academicJournals

Vol. 13(38), pp. 3928-3934, 17 September, 2014
DOI: 10.5897/AJB12.2181
Article Number: 093956247610
ISSN 1684-5315
Copyright © 2014
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Simple and enhanced production of lignocellulosic ethanol by diluted acid hydrolysis process of pineapple peel (*Ananas comosus*) waste

Chatchanun Niwaswong[1,2], Patiwat Chaiyamate[1], Peerapong Chotikosaikanon[1] and Chalerm Ruangviriyachai[1,2]*

[1]Department of Chemistry and the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Khon Kaen University, Khon Kaen, 40002 Thailand.
[2]National Research University Project of Thailand, Biofuel Cluster, Khon Kaen University, Khon Kaen, 40002Thailand.

Received 17 June, 2012; Accepted 4 September, 2014

Ethanol can be produced from a fermentation process using raw materials obtained from highly economically important plants such as corn, cassava and sugarcane, and used as an alternative energy source. These economical plants are being used less because their initial cost is still increasing. However, lignocellulosic ethanol production can alternatively be done from agricultural wastes such as corn stover, sugarcane bagasse and rice straw. In this work, a solution of hydrolyzed pineapple peel was the substrate and was converted to ethanol via batch fermentation. The preparation and characterization of the cellulose obtained from the modified TAPPI T203 test method for the enhancement of the ethanol production was investigated. The results show that the FTIR spectra of their removable lignin and hemicellulose disappeared at 1590, 1475, 1250 and 1164 cm^{-1}, respectively. The percentage of cellulose obtained was 20.44. The maximum percentage yield of total reducing sugars in the diluted acidic hydrolysis of pineapple peel by 0.2 M H_2SO_4 was 82.10±2.30. The fermented broth using *Saccharomyces cerevisiae* TISTR 5048 gave the highest percentage of bioethanol yield which was 65.27±2.45%. This process is not complicated, simple and low cost for ethanol production industries.

Key words: Lignocellulosic ethanol, diluted acid hydrolysis, pineapple peel waste, total reducing sugars.

INTRODUCTION

Currently, a continuous increase in oil price as a result of the global energy crisis is an urgent problem awaiting a solution in many countries. Renewable energy is being discussed, including alternative raw materials, such as municipal waste and waste paper or particular crops. The economical use of energy is the first issue which can

*Corresponding author. E-mail: chal_ru@kku.ac.th. Tel: + 66 81 9649461. Fax: + 66 43 202373.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

decrease the energy expenditure by reducing crude petroleum fuel imported from the Organization of Petroleum Exporting Countries (OPEC). In addition, alternative fuels from renewable resources are subjects for energy conservation which can replace petroleum fuel resources (Wyman, 1994). A renewable energy source such as ethanol was used in Brazil and the USA by mixing fuel oils to increase the octane number of fuel oil (Laluce, 1991). Ethanol can be produced through fermentation of lignocellulosic biomass such as sugar cane and corn using microorganisms (Morais et al., 1996).

Lignocellulosic biomass is a potential source of cheap sugars for producing fuels and chemicals, and a pretreatment stage is essential to make the cellulose accessible to hydrolysis by a dilute acid (Mohagheghi et al., 2004). Cazetta et al. (2007) studied the utilization of lignocellulosic biomass which has been closely associated with a new technological concept, the so called Biorefinery. Therefore, ethanol is one of the most important renewable fuels contributing to the reduction of negative environmental impacts generated by the utilization of fossil fuels (Dale, 1999).

Thailand is an agricultural country which has plentiful agricultural wastes. They can be used as material sources for ethanol production such as sugarcane bagasse, cassava stem, corn stover and rice straw. Therefore, Lin and Tanaka (2006) have developed a new material which has a high content of lignocellulosic biomass and possesses a reduced demand for supplying the ethanol production process, the so called lignocellulosic ethanol. Pineapple peel is one of the agricultural wastes which has lignocellulosic biomass. Although most of lignocellulosic biomass is composed of 38 to 50% cellulose, 23 to 32% hemicellulose, and 13 to 30% lignin (Sierra et al., 2008), pineapple peel is an interesting biomass resource for lignocellulosic ethanol production because there is a lot of peel waste. However, the production of lignocellulosic ethanol is a relatively complicated process. Reddy and Reddy (2005) have developed the transformation of biological resources as rich energy crops requiring the optimum conditions for conversion as lignocellulosic ethanol by fermenting organisms. Additionally, aqueous solutions of ethanol should be concentrated for obtaining hydrous ethanol (Maiorella et al., 1984). The ethanol in gasoline was used as an oxygenated fuel. Cardona and Oscar (2007) studied the complexity of this process partly to explain why fuel ethanol has not played a leading role in comparison to cheaper oil derived fuels. Due to rising environmental concerns and the periodic crises in some of the larger oil exporting countries, it has become a viable and realistic alternative in the energy market (Bayrock and Ingledew, 2001).

In addition, the main components of lignocellulosic biomass and type of microorganisms can also affect lignocellulosic ethanol production (Boerjan et al., 2003). Thomas and Rose (1979) have developed non-pretreated lignocellulosic biomass and *Saccharomyces cerevisiae* for use in lignocellulosic ethanol production with the advantage of simple ethanol production. It can grow in aerobic conditions and is used in the baking and brewing industries (Lynd et al., 1991; Narendranath and Power, 2005). Grosz and Stephanpoulos (1990) have developed ethanol producing organisms used in industrial processes. In previous works, lignocellulosic ethanol from pineapple peel by the enzymatic hydrolysis process via Simultaneous Saccharification and Fermentation (SSF) was studied (Itelima et al., 2013). Although the enzymatic hydrolysis process can give high yield bioethanol, it is difficult to perform it at a large scale for ethanol production industries as a result of the complicated procedure and high cost. The aim of this study was to produce the lignocellulosic ethanol via batch fermentation from the dilute acid hydrolysis process of pineapple peel waste. This work was conducted to provide an added value to this waste for the canned fruit industries. The main components (lignin, hemicellulose and cellulose) were characterized by Fourier Transform Infrared (FTIR) and TG/DTA techniques. In addition, the contents of total reducing sugars and lignocellulosic ethanol obtained were investigated by the spectrophotometric technique and gas chromatography - flame ionization detector (GC-FID), respectively.

MATERIALS AND METHODS

Fresh pineapple peel was collected from the Food Service Center in Khon Kaen University (KKU), Khon Kaen province, Thailand. Firstly, the pineapple peel was washed thoroughly with distilled water, minced and dried at 60°C in a hot-air oven. After that, it was ground and sieved to obtain particle sizes of less than 500 µm. This sample was stored in a plastic box before use and then characterized by a FTIR spectrometer (Spectrum One; Perkin Elmer, Germany) with the KBr pellet method.

Determination of main components

The main components in the pineapple peel such as lignin, hemicellulose and cellulose were determined by a Perkin Elmer Thermogravimetry (TG) (Pyris Diamond TG/DTA 6300, Germany) with temperature ranging from 30 to 830°C and heating rate of 10°C/min under nitrogen atmosphere (Nishiyama et al., 2002).

Removal of main components

This method was modified from the Technical Association of Pulp and Paper Industrial T203 test method (TAPPI, 1994-1995). Briefly, 10 g of raw pineapple peel powder was extracted with the solvent, a mixture of hexane: methanol of 2:1 by volume to remove ester compounds by shaking at 180 rpm for 30 min and drying in a fume hood for solvent disposal (assigned as Sample I). The lignin removal was done by soaking Sample I in 150 mL deionized water with the addition of 1.5 g $NaClO_4$ and 10 drops of 18 M CH_3COOH

in a water bath at 70°C for 1 h. Then, it was washed thoroughly with distilled water, dried in an oven at 80°C for 1 h and weighed (assigned as Sample II). Then, the powder of Sample II had the hemicellulose removed with soaking in 0.25 M NaOH for 24 h and then it was boiled at 70°C for 1 h as modified from the TAPPI T203 test method. It was then washed thoroughly with distilled water, dried in an oven at 60°C and weighed to achieve dried cellulose (assigned as Sample III) at the end. All three samples were characterized by using a FTIR spectrometer with the KBr pellet method.

Diluted acid hydrolysis

Ten (10) g of samples were weight hydrolyzed with 100 mL 0.2 M H_2SO_4 using an electrical autoclave (All American Pressure Sterilizer, U.S.A.) at 120°C, 15 psi for 90 min. The working conditions for diluted acid hydrolysis were studied (Xu et al., 2003). Then, the hydrolyzed solution obtained was filtered through filter paper. The total reducing sugars of the hydrolyzed solution were determined according to the dinitrosalicylic acid method at wavelength 570 nm by a UV-VIS spectrophotometer (Agilent 8453 UV-Visible Spectroscopy System, Germany) (Miller, 1959).

Fermentation

A pure yeast strain of *S. cerevisiae* TISTR 5048 in this experiment was purchased from the Microbiological Resources Center, Thailand Institute of Science and Technological Research (TISTR), Pathum Thani Province, Thailand. For the batch fermentation process, the hydrolyzed solution of pineapple peel was neutralized to pH 7.0 using 2.0 M NaOH and filtered through filter paper. Then, this solution was added into the synthetic medium (consisting of 1.0 g/L yeast extract, 1.0 g/L $MgSO_4$, 2.0 g/L $(NH_4)_2SO_4$ and 0.5 g/L KH_2PO_4 in 1 L of distilled water) (Brown et al., 1981). After that, it was sterilized using an electrical autoclave at 120°C, 15 psi for 30 min. Then, 10.0 mL of *S. cerevisiae* TISTR 5048 broth was loaded into this medium. Finally, the batch fermentation was carried out by a rotary shaker with speed 150 rpm at 30°C for 72 h. by sampling every 6 h.

Ethanol analysis

The fermented broth was obtained, centrifuged at 3000 rpm for 10 min and filtered through a 0.45 µm filter membrane. The bioethanol was monitored by a (GC-FID) (TraceGC, Thermo Finnigan, Italy) using a DB-5 column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The temperature of the injector was set at 250°C. The flame ionization detector was kept at 280°C. The temperature was programmed at 50°C for 2 min, from 50 to 100°C at 10°C/min, then held for 2 min at 100°C. The internal standard used was n-butanol (Caylak and Vardar, 1998).

RESULTS AND DISCUSSION

Identification of main components in pineapple peel

The IR spectra of the raw pineapple peel from Figure 1a for mode assignment showed O-H stretching of acid and methanol in reducing sugars at 3500 to 3200 cm^{-1}, C–H_n stretching of alkyl, aliphatic and aromatic occurring at 1750 cm^{-1}, C=O stretching of ketone and carbonyl occurring at 1590 cm^{-1}, C=C stretching of aromatic skeletal mode in lignin occurring at 1475 cm^{-1}, C-O-C stretching of aryl-alkyl ether linkage occurring at 1250 cm^{-1} and C-O-C stretching vibration in hemicellulose occurring at 1164 cm^{-1} (Yang et al., 2007). These confirmed main components were present in the raw pineapple peel such as the reducing sugars in cellulose and hemicellulose, the ester compounds, and the lignin portion. From the main components removal with the modified TAPPI T203 test method, Sample I had alkyl, aliphatic and aromatic compounds removed from raw pineapple peel as shown in Figure 1b in which there is no peak at 1750 cm^{-1}. In Figure 1c of Sample II spectra peaks disappeared at 1590 and 1475 cm^{-1} due to the absence of lignin. In Sample III due to the hemicellulose removal the peak of arabinose disappeared at 1250 and 1164 cm^{-1} (Figure 1d). The characterization of the main components was successfully achieved by FTIR at each step.

Determination of main components by the TG/DTA technique

The DTG curve in Figure 2a shows that the first event was moisture removal (1) up to around 100°C followed by the second, third and fourth events around 150 to 300°C were the evolution of hemicelluloses (2) and cellulose (3) degradation, respectively. Degradation of lignin took place slowly over a wide temperature range and rose to a higher temperature (4) (Yang et al., 2006; Yang et al., 2007). The TG curve shows a maximum percentage of weight loss occurring in the temperature range of 150 to 450°C. The first step that could be attributed to decomposition begins with moisture about 100°C. The second mass loss step was hemicellulose degradation of 9.50±0.77%. It has an amorphous structure and linear polymer structure with short side chains which are easier to remove than cellulose and some hydrocarbons at the lower temperature. The third step, related to cellulose, mainly consists of a semicrystalline arrangement of chains associated with others which is a strong structure degrading by 21.16±0.73% and the final step was 42.11±0.85% for the lignin degradation because it is complex and has a strong structure of a phenolic polymer covering the polysaccharides of the cell walls as shown in Figure 2b (Wanitwattanarumlug et al., 2012).

Determination of main components by removal processes

The modified TAPPI T203 test method can be used to determine the main components in pineapple peel. It can be performed from the weight loss of three samples by

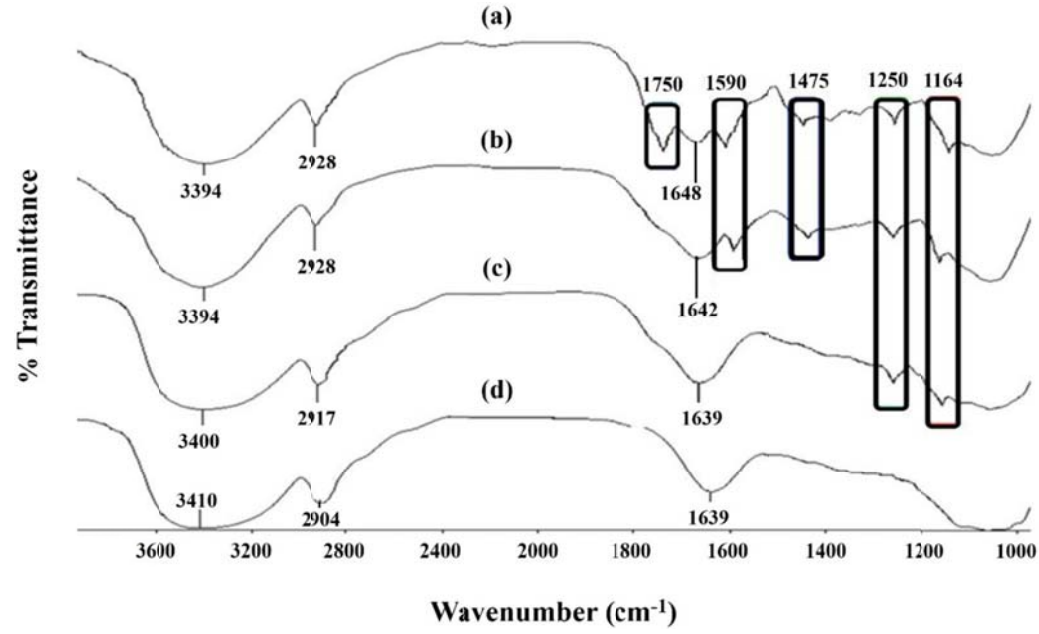

Figure 1. The IR spectra of (a) raw pineapple peel, (b) Sample I = lignin + hemicellulose + cellulose, (c) Sample II = hemicellulose + cellulose and (d) Sample III = cellulose. The mode assignment of the functional groups in each main component was removed.

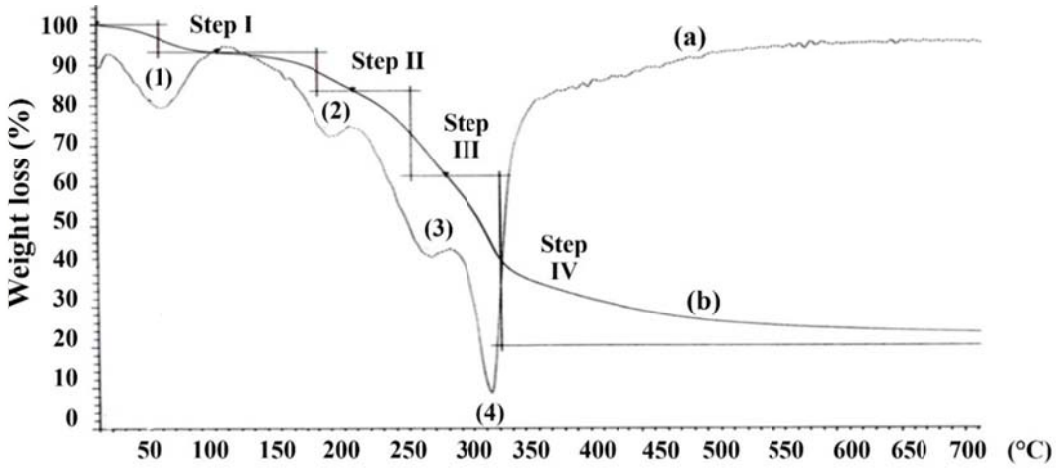

Figure 2. The thermal gravimetric analyzer (TG/DTA) curve (a) and TG curve (b) show typical weight losses of components in raw pineapple peel monitored in the range of 30-830 °C. Step I: moisture removal (I), Step II: hemicellulose degradation (II), Step III: cellulose degradation (III) and Step IV: lignin degradation (4).

the classical gravimetric method. It is interesting to note that raw pineapple peel was composed of 9.43±1.51% hemicellulose, 20.44±1.45% cellulose and 41.21±3.07% lignin. Figure 3 shows a comparison of the main components between removal processes and the TG/DTA technique showing that there is no significant difference between the removal processes and the TG/DTA data.

Determination of total reducing sugars

The standard curve for glucose solution was achieved between 0.01 to 0.10%, w/v by using the dinitrosalicylic acid method by a UV-VIS spectrophotometer. The percentage of total reducing sugars in all of the hydrolyzed samples is shown in Figure 4. The hydrolyzed solution of Sample II and Sample III gave the total

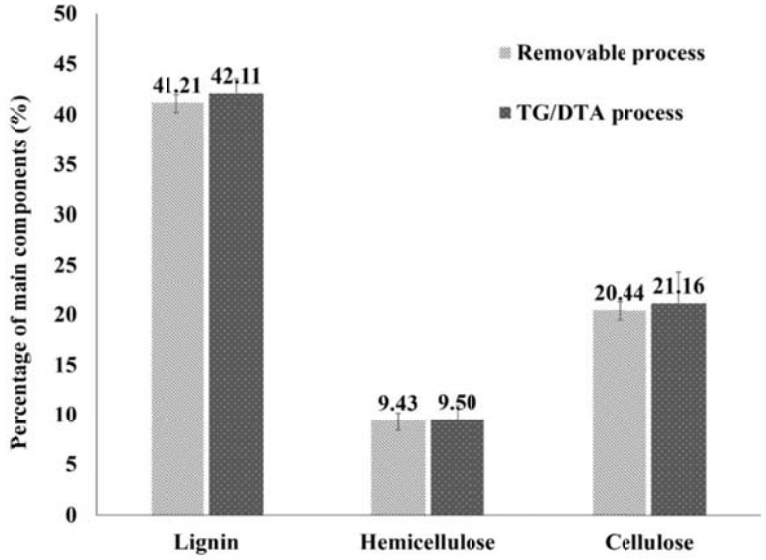

Figure 3. The percentage of components in raw pineapple peel obtained from each removal process compared with TG/DTA data.

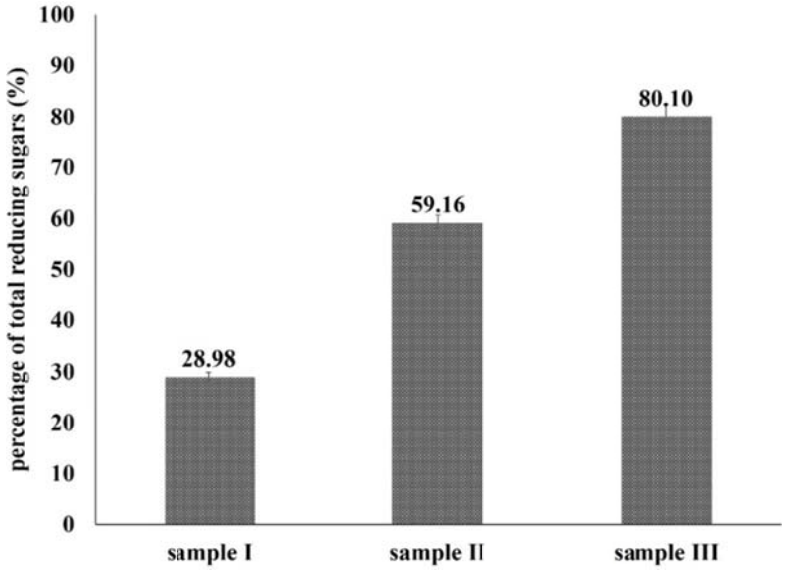

Figure 4. The percentage of the total reducing sugars obtained from the hydrolyzed pineapple peel samples. Sample I = lignin + hemicellulose + cellulose; Sample II = hemicellulose + cellulose; Sample III = cellulose.

reducing sugars of 59.16±1.48 and 82.10±2.30%, respectively. For the hydrolyzed solution, Sample III had the highest amount of total reducing sugars which was converted from all glucose, because of the highest amount of cellulose from the removal process. Normally, the structure of the cellulose is linear chain polymers which can be hydrolyzed with diluted sulfuric acid more easily than the others (Updegraff, 1969). The hydrolyzed solution of Sample II also contained some glucose and other reducing sugars from the hydrolyzed hemicellulose. In fact, the structure of hemicellulose is long and has many branches which could be a blocker to diluted sulfuric acid hydrolysis (Ebringerova et al., 2005). While the hydrolyzed solution of Sample I gave 28.98±0.96%

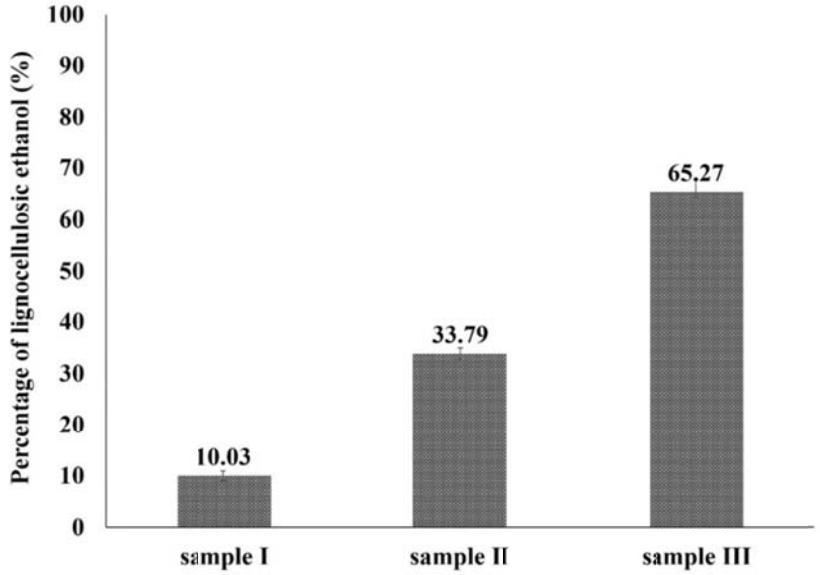

Figure 5. The percentage of bioethanol obtained from three pineapple peel samples via batch fermentation.

of total reducing sugars; it was quite low because the effect of the amount and the complicated structure of lignin, including long and many branches of hemicellulose.

Determination of lignocellulosic ethanol

The standard curve of ethanol was obtained in the range of 0.01 to 0.09% v/v and the percentage of lignocellulosic ethanol obtained from each fermented broth sample is shown in Figure 5. The fermented broth of Sample III gave the highest bioethanol of 65.27±2.45%. This sample contained most glucose for producing bioethanol fermented by *S. cerevisiae TISTR 5048*, which was better than others and there was no interference from other components (Lui and Shen, 2008). The percentage of lignocellulosic ethanol in the fermented Sample II was 33.79±1.16%. The other reducing sugars (such as xylose and arabinose) from this sample could not be converted to ethanol by *S. cerevisiae* TISTR 5048 (Marek et al., 2007). Finally, the fermented broth of Sample I attained 10.03±0.93% only. The lowest percentage of lignocellulosic ethanol obtained was due to inhibition from the high content of the lignin. A regular elevation in production of lignocellulosic ethanol was observed until 18 h of batch fermentation and declined thereafter. *S. cerevisiae* TISTR 5048 has been promising in utilization of maximum fermentable glucose presented in hydrolyzed lignocellulose which is reflected in higher bioethanol production and a greater yield. The lignocellulosic ethanol production from pineapple peel can be enhanced with the preparation of cellulose in the removal processes using modification from the TAPPI T203 test method. However, our process may give less ethanol yield than others processes. Nonetheless, it was found that this diluted acid hydrolysis process for this pineapple peel waste is quite simple, uncomplicated and low cost. So it can be applicable in the ethanol production industries.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

The authors would like to thank the Department of Chemistry, Faculty of Science, Khon Kaen University, Thailand, for all supporting facilities. The Center of Excellence for Innovation in Chemistry (PERCH-CIC), the Higher Education Research Promotion and National Research University Project of Thailand, Office of the Higher Education, through the Biofuel Cluster of Khon Kaen University, Thailand, are also gratefully acknowledged for the financial support and chemicals.

REFERENCES

Bayrock DP, Ingledew WM (2001). Application of multistage continuous fermentation for production of fuel alcohol by very high gravity fermentation technology. J. Ind. Microbiol. Biotechnol. 27: 87-93.

Boerjan W, Ralph J, Baucher M (2003). Lignin biosynthesis. Annu. Rev. Plant Biol. 54: 519-549.

Brown SW, Oliver SG, Harrison DEF (1981). Ethanol inhibition of yeast growth and fermentation: differences in the magnitude and complexity of the effect. Eur. J. Appl. Microbiol. Biotechnol. 11:151-155.

Cardona AC, Oscar JS (2007). Fuel ethanol production: process design trend and integration opportunities. Bioresour. Technol. 98:2415-2457.

Caylak B, Vardar SF (1996). Comparison of different production processes for bioethanol. Turk. J. Chem. 22:351-359.

Cazetta ML, Celligoi MAPC, Buzato JB, Scarmino IS (2007). Fermentation of molasses by *Zymomonas mobilis*: effect of temperature and sugar concentration on ethanol production. Bioresour. Technol. 98:2824-2838.

Dale BE (1999). Biobased industrial products, bioprocess engineering, when cost really counts. Biotechnol. Prog. 15: 775-776.

Ebringerova A, Hromadkova Z, Heinze T (2005). Hemicellulose. Adv. Polym. Sci. 186:1-67.

Grosz R, Stephanpoulos G (1990). Physiology, biochemistry, and mathematical studies of micro-aerobic continuous ethanol fermentation by *Saccharomyces cerevisiae* I: hysteresis, oscillations and maximum specific ethanol productivities in chemo stat culture. Biotechnol. Bioeng. 36:1006-1019.

Itelima J, Onwuliri F, Onwuliri E, Onyimba I. Oforji S (2013). Bio-ethanol production from banana, plantain and pineapple peels by simultaneous saccharification and fermentation process. Int. J. Environ. Sci. Dev. 4(2): 213-216.

Laluce C (1991). Current aspects of fuel ethanol production in Brazil. Crit. Rev. Biotechnol. 11:149-161.

Lin Y, Tanaka S (2006). Ethanol fermentation from biomass resource: current state and prospects. Appl. Microbiol. Biotechnol. 69:449-453.

Lui R, Shen F (2008). Impacts of main factors on bioethanol fermentation from stalk juice of sweet sorghum by immobilized *Saccharomyces cerevisiae* (CICC 1308). Bioresour. Technol. 99:847-854.

Lynd LR, Ahn HJ, Anderson G, Hill P, Kersey DS, Klapatch T (1991). Thermophilic ethanol- production-investigation of ethanol yield and tolerance in continuous culture. Appl. Biochem. Biotechnol. 28-29:549-570.

Maiorella BL, Blanch HW, Wilke CR (1984). Economic evaluation of alternative ethanol fermentation processes. Biotechnol. Bioeng. 26:1003-1025.

Marek S, Wojciech K, Malgorzata L (2007). Ethanol production from whey in bioreactor with co-immobilized enzyme and yeast cells followed by pervaporative recovery of product: kinetic model predictions. J. Food. Eng. 82: 618-625.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal. Chem. 5:426-428.

Mohagheghi A, Dowe N, Schell D, Chou Y, Eddy C, Zhang M (2004). Performance of a newly developed integrant of *Zymomonas mobilis* for ethanol production on corn stover hydrolysate. Biotechnol. Lett. 26:321-325.

Morais PB, Rosa CA, Linardi VR, Carazza F, Nonato EA (1996). Production of fuel alcohol by *Saccharomyces cerevisiae* from tropical habitats I. Biotechnol. Lett. 18:1351-1354.

Narendranath NV, Power R (2005). Relationship between pH and medium dissolved solids in terms of growth and metabolism of lactobacilli and *Saccharomyces cerevisiae* during ethanol production. Appl. Environ. Microbiol. 71:2239-2243.

Nishiyama Y, Langan P, Chanzy H (2002). Crystal structure and hydrogen bonding system in cellulose Iβ from synchrotron x-ray and neutron fiber diffraction. J. Am. Chem. Soc. 124: 9074-9082.

Reddy LVA, Reddy OVS (2005). Improvement of ethanol production in very high gravity fermentation by horse gram (*Dolichos biflorus*) flour supplementation. Lett. Appl. Microbiol. 88:440-444.

Sierra R, Smith A, Granda C, Holtzapple MT (2008). Producing Fuels and chemicals from Lignocellulosic Biomass. Chem. Eng. Prog. 104(8):S10-S18.

TAPPI Test Methods (1994). T 222 OM-88. In Acid-insoluble lignin in wood and pulp, TAPPI, Atlanta, GA.

Thomas DS, Rose AH (1979). Inhibitory effect of ethanol on growth and solute accumulation by *Saccharomyces cerevisiae* as affected by plasma membrane lipid composition. Arch. Microbiol. 122:49-54.

Updegraff DM (1969). Semimicro determination of cellulose in biological materials. Anal. Biochem. 32:420-424.

Wanitwattanarumlug B, Luengnaruemitchai A, Wongkasemjit S (2012). Characterization of Corn Cobs from Microwave and Potassium Hydroxide Pretreatment. Int. J. Chem. Biol. Eng. 6:354-358.

Wyman CE (1994). Ethanol from lignocellulosic biomass: technology, economics and opportunities. Bioresour. Technol. 50:3-16.

Xu H, Templeton AC, Reed RA (2003). Quantification of 5-HMF and dextrose in commercial aqueous dextrose solutions. J. Pharm. Biomed. Anal. 2:451-459.

Yang H, Yan R, Chen H, Lee D H, Liang D T, Zheng C (2006). Pyrolysis of palm oil wastes for enhanced production of hydrogen rich gases. Fuel Proc. Technol. 87:935-942.

Yang H, Yan R, Chen H, Lee D H, Zheng C (2007). Characteristics of hemicellulose, cellulose and lignin pyrolysis. Fuel 86:1781-1788.