

Preliminary Study on Factors that Enhanced the Production of Uricase by *Aspergillus Flavus*

M. Siti Hatijah and W. R. Wan Ruhayu

Abstract—Urate oxidase or uricase is an enzyme that catalyses the oxidation of uric acid to allantoin and plays important role in purine metabolism. The first important application discovered for uricase was in clinical biochemistry as diagnosis reagent for measurement of uric acid in blood. The precipitation of uric acid can leading to gout symptom. The main purpose of this research is to optimize the culture condition for maximum uricase production by *Aspergillus flavus*. The parameters studied were pH (ranged pH4 to pH8), the sucrose concentration (10g/l to 50 g/l) and the agitation rate (100 to 300 rpm). The *Aspergillus flavus* were inoculated in yeast extract with sucrose and incubated for 24 hours at 200 rpm and 30°C. The maximum enzyme activity obtained from the experiment is 0.03974 U/ml at pH 6, 200 rpm and 30 g/l sucrose concentration.

Index Terms— *Aspergillus flavus*, gout, uric acid, uricase.

I. INTRODUCTION

In the past few decades, gout has markedly increased in incidence and prevalence in Malaysia and elsewhere. Gout patients nowadays are more clinically complex than in past due to combinations of advanced age and drug-drug interactions [1]. Fortunately, recent research on novel advances in treatments highlights how gout can be better managed with cost effective and well established therapies using therapeutic enzyme called urate oxidase.

Urate oxidase (uricase) is an enzyme found in liver peroxisomes of most mammalian species, converts uric acid to a more soluble and easily excreted compound, i.e., allantoin [2]. Although this enzyme is widely present in most vertebrates, it was found absent in human [3]. When the level of uric acid increases in blood over than the normal value it can cause renal failure and may contribute to a group of diseases such as gout, leukimia, toxemia of pregnancy, severe renal impairment and idiopathic calcium urate nephrolithiasis [4]. The first application of uricase in clinical biochemistry is as diagnostic reagents for measurement of uric acid in blood and other biological fluids [5]. Purified uricase from *Asperfillus flavus* has been found effective in the treatment of hyperuricemia and gout [6].

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II. METHODOLOGY

A. Culture Condition of Fungi

The *Aspergillus flavus* strain use in this research was obtained from Malaysian Agricultural Research and Development Institute (MARDI). The cultures are maintaining on potato dextrose agar (PDA) and stored at 5 °C.

B. Inoculum Preparation

Cultures were transferred in to 250 ml conical flasks containing 100 ml aliquots which are 1% of yeast extract, 10% sucrose and distilled water. It was incubated in an incubator shaker operating 200 rpm at 30 °C for 24 hours. At the end of incubation, 1.5 ml of the culture was monitored at 540 nm using spectrophotometer. The optical density was set at 0.60.

C. Fermentation Medium

Inoculum with 10 ml culture was transferred into 250 ml conical flask containing 90 ml of medium, and was used for the study of the condition of the fermentation with following medium used: 20 g/l peptone, 30 g/l sucrose, 1 g/l KH_2PO_4 , 0.5 g/l $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.3 g/l uric acid. The culture condition experiments were performed in 12 samples. During the fermentation, a sample of the culture was withdrawn at 4 hours intervals for 48 hours. Then, the entire cells were collected by centrifugation at 8000 rpm for 2 minutes. Then, the supernatants were used for enzyme analysis and the cell was dried at 60 °C for overnight.

D. Effect of pH

The effect of pH on uricase production was examined using the 250 ml conical flask containing 100 ml of medium and incubated as described above. To determine the optimal pH for uricase production, the inoculum were cultured in the pH media, of which the pH adjusted to 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0.

E. Effect of Sucrose Concentration

The effect of sucrose concentration on uricase production was examined using the 250 ml conical flask containing 100 ml of medium and incubated as described above. To determine the optimal sucrose concentration for uricase production, the inoculum were cultured in the media, of which the sucrose concentration adjusted to 10, 20, 30, 40 and 50 g/l.

F. Effect of Agitation Rate

The effect of agitation rate on uricase production was examined using the 250 ml conical flask containing 100 ml of

medium and incubated as described above. To determine the optimal agitation rate for uricase production, the inoculum were cultured in the media, of which the agitation rate adjusted to 100, 150, 200, 250 and 300 rpm.

G. Enzyme Assay

The principle of enzyme measurement was as follow: uricase could catalyze the oxidation of uric acid into allantoin and H₂O₂ which was then measured by using a reaction system containing 4-aminoantipyrine, phenol and peroxidase as chromogen. In practical analysis, 0.1ml enzyme solution was incubated with the mixture of 0.6 ml sodium borate buffer (pH 8.5, 0.1 M) containing 2 mM uric acid, 0.15 ml 4-aminoantipyrine (30mM), 0.1 ml phenol (1.5%), 0.05 ml peroxidase (15 U/ml) at 37 °C for 20min. The reaction was stopped by addition of 0.1 ml ethanol. Then, the sample was followed modified DNS method to determine glucose concentration at 540 nm was read against the blank by a spectrometer. One unit of enzyme was defined as the amount of enzyme that produces 1.0 mmol of H₂O₂ per minute under the standard assay conditions.

III. RESULTS AND DISCUSSION

A. Effect of pH

The result in Fig. 1 shows pH 6 of fermentation medium give the highest enzyme activity of the extracellular product with 0.03974 U/ml. For pH 4 and pH 5, enzyme activity obtained was 0.02662 U/ml and 0.02932 U/ml while for pH 7 and pH 8 were 0.03549 U/ml and 0.03433 U/ml. Acidic fermentation medium give higher production medium as compared to more basic fermentation medium. The result was coincided with those of Tohamy and Shindia [7] and Yazdi *et al.* [5] who found that pH 6.0 was optimum pH medium for uricase production from *Aspergillus flavus* and *Mucor hiemalis*, respectively. However, this result is in disagreement by Ammar *et al.* [8] which reported optimum pH for uricase by *Aspergillus flavus* was 9.2. Besides that, Tohamy and Shindia [7] reported that species *Aspergillus terreus* produced maximum uricase production at pH 6 and 30 °C.

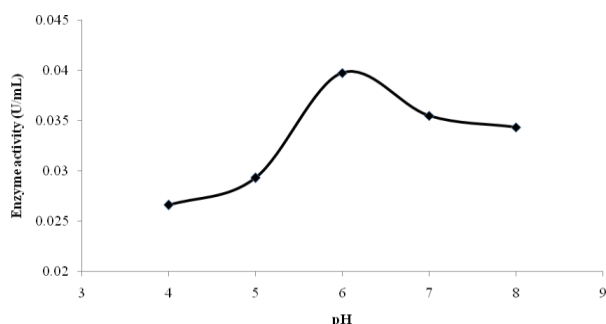


Fig. 1. Effect of pH on uricase activity.

The optimal pH of most enzymes is from 6-8, but there may be exceptions. Most extremely acidic or basic environments cause the enzyme to denature, unravelling the protein structure [9]. Once enzymes are denatured, they are rendered inactive. This is important to biology because if there are too many acidic environments in the body certain

functions may work inappropriately because of unusual pH levels. If the medium contains a lot of hydrogen ions (H⁺), then the medium will be acidic.

Strain *Bacillus thermocatenulatus* was proved by Lotfy [10] give highest uricase production of 1.25 U/ml at pH 7. The highest dried cell weight (1.85 g) for uricase production was also observed at pH 6. Anderson and Vijayakumar [11] found pH 8.5 give highest production of uricase using *Pseudomonas aeruginosa* while Zhou *et al.* [3] found that pH 7.5 was optimum pH for *Microbacterium* for production of uricase.

B. Effect of Sucrose Concentration

The fermentation medium was prepared at different sucrose concentration (10, 20, 30, 40 and 50 g/l) at pH 6 and 200 rpm. Study on effect of sucrose concentration on uricase production showed that the enzyme productivity decreased in the following order: 30>40>50>20 and 10 g/l. Fig. 2 shows that at 30 g/l sucrose concentration, the highest uricase activity and dried cell weight were detected at 0.03974 U/ml and 1.85 g/l, respectively. For 40 g/L concentration, enzyme activity obtained 0.03395 U/ml and 1.342 g of dried cell weight while for concentration 50 g/l and 20 g/l give the enzyme activity 0.02315 U/ml and 0.02276 U/ml. However, dried cell weight of 20 g/l was higher with 0.982 g than 50 g/l with 0.895 g of dried cell weight. For 10 g/l, it gave the lowest enzyme activity with 0.01312 U/ml and lowest dried cell weight with 0.873 g.

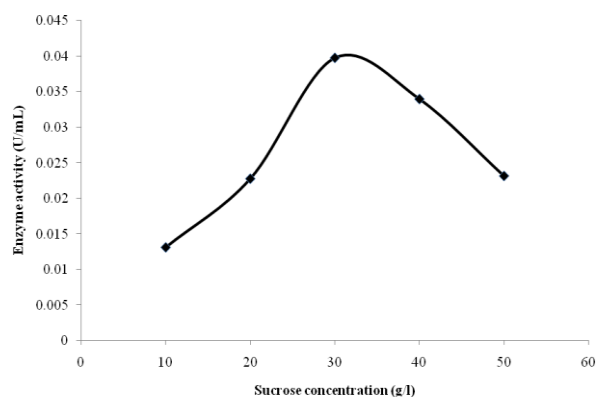


Fig. 2. Effect of sucrose concentration on uricase activity.

Substrate concentration affects the rate of enzymatic reactions. At lower substrate concentrations, the reaction rate is strictly proportional to the substrate concentration. However, once the substrate molecule concentrations increase beyond a certain level, there are no more binding sites available for them. This is called saturation, when enzymes catalyze as fast as they can, and reaction rate reaches its maximum potential. Without substrate, enzymes cannot function, and without the appropriate amount of substrate, the velocity of the reactions would take place very slowly [12]. When this saturation point is reached, then adding extra substrate will make no difference. The reaction will not speed up, no matter how much additional substrate is added. The graph of the reaction rate will plateau.

C. Effect of Agitation Rate

The experiment was designed determine agitation rate that give highest production of uricase. The agitation uses were

100, 150, 200, 250 and 300 rpm. From the result, the production of uricase was highest at agitation rate 200 rpm with 0.03974 U/ml. After that, agitation rate of 250 rpm gave the second highest enzyme activity with 0.03781 U/ml and followed by agitation rate at 300 rpm with 0.02701 U/ml. At 150 rpm, the enzyme activity obtained was 0.02623 U/ml and 100 rpm gave the lowest enzyme activity with 0.02122 U/ml. Some agitation rate increases the hydrolysis rate and yield, but excessive mixing can deactivate the enzyme and reduce the conversion yield [13], [14]. The deactivation effect has been attributed to shear force generated by the mixer and the entrapment of air bubbles into the medium at the air-liquid surface. In fact, the shear deactivation effect is a deterring factor in applying a more intensive agitation. However, a more properly designed and more intensive mixing regime should facilitate a better mass transfer inside the reactor, reducing a potentially high local product concentration surrounding the enzyme and active sites [15].

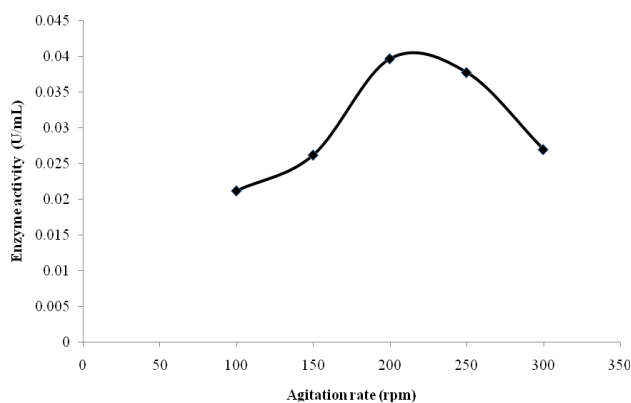


Fig. 3. Effect of agitation rate on uricase activity.

IV. CONCLUSION

As conclusion, the optimum parameters for uricase production had been determined. The maximum enzyme activity obtained from the experiment is 0.03974 U/ml with dried cell weight 1.85 g at pH 6, 200 rpm and 30 g/l sucrose concentration. Further study on the optimization study using statistical method will carried out later to validate the results from this preliminary studies.

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