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The effect of gas double-dynamic on mass distribution in solid-state fermentation

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ABSTRACT

The mass distribution regularity in substrate of solid-state fermentation (SSF) has rarely been reported due to the heterogeneity of solid medium and the lack of suitable instrument and method, which limited the comprehensive analysis and enhancement of the SSF performance. In this work, the distributions of water, biomass, and fermentation product in different medium depths of SSF were determined using near-infrared spectroscopy (NIRS) and the developed models. Based on the mass distribution regularity, the effects of gas double-dynamic on heat transfer, microbial growth and metabolism, and product distribution gradient were systematically investigated. Results indicated that the maximum temperature of substrate and the maximum carbon dioxide evolution rate (CER) were 39.5 °C and 2.48 mg/(h g) under static aeration solid-state fermentation (SASSF) and 33.9 °C and 5.38 mg/(h g) under gas double-dynamic solid-state fermentation (GDSSF), respectively, with the environmental temperature for fermentation of 30 ± 1 °C. The fermentation production (cellulase activity) ratios of the upper, middle, and lower levels were 1:0.90:0.78 at seventh day under SASSF and 1:0.95:0.89 at fifth day under GDSSF. Therefore, combined with NIRS analysis, gas double-dynamic could effectively strengthen the solid-state fermentation performance due to the enhancement of heat transfer, the stimulation of microbial metabolism and the increase of the homogeneity of fermentation products.

1. Introduction

Fermentation has played a major role in the production of food, alcoholic beverages, enzymes, food additives and supplements for a long period [1]. There are essentially two kinds of fermentation modes, either in solid state or submerged in liquid. In submerged fermentation (SmF), the microorganism and nutrient source are normally suspended or dissolved in a liquid medium and the growth takes place in a dispersed cell suspension [2]. While referring to solid-state fermentation (SSF), it is defined as the growth of microorganisms in absence or near absence of free water [3].

In recent years, SSF has caused much more attention from researchers since SSF offers numerous opportunities in processing of agro-industrial residues. And its processes have environmental-friendly advantages of lower energy requirements and less wastewater production [4,5]. However, since the continuous phase in SSF is the gas phase with low thermal conductivity and the culture medium is solid phase rather than the liquid phase [6], the heat and mass transfers in SSF medium are more difficult than those in SmF [7,8]. Thus, the temperature, water, biomass and product distribution gradients are easily formed in the SSF medium [9,10]. Gradient phenomena result in unstable product quality and reactor's low production efficiency. At present, much attention to the research of reducing heat and mass distribution gradients is directed toward designs involving mechanical agitation and rotation [11]. However, the mechanical agitation and rotation may also lead to microbial mycelium damage while enhancing heat and mass transfers. Sometimes this negative effect and positive effects of enhancing heat and mass transfers will cancel each other out [12,13].

In order to reduce the heat and mass gradients and avoid the microbial mycelium damage, a novel SSF bioreactor, gas double-dynamic solid-state fermentation bioreactor (GDSFB) was devised by our research group [14,15]. It consists of periodic pulsation of air pressure and internal air circulation. Pressure pulsation occurs

Abbreviations: SSF, solid-state fermentation; NIRS, near-infrared spectroscopy; CER, carbon dioxide evolution rate; SASSF, static aeration solid-state fermentation; GDSSF, gas double-dynamic solid-state fermentation; SmF, submerged fermentation; GDSFB, gas double-dynamic solid-state fermentation bioreactor; NIR, near-infrared reflectance; PLS, partial least-squares; PDA, potato dextrose agar; RH, relative humidity; SEM, scanning electron microscope.

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through feeding and exhaling sterile air in the reactor, not only promoting evaporation and cooling, but also enhancing biological activity by outside periodic stimulation. Internal air circulation is another dynamic process. Its main purpose is to strengthen the internal air flow and circulation [16].

Early studies have proven that gas double-dynamic can reduce the temperature gradient in solid state substrate [15] and the effect of gas double-dynamic on cellulase production were studied roughly [17]. However, limited to traditional analytical tools and the complexity and heterogeneity of the solid medium [18], a more in-depth study on the effect of gas double-dynamic on mass distribution gradients in medium has not been carried out.

Recently, the development of near-infrared spectroscopy (NIRS) techniques and chemometrics have resulted in rapid detection for chemical components and have been widely applied in the fields of food chemistry [19], pharmaceutical technologies [20] and determination of chemical compositions of straw [21,22]. Based on the C—H, N—H, and O—H absorption frequencies by functional groups and scattering at specific wavelength in the near-infrared (NIR) region, near-infrared reflectance spectroscopy in the range of 11,000–7000 cm^{-1} has been used for the analysis of protein, oil, and moisture in many agricultural products, such as cereal grains, forage, and rice straw [22–25]. This method is based on the construction of multivariate calibration models combining spectrometric data and traditional chemical composition results obtained with conventional laboratory methods, normally consisting of the following three procedures. First, calibrating models, that is, to construct models or called databases combining NIR spectra properties and conventional analysis results of large numbers of samples using chemometrics. For example, as reference data obtained by conventional methods, moisture content can be measured by the constant weight method, drying at 105 °C [21]. The biomass can be obtained by the chemical analysis of biomass components, glucosamine [26]. Cellulase activity can be determined by filter paper assay as described by Ghose [27]. In order to relate the spectral data to the reference data, multivariate analysis could be performed with a commercial spectral analysis program (TQ Analyst 6.2) and partial least-squares (PLS) regression could be used to construct models [21]. Second, validation. Student's *t*-test, internal validation and external validation or other validation methods can be performed to check the prediction capacity and robustness of the obtained models. At last, prediction. After the models being checked to be efficient, the variables such as moisture content, biomass and cellulase activity of new samples could be predicted through inputting the collected spectra to the models. In our previous study, the models were developed based on 50 samples for water content, biomass and cellulase activity determination, giving R^2-value of 0.994, 0.999 and 0.984, respectively [28], and showing the accuracy of NIRS method.

In this study, the distribution gradients of water, biomass, and product (cellulase) at different medium depths in gas double-dynamic solid-state fermentation (GDSSF) versus static aeration solid-state fermentation (SASSF) were studied and compared using NIRS and the developed models. Based on the distribution regularity, the effects of air pressure pulsation and air circulation on microbial growth and metabolism in SSF medium were analyzed and discussed.

2. Materials and methods

2.1. Microorganism and medium

Trichoderma reesei YG3 used in this study was from School of Life Sciences in Qufu Normal University, Shandong, China. The strain was preserved on the potato dextrose agar (PDA) slant at 4 °C. To prepare the inoculum, spores from the slant were suspended in 2 mL of 9 g/L NaCl (10^6–10^7 spores/mL) and transferred into a 250 mL Erlenmeyer flask containing 50 mL of substrate. The culture medium was Mandels' salt solution [29] supplemented with 10 g/L wheat bran and 1.5 g/L peptone. Fungal cells were cultured in an orbital shaker (175 rpm) at 30 °C for 48 h before the mycelium was used for inocula.

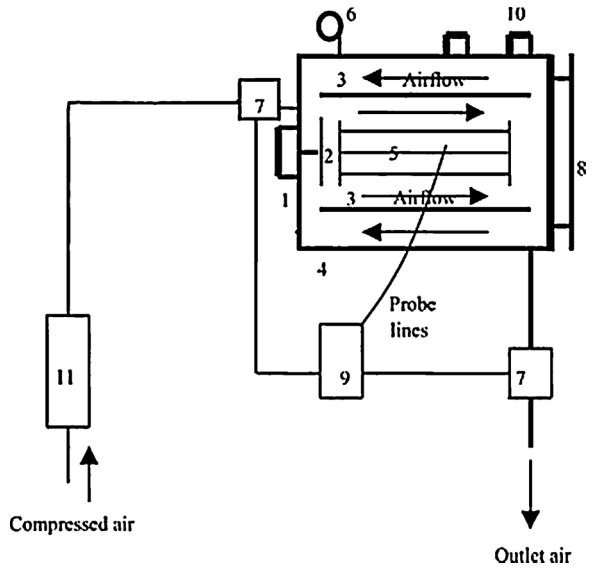

Fig. 1. Schematic diagram of gas double-dynamic solid-state fermentation bioreactor (GDSFB). Key parts to the bioreactor: (1) variable speed motor; (2) fan; (3) air baffle; (4) fermentation vessel (50 L); (5) trays (containing temperature probes); (6) air pressure meter; (7) electric valve; (8) handle; (9) relay and temperature data logger; (10) observation ports; (11) filter [15].

For cellulase production, the medium composition was 100 g of dry steam-exploded wheat straw [30] and 25 g of wheat bran supplemented with 325 mL salt solution [15 g/L $(NH_4)_2SO_4$, 6 g/L $MgSO_4$, 3 g/L KH_2PO_4]. The medium was put into a 22 cm × 15 cm × 4.5 cm enamel tray and autoclaved at 121 °C for 20 min. After sterilization and inoculation of 50 mL of spore suspension, the medium was put into the GDSFB for fermentation.

2.2. Static aeration solid-state fermentation (SASSF) and gas double-dynamic solid-state fermentation (GDSSF)

GDSFB used in the present study was devised by our research group [15]. A schematic diagram of the experimental set-up mainly consists of a fermentation vessel, a control system (electric valves and a relay) and a dynamic system (a variable speed motor and a fan) (Fig. 1). The temperature and relative humidity (RH) of air entering into the fermenter were maintained at 30 ± 1 °C and 90–97% RH. Two types of SSF were carried out in GDSFB: SASSF and GDSSF.

SASSF was operated as follows: The air inlet valve and the air outlet valve were both open, air entered the GDSFB from the air inlet and was discharged out from the air outlet. The air rate in this experiment was set to 1.5 m/s through turning speeds of the motor and the fan power. During the entire experiment process, the atmospheric pressure inside the reactor was maintained at near 0.0 MPa (gauge pressure). Under the given conditions, the strain was incubated for 7 days.

GDSSF was operated as follows: when the air inlet valve was on and the air outlet valve was off, the compressed and sterilized air entered into the GDSFB rapidly until the air pressure reached the set value (0.2 MPa); when the inlet valve was off and the air outlet valve was also off, the air pressure was maintained for given time (1 min); when the set time was over, the outlet valve was on and the inlet valve was still off and the air left out of the fermenter rapidly until the air pressure reached the set value (0.0 MPa); at the same time, the outlet valve and the inlet valve were both off and the air pressure was maintained for a while (20 min); and when the set up time was over, the air would start to repeat the above process of air pulsation. The air circulation rate was set to 1.5 m/s. Since the fermentation period was shortened by nearly 30% under GDSSF [2,31], the strain was incubated for 5 days.

2.3. Near-infrared spectrometer set-up and spectra collection

A Nicolet Nexus FT-NIR spectrometer (Thermo Nicolet Corporation, USA) was used to obtain NIRS spectra. The system TQ Analyst 6.2 (Thermo Nicolet Corporation, USA) was used for calculation and analysis of the spectra.

Remove the tray at the end of fermentation. As shown in Fig. 2, the medium was carefully divided into three layers accordance with the average height. The height of each layer was about 1.5 cm. In each layer, layout 11 × 7, total 77 collection points, the row spacing and line spacing were 2 cm. The fiber optic probe was pointed directly to the collection point to collect NIR spectra.

The settings of the parameters in the experiment were as follows: the spectrum data range was 4000–10,000 cm^{-1}, number of scans was 64, and the resolution was