International Journal of Technology (2015) 1: 3-10
ISSN 2086-9614 © IJTech 2015

# EFFECT OF ACID PRETREATMENT ON ENZYMATIC HYDROLYSIS IN BIOETHANOL PRODUCTION FROM RICE STRAW

H. B. Aditiya[1,2*], K. P. Sing[1], M. Hanif[1], T. M. I. Mahlia[1,3]

[1]*Department of Mechanical Engineering, College of Engineering, Universiti Tenaga Nasional, 43000 Selangor, Malaysia*
[2]*Department of Mechanical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia*
[3] *Department of Mechanical Engineering, Syiah Kuala University, Banda Aceh 23111, Indonesia*

(Received: June 2014 / Revised: December 2014 / Accepted: December 2014)

## ABSTRACT
Clean, safe and sustainable energy sources must be found to minimize all side-effects of fossil fuel consumption. Second generation bioethanol possesses a great potential as an alternative energy source especially in the transportation sector. In this study, rice straw was selected to be studied as a conversion of potential lignocellulosic biomass into bioethanol. Firstly, rice straw was processed with mechanical pretreatment using a home blender, followed by acid pretreatment using 2.0 M sulphuric acid ($H_2SO_4$) at 90ºC for 60 minutes. The glucose yield was found to be 9.71 g/L. Then, rice straw pretreated with acid was hydrolyzed using 24 mg of cellulase from Tichoderma Ressei ATCC 26921 over a 72-hour duration, which yielded a total glucose count of 11.466 g/L. After fermentation with Saccharomyces cerevisiae, it was found that by combining enzymatic hydrolysis with acid pretreatment yielded a higher ethanol content after fermentation (0.1503% or 52.75% of theoretical value) compared to acidic pretreatment alone (0.013% or 11.26% of theoretical value).

*Keywords:* Acid pretreatment; Bioethanol; Enzymatic hydrolysis; Rice straw; Second generation biofuel production

## 1. INTRODUCTION

Due to factors such as the increasing depletion of world primary energy supplies related to fossil fuels and the resulting high $CO_2$ emissions, sustainable, cleaner and safe alternative energy sources must be found. Bioethanol possesses a great potential as an alternative fuel in various energy sectors especially in the transportation sector. In 2007, the United States of America became the world's largest bioethanol producer with a production capacity of fuel alcohol of 51.5 billion litres from 180 plantations with bio-refineries (Walker, 2010). Bioethanol has been widely used as a blending fuel in gasoline in countries such as Brazil, the USA and many others. According to a British Petroleum (BP) statistical review of world energy production, in 2006 Malaysia started to produce biofuels (bioethanol and biodiesel) with production amount of 48,000 toe (tonne of oil equivalent) and it has risen to 97,000 toe in 2011 (BP, 2012). In 2012, the company; Sime Darby Berhad announced its plans to invest MYR 2 billion for the setting up of 616 bio-refinery module lines in the next five years in Malaysia and Indonesia (Choong, 2012).

* Corresponding author's email: aditharjon@gmail.com, Tel. +60-133-19016, Fax. +60-133-19016
Permalink/DOI: http://dx.doi.org/10.14716/ijtech.v6i1.778

Malaysia's biofuel research community has been working on the development of the processes that turn different sources of cellulosic biomass into bioethanol as an alternative transportation fuel, replacing gasoline and natural gas. One of the potential lignocellulosic biomass feedstock for bioethanol production that is abundant and cheap is rice straw waste.

The predominant composition of rice straw is cellulose (32–47%), hemicellulose (19–27%) and lignin (5–24%) (Karimi et al., 2006). Cellulose is a polymer of repeating β-D-glucopyranose units and is a chief constituent of feedstock; while hemicellulose, a polymer of C5 and C6 sugars, is similar to cellulose, a polysaccharide but less complex and it can be easily hydrolyzed by acids to monomer components such as xylose, mannose, glucose, galactose, arabinose (Dwivedi et al., 2009). The sugar content of rice straw is presented in Table 1.

Table 1. Sugars presented in rice straw (Nutawan et al., 2010)

| Component | Content (%) |
|---|---|
| Glucose | 41 – 43.4 |
| Xylose | 14.8 – 20.2 |
| Arabinose | 2.7 – 4.5 |
| Mannose | 1.8 |
| Galactose | 0.4 |

According to Roslan et al. (2011), only cellulose and hemicellulose have the potential to be converted to sugar by chemical and/or enzymatic hydrolysis before fermentation. Table 2 shows an example of the composition of carbohydrate polymers (cellulose and hemicellulose) with the theoretical ethanol yield of rice straw, assuming hemicellulose fractions are all polymers of xylose (Binod et al., 2010).

Table 2. Carbohydrate composition and theoretical ethanol yield of rice straw (Binod et al., 2010)

| Component | Amount |
|---|---|
| Cellulose content (%) | 38.6 |
| Hemicellulose content (%) | 19.7 |
| Theoretical ethanol yield (L/kg dry) | 0.42 |

According to Lim et al. (2012), "Rice demand is expected to remain strong in the next few decades due to the economic and population growths in many countries across Africa and Asia." For every kilogram of harvested paddy, there will be rice straw waste ranging between 0.396 to 0.41 kg. Unfortunately, the farmers in many Asian countries are still practicing open field burning of rice straw after the harvesting season. In Malaysia, it is estimated that the amount of rice straw produced yearly is approximately two million tones from harvesting paddy fields with an area of 350,000 ha. There is an attempt to increase the yield of rice to 10 t/ha (M. Sashikala & Ong, 2009). Therefore, the increase in rice yield offers the promise of a huge and continuous supply of rice straw for bioethanol production in Malaysia.

Due to the promising yields of rice straw, rice straw is chosen as the material in this study. This research aimed to analyse and compare the ethanol extracted from rice straw with methods to combine and compare acid pretreatment with enzymatic hydrolysis and acid pretreatment alone.

## 2. METHODOLOGY

The methodology of this study consists of feedstock preparation, pretreatment of rice straw, enzymatic hydrolysis of rice straw, and fermentation, as shown in Figure 1.

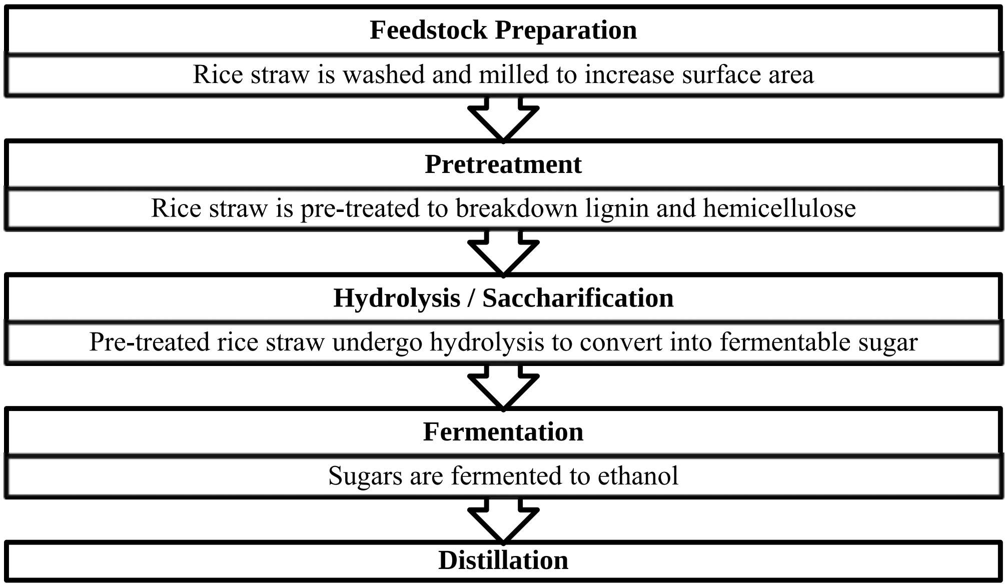


Figure 1 General methodology of the experiment

### 2.1. Preparation of Raw Material

Rice straw used throughout the study was obtained from Sekinchan, Selangor during harvesting season on July. The rice straw obtained was cleaned with running tap water to remove dust and any other unwanted impurities. Then, the washed rice straw was air dried using a hot air oven for 6 hours at $60^{o}$C.

### 2.2. Pretreatment of Rice Straw

The pretreatments involved mechanical pretreatment and acid pretreatment. Mechanical pretreatment aims to increase the total accessible surface area and size of lignocellulosic pores. Furthermore, mechanical pretreatment will also aid in decreasing the crystallinity and degrees of polymerization of the cellulose. Acid pretreatment was carried out under diluted concentration conditions. Acid pretreatment allows cellulose to have better accessibility to the enzymes action during hydrolysis and has little effect on degrading lignin (Binod et al., 2010). In this study, sodium hydroxide was used to neutralize the acidic solution in the acid pretreatment process.

*2.2.1. Pretreatment of rice straw*

The cleaned rice straw was physically treated by using a high speed home blender. The physically pretreated rice straw was stored inside a container at room temperature for further use.

*2.2.2. Acid pretreatment*

Six sets of samples, each with 3 g of physically pretreated rice straw, were weighed and transferred into respective conical flasks. The samples were labeled accordingly with two sets for a retention time of 30 minutes, two sets for a retention time of 60 minutes and two sets for a retention time of 90 minutes. A 100 ml measure of prepared 1 Molar (M) sulfuric acid ($H_2SO_4$) was added to each conical flask. The samples were heated using a water bath at 90℃ for retention time of 30, 60 and 90 minutes respectively. The samples were taken out after the retention time and were left to cool down at room temperature. A 100 ml of prepared 2 M sodium hydroxide was added to each conical flask for neutralization purposes. The procedures were repeated with 1.5 M and 2.0 M of sulfuric acid and 3.0 M and 4.0 M sodium hydroxide respectively to obtain different samples.

### 2.3. Enzymatic Hydrolysis of Pretreated Rice Straw

The conditions for highest yield of glucose from acid pretreatment were carried forward to enzymatic hydrolysis and the yielded solution was separated into solid and liquid phases by pressing through conventional cheesecloth. The liquid phase was brought forward for fermentation with the acid pretreatment solution.

Cellulase from Tichoderma Ressei ATCC was used as an enzyme in this study. There were 3 samples of pretreated rice straw (acid pretreatment and mechanical pretreatment) and 1 sample with only mechanically pretreated rice straw. A 100 ml measure of Sodium Acetate was added into each flask. Then, 0.8% w/w (24 mg for 3 g of substrate) of cellulase enzyme was added into each flask. The pH value for the samples was adjusted to 5.0. The samples were then incubated in a shaking incubator for up to 96 hours.

### 2.4. Fermentation of Reducing Sugars

The yeast used for the study was *Saccharomyces cerevisiae*. The liquid phase from the acid pretreatment and enzymatic hydrolysis was fermented and studied separately for a better understanding.

#### *2.4.1. Fermentation of acid pretreated solution*

Nutrients, namely yeast extract, ammonium chloride ($NH_4Cl$) and potassium dihydrogen phosphate ($KH_2PO_4$) with a ratio of 1:0.2:0.4 gram/100mL solution were respectively added to the acid pretreated samples. The pH value was then adjusted to 5.5. The samples were then autoclaved at 121 ℃ for 15 minutes. After autoclaving, *Saccharomyces cerevisiae* was introduced into all the samples, except the one which acted as the control for the experiment. Then, the samples were incubated using an incubator shaker for up to 120 hours at a setting of 100 rpm and 37$^{o}$C.

#### *2.4.2. Fermentation of acid pretreated and hydrolyzed solution*

Samples of acid pretreated and hydrolyzed solution were added with the nutrients as mentioned in the previous section. Likewise, the pH values of these samples were adjusted to 5.5 and autoclaved at 121$^{o}$C for 15 minutes. After autoclaving, Saccharomyces cerevisiae was also introduced into the samples, except the one which acted as the control for the experiment. Then, the samples were incubated using an incubator shaker for 72 hrs at setting of 100 rpm and 37$^{o}$C.

### 2.5. Distillation of Fermented Solution

The fermented solution from the acid pretreatment and enzymatic hydrolysis was then distilled using a rotary evaporator where the distillation process was carried out in a vacuum condition. The temperature of the heating was set to be at 73$^{o}$C and the distillation was left to run for 30 minutes under vacuum conditions. The pressure achieved was 80 kPa.

### 2.6. Analysis of Sugar Content using DNS Method

A 2 ml measure from each sample in the centrifuge tubes was transferred into different test tubes accordingly and 2 ml of the DNS reagent was added into each test tube. A control solution was prepared by mixing 2 ml of RO water with 2 ml of DNS reagent. The test tubes were heated in a water bath for 5 minutes at 90$^{o}$C. A spectrophotometer with a wavelength setting of 540 nm was used to obtain the data for level of absorbance.

### 2.7. Analysis of Ethanol Content by High Performance Liquid Chromatography (HPLC)

All samples were filtered using filter attached syringe and 30 µL of each sample was injected into the HPLC injection inlet. The running time of HPLC was set at 20 minutes. The values resulting from the HPLC were shown in the peak area unit and they were converted into the percentage of ethanol concentration through a standard curve made from several known ethanol concentrations. Furthermore, mechanical pretreatment will also aid in decreasing the crystallinity and degrees of polymerization of the cellulose. Acid pretreatment was carried out under diluted concentration conditions. Acid pretreatment allows cellulose to have better accessibility to the enzymes action during hydrolysis and has little effect on degrading lignin (Binod et al., 2010). In this study, sodium hydroxide was used to neutralize the acidic solution in the acid pretreatment process.

## 3. RESULTS AND DISCUSSION

### 3.1. Glucose Yield from Acid Pretreatment on Rice Straw

In the study, rice straw samples had been treated by varying two different parameters which were acid concentration and retention time of pretreatment. The total reduced sugar yield from each of the parameters is as shown in Figure 2. From Figure 2, all curves are shown to have a positive trend. However, the highest sugar yield was at 2.0 M at a retention time of 60 minutes, which was 9.71 g/L or 32.37 g/g%. Further retention time after 60 minutes at 2.0 M gave no meaningful increase in sugar yield. Although 1.5 M of acid concentration yielded 9.63 g/L or 32.11 g/g% of glucose which closely high as by 2.0 M, this was achieved after 90 minutes of pretreatment. The shorter pretreatment period is favorable since it indicates time and energy consumption efficiency in general.

### 3.2. Glucose Yield from Enzymatic Hydrolysis on Rice Straw

The acid pretreated rice straw under the best condition (2M of $H_2SO_4$ with retention time of 60 minutes) was carried forward for an enzymatic hydrolysis process with an enzyme loading of 0.8% w/w of a dry rice straw basis. The main varying parameter is time. Furthermore, a sample without acid pretreatment (only mechanical pretreatment) was also tested to study the effect of acid pretreatment on enzymatic activity.

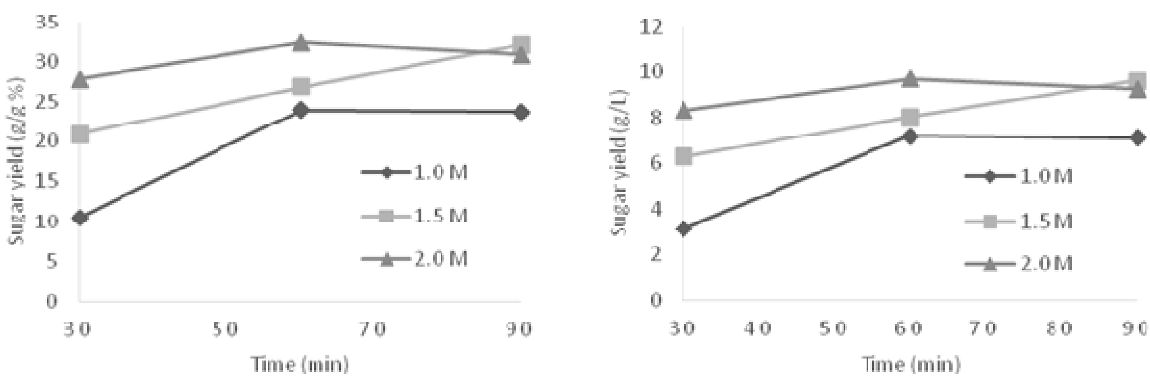


Figure 2 Effect of different acid concentrations with pretreatment period on sugar yield: (a) in g/g% unit and; (b) in g/L unit

Figure 3 shows the results obtained by using samples with a different pretreatment process. Rice straw without acid pretreatment (only mechanical pretreatment) showed almost negligible sugar yield even after undergoing enzymatic hydrolysis for 96 hours, while rice straw with additional acid pretreatment showed a good yield from enzymatic hydrolysis. The highest sugar yield for the mechanical pretreated only sample was 0.099 g/L, which occurred after 72 hours of hydrolysis.

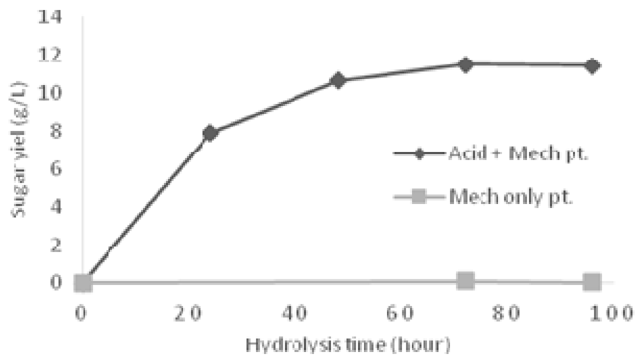


Figure 3 Effect of additional acid pretreatment on enzymatic hydrolysis to the glucose yield

Meanwhile, a combination of acid and mechanical pretreatment yielded as high a result as 11.466 g/L of glucose over the same hydrolysis period. The results indicate that acid pretreatment improves the glucose yield in the hydrolysis process and these results are aligned with other studies. A comparable study on the acid pretreatment effect on durian seed in bioethanol production was performed and resulted in a level of 50.09 g/L sugar higher than the one without it (only mechanical) in an enzymatic hydrolysis, which was only 23.66 g/L (Aditiya et al., 2014). Another study also stated that acid solution degrades the hemicellulose component in lignocellulosic material by cutting off the van de Waals forces of covalent and hydrogen bonds that made up the component (Li et al., 2010). By having a simpler form of the hemicellulose, the enzymes are expected to have better access to perform the hydrolysis and yield more sugar.

### 3.3. Analysis of Ethanol Produced

Figure 4 shows the effect of different pretreatment methods on fermentation based on the amount of glucose consumed. An acid pretreated only sample required more time for the yeast to consume more glucose, from initial measure of 7.664 g/L to 0.926 g/L glucose after 120 hours of fermentation. On the contrary, acid pretreatment with an enzymatic hydrolysis sample only required 72 hours for the yeast to consume 11.466 g/L to 0.266 g/L of glucose. Both samples indicate a drastically decreasing pattern, but the latter sample is preferable for its faster conversion rate (97.68%).

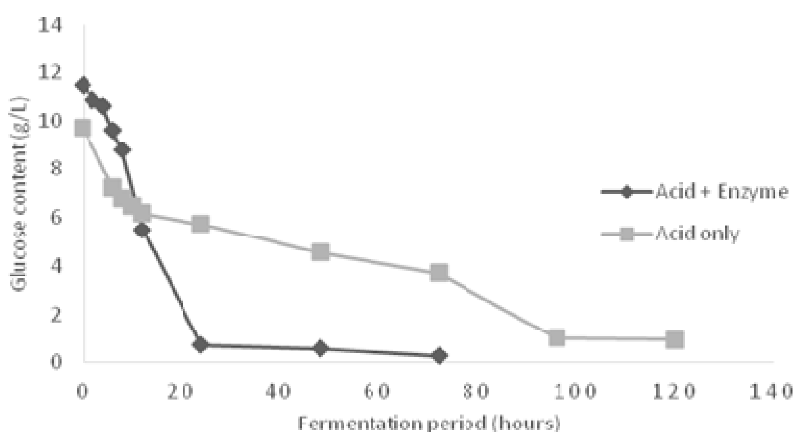


Figure 4 Glucose reduction on fermentation process of different samples

Experimental results obtained from the HPLC were recorded from the conversion of peak areas generated by the HPLC through the ethanol standard curve. From the HPLC method, the acid only pretreated sample produced 0.013% of ethanol. Meanwhile, additional enzymatic hydrolysis fermentation resulted in a rating of 0.1503% of ethanol. When experimental values were obtained through the HPLC testing method, theoretical values were also observed in this study through stoichiometry. Since in theory fermentation converts one glucose monomer to two ethanol compounds, 11.26% ethanol was recorded from the acid only pretreated sample while 52.75% ethanol was from the combination of an acid pretreated and enzymatic hydrolyzed sample. The results are in agreement with another similar study, as acid pretreatment combined with enzymatic hydrolysis also gave an improvement of ethanol content from durian seed (Aditiya et al., 2014). The literature also mentioned that acid pretreatment gave a finer form of sugar for the yeast to consume the substrate than the one without it. The finer form of sugar (glucose) is necessary since *Saccharomyces cerevisiae* is only able to ferment glucose, other form of sugars are able to be fermented by other types of microorganisms (Kumar & Wyman, 2009). By combining both acid pretreatment and enzymatic hydrolysis, it is expected to have better glucose yield and ethanol produced due to the combination of both favorable characteristics, compared to the one with only acid pretreatment (Figure 4).

## 4. CONCLUSION

Glucose concentration (formed from breaking lignocellulosic material into reducing sugar) and ethanol grade (formed from microorganism activity of converting sugar into ethanol) are desirable and as studied are considered in both methods observed as acid only pretreatment and acid pretreatment with enzymatic hydrolysis. The cellulase enzyme from Tichoderma Ressei ATCC 26921 was used in enzymatic hydrolysis, yielding 0.099 g/L after retention time of 72 hours, while the additional acid pretreatment in hydrolysis resulted in a yield of 11.466 g/L of glucose. In conclusion, the combination of acid pretreatment with enzymatic hydrolysis is more desirable for its higher yield of ethanol content after 72 hours fermentation (0.1503% or 52.75% of the theoretical value) compared to the acid only pretreated sample after 120 hours fermentation (0.013% or 11.26% of the theoretical value).

Further and deeper research on the conversion of rice straw biomass into bioethanol is strongly advised as it offers a high potential for bioethanol production from agricultural waste. Moreover, conducting an optimization approach in this study is definitely recommended for the second generation of biofuel research. Although second generation bioethanol production is not yet commercially viable, it is believed that bioethanol would be able to make a major contribution to society especially in transportation sector.

## 5. ACKNOWLEDGEMENT

Authors would like to acknowledge this research as supported by Exploratory Research Grant Scheme (ERGS) through the Ministry of Higher Education of Malaysia (MOHE) with reference number ERGS/1/2013/TK07/UNITEN/01/01.

## 6. REFERENCES

Aditiya, H.B., Chong, W.T., Ghazali, K.A., Mahlia, T.M.I., 2014. Acid Pre-treatment of Durian Seed to Improve Glucose Yield as Second Generation Bioethanol. *REEGETECH Proceeding*, 2014 Bandung, Indonesia. pp. 100–104

Binod, P., Sindhu, R., Singhania, R.R., Vikram, S., Devi, L., Nagalakshmi, S., Kurien, N., Sukumaran, R.K., Pandey, A., 2010. Bioethanol Production from Rice Straw: An Overview. *Bioresource Technology*, Volume 101, pp. 4767–4774

Bp, 2012. *BP Statistical Review of World Energy*, June 2012. In: P.L.C, B. (ed.)

Choong, M.Y., 2012. *'Useless' Bioethanol Now Finds Wide Uses*. TheStar, October 2

Dwivedi, P., Alavalapati, J.R.R., Lal, P., 2009. Cellulosic Ethanol Production in the United States: Conversion Technologies, Current Production Status, Economics, and Emerging Developments. *Energy for Sustainable Development*, Volume 13, pp. 174–182

Karimi, K., Kheradmandinia, S., Taherzadeh, M.J., 2006. Conversion of Rice Straw to Sugars by Dilute-acid Hydrolysis. *Biomass and Bioenergy*, Volume 30, pp. 247–253

Kumar, R., Wyman, C., 2009. Effect of Enzyme Supplementation at Moderate Cellulase Loadings on Initial Glucose and Xylose Release from Corn Stover Solids Pretreated by Leading Technologies. *Biotechnology and Bioengineering*, Volume 102, pp. 457–467

Li, C., Knierim, B., Manisseri, C., Arora, R., Scheller, H., Auer, M., 2010. Comparison of Dilute Acid and Ionic Liquid Pretreatment of Switchgrass: Biomass Recalcitrance, Delignification and Enzymatic Saccharification. *Bioresource Technology*, Volume 101, pp. 4900–4906

Lim, J.S., Abdul Manan, Z., Wan Alwi, S.R., Hashim, H., 2012. A Review on Utilisation of Biomass from Rice Industry as a Source of Renewable Energy. *Renewable and Sustainable Energy Reviews*, Volume 16, pp. 3084–3094

Nutawan, Y., Phattayawadee, P., Pattranit, T., Eshtiaghi, M.N., 2010. Bioethanol Production from Rice Straw. *Energy Research Journal*, Volume 1, 26–31

Roslan, A.M., Yee, P.L., Shah, U.K.M., Aziz, S.A., Hassan, M.A., 2011. Production of Bioethanol from Rice Straw using Cellulase by Local Aspergillus sp. *International Journal of Agricultural Research*, Volume 6, pp. 188–193

Sashikala, M., Ong, H.K., 2009. Synthesis, Identification and Evaluation of Furfural from Rice Straw. *Tropical Agriculture and Food Science*, Volume 37, pp. 95–101

Walker, G.M., 2010. *Bioethanol: Science and Technology of Fuel Alcohol*. Global Production of Bioethanol. Graeme M. Walker & Ventus Publishing ApS