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Physical structure changes of solid medium by steam explosion sterilization

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HIGHLIGHTS

- Revealed mechanisms of improving SSF by SES contained chemical and physical effects.
- Pores where capillary water located were found to be active sites for SSF.
- Steam explosion enlarged effective pores for microbial cells and thus improved SSF.
- Proposed a model well elucidating SM structure changes during sterilization and SSF.

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ABSTRACT

Physical structure changes of solid medium were investigated to reveal effects of steam explosion sterilization on solid-state fermentation (SSF). Results indicated that steam explosion changed the structure of solid medium at both molecular and three-dimensional structural levels, which exposed hydrophilic groups and enlarged pores and cavities. It was interesting to find that pores where capillary water located were the active sites for SSF, due to the close relationship among capillary water relaxation time, specific surface area and fermentation performance. Therefore, steam explosion sterilization increased the effective contact area for microbial cells on solid medium, which contributed to improving SSF performance. Combined with the previous research, mechanisms of SSF improvement by steam explosion sterilization contained both chemical and physical effects.

1. Introduction

Solid-state fermentation (SSF) is an environmental friendly process in which microorganisms grow on solid substrates in the absence (or near absence) of free water (Pandey, 2003). Its applications have been expanded to feed additives, biofertilizers, biopesticides due to the advantages of high products concentration, low energy requirement and little wastewater discharge (Hölker et al., 2004; Zhao et al., 2015b). Sterilization of solid medium (SM) is a crucial procedure for pure culture in SSF, which determines the success of fermentation (Mei et al., 1999). Conventional thermal sterilization (CTS) using steam treatment at 121 °C for 20–30 min requires long time for the whole sterilization operation. Meanwhile, it reduces the nutritional value of SM since thermal degradation of nutrients occurs during the long-time steam heating (Lund, 1988). A new sterilization technique for SM, steam explosion sterilization (SES), has been developed in the previous study (Zhao et al., 2015a). High steam pressure and instant pressure relief in SES was conductive to destroying microbial cells and improving SM nutrition efficiently. Complete sterilization can be achieved when SES conditions were above 172 °C for 2 min and above 128 °C for 5 min. Viable cell number on per gram of dry SES medium after fermentation increased by 2.83 times than that on per gram of dry CTS medium. Therefore, SES improved SM sterilization efficiency and enhanced SSF performance effectively, which was proved to be a promising method for SM sterilization. However, effects of SES on physical-structural properties of SM have not been elucidated, which limited the understanding of mechanisms of SSF improvement by SES comprehensively.

The solid medium is generally consisted of gas, solid and liquid. Its structural properties have significant effect on microbial growth and thus determine the SSF performance (Duan and Chen, 2012). Low-field nuclear magnetic resonance (LF-NMR) is a promising analytical technique, in which the relaxation times of the proton

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can be used to assess different states of water in substrates (Felby et al., 2008). Due to the different microenvironments that the protons experience, LF-NMR can be regarded as a powerful method to study the microstructure properties of solid matrix (Gianferri et al., 2007). Li et al. (2015b) studied the water dynamics and microstructure variations during the soaking, steaming and SSF of glutinous rice by LF-NMR. Three different proton fractions, T_{2b}, T_{21} and T_{22}, were identified with different mobilities. The proton distribution was changed by soaking, steaming and SSF processes, implying the medium microstructure variations. Felby et al. (2008) used LF-NMR to study the cellulose–water interactions during enzymatic hydrolysis. Structural changes of cellulose fiber upon enzymatic hydrolysis were elucidated by the state and location of water. According to the structural changes of substrate, the synergistic mechanism of endo- and exo-glucanase on lignocelluloses was revealed. Han et al. (2014) applied LF-NMR to investigate the water mobility and microstructure changes during heat-induced gelation process of pork myofibrillar proteins. Great differences in microstructure depending on temperature were observed. The LF-NMR data was significantly related to the microstructure characteristics and helped to elucidate the structural changes of pork myofibrillar proteins during the heat-induced gelation process. Therefore, LF-NMR is an effective technique to characterize the structural properties of solid medium.

In the present study, LF-NMR was adopted to investigate the effects of SES on microstructure changes of solid medium and further on SSF performance. Water states, microstructure and fermentation performance of different sterilized media were compared systematically. Principal component analysis (PCA) was performed to find the relationship among fermentation performance with physical properties and LF-NMR data. More insights into the mechanisms of SSF improvement by SES besides nutrition aspect were presented.

2. Methods

2.1. Experimental materials

SM consisted of 200 g wheat bran, 20 g soybean meal, 5 g glucose, 10 g $CaCO_3$ and 200 mL of 2.5% KH_2PO_4, 1.25% $(NH_4)_2SO_4$, and 0.05% $MnSO_4$ inorganic salt solution. Initial moisture content (MC) of SM was adjusted to 60% (w/w) by adding deionized water. Chemicals were purchased from Beijing Chemical Works.

2.2. Steam explosion sterilization (SES) and conventional thermal sterilization (CTS)

Steam explosion sterilization (SES) was conducted in a 2.0 L self-designed apparatus which mainly consisted of a reactor chamber, a reception chamber, and a steam generator. SM was top-loaded into the reactor chamber. High-pressure steam supplied by the steam generator was rapidly injected into the reactor until the holding temperature reached to desired value (177 °C, 0.9 MPa gauge pressure). After maintaining the set residence time (2 min), SM was exploded into the sterilized reception chamber by a ball valve (Liu and Chen, 2015; Zhao et al., 2015a). The sterilized materials were cooled and prepared for subsequent fermentation and analysis. Conventional thermal sterilization (CTS) was performed as control. SM was loaded into a 2.0 L beaker covered with 8 layers of gauze and then put into a vertical autoclave (YXQ-LS-50A, Shanghai Boxun Industry & Commerce Co., Ltd). The temperature and holding time were set to 121 °C for 20 min as common. After sterilization, the materials were cooled and prepared for subsequent fermentation and analysis. MC of medium after SES increased to 66% while that after CTS remained 60%. To highlight the influence of SM structure change and eliminate interference of MC on SSF, some CTS medium was added with sterile water to keep the same MC value with SES medium of 66%, which were defined as CTSW medium as control. All these samples were prepared with two replicates.

2.3. Solid-state fermentation (SSF) and microbial growth curve determination

Bacillus subtilis PFK1302 used in this study was kindly provided by Hunan PERFLY-Bio Co., Ltd., Changsha, China. The strain was stored in LB medium slant at 4 °C. *B. subtilis* PFK1302 was precultivated in 100 mL liquid medium containing 4% (w/w) glucose, 1% (w/w) peptone, 1% (w/w) yeast extract, 1% (w/w) $CaCO_3$ and 0.05% (w/w) $MgSO_4$ at 37 °C and 150 rpm for 24 h. 31–50 g of SES, CTS, CTSW media were sampled and inoculated by adding 0.4 mL seed solution per gram of dry medium. SSF were carried out in 100 mL Erlenmeyer flasks and cultured at 37 °C for 72 h.

B. subtilis PFK1302 is a kind of probiotics for animals, which is widely used in feed additives. Bacteria number is a key factor indicating the fermentation product quality. Viable cell numbers were determined to evaluate the fermentation performance during SSF at 12, 24, and 48 h, respectively. 3–5 g of SM were sampled and mixed with 50 mL of sterile water in 250 mL Erlenmeyer flasks. The Erlenmeyer flasks were shaken at 150 rpm for 30 min at 37 °C. 100 μL of the supernatant was serially diluted with sterile water down to 10^7. The cells counts were determined by spread plate method in duplicate on LB plates. After 36 h of incubation at 37 °C, the number of colonies was determined and expressed as colony-forming units (CFU).

2.4. Microstructure characterization

The Brunauer–Emmett–Teller (BET) method was utilized to determine the specific surface area (SSA) variations of SM during SSF at intervals of 24 h. 3–5 g of SM were sampled and washed with a volume of water equal to 25 times the wet weight of SM. Then the SM samples were oven dried for 4 h at 105 °C to the absolute dry state. 0.2 g of the dry SM was sampled to determine SSA by nitrogen adsorption/desorption isotherm on an Automatic Intelligent 3H-2000A Volumetric System (Beishide Instrument S&T Co., Ltd., Beijing, China).

2.5. Low-field nuclear magnetic resonance (LF-NMR) measurements

Water distribution and dynamics of SM were detected by LF-NMR on a 20 MHz NMI20-Analyst (Niumag Co., Ltd., Shanghai, China). Approximately 3 g of different SM samples were obtained from the central part of the substrates and placed into 15 mm NMR glass tubes. The tubes were then inserted into the NMR-probe. T_2 relaxation times were measured using Carr–Purcell–Meiboom–Gill (CPMG) sequence with a τ-value of 500 μs (time between 90° and 180° pulse). 1500 echoes were collected as 4 scan repetitions, and the repetition time between two successive scans was 1.5 s. The relaxation measurements were performed at 32 °C. Data analysis was performed with the NMR Analyzing System Ver 1.0 (Niumag Co., Ltd., Shanghai, China). The inverse Laplace transformation method was used. A continuous distribution of exponentials for a CPMG experiment is defined by the following equation:

$$M(t) = \sum_{i=1}^{n} P_i \exp\left(-\frac{t}{T_{2i}}\right) + L \quad (1)$$

where $M(t)$ is the residual magnetization as a function of acquisition time t; P_i and T_{2i} are the spin–spin relaxation amplitude and time,