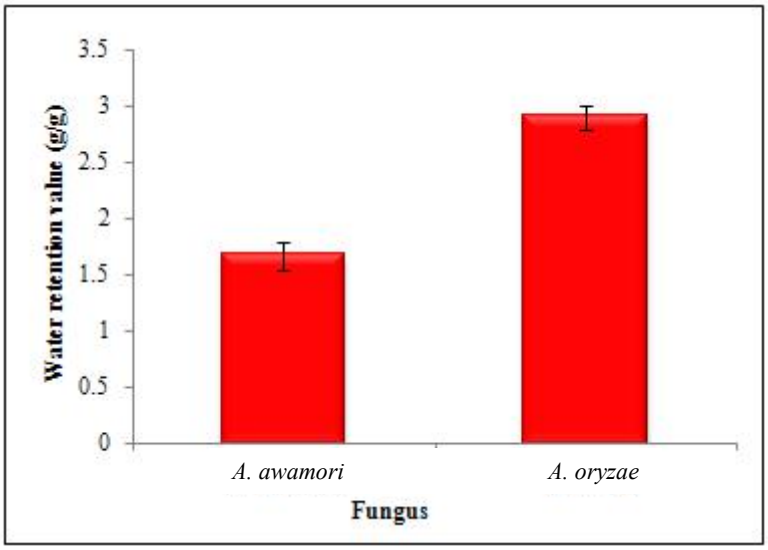

Fig. 3 WRV obtained for fungal mycellium mat of *A. awamori* and *A. oryzae*.

The WRV for *A. awamori* and *A. oryzae* was determined (Figure 3) and each fungus was tested using four replicates. The average *WRV* for *A. awamori* was 1.67 g/g (range between 1.53 and 1.79 g/g) and it had an average coefficient of variation between replicates of 1.37%. The fungus *A. oryzae* showed an average WRV of 2.90 g/g (range between 2.79 and 3.01 g/g) and had an average coefficient of variation between replicates of 1.16%. Briefly, these results show that the ability of *A. oryzae* to retain water in the cells was higher (73% higher) than that of *A. awamori*.

To support this finding, another experiment was carried by measuring the decrease of moisture content of fungal mycellium mat of *A. awamori* and *A. oryzae* at 80 °C (at different times). A mature fungal mycellium mat (after 7 days) was submerged in water for 24 h at 30 °C. The mat was removed, pat dried with a lint free cloth and weighed. This gives initial moisture content to the fungal mycellium mat. Subsequently, the fungal mat was placed in an oven and heated at 80 °C. At regular time intervals, the fungal mat was weighed as quickly as possible and then immediately placed back into the oven. This allows measuring the moisture content at those particular times. The process was repeated until the weight of fungal mycellium mat reached a constant value indicating that all the water from fungal mycellium mat was removed.

The rate of removal of the moisture content (through drying process) from fungal mycellium mat is illustrated in Figure 4. The initial removal of moisture content (AB) occured as the fungal mat and the water within it experienced a slight temperature increase. During this period, a heating process accoured whereby the fungal mycellium mat was still able to absorb heat from the environment. Following the initial stages of drying, significant reductions in moisture content occurred at a constant rate. The constant rate drying period (BC) matched the temperature of the oven. At this stage, the constant rate during the drying period continued until the moisture content was reduced to a critical moisture content (after 120 min). The diffusion rate drying period (CD) then followed. At this stage, the critical moisture content was further defined due to the abrupt change in the rate of moisture removal.

Moisture content of the fungal mycellium mat of *A. awamori* drastically decreased from 86.63% to 4.6%, while moisture content of *A. oryzae* fungal mycellium mat was still high at 25.6% at 120 min and only reached 1.22% after 160 min. Here, it can be seen that *A. oryzae* is able to retain water content about 5 times higher than *A. awamori*. In addition, moisture content

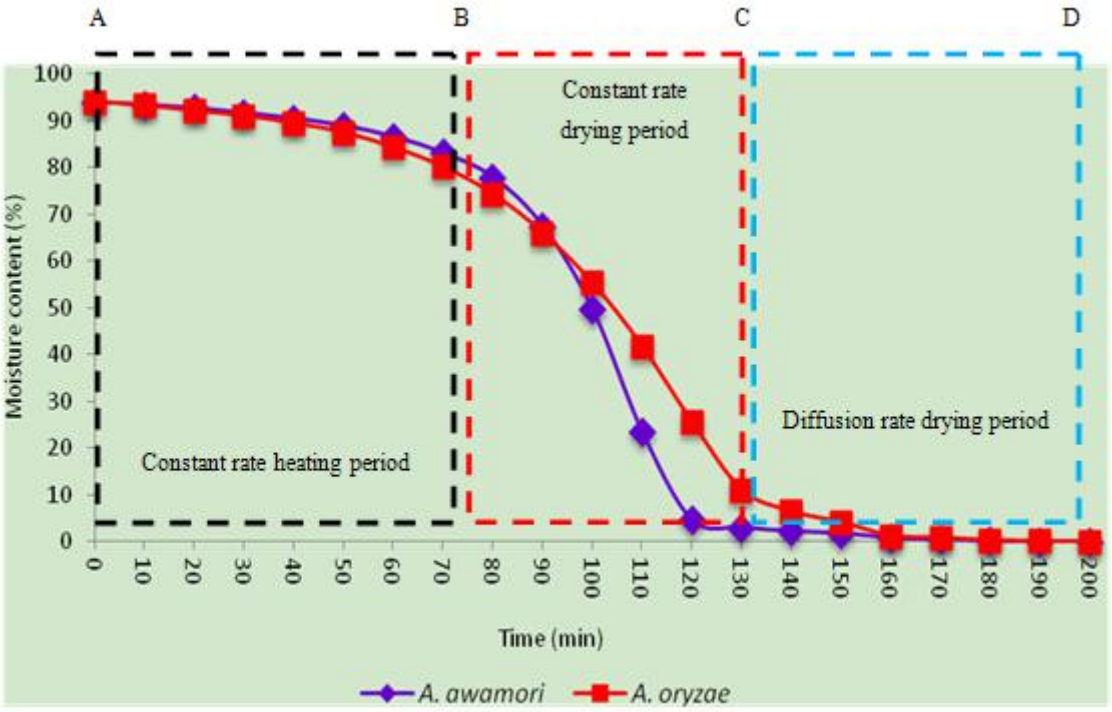

Fig. 4 Profiles of moisture content removal for the fungal mat of *A. awamori* and *A. oryzae*.

loss from *A. awamori* is 46% higher than that from *A. oryzae*.

On top of that, through our naked eye observation, fungal mycellium mat from *A. awamori* is soft, fragile and non-elastic compared to fungal mycellium mat from *A. oryzae*, which is hard, elastic and not easy to break. The filamentous fungal mycellium mat of *A. awamori* is difficult to handle and can be easily damaged (Figure 5). This is possibly due to the higher levels of chitin in *A. oryzae*. In this case, glucosamine was measured based on the fact that glucosamine is a monomer component of chitin, present as acetylglucosamine [9]. Table 1 shows the concentration of glucosamine in fermented wheat bran, soybean hulls, rapeseed meal and fungal mat obtained from solid agar medium. It was observed glucosamine concentration in *A. oryzae* samples were higher between 7-16.83% compared to *A. awamori*. According to Peter [12], variations in the concentrations of glucosamine (amounts of chitin) may depend on physiological parameters in natural environments as well as on the fermentation conditions in biotechnological processing or in cultures of fungi. In addition, chitin serves as a fibrous strengthening element responsible for cell wall rigidity.

Examined under Environmental Scanning Electron Microscope (ESEM), fungal mycelium mat from *A. oryzae*, whether from a wet sample or after being treated with gold, showed a tightly fused and rigid structure. The abundant fungal mycelium in *A. oryzae*, which is fused and tightly bound, has several advantages as it is more hydrophilic and can hold water for moisture content maintenance. This can be observed in micro-images of fungal mycelium mat of *A. oryzae* when treated with gold (Figure 6 (b)—AO: MS [1] and AO: LS [2]: at magnification 1000X and 8000X, respectively) with some fibre-like structure but mainly a smooth surface. Micro-images of fungal mycelium mat of *A. awamori* treated with gold (Figure 6 (a)—AA: MS [1] and AA: LS [2]: at magnification 600X and 8000X, respectively) showed micro-structures of a porous body (mycelium) with abundant, large pores and interconnected mycelium.

Fig. 5 Live and wet images of fungal mat surface (MS) and the lower surface (LS) of *A. awamori* (AO) and *A. oryzae* (AO).

Table 1 Glucosamine concentration.

Fungus	Fermented wheat bran (mg/g) [db]	Fermented soybean hulls (mg/g) [db]	Fermented rapeseed meal (mg/g) [db]	Fresh fungal mycelium from solid agar (mg/g) [db]
A.awamori	120.35 ± 0.01	85.69 ± 0.01	93.02 ± 0.01	100.46 ± 0.02
A. oryzae	132.57 ± 0.02	92.14 ± 0.01	104.92 ± 0.01	120.79 ± 0.01

[db]: dry basis.

Each figure represents the means of three replicates ± s.d.

4. Conclusions

Results of this study indicate that different microorganism and solid substrates had different value of *WRV*. This work shows that fungal mycellium of *A. oryzae* has better ability to retain water in its cells. *A. oryzae* can therefore retain moisture content during the fermentation process better than *A. awamori*. This work has also found that, in addition to the ability of fungal mycelium to absorb and retain water, solid substrates also plays important role in retaining water within the particles. As already known the water content in the SSF is the most important parameter that need to be addressed before the start of SSF. This property will lead to determine how combination of cells of the fungus and solid substrates play an important role in maintaining moisture content. The information obtained will lead to design experiment in a successful SSF process, which will influence the behavior and the productivity of microorganisms involved for substrate utilization and product formation. Further motivation for this study should be carried out with varies solid substrates and microorganisms to explore details of the information of *WRV* before and during SSF.

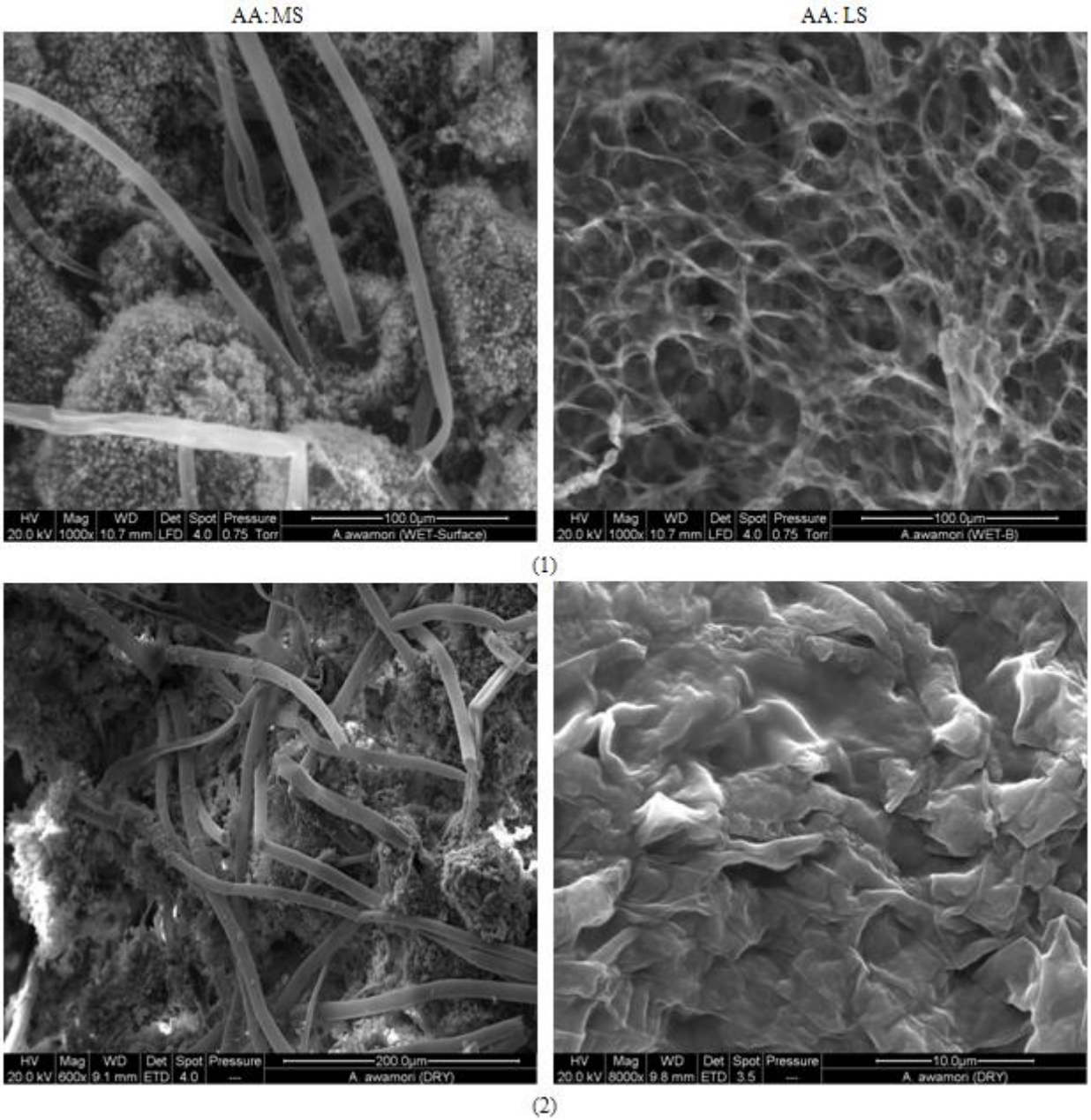

Fig 6 (a) ESEM images of fungal mat surface (AA: MS) and the lower surface (AA: LS) of *A. awamori* (AA). Image 1 from untreated wet sample and image 2 from sample treated with gold.

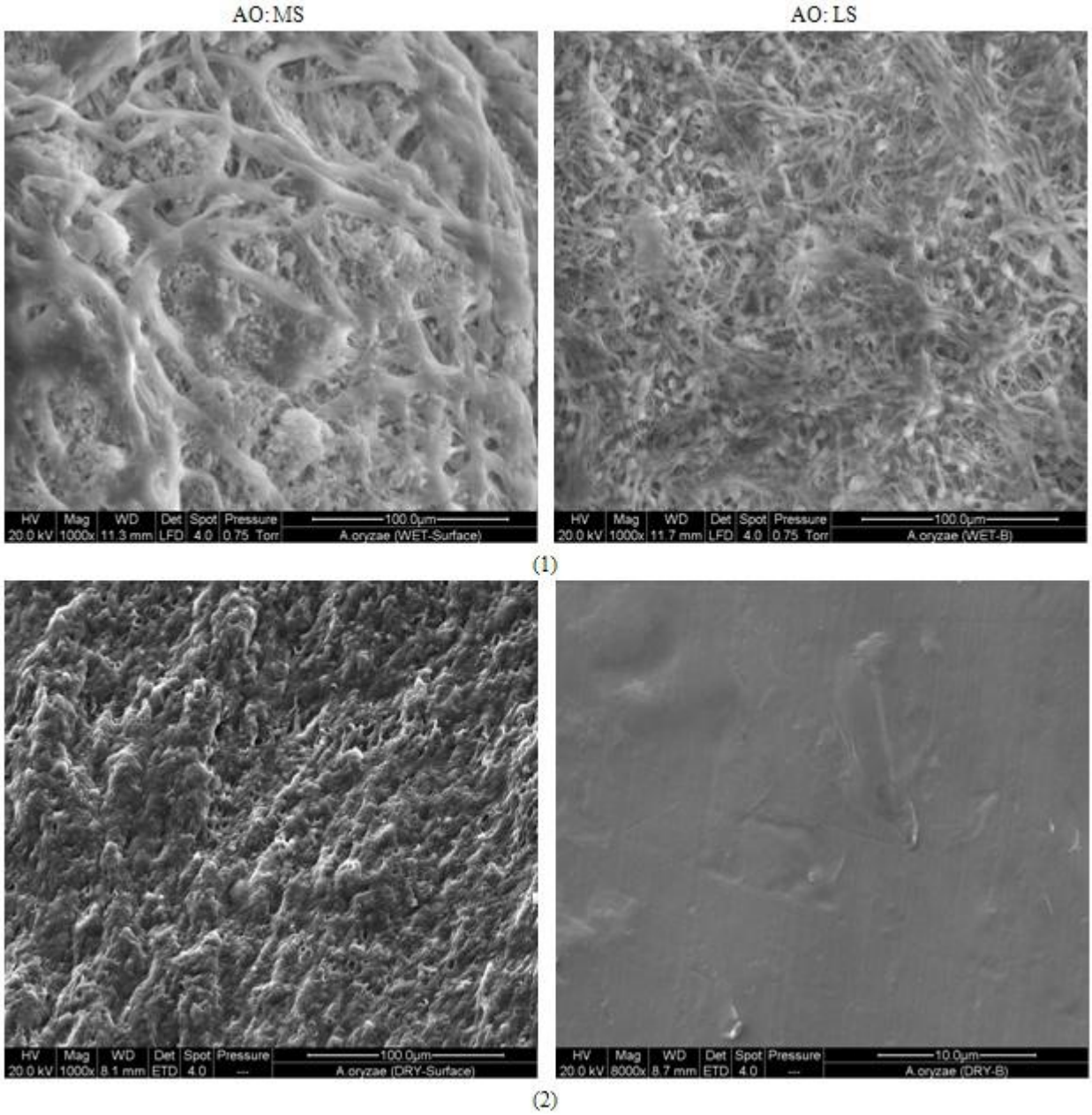

Fig 6 (b) ESEM images of fungal mat surface (AO: MS) and the lower surface (AO: LS) of *A. oryzae* (AO). Image 1 from untreated wet sample and image 2 from sample treated with gold.

Acknowledgments

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