ENZYMATIC SACCHARIFICATION OF HYDROGEN PEROXIDE PRETREATED OF RICE STRAWFOR BIOETHANOL PRODUCTION

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SUMMARY

Rice straw contains more than 35% of cellulose.This material presents a potential feedstock for bioethanol production. In this study, the two-stage pretreatment of Q5 specie rice straw was used for preparation of cellulosic pulp for enzymatic saccharification. Firstly, rice straw was treated by mixture of hydrogen peroxide with sulfuric acid and sodium molipdate in conditions of liquor-to rice straw ratio (10:1) ml/g, temperature 120°C and 120 mins of cooking. Secondary, pretreated rice straw was submerged in sodium hydroxide solution (2.5% w/w), at temparature of 100°C, for 30 min. Enzymatic saccharification of pretreated rice straw by Novozymes commercial enzyme Cellic®CTec2 and Cellic®HTec2 with dosage of 80 FPU/g and 18 FPU/g of pretreatred rice straw achieved the reduce sugar yield an average 40.0% - 41.0% of over dry rice straw. Fermentation of sugars has been succesful.

Keywords: Bioethanol, enzymatic saccharification, reduce sugars, rice straw, pretreatment.

I. INTRODUCTION

Ethanol from renewable resources has been of interest in recent decades as an alternative fuel to the current fossil fuels. Moreover, a promising scenario to reduce the negative consequences of the greenhouse effect is to produce fuel from biomass. Lignocellulosic biomass materials have especially great potential since they are ubiquitous and can serve as substrates for bioethanol production. However, the production of bioethanol from food crops such as grains (first generation biofuels) has resulted in an undesirable direct competition with food supply. A switch to a more abundant inedible plant material should help to reduce pressure on the food crops.

Rice straw is a by-product of rice production, great bio-resource and is one of the abundant lignocellulosic waste materials in the world. Annually Vietnam produces about 45 million tons of rice and hence produces about 50 million tons of rice straw biomass. A large part of this rice straw is going as cattle feed, combustion in life, making the compost and rest is eliminated to the surrounding environment as waste.

Utilization of rice straw as a renewable source for the bioethanol production has attracted the interest of many researchers in the past two decades, with a series of studies on pretreatment, saccharification and fermentation using different microorganisms and commercial enzymes. Rice straw has several characteristics that make it a potential feedstock for fuel ethanol production. Large parts of rice straw material are made up of complex carbohydrates such as cellulose and hemicelluloses which can be readily converted to fermentable sugars. In particularly, rice straw predominantly contains cellulose 32-47%, hemicelluloses 19-27%, lignin 5-24%, and ashes 10-18%.

A number of pretreatment technologies based on numerous physical, chemical and biological methods have been developed for pretreatment of rice straw such as steam explosion, hot water treatment, solvent treatment, alkaline treatment and acid treatment. Among these pretreatment methods the alkaline treatment appears to be the most effective method for rice straw pretreatment in dissolution of ash and breaking the ester bonds between lignin, hemicellulose and cellulose as well as avoiding fragmentation of the cellulose polymers. One of the major challenges in developing technology for bioethanol production from rice straw is selection of an appropriate pretreatment technique. Notably, in these previous reports, relatively effective method for rice straw is alkaline pretreatment but at mild treatment condition such as at low temperatures but with a relatively long time, which gives low yield of reducing sugars and could not fully remove of lignin and ash in raw material. While many factors contribute to alkaline pretreatment such as alkali use, temperature as well as other processing factors should be optimized to accomplish pretreatment, minimize waste treatment, and minimize working capital investment resulting from raw material costs. Thus the aim of this study is to optimize the pretreatment process of rice straw for improving of enzymatic saccharification. The results present herein originated form an investigation to determine the optimum alkaline pretreatment process to efficiently break down the close association between carbohydrates and lignin in rice straw, disolve the silica and to make the substrates more accessible for the enzyme.

II. MATERIAL AND METHODS

2.1. Materials

Q5 specie rice straw (*Oryza sativa L.*), which was harvested in 2013 from 300-400 m^2 planted area in Tien Hai Dist, Thai Binh province, than has dried and prevented, after 15-20 days was used for experiments. The compositions of raw rice straw are cellulose content of 35.9%, pentose of 23.3%, lignin of 17.2%, ash content of 11.2% and extractives in one percent sodium hydroxide solution of 3.5%. The over dry rice straw was grinded and screened. The fraction that was retained on 24 mesh screen was used for the study. A commercial enzyme, Cellic®CTec2 and Cellic®HTec2 were obtained from Novozymes, Denmark. Their activities were determined to be 14 FPU/ml as cellulase and 11 FPU/ml as xylanase. Other chemicals were purchased from Merck, Germany and China.

2.2. Hydrogen peroxide pretreatment

Rice straw pretreatments were carried out with solid/liquid ratio of 1:10 in 1 litter autoclave (Fig.1). The autoclave was kept in oil bath and heated up from 60^{o}C to desired temperature. Amount of hydrogen peroxide, sulfuric acid and sodium molipdate used for experiment varied in certain range to choose the optimal conditions. Residual solid sample was then separated from the pretreatment liquid by filtration through predried and preweighed glass fiber. Pretreated rice straw was treated by sodium hydroxide solution with dosage 2.5% of over dry rice straw at 100^{o}C within 30 min. Obtained pulp was washed by DI water several time, neutralized by acetic acid and collected for enzymatic saccharification.

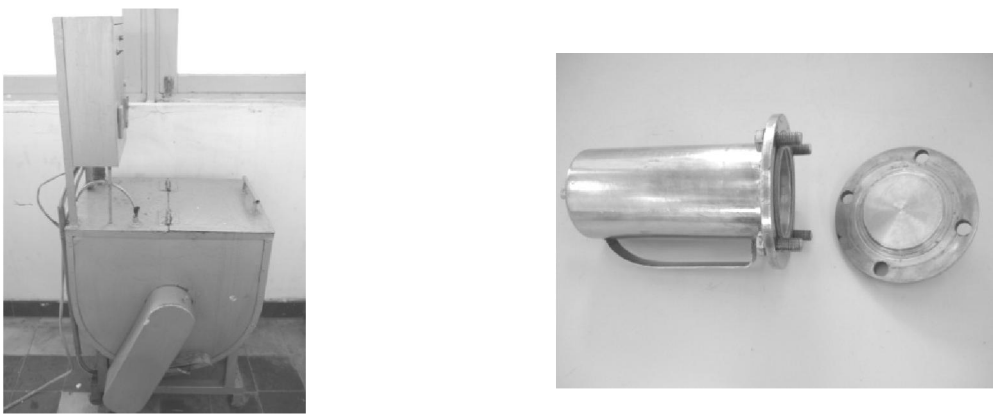

Fig.1. Laboratory autoclave for tretreatment of rice straw

2.3. Enzymatic saccharification

Samples of pretreated biomass solid was taken and placed in 250 ml flask for enzymatic hydrolysis with liquid/solid ratio of 10:1 at 50^oC in 120 hrs. 0.05 M sodium citrate solution with pH of 5 was used as buffer. Cellic® CTec2 and Cellic® HTec2 enzyme dosage were 80 FPU/g and 18 FPU/g dry biomass matters, respectively. After hydrolysis the slurry was filtered to get the solid residue and the liquid hydrolysate.

2.4. Analysis

Basic chemical composition of rice straw (moisture, ash content, lignin content of biomass) were characterized following the TAPPI methods, cellulose content was determined by Kushner – Hoff method.

Yield of reducing sugar was calculated followed equation: Y (%) = Msg/Mrr * 100%

Whereas: Msg was amount of reducing sugar recovered after enzymatic hydrolysis (g); Mrr was amount of dry raw rice straw (g).

Liquid hydrolysate was characterized by spectrophotometer (Optima-sp 300) to determine the yield of reducing sugar following the methods that reported by Miller (Dinitro salicylic acid (DNS) method).

III. RESULTS AND DISCUSSION

3.1. Influence of hydrogen peroxide dosage to yield of reducing sugar

In this case, rice straw was pretreated by hydrogen peroxide in acid solution (sulfuric), with adding of sodium molipdate as a catalyst for reaction of hydrogen peroxide conversion by sulfuric acid to the hydrogen peroxyl anion HOO^-, which as delignification reagent. This a new environmental friendly low temperature method that can be used for pulping. Several experiments with hydrogen peroxide pretreatment of rice straw (data not shown) to study on influence of experiment factors to enzymatic saccharification were carried out. It's found that the suitable pretreated time is about 120 min for cooking at 120^oC. Therefore, this pretreated time was chosen for next experiments.

In order to examine the effect of composition of pretreatment reagents on the damage of rice straw structure and enzymatic saccharification, different hydrogen peroxide dosages were used to treat the raw material. The sulfuric acid dosage was 1% and the sodium molipdate dosage was 0,1% w/w over dry rice straw. The results were shown in Fig.2. It could be seen that higher content of hydrogen peroxide led to better deconstruction of lignocelluloses biomass and increasing the obtained sugar in sample. In particular, using low amount of hydrogen peroxide (< 5% w/w over dry rice straw) gave low yield of reducing sugar, about 8% w/w of the raw material. Yield of reducing sugar increased when the hydrogen peroxide content increased. Yield of reducing sugar reached to maximum of 40-41% w/w at hydrogen peroxide content of 12-15%. Therefore, hydrogen peroxide dosage of 12-15% w/w dry raw material could be considered as optimum condition for alkaline pretreatment of rice straw.

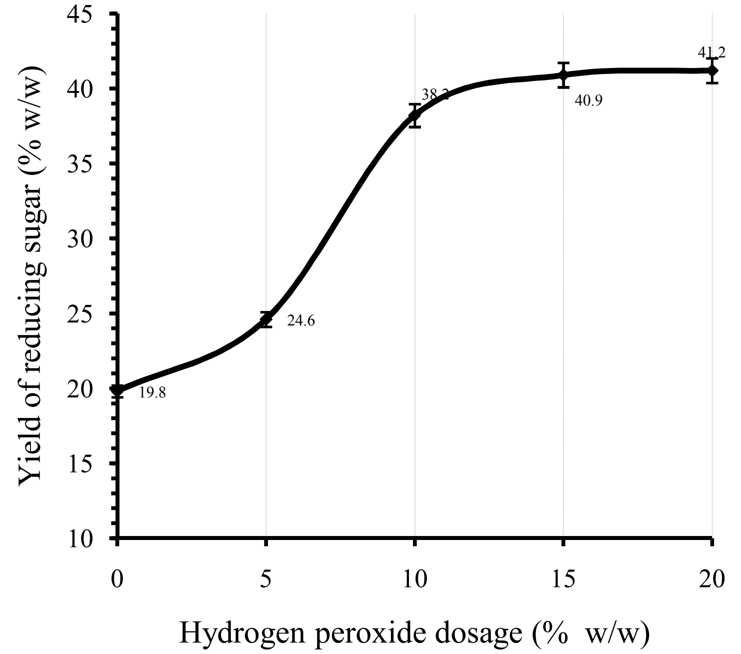

Fig.2. The effect of amount of hydrogen peroxide on yield of reducing sugar after enzymatic hydrolysis

3.2. Influence of sodium molipdate dosage to yield of reducing sugar

We have conducted the experiments to find out the suitable condition for pretreatment of rice straw with hydrogen peroxide dosage 15% w/w dry rice straw by changing the sodium molipdate dosage. After pulping, the efficiency of the process was determined based on the yield of reducing sugar. Results of this experiments were showed in Fig.3.

It could be seen that by keeping the solid/liquid ratio of 1:10, temperature of 120^{o}C, treatment time of 120 min with the increase of sodium molipdate charge in range of 0 - 0.2% (w/w dry rice straw), the yield reducing sugar also increased from about 21.6% to 41.3 %. It's found in sodium molipdate dosage level more than 0.025% w/w the yield of reducing sugar increased not significantly. Thus the sodium molipdate charge of 0.025% was considered as suitable for pretreatment.

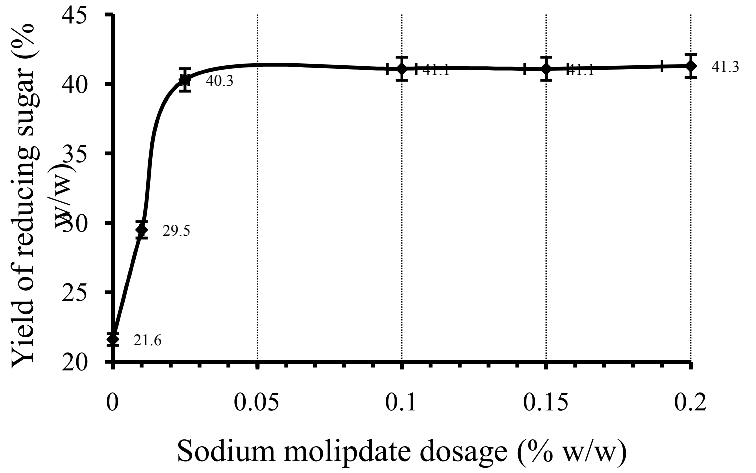

Fig.3. The effect of amount of sodium molipdate on yield of reducing sugar after enzymatic hydrolysis

3.3. Influence of sodium molipdate dosage to yield of reducing sugar

The effect of sulfuric acid dosage on yield of reducing sugar had similar trend with hydrogen peroxide and sodium molipdate charge. Next experiments have conducted with hydrogen peroxide dosage 15% w/w and sodium molipdate dosage 0.025% w/w. When sulfuric acid dosage increased from 0.25% to 2.0% w/w dry rice straw, the yield of reducing sugar increased from about 21.2% up to 41.6%. However, when sulfuric acid dosage increased further from 1.5% to 2.0%, the yield of reducing sugar increased slightly (Figure 4). Therefore, by consideration of economic and technical efficiency the sulfuric acid dosage for pretreatment of rice straw was chosen to be 1-1,5%.

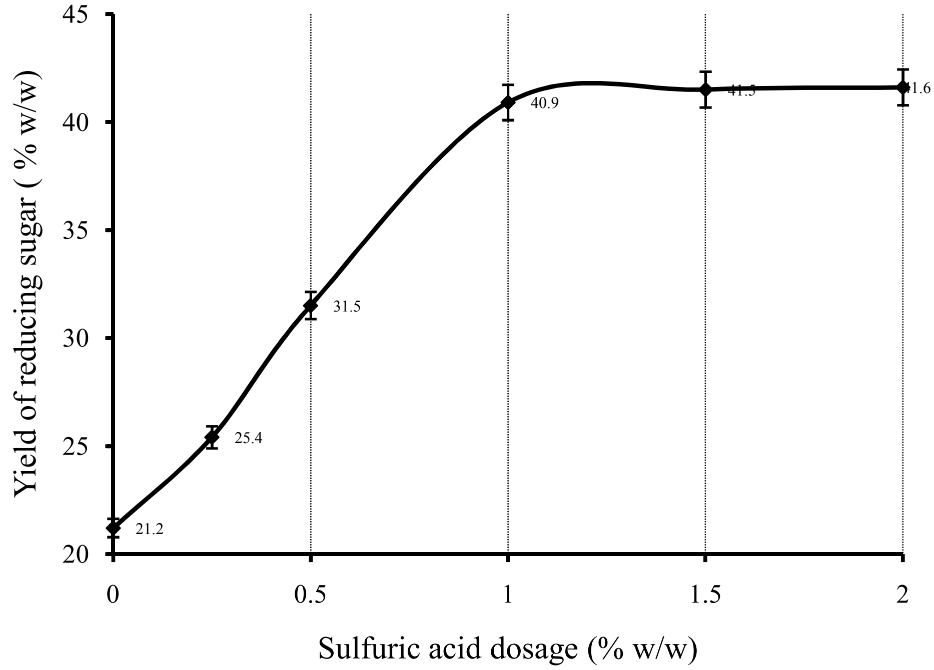

Fig.4. The effect of amount of sulfuirc acid on yield of reducing sugar after enzymatic hydrolysis

IV. CONCLUSION

Studies were performed for the Q5 specie rice straw two-stage pretreatment process by hydrogen peroxide in sulfuric acid solution with adding of sodium molipdate and the results indicated that the experiments were reliable and significant.

The optimal conditions of first-stage were found to be a temperature of 120°C, hydrogen peroxide dosage of 10-15%, sodium molipdate dosage of 0.025-0.05%, sulfuric acid dosage of 1.0-1.5% w/w dry rice straw and a pretreated time of 120 min.

The following second-stage was carried out at temperature of 100°C within 30 min., with sodium hydroxide dosage of 2.5% w/w dry rice straw. These conditions can be optimized.

When the pretreatment was carried out under optimal conditions, approximately 40.0-41.0 % (w/w dry rice straw) of reducing sugar was recovered.

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ĐƯỜNG HÓA RƠM RẠ ĐÃ QUA TIỀN XỬ LÝ BẰNG HYDROPEOXIT CHO SẢN XUẤT ETANOL SINH HỌC

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TÓM TẮT

Rơm rạ chứa trên 35% xenlulozơ. Đây là nguyên liệu tiềm năng cho sản xuất etanol sinh học. Trong nghiên cứu này, phương pháp tiền xử lý hai công đoạn rơm rạ giống lúa Q5 đã được sử dụng để thu nhận xenlulozơ cho quá trình đường hóa bằng enzyme. Trước tiên, rơm rạ được xử lý bằng hỗn hợp hydropeoxit, axit sunfuric và natri molipdat, với tỉ dịch (lỏng:rắn) là 10 ml/g, ở nhiệt độ 120^{o}C, trong 120 phút. Sau đó, rơm rạ đã qua xử lý được phối trộn với dung dịch NaOH với tỉ lệ 2,5% so với rơm rạ, ở nhiệt độ 100^{o}C, trong 30 phút. Đường hóa rơm rạ đã qua xử lý bằng enzyme thương phẩm Cellic®CTec2 và Cellic®HTec2 của Novozymes, được tiến hành với mức sử dụng tương ứng là 80FPU/g và 18FPU/g so với rơm rạ đã qua tiền xử lý, cho hiệu suất đường khử đạt 40,0-41,0% sơ với rơm rạ khô gió. Đường có khả năng lên men tốt.

Từ khóa: Đường hóa enzyme, đường khử, etanol sinh học, rơm rạ, tiền xử lý.

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