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Air pressure pulsation solid state fermentation of feruloyl esterase by *Aspergillus niger*

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ABSTRACT

Air pressure pulsation solid state fermentation (APP-SSF) was applied to produce feruloyl esterase (FAE) by *Aspergillus niger*. With the optimization of some variables by orthogonal design, the optimal condition obtained was 0.2 MPa (gauge pressure) of high pressure intensity, 30 min of low pressure duration and 20 s of high pressure duration. Based on the optimized condition, the APP-SSF achieved the reasonable enzyme yield of 881 mU/g at 48 h, which was 58% more than that by static solid state fermentation (static SSF) at 72 h. By comparison of two fermentation methods in temperature, O_2 and CO_2 concentration, and respiration intensity, it was concluded that APP-SSF enhanced heat and mass transfer of fermentation system and strengthened the metabolism of microorganisms. The APP-SSF had a greatly positive effect on FAE production by *A. niger*, by enhancing mass and heat transfer and activating growth and metabolism.

1. Introduction

Ferulic acid (FA) is one of the most abundant hydroxycinnamic acids in the plant world (Mathew and Abraham, 2004), which is commonly covalently linked to polysaccharides, such as hemicellulose, through ester linkages in cell walls of straw (Saulnier and Thibault, 1999). It has been reported to have many physiological functions, including antioxidant, antimicrobial, anti-thrombosis and anti-cancer activities (Ou and Kwok, 2004). Therefore, extracting FA from straw and other lignocellulose materials has a great significance for high-value conversion of straw resources. As an effective extraction method, feruloyl esterase (FAE) can release FA present in plant cell walls, by hydrolyzing the ester linkages between FA and polysaccharides (Mathew and Abraham, 2004). Besides, in Topakas et al.'s review, it was discussed that FAEs have many potential applications in pulp and paper industry, production of fuel ethanol, farming industry and so on (Topakas et al., 2007). Accordingly, much attention has been focused on microbial fermentation of FAE (Faulds et al., 1997; Mathew and Abraham, 2005).

It was in the culture of *Streptomyces olivochromogenes* that FAEs were first detected releasing FA from wheat bran (Mackenzie et al., 1987). Since then it has been recognized that many microorganisms' enzyme systems contain FAEs, such as *Aspergillus flavipes* (Mathew and Abraham, 2005), *Aspergillus niger* (Johnson et al., 1989), *Sporotrichum thermophile* (Topakas et al., 2003), and *Penicillium brasilianum* (Panagiotou et al., 2006). Mukherjee et al. obtained 2.0 mU/ml of enzyme yield under agitated submerged fermentation (SmF) from the mineral basal salts (MBS) medium by *Streptomyces S10* (Mukherjee et al., 2007). Moreover, Topakas et al. reported the FAE production of 156 mU/g by *S. thermophile* under solid state fermentation (SSF) with wheat straw and wheat bran as carbon source (Topakas et al., 2003). By comparison, SSF of FAE would be more eco-friendly and economical than SmF because of less consumption of water and lower cost of substrates and equipment (Pandey et al., 2000).

However, the traditional static SSF has difficulties in mass and heat transfer (Raghavarao et al., 2003). Though agitation and rotation were carried out to solve the problem, the shearing forces caused by them often affected medium porosity and disrupted fungal mycelia (Raghavarao et al., 2003). For this problem, our coworkers have devised a novel strategy of SSF, air pressure pulsation, which has been proved to improve the enzyme yield effectively in the SSF production of cellulose (Sun et al., 1999; Xu et al., 2002a) and B.t. (Chen et al., 2002). According to Xu's result, air pressure pulsation increased the average cellulose activity from 10.8 IU/g to 20.4 IU/g in SSF (Xu et al., 2002a). And the virulence of B.t produced was maintained above 18,000 IU/mg in a novel industrial-level bioreactor (70 m^3) of APP-SSF designed by Chen et al. (2002). Since then, many attempts have been made to explore why the air pressure pulsation has such a great positive influence in SSF (Zhao et al., 2001a). It is argued that air pressure pulsation may be able to improve mass and heat transfer effectively, or

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stimulate the growth and metabolism of microorganisms positively (Xu et al., 2002b).

In this paper, *A. niger* was adopted to produce FAE by APP-SSF, and orthogonal experimental method was applied to investigate APP-SSF of FAE, with three different factors at three different levels, to determine the optimum condition of air pressure pulsation. Furthermore, some key variables during the fermentation process, such as temperature, O_2 and CO_2 concentration, and respiration intensity, were determined in order to explore the effect of the APP-SSF on heat and mass transfer and strain growth.

2. Methods

2.1. Microorganism

A. niger WH-4, isolated by our coworkers (Wang and Chen, 2007), was used in the present study. The strain was preserved on potato-dextrose-agar (PDA) at 4 °C for storage.

2.2. Steam-exploded rice straw

Steam-exploded rice straw was prepared by putting the rice straw (3–4 cm, containing 15% water) into the steam-exploded vessel at 1.7 MPa (gauge pressure) for 4 min (Chen, 1998).

2.3. APP-SSF bioreactor system

APP-SSF bioreactor of 25 L used for experiments was invented and manufactured by the National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences (Beijing, China). It was made up of a stainless fermentation vessel and a control system (Xu et al., 2001). Fermentation process was controlled by MCGS industrial control configuration software.

The air pressure pulsation was regulated by the control system as follows: when the air inlet valve was on and the air outlet valve was off, the compressed and sterilized air entered into the fermentation vessel rapidly until the air pressure reached the set value (high pressure intensity); when the inlet valve was off, the air pressure was maintained for given time (high pressure duration); when the set time was over, the outlet valve was on and the air left out of the fermenter rapidly until the air pressure reached the set value (low pressure intensity); at the same time, the outlet valve was off and the air pressure was maintained for a while (low pressure duration); and when the set up time was over, the air would start to repeat the above process of air pulsation.

2.4. Inoculum preparation

The medium used for inoculum preparation contained potato extract and 2% glucose. Fifty milliliter of medium was taken in a 250 ml Erlenmeyer flask and autoclaved at 121 °C for 20 min. After cooling to room temperature, several flasks were inoculated and cultivated on a shaker at 28 °C and 150 rpm for 24 h.

2.5. Solid state cultivation

The solid state medium (SSM) was composed of steam-exploded rice straw, mixed with wheat bran (4:1, w/w) as carbon source. The moisture content of the SSM was adjusted to 75% (w/w, wet weight basis) by adding distilled water without pH adjustment. After heat sterilization (123 °C, 30 min), the SSM was added with inoculum of mycelial suspension (10%, v/w).

Five gram (dry weight) of SSM was added into a 250 ml Erlenmeyer flask. Several flasks were put in APP-SSF bioreactor or a static incubator for culturing at 28 °C. (When investigating the temperature variations caused by the microorganism metabolism, the cultivation temperature was not manipulated, and the room temperature was kept at 20 °C all the time.)

All the results were average of three repeated experiments, and the standard errors were less than 3.5%.

2.6. Enzyme extraction

After suitable periods of cultivation time, enzyme was extracted from the fermented carbon source with 10-fold (v/w) distilled water by shaking (150 rpm) at room temperature for 60 min. Solids were then separated from the solution by filtering through a nylon cloth sieve. The solution was centrifuged at 10,000 g for 20 min at 4 °C in a refrigerated centrifuge. The supernatant was collected and used as the source of crude enzyme.

2.7. Enzyme assay

FAE activity was assayed by analysis of free FA released from de-starched wheat bran (DSWB) (Johnson et al., 1988). The assay was carried out in 100 mM MOPS buffer, pH 6.0, 50 °C (Topakas et al., 2003). FA release was analyzed by HPLC using a Nucleosil C18 column. One unit (U) of enzyme activity was defined as the amount of enzyme which released 1 μmol of FA per min under the above assay conditions. All assay results were expressed on a dry weight basis.

2.8. Orthogonal design method

With the highest enzyme activity in the whole fermentation process taken as an index and low pressure intensity of 0 MPa (gauge pressure), three operation parameters of APP-SSF, including high pressure intensity (0.1 MPa, 0.2 MPa, 0.3 MPa), high pressure duration (10 s, 20 s, 30 s), and low pressure duration (10 min, 20 min, 30 min), were chosen as three factors in Orthogonal experiment. According to orthogonal table L_9 (3^4), 9 groups of experiments were arranged (Table 1).

Table 1
Orthogonal test of APP-SSF condition

Serial number of the experiments	(A) High pressure intensity (MPa, gauge pressure)	(B) High pressure duration (s)	(C) Low pressure duration (min)	The highest enzyme activity (mU/g)
1	0.1	10	10	491
2	0.1	20	20	590
3	0.1	30	30	701
4	0.2	10	20	851
5	0.2	20	30	881
6	0.2	30	10	786
7	0.3	10	30	549
8	0.3	20	10	535
9	0.3	30	20	517
I	1782	1891	1812	
II	2518	2006	1958	
III	1601	2004	2131	
R	917	115	319	

The low 2–10 of the columns 1–4 were the design of this orthogonal test, which contained 9 experiments. The column 5 was the result of each experiment. The last 4 lows were the traditional statistic analysis of the orthogonal test results. For example, the low I of the column 2 was the sum of the results of the experiments of high pressure intensity of 0.1 MPa; the low I of the column 3 was the sum of the results of the experiments of high pressure duration of 10 s; the low I of the column 4 was the sum of the results of the experiments of low pressure duration of 10 min. The low II and low III were the same. The low R was the maximal difference among the low I, low II, low III.