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Influence of Agitation Speeds and Aeration Rates on the Xylanase Activity of *Aspergillus niger* SS7

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ABSTRACT

In this study, the effect of agitation and aeration rates on xylanase activity of Aspergillus niger *SS7 in 3-litre stirred tank bioreactor was investigated. The agitation rates tested were 100, 200 and 300 rpm at each airflow rates of 0.5, 1.0 and 1.5 vvm. The maximum xylanase activity in mono- agitator system was at the agitation speed of 200 rpm and aeration rate of 1.0 vvm. In bi-agitator system, at low agitation speed (100 rpm), the xylanase activity was enhanced by 13% compared to mono- agitator system for an aeration rate of 1.0 vvm. Xylanase productivity in continuous culture was higher by approximately 3.5 times than in batch culture.*

Key words: *Aspergillus niger*, agitation, aeration, xylanase

INTRODUCTION

Xylanolytic enzymes are a group of enzymes that hydrolyze xylan and arabinoxylan polymers. This enzyme group includes endo-β-1,4-xylanase, β-xylosidase, arabinofuranosidase and acetylxylan esterase (Biely, 1993). Xylanolytic enzymes are produced by a wide variety of microorganisms, among which the filamentous fungi are especially interesting as they secrete these enzymes into the medium and their xylanase activities are much higher than those found in yeast and bacteria (Guimarães et al., 2006). Endo-β-1, 4-xylanase plays important roles in the animal feed, increasing the body weight gains of the animals (Medel et al., 2002). In pulp and paper industry, xylanase are employed in the prebleaching process to reduce the use of the toxic chlorine chemicals (Wong et al., 2000). In bread and bakery industry, xylanases are used to increase the dough viscosity, bread volume, and shelf life (Romanowska et al., 2003). Other potential applications of xylanases include the conversion of xylan in wastes from agriculture and food industries into xylose, and the production of fuel and chemical feedstocks (Sunna and Antranikian, 1997).

Aeration and agitation are basic problems of fungal aerobic fermentation processes. Aeration of growing microbial culture is undertaken primarily to supply its oxygen requirements and at the same time to remove waste products. The necessary oxygen for the growth and production of fungal culture can be ensured by agitation and aeration of the culture. The efficiency of aeration can be improved by agitation, resulting in an increased interface between gas and liquid. It is known that the intensive flow of liquid, caused by agitation, forces the air bubbles to disintegrate into a large number of small bubbles. An additional beneficial effect of agitation is to diminish the size of

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mycelial aggregates, making oxygen more easily accessible to the cells (Jafari et al., 2007).

The enhancement of xylanase production with increase of aeration rates has been was reported by Hoq et al., (1994) and Reddy et al., (2002). It is appropriate combination of impeller speed and aeration rate which is more important for the enhancement of specific xylanase production (Gaspar et al., 1998; Bakri et al., 2002). When designing the industrial production processes, these different behaviors must be defined in the laboratory in order to achieve the optimal production conditions.

In the design of a fermentation process, many operation parameters, such as agitation and aeration, pH, dissolved oxygen, and inoculum levels have to be investigated (Jüsten et al., 1996). Among them, agitation and aeration rates are the most critical parameters used for process scale-up (Felse and Panda, 2000) and play significant roles in determining the productivity of the process (Jüsten et al., 1996).

The major goal of this work was to enhance the xylanase production by different optimization techniques. In the first phase, a study was carried out to examine the effect of agitation and aeration conditions on the production level of xylanase.

MATERIALS AND METHODS

Microorganism

Aspergillus niger SS7 was isolated from the local soil, and identified by the Paris Natural History Museum (PNHM), Paris, France. It was previously reported as xylanase producing strain in submerged culture (Bakri et al., 2008). The stock cultures were maintained on potato-dextrose-agar (PDA) at 4 $^{\circ}$C.

Culture Medium

The culture medium used for xylanase production in submerged culture composed of 30 g/L of corn cob hulls in mineral salt medium. The mineral salt medium contained (%, w/v) K_2HPO_4 0.15, MgSO4. $7H_2O$ 0.05, peptone 0.2 and yeast extract 0.4. For comparison between the batch and continuous culture, soluble sugars extract from corn cob hulls was used. In order to prepare the hyrolysate, 10% (w/v) of milled corn cob hulls was soaked in distilled water at 30°C for 24 h. The liquid hydrolysate obtained after the centrifugation was used as a carbon source for xylanase production. The nitrogen source and mineral salts were used as the same in submerged culture. The pH in all the experiments was adjusted to 6.5 before sterilization.

Bioreactor

A 3-L -lab fermenter (Electro-lab limited, UK) was used as the base mechanical vessel for xylanase production by *A. niger* SS7. The agitation system in the fermenter was made of Rushton turbine DT6 whose diameters (d) was 50 mm. The clearance above the base of the bioreactor and the distance between the two impellers were equal to turbine diameter (d). The bioreactor was filled with 1.5 L of culture medium and then sterilized by autoclaving at 121°C for 20 min. A regulation system was used to control the temperature at 30°C throughout of the experiment after sterilization. Foaming was controlled with the addition of 0.05% (v/v) Tego antifoam KS911 (Goldschmidt, Essen, Germany).

The bioreactor was inoculated with a 5% (v/v) of inoculum under aseptic conditions. The inoculum was prepared in a 250-mL Erlenmeyer flask in the medium containing 2% glucose, 1% yeast extract, and 1% peptone. The inoculum had a spore concentration of 10^6 spores/mL. The incubation was carried out at 30°C under shaking (150 rpm) for 48 h. The spore concentration in the suspension was determined in a Neubauer counting chamber by microscope. The air flow rates studied were 0.5, 1.0, and 1.5 vvm at agitation speeds of 100, 200 and 300 rpm corresponding to the following peripheral speeds of 0.26, 0.52 and 0.79 m/s, respectively.

Xylanase assays

Xylanase activity was determined according to Bailey et al. (1992) using 1% birchwood xylan in 0.05 M citrate buffer (pH 5) as substrate. The xylan solution and the enzyme at appropriate dilution were incubated at 55°C for 5 min and the reducing sugars were determined by the dinitrosalicylic acid procedure (Miller, 1959), with xylose as standard. The realeased xylose was measured spectrophotometrically at 550 nm. One international unit (IU) of enzyme activity is defined as the amount of enzyme releasing 1 µmol xylose/mL in 1 minute under the described assay conditions. Results given are the mean of triplicate experiments.

RESULTS AND DISCUSSION

Effect of agitation speeds and aeration rates on xylanase activity

The results obtained after 96 h of culture in mono-agitator system at different agitation speeds for aeration rates of 0.5, 1.0 and 1.5 vvm are presented in Fig. 1. At all the peripheral speeds tested, improvement in xylanase activity was observed when aeration rates increased from 0.5 to 1.0 vvm. Increases in the aeration rate more than 1.0 vvm resulted in reduced enzymatic activity. The optimum xylanase activity of 162 (IU/mL) was obtained in 1.0 vvm aeration rate at 0.52 m/s peripheral speed. At high peripheral speed (0.79 m/s), the decrease in xylanase production has been attributed to the effect of hydrodynamic stress, which may cause hyphal disruption and leakage of intracellular compounds (Chipeta et al., 2008). The harmful effect of the shear forces as a result of agitation intensity has been reported to cause decreased enzyme production in some filamentous fungi (Palma et al., 1996; Lenartovicz et al., 2003; Techapun et al., 2003).

Xylanase activity in 1.5 vvm was higher than 0.5 vvm aeration rate. This indicated that low aeration rate had more negative effect on xylanase activity than high aeration rate. However, it has been reported that improving aeration rate has a positive effect on enzyme activity from aerobic microorganism (Bakri et al., 2002; Jafari et al., 2007; El-Enshasy et al., 2008).

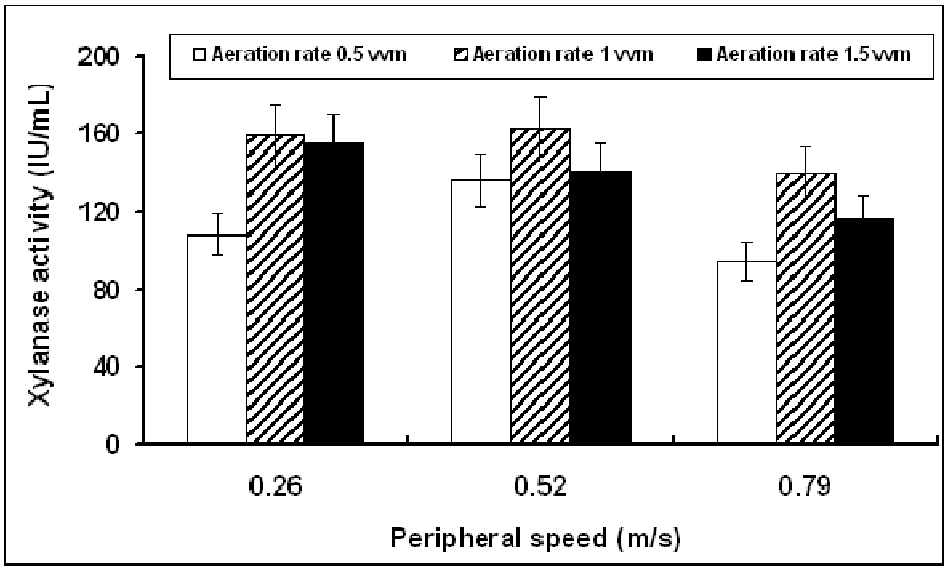

Figure 1 - Xylanase activity (IU/ml) after 96 h in batch cultivation of *Aspergillus niger* SS7 in a 3-L bioreactor fitted with DT6, at different peripheral speeds. The aeration rates were 0.5, 1.0 and 1.5 vvm.

These results agreed well with the results reported by Reddy et al. (2002), where an aeration rate higher than 0.75 vvm decreased xylanase production by *Thermomyces lanuginosus* SSBP. Similar results were obtained with *Thermomyces lanuginosus* RT9 by Hoq et al. (1994) who observed an increase in β-xylanase production with increasing aeration to 1.0 vvm. However, when aeration was increased to 1.5 vvm, β-xylanase titre decreased 1.5-fold. Also, Techapun et al. (2003) found that an increase in the aeration rate from 0 to 1.0 vvm increased the xylanase production from *Streptomyces sp.* Ab106. However, when the aeration rate was increased to more than 1.0 vvm, xylanase production gradually decreased. The higher enzyme inactivation in highly aerated culture may be due to irreversible oxidation of amino acid residues of the enzyme structure (Cabiscol et al., 2000).

In bi-agitator conditions, the influence of airflow rate and agitation speed on xylanase production was studied at 0.26 and 0.52 m/s peripheral speeds and three different airflow rates (0.5, 1.0 and 1.5 vvm) in fermenter mounted with two turbines

DT6–DT6. Xylanase production after 96 h of culture is shown in Fig. 2. In bi- agitator conditions**,** xylanase production was higher at 0.26 m/s than 0.52 m/s for 0.5, 1.0 and 1.5 vvm. The maximum xylanase production was obtained at 0.26 m/s for 1vvm (182 IU/mL) after 96 h of culture. At 0.52 m/s, higher shear force might have caused lower xylanase production (Fig. 2).

In bi-agitator system, at low peripheral speed 0.26 m/s, the xylanase production enhanced by 13% in comparison with mono-agitator system for an aeration rate of 1.0 vvm (Fig. 3). Multiple impeller systems are more efficient in mass transfer than the single-impeller. Single-impeller stirred tanks are often criticized for the uneven distribution of shear and energy dissipation which are known to be harmful, especially to the microorganisms in the bioreactors. Hence, multiple impeller systems should be favored where microorganism is sensitive to shear effect (Gogate et al., 2000).

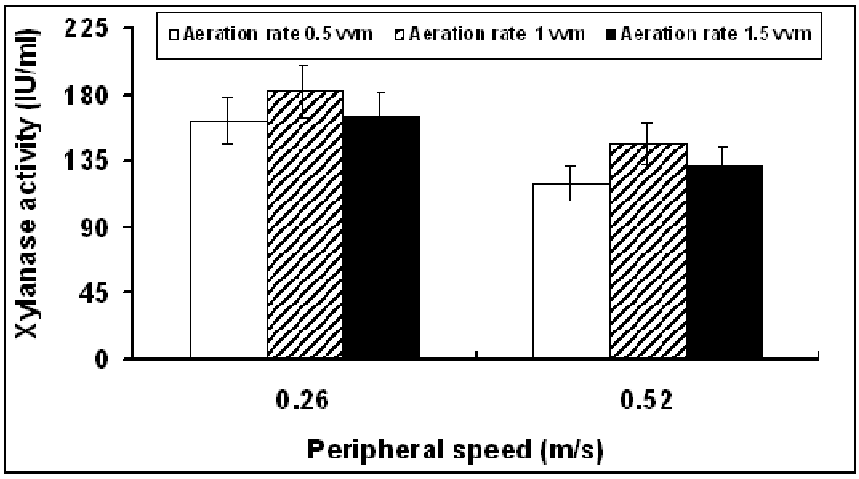

Figure 2 – xylanase activity (IU/ml) after 96 h in batch cultivation of *Aspergillus niger* SS7 in a 3-L bioreactor fitted with two DT6 at different aeration rates. The peripheral speeds were 0.26 and 0.52 m/s.

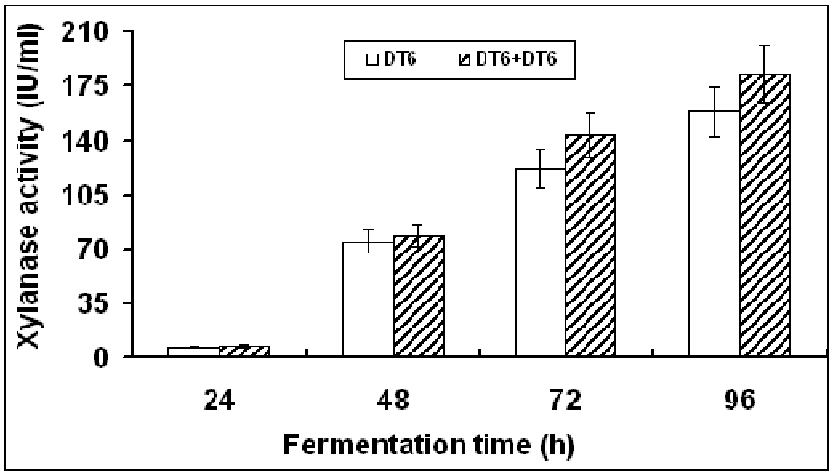

Figure 3 - Evolution of xylanase activity (IU/mL) during batch culture of *Aspergillus niger* SS7 in a 3-L bioreactor fitted with one or two DT6 at 1.0 vvm and 0.26 m/s peripheral speed

Batch and continuous culture

Using the optimum conditions of agitation and aeration selected from pervious experiments, batch and continuous culture were carried out on a corn cob hulls hyrolysate medium. Xylanase productivity from *A.niger* SS7 in batch and continuous processing is shown in Fig. 4. It was observed that enzyme productivity obtained in continuous culture (0.255 IU/ml/h) was significantly higher than that obtained in batch culture (0.077 IU/ml/h) by approximately 3.5 times. These results confirmed the benefit of this culture type for xylanase production. Many researchers have reported the increase of the enzyme productivity in the continuous culture compared with the batch one (Mamo and Gessesse, 2000; Cheng et al., 1989; Montesinos et al., 1996).

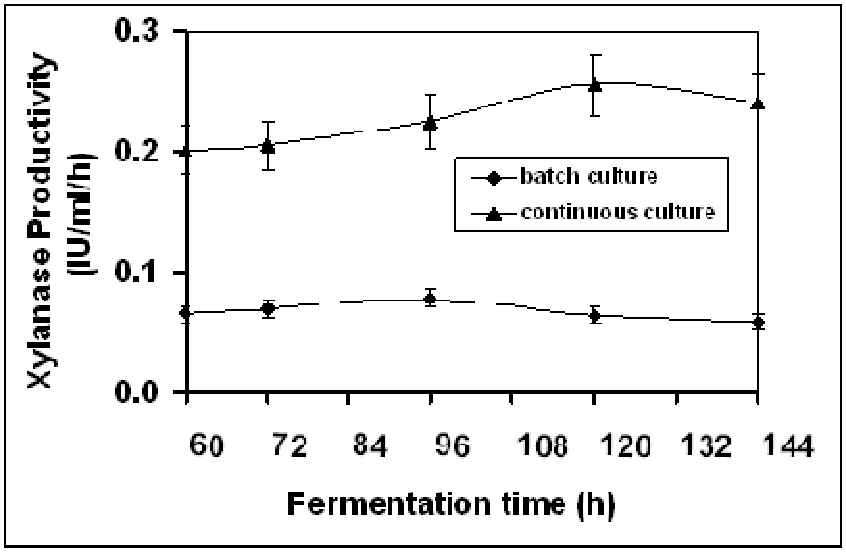

Figure 4 - Evolution of xylanase productivity (IU/mL/h) during batch and continuous liquid culture of *Aspergillus niger* SS7 in a 3-L bioreactor fitted with two DT6 at 1.0 vvm and 0.26 m/s peripheral speed.

CONCLUSION

Mixing is very crucial for the maximum productivity in microbial fermentation and it could be achieved by means of aeration and agitation. But agitation at higher stirring speeds may cause disruption of free cells in the reactor by forces and formation of vortex which may result in poor mass transfer (oxygen/substrate). Therefore, it is important to provide optimum combination of aeration and agitation in free cell batch bioreactor operation. From the results, it could be concluded that agitation and aeration had a significant effect on xylanase production. The maximum xylanase production was obtained at 0.26 m/s for 1vvm in DT6-DT6 combination system, and the xylanase productivity in continuous culture was higher by approximately 3.5 times than in batch culture.

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