การใช้ไมโครเวฟช่วยในกระบวนการปรับสภาพเบื้องต้นพร้อมการย่อยกากเมล็ดสบู่ดำเพื่อผลิตไบโอเอทานอล

Microwave-Assisted Simultaneous Pretreatment and Hydrolysis of Physic Nut Seed Cake for Bioethanol Production

วิมลรัตน์ ทองภูธร[1] วีไลพร สุปัญโญ[1] สุภาวดี แทนกุดเรือ[1] มานพ ศรีอุทธา[2]

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บทคัดย่อ

วัตถุประสงค์ของงานวิจัยนี้เพื่อนำเสนอวิธีการปรับปรุงกระบวนการผลิตไบโอเอทานอล โดยใช้ไมโครเวฟช่วยในการปรับสภาพเบื้องต้น พร้อมการย่อยกากเมล็ดสบู่ดำ ซึ่งเป็นวัตถุดิบที่สามารถนำมาผลิตเอทานอลได้ เนื่องจากมีองค์ประกอบส่วนใหญ่เป็นเซลลูโลสร้อยละ 93 เฮมิเซลลูโลส 3.55 และลิกนิน 2.92 โดยมวล ศึกษาสภาวะที่เหมาะสมของการปรับสภาพเบื้องต้นพร้อมการย่อยโดยใช้กรดซัลฟูริคที่มีความเข้มข้นร้อยละ 1 - 4 โดยปริมาตร ทำไมโครเวฟเป็นเวลา 1, 3, 5 และ 7 นาที ด้วยกำลังวัตต์ 300, 400 และ 500 วัตต์ ภายใต้สภาวะที่เหมาะสมคือใช้กรดซัลฟูริคที่มีความเข้มข้นร้อยละ 4 โดยปริมาตร ด้วยกำลัง 500 วัตต์ เป็นเวลา 7 นาที จะได้น้ำตาลรีดิวซ์ 7.702 มิลลิกรัมต่อมิลลิลิตร นอกจากนี้เมื่อเปรียบเทียบผลการทดลองที่ได้จากการปรับสภาพเบื้องต้นพร้อมการย่อยด้วยกรดหรือการทำไมโครเวฟเพียงอย่างเดียว พบว่าการใช้กรดร่วมกับการทำไมโครเวฟจะช่วยเพิ่มผลผลิตน้ำตาลรีดิวซ์ได้มากกว่า ทั้งนี้อาจเนื่องมาจากกระบวนการดังกล่าวทำให้เกิดปฏิกิริยาได้มากกว่า และสามารถเปลี่ยนเฮมิเซลลูโลสและเซลลูโลสให้เป็นน้ำตาลที่ละลายได้มากกว่า นอกจากนี้ได้ศึกษาสภาวะที่เหมาะสมของการหมักแบบกะโดยศึกษาความเข้มข้นของยีสต์สายพันธุ์ *Saccharomyces Cerevisae* (ร้อยละ 1 - 4 โดยมวลต่อปริมาตร) และระยะเวลาในการหมัก (1-4 วัน) พบว่า ได้เอทานอลสูงสุดที่ความเข้มข้นร้อยละ 0.39 โดยปริมาตร

[1] *คณะวิศวกรรมศาสตร์ มหาวิทยาลัยเทคโนโลยีราชมงคลอีสาน วิทยาเขตขอนแก่น*

[2] *คณะวิทยาศาสตร์ มหาวิทยาลัยขอนแก่น วิทยาเขตหนองคาย*

E - mail : aammyytt@hotmail.com

เมื่อหมักโดยใช้ยีสต์ที่มีความเข้มข้นร้อยละ 3 โดยมวลต่อปริมาตร เป็นเวลา 4 วัน ดังนั้นจึงสรุปได้ว่าการใช้กรดร่วมกับการทำไมโครเวฟในกระบวนการปรับสภาพเบื้องต้นพร้อมการย่อยจะช่วยให้ได้น้ำตาลรีดิวซ์ซึ่งสามารถหมักให้เป็นเอทานอลได้ในเวลาอันสั้น

คำสำคัญ : ไบโอเอทานอล; ไมโครเวฟ; กากเมล็ดสบู่ดำ; การปรับสภาพเบื้องต้น; การย่อย; น้ำตาลรีดิวซ์

Abstract

The aim of this research was to investigate the potential for improvement of bioethanol production from physic nut seed cake by microwave assisted in simultaneous pretreatment and hydrolysis. Physic nut seed cake is a renewable resource that can be used to produce ethanol because it consisted of mainly cellulose (93.55% by mass), hemicelluloses (3.55% by mass) and lignin (2.92% by mass). The optimized conditions for simultaneous pretreatment and hydrolysis including concentration of sulfuric acid (H_2SO_4) in the range of 1 - 4% (v/v), time for microwave (1, 3, 5 and 7 min) and power of microwave (300W, 400W and 500W) were studied. The results showed that the highest content of reducing sugar as 7.702 mg/mL was obtained by using 4% (v/v) of H_2SO_4 with 500 W for 7 min. Moreover, compared with treated sample without acid or microwave, combination of acid with microwave treatments promoted an increase in reducing sugar yield. It may be due to it able to achieve high reaction rates and effectively convert of the hemicelluloses and cellulose to dissolved sugars. In addition, concentration of yeast strain *Saccharomyces cerevisiae* (1 - 4% w/v) and fermentation time (1 - 4 day) in batch fermentation step were optimized. The results showed that by using 3% (w/v) of yeast and fermentation time for 4 days, it gave the highest ethanol concentration as 0.39% by volume. In conclusion, using acid together with microwave can be assisted effectively for increase reducing sugar yield that can be fermented into ethanol in short time.

Keywords : Bioethanol; Microwave; Physic nut seed cake; Pretreatment; Hydrolysis; Reducing sugar

Introduction

The research in development of renewable and sustainable fuels is an important effort in order to reduce the total tendency on fossil fuels. Recently, bioethanol obtained from biomass and bioenergy crops has become an increasingly popular alternative fuel. However, first-generation bioethanol that is derived from edible sources, for instance, corn and sugarcane that may not be desirable due to their feed value and competition with food supply. In this respect, bioethanol converted from non-edible sources such as lignocellulosic biomass, has offered a great promise to replace fossil fuels without causing the dispute of food-fuel supply. This kind of bioethanol is known as second-generation bioethanol or cellulosic ethanol (Kar, A. and Kanoria, M., 2009). Therein, physic nut (*Jatropha curcas L.*) has gained attention as a perennial culture that produces seeds with high oil content and excellent properties. This has created a great deal of attention, resulting in the planting of large plantations in Asia, Africa and the Americas (A. S. Appiah, H. et al., 2012). However, it is still lack of information reported on the conversion of physic nut seed cake (PSC) into sugar for bioethanol production. The previous study of Sricharoenchaikul et al. (Sricharoenchaikul, V. et al., 2007) has been reported that the chemical composition of physic nut waste consisted of hemicelluloses (17.47%wt), cellulose (56.31%wt) and lignin (23.91%wt) (Sricharoenchaikul, V. et al., 2007). With high cellulose content, therefore, PSC has great interested to use it as feedstock for cellulosic ethanol production.

The bioethanol production from lignocellulosic biomass involves different steps such as pretreatment, hydrolysis, fermentation and ethanol recovery (Balat, M. et al., 2008). A pretreatment step is essential to overcome the natural recalcitrance of lignocellulosic biomass to hydrolysis to sugars through opening up the lignocellulosic complex and making high sugar yields possible (Mosier, N. et al., 2005). A variety of methods of pretreatment have been developed. They include mechanical pretreatment, alkali or acid pretreatment, steam explosion, ammonia fiber explosion, hot water, supercritical CO_2 treatment, ozone pretreatment, biological pretreatment and others (Monte, J.R. et al., 2011). Among all the treatments, the dilute acid, especially for dilute sulfuric acid is one of the most studied and widely used methods (Zheng, Y. et al., 2009). To achieve the dilute acid pretreatment, the process is usually carried out in a high temperature environment using conventional heating. This method is not very often satisfactory if used individually because it gave low yield of sugar. Thus, many times are employed in combination with microwave heating in order to improve the process efficiency (Chen, W.H. et al., 2011; Chen, C et al., 2012; Kannan, T.S. et al., 2013).

Furthermore, hydrolysis is an essential step to produce fermentable sugars which are then fermented into ethanol by microbial biocatalyst (Balat, M. et al., 2008). However, the conversion of lignocellulosic material into fermentable sugars using energy efficient, economic and faster way is the greatest concern for commercial fuel ethanol production.

In the present study, in order to identify the feasibility of ethanol production from PSC, the chemical composition of PSC was determined. Microwave-assisted dilute acid simultaneous pretreatment and hydrolysis was proposed to enhance the saccharification of physic nut seed cake. The effects of sulfuric concentration, reaction time and power of microwave on reducing sugar were investigated. Moreover, the effects of acid and microwave on the releasing of sugar were studied. Additionally, the amounts of yeast and fermentation time were also optimized.

Materials and Methods

Sample preparation

The physic nut seed cake (PSC) was collected from Koksree area in Khonkaen province. It was dried by oven at 60°C for 6 h. Then, it was milled using domestic blender and sieved into less than 0.2 mm size powder. The ground sample was stored in sealed plastic bag prior use. The chemical composition of PSC was determined by using Technical Association of the Pulp and Paper Industry (TAPPI) standard method with little modification (TAPPI, 1994).

Optimized condition for simultaneous pretreatment and hydrolysis

Microwave-assisted in simultaneous pretreatment and hydrolysis was carried out using a modified domestic microwave oven with a frequency setting of 2450 MHz. The sample and acid solution loading ratio was fixed at 1:10 for the experiment. Ten grams of dried PSC was submerged in 100 mL of sulfuric acid in a 500-mL round. The condition for simultaneous pretreatment and hydrolysis was optimized by following, ten grams of dried PSC bottom flask. Then, the flask was placed at the center of rotating circular plate in the microwave oven. Microwave with various acid concentrations (1%, 2%, 3% and 4%v/v) was carried out at different microwave power rating of 300, 400 and 500 W for 3, 5 and 7 min of microwave heating. Samples were filtered and taken out to determine the concentration of reducing sugar by 3,5 dinitrosalicylic acid (DNS) method as described in previous studies (Miller, G.L., 1959). Sample was mixed with 3 ml DNS and boiled for exactly 5 min. The optical density was checked at 540 nm to measure the color intensity. The reducing sugar as glucose was expressed in mg/ml. A standard curve of glucose was used for reducing sugar measurement.

The residue obtained from the optimum condition of simultaneous pretreatment and hydrolysis was characterized by using Furrier Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). The effect of acid and microwave on the releasing of sugar was carried out under optimum condition. This study can be providing into three methods of simultaneous pretreatment and hydrolysis. First method, sample was treated in water under microwave. Second method, the experiment was done by using acid together with conventional heating and third method was carried out by using acid combined with microwave.

Optimized condition for fermentation

Fermentation was carried out by transferring 100 mL of hydrolyzates obtained after filtered to 250 mL bottles and adjusted pH to 4 – 5. Various concentration of yeast strain *saccharomyces cerevisiae* (1 – 4% w/v) was added and the samples were incubated at 40°C for 24, 48, 72 and 96 h. After reach each fermentation time, the samples were filtered and determined ethanol concentration by using gas chromatography (GC).

Characterization of PSC

The structure changes of untreated and treated PSC were analyzed using FT-IR spectroscope (Spectrum One, Perkin Elmer, USA). The ground sample was mixed with spectroscopic grade KBr and FT-IR spectrum was recorded between 4000 and 450 cm^{-1} using Shimadzu spectrometer, spectrum one model (Shimadzu, Japan) at 4 cm^{-1} resolution and 10 scans per sample.

The different in the lignocellulosic structure of untreated and treated PSC was evaluated by scanning electron microscope, SEM (HITACHI S-3000N, Japan). The samples were mounted on a double sided conductive tape on precut brass sample stubs and a sputter coated with palladium using auto fine coater. The images of raw and pretreated PSC were acquired with a 5 kV accelerating voltage at a magnification of 1000x.

GC analysis

Ethanol was purified after fermentation and then its concentration was determined by internal standard method using Gas chromatograph (Varian 450-GC, UK) with auto injector Varian CP8400. The WCOT fused silica column coating CP-SIL 8 CB (30 m x 0.25 mm) was used. Oven temperature was set initially at 50°C and then increased to the final temperature of 100°C in 5 min at the rate of 10°C/min and

maintained for 1 min. Injection volume was limited to 0.2 μL. Splittless injection mode was selected. The flow rates of H_2 and air were set at 28 and 300 mL/min, respectively. The temperature of the flame ionization detector (FID) and the injection port was set at 275°C, and 250°C, respectively. Helium (He) in the flow rate of 2 mL/min was used as the carrier gas.

Results and discussion

Chemical compositions of PSC

The results of chemical composition analysis present that PSC consisted of mainly cellulose (93.55% by mass), hemicelluloses (3.55% by mass) and lignin (2.92% by mass), respectively. Its comparatively high cellulose and low lignin content exhibited great potential on cellulosic ethanol production (Tye, Y. Y. et al., 2012).

Characteristic of samples

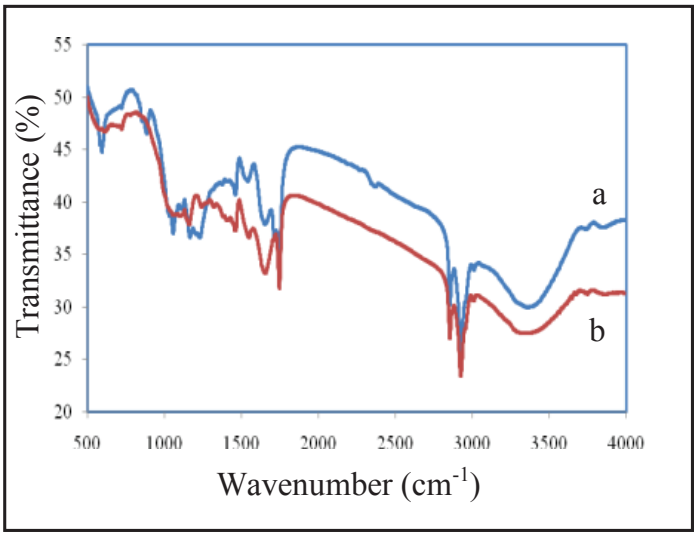

Figure 1 FT-IR spectra of (a) untreated and (b) treated PSC

The effect of pretreatment and hydrolysis on the characteristics of sample was evident from the FT-IR spectra of untreated and treated PSC as shown in Figure 1.

Table 1 Identified peaks in FT-IR spectra for PSC

Peak(cm^{-1})	Assignment
3350-3500	H-bonded OH stretching associated with the breakage of H bonds in cellulose
2850-3010	-C-H stretching associated with cellulose
2350	CH_2 stretching associated with amorphous cellulose
1710-1744	-C-O-C ether bonds associated with lignin
1650	-C=O carbonyl group with intra-molecular hydrogen bonds associated with lignin
1600	Aromatic skeleton vibration in lignin
1425	-O-CH_3 methoxide group present in lignin and hemicelluloses
1350	Phenolic hydroxyl groups associated with the structure of lignin
1250	-C-O-C ether associated with lignin-carbohydrate complexes
1050	-C-OH bending in hemicelluloses and lignin
885	β-glucosidic linkages in cellulose and hemicelluloses
675	Characteristic feature of lingo-sulphates

The identified peaks on the FT-IR spectrum based on the research of Marx et al. (Marx, S., et al., 2014) is summarized in Table 1. The different between the two spectra in Figure 1 shows the extent to which sulfuric acid liberated components from the raw material of PSC. The broad absorption at 3350-3500 cm^{-1} which is the O-H stretching band of the hydroxyl group associated with the breakage of hydrogen bonds in cellulose. The many lignin bands show that not all of the lignin was dissolved during pretreatment. However, the FT-IR spectrum of treated PSC showed dropping absorption bands at 1710, 1650, 1600 and 1350 cm^{-1}. This suggests that removal of lignin during pretreatment and hydrolysis was occurred. In other words, this method caused the lignin structures to expand and eventually break into small molecular compounds. The peak at 2350 cm^{-1} is due to CH_2 stretching associated with amorphous cellulose and which is unaffected by the change in crystallinity of the biomass. This peak is sharper for the untreated PSC and almost disappears after pretreatment and hydrolysis, showing that this portion of the cellulose was converted by the treatment. The β-glucosidic linkages observed at 897 cm^{-1} associated with hemicelluloses presence are evident in the spectra for untreated PSC was sharper than that of treated sample. It was indicated that some hemicelluloses was liberated or broken down by the treatment.

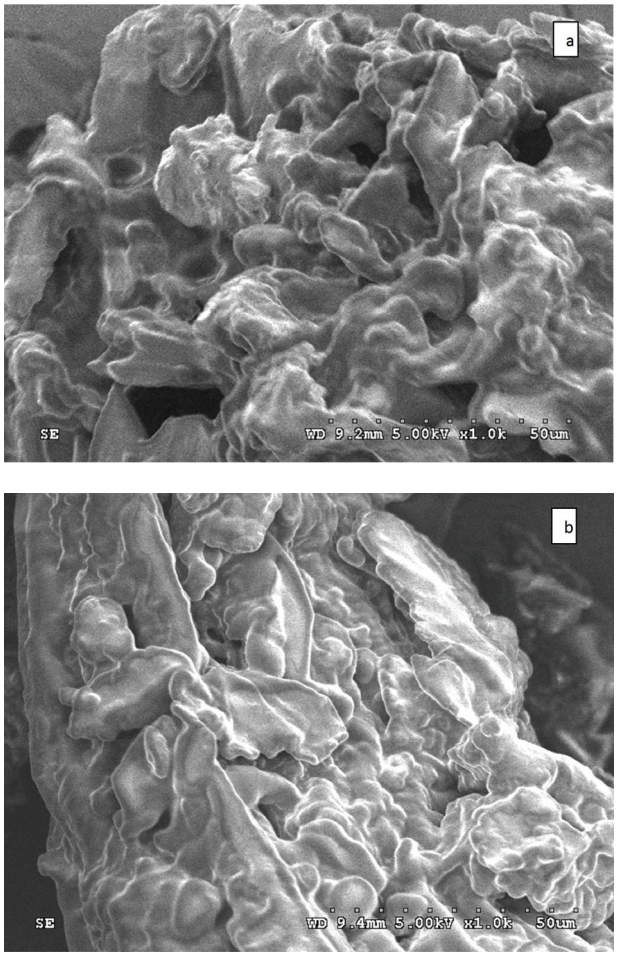

Figure 2 SEM micrographs of (a) untreated PSC and (b) treated PSC under optimum condition

Moreover, the FT-IR spectra thus confirmed the lowing in crystallinity of cellulose and subsequent conversion to glucose as well as the fact that hemicellulose was not completely liberated from the PSC structure. Furthermore, SEM was used to study the morphological features and surface characteristics of materials after pretreatment and hydrolysis compared with untreated PSC.

As illustrated SEM images in the Figure 2, comparison of untreated and treated PSC showed that the pretreatment and hydrolysis induced physical changes in sample. For the untreated PSC, a compact lignocellulosic structure is clearly observed. Moreover, the untreated PSC has smooth and continuous surface whereas the treated PSC has rough surface. It was noticed that the structure of treated PSC was opened up and generated pores were observed, which can provide higher surface area for subsequent acid reactions. So that cellulose becomes more accessible.

Optimized condition for simultaneous pretreatment and hydrolysis

The results of the study on optimized condition for simultaneous pretreatment and hydrolysis are shown in Figure 3, Figure 4 and Figure 5. The results illustrated in Figure 3 show that by increasing the concentration of acid at constant time of microwave, an increase in reducing sugar release was obtained and highest glucose yields were achieved at concentration of 3%. The functions of dilute acid pretreatment are to convert hemicelluloses contained in It can be described that the functions lignocellulosic biomass to soluble sugars and to facilitate the subsequent hydrolysis of cellulose. In other words, the disruption of recalcitrant structure of biomass is closely relevant to the performance of dilute acid pretreatment (Chen, W.H. et al., 2011). Our results could be supported by Tasic et al. (Tasic, B.M., 2009) who investigated the hydrolysis of starch from fresh potato tubers by HCl and H_2SO_4 and concluded that the rate of hydrolysis increased with increasing acid concentration. This is probably due to the increase in the activity of hydrogen ions participating in the reaction as catalyst resulting in higher releasing of reducing sugar (Tasic, B.M., 2009).

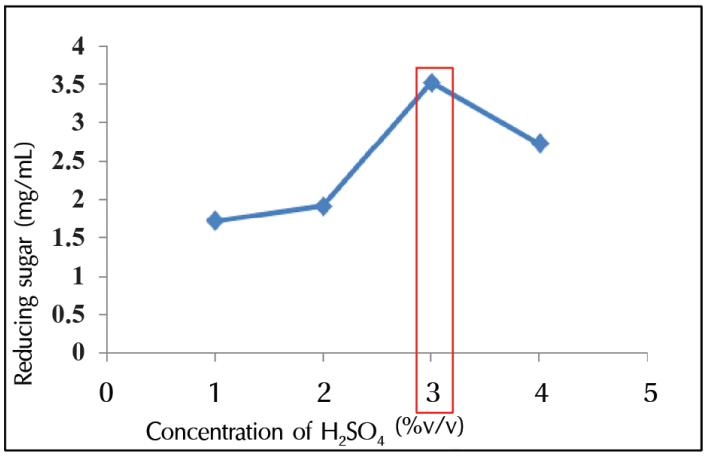

Figure 3 The relation between concentration of H_2SO_4 and reducing sugar release

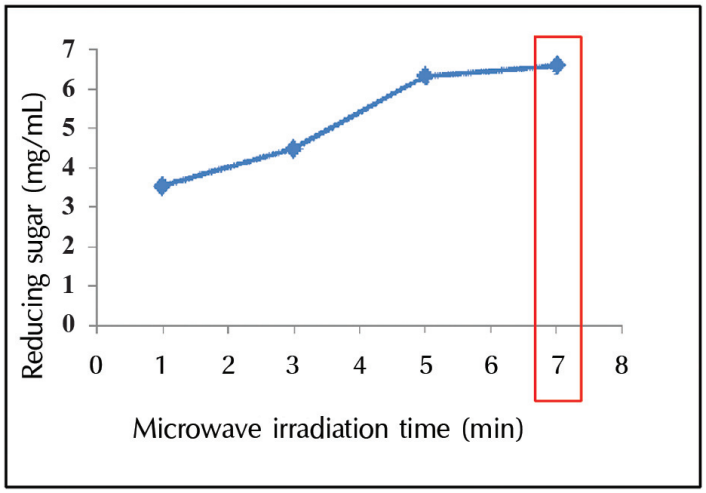

Figure 4 The relation between microwave irradiation time and reducing sugar release

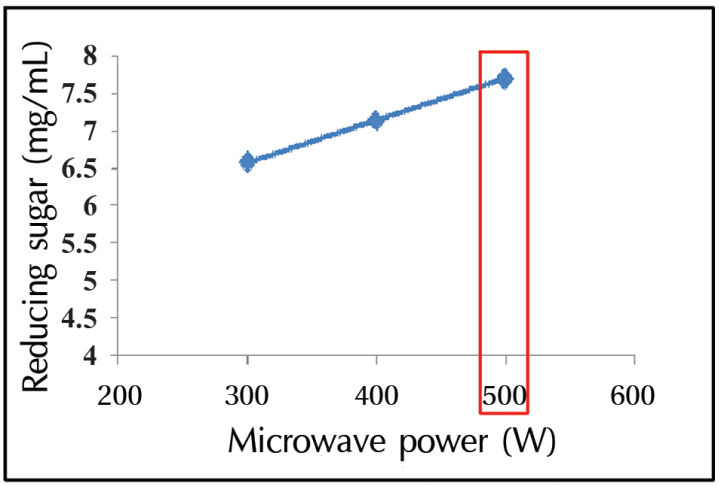

Figure 5 The relation between microwave power and reducing sugar release

However, it was found that at concentration of H_2SO_4 over 3% v/v, the reducing sugar release was decreased. It may be due to the decomposition of sugar into several of inhibitors like acetic acid, furfural and 5 hydroxymethylfurfural. These products are growth inhibitor of microorganisms (Sarkar, N et al., 2012). Thus, 3%v/v of H_2SO_4 was the optimum concentration for simultaneous pretreatment and hydrolysis, which gave the maximum reducing sugar of 3.532 mg/mL and used for further studied. The comparison of reducing sugar yield obtained for different microwave time and power is shown in Figure 4 and Figure 5, respectively. It was found that when microwave irradiation time rised from 1 to 7 min at microwave power of 300 W, the reducing sugar release increased. Similar trends were observed when microwave power

increased from 300 to 500 W for 7 min. Under optimum condition (7 min, 500W), 7.702 mg/mL of reducing sugar release was obtained.

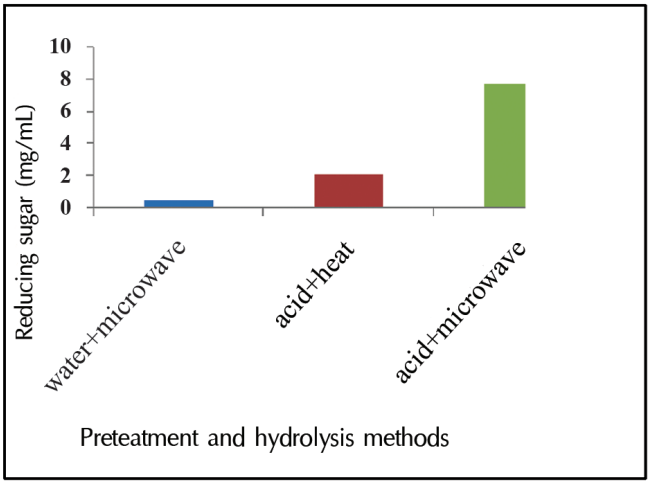

Figure 6 Reducing sugar release by different methods of pretreatment and hydrolysis

The comparison of maximum reducing sugar release obtained for different method is shown in Figure 6. This shows that irradiation combined with acid enhanced the releasing of reducing sugar more than that pretreatment and hydrolysis without acid or microwave. This finding may be attributed to the role of microwave irradiation in the digestion of lignocellulosic compositions in material. Microwave vibration caused polar water molecules to rub against and collide with each other, thereby producing great heat to effectively decompose lignocelluloses matrices and crystal structures.

Furthermore, microwave is able to penetrate into the solutions and can provide a rapid and energy efficient heating on biomass pretreatment. The electromagnetic field also induced the degradation of lignocellulosic material (Xia, A et al., 2013). Unlike conventional heating, microwaves generate higher power densities, enabling higher production rates (Wei-Hsin, C et al., 2012). These accelerate the reaction of acid with biomass, which lead to a higher yield of reducing sugar.

Optimized condition for fermentation

As the results present in Figure 7, it was found that the concentration of ethanol was found to be increase with respect to time. Moreover, the rate of the ethanol production was increased rapidly after 2 days and relative slow thereafter. The reason for the observed results is that shorter time in fermentation causes inadequate growth of microorganisms eventually causing inefficient fermentation.

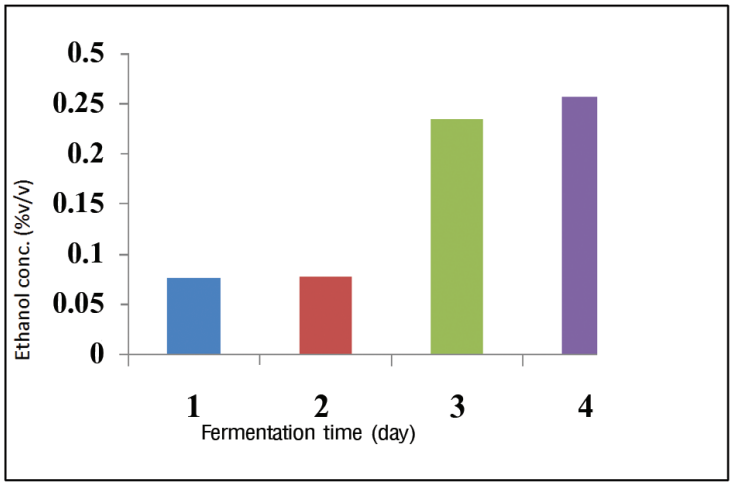

Figure 7 The relation between fermentation time and ethanol production

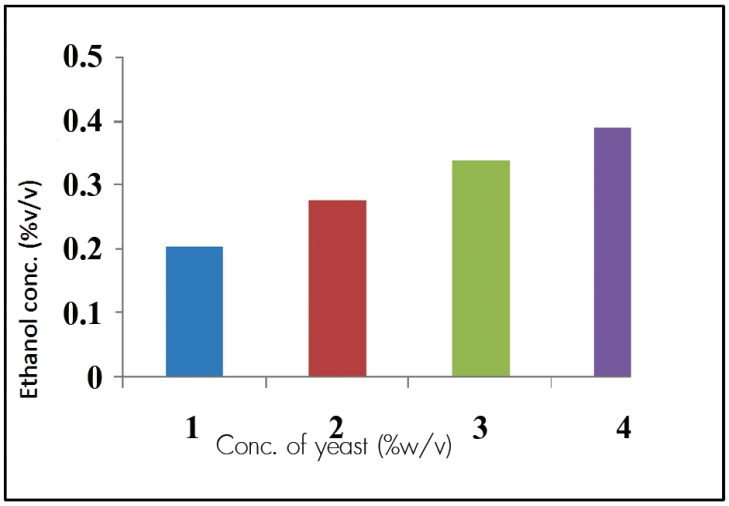

Figure 8 The relation between concentration of yeast and ethanol production

Moreover, the results suggest that more time required for complete fermentation. Thus, 3 days was the optimized fermentation time which gave ethanol concentration of 0.24%v/v. The results in Figure 8 show that ethanol concentration increase as the concentration of yeast increase. It can be described that lower concentration of yeast may not be sufficient for initiating of the cell growth whereas the higher concentration of yeast makes it possible for cell to multiply rapidly in fermentation medium resulting in an increment on ethanol yield. The maximum concentration of ethanol (0.39%v/v) was obtained by using 4% of yeast concentration.

Conclusions

PSC has potential in application as raw material for ethanol production because of its acceptable content of cellulose and hemicelluloses and low lignin content. The results showed that the maximum reducing sugar release was 7.702 mg/mL, achieved under optimum condition of 3% v/v of H_2SO_4 at 500W microwave power and 7 min microwave irradiation time. Moreover, the pretreatment and hydrolysis of PSC by using microwave combined with acid led to a higher reducing sugar release than the corresponding that value obtained from the conventional heating. The structural changes of PSC mainly came from the combination of microwave disruption and chemical dissolution of hemicelluloses by dilute sulfuric acid. It was outlined that microwave-assisted in acid condition could led to higher yield of reducing sugar, shorter reaction time and lower energy consumption, so that it was suitable technique for the saccharification process of PSC. Additionally, under optimum condition of fermentation by using 3% w/v of yeast and fermentation time of 3 days, the maximum ethanol concentration (0.39% v/v) was obtained. Although, the ethanol production under this condition was comparatively low. However, this simultaneous pretreatment and hydrolysis method utilized less energy and cost in short time which signify a promising technique for the formation of reducing sugar.

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