The effect of dilute acid pre-treatment process in bioethanol production from durian (*durio zibethinus*) seeds waste

K A Ghazali[a], S F Salleh[a, 1], T M I Riayatsyah[b], H B Aditiya[c], T M I Mahlia[a]
[a]Centre of Renewable Energy,Universiti Tenaga Nasional, 43000 Kajang, Selangor, Malaysia

[b]Department of Mechanical Engineering, Faculty of Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia

[c]Department of Mechanical Engineering, The University of Melbourne, VIC 3010, Australia

Abstract. Lignocellulosic biomass is one of the promising feedstocks for bioethanol production. The process starts from pre-treatment, hydrolysis, fermentation, distillation and finally obtaining the final product, ethanol. The efficiency of enzymatic hydrolysis of cellulosic biomass depends heavily on the effectiveness of the pre-treatment step which main function is to break the lignin structure of the biomass. This work aims to investigate the effects of dilute acid pre-treatment on the enzymatic hydrolysis of durian seeds waste to glucose and the subsequent bioethanol fermentation process. The yield of glucose from dilute acid pre-treated sample using 0.6% H_2SO_4 and 5% substrate concentration shows significant value of 23.4951 g/L. Combination of dilute acid pre-treatment and enzymatic hydrolysis using 150U of enzyme able to yield 50.0944 g/L of glucose content higher compared to normal pre-treated sample of 8.1093 g/L. Dilute acid pre-treatment sample also shows stable and efficient yeast activity during fermentation process with lowest glucose content at 2.9636 g/L compared to 14.7583g/L for normal pre-treated sample. Based on the result, it can be concluded that dilute acid pre-treatment increase the yield of ethanol from bioethanol production process.

1. Introduction

Bioethanol is a renewable energy source which offers a sustainable solution to the rise in the global energy demand and depleting oil reserves. The current trend of producing bioethanol from food crops such as corn, barley, wheat and sugarcane has led to concern over food security. Therefore, lignocellulosic biomass which is defined as lignocellulosic material with cheap and abundant properties derived from plant is deemed as a better feedstock in the second generation of biofuel [1]. Durian is a seasonal fruit which belongs to *Bambaceae* family, found typically in the South East Asia. Malaysia as one of the biggest producer and exporter of durian generates large amount of agricultural wastes with an average of 90,141 metric tonnes of waste produced annually from 2008 to 2010 [2]. Currently, durian seeds are not being utilised for commercial purposes and usually thrown away as a waste product. Nevertheless, the raw seeds contain high percentages of carbohydrate (43.6%) [3] and therefore, can potentially be used as biomass feedstock for bioethanol production.

[1] Author of correspondence: sitifatihah.salleh@gmail.com

The bioethanol production process of lignocellulosic biomass starts from pre-treatment, hydrolysis, fermentation, distillation and finally obtaining the final product, ethanol [4]. Different pre-treatment methods are available to prepare the raw materials for enzymatic hydrolysis. One of them is the chemical pre-treatment method which mainly uses acids and bases of different strengths and under different conditions to effectively break down the hemicellulose [5]. This is so that the cellulose becomes easily accessible to enzymes during the subsequent hydrolysis process where it is converted to glucose. In this work, dilute acid was used during the pre-treatment process of durian seeds wastes since it is less corrosive than concentrated acid and less inhibitive to the yeast during the fermentation process. The effects of this pre-treatment process towards the production of sugar and bioethanol were investigated.

2. Methodology

2.1. Preparation of raw material

Durian seeds waste was obtained from local fruits stalls around Kajang, Selangor. The seeds were later washed with plain water and dried in the oven at 70°C for 3 hours to remove excess moisture from the outer layer of the seeds. Then, the outer skins of the seeds were peeled to remove the lignocellulosic material as only the inner part contain high amount of carbohydrates useful for ethanol production. The peeled seeds were then shredded by vegetable grater, followed by drying at 80°C for 4 hours and grinding until it become granulated-powdery durian seeds.

2.2. Dilute acid pre-treatment of durian seeds

Four sets of samples, each with 100 ml of H_2SO_4 of different concentrations 0.019 M, 0.056 M 0.113M and 0.167 M were prepared inside conical flasks. 5 g of physically pre-treated durian seeds, were weighed and transferred into respective conical flasks. The prepared flasks were then sealed and heated in an autoclave at 130°C for a retention time of 30 minutes and left to cool down to room temperature. 100 ml of prepared 0.038M, 0.113M, 0.225M and 0.338M sodium hydroxide solutions were added to each conical flask respectively for neutralization purpose.

2.3. Enzymatic hydrolysis of dilute acid pre-treated durian seeds

0.025g of sodium chloride was added to the acid pre-treated samples for stabilization. Then, α-amylase enzymes (Sigma-Aldrich) was added into the samples and incubated in a shaking incubator for 90 minutes.

2.4. Fermentation of reducing sugars

Nutrients, *Saccharomyces cerevisiae* 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 added to the acid pre-treated and enzyme hydrolysed sample. The samples were then autoclaved at 121 ^oC 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 48 hours at a setting of 160 rpm and 37 ^oC.

2.5. Analysis of sugar content using DNS method

1 ml solution was collected from each samples into test tubes containing 1 ml of 0.1% DNS reagent [6]. A control solution was prepared by mixing the DNS reagent with 1 ml of RO water. The test tubes were heated in a water bath at 90 ^oC for 5 minutes. The corresponding absorbance values (A_{540}) were measured using a spectrophotometer.

2.6. Analysis of ethanol content by High Performance Liquid Chromatography (HPLC)
20 μL samples were filtered by using 0.50 μm fibre meshes and later injected into the HPLC. 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.

3. Results and discussion

3.1. Glucose yield from acid pre-treatment on durian seeds
The total reduced sugar yield after 90 min from different concentration of acid was plotted in Figure 1. The highest sugar yield was at 0.113 M which was 23.5 g/L. Further increase in acid concentration gave no meaningful increase in sugar yield.

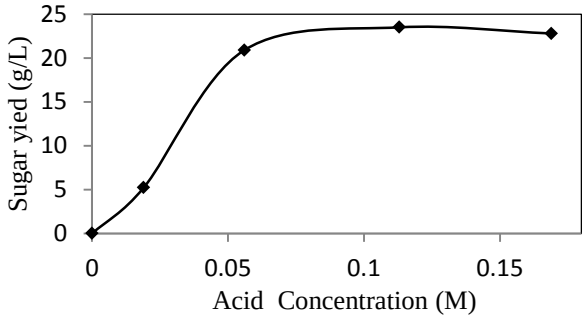

Figure 1. Effect of different acid concentrations on sugar yield

3.2. Glucose yield from enzymatic hydrolysis on durian seeds
The sample containing durian seeds that were pre-treated with 0.113 M H_2SO_4 was carried forward for the enzymatic hydrolysis process. The hydrolysis was also repeated with durian seeds without acid pre-treatment (only mechanical pre-treatment) as a control. Figure 2 shows the results obtained by each sample. Durian seeds without acid pre-treatment showed almost negligible sugar yield of 5.56 g/L at 90 min. On the other hand, additional acid pre-treatment enhances the yield from enzymatic hydrolysis significantly to 50.1 g/L at 90 min.

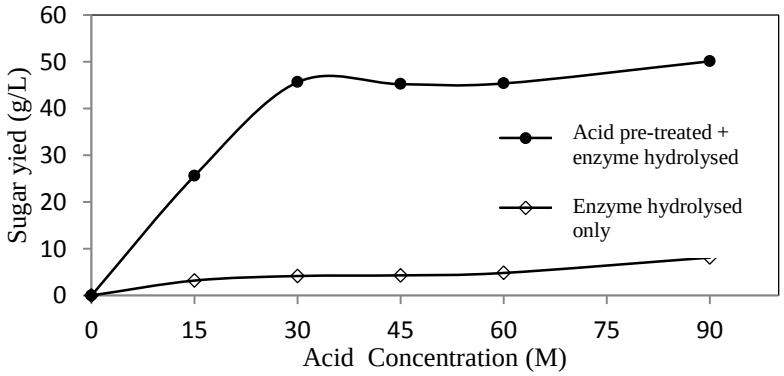

Figure 2. Effect of additional acid pre-treatment on sugar yield from enzymatic hydrolysis

3.3. Analysis of ethanol produced

Figure 3 compares the fermentation profiles of the enzyme hydrolysed with additional acid pre-treatment sample and enzyme hydrolysed sample. The reduction in the glucose content signifies the amount of glucose being fermented by yeast to produce ethanol. The fermentation activity of the yeast is significantly lower than the acid pre-treated sample, from initial measure of 23.1 g/L to 13.9 g/L glucose after 30 hours of incubation. On the other hand, the yeast in acid pre-treated with an enzymatic hydrolysis sample takes only 24.2 hours to consume 34.5 g/L of glucose. From the HPLC method, the enzyme hydrolysed with additional acid pre-treatment sample produced 0.47% of ethanol.

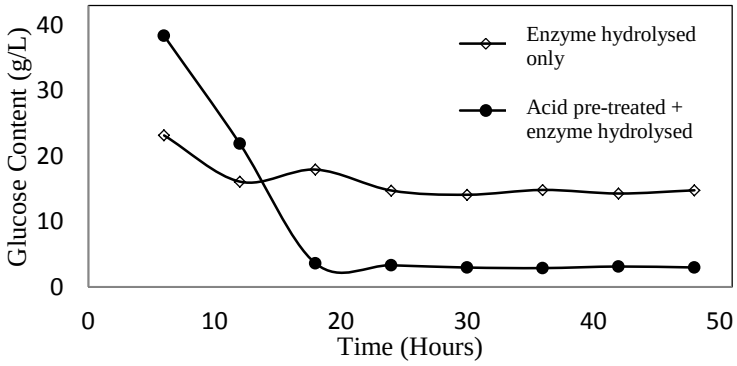

Figure 3. Fermentation profiles of the enzyme hydrolysed with additional acid pre-treatment sample and enzyme hydrolysed sample.

4. Conclusion

The pre-treatment of durian seeds with dilute H_2SO_4 (0.113 M) has significantly enhanced the yield of sugar obtained in enzymatic hydrolysis (50.1 g/L) and ethanol in the fermentation process (0.47%).

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

[1] Gomez L D 2008 Sustainable liquid biofuels from biomass: the writing's on the walls *New Phytol*, pp 473-485
[2] Omar A A M F Z Zakaria Z Long H D Abdullah A G 2011 Durian skin machine *6th MARDI Science & Technology Exhibition*, p 75
[3] Brown M J 1997 *Durio* - a bibliographic review (Canada: International Plant Genetic Resources Institute)
[4] Mussatto S I Dragone G Guimarães P M R Silva J P A Carneiro L M Roberto I C Vicente A Domingues L Teixeira J A 2010 Technological trends, global market, and challenges of bio-ethanol production. *Biotechnol Adv* , pp 817–830
[5] Zheng Y Pan Z Zhang R 2009 Overview of biomass pretreatment for cellulosic ethanol production *Int J Agric & Biol Eng*, **2** (3) p 51
[6] Miller G L 1959 Use of dinitrosalicylic acid reagent for determination of reducing sugar *Anal Chem*, pp 426-428