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Bioethanol from Food Waste Fermentation and Its Application for Direct Ethanol Fuel Cell

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ABSTRACT

This research aims to apply food waste for bioethanol production through biological process. In order to increase the fermentation efficiency, the fungal mash of *Aspergillus oryzae* TISTR 3018 was used for saccharification of food waste. The suitable hydrolysis conditions were 5% (w/v) of fungal spore loading under semi-solid state at room temperature (28.92 ± 0.56°C) for 72 h. Using those optimal conditions, the reducing sugar concentration was obtained, 59.23 ± 0.36 g/L or 0.30 g/g dried food waste. Non-sterilized food waste hydrolysate without solid residue separation was used for bioethanol fermentation by *Saccharomyces cerevisiae*. The highest bioethanol (crude bioethanol) of 33.4 g/L which calculated as 0.17 g/g dried food waste was achieved at 120 h of fermentation. The crude bioethanol was further utilized as biofuel for electricity generation by direct ethanol fuel cell. It was found that crude bioethanol exhibited the maximum current density of 0.48 $mA.cm^{-2}$ at 0.57 V. This research demonstrated that food waste was usable as renewable raw material for bioethanol production and can also solve the environmental problem and energy scarcity problem in the future.

Keywords: Food waste, *Aspergillus oryzae*, Direct ethanol fuel cell

INTRODUCTION

Food waste is a discarded edible material along the food supply chain (harvesting, transportation, manufacturing, storage and consumption). It is generated with the unintentionally wasting of food or removal of non-edible part (Zhang and Jahng, 2012). Food waste can be made the negative impact on environmental, social and economic level in many countries by improper handling of food waste such as landfilling and incineration (Eriksson et al., 2018). The amount of food waste in Asian countries are estimated to increase to 416 million tons per year in 2025 due to the rapid population growth, economic and industrial development (Melikoglu et al., 2013). In Thailand, food waste is generated more than 0.73 kg/person/day which equal to 64% of total municipal waste (Pollution Control Department, 2018). Thus, the proper food waste treatment need to be investigated. The utilization as low cost raw material for value-added products production via biorefinery process is the promising method and sustainable development.

Food waste is the source of organic material which consists of varied chemical composition depending on the origin. It mainly contains carbohydrate, protein and lipid which can be used as nutrients source in fermentation process. Many researches have been focused on food waste utilization for valuable products production such as ethanol (Kim et al., 2008; Matsakas et al., 2015; Hafid et al., 2017), methane (Kiran et al., 2015; Wu et al., 2018), biodiesel (Ma et al., 2018; Priyadarshi and Paul, 2018), lactic acid (Pleissner et al., 2017), succinic acid (Sun et al., 2014), etc. However, the pretreatment step of food waste before fermentation is necessary in order to recover nutrients because of the microorganisms cannot directly assimilate nutrients in food waste mixture. Although there are many approaches that have been applied for food waste pretreatment, the biological is an interesting method due to environmentally friendly, mildly conditions and specific reaction. Glucoamylase, α-amylase, protease, cellulase, β-glucosidase and xylanase are commercial enzymes that have been subjected in enzymatic hydrolysis of food waste. Moon et al. (2009) used commercial enzymes of carbohydrase, amyloglucosidase and mixture of them for food waste hydrolysis to produced ethanol. The highest glucose yields of 0.35, 0.41 and 0.46 g/g of dry food waste were obtained, respectively. Hafid et al. (2014) examined food waste hydrolysis by using crude commercial *Aspergillus niger* glucoamylase. It was found that the glucose concentration was increased to 99.56 ± 4.37 g/L after 6 h.

In addition, food waste pretreatment by using secreted hydrolytic enzymes from fungal such as *Aspergillus awamori* and *Aspergillus oryzae* are an alternative method for fermentable sugar production. Sun et al. (2014) produced food waste hydrolysate via fungal mash, *A. awamori* and *A. oryzae*. The results showed that the hydrolysate with the amount of glucose of 31.9 g/L was appropriated for succinic acid production by *Escherichia coli*. Yin et al. (2016) also utilized the fungal mash of *A. awamori* for hydrolysis of food waste. Food waste hydrolysate containing glucose of 3959.8 ± 128.3 mg/L and free amino nitrogen of 74.1 ± 13.9 mg/L were obtained at 24 h of pretreatment, and was then subjected for anaerobic digestion.

Bioethanol is an alternative biofuel that is important in many industrial and automotive sectors. It has high octane number, flame speed, vaporization heat, and broader flammability limit which lead to no particulate emissions from engine (Krylova et al., 2008; Liu et al., 2019). It can also be used as starting material for hydrogen production and electric energy generation which can be solved the environmental problems (Lazar et al., 2019). Bioethanol can be produced by the fermentation of organic substrate rich in sugar by microbial. There are many researches interested in the utilization of food waste hydrolysate as a raw material for bioethanol production. *Saccharomyces cerevisiae* is the microorganism that has been used to produce bioethanol because of it has high growth rate, capable in large scale cultivation and effective waste utilization (Reis et al., 2013). According to Kim et al. (2008), the ethanol fermentation was done by using *S. cerevisiae* KA4, the highest ethanol concentration of 57.5 g/L was obtained after 14 h of fermentation. Yan et al. (2012) obtained the maximum ethanol concentration of 82.23 ± 2.03 g/L (0.42 ± 0.02 g ethanol/g glucose) within 60 h of fermentation by *S. cerevisiae* CGMCC 2982 on food waste hydrolysate. However, many researchers have interested in the common yeast strain (baker's yeast) as the ethanol producer due to inexpensive cost and convenient in inoculum preparation and preservation. As a report of Uncu and Cekmecelioglu (2011) who applied the commercial dried baker's yeast (*S. cerevisiae*) for ethanol production by using kitchen waste hydrolysate as culture medium. The maximum ethanol concentration of 32.2 g/L was obtained under the optimum pH of 4.5, 30°C and 150 rpm within 58.8 h. In addition, Kiran and Liu (2015) used dried baker's yeast, *S. cerevisiae*, for ethanol fermentation on food waste hydrolysate. The ethanol concentration of 58 g/L and yield of 0.5 g/g glucose were achieved.

Fuel cell is a device that converts the chemicals energy of fuel containing hydrogen atom such as ethanol and methanol into electricity through chemical reaction (Gomes and Bortoli, 2016). Direct ethanol fuel cell

(DEFC) is the type of fuel cell that uses ethanol as fuel for electricity generation. The advantages of ethanol are rich in hydrogen, high specific energy (8.00 kWh/kg), less toxic and easily produce from organic waste materials through fermentation process. Currently, DEFC have widely interested and expected to replace commercial batteries (Akhairi and Kamarudin, 2016).

This research aims to utilized food waste for fermentable sugar production by biological pretreatment. Optimization of the amount of fungal spore for saccharification was investigated. Food waste hydrolysate with sterilized and non-sterilized were used as starting materials for bioethanol fermentation. The produced bioethanol was subjected as an initial substance for electricity generation via fuel cell. The advantages of this research is not only to obtain the low priced bioethanol from discarded food, but the problems of food waste accumulation is also figured out.

MATERIAL AND METHODS

Materials

Food waste Food waste used in this study was collected from canteen of Department of Biology, Faculty of Science, Chiang Mai University, Thailand. The sampling was carried out within 4 months (September to December, 2017). Plastic, bones and inorganic objects were separated out from food waste. The remaining fraction was dried in hot air oven at 70°C for 1 week and homogenized into small particle using mechanical blender. Then, the homogenized food waste was kept in plastic bag for further study.

Microorganisms *Aspergillus oryzae* TISTR 3018 was purchased from Thailand Institute of Scientific and Technological Research (TISTR). For the preparation of fungal spore, the spore of *A. oryzae* was strewed on cooked Thai jasmine rice which is contained in a plastic bag and placed at room temperature for 7 days. After that, fungal spore was dried in hot air oven at 45 ± 5°C and blended into powder. This fungal spore was kept in plastic bag at 4°C.

Saccharomyces cerevisiae was purchased from Greathill Co., Ltd., Thailand. The pre-culture of *S. cerevisiae* was prepared by cultivation at room temperature (28.92 ± 0.56°C) for 24 h on culture medium containing the following components: 10 g/L glucose, 2.5 g/L yeast extract and 5 g/L malt extract. The pre-culture was transferred to the new culture medium until 2.35 × 10^7 cells/mL (enriched *S. cerevisiae*) was obtained and used as an inoculum in bioethanol fermentation.

Biological pretreatment

Fungal spore pretreatment Fungal pretreatment of food waste was conducted in 250 mL Erlenmeyer flasks containing 20 g of food waste under semi-solid state condition. Various parameters of pretreatment including fungal spore loading and incubation time were evaluated. The experiment was applied from Kiran and Liu (2015). Varied fungal spore loading of 0, 1, 3, 5, 7 and 9% (w/w) were added into 20% (w/v) sterilized food waste. The experimental was carried out at room temperature (28.92 ± 0.56°C) for 96 h. The obtained supernatant was collected by centrifugation at 3500 rpm, 4°C for 10 min. The supernatant was collected for reducing sugar determination.

Bioethanol fermentation

Comparison of sterilized and non-sterilized hydrolysate for bioethanol fermentation The bioethanol fermentation was compared between the food waste hydrolysate obtained from fungal spore pretreatment with sterilized (by autoclaving; pressurized saturated steam at 121 °C with a pressure of 15 psi for 15 min) and non-sterilized. Both food waste hydrolysate were used directly (without the separation of solid residue) as medium in bioethanol production. Enriched *S. cerevisiae* was inoculated to food waste hydrolysate and then conducted at room temperature without agitation for 168 h. After fermentation, the supernatant was collected for reducing sugar and bioethanol determination.

Scale-up of fungal spore pretreatment and bioethanol fermentation According to fungal spore pretreatment, the ratio of food waste to water was adjusted by the up-scaling by twofold, 1:5 to 2:10. The experimental was conducted in 500 mL Erlenmeyer flask. 5% (w/w) fungal spore was added and placed at room temperature for 72 h. After that, enriched *S. cerevisiae* was added and incubated at room temperature for 168 h. The sample was harvested every 24 h and the supernatant was collected for reducing sugar and bioethanol determination.

Bioethanol fuel cell

Crude bioethanol was used for evaluation of electricity production by fuel cell. The crude bioethanol of 0.5 M was prepared in 0.5 M H_2SO_4, and was then purged by nitrogen gas for 15 min. The glassy carbon electrode (3 mm of diameter) as working electrode, Ag/AgCl (saturated KCl) electrode as reference electrode and platinum wire as auxiliary electrode were used as the conventional electrochemical electrodes. Pt/rGO-CNT (Platinum (Pt) on

reduced graphene oxide (rGO) as a catalyst was dropped onto glassy carbon electrode and dried. Then, all of the electrodes were combined and observed the electric current.

Analytical methods

The chemical composition analysis of dried food waste is based on the standard method of AOAC (1990). Moisture content was determined by drying in a hot air oven at 105°C until constant weight. Lipid content was analyzed using Soxhlet extraction method. The measurement of protein and nitrogen content was according to the Kjeldahl method (conversion factor of 6.25). The fiber content was determined using acid base digestion and solubilization. The total ash content was determined by combustion at 600°C for 3 h. The remaining percentage was assumed to be the total carbohydrate content.

The reducing sugar concentration was determined via the 3,5-dinitrosalicylic acid (DNS) method (Miller et al., 1959). The sugars composition was analyzed by using high performance liquid chromatography (HPLC) (Agilent, USA) equipped with a refractive index (RI) detector and an Econosphere NH_2 5u (Alltech Associates, Inc.) capillary column (250 mm × 4.6 mm). 90% acetonitrile was used as mobile phase. The sample (10 µl) was injected at a flow rate of 1 mL/min. Xylose, arabinose, fructose, glucose, galactose, sucrose, maltose and lactose were used as standard sugars.

Bioethanol was quantified by gas chromatography (GC). GC was done with the following equipment: GC-17A gas chromatography (Shimadzu), equipped with a flame ionization detector (FID) and HP-INNOWAX (Agilent Technologies, USA) capillary column (30 m × 250 µm × 0.25 µm). Helium was used as a carrier gas, at a flow rate of 1 mL/min. The injector and detector temperatures were set at 200 and 280°C, respectively. The oven temperature was held at 40°C for 4 min, and then programmed to 110°C at a rate of 15°C/min.

The alcohol and aldehyde compositions were measured by GC. The injector and detector temperatures were set at 200 and 280°C, respectively. The oven temperature was held at 40°C for 4 min, and then programmed to 150°C at a rate of 15°C/min and held for 5 min. The total running time was 16.67 min. Helium was used as a carrier gas, at a flow rate of 1 mL/min. Sample of 1 µL was injected with the split mode at ratio of 200:1. Methanol, ethanol, propanol, isobutanol, butanol, 3-methyl-1-butanol and acetaldehyde were used as standard substances.

Fatty acid was analyzed by GC and used AT-5, 30 m × 250 µm × 0.25 µm as a column (Alltech). The oven temperature was held at 150°C for 1 min,

and then programmed to 250°C at a rate of 10°C/min and held for 3 min. The injector and detector temperatures were set at 220°C and 270°C, respectively. The total running time was 14 min. Nitrogen was used as a carrier gas. Sample of 1 µL was injected with the split mode at ratio 150:1 and pressure of 19.32 psi. Lauric acid, palmitic acid, stearic acid, oleic acid and linoleic acid prepared in ethyl ester form were used as standard fatty acids.

The efficiency of ethanol fuel cell could be observed by the cyclic voltammetry (CV) measurement. These following parameters was input into computer installed with EChem V.2.2.2 and connected to eDAQ potentiostat:

- Mode : Cyclic
- $E_{Initial}$: -400 mV (To study hydrogen adsorption/desorption)
  : 0 mV (To study ethanol oxidation reaction)
- E_{Final} : -400 mV (To study hydrogen adsorption/desorption)
  : 0 mV (To study ethanol oxidation reaction)
- Rate : 50 mV/s
- Step W : 20 ms

The CV for hydrogen adsorption-desorption in 0.5 M H_2SO_4 with saturated nitrogen gas at potential range -400 to 1000 mV was determined to study the electrochemical characterization of catalyst. The CV at the potential range from 0 to 1000 mV was used to measure the electrode activity for ethanol oxidation reaction in 0.5 M CH_2CH_3OH + 0.5 M H_2SO_4 solution with saturated nitrogen gas. The CV curves was plotted by Origin 8.0 program of current density (mA/cm^2) against the potential (V).

RESULTS

Chemical composition of food waste

The characteristic of food waste before and after drying is shown in Figure 1. Pre-drying food waste consisted of rice and noodle higher than the other compositions (meat, bone, vegetable and fruit peel). The food waste after drying and blending become fine powder, brown color with a size about 12 meshes. The chemical composition of food waste is presented in Table 1. The results showed that food waste was mainly composed of carbohydrate approximately 48.11% (w/w) of dry mass. The amount of lipid and protein were 18.11% (w/w) and 17.53% (w/w), respectively. The less component was fiber content, 0.48% (w/w) containing in food waste sample.

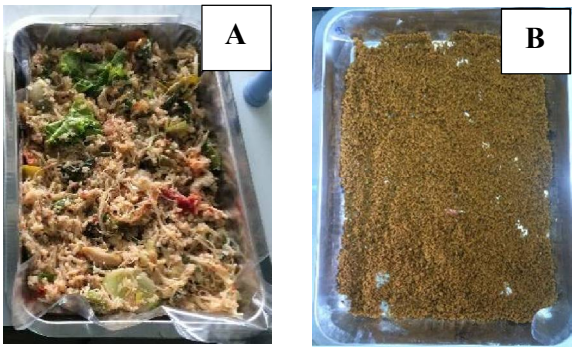

Figure 1. Physical characteristic of food waste before (A) and after drying and blending (B).

Table 1. Chemical composition of collected food waste (dry mass).

Compositions	% (w/w)
Carbohydrate	48.11 ± 1.24
Lipid	18.11 ± 1.06
Protein	17.53 ± 0.35
Moisture	10.60 ± 0.60
Ash	5.18 ± 0.31
Fiber	0.48 ± 0.16

Results are presented as means ± S.D.

Biological pretreatment

Fungal spore pretreatment

The results of fungal spore pretreatment of food waste is shown in Figure 2. It was found that the reducing sugar concentration of all treatments (1 to 9% of spore loading) were highly increased after the addition of fungal spore and reached to constant value at 72 h. The pretreatment with 5% (w/w) fungal spore loading for 72 h showed highest reducing sugar concentration of 59.23 ± 0.36 g/L with yield of 0.30 g/g food waste.

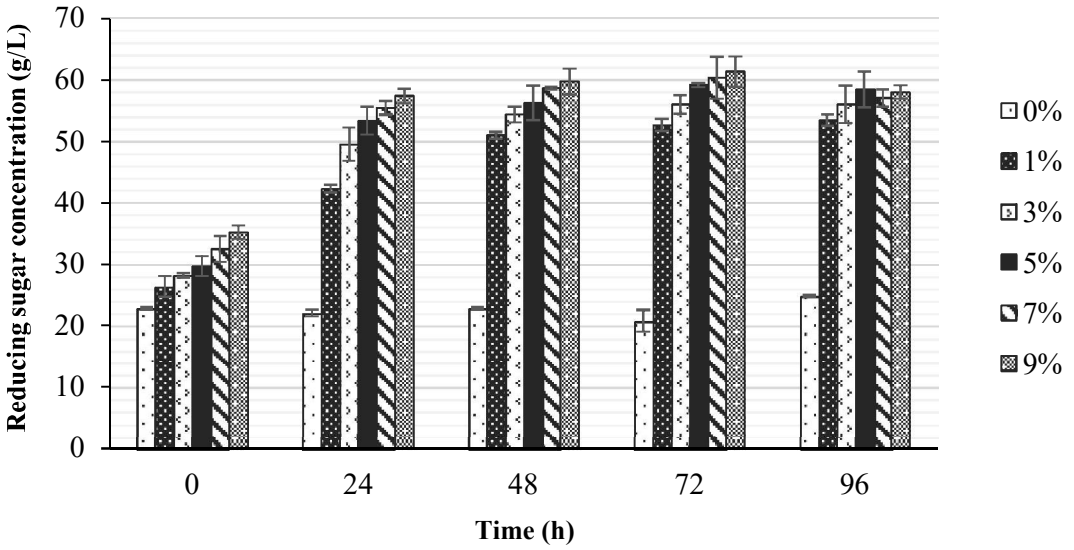

Figure 2. Reducing sugar concentration from food waste pretreatment using various fungal spore loading (%, w/w)

The sugar composition derived from fungal spore pretreatment of food waste by HPLC analysis is shown in Table 2. Food waste hydrolysate was consisted of maltose, glucose and fructose. Glucose was detected as the major sugar component which as high as 51% of total sugars.

Table 2. The sugar composition of food waste hydrolysate from fungal spore pretreatment.

Sugar composition	Content (%)
Maltose	31.68
Glucose	51.04
Fructose	17.28

Bioethanol fermentation

Comparison between non-sterilized and sterilized food waste hydrolysate in bioethanol fermentation

The results of bioethanol production on non-sterilized and sterilized food waste hydrolysate is shown in Figure 3. The highest bioethanol concentration of 4.23 ± 0.12% (v/v) was obtained from non-sterilized food waste hydrolysate at 120 h of the fermentation. The remaining reducing sugar

concentration of non-sterilized process was lower than 10 g/L after 120 h of the fermentation. The sterilized process presented the highest bioethanol concentration of 2.59 ± 0.14% (v/v) at 24 h, and the amount stayed constantly through 168 h of the fermentation. The reducing sugar concentration of sterilized process was increased to maximum approximately 30 g/L in 24 h of fermentation throughout 168 h.

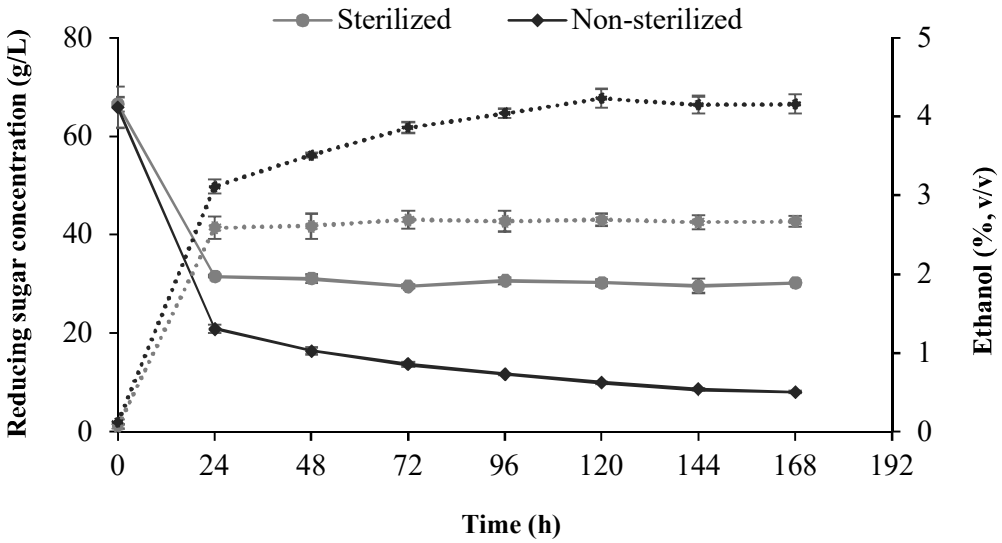

Figure 3. Bioethanol concentration (dashed line) and the remaining reducing sugar concentration (solid line) in the fermentation of non-sterilized and sterilized food waste hydrolysates.

Scale-up of fungal pretreatment and bioethanol fermentation

The reducing sugar and bioethanol concentration from scale-up of pretreatment and fermentation is presented in Figure 4. After scale-up, the produced reducing sugar concentration at 72 h of pretreatment was 62.56 ± 1.02 g/L, which was not different from before scale-up (65.85 ± 4.22 g/L). For bioethanol production, it should be noted that the bioethanol concentration from scale-up fermentation was slightly higher than small scale. The highest bioethanol concentration from scale-up and non-scale-up were achieved at168 h with 4.61 ± 0.04 and 3.34 ± 0.18% (v/v), respectively.

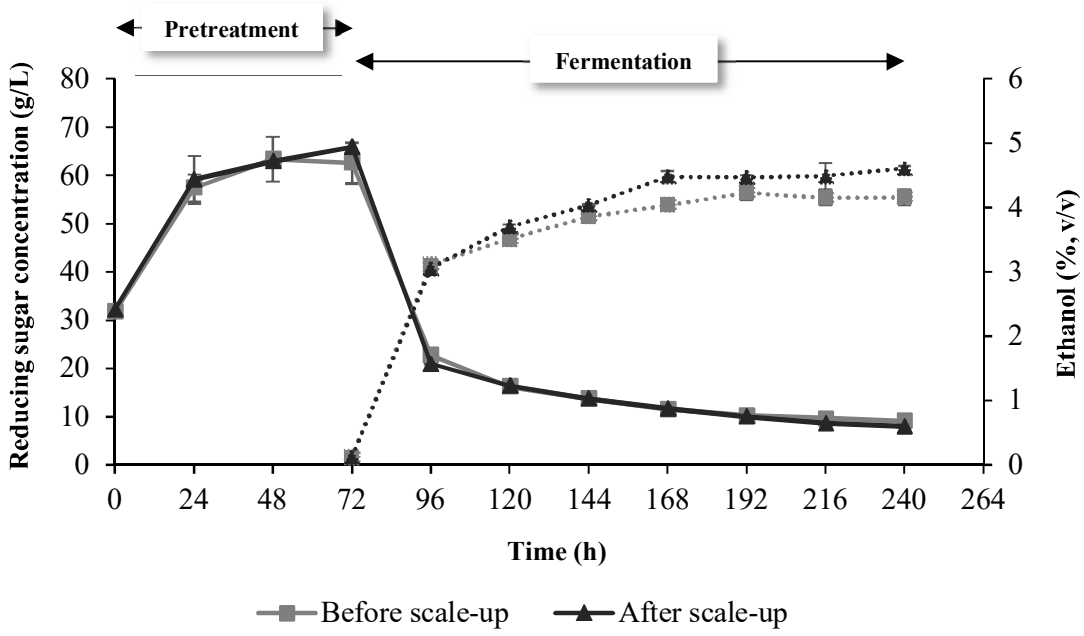

Figure 4. Reducing sugar concentration (solid line) and bioethanol concentration (dashed line) of food waste fermentation with different scale of solid-liquid proportion.

Characteristic of bioethanol production

The characteristics of crude bioethanol including sugar, aldehyde and alcohol and fatty acids are shown in Figure 5, 6 and 7, respectively. Crude bioethanol was consisted of various components including sugar (glucose), aldehyde (acetaldehyde), alcohols (methanol, ethanol, propanol, isobutanol, butanol and 3-methyl-1-butanol) and fatty acids (lauric acid, palmitic acid, linoleic acid, oleic acid and stearic acid). In addition, fatty acids composition in crude bioethanol were similar to food waste feedstock (data not shown). However, crude bioethanol from food waste might be contained with other substances besides the standard compounds used in GC analysis

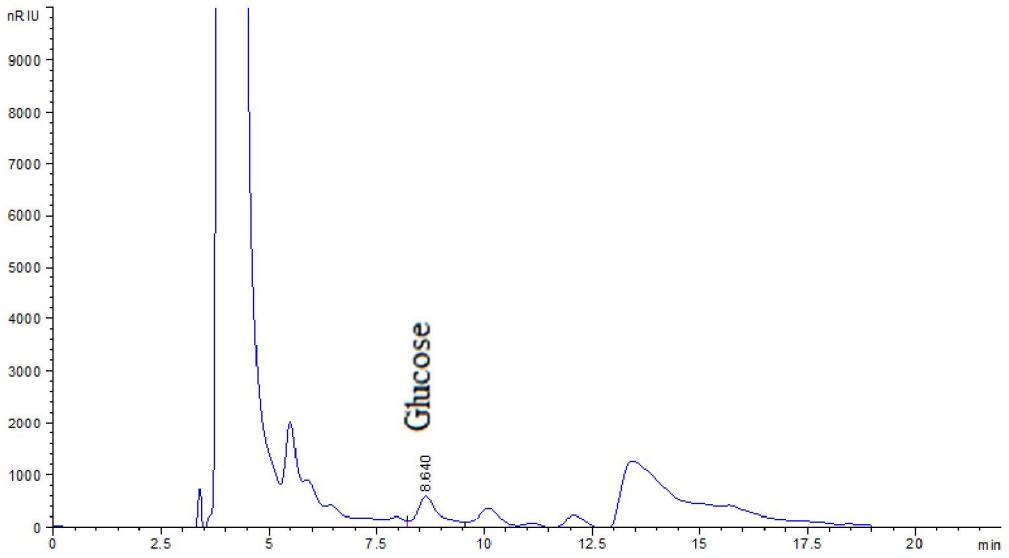

Figure 5. HPLC chromatogram of sugar in crude bioethanol

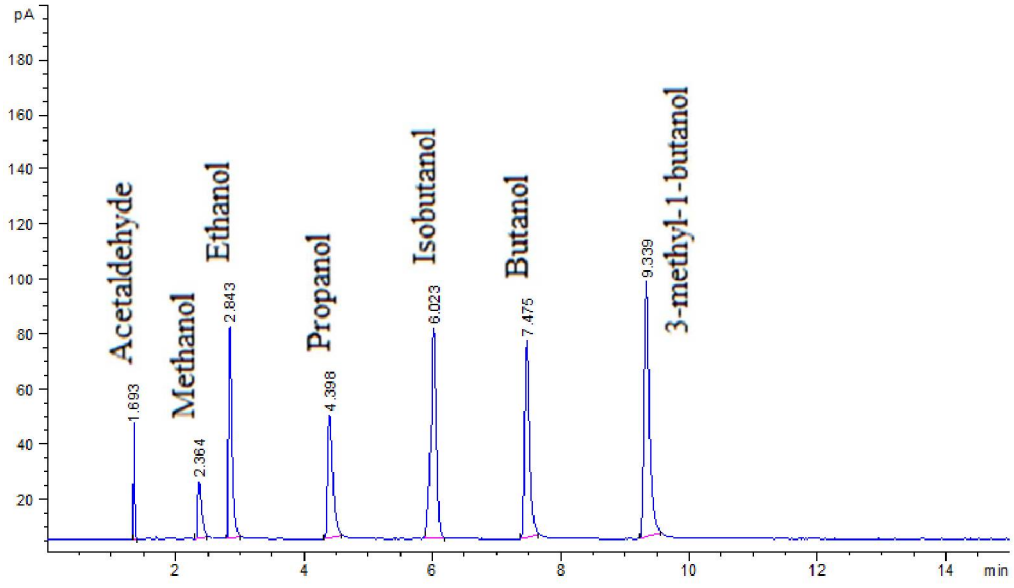

Figure 6. GC chromatogram of aldehyde and alcohol in crude bioethanol

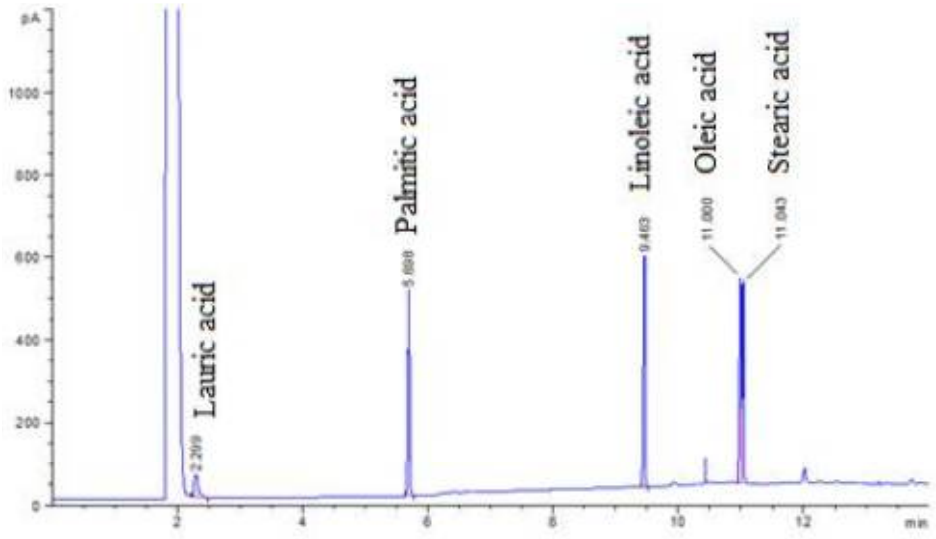

Figure 7. GC chromatogram of fatty acid in crude bioethanol

Bioethanol fuel cell

Crude bioethanol was used for electricity generation through electrochemical reaction by fuel cell prototype. Figure 8 showed the cyclic voltammetry (CV) curves of current density and potential correlation which obtained from crude bioethanol. The characteristics peak of CV curve of crude bioethanol presented the maximum value of 0.48 $mA.cm^{-2}$ at 0.57 V.

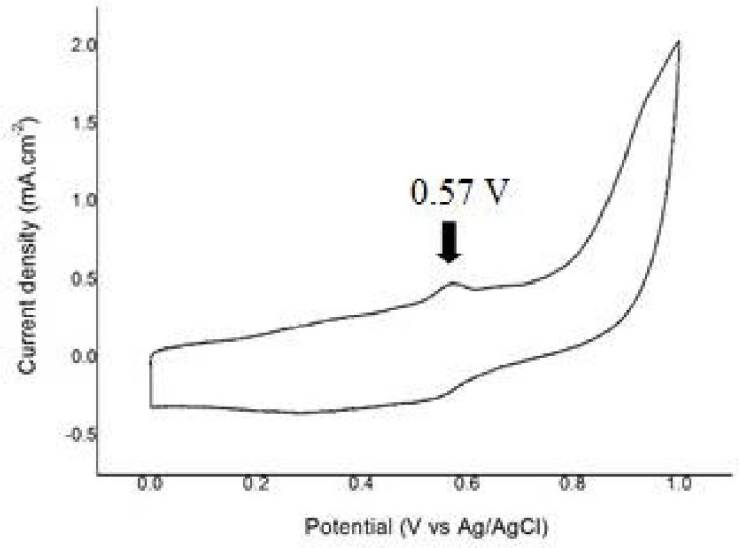

Figure 8. CV curve of crude bioethanol in 0.5 M H_2SO_4 solution; potential range from 0.00 V to 1.00 V at a scan rate 50 mV/s.

DISCUSSION

The composition of food waste used in this research was close agreement to the other reports that presented the content of carbohydrate, protein and lipid in range of 47.7-64.5%, 14.86-21.1% and 8.3-17.46% of dry weight basis, respectively (Ferris et al., 1995; Moon et al., 2009). The food waste containing high carbohydrate and protein can be hydrolyzed for recovery of nutrients such as glucose, fructose and amino acids which subsequently used as substrate in fermentation process. However, the composition of food waste could be varied depending on the consumption behavior and source of food waste (Cerda et al., 2018).

Fungal spore of *A. oryzae* was usable as biological pretreatment of food waste since it was directly hydrolyzed food waste containing high carbohydrate (starch) resulting in fermentable sugar. 5% fungal spore loading was an effective in reducing sugar production. It was found that the fungal grew well on food waste and provided sugar yield of 0.30 g/g food waste under semi-solid state condition. This was quite similar to Pleissner et al. (2014) who obtained glucose yield of 0.29 g/g food waste from fungal spore *A. oryzae* hydrolysis of food waste. The sugars composition of food waste hydrolysate was highly consisted of glucose and maltose. This demonstrated that the fungal can be produced amylolytic enzymes for starch hydrolysis. *A. oryzae* is hydrolytic enzymes producing microorganism including amylase, protease, lipase and nuclease. These enzymes can be hydrolyzed carbohydrate, protein and lipid in food waste into smaller molecules (Gomi, 2014). The remaining of maltose indicated that oligosaccharide hydrolysis by fungal spore was not completed. However, the sugar composition may differ from other research because of the difference in efficiency of hydrolysis process and food waste characteristic. In addition, the long pretreatment period should be avoided due to sugar consumption of fungal could be occurred for its growth (Sun et al., 2014).

Typically, culture media was sterilized before using in the fermentation in order to prevent the contamination and increase the product yield (Salakkam et al., 2017). This study, the bioethanol production on sterilized and non-sterilized food waste hydrolysate were conducted to examine the effect of thermal from sterilization on food waste hydrolysate. It was found that non-sterilized food waste hydrolysate presented the bioethanol concentration higher than sterilized food waste hydrolysate. This could be due to the autoclaving process generated of unfavorable by-products such as furfural derivatives, which can be inhibited the growth of *S. cerevisiae* during fermentation. In addition, the Maillard reaction could be occurred under high temperature

condition and resulting in the decreasing of desired products such as fermentable sugar, amino acids and other nutritional elements (Klinke et al., 2004; Sakai and Ezaki, 2006; Pilli et al., 2015). Thus, the sterilization of food waste hydrolysate was not required for bioethanol fermentation. This can also be reduced the energy consumption by autoclaving.

Generally, various parameters for scaling up of the fermentation are such as cell growth, mutation probability, sterilization process, temperature and pH control, agitation, surface aeration and pressure, which consequently results productivity reduction (Thiry and Cingolani, 2002). However, this study demonstrated that the scale-up of food waste pretreatment and bioethanol fermentation had no affected on the reducing sugar and bioethanol production. Moreover, the yield of bioethanol of 0.17 g/g food waste was comparable to Wang et al. (2008); Moon et al. (2009); Uncu and Cekmecelioglu (2011) and Yan et al. (2012) who obtained yield of ethanol from fermentation by *S. cerevisiae* in range of 0.16 to 0.23 g/g of food waste.

Preliminary experiment of electricity generation by fuel cell, the crude bioethanol exhibited the current density lower than commercial ethanol (data not shown). This suggested that the purity of the fuel (crude bioethanol) potentially influences on electricity generation. It could be assumed that some impurities containing in crude bioethanol was coated on the surface area of working cell resulting in hindering the electrochemical reaction of bioethanol and catalyst. In addition, the current density from produced bioethanol can be varied depending on the type of catalysts on electrode fuel cell and temperature of the system (Devrim and Arıca, 2019). For improvement of the electricity generation efficiency, the contaminated substances contained in crude bioethanol need to be investigated.

CONCLUSION

The semi-solid state fungal spore (*A. oryzae*) pretreatment of food waste rich in carbohydrate (48.11%, w/w) was an alternative method for fermentable sugar production. The non-sterilized food waste hydrolysate was appropriated for using as feedstock in the bioethanol fermentation by *S. cerevisiae*. The maximum bioethanol concentration and yield of 4.23% (v/v) and 0.17 g/g food waste, respectively, were obtained at 120 h of the fermentation. Crude bioethanol is a promising biofuel for fuel cell prototype which can be generated the electric current density of 0.48 $mA.cm^{-2}$ at 0.57 V. The process presented in this study could be an environmentally friendly and low-cost process for food waste treatment and bioenergy production.

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