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Research Article

## Phytase Production by *Aspergillus niger* in Solid-State Fermentation using a Rotating Drum Bioreactor

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### ABSTRACT

Phytase production by *Aspergillus niger* in solid-state fermentation on mixed substrate was investigated at the rotational speed of 1 rpm and air velocity of 1 m/s using a 200 liter rotating drum bioreactor (RDB). Tapioca pulp and soybean pulp at the ratio of 4:6 with 60 percent initial moisture content were used as the main substrate. The effect of the rotational speed and air velocity on the substrate temperature, the substrate moisture content, growth and phytase production by *Aspergillus niger* were estimated. The highest growth and phytase production was obtained with the highest biomass concentration of 0.22 g/g substrate dry weight and the phytase production of 500 unit/g substrate dry weight. This knowledge will lead to the better operation, design and scale-up of solid-state fermentation of the bioreactor.

**Key words:** Solid-state fermentation, *Aspergillus niger*, Phytase, Rotating drum bioreactor.

### INTRODUCTION

Plant feedstuff is the major storage of phosphorus in the form of phytic acid and phytate, as found in seeds and cereals. Moreover, the phosphorus in the phytate form can be combined with cat-ion groups in the proteins, amino acids, starch and lipids in animal feed [22,28]. This complex molecule with essential nutrients for growth affects the utilization efficiency of minerals, proteins and nutrients. The source of animal feed is mostly composed of agro-industrial residue, which is a major storage of phosphorus in the form of phytic acid.

Phytase (myo-inositol hexaphosphate phosphohydrolases) is an enzyme which hydrolyses phytic acid in plants into myo-inositol phosphates and inorganic orthophosphates [11]. This enzyme is widely used in the animal feed industry to improve the availability of nutrients in animal feed, reduce anti-nutritional factors, reduce environmental pollution and reduce the feed cost. Commercial phytase is popularly derived from filamentous fungi, especially the *Aspergillus* species. *Aspergillus niger* is most commonly used for the production of phytase on a commercial scale [19,7].

Monogastric animals or non-ruminant animals (such as swine, poultry and fish) are unable to produce phytase at the gastrointestinal level. Thus, they are unable to digest phytic acid and unabsorbed phosphorus passes through the gastrointestinal tract. Therefore, monogastric animals can excrete phosphorous in the manure form, which can lead to phosphorous accumulation in the soil which causes environmental problems such as eutrophication. This problem affects future surface and ground water [26,35]. However, phytase is important for animal feed supplements to promote growth rate and animal performance as well as to reduce environmental problems.

The large scale bioreactor has difficulty controlling phenomena that occur in the system of solid-state fermentation (SSF). The phenomena in SSF depend on the microorganisms, the stage of growth, the substrate used, the bioreactor design and the operated bioreactor. However, the moisture content is a factor that is necessary for new cell synthesis, which will limit the diffusion rate of oxygen to the cell. The optimum moisture content in the SSF process depends on the nature of the substrate, microorganisms and product formation [12,20,14,3]. High temperature affects spore

germination, cell growth and product formation. The temperature and moisture content for cell growth is essentially controlled in the bioreactor, is one of the main problems in the large scale and affects fungi growth.

The substrate temperature and moisture content are critical for growth and enzyme production of *A. niger* in SSF. However, the metabolic heat generated during cell growth can lead to heat accumulation in the bioreactor, which causes high temperatures in the substrate bed. Therefore, the high temperature due to the metabolic heat generated must be inhibited by cell growth and enzyme formation during the SSF process in the bioreactor. The mathematical model was applied to control the heat transfer during SSF [21,26,15,30]. Hence, heat removal and oxygen diffusion through the substrate bed are important in controlling the performance of rotating drum bioreactors [27,6,31,10]. Heat removal can be applied to solve the major problem of heat accumulation and control the optimal temperature of substrate in RDB. Therefore, the air velocity and the rotational speed of the bioreactor are considered to be able to maintain the temperature and the moisture content during fermentation in the rotating drum bioreactor (RDB). High temperature can potentially inhibit cell growth and enzyme production of *A. niger* in SSF. In the case of SSF, the optimal temperature of growth and enzyme production by *A. niger* was within the range of 25-38 °C and the substrate moisture content ranged from 40-80% [16,18,33,9,8]. A previous study has been done by Saithi *et al.* [24], who observed the highest growth and phytase production of *A. niger* when cultured using the substrate moisture content of 60% at 30 °C. In the current work, we investigated the effect of air velocity on growth and phytase production of *A. niger* in SSF using a 200 L RDB. Specifically, the inlet air velocity was used to control the substrate temperature and the moisture content of the substrate in a RDB at the optimal level for maximum growth and phytase production by *A. niger*.

## Materials and Methods

### 1. Microorganism and spore preparation:

A wild type strain of *Aspergillus niger* was obtained from the Division of Biotechnology (King Mongkut's University of Technology Thonburi, Thailand). The culture was maintained on potato dextrose agar (PDA) at 30 °C for 5 days and stored at 4 °C until used. The spores were harvested from the surface by adding a sterile 0.10% Tween 80 (w/v) solution and scraping the surface with a sterile spatula. The spore suspension was measured with a haemocytometer and adjusted to  $2 \times 10^7$  spores/ml.

### 2. Rotating drum bioreactor (RDB):

The solid-state fermentation was carried out in a 200 L RDB, which is a horizontal stainless steel bioreactor of 2 mm thickness, 50 cm diameter, 100 cm length and internal volume capacity of 200 liter. The RDB is equipped with an air compressor to control the inlet air velocity, a motor to control rotation speed, thermocouples to monitor the substrate temperature, and air temperature at 3 positions along the length of the bioreactor. The process parameters were performed using the LabVIEW7 Express program.

### 3. Culture conditions:

The tapioca pulp and soybean pulp (residue from the production of soybean milk) at the ratio of 6:4 by weight were used as the substrate. The moisture content of the mixed substrate was adjusted to about 60%. The mixed substrate was added to a RDB at 10% of the total volume and steamed at 100 °C for 2 hours. Then, the substrate was cooled and mixed with 10% of the spore suspension (w/w) and fermented at 30 °C by the temperature of the air inlet bioreactor. The RDB during fermentation was controlled by the inlet air velocity of 0.3-1.0 m/s and the rotational speed of 1 rpm. During fermentation, samples were taken in triplicate for measurement.

### 4. Moisture content determination:

The moisture content of the culture medium was determined by drying in a hot-air oven at 80 °C to derive a constant weight.

### 5. Biomass determination:

The biomass concentration is an estimation of the ratio of the glucosamine content to the dry weight of the fungal biomass. Therefore, the glucosamine content was converted to the fungal biomass. The glucosamine content was determined by the colorimetric reaction using the method of Aidoo *et al.* [2]. The results were expressed as mg glucosamine per gram dry weight.

### 6. Phytase assay:

The phytase activity was examined by measuring the amount of inorganic phosphorus released from sodium phytate using the method of Engelen *et al.* (1994). One unit of phytase activity was defined as the amount of phytase required to release 1 µg of inorganic phosphorus in 1 minute at 37 °C, pH 5.50.

## Results and Discussion

### 1. Effect of the inlet air velocity on phytase production:

The various inlet air velocities on growth and phytase production of *A. niger* in a 200 L RDB are summarized in Table 1. In all cases, the effect of different inlet air velocities of 0.30-1.00 m/s and the bioreactor rotation speed of 1 rpm on the growth and phytase production in a 200 L RDB for a total period of 72 h were investigated. At 42 h, the maximum phytase production was 500 unit/g substrate dry weight at the inlet air velocity of 1 m/s and the bioreactor rotation speed of 1 rpm. The phytase production of *A. niger* by SSF on the mixed substrate decreased at different inlet air velocities in a 200 L RDB.

The optimum air flow rate in the SSF process depends on the microorganisms, the oxygen

requirement for product formation, the thickness and the porosity of the substrate, the removal of heat and the removed carbon dioxide from the bioreactor [13]. The maximum growth and phytase production of *A. niger* on the mixed substrate were observed at the highest air velocity using a 200 L RDB.

The rotation of the bioreactor was performed to improve oxygen, remove carbon dioxide and remove the metabolic heat generated during SSF [17,3]. In addition, the mixing affected the shear force, which damaged fungal mycelia and reduced the porosity of the substrate. The lack of a rotation system can lead to heterogeneous conditions during inoculation and fermentation.

**Table 1:** The effect of the inlet air velocity on the maximum phytase activity and biomass of *A. niger* using a 200 L RDB.

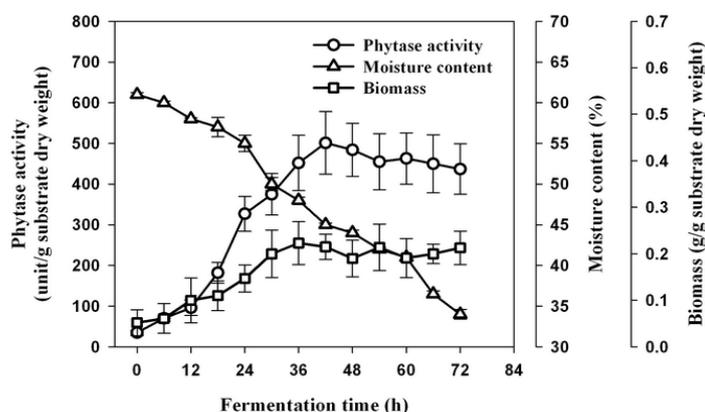
Air velocity (m/s)	Phytase activity (unit/g substrate dry weight)	Biomass (g/g substrate dry weight)
0.30	248±75	0.17±0.02
0.60	395±86	0.20±0.06
1.00	500±102	0.22±0.07

For the time course of phytase production, the biomass and moisture content at the inlet air velocity of 1m/s and the bioreactor rotational speed of 1 rpm using a 200 L RDB are shown in Fig. 1. These profiles were estimated at the best aeration for the highest phytase production. In this study, the profile of phytase production increased slowly until 12 h, after which the phytase production started to increase sharply, reached about 500 unit/g substrate dry weight at 42 h of fermentation and then remained constant until the end of fermentation. This increase of phytase production for *A. niger* during the SSF process followed the same profile of the growth, which is associated with growth.

The biomass is defined as the indicator parameter of the cell growth of *A. niger*. In this study, biomass concentration at the surface increased with the time. The biomass increased sharply until 42 hours, after which he biomass started to remain stable at approximately 0.22 g/ g substrate dry weight

(Fig. 1). A similar increase was also observed in phytase production.

In contrast, the moisture content, adjusted to 60% at the beginning of the fermentation, decreased slowly until 24 h, after which it started to decrease sharply and reached approximately 45% moisture content at 42 h. However, this level of moisture content is still suitable for the growth and enzyme formation by *A. niger* on SSF. This agrees well with the results of Acuna-Arguelles *et al*. [1] and Saithi *et al*. [24], who noted that the optimum moisture contents for the growth of *A. niger* were within the range of 40-60%. The moisture content of the substrate decreased during the exponential phase of fermentation loss with evaporation cooling due to high temperatures in the system. However, the optimal substrate moisture content and temperature of *A. niger* during SSF agrees with results for phytase production were within the range of 40-74% substrate moisture content and temperature at 28-34 °C [4,34].

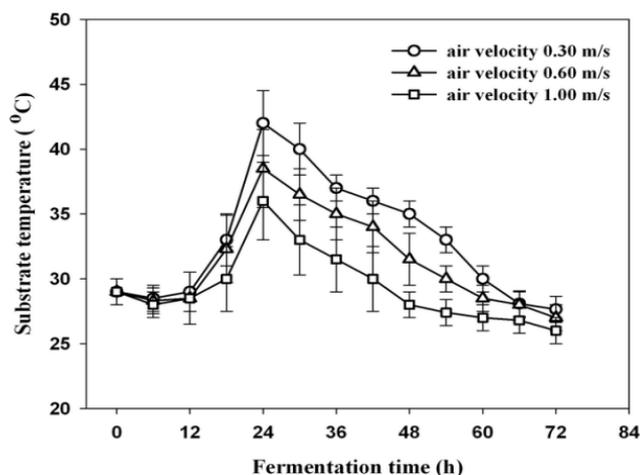


**Fig. 1:** Time profile of phytase production, biomass and moisture content of *A. niger* at the inlet air velocity of 1m/s and rotation speed of 1 rpm using a 200 L RDB.

## 2. Effect of the inlet air velocity on the substrate temperature:

The time course of the substrate temperature at different inlet air velocities using a 200 L RDB is compared in Fig. 2. In all cases, the substrate temperature profiles during fermentation had similar patterns. The substrate temperature started to increase sharply and reached the peak temperature at 24 h. After that, the substrate temperature slightly decreased and remained constant until the end of fermentation. The substrate temperature depended on the inlet air velocity. The maximum substrate temperature measured during fermentation was recorded at 36 °C with the air velocity of 1 m/s, 38 °C with the air velocity of 0.6 m/s and 42 °C with the air velocity of 0.3 m/s, respectively. In previous studies, the air flow rate had an effect on the temperature and moisture content of the substrate in SSF using the bioreactor [25,23,32].

The heat generated due to cell growth can increase the temperature and heat accumulated in the substrate bed during fermentation in a RDB. Hence, the high temperature must have inhibited cell growth and led to a decrease in phytase production. Therefore, the temperature in the system can be controlled by using the air velocity during fermentation. The inlet air velocity is an important factor that must be considered in the scale-up of the bioreactor. In addition, the air velocity was required to maintain the substrate temperature and cool the system during fermentation. However, the air flow rate was unable to remove all the metabolic heat from the bioreactor. Increasing the air velocity can reduce the temperature in a RDB. In addition, the air flow through the bed can help promote evaporation of water from the bed. In contrast, the moisture content of the substrate was rapidly decreased from 60% to 35% at the end of fermentation, which occurred by evaporation of water during exponential growth.



**Fig. 2:** The substrate temperature profiles during SSF of *A. niger* in a 200 L RDB at different inlet air velocities.

The results obtained in this study show that the optimal growth and phytase production of *Aspergillus niger* in solid-state fermentation on mixed substrate was investigated at the rotational speed of 1 rpm and air velocity of 1 m/s using a 200 L RDB. Although this study shows a considerable the optimal phytase production, further study focuses on the mathematical model of heat and mass transfer phenomena occurring in the various rotating drum bioreactors.

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