JFHB Journal of Food Health and Bioenvironmental Science

Journal of Food Health and Bioenvironmental Science

Journal homepage : http://jfhb.dusit.ac.th/

Ethanol Production from Sago Palm Residue Pretreated with Two-Stage Chemical Process by Using Seed Sludge

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Article info

Article history:
Received : 27 October 2018
Revised : 3 January 2019
Accepted : 24 January 2019

Keywords:
Sago Palm Residue, Ethanol Production, Two-Stage Chemical Pretreatment, Cellusoft ® CR conc

Abstract

The objectives in this work are to study the pretreatment of sago palm residue via two-stage chemical pretreatment process and to investigate the optimum fermented temperature for ethanol production by using seed sludge from wastewater treatment plant. The two-stage chemical pretreatment process with 0.5 %w/v sodium hydroxide followed by 0.26 %w/v sulfuric acid of sago palm residue was correlated with the removal of lignin and the disruption of cellulose structure, respectively. The pretreated sago palm residue was hydrolyzed with cellulase enzyme (Cellusoft ® CR conc) in order to convert cellulose and hemicellulose of sago palm residue to reducing sugar. The reduction of sugar was further fermented to produce ethanol by seed sludge at different temperature. From the study, it was found that the optimum hydrolysis time for pretreating sago palm residue was 72 hours. The pretreated sago palm residue via two-stage chemical pretreatment process gave a 49.63 % decrease in lignin and 81.57 % decrease in crystalline cellulose. It is suggested that the pretreated sago palm residue was easier to digest in reducing sugar by cellulase enzyme (Cellusoft ® CR conc) resulting in a fermented ethanol that was enhanced 3.2 times. Under a fermented temperature of 45°C, the process performance was more efficient in terms of the maximum ethanol yield.

Introduction

Currently, energy is a basic factor in daily life with the main energy source being petroleum. There is continuous population growth, that causes a high demand for energy resulting in diminishing energy resource and unstable oil prices. In order to solve this problem, most researcher try to search for alternative energy that is sustainable and environmentally friendly such as biogas, ethanol, butanol and biodiesel (Kuiprasut, 2008). Ethanol and butanol are the most attractive alternative energy because they have the potentialfor replacing gasoline (Ouephanit et al., 2012). Butanol has overcome ethanol in term of 7.5 times lower of Reid vapor pressure (RVP) and low volatility that is important relating to the function and operation of gasoline-powered, especially carbureted (Ouephanit et al., 2012). Due to high flash point, hence it is safe for use. Moreover, butanol has a high energy

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value of 24 % (Ouephanit et al., 2012). However, the production of butanol has not been popular among researchers because of the high investment and the limitation of process production that includes giving a lower yield than ethanol (Sonil et al., 2014). For butanol production, the specific cultures required are *Clostridium* sp. and *Bacillus* sp. (Watchara & Benjamas, 2011). Both *Clostridium* sp. and *Bacillus* sp. are expensive microorganism and the optimum condition should be a completely anaerobic environment and sterile which is difficult to control when the organic waste used is a raw material (Sonil et al., 2014).

Gasohol is an alternative fuel for gasoline engines. It is a mixture between gasoline and ethanol in a different ratio, for example 80:20 (National Science and Technology Development Agency: NSTDA, 2010) and 85:15 (Kuiprasut, 2008). The addition of ethanol not only tends to increase the octane number but also could reduce the use of fossil fuel and be environmentally friendly. Ethanol is the substance produced from lignocellulosic material which is mostly agricultural waste via a fermentation process under oxygen limit condition (Pawongrat, 2015). The purification of produced ethanol is obtained as high as 99.5 % by a distillation process.

Ethanol can be produced from various types of waste material such as agriculture, wood and crop residue (Klaichom, 2011) which consists of cellulose, hemicellulose and lignin. Lignin is the composition enveloping cellulose and hemicellulose. Therefore, the pretreatment is a necessary process for lignin removal and microorganism can digest both cellulose and hemicellulose and further converts to reducing sugar: monosaccharides for example glucose and xylose (Pawongrat, 2015). Reducing sugar is fermented to ethanol simultaneously (Ditkunchaimongkol, 2015).

Large quantities of agricultural waste are abandoned, especially sago palm residue which not only causes problems of odor but also water pollution during the rainy season. For 100 kg of sago starch produced, 40 kg of sago palm residue is generated. Moreover, there is some starch remaining in sago palm residue (54-60%) and it has high cellulose content (Pitchayapon, 2018). Therefore, sago palm residue was considered as one suitable lignocellulosic raw material for ethanol production. Naturally, many species of microbes coexist by interacting with each other, whereas many species of microbes are most effective only when they are present in association with other groups of microbes. In this study, sago palm residue was used as a raw material to produce ethanol by using seed sludge. The optimum condition for pretreatment of sago palm residue and the hydrolysis process of pretreated sago palm residue by Cellusoft ® CR conc enzyme was also studied. The ethanol production by seed sludge was also attempted.

Materials and methods

1. Substrate and seed sludge preparation

The sago palm residue used in this study obtained from Yai Chui Farm, Phatthalung, Thailand. It was dried under sun light for 7 days. The dried sago palm residue was crushed with electrical blender at 14,000 round per minute (rpm) for 3 minutes. The crushed sago palm residue was screened by a 60 mesh sieve. The powder of sago palm residue was kept in desiccator before use.

Cellusoft ® CR conc enzyme was obtained from Brenntag Ingredients (Thailand) Pub Co., Ltd., Bangkok, Thailand. The seed sludge was collected from the biogas plant treating ethanol wastewater of Sapthip Co., Ltd., Lopburi, Thailand, which was first concentrated by sedimentation, and the concentrated sludge was then ground and screened by sieving to remove large solid particles. The sieved seed sludge without heat treatment was directly added to a reactor. The microbial concentration, in terms of the mixed liquid volatile suspended solids (MLVSS) for the start-up in this study was about 71,000 mg/L. The genus of microbes might be found in this study seed sludge was facultative anaerobes such as acidogen and methanogen.

2. Proximate analysis and chemical composition

The proximate analysis and chemical composition of sago palm residue were investigated by using standard method that have been reported in the previous work (Sengar et al., 2012). Proximate analysis is covering with organic and inorganic compound in sago palm residue. Volatile matter (VM) was mostly found in sago palm residue indicating that this studied sago palm residue contained mainly organic compound which was easily digested by microbes. Moreover, the significant chemical composition is cellulose, hemicellulose and lignin. The order of fractions in the sago palm residue sample was hemicellulose > cellulose > lignin > ash.

3. Two-stage chemical pretreatment process

10.00 g of sago palm residue powder was immersed in 0.05% w/v NaOH solution in the ratio of 1:10. The solution of sago palm residue was refluxed at 100°C for 2 hours. After refluxing, 0.05 M H_2SO_4 was added to the solution in the ratio of 1:10 and left for 24 hours. The

solution was allowed to reflux again at 100°C for 30 minutes. The crystalline structure of cellulose in untreated and pretreated sago palm residue were determined by X-ray diffraction method.

4. Hydrolysis

The pretreated sago palm residue was hydrolyzed by Cellusoft ® CR conc in order to break down cellulose to reduce to sugar. Before hydrolysis process, the solution from two-stage chemical pretreatment process was adjusted to pH 5.00 with 0.1 M NaOH. The added enzyme solution was shaken at 150 rpm under the temperature of 50°C for 72 hours. The sample was taken out every 12 hours for analyzing the reduction of sugar by dinitrosalicylic colorimetric (DNS) method (Michael, 1988).

Dinitrosalicylic colorimetric method was analyzed by adding 1.00 ml of 0.03 M dinitrosalicylic solution to 1.00 ml of sample from the hydrolysis step. Then, the reaction was stopped by heat for 10 minutes. Spectrophotometer with the wavelength of 540 nm measured the amount of reduced sugar (Young et al., 2013).

5. Ethanol production

Ethanol was produced by using batch process with a working volume of 220 ml. The reactor was under closed-system for allowing an anaerobic condition. The oxygen was a factor that directly affected the activity of facultative anaerobes in seed sludge (Kanchanatawee, 2012). The solution from hydrolysis step was fed to the reactor containing 20%v/v of the seed sludge. Fermentation process was operated under shaker bath at 100 rpm for 7 days. The system was performed at different fermented temperature of 37, 45 and 55°C without pH control in order to determine the optimum fermented temperature for ethanol production. For each fermented temperature, the sample was quickly taken out of the system for detecting the produced ethanol by HPLC every 4 hours.

Results and discussion

1. Two-stage chemical pretreatment process

Fig. 1 shows the X-ray diffraction patterns of untreated, basic pretreated and two-stage pretreated sago palm residue. The crystalline structure of cellulose in untreated sago palm residue was assigned a sharpen diffractogram at 15.6° and 22.3° (2θ) (Banik et al., 2015). On the other hand, the crystalline structure of cellulose in pretreated sago palm residue gradually disappeared which was consistent with the sharpen diffractogram at 15.6° and 22.3° which became broad. It can be suggested that the two-stage chemical pretreatment process not only has an effect to the decrease in lignin composition but also the crystalline structure of cellulose showed disruption causing intermolecular force: hydrogen bond and β-1,4-glycosidic linkage was weak (Piakong, 2014) therefore, the cellulose and hemicellulose content of pretreated sago palm residue was 81.57 and 28.25% higher than that the untreated sago palm residue, respectively.

Sago palm residue is lignocellulosic material consisting of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are significant components for the production of ethanol. Whereas, the lignin performs as a wall that prevents enzyme to digest both cellulose and hemicellulose in reducing sugar. Therefore, the pretreatment process was not only necessary for removing lignin but also for disrupting the crystalline structure of cellulose simultaneously. When the sago palm residue was pretreated with $NaOH/H_2SO_4$, it had 49.63% lower of lignin and the crystalline structure of cellulose became loosen compared to the untreated sago palm residue. Due to β-1,4-glycosidic linkage in cellulose and hemicellulose structure, they can be broken down to reduce sugar by cellulase enzyme (Charles et al., 2005; Ram & Roshan, 2018; Sonil et al., 2014), which will be discussed in the next section.

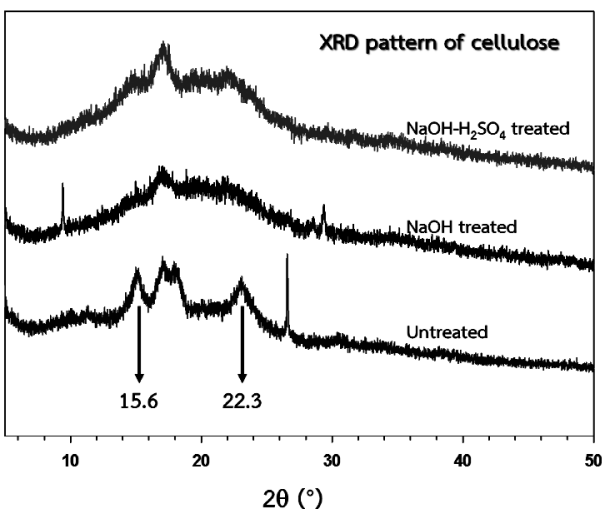
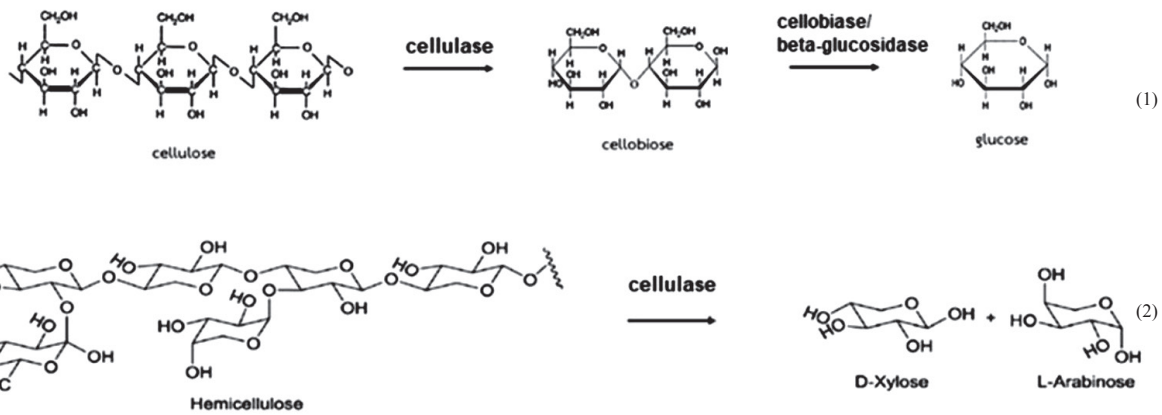


Fig. 1 X-ray diffraction patterns of untreated, basic treated and two-stage pretreated sago palm residue

2. Hydrolysis process

The pretreated sago palm residue was adjusted to pH around 5.0 with 0.1 M NaOH because this pH was an optimum condition for the activity of Cellusoft ® CR

conc enzyme (Kai & Lars, 2014). According to ethanol production process that requires a specific pathway, the Cellusoft ® CR conc enzyme was selected. Cellusoft ® CR conc enzyme can hydrolyzes (1,4)-beta-D-glucosidic linkages in cellulose and hemicellulose structure. The hydrolysis process was operated at a temperature of 50°C with shaking 150 rpm (Tabka et al., 2006). The reduced sugar was analyzed by using spectrophotometer at wavelength of 540 nm with DNS method. From the result, it was found that the concentration of reducing sugar increased with hydrolysis time and attained a maximum value of 19.53 mg/L (or 229.78 mg/g biomass) at a hydrolysis time of 96 hours. The production of reducing sugar from pretreated sago palm residue was about 3.2 time higher than that untreated sago palm residue. This was the same result with other related work (Farzad et al., 2013). Beyond a hydrolysis time of 96 hours, the reduced sugar concentration was constant (Fig. 2). The two-stage chemical pretreatment process result shows sago palm residue had more cellulose and hemicellulose content. Therefore, when pretreated sago palm residue went into the hydrolysis process, it easily converted cellulose and hemicellulose in reducing sugar (Equation 1-2) (Anthonia & Philip, 2015). In this study, it can be concluded that the optimum hydrolysis time for the production of reducing sugar was 96 hours.



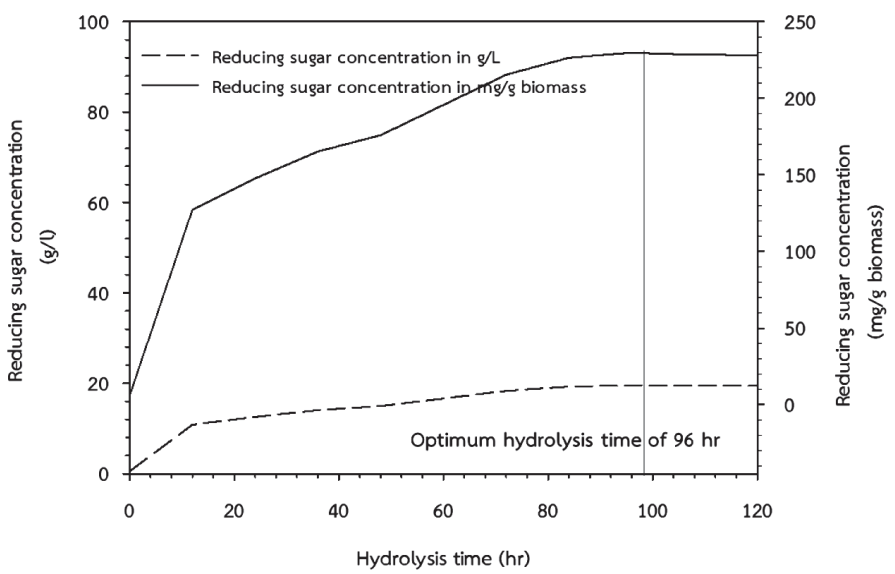


Fig. 2 Reducing sugar concentration at various hydrolysis time at 50°C for 120 hours ethanol production

Fig. 3 shows ethanol concentration at different fermented temperature. Ethanol concentration increased with increasing fermented temperature from 37°C to 45°C. The maximum ethanol concentration was found at a fermented time of 72 hours. However, the ethanol concentration decreased when fermented temperature increased from 45°C to 55°C. Hence, the temperature might be a factor that has an effect on the activity of microbes. The condition for producing ethanol was the same as biogas production system (Laowansiri et al., 2018), which is anaerobic environment, with various factors that have an effect on microbial activity such as temperature, pH, and oxygen content. Among those factors, temperature had the most significant effect on the microbe activity. At a higher temperature (beyond 50°C), the microbial activity particularly non-spore forming microorganisms (facultative anaerobes) was suppressed (Niamsup et al., 2009) because they could not withstand high heat (Lin & Hung, 2008). In this study, a fermented temperature of 45°C is considered an optimum condition for ethanol production using seed sludge.

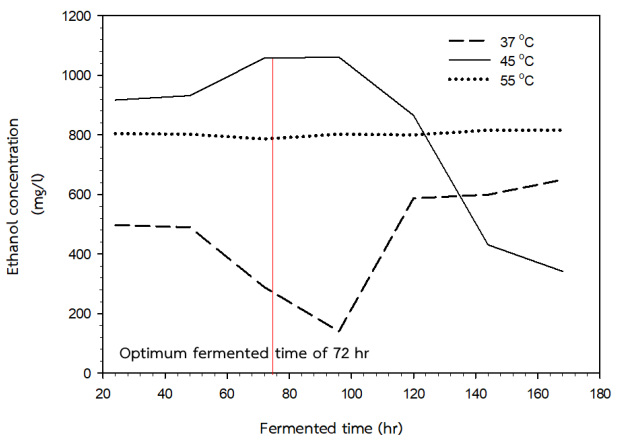


Fig. 3 Ethanol concentration at fermented temperature of 37°C, 45°C and 55°C

Under optimum fermented temperature of 45°C, the ethanol concentration increased with increasing fermented time from 24 to 96 hours which is consistent with the decrease in reduced sugar concentration (Fig. 4). It was suggested that reducing sugar was digested by microbes and further converted to ethanol (Kanchanatawee, 2012). The produced reduction of sugar such as glucose and xylose could be a carbon source for an ethanol producer (Gregory et al., 1998; Sonil et al., 2014). Both glucose and xylose are converted to ethanol via glycolysis which has pyruvate as a main product. The pyruvate changed to acetaldehyde under decarboxylation pathway by decarboxylase enzyme of anaerobe (Shang-Tian et al., 2007; Gregory et al., 1998). The acetaldehyde was transformed to ethanol via the reduction of acetaldehyde pathway by alcohol dehydrogenase enzyme (Shang-Tian et al., 2007; Gregory et al., 1998). Beyond a fermented time of 96 hours, both ethanol and reducing sugar decreased. The result from reducing sugar degraded to small organic acids which were acetic acid (HAc), propionic acid (HPr), butyric acid (HBu) and lactic acid (HLa) (Equation 3-6) under oxidative fermentation of ethanol (Mani et al., 2016; Pongdam, 2017). The produced organic acid might be accumulated in the system and further toxic to the growth of microbe (Kanchanatawee, 2012). Moreover, the pH of the system also decreased from 6.50 to 4.45 whereas, the optimum pH for the growth of microbe was around 6.00-7.00 (Kvesitadze et al., 2012). The toxicity from organic acids accumulation resulted from associated acid and most of produced organic acid found in this study was mainly weak acids that were difficult to break down (Kanchanatawee, 2012; Patcharee et al., 2012).

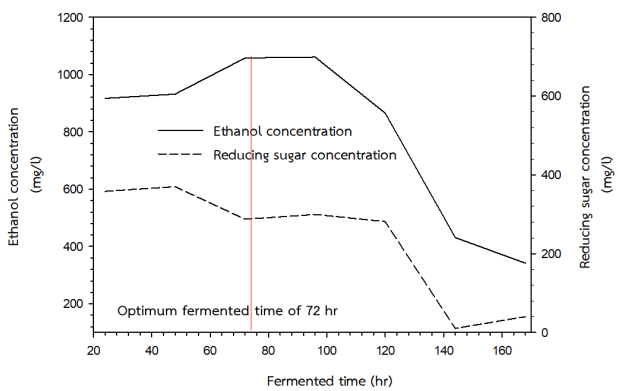


Fig. 4 Ethanol and reducing sugar concentration at an optimum fermented temperature of 45°C

Fig. 5 shows the concentration of small organic acids at different fermented time. The composition of small organic acids found in this study was acetic acid (HAc), propionic acid (HPr), butyric acid (HBu), and lactic acid (HLa). All acid concentrations tended to increase at any given fermented time. Not only was ethanol produced but the gaseous product was also obtained which were methane and carbon dioxide. It was suggested that reducing sugar was a small soluble organic molecule which easily degraded anaerobically by microbes. The

reducing sugar can be degraded to both ethanol and biogas, according to Equation 4-8, respectively (Piakong, 2014; Laowansiri et al., 2018).

$$C_6H_{12}O_6(l) + 2H_2O(l) \rightarrow 2CH_3COOH(l) + 4H_2(g) + 2CO_2(g) \quad (3)$$

$$C_6H_{12}O_6(l) + 2H_2O(l) \rightarrow 2CH_3CH_3COOH(l) + 2H_2O(l) \quad (4)$$

$$C_6H_{12}O_6(l) \rightarrow CH_3(CH_2)_2COOH(l) + 2H_2(g) + 2CO_2(g) \quad (5)$$

$$C_6H_{12}O_6(l) \rightarrow CH_3CHOHCOOH(l) \quad (6)$$

$$CH_3COOH(l) \rightarrow CH_4(g) + CO_2(g) \quad (7)$$

$$CO_{2(g)} + 4H_2(g) \rightarrow CH_4(g) + 2H_2O(l) \quad (8)$$

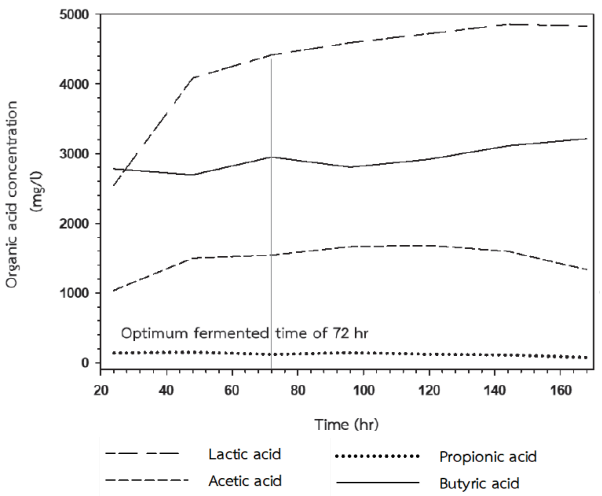


Fig. 5 Organic acid concentration at an optimum fermented temperature of 45°C

The comparison of ethanol production with different types of raw material, fermented temperature and pretreatment process is shown in Table 1. The pretreated raw material gave a higher ethanol concentration than the untreated raw material. The pretreated raw material was easily utilized by microbe since lignin composition as a thick wall enveloped cellulose and hemicellulose was eliminated (Farzad et al., 2013). In addition, the system operated at a higher fermented temperature of 45°C gave a higher ethanol production than that at a low fermented temperature of 37°C, according to Arrhenius' equation. At a higher temperature, the rate constant was higher resulting in a faster reaction rate causing a higher ethanol production performance. Under the same fermented temperature of 37°C, the produced ethanol found in this study was higher than the previous study (Farzad et al., 2013; Kim et al., 2017). Due to the use of sago palm residue as a raw material, the mixed culture from seed sludge was more suitable than that of single culture (Niamsup et al., 2009). The sago palm residue is an agricultural waste in the group of lignicellulosic material. It contains many complex structures such as starch, cellulose and hemicellulose which is difficult to digest by single microorganisms. In this study, seed sludge contained various types of microorganisms: cellulolytic microorganisms, amylolytic microorganisms, microorganisms that use sugar as food and acid-based microorganisms (Songrit & Kositanont, 2014). Therefore, the synergistic property of various kinds of microorganisms helps to degrade and digest sago palm residue affecting to the system by obtaining more ethanol production performance in terms of ethanol yield.

Table 1 Reducing sugar and ethanol concentration produced from different biomass, fermented temperature and pretreatment process

Biomass	Pretreatment process	Fermented temperature (°C)	Time (hr)	Reducing sugar concentration (greducing sugar/ kgbiomass)	Ethanol concentration (gethanol/ kgbiomass)	Ref.
Sago palm residue	NaOH/ H_2SO_4 (0.5 %w/v/0.26 %w/v)	45	96	229.78	62.18	Present study
Sago palm residue	NaOH/ H_2SO_4 (0.5 %w/v/0.26 %w/v)	37	48	229.78	32.82	Present study
Rice straw	-	37	72	102.00	<0.10	Farzad et al., 2013
Rice straw	Alkali	37	72	163.50	1.20	Farzad et al., 2013
empty fruit bunches	Dilute H_2SO_4 (1 %w/v)	30	72	68.00	18.50	Young et al., 2013
Spoilage date palm	-	30	72	915.80	1.15	Hemida & Elsadek, 2012
Coffee residue	H_2SO_4 (4 %w/v)	37	72	229.00	266.00	Kim et al., 2017

Conclusion

In this work, the ethanol production from sago palm residue by using seed sludge was investigated. The sago palm residue was chemically pretreated with 0.5 %w/v sodium hydroxide followed by 0.26 %w/v sulfuric acid via two-stage process. The pretreated sago palm residue gave a 49.63 % decrease in lignin, 44.92 % increase in cellulose and 22.02 % increase in hemicellulose. The maximum reduction in sugar concentration was as

high as 229.78 g glucose/kg biomass at optimum hydrolysis time of 96 hours. The use of dilute acid (<0.50% w/v) was more efficient to break β-1,4-glycosidic linkage of cellulose and hemicellulose structure causing the increase in ethanol production performance in term of ethanol yield. The optimum fermented temperature for maximize ethanol concentration was found at 45°C.

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