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# Screening of Process Parameters to Produce Xylanase from *Aspergillus niger* for Secondary Bioethanol Production

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**Abstract.** In recent years, the biotechnological use of xylanases has grown remarkably. Xylanase is a hydrolytic enzyme with a broad industrial application. In specific, xylanase can convert xylan into xylose, a fermentable sugar source for secondary bioethanol production. The objective on this study is to investigate the significance of different parameter effects for an efficient xylanase production from *Aspergillus niger* (*A. niger*). In this study, four factors: incubation temperature, medium pH, incubation time, and agitation speed were screened by performing One-factor-at-a-time (OFAT) analysis. Xylanase production with the maximal enzyme activity was successfully obtained from OFAT analysis under condition of 32 °C, pH 5.0, 5 days, and 150 rpm.

## 1. Introduction

Agricultural and forest industries produce hundred-million tons of lignocellulose wastes yearly, which contain approximately 30–40% of hemicellulose in dry weight [1]. Due to improper handling and lack of technical know-how, these waste substances could be a cause of environmental pollution [2]. Many processing industries which are generating agro-waste substances or by-products are struggling to challenge for their conversion into value-added products. Therefore, enzyme, carbo-hydrolase is the excellent alternative for conversion of cellulose and hemicellulose into value added products [3]. From many research developed, the use of xylanases promised such processes to be run with fewer chemicals, under less harsh conditions, and with less severe side reactions [4]. Xylanases can be included in bioconversion of lignocellulosic material such as wheat bran, rice husk, rice straw, sugarcane bagasse and other agro-wastes into fermentative products [5]. This process can occur under simultaneous saccharification and fermentation. This application can recycle the wasted biomass into



useful energy such as bioethanol. Thus, it is indirectly minimizing the amount of waste from industry [6].

Yet, the overly priced commercial xylanases are known to be a bottleneck for the secondary bioethanol production. Furthermore, the cost of enzyme production is one of the predominant factors determining the economics of a process [7]. Reducing the costs of enzyme production by optimization of the fermentation medium and the process is the goal of basic research for industrial application. The microorganisms used for xylanase production must be non-pathogenic, capable of producing high levels of extracellular enzymes and most important is it can be cultivated very easily. Studied data have shown that xylanases can be produced by a diverse variety of microorganism [8]. From an industrial point of view, filamentous fungi such as *A. niger* is a preferred producer of extra-cellular xylanase enzyme as compared to bacteria and yeast [9]. Moreover, the mentioned fungi also produce numerous auxiliary enzymes, which are fundamental for debranching of the substituted xylan [9]. However, research on the screening condition of *A. niger* in submerged state fermentation to produce maximum xylanase is limited. Hence, it is important to study on the screening process for a better knowledge on the factors that have a significant role in maximizing the xylanase production from *A. niger*.

## 2. Material and Methods

### 2.1. Materials

All the chemicals used in this research work were analytical grade. These chemicals were purchased from Sigma-Aldrich, St. Louis, MO USA except otherwise stated.

### 2.2. Microorganism Maintenance

Fungal strain identified as *A. niger* was obtained from Universiti Tunku Abdul Rahman, (UTAR). The strain was maintained on Potato Dextrose Agar (PDA) slants by monthly transfer and was stored at 4°C.

### 2.3. Inoculum Development

A 10 mL of 1% (v/v) Tween-80-sterile distilled water was added to a 5 days old pure *A. niger* culture on agar plate and the agar surface was rubbed with a sterile L-shaped spreader to obtain spore suspension. The suspension was sieved through Whatman no.1 paper to remove mycelia. After appropriate dilutions, inoculum sizes ( $2 \times 10^6$  spores/mL) were determined by direct microscopic observation using hemocytometer.

### 2.4. Submerged Fermentation (SmF)

Xylanase biosynthesis were performed in a 250 mL Erlenmeyer flask using SmF in a production medium containing 100 mL of Mandels and Sternburg's basal medium and 5.0 g/L of xylan as carbon source. Composition of the growth medium includes: 2.0 g/L  $\text{NH}_4\text{NO}_3$ , 2.0 g/L  $\text{K}_2\text{HPO}_4$ , 1.0 g/L  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.3 g/L  $\text{CaCl}_2$ , and 0.012 g/L trace elements: 5.0 mg/L  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.6 mg/L  $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ , 3.45 mg/L  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  and 2.0 mg/L  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  and 0.1% (v/v) Tween 80. The medium was sterilized at 121°C for 15 mins. Aliquot of 1mL of standardized *A. niger* inoculum ( $2 \times 10^6$  spores/mL) was aseptically inoculated into the medium and fermentation was allowed to take place at different selected incubation temperature, incubation period and agitation speed. Crude enzyme was extracted with 0.05 M citrate buffer pH 4.8 (50 mL). After the addition of the buffer, the whole flask was kept in a rotary incubator at 150 rpm (32°C) for 15 min. The cell was filtered through Whatman no.1 filter paper to extract the filtrate which was then centrifuged at 10,000 rpm for 15 min at 4°C. The filter paper was dried at 60°C for three consecutive days in an oven (Memmert, Germany). Mycelial Dry Mass per Volume (MDMV) was determined by weighing the filter paper on an analytical balance (Sartorius, Germany). The supernatant was collected as the crude enzyme for xylanase activity analysis [10].

### 2.5. Screening Factors Affecting Xylanase Production

Factors that affect xylanase production was screened by performing one-factor-at-time (OFAT) experiments, which vary only one factor or a variable at a time while keeping others fixed. Four parameters that manipulated were incubation temperature, medium pH, agitation speed, and incubation time. Each parameter was varied into a few sets of experiments. The pH used in this study was 3.0, 5.0, 7.0 and 9.0. Meanwhile, the temperatures used were at 25, 28, 32, and 37°C respectively for the screening study. Medium agitation speed used were 50, 100, 150, and 200 rpm. The culture incubation time was varied from day intervals. The temperature, pH, incubation period and agitation speed were kept constant at 32°C, pH 5, 5 days and 150 rpm, respectively when not set as a manipulative variable.

### 3. Result and Discussion

Screening analysis was done via OFAT technique to determine the parameters that significantly influenced the xylanase production. The effect of temperature on xylanase production in the range of 25 to 37°C was examined (Figure 1(a)). The temperature of the medium has governed role in a fermentation process. An extremely low or high temperature setting could denature microbial growth and enzyme synthesis [11]. Figure 1(a) indicates that mycelial dry mass per volume (MDMV) of *A. niger* increased from 25°C, until optimal MDMV of 0.270 mg/mL was attained at 32°C. In common, the temperature with the highest growth of fungi results the maximal xylanase production [12]. The enzyme activity displayed a similar trend as the MDMV toward the incubation temperature. The highest enzyme activity (0.524 U/mL) was detected at the optimal *A. niger* growth temperature of 32°C.

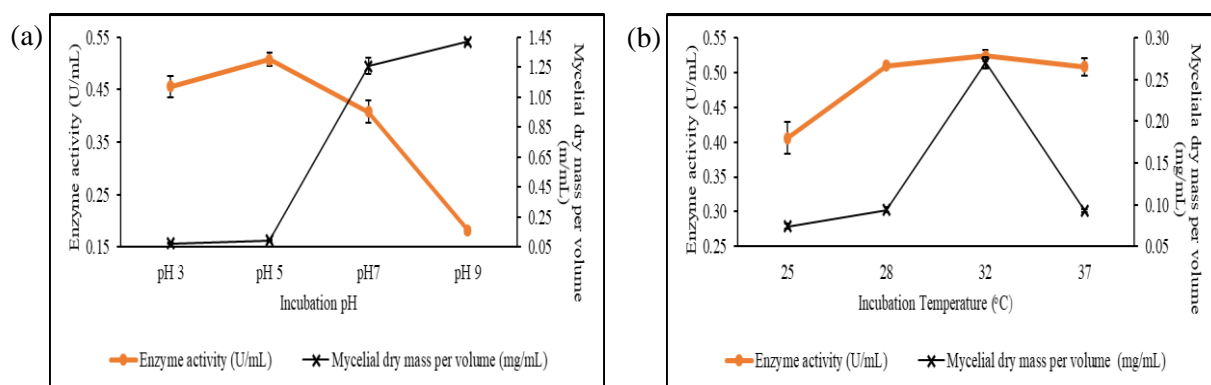


Figure 1: (a) Effect of incubation temperature on xylanase production and (b) Effect of incubation pH on xylanase production

pH is a key parameter in most biological processes and have various effects on the biomass, including on the pellet formation, the growth of biomass; synthesis of extracellular enzyme, its secretion and the stability of the enzymatic system [13]. The result of different medium pH on enzyme production by *A. niger* is illustrated in Figure 1(b). From Figure 1(b), it can be concluded that pH 5.0 was the most favourable xylanase production condition as highest enzyme activity (0.508 U/mL) was recorded. It can be also observed that MDMV at alkaline pH (7 and 9) was greater than at pH 5 but this response did not boost the enzyme activity. This was due to the fact that an alkaline fermentation media can affect enzyme stability, which caused enzyme denaturation occurred [9].

Figure 2(a) illustrates the effect of the incubation time on the xylanase production. The enzyme activity was at its peak of 0.675 U/mL with maximum MDMV (0.083 mg/mL) when the incubation time was prolonged to 5 days. This was due to the fact that *A. niger* achieved its maximum growth in the stationary phase, which is approximately after 5 days [11]. Subsequently, the production of xylanase was at optimum state. There was a sharp decrement in MDMV at the incubation was continued for 7

and 9 days as the growth of the *A. niger* was entering death phase whereby there will be poor availability of oxygen and toxic accumulation in the fermentation media [14].

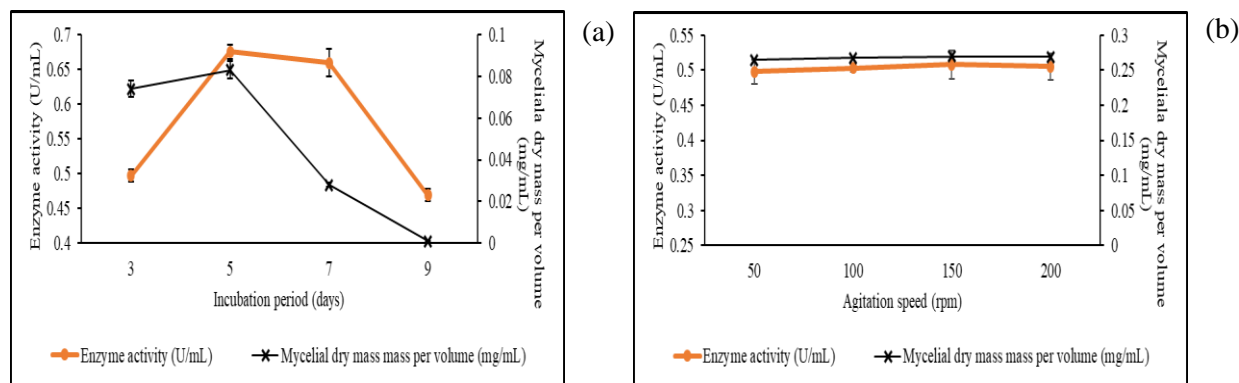


Figure 2: Effect of incubation time on xylanase production and (b) Effect of agitation speed on xylanase production

Last but not least, the effect of agitation speed was selected to be investigated. This is because the agitation speed has an influence on the mixing extent and nutrient availability in the xylanase production media was investigated [15]. Figure 2(b) shows that the effect of the agitation speed on the xylanase production. From the Figure 2(b), it can be seen that the enzyme activity and MDMV increased with the increase in agitation until the optimal reading of 0.508 U/mL and 0.270 mg/mL, respectively was achieved at 150 rpm. Additionally, a slight decrease was observed for the MDMV (0.269 mg/mL) and enzyme activity (0.505 mg/mL) when the agitation speed was further raised to 200 rpm. The adequate aeration and nutrient such as dissolved oxygen (DO) by increased agitation speed might cause the fungus to grow well, but shear forces and cell damage due to collision among the fungal pellets finally had a negative effect on enzyme production [16].

#### 4. Conclusion

This research work focused on evaluation of the effect of process parameters for xylanase production. There were four main parameters that affect the xylanase yield from *A. niger* in production medium which were incubation temperature, medium pH, incubation period, and agitation speed. The highest enzymatic activity was recorded at 32 °C (0.525 U/mL), pH 5.0 (0.508 U/mL), 5 days (0.675 U/mL), and 150 rpm (0.508 U/mL), respectively. Finally, it can be concluded that through OFAT analysis, incubation temperature and pH were the two most influential parameters on the xylanase production process. The notable deviation in enzymatic activity within the range set for incubation temperature (25, 28, 32, and 37°C) and pH (3, 5, 7 and 9) contributes the significance of these parameters. Nevertheless, the results obtained from OFAT analysis was only a surface guidance to individually recognize the specific optimal enzymatic activity and significance of each analyzed parameter. Hence, in future, optimization based research work should be conducted for the xylanase production through a Response Surface Methodology (RSM). With this, the combined interaction effect of the parameters and an overall optimized condition for maximized xylanase production to be aided in xylan to bioethanol conversion could be identified.

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