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# Comparison of Sodium Hydroxide and Potassium Hydroxide Followed by Heat Treatment on Rice Straw for Cellulase Production under Solid State Fermentation

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**Abstract:** Rice straw is the major agricultural by-product in the world. Its low digestibility made it unsuitable as livestock feed which lead to the mass disposal and burning of rice straw. The main objective of this study were to optimise the alkali concentration and soaking time on rice straw for the alkali pretreatment followed by heat treatment for cellulose production and to compare the effectiveness of both alkali used. The rice straws were subjected to two treatments, which were Treatment A and Treatment B. In Treatment A, the rice straws were treated with sodium hydroxide (NaOH) followed by heat treatment (autoclaving at 121°C for an hour), whereas the rice straws were treated with potassium hydroxide (KOH) followed by heat in Treatment B. Four different alkali concentrations (5, 10, 15 and 20%) at four different soaking times (1, 2, 3 and 4 h) were investigated. The treated rice straws were subjected to Solid State Fermentation (SSF) with the condition of 1:1 moisture content, 1% ammonium sulphate and $1\times10^7$ spores $mL^{-1}$ of locally isolated *Aspergillus niger*. The highest yield of filter paper enzyme (FPase) and carboxymethyl cellulase (CMCase) were obtained after 6 days of SSF, which were 7.85±0.18 U $g^{-1}$ substrate and 11.73±0.27 U $g^{-1}$ substrate, respectively; when the rice straw was pre-treated with 15% KOH with 1 h soaking time followed by heat. Conclusively, rice straw can be effectively bio-converted into valuable product such as cellulase in SSF.

**Key words:** Rice straw, alkali/heat pretreatment, *Aspergillus niger*, solid state fermentation

## INTRODUCTION

Nowadays, the world population has grown steadily to a mass number. This eventually leads to an increase in the world's food demand. Rice is known as the most consumed staple food crops followed by wheat in all the modern and developing countries today. Rice-producing countries such as China and Nigeria produce hundred million tones of agricultural products annually, resulting in the generation of enormous amount of wastes such as straws, hulls and husks which may cause the deterioration of environment (Sharma *et al*., 2001). As in 2007, the most generated agricultural waste materials are rice straw, wheat straw, sugarcane bagasse and corn cob (Badhan *et al*., 2007).

These large amounts of agro-industrial residues generated increasing the biotechnological interest on the utilization of these residues as raw-materials. Such lignocellulosic waste materials can be exploited as a source or substrate for the production of a number of value added products such as food, fuel, feed, chemicals and a wide variety of enzymes (Someet *et al*., 2001). The conversion process of this cellulosic biomass into reactive intermediates and useful products can be achieved through Solid State Fermentation (SSF), which serves as an attractive process due to its economical cost and simple procedures (Chandra *et al*., 2007). Appropriate strains of microorganisms involved in SSF are needed to be taken into consideration to ensure that the best species is chosen for maximum yields of a particular product. Recent researches involved the use of pretreatment methods on the lignocellulosic substrates has been extensively studied as to accelerate the bioconversion process (Zaheh and Karimi, 2008). Hence, the objectives of this study were to determine the optimum conditions of potassium hydroxide and sodium hydroxide followed by heat pre-treatment for rice straw for cellulase production under solid state fermentation and compare the effectiveness of both alkali used for the cellulose production.

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## MATERIALS AND METHODS

**Rice straw:** Raw rice straws were obtained from Muda Agricultural Development Authority (MADA) Pendang, 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.

**Microorganism:** Locally isolated *Aspergillus niger* obtained from the Department of Biotechnology Culture Collection, AIMST University, Bedong, Kedah was used in this study. Potato dextrose agar slant were used for sporulation and storage. A suspension of $1\times10^7$ spores $mL^{-1}$ was prepared in sterile 0.1% Tween 80 as an inoculum.

**Pretreatment of rice straw**

**Alkali pretreatment:** For Treatment A, the rice straws were pretreated with different percentage of sodium hydroxide (NaOH), i.e., 5, 10, 15 and 20% each with different soaking time of 1, 2, 3 and 4 h, respectively. Whereas in Treatment B includes the treatment of rice straws with different percentage of potassium hydroxide (KOH), i.e., 5, 10, 15 and 20% concentration each with soaking time of 1, 2, 3 and 4 h, respectively. After the alkali treatment, the treated rice straws were then washed with distilled water as much as possible and the pH was adjusted to 7.8 using 1 M hydrochloric acid (HCl).

**Heat pretreatment:** After the alkali treatment, the treated rice straws were subjected to the heat treatment for 1 h at 121°C. Then, the fully treated rice straws were dried in the oven and kept for further use.

**Solid state fermentation:** Solid State Fermentation (SSF) was carried out in 250 mL Erlenmeyer flask, where it consisted of 5 g treated rice straw, 1.0:1.0 moisture level with the addition of 1% ammonium sulphate as nitrogen source. The mixture was sterilized at 121°C for 15 min. Then, it was cooled to room temperature before the inoculation of 10% spores suspension with the concentration of $1\times10^7$ spores $mL^{-1}$. After that, it was incubated at room temperature for 6 days where it was the highest day of cellulose production (Chuah, 2008; Rajamohan, 2008; Rajandas, 2008; Wong, 2008).

**Enzyme extraction:** After the fermentation process, 50 mL of cooled sterile distilled water was added for the extraction of crude cellulase and was agitated at 150 rpm for 1 h. Then, they were filtered through muslin cloth and centrifuged at 10,000 rpm at 4°C for 15 min. The supernatants were collected and kept for further analysis.

**Analysis:** Filter paper enzyme (FPase) activity was assayed according to the method proposed by Ghose (1987), using Whatman filter paper No. 1 strip (1×3 cm) as substrate. The reducing sugar was determined by dinitrosalicylic acid method using glucose as a standard according to Miller (1959). The carboxymethyl cellulose (CMCase) activity was assayed according to the method proposed by Wood and Bhat (1998), using 1% of sodium carboxymethyl cellulose (medium viscosity) in sodium citrate buffer (50 mM at pH 4.8). The reducing sugar was determined by dinitrosalicylic acid method using glucose as a standard according to Miller (1959).

## RESULTS

Figure 1 and 2 show the FPase yield and CMCase yield after 6 days of SSF which carried out with four different percentage of NaOH, i.e., 5, 10, 15 and 20% at four different soaking times (1, 2, 3 and 4 h). The yield of FPase and CMCase were higher when rice straws were pretreated with NaOH followed by heat treatment as compared to untreated rice straws. The highest yield of Fpase and CMCase can be observed when the rice straws were subjected to 15% NaOH and soaked for 1 h followed by heat treatment, where the enzyme activities were 7.69±0.08 U $g^{-1}$ substrate and 11.55±0.22 U $g^{-1}$ substrate, respectively. Figure 1 shows the FPase activity obtained from the treated rice straw with 15% NaOH for

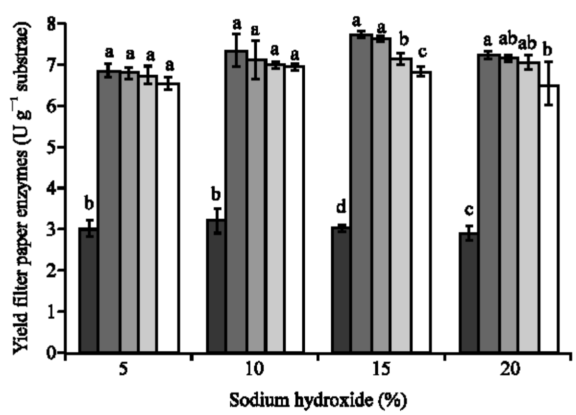


Fig. 1: Fpase production by locally isolated *A. niger* after pretreated with different percentage of NaOH at different soaking time 1 h (■), 2 h (■), 3 h (■) and 4 h (■) followed by heat compare with untreated (■) rice straw. The same letters above each vertical columns of the graph within each individual concentration were not significantly (p≥0.05)

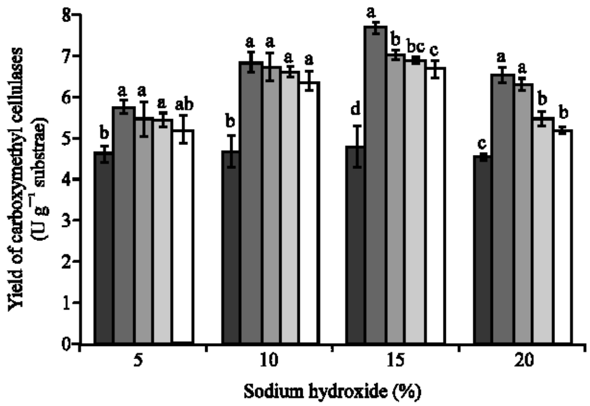


Fig. 2: CMCase production by locally isolated *A. niger* after pretreated with different percentage of NaOH at different soaking time 1 h (■), 2 h (■), 3 h (■) and 4 h (■) followed by heat compare with untreated (■) rice straw. The same letters above each vertical columns of the graph within each individual concentration were not significantly ($p\geq 0.05$)

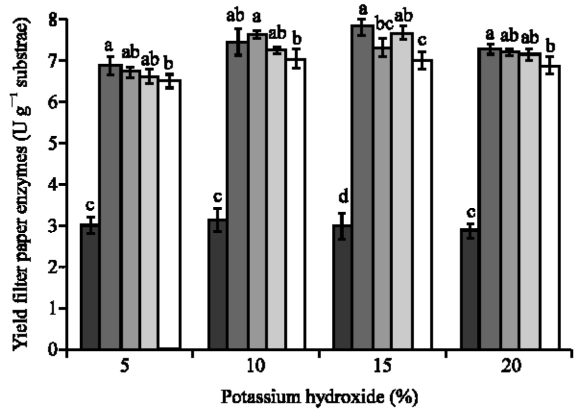


Fig. 3: Fpase production by locally isolated *A. niger* after pretreated with different percentage of KOH at different soaking time 1 h (■), 2 h (■), 3 h (■) and 4 h (■) followed by heat compare with untreated (■) rice straw. The same letters above each vertical columns of the graph within each individual concentration were not significantly ($p\geq 0.05$)

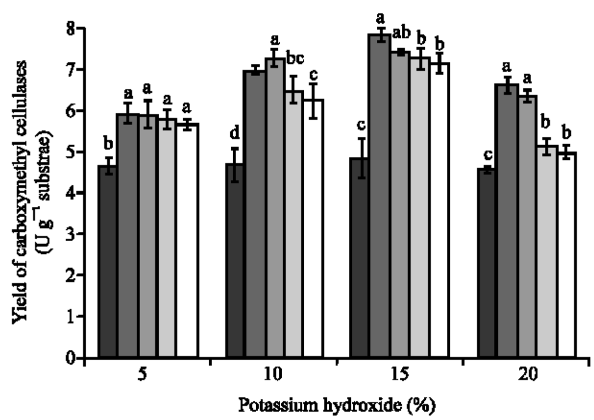


Fig. 4: CMCase production by locally isolated *A. niger* after pretreated with different percentage of KOH at different soaking time 1 h (Error! Bookmark not defined.), 2 h (Error! Bookmark not defined.), 3 h (■) and 4 h (■) followed by heat compare with untreated (■) rice straw. The same letters above each vertical columns of the graph within each individual concentration were not significantly ($p\geq 0.05$)

1 h followed by heat treatment has increase 2.54 fold as compared to the untreated rice straw. While the CMCase activity obtained from the treated rice straw with 15% NaOH for 1 h followed by heat treatment has increase 60.16% as compared to the untreated rice straw as shown in Fig. 2.

Figure 3 and 4 show the FPase yield and CMCase yield after 6 days of SSF which carried out in Treatment B with four different percentage of KOH, i.e., 5, 10, 15 and 20% at four different soaking times (1, 2, 3 and 4 h). The yield of FPase and CMCase were higher when rice straws were pretreated with KOH followed by heat treatment as compared to the untreated rice straws. The rice straws which were subjected to 15% KOH followed by heat treatment gave the highest yield of FPase and CMCase as compared to the untreated rice straw, which were $7.85\pm0.18$ U $g^{-1}$ substrate and $11.73\pm0.28$ U $g^{-1}$ substrate, respectively. Figure 3 shows the FPase activity obtained from the treated rice straw with 15% KOH for 1 h followed by heat treatment has increase 2.60 fold as compared to the untreated rice straw. While, the CMCase activity obtained from the treated rice straw with 15% KOH for 1 h followed by heat treatment has increase 62.60% as compared to the untreated rice straw as shown in Fig. 4.

## DISCUSSION

Analysis of variance on the effect of NaOH and KOH followed by heat treatment on cellulase yield showed that there is a significant ($p<0.05$) in the enzyme yield between the chemicals used, percentage of alkai used and soaking period. This is due to pretreated rice straw with alkali degraded the lignin, cellulose and a part of the hemicelluloses contained within the rice straw. Alkali pretreatment also caused the swelling of the rice straw particles resulted in the increase in internal surface area. The swelling of the particles was believed to be caused by the signification of the inter medullar ester bonds as well as the separation of the hydrogen bonds holding the cellulose in the rice straw. This in turn caused the depolymerization of the cellulose (Fan *et al*., 1982). The heat treatment applied further expanded the cellulose and increased the water holding capacity of the rice straw, leading to the further swelling of the rice straw. Hence, resulted in the increase porosity of the rice straw allowing the better accessibility of the cellulase enzyme to the cellulose (Zhang and Cai, 2008).

However, both FPase and CMCase yield decreased at 20% NaOH and KOH, respectively. This is mainly caused by the production and accumulation of organic acids, pentoses, hexoses and some phenolic compounds in the rice straw that might interfere and inhibit the growth of microorganisms in the fermentation process (Bharathi and Ravindra, 2006). Hence, the production of cellulases were interrupted and decreased at high alkali concentration. Miyamoto (1997) reported that production of cellulases and reducing sugars will decrease at high alkali concentration as it will cause the simultaneous dissolution of pentosan; thus, leading to a reduction in cellulases production as well as the reducing sugars recovery. This occurrence might be due to the insufficient washing of the rice straw soaked in the alkali solution for longer retention times. Longer soaking tine caused the difficulty to wash away the alkalinity on the rice straw and it was harder to adjust the rice straw to neutral pH (Bharathi and Ravindra, 2006). The fungus cannot really grow well on the rice straw as they are very sensitive to the pH changes. Hence, the production of cellulases decreased at longer soaking times. The combination of alkali and heat pretreatment generally resulted in the higher cellulases production, where heat treatment applied further removed more hemicellulose and lignins in the rice straw completely resulting in a better degradation of the celluloses by the cellulases. Rice straw pretreated by alkali followed by heat showed higher cellulose content and lower lignins as well as hemicellulose content than the rice straw pretreated with only alkali treatment alone (Zhu *et al*., 2005).

## CONCLUSION

The production of cellulase has been increased after chemical/physical pretreatment on the rice straw before SSF using locally isolated *A. niger*. The optimum pretreatment conditions for rice straw were treated with 15% KOH and soaked for one hour followed by heat treatment where the FPase and CMCase obtained were 7.85±0.18 U $g^{-1}$ substrate and 11.73±0.28 U $g^{-1}$ substrate, respectively.

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