



Correlations of medium physical properties and process performance in solid-state fermentation



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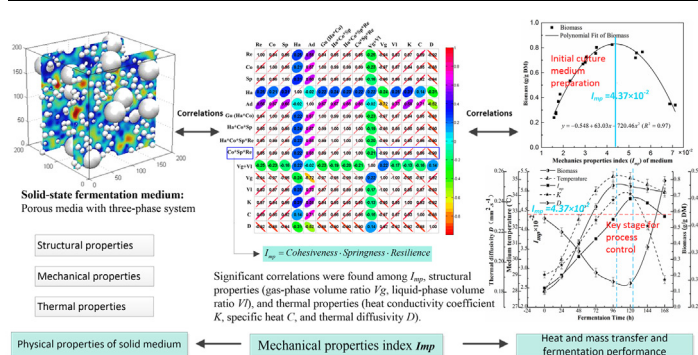
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HIGHLIGHTS

- A mechanical index I_{mp} was built to fully characterize medium physical properties.
- I_{mp} was the product of medium resilience, cohesiveness and springiness.
- I_{mp} significantly correlated with heat and mass transfer in SSF.
- I_{mp} significantly correlated with biomass content in SSF.
- Results are useful for guiding culture medium preparation and SSF process control.

GRAPHICAL ABSTRACT



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ABSTRACT

The medium of solid-state fermentation (SSF) is a multiphase system, whose complex physical properties cause difficulties of the cognition of SSF process and the process control. This paper aimed to deeply analyze the mechanical, structural and thermal properties of solid-state medium, explored relationships between the medium's physical properties and fermentation performance, then to guide the process control and culture medium optimization for SSF. A mechanical property index (I_{mp}), which was the product of resilience, cohesiveness, and springiness, was established to fully characterize physical properties of medium. Results showed that at the initial stage of SSF, there were positive correlations of I_{mp} with thermal conductivity and water retention, negative correlations with gas permeability and thermal diffusivity, and a parabolic relationship with biomass content with a symmetry-axis at $I_{mp} = 4.37 \times 10^{-2}$. Correlations were further verified in SSF dynamic process. During the first 100 h, $I_{mp} \leq 4.37 \times 10^{-2}$ was positively correlated with heat conduction and water retention in medium, which was beneficial for cell growth; while when $I_{mp} > 4.37 \times 10^{-2}$ during 100–168 h, medium heat accumulation, poor heat diffusivity and poor gas permeability indicated the reduction of fermentation performance. Results revealed medium physical properties significantly affected heat and mass transfer and further influenced fermentation performance in SSF. Mechanical property index I_{mp} could well comprehensively characterize physical properties of medium, which could be useful for guiding initial culture medium preparation and SSF process control.

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1. Introduction

Solid-state fermentation (SSF) is defined as a microbial culture that develops on natural solid substrates or impregnated inert

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supports in absence or near absence of free water (Pandey et al., 2008; Chen, 2013).

The porous medium of SSF is a multiphase system consisted of gas, water, solid matrix and microorganism (Chen, 2013; Duan and Chen, 2012; Khanahmadi et al., 2004). Gas is the mobile phase and the liquid film attached with the solid matrix is the stationary phase, which is the essential difference between SSF and submerged fermentation (Chen, 2013). From the perspective of mass transfer (nutritional supplement), whether sugar or other small molecules could only migrate in water (Laukevics et al., 1985), but not transfer in gas, and the nutrients only dissolved in water could be utilized by the microorganism (Krishna, 2005). From the perspective of heat transfer, the thermal conductivity of gas is much lower than that of the water (Chen, 2013; Mitchell et al., 2004), so it is more difficult to achieve rapid cooling of the medium when the gas is served as the mobile phase in SSF. Thus, the difficulty of heat and mass transfer caused by physical characteristics of porous medium in SSF is the fundamental reason that restricts the development of SSF (Ashley et al., 1999). Meanwhile, the complex physical characteristics of solid-state medium cause the challenges of the cognition of SSF process and its control (Chen, 2013).

The mechanical properties of substrate (such as strength, hardness, plasticity, springiness and viscosity) in SSF have been considered as the primary factors influencing the heat and mass transfer in SSF process, as well as fermentation performance. In Chinese traditional liquor industry, a verbal statements saying “rice husk was added to increase the mechanical strength of fermented grains in liquor fermentation” have been often heard. As support carrier, the addition of rice husks might improve the resistance of the matrix to compaction, and could keep a relatively high level of porosity of medium during fermentation, which is beneficial to gas transfer and heat removal. Besides, in Chinese Maotai liquor production, the whole sorghum grain was not grinded but directly used for fermentation. This behavior was considered to increase the mechanical strength of matrix and improve the heat and mass transfer in the fermentation process (Wu and Tian, 2014). In western country, Maheva et al. (1984) reported that the excellent mechanical properties of buckwheat (retention of structure, lack of agglomeration) accompanied the high spore yields of *Penicillium roqueforti*. Richard et al. (2004) reported that mechanical strength determines the resistance of the matrix to compaction, thus determining bulk density, air filled porosity, and permeability, as overburden stress increases with bed depth. Angel-Cuapio et al. (2015) indicated that the use of water hyacinth as a texturizer in solid-state cultures was effective to reduce the compaction of the substrate, to increase the porosity fraction and improve exchange gaseous, thus the conidial yields were improved to 1.33–1.55 times.

The tray bioreactor is the mainstream configuration for SSF at present (Demir and Tari, 2016; Figueroa-Montero et al., 2011; Mohseni et al., 2012; Pouryafar et al., 2015; Vaseghi et al., 2013), and the loading coefficient and the equipment utilization of which are not high. For the reason that the low thermal conductivity of the medium causes difficulties for heat removal and also results in the need to increase the area of heat exchange or to use forced aeration for the removal of excess metabolic heat (Castro et al., 2015; Chen, 2013; Figueroa-Montero et al., 2011). If the bed depth is increased, the porosity of the medium might be reduced by self-weight stress along with the direction of bed depth, resulting in the limitations of oxygen transfer and heat exchange (Angel-Cuapio et al., 2015). Therefore, if optimization of SSF medium is based on mechanical properties, then taking measures to try to keep the mechanical strength and porosity of substrate in constant in SSF process, and loading coefficient and equipment utilization are expected to improve. Moreover, it's a novel method for guiding SSF medium optimization, which could make up for the deficiency of the traditional medium optimization methods, which mainly

refer to the nutrient supply (Mizumoto and Shoda, 2007; Naveena et al., 2005; Senthilkumar et al., 2005).

In addition, the mechanical properties of substrate may contribute to the process control in SSF. During SSF, the physical properties of the medium are changed along with microbial growth and substrate decomposition (Duan et al., 2012; Hölker and Lenz, 2005; Krishna, 2005). These physical properties include agglomeration of substrate particles, reduction of porosity and springiness, and increased viscosity of medium. Dynamic changes in the physical properties of the medium affect heat and mass transfer and microbial growth and metabolism consequently during SSF (Casciatori et al., 2014; Hölker and Lenz, 2005; Karimi et al., 2014). Thus it requires the adjustment of process parameters, such as proper ventilation, inlet temperature of air and humidity. Therefore, understanding the relationships among the medium, heat and mass transfer, and the fermentation performance of the mechanical properties in SSF will make a significant contribution to culture medium optimization, loading coefficient improvement and process control for SSF.

To date, the process optimization for SSF have mainly always concerned about several factors, such as temperature (Mitchell et al., 2002; Nagel et al., 2001), oxygen diffusion (Oostra et al., 2001), moisture content (Lu et al., 2003; Oriol et al., 1988; von Meien and Mitchell, 2002), particle size (Barrios-González et al., 1993; Casciatori et al., 2014; Zadrzil and Puniya, 1995), and bulk properties (Angel-Cuapio et al., 2015; Barrios-González et al., 1993; Saw et al., 2012). There lacks a systematic cognition on the correlations among these physical properties (mechanical properties, structural properties and thermal properties) and researches on their effects on heat and mass transfer and fermentation performance of SSF. In the present work, we systematically studied several physical factors in SSF (mechanical properties, structural properties and thermal properties), established an integrated index to comprehensively characterize the medium physical properties, and explored the relationships among the integrated index, heat and mass transfer and fermentation performance during SSF, for guiding culture medium preparation and process control in SSF.

2. Materials and methods

2.1. Microorganism and medium

Trichoderma harzianum 3.5314, obtained from Institute of Microbiology, Chinese Academy of Sciences, was used in the present study. The culture was maintained on potato-dextrose agar (PDA) and subcultured fortnightly. Slants were incubated for 7 days at 30 °C and stored at 4 °C. The spores of a fully sporulated slant were dispersed in 0.1% Tween 80 solution by dislodging them with a sterile loop under aseptic conditions. The spore suspension obtained was used as the inoculum (Nampoothiri et al., 2004). Spores present in the suspension were determined by serial dilution followed by plate count (Nampoothiri et al., 2004).

Corn straw (collected from Hebei Province, China) was harvested at maturity in September 2015 and air-dried at room temperature. 30 kg of corn straw was cut into 5–10 cm and then steam-exploded at 1.5 MPa for 5 min using saturated steam in a 0.5-m³ batch reactor (Zhang et al., 2012). Subsequently, the sample was water-washed for detoxification and air-dried, and steam-exploded straw (SES) was obtained.

SES was ground and sieved, to obtain the samples with 5–14, 14–30 and 30–50 mesh with the same compositions. The particle size of samples with 5–14, 14–30 and 30–50 mesh were 1.41–4.28, 0.55–1.41 and 0.31–0.55 mm, respectively. 20 g of sample with one size was placed in a 250-mL Erlenmeyer flask and supplemented with 10 mL of nutrient solution, containing 1 g Glucose, 1 g Peptone, 0.5 g NH₄NO₃, 0.1 g KH₂PO₄, 0.1 g

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