Enzymatic Hydrolysis of Rice Straw: Process Optimization

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***Abstract*—Rice straw consists of cellulose, hemicellulose and lignin. Cellulose is the most abundant component that present in the rice straw. Through enzymatic hydrolysis of cellulose, fermentable sugars are produced in the present of cellulases. In this study, untreated rice straw that is abundance in Malaysia was used as feedstock for exoglucanase production and enzymatic hydrolysis. Under solid substrate fermentation, exoglucanase was produced by locally isolated *Aspergillus niger* using untreated rice straw as sole carbon source. Subsequently, the untreated rice straw was hydrolyzed by the extracted exoglucanase, which obtained from solid substrate fermentation (SSF) to optimize the production of fermentable sugar based on three different factors, i.e. initial pH, substrate concentration, and enzyme concentration. An exoglucanase with a maximum activity of 46.45 FPU/g of rice straw was obtained from SSF produced by locally isolated *A. niger*. The optimum parameters obtained for the hydrolysis were at pH 6.0, 12% (w/v) of substrate concentration and with enzyme activity of 10 U/g of rice straw. The fermentable sugar concentration obtained with these parameters was 3.62 g/L.**

***Index Terms*—*Aspergillus niger*, enzymatic hydrolysis, exoglucanase, rice straw**

I. Introduction

In 2012, FAO has set its first forecast of global paddy production at 732.3 million tones, 1.7% above the revised 2011 estimate [1]. Malaysia's average yield of paddy production was 3.7 tones per hectare [2]. Cellulase is a group of enzymes which have the capability of hydrolyzing cellulose into fermentable sugars such as glucose [3], which can be used for producing many useful products such as ethanol, biofuel and other useful chemicals from the cellulosic feedstocks [4], [5], [6]. Cellulase could be produced by many lignocelluloytic feed stocks such as straws, bagasse, wheat bran, corn stover, corncob, etc [7], [8], [9].

Enzymatic hydrolysis of such cellulosic material by cellulase is the most promising approach to obtain high product yields vital to economic success [10]. The high cost of cellulase production hinders the application of the enzymes in bioethanol production [10], [11]. Many bacteria and filamentous fungi can produce cellulose degrading enzymes. Most bacteria cannot utilize crystalline cellulose, which can be degraded by many filamentous fungi [12].

Therefore, the objectives of this study were to produce exoglucanase from solid substrate fermentation using locally isolated A. niger and optimize the parameters of enzymatic hydrolysis for the production of fermentable sugar from rice straw.

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II. Materials and Methods

A. Rice Straw

Rice straws were obtained from commercial rice processing plant in Jitra, Kedah. The rice straws were cut into 2 to 3 cm in length and ground with a blender. After that, the grinded rice straws were sieved and collected at the range of 0.36-1.00 mm.

B. Exoglucanase Production

Exoglucanase was produced under SSF in a 250 mL Erlenmeyer flask cover with a cotton plug, where ammonium sulphate (1% of N) as additional nitrogen source was mixed with 10 g of rice straw. The mixture was adjusted to 70% of initial moisture content and sterilized at 121°C for 15 min. After that, the substrate was inoculated spore suspension of locally isolated *A. niger* (10% v/w) which consist of 1×10^7 spore/mL and cultivated at room temperature for 4 days. The enzyme produced via SSF was extracted by adding 100 mL of cooled distilled water and agitating at 200 rpm for 30 min. Solids were removed by filtration using cheese cloth. The clear filtrate was collected.

C. Exoglucanase Assay

The exoglucanase assay was based on the method proposed by Ghose [13], 1 cm× 1 cm of Whatman No. 1 filter paper strip was used as substrate. The reducing sugar release was determine using the 3,5-dinitrosalicylic acid method [14]. The enzymatically liberated reducing sugar was calculated from a previous established standard curve using glucose as a standard. One unit of filter paper assay enzyme (FPU) was defined as the amount of enzyme releasing 1μmole of reducing sugar from filter paper per mL per min.

D. Enzymatic Hydrolysis

The hydrolysis of rice straw was carried out in a 100 mL Erlenmeyer flask with 6% of rice straw in 0.1 M phosphate buffer (pH 6), and 10 mL of exoglucanase (8 FPU/g of rice straw), which was produced via SSF. Then, the mixture was incubated at 45°C, and 150 rpm in for four days. The sample was collected daily.

Different initial pH: The initial pH values used for the hydrolysis process were 5.5, 6.0, 6.5, and 7.5. The pH was

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adjusted using phosphate buffer (0.1 M). The temperature was at 45°C, concentration of the substrate was 6%, and the concentration of enzyme was 8 FPU/g of rice straw.

Different substrate concentration: Various substrate concentrations were used for the hydrolysis process, which were 6%, 8%, 10% and 12%. The initial pH was the optimum condition obtained from a previous experiment, at 45°C, and the concentration of the enzyme was 8 FPU/g of rice straw.

Different enzyme concentration: Different enzyme concentrations were used for the hydrolysis process, which were 4, 6, 8, and 10 FPU/g of rice straw. The initial pH and substrate concentration were the optimum conditions obtained from a previous experiment. This work was carried out at 45°C.

Sample analysis: The fermentable sugar was determine using the 3,5-dinitrosalicylic acid method [14].

III. Results and Discussion

The production of exoglucanase was carried out under SSF. There are numerous advantages of SSF over submerged fermentation (SmF), including superior productivity, simple technique, low capital investment, low energy requirement and less wastewater output, and better product recovery [15]. Exoglucanase with an activity of 46.45 FPU/g of rice straw was extracted after 4 days of SSF. Exoglucanase produced by *Phanerochaete chrysosporium* exhibit 30.18 U/g of rice straw [16]. The locally isolated *A. niger* able to produce higher enzyme concentration as compare with *P.*, this indicated that *A. niger* able to produce more enzyme when 1 g of substrate was used. When alkali-treated rice straw was used for the exoglucanase production as reported by Ong et al. [17] and Fatma and Fadel [18], the enzyme activity obtained were 7.85 FPU/g of rice straw and 16.2 U/g of rice straw, respectively. This showed that untreated rice straw has higher enzyme activity as compared to treated rice straw. During the alkali pretreatment reaction, the alkali added will be converted into irrecoverable salts and/or the incorporation of salts into the biomass [19]. This phenomenon might be unfavorable for the fungal growth.

The fermentable sugar concentration produced through the hydrolysis of rice straw using exoglucanase obtained via SSF at pH 5.5, 6.0, 6.5, and 7.5 within 4 days was shown in Fig. 1. Basically, the glucose produced increased with number of days for all the pH values investigated, even though it did not show a large increment. During the period of the hydrolysis process, the highest glucose concentration was achieved at pH 6.0 (2.40 g/L), followed by pH 5.5 (2.14 g/L) and 6.5 (2.10 g/L). Based on the results obtained, the productivity of the fermentable at pH 5.5 and 6.5 were not significant different. The lowest glucose production (1.64 g/L) was at pH 7.5.

Fig. 2 shows the fermentable sugar produced via the hydrolysis of rice straw carried out with four different substrate concentrations, i.e. 6%, 8%, 10%, and 12% (w/v) within 4 days. Basically, the fermentable sugar produced through the hydrolysis process was increased with the number of days. The highest fermentable sugar concentration was achieved at 12% (w/v) of substrate concentration (3.83 g/L), followed by 10% and 8% (w/v) of substrate concentration, which was 3.61 g/L and 3.29 g/L, respectively. To increase the production of fermentable sugar and economically feasible for cellulosic ethanol production, high solid loading is required [20].

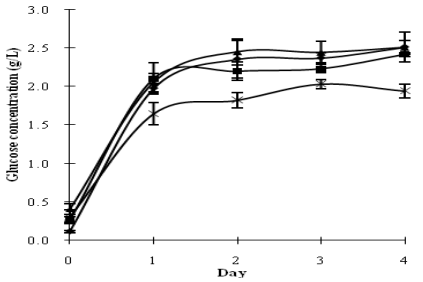

Fig. 1. The effect of different pH on the enzymatic hydrolysis of rice straw using cellulases produced from the SSF with the condition of 8 FPU/g of rice straw of enzyme concentration, 6% of substrate concentration at 45°C. Symbols: (■) 5.5; (♦) 6.0; (σ) 6.5; (5) 7.0.

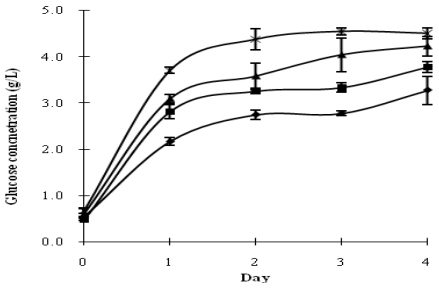

Fig. 2. The effect of different substrate concentration on the enzymatic hydrolysis of rice straw using cellulases produced from the SSF with the condition of 8 FPU/g of rice straw of enzyme concentration, at pH 6.0 and 45°C. Symbols: (■) 6%; (♦) 8%; (σ) 10%; (5) 12%.

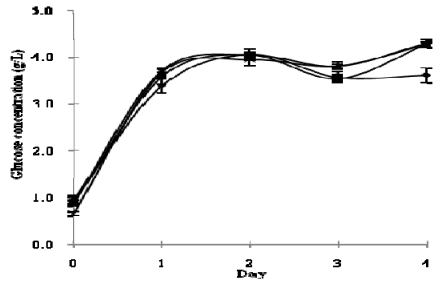

Fig. 3. The effect of different enzyme concentration on the enzymatic hydrolysis of rice straw using cellulases produced from the SSF with the condition of 12% substrate concentration, at pH 6.0 and 45°C. Symbols: (■) 4 FPU/g of rice straw; (♦) 6 FPU/g of rice straw; (σ) 8 FPU/g of rice straw; (5) 10 FPU/g of rice straw.

Fig. 3 shows the fermentable sugar concentration produced through the hydrolysis of rice straw with different enzyme concentrations (4, 6, 8, and 10 FPU/g of rice straw) within 4 days. The enzyme concentration at 10 FPU/g of rice straw used for the hydrolysis produced the highest fermentable sugar concentration, which was 3.62 g/L and it showed a significant difference with 4 FPU/g of rice straw and 6 FPU/g of rice straw of enzyme loading. It was observed that the fermentable sugar productivity from 6 and 8 FPU/g of rice straw of enzyme concentration do not show any significant among each other.

The enzymatic activity is usually directly proportional to enzyme concentration; however, the concentration of enzyme is usually much less than that of the substrate, the enzyme becomes limiting at saturating substrate concentrations. Thus,

at high concentrations of substrate, activity no longer changes with increasing substrate. On the other hand, as substrate is converted to product, the enzyme's active sites are no longer saturated, and substrate concentration becomes rate limiting. Once a saturation level of substrate or enzyme is reached, there will be no additional increase in enzymatic activity no matter how much more enzyme or substrate is added. This also means, at the saturation point, that the enzymatic activity will not change, no matter how much additional substrate or enzyme is added [21]. Other than that, accumulation of cellobiose can caused severs feedback inhibition to cellulose reaction, as the enzyme is more susceptible to end-product-inhibition caused by cellobiose than glucose [22].

IV. Conclusion

Exoglucanase (46.45 FPU/g of rice straw) were produced via SSF using locally isolated *Aspergillus niger*. From the investigation on the effect of pH, substrate concentration, and enzyme concentration for the hydrolysis of rice straw, the optimum conditions were at pH 6.0, with 12% of substrate concentration, and 10 FPU/g of rice straw of enzyme concentration. For that reason, the reducing sugar produced from enzymatic hydrolysis process can be used in the next step for bioethanol production from untreated rice straw.

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