

# Sodium Hydroxide-Steam Explosion Treated Oil Palm Empty Fruit Bunch: Ethanol Production and Co-Fermentation with Cane Molasses

Trinset Weeraphan,<sup>a</sup> Vasana Tolieng,<sup>b</sup> Vichien Kitpreechavanich,<sup>c</sup>  
Somboon Tanasupawat,<sup>d</sup> and Ancharida Akaracharanya<sup>a,\*</sup>

Oil palm empty fruit bunch (OPEFB) was pretreated by NaOH-steam explosion and then fermented to ethanol by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) processes using *Kluyveromyces marxianus* G2-16-1 at 40 °C. The maximum ethanol production by the SHF and SSF processes was 8.09 g/L (22.21 g/L reducing sugar, 0.08 g/g OPEFB) and 13.658 g/L (0.136 g/g OPEFB), respectively, at 48 h. The OPEFB hydrolysate mixed with molasses to 22% (w/v, total sugar) gave an ethanol yield of 61.60 g/L (0.38 g/g total sugar) at 72 h, while molasses alone gave 53.89 g/L (0.34 g/g total sugar). The OPEFB slurry (OPEFBS; OPEFB hydrolysate containing the solid residue of pretreated OPEFB) gave a maximum ethanol yield of 68.77 g/L (0.44 g/g total sugar) when it was mixed with molasses. Scanning electron micrographs of the solid OPEFB residue in the OPEFBS showed yeast cells adsorbed to the OPEFB fibers. The results indicated that ethanol production from molasses mixed with OPEFB hydrolysate was equal to the cumulative sum of ethanol production from each raw material, and the solid OPEFB residue in the OPEFBS increased the ethanol production in the co-fermentation of molasses and OPEFB hydrolysate.

*Keywords:* Oil palm empty fruit bunch; Steam explosion; Ethanol; Co-fermentation; Cane molasses

*Contact information:* a: Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand; b: Institute of Biotechnology and Genetic Engineering, Chulalongkorn University, Bangkok 10330, Thailand; c: Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand; d: Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Science, Chulalongkorn University, Bangkok 10330, Thailand;

\* Corresponding author: [sanchari@chula.ac.th](mailto:sanchari@chula.ac.th)

## INTRODUCTION

Oil palm empty fruit bunch (OPEFB), a lignocellulosic waste of the palm oil industry, is the oil palm fruit bunch after its fruits are removed by steam treatment. More than a million tons of OPEFB are generated annually in Thailand (DIT 2014). OPEFB is typically composed of 62.9% cellulose (w/w), 28% hemicellulose (w/w), and 18.5% acid-insoluble lignin (w/w) (Law *et al.* 2007). OPEFB is an interesting source of fermentable sugar for bio-ethanol production due to its high cellulose content, and it is available year-round as a non-food waste product. Therefore, a high efficiency pretreatment method for OPEFB is important to maximize its subsequent enzymatic saccharification to ethanolic fermentable sugars. Lignin encases cellulose and hemicellulose in a structure that protects them from enzyme digestion (Ariffin *et al.* 2008). The lignin content of a lignocellulosic biomass has the highest impact on its enzymatic digestion, as lignin reduces enzyme efficiency (Choi *et al.* 2013).

Alkaline pretreatment by using sodium hydroxide (NaOH) is one of the most effective pretreatment methods for lignocellulosic biomass because it directly reduces the lignin content. It breaks cross-linkage between lignin and xylan, resulting in an increase of porosity in the biomass (Silverstein *et al.* 2007), and it causes the lignocellulosic structure to swell, which increases an internal surface of the biomass and decreases the polymerization degree and crystallinity of cellulose (Sun and Cheng 2002). Although NaOH pretreatment takes much more time than acid pretreatment, the NaOH doesn't degrade the desired fermentable sugar (Mosier *et al.* 2005). Moreover, the NaOH-dissolved lignin could be recovered by acid precipitation for using as value substrate in other applications (Ramos 2003).

Steam pretreatment is one of promising pretreatment methods for OPEFB. The tightly packed structure of OPEFB changes to a loosened structure after treatment with steam at either 121 °C and 15 psi or 240 °C and 40 psi, where both the lignin and hemicellulose contents are removed. In addition, higher temperature removes greater amounts of lignin and hemicellulose (Ariffin *et al.* 2008). Likewise, steam treatment at 180 °C or above removes silica bodies from the OPEFB structure, increasing enzyme accessibility to the internal layers of OPEFB (Bahrin *et al.* 2012). A combination of NaOH and steam pretreatment has an even greater effect (Ariffin *et al.* 2008); lignin is effectively removed from OPEFB treated with NaOH and steam at 160 °C (Choi *et al.* 2013). Subsequent fermentation to ethanol of the pretreated OPEFB by the simultaneous saccharification and fermentation (SSF) process yields ethanol at 29.4 g/L or 0.18 g/g OPEFB. The steam explosion was also reported to be an effective pretreatment method for other lignocellulosic biomass. Steam explosion pretreated olive tree pruning (230 °C, 5 min) and vineyard pruning residues (210 °C, 10 min) gave maximum ethanol 0.072 and 0.089 g ethanol/g by the SSF process (Cara *et al.* 2008) and the separate hydrolysis and fermentation (SHF) process (Buratti *et al.* 2014), respectively. In this study, OPEFB was pretreated with NaOH followed by steam explosion. Ethanol was fermented by separate hydrolysis and fermentation (SHF) and SSF processes using *Kluyveromyces marxianus* G2-16-1, a thermotolerant yeast strain that hydrolyzes cellobiose, a feed-back inhibitor of cellulose hydrolysis, to glucose. Due to the low sugar content of the OPEFB hydrolysate, the pretreated OPEFB hydrolysate was co-fermented with molasses for an economic optimization, and ethanol production was investigated after adding the solid fiber residue after pretreatment of OPEFB to the molasses/OPEFB hydrolysate mixture.

## EXPERIMENTAL

### Materials

#### *Source of OPEFB*

The OPEFB was collected from the Thai Tallow and Oil Co. Ltd., Surajthani Province, Thailand. It contained 47.9% (w/w) cellulose, 16.8% (w/w) hemicellulose, and 18.3% (w/w) acid-insoluble lignin. The OPEFB was shredded, hammer-milled, sieved to yield fibers of 0.25 to < 2 mm, 2 to < 10 mm, or 10 mm in length, and stored at 4 °C.

#### *Microorganism*

*Kluyveromyces marxianus* G2-16-1 is a cellobiase-producing yeast that was isolated at 40 °C from a sugar factory in Thailand. A single colony of *K. marxianus* G2-

16-1 grown on yeast extract peptone dextrose (YPD) medium (10% (w/v)) glucose, 0.3% (w/v) peptone, 0.3% (w/v) yeast extract, and 2% (w/v) agar, pH 5.0) at 40 °C for 24 h was inoculated into 50 mL of YPD broth and incubated at 40 °C and 200 rpm for 24 h. The culture was centrifuged, and the precipitated cells were used as the inoculum.

## Methods

### *Optimal conditions for the steam explosion pretreatment of OPEFB*

OPEFB was soaked in 0.5 M NaOH at 10% (w/v) for 16 h and then recovered via a stainless steel sieve. The NaOH-treated OPEFB was then pretreated by the steam explosion method using a high-pressure reactor (Parr Instrument Company, model 4523, USA) at 3% (w/v) substrate loading and 200 °C for 2 min. The pretreated OPEFB was separated from the pretreatment hydrolysate by filtration, washed with distilled water until the pH reached 7.0, and then hydrolyzed by cellulase (Accellerase™ 1500; Genencor, Finland) at 298 carboxymethyl cellulose (CMC) units (U)/g and 77.44 p-nitrophenyl-glucoside (pNG) U/g OPEFB dry weight (DW). The enzymatic hydrolysis was performed by suspending the pretreated OPEFB at 10% (w/v) DW in 100 mM sodium citrate buffer pH 6.0 and incubating at 50 °C for 6 h. The amount of reducing sugar liberated into the enzymatic hydrolysate was analyzed by the Somogyi-Nelson method (Somogyi 1952). The pretreatment condition was optimized by sequential univariation of the OPEFB fiber length (0.25 to <2 mm, 2 to <10 mm, and 10 mm), substrate loading level (2, 3, and 4% (w/v) final), NaOH concentration (0 to 2.5 M), pretreatment time (2, 5, and 10 min), and pretreatment temperature (160, 200, and 240 °C). In each case, the condition that produced the highest reducing sugar was used in subsequent experiments.

### *Optimal conditions for enzymatic hydrolysis of OPEFB*

Cellulase hydrolysis of the pretreated OPEFB was optimized by adjustment of the pH (4 to 6) and enzyme dosage (298 to 1192 CMC U/g OPEFB). The condition that produced the highest reducing sugar was used in the subsequent experiments.

### *Ethanol production from OPEFB by the SHF process*

Pretreated OPEFB at 10% (w/v) in 50 mL of 100 mM sodium-citrate buffer (pH 4.5) was hydrolyzed by the optimized dose of Accellerase™ 1500 at 50 °C for 6 h. Next, the hydrolysate was separated from the solid OPEFB residue by centrifugation, supplemented with 0.6% (w/v) yeast extract and 0.9% (w/v) peptone, and then sterilized by autoclaving (121 °C, 15 lb/in<sup>2</sup> for 3 min). The *K. marxianus* G2-16-1 inoculum was added to 10<sup>8</sup> cells/mL, and the culture was incubated at 40 °C and 130 rpm under an oxygen-limited condition for 72 h. After centrifugation, the supernatant was analyzed for ethanol content by gas chromatography (GC) as reported by Jutakanoke *et al.* (2012) and for the residual reducing sugar concentration. The oxygen-limited condition was performed by closing the airtight screw cap of the 100-mL fermentation (Duran) bottle.

### *Ethanol production from OPEFB by the SSF process*

The pretreated OPEFB suspension at 10% (w/v) in 50 mL of 100 mM sodium citrate buffer (pH 5.0) was supplemented with 0.6% (w/v) yeast extract and 0.9% (w/v) peptone and then sterilized by autoclaving (121 °C, 15 lb/in<sup>2</sup>, 3 min). Accellerase™ 1500 at the optimized dose was added along with the *K. marxianus* G2-16-1 inoculum (to 10<sup>8</sup> cells/mL final concentration) and incubated at 40 °C and 130 rpm with limited oxygen for

72 h. After incubation, the culture was centrifuged (4 °C, 8000 rpm, 15 min), and the supernatant was analyzed for ethanol and residual reducing sugars.

#### *Co-fermentation of cane molasses and OPEFB hydrolysate to ethanol*

Cane molasses diluted to 22% (w/v) total sugar in 100 mM sodium-citrate buffer (pH 4.5) was supplemented with 0.2% (w/v) (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.6% (w/v) yeast extract, and 0.9% (w/v) peptone. It was fermented to ethanol by *K. marxianus* G2-16-1 (10<sup>8</sup> cells/mL) at 40 °C, 130 rpm, and limited oxygen for 96 h. The supernatant obtained after centrifugation was analyzed for the ethanol and residual sugars.

Co-fermentation of cane molasses and OPEFB hydrolysate was performed by mixing the OPEFB hydrolysate or OPEFB slurry (OPEFBS; hydrolysate containing the solid residue of pretreated OPEFB) with the diluted molasses. Ethanol fermentation was performed by the same procedure as above, and the total sugar concentration was analyzed by the phenol sulfuric method (Dubois *et al.* 1956).

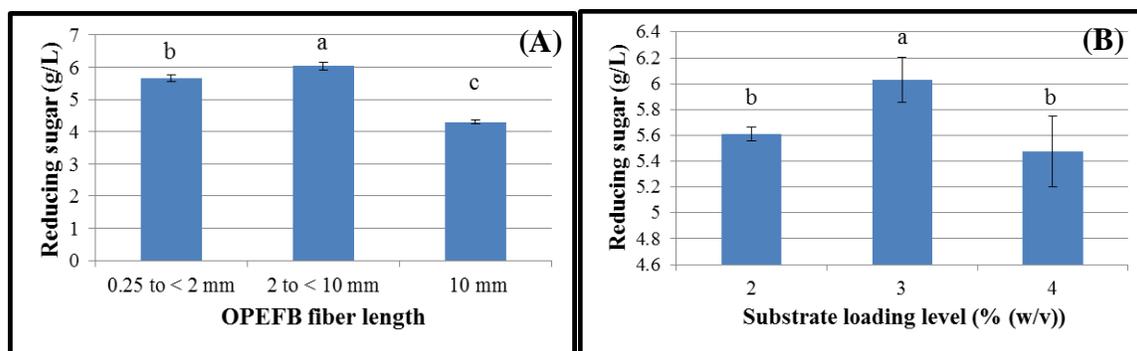
#### *Analytical procedures*

The cellulose and hemicellulose contents were analyzed by the TAPPI T203 cm-99 method (1999), while the lignin contents of OPEFB were analyzed by TAPPI T222 om-02 (2002).

## RESULTS AND DISCUSSION

### Optimal Conditions for the Steam Explosion Pretreatment of OPEFB

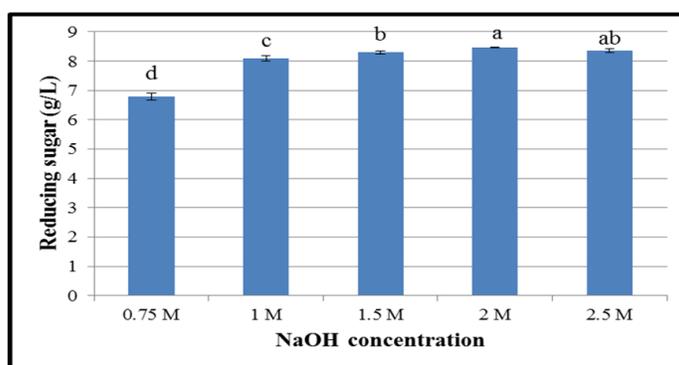
OPEFB fibers of different lengths (0.25 to < 2 mm, 2 to < 10 mm, and 10 mm) were soaked in 0.5 M NaOH at 10% (w/v, DW) for 16 h and then subjected to steam explosion pretreatment. Then the OPEFB fibers were washed with distilled water and hydrolyzed by Accellerase™ 1500 for 6 h. The 2 mm to < 10 mm long OPEFB fibers liberated the most reducing sugar (6.031 g/L), which was dramatic more than the other fiber lengths (Fig. 1); these fibers were used thereafter. These differences reflect that the longer OPEFB fibers (10 mm) and the short (0.25 to < 2 mm) OPEFB fibers which formed clump had a lower total surface area for enzyme activity than (2 mm to < 10 mm) OPEFB fibers.



**Fig. 1.** Effect of the (A) OPEFB fiber length and (B) the substrate loading level on the amount of reducing sugar liberated by cellulase digestion. Data are shown as the means ± SD derived from 3 independent repeats. Means with different letters are significantly different ( $p < 0.05$ ). Digests were performed using OPEFB fibers of (A) the indicated size or (B) 2 to < 10 mm length (A) 3% (w/v) or (B) the indicated dose.

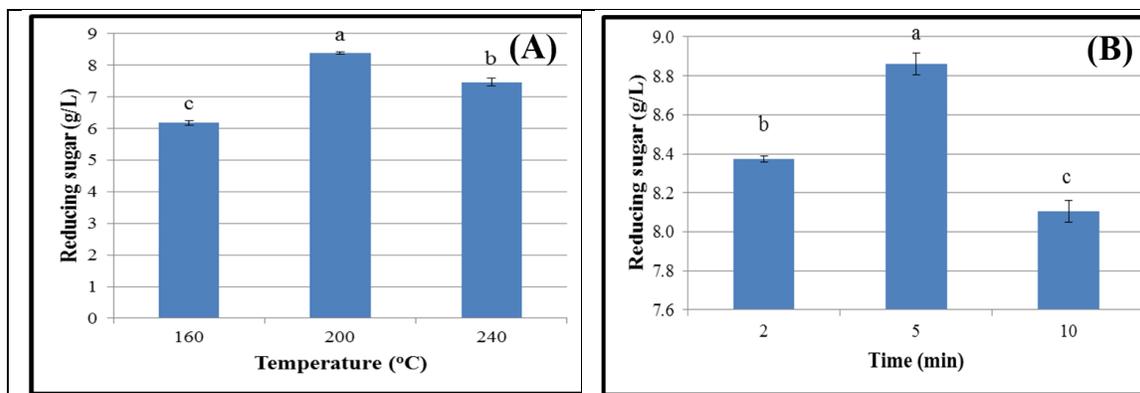
The NaOH-treated OPEFB fibers (2 to < 10 mm in length) were subjected to steam explosion pretreatment at substrate loading levels of 2, 3, or 4% (w/v) and analyzed for reducing sugar. Because the highest reducing sugar (6.03 g/L) was found at a 3% (w/v) substrate loading (Fig. 1); this condition was used thereafter. The lowest substrate loading level (2%, w/v) could generate low reducing sugar level, while the highest substrate loading level (4%, w/v) generated the lowest reducing sugar, which is likely due to reduction of heat transfer into substrate reduced efficiency of the steam treatment.

OPEFB fibers (2 to < 10 mm) were soaked in various concentrations of NaOH (0, 0.25, 0.5, 0.75, 1, 1.5, 2, and 2.5 M) and then pretreated by steam explosion at a 3% (w/v) substrate loading level. The reducing sugar liberated by cellulase hydrolysis was highest when the OPEFB was soaked in 2 M NaOH (Fig. 2), which reflects that the removal of lignin from the OPEFB fibers increased with higher concentrations of NaOH (Choi *et al.* 2013). Thus, pretreatment with 2 M NaOH was used for subsequent experiments.



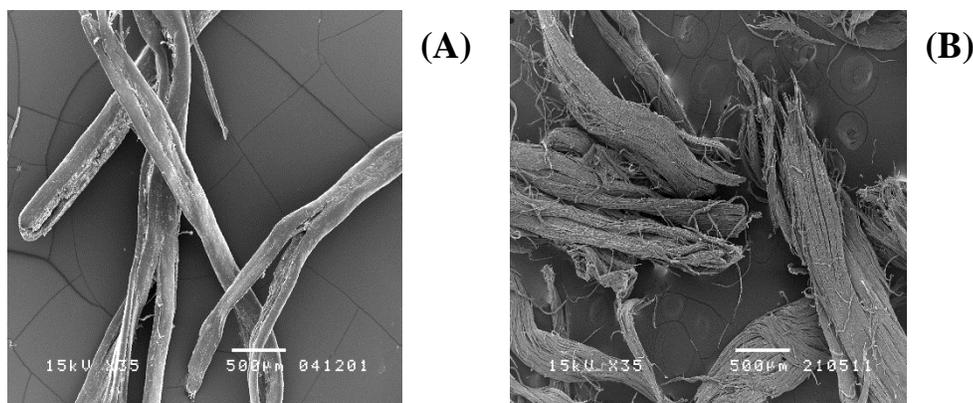
**Fig. 2.** Effect of the NaOH concentration used for soaking OPEFB fibers on the reducing sugars liberated by cellulase digestion. Data are shown as the means  $\pm$  SD derived from 3 independent repeats. Means with a different letter are significantly different ( $p < 0.05$ ).

The OPEFB fibers (2 to < 10 mm length) treated with 2 M NaOH were then treated by steam explosion with the temperature varied at 160, 200, or 240 °C. The highest reducing sugar level (8.40 g/L) after cellulase hydrolysis was obtained using the steam explosion treatment at 200 °C (Fig. 3A).



**Fig. 3.** Effect of the pretreatment (A) temperature and (B) time on the amount of reducing sugar liberated by cellulase digestion. Data are shown as the means  $\pm$  SD derived from 3 independent repeats. Means with a different letter are significantly different ( $p < 0.05$ ).

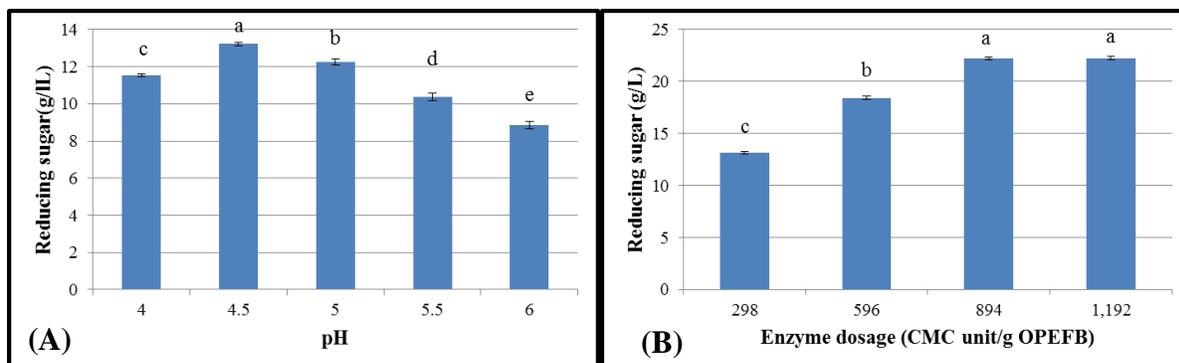
In addition, the extension of pretreatment time from 2 to 5 min increased the amount of reducing sugar to 8.86 g/L (Fig. 3B). Thus, a higher temperature and longer reaction time promoted more lignin removal (Ariffin *et al.* 2008; Choi *et al.* 2013). However, as more lignin was released into the solution (at 240 °C, 10 min), it was re-adsorbed into the cellulose/hemicellulose scaffold of the treated OPEFB fibers. This re-adsorbed lignin inhibited and unproductively adsorbed the cellulase enzyme and decreased the reducing sugar yield (Zheng *et al.* 2013). Regardless, the tightly packed OPEFB structure became more swollen and loose after pretreatment in optimized conditions (Fig. 4).



**Fig. 4.** SEM (35x magnification) of the OPEFB fibers (A) before and (B) after the NaOH-steam treatment in optimized conditions (2 M NaOH at 10% (w/v) DW for 16 h and steam-treated at 3% (w/v) at 200 °C for 5 min). Scale bars represent 500 µm. The micrographs shown are representative of at least 10 fields of view per sample and 3 independent samples.

### Optimal Condition for Enzymatic Hydrolysis of Pretreated OPEFB Fibers

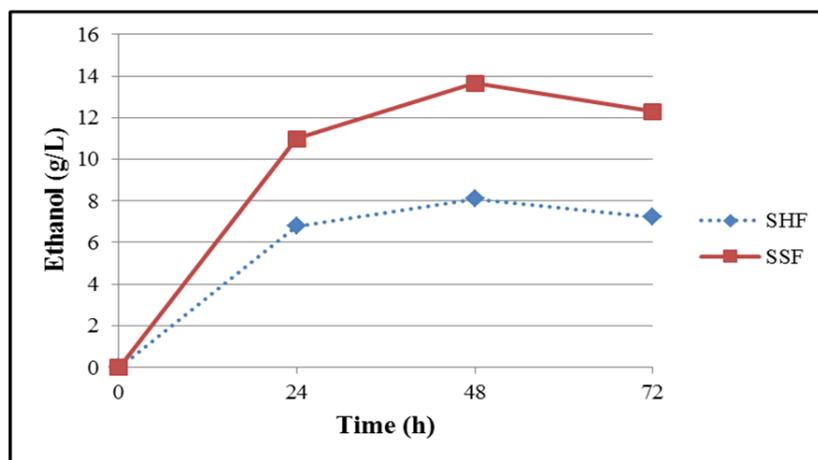
The OPEFB fibers (2 to <10 mm length) pretreated at optimized conditions were hydrolyzed by 298 CMC U/g of Accellerase™ 1500 at various pH values (4.0, 4.5, 5.0, 5.5, or 6.0). At pH 4.5, the pretreated OPEFB liberated the highest amount of reducing sugar (13.23 g/L), which decreased with increased or decreased pH (Fig. 5A). Increasing the amount of Accellerase™ enzyme from 298 to 894 CMC U/g (232.4 PNG U/g) DW at pH 4.5 increased the reducing sugar yield to 22.21 g/L (0.22 g/g OPEFB) (Fig. 5B). Increasing the amount of enzyme above 894 CMC U/g DW had no noticeable effect on the reducing sugar, suggesting that the substrate was saturated at that enzyme dose.



**Fig. 5.** Effect of the (A) pH and (B) amount of cellulase enzyme on the reducing sugar yield. Data are shown as the means  $\pm$  SD derived from 3 independent repeats. Means with a different letter are significantly different ( $p < 0.05$ ). Digests were performed with (A) 298 CMC U/g enzyme at various pH values or (B) various enzyme loadings at pH 4.5 for 6 h at 40 °C.

## Ethanol Production from OPEFB by the SHF and SSF Processes

Ethanol was produced from the NaOH- and steam-pretreated OPEFB by the SHF process; the pretreated OPEFB was hydrolyzed with the optimal dose of cellulase (894 CMC U/g). After solid residue was removed, the pretreated OPEFB hydrolysate was supplemented with yeast extract and peptone, sterilized, and inoculated with *K. marxianus* G2-16-1. The maximum ethanol production (8.09 g/L or 0.39 g ethanol/g reducing sugar) occurred after 48 h of incubation (Fig. 6). For the SSF process, the same optimized dose of Accellerase™ 1500 and *K. marxianus* G2-16-1 were added simultaneously to ferment the pretreated OPEFB. The maximum ethanol level (13.66 g/L) was also obtained after 48 h, but it was 1.7-fold higher than that obtained by SHF.



**Fig. 6.** Ethanol production from OPEFB by the SHF and SSF processes. Data are shown as the means  $\pm$  SD derived from 3 independent repeats. Fermentation was performed with 894 CMC U/g Accellerase™ 1500 at 40 °C and pH 4.5.

The optimal condition for the NaOH-steam pretreatment was soaking the 2 to < 10 mm long OPEFB fibers at 10% (w/v) in 2 M NaOH for 16 h, with steam explosion treatment at a 3% (w/v) substrate loading, and 200 °C for 5 min. These conditions resulted in the maximum yield of reducing sugars and glucose (22.21 and 15.31 g/L, respectively) during a 6-h hydrolysis with 894 CMC U/g (232.35 pNG U/g) cellulase. The maximum ethanol production amounts by *K. marxianus* G2-16-1 using the SHF and SSF processes were 8.09 and 13.66 g/L (0.08 and 0.136 g/g OPEFB), respectively, after a 48-h incubation.

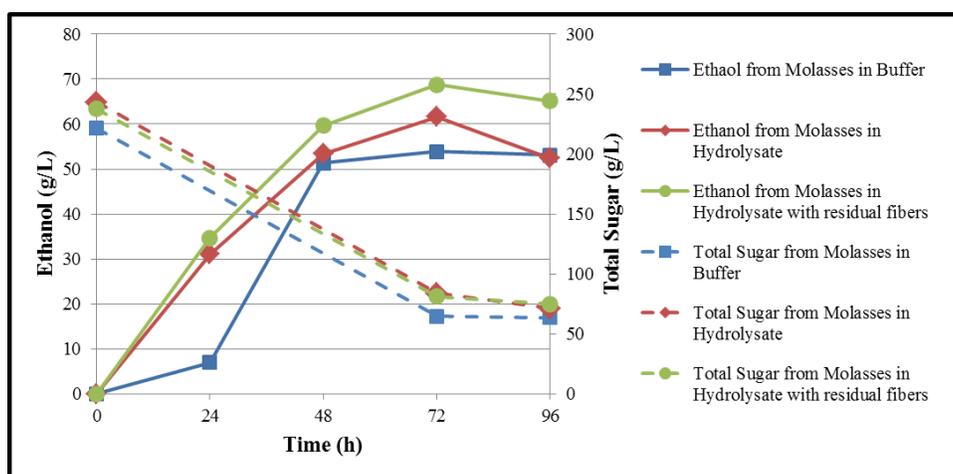
These values are lower than those previously reported for OPEFB. When OPEFB was treated with NaOH and steam treatment at 160 °C for 11 min 20 s and fermented by SSF using *Saccharomyces cerevisiae* L3262a and CTec2 cellulase (40 FPU/g glucan), 0.18 g ethanol/g OPEFB was produced after 30 h (Choi *et al.* 2013). The different enzyme and dose used, hydrolysis time, fermentation temperature, and yeast strain might account for the differences in reducing sugars, ethanol yield, and ethanol productivity.

## Co-fermentation of the Molasses/OPEFB Hydrolysate Mixture

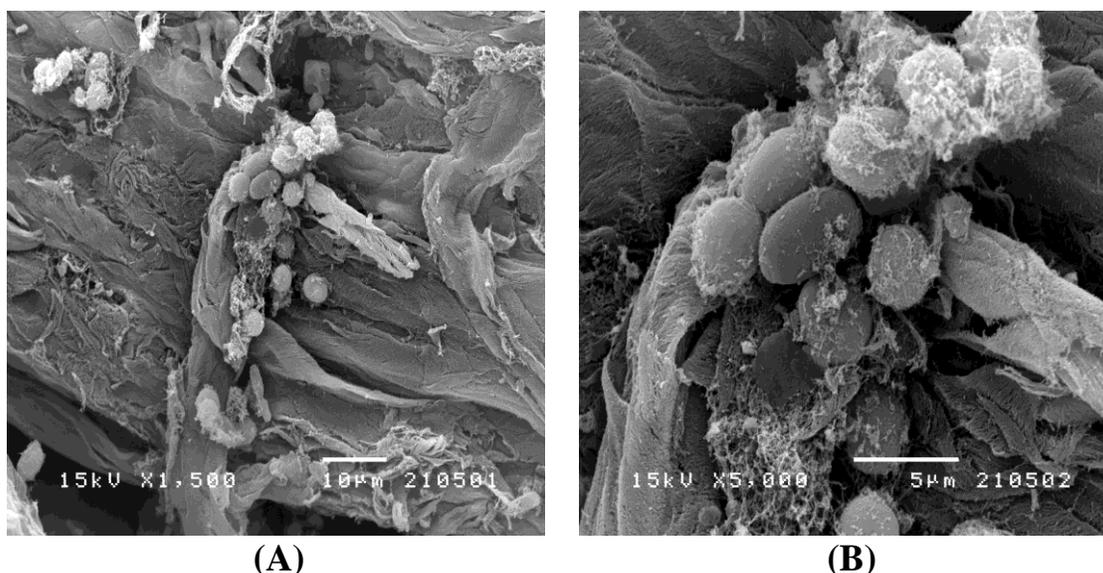
Cane molasses diluted in 100 mM sodium-citrate buffer to 22% (w/v) total sugar and supplemented with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, yeast extract and peptone was fermented to ethanol by *K. marxianus* G2-16-1, as described above. The maximum ethanol concentration obtained was 53.89 g/L (0.34 g/g total sugar) at 72 h (Fig. 7), and with the OPEFB hydrolysate, the mixture gave a maximum ethanol concentration of 61.60 g/L (0.39 g/g total sugar) at 72 h (Fig. 7). As the SHF ethanol production from the OPEFB hydrolysate alone was 8.09 g/L

(Fig. 6), the ethanol production from the molasses/OPEFB hydrolysate mixture was equal to the cumulative sum of ethanol production from the molasses and the OPEFB hydrolysate (61.98 vs. 61.6 g/L). Thus, there did not appear to be inhibitors of yeast growth or fermentation in the OPEFB hydrolysate.

The mixture of the molasses and OPEFB hydrolysate containing solid residue of treated OPEFB fibers (OPEFBS) gave a maximum ethanol concentration of 68.77 g/L (0.44 g/g total sugar) at 72 h (Fig. 7). The ethanol production was 1.13-fold higher than that of the molasses-OPEFB hydrolysate because the solid residue of treated OPEFB fibers in the OPEFBS acted as an immobilization support to protect the yeast cells from environmental stresses during fermentation. The solid residue of treated OPEFB fibers was examined after fermentation by scanning electron microscopy; the yeast cells were adsorbed onto the solid residue of OPEFB (Fig. 8).



**Fig. 7.** Ethanol fermentation of molasses alone, molasses plus OPEFB hydrolysate, and molasses plus OPEFB hydrolysate with residual solids. Data are shown as the means  $\pm$  SD derived from 3 independent repeats.



**Fig. 8.** SEM of the solid pretreated OPEFB residue after ethanol fermentation at (A) 1500x and (B) 5000x magnification. Scale bars represent (A) 10  $\mu$ m and (B) 5  $\mu$ m. The micrographs shown are representative of at least 10 fields of view per sample and 3 independent samples.

This result is consistent with previous reports where, for example, ethanol production by *Candida shehatae* was increased in the presence of palm-pressed fiber (PPF), a solid waste extracted from OPEFB through decortication, and the *C. shehatae* cells were immobilized on the PPF (Riansa-ngawong *et al.* 2012). The pretreatment of the PPF by size reduction and delignification improved its immobilization support. In addition, several agro-industrial wastes, such as wine pomace, corncobs, and sugar beet pulp, function as immobilization supports in ethanol production (Genisheva *et al.* 2011; Vucurovic and Razmovski 2012). The advantages of using cells immobilized to a natural immobilization support are the ease of operation, less adverse affects, and the natural replacement of old cells with new active fermenting ones.

## CONCLUSIONS

1. Ethanol was economically produced from NaOH-steam explosion pretreated oil palm empty fruit bunch (OPEFB) hydrolysate by co-fermentation with molasses.
2. Increases in ethanol production from the co-fermentation of OPEFB hydrolysate and molasses were achieved by including solid residue from pretreated OPEFB.

## ACKNOWLEDGMENTS

The authors thank Dr. Robert Butcher for critically reading the manuscript. This study was financially supported by the 90<sup>th</sup> Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund).

## REFERENCES CITED

- Ariffin, H., Hassan, M. A., Umikalsom, M. S., Abdullah, N., and Shirai, Y. (2008). "Effect of physical, chemical and thermal pretreatments on the enzymatic hydrolysis of oil palm empty fruit bunch (OPEFB)," *Journal of Tropical Agriculture and Food Science* 36(2), 259-268. DOI: 10.1016/j.biombioe.2011.10.040
- Bahrin, E. K., Baharuddin, A. S., Ibrahim, M. F., and Razak, M. N. A. (2012). "Physicochemical property changes and enzymatic hydrolysis enhancement of oil palm empty fruit bunches treated with superheated steam," *BioResources* 7(2), 1784-1801. DOI: 10.15376/biores.7.2.1784-1801.
- Buratti, C., Barbanera, M., and Lascaro, E. (2014). "Ethanol production from vineyard pruning residues with steam explosion pretreatment," *Environmental Progress & Sustainable Energy* 34(3), 802-809. DOI: 10.1002/ep.12043
- Cara, C., Ruiz, E., Ballesteros, M., Manzanares, P., Negro, M. J., and Castro, E. (2008). "Production of fuel ethanol from steam-explosion pretreated olive tree pruning," *Fuel* 87, 692-700. DOI: 10.1016/j.fuel.2007.05.008
- Choi, W.-I., Park, J.-Y., Lee, J.-P., Oh, Y.-K., Park, Y. C., Kim, J. S., Park, J. M., Kim, C. H., and Lee, J.-S. (2013). "Optimization of NaOH-catalyzed steam pretreatment of empty fruit bunch," *Biotechnology for Biofuels* 6:170. DOI: 10.1186/1754-6834-6-170

- Department of Internal Trade of Thailand (DIT). (2014). *Palm oil situation*, ([http://agri.dit.go.th/web\\_dit\\_sec4/home/view\\_multi.aspx?menu\\_id=576&name](http://agri.dit.go.th/web_dit_sec4/home/view_multi.aspx?menu_id=576&name))
- DuBois, M., Gilles, K., Hamilton, J., Rebers, P., and Smith, F. (1956). "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry* 28(3), 350-356. DOI: 10.1021/ac60111a017
- Genisheva, Z., Mussatto, S. I., Oliveira, J. M., and Teixeira, J. A. (2011). "Evaluating the potential of wine-making residues and corn cobs as support materials for cells immobilization for ethanol production," *Industrial Crops and Products* 34, 979-985. DOI: 10.1016/j.indcrop.2011.03.006
- Jutakanoke, R., Leepipatpiboon, N., Tolieng, V., Kitpreechavanich, V., Srinorakutara, T., and Akaracharanya, A. (2012). "Sugarcane leaves: Pretreatment and ethanol fermentation by *Saccharomyces cerevisiae*," *Biomass & Bioenergy* 39, 283-289. DOI: 10.1016/j.biombioe.2012.01.018
- Law, K. N., Wan Daud, W. R., and Ghazali, A. (2007). "Morphological and chemical nature of fiber strands of oil palm empty-fruit-bunch (OPEFB)," *BioResources* 2(3), 351-362.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., and Ladisch, M. (2005). "Features of promising technologies for pretreatment of lignocellulosic biomass," *Bioresour. Technol.* 96, 673-686. DOI: 10.1016/j.biortech.2004.06.025
- Ramos, L. P. (2003). "The chemical involved in the steam treatment of lignocellulosic material," *Química Nova* 26(6), 863-871. DOI: 10.1590/S0100-40422003000600015
- Riansa-ngawong, W., Suwansaard, M., and Prasertsan, P. (2012). "Application of palm pressed fiber as a carrier for ethanol production by *Candida shehatae* TISTR5843," *Electronic Journal of Biotechnology* (15)6. DOI: 10.2225/vol15-issue6-fulltext-1
- Silverstein, R. A., Chen, Y., Sharma-Shivappa, R. R., Boyette, M. D., and Osborne, J. (2007). "A comparison of chemical pretreatment methods for improving saccharification of cotton stalks," *Bioresource Technology* 98, 3000-3011. DOI: 10.1016/j.biortech.2006.10.022
- Somogyi, M. (1952). "Notes on sugar determination," *Journal of Biological Chemistry* 195, 19-23.
- Sun, Y., and Cheng, J. (2002). "Hydrolysis of lignocellulosic materials for ethanol production: A review," *Bioresource Technology* 83, 1-11. DOI: 10.1016/S0960-8524(01)00212-7
- TAPPI T203 cm-99. (1999). "Alpha-, beta- and gamma-cellulose in pulp" TAPPI Press, Atlanta, GA.
- TAPPI T222 om-02. (2002). "Acid-insoluble lignin in wood and pulp," TAPPI Press, Atlanta, GA.
- Vucurovic, V. M., and Razmovski, R. N. (2012). "Sugar beet pulp as a support for *Saccharomyces cerevisiae* immobilization in bioethanol production," *Industrial Crops and Products* 39, 128-134. DOI: 10.1016/j.indcrop.2012.02.002
- Zheng, Y., Zhang, S., Miao, S., Su, Z., and Wang, P. (2013). "Temperature sensitivity of cellulase adsorption on lignin and its impact on enzymatic hydrolysis of lignocellulosic biomass," *Journal of Biotechnology* 166, 135-143. DOI: org/10.1016/j.jbiotec.2013.04.018

Article submitted: Nov. 9, 2015; Peer review completed: Feb. 19, 2016; Revised version received: March 17, 2016; Accepted: March 18, 2016; Published: August 4, 2016.  
DOI: 10.15376/biores.11.3.7849-7858