# Solid State Fermentation for Glucanase Production Using Acid/Heat Treated Rice Straw

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***Abstract*—Rice straw that has been treated with hydrochloric acid or sulfuric acid followed by heat treatment was used for the production of endo- and exo-glucanase. Solid state fermentation was carried out with the following condition: inoculum size, 10%; moisture content, 50%; ammonium sulphate, 1%. After 6 days of fermentation, endo-glucanase and exo-glucanase were extracted; subsequently, enzyme assays were carried out. The highest exo-glucanase and endo-glucanase activity obtained were 8.16 ± 0.12 U/$g_{rice\ straw}$ and 11.25 ± 0.14 U/$g_{rice\ straw}$, respectively using the acid/heat treated rice straw which was soaked in 15% of sulfuric acid for 2 hours. Conclusively, there was interaction between soaking time, concentration of acid and type of acid, and significant ($p$ < 0.05) improvement in glucanase yield when compared to untreated rice straw.**

***Index Terms*—rice straw, solid state fermentation, endo-glucanase, exo-glucanase, acid/heat treatment**

## I. Introduction

Lignocellulosic materials are one of the most abundant and underutilized source of renewable energy found, which include various agricultural residues, herbaceous crops, woods and its residues. Bioconversion of the lignocellulosic materials into fermentable sugar, alternative fuels and chemical feedstock is gaining research attention [1]. Lignocellulosic materials consist of 40-60% cellulose, 20-30% hemicellulose and 15-30% lignin [2]. To hydrolyze β 1, 4-glycosidic linkage in the plant cell wall, cellulase which consist of endo-glucanase, exo-glucanase and β-glucosidase, plays a key role. Suitable filamentous fungi with solid state fermentation (SSF) is able to further improve the production economics by using cheap biomass resources as substrate for cellulase production that later leads to bio-ethanol production [3]-[5].

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With the annual production of 731 million tons, rice straw has recently gained considerable interest in Asian countries as a potential biomass for bio-ethanol production [3], [6]. Currently, most Asian countries practice open burning as an easy and cheap method of disposing the rice straw that was left behind after the harvest season. Open burning of rice straw results in incomplete combustion producing pollutants such as carbon monoxide (CO), volatile organic compounds (VOC), carcinogenic polycyclic aromatic hydrocarbons and fine, inhalable particles [7]. These pollutants are toxic and can cause harm to human health and environment. Open burning of rice straw not only reduces air quality, it also releases green house gases which further contribute towards global warming [8]. Consequently the application of rice straw as a substrate for enzyme production is not only a cost effective alternative; it also helps to relieve the problem of environmental contamination.

However, in the presence of lignin (12%) in rice straw, cellulase cannot effectively convert it to fermentable sugars without pre-treatment. Cellulose digestibility could be increased when the rice straw is treated with diluted acid at high temperature [9]. With this, the objective of this study was to investigate the soaking time and percentage of acid used to pre-treat the rice straw for endo- and exo-glucanase production under solid state fermentation.

## II. Materials and Methods

### A. Rice Straw

Rice straw was collected from Muda Agricultural Development Authority (MADA) Pendang, Kedah. The rice straw was cut into 2 to 3 cm in length and ground with a blender. After that, the ground rice straw was sieved and collected in the range of 0.36-1.00 mm [10].

### B. Microorganism

Locally isolated *Aspergillus niger* was obtained from



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the culture collection in AIMST University and maintained in potato dextrose agar (PDA) slant. The sporulated fungus was harvested with sterilized Tween 80 (0.1%) for further use.

### C. Pretreatment of the Rice Straw

Rice straw was soaked in different concentrations (5, 10, 15, 20%, v/v) of HCl or $H_2SO_4$ for different soaking time (1, 2, 3, 4 hours) using factorial design. After the acid treatment, the rice straw was washed with tap water as much as possible and the pH was adjusted to 7.8 with 1 M NaOH. Subsequently, the treated rice straw was subjected to heat treatment at 121 °C for 1 hour before drying in the oven at 60 °C for 48 hours.

### D. Enzyme Production by Solid State Fermentation

Five grams of treated rice straw was weighed in 250 ml Erlenmeyer flask and moisten with 1% $(NH_4)_2SO_4$ as nitrogen source with the moisture:substrate ratio of 1:1. The flask was autoclaved at 121°C for 15 minutes for sterilization. After that, the flask was inoculated with 10% of spore suspension ($1\times10^7$ spore/ml) and incubated at room temperature for 6 days which gave the highest cellulase production [10].

### E. Enzyme Assays

Endoglucanase activity was assayed using 1% of sodium carboxymethly cellulose (medium viscosity) in sodium citrate buffer (50 mM, pH 4.8) as substrate [11]. The crude enzyme (0.1 ml) was added into 0.9 ml of substrate and incubated at 30 °C for 30 minutes. Reducing sugar released was determined by the dinitrosalicylic acid method by adding 1.5 ml of 3, 5-dinitrosalicylic acid reagent to 1.0 ml of the reaction mixture and placing them in boiling water for 15 minnutes [12]. The mixture was then cooled down to room temperature and the absorbance was read at 575 nm. Exoglucanase activity was assayed using filter paper Whatman No. 1 strip (1 × 3 cm) as substrate, where 0.1 ml of crude enzyme was added with 0.9 ml of sodium citrate buffer (50 mM, pH 4.8) [13]. The reaction mixture was incubated at 30 °C for 60 minutes. Reducing sugars released was determined by the dinitrosalicylic acid method [12]. The enzymatically liberated reducing sugars were calculated based on a standard curve using glucose as a standard. One unit of enzyme activity was defined as the amount of enzyme releasing 1 µmole of reducing sugar per ml per minute. Enzyme activity was calculated based on the following formula:

$$EA = \frac{x-c}{m} \times \frac{df}{V} \times \frac{1}{t} \times \frac{1}{180.16} \times \frac{v}{w} \times \frac{1000}{1}$$

$EA$ = enzyme activity (U/g)
$x$ = final absorbance
$c$ = intercept at axis-y
$m$ = slope
$df$ = dilution factor
$V$ = sample volume (ml)
$T$ = reaction time (minutes)
$v$ = extraction volume (ml)
$w$ = weight of the substrate (g)

### F. Statistical Analysis

Pre-treatments were carried out in triplicates and the experimental data obtained from the study was subjected to two-way ANOVA and one-way ANOVA Tukey test. *P* value less than 0.05 was considered as significantly different.

## III. Results and Discussion

Rice straw was prior treated with different concentration and soaking time of HCl or $H_2SO_4$ before heat treatment and later proceeded to SSF for the production of endo- and exo-glucanase. The enzyme activities produced using the HCl/heat or $H_2SO_4$/heat treated rice straw were higher as compared to untreated rice straw which served as a control.

The results indicated that with the increase of HCl percentage (0-15%), the enzyme activity of endo- and exo-glucanase also increased. However, the enzyme activities started to drop when 20% of HCl/heat was used for all the treatment time. The highest endo- and exo-glucanase activities can be observed when the rice straw was soaked in 15% HCl/heat for 2 hours (Fig. 1). For endo-glucanase, the activity was 1.94 times higher compared to the untreated rice straw with the activity of 5.65 ± 0.28 U/$g_{rice\ straw}$. On the other hand, the activity of exo-glucanase for the untreated rice straw was 3.53 ± 0.05 U/$g_{rice\ straw}$ and 15% HCl/heat treated rice straw soaked for 2 hours was 7.76 ± 0.14 U/$g_{rice\ straw}$.

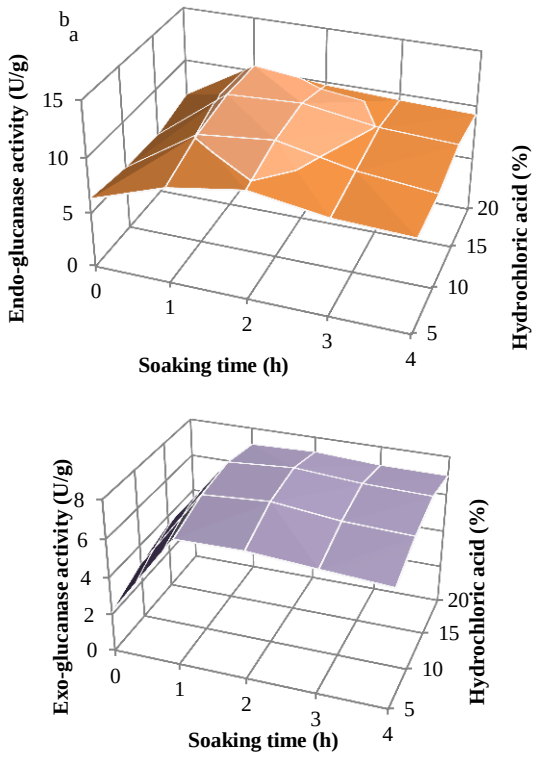


Figure 1. Production of (a) endo-glucanase, (b) exo-glucanase after 6 days of SSF with HCl/heat treat rice straw.

Bharathi and Ravindra [12] also carried out similar study and they found that using rice bran pre-treated with 1 N HCl followed by 10 minutes heat treatment gave the highest yield of reducing sugar (6.04 mg/g). The reducing sugar content yield reduced as the concentration of HCl increased [14] and this result coincided with the findings of the present study, whereby the exo-glucanase activity declined as the concentration of HCl increased above 15% HCl. Basically, exo-glucanase activity results in reducing sugar which serves as an indication of cellulose catabolism. Therefore, the production of exo-glucanase is directly proportional to the reducing sugar yield.

When the rice straw was treated with 15% $H_2SO_4$/heat for 2 hours (Fig. 2), the highest endo-glucanase activity obtained was 11.25 ± 0.14 $U/g_{rice\ straw}$. Compared to the HCl/heat treated rice straw with the same parameters, the endo-glucanase activity increased 2.4% when $H_2SO_4$/heat treated rice straw was used. The exo-glucanase activity obtained from the rice straw treated with 15% $H_2SO_4$/heat for 2 hours was 2.31 times higher compared to the untreated rice straw (3.53 ± 0.05 $U/g_{rice\ straw}$) and 1.03 times higher compared to the HCl/heat treated rice straw with the same parameters. The enzyme activities for both endo- and exo-glucanase dropped when rice straw was treated with 20% of $H_2SO_4$ for all the soaking time.

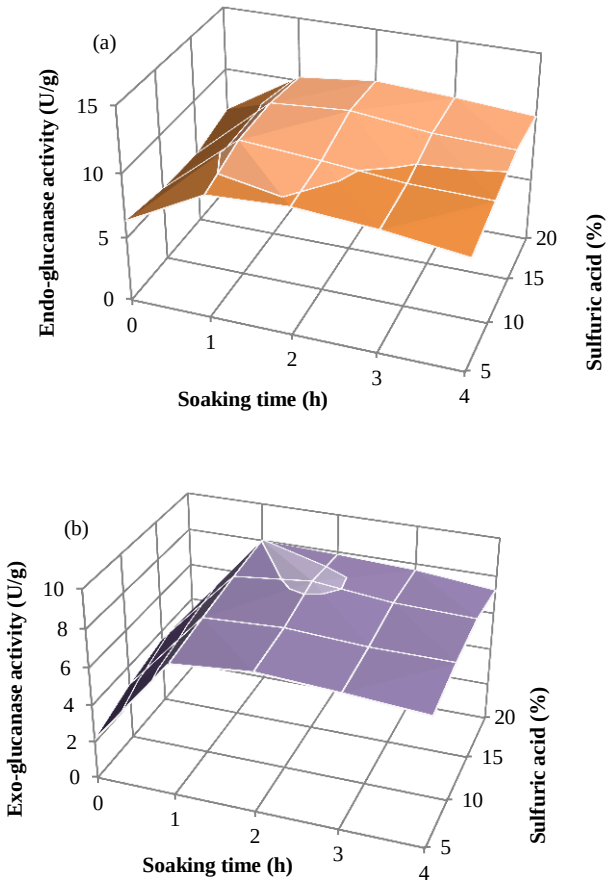


Figure 2. Production of (a) endo-glucanase, (b) exo-glucanase after 6 days of SSF with $H_2SO_4$/heat treat rice straw.

When the rice straw was treated with 20% acid/heat, decline in the enzyme activities was observed. Theoretically, one may expect to have increased enzymatic yield at higher acid concentrations as more lignin will be broken down and more hemicelluloses removed, but this was not the case in this study. This is may be because at very high acid concentrations, excessive breakdown of cellulose to glucose was followed by the breakdown of glucose to 5-hydroxylmethyl-furfural (HMF) and furfural [15]. Further breakdown to the furan derivatives will lead to the generation of organic acids such as levulinic acid, formic acid and other degradation products which act as potent microbial inhibitors. These by-products inhibit the growth of the fungi and the subsequent fermentation process, thereby yielding a lower enzyme activity and soluble protein concentration at very high acid concentration. HMF and furfural have been long noted as common by-products formed in acid hydrolysis of lignocellulosic materials [16]. However, further degradation of sugar to furfural and HMF can be terminated with diluted acid pretreatment at low temperature (121°C).

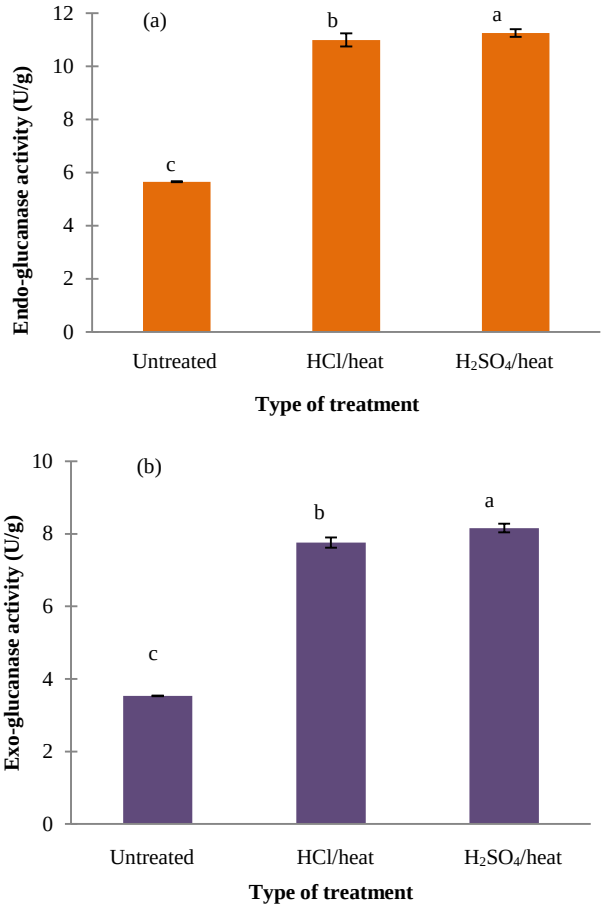


Figure 3. Comparison of (a) endo-glucanase, (b) exo-glucanase yields between untreated rice straw, HCl/heat and $H_2SO_4$/heat treated rice straw (acid treatment for 2h at 15% concentration). *The same letters above each vertical column of the graph are not significantly different ($p \geq 0.05$).

Acid/heat treatment for 2 hours at 15% concentration gave the best endo- and exo-glucanase yield for both $H_2SO_4$ and HCl. Fig. 3 shows a comparison between the optimum overall yields of endo- and exo-glucanase activities after acid treatments. $H_2SO_4$/heat treated rice straw had significantly higher ($p$<0.05) cellulase yield than HCl/heat pre-treatment according to the statistical analysis. This can be explained by the fact that $H_2SO_4$ is a stronger acid, it has double hydrogen ions and so more hydrolysis reactions occurred. Therefore, pre-treatment with $H_2SO_4$ followed by heat pre-treatment resulted in a more efficient removal of hemicellulose and disruption of lignin which then led to higher endo- and exo-glucanase activities [17].

## IV. Conclusion

Rice straw treated with 15% of $H_2SO_4$/heat for 2 hours gave the highest endo- and exo-glucanase activities. With proper pretreatment, rice straw can serve as a potential alternative towards process development for the production of 'second generation biofuel'.

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