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Ethanol production by co-fermentation of molasses and oil palm empty fruit bunch hydrolysate

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Abstract

Maximum ethanol production by co-fermentation of molasses (22%, w/v total sugar) and oil palm empty fruit bunch (OPEFB) hydrolysate was 61.60 g/l (0.38 g/g sugar) at 72 h, while maximum ethanol produced from the molasses was 53.89 g/l (0.34 g/g sugar). OPEFB slurry (the OPEFB hydrolysate which contained solid residue of pretreated OPEFB) gave maximum ethanol 68.77 g/l (0.44 g/g sugar) when it was co-fermented with the molasses. After fermentation, scanning electron micrograph of pretreated OPEFB in the OPEFB slurry revealed yeast cells adsorped to the pretreated OPEFB. The results indicated that ethanol production by co-fermentation of molasses and OPEFB hydrolysate was cumulative sum of ethanol produced from each raw material, and pretreated OPEFB suspended in OPEFB hydrolysate increased ethanol production in the co-fermentation of molasses and OPEFB hydrolysate.

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

1. Introduction

Oil palm empty fruit bunch (OPEFB), a lignocellulosic waste of palm oil industry, is an oil palm fruit bunch which its seeds are removed after steam treatment. There are more than million tons of OPEFB generated annually in Thailand [1]. It is interesting source of fermentable sugar for bio-ethanol production because it has high cellulose content [2] and available all year-round [3-5]. In this study, OPEFB was pretreated with NaOH followed by steam explosion. The pretreated OPEFB was subsequently saccharified by commercial cellulase and fermented to ethanol by *Kluyveromyces marxianus* G2-16-1. The *K. marxianus* G2-16-1 is a thermotolerant yeast which hydrolyzes cellobiose, a feed-back inhibitor of cellulose hydrolysis, to glucose [6]. The OPEFB hydrolysate obtained with and without solid residue of pretreated OPEFB were co-fermented with molasses for an economic optimization. Effect of the solid residue of pretreated OPEFB in the OPEFB hydrolysate on ethanol production was investigated.

2. Materials and methods

2.1 Oil palm empty fruit bunch (OPEFB)

OPEFB was collected from 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 for 2-10 mm fiber length, then kept at 4°C until used.

2.2 Kluyveromyces marxianus G2-16-1

Single colony of the *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, 200 rpm for 24 h. Cells obtained from centrifugation of the culture was used as inoculum.

2.3 Preparation of OPEFB hydrolysate

The OPEFB (2–10 mm length) was soaked in 2 M NaOH at 10% (w/v) for 16 h, and filtered. The NaOH-treated OPEFB was further pretreated by the steam explosion method using a high pressure reactor (Parr Instrument Company, model 4523, USA) at a 3% (w/v) substrate loading, 200 °C for 5 min. The pretreated OPEFB was separated from the pretreatment hydrolysate by filtration,

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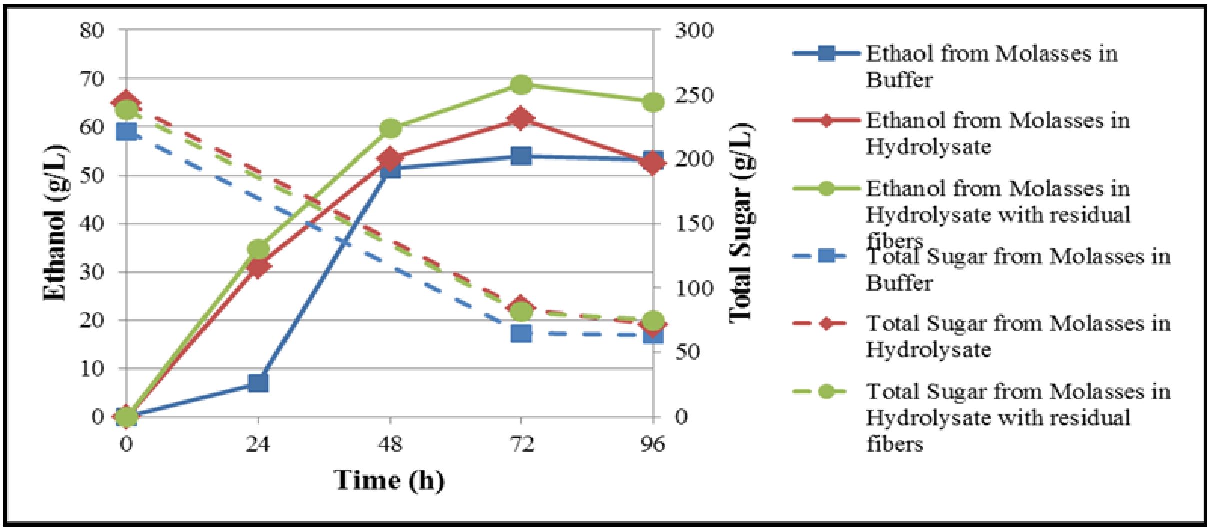


Figure 1 The ethanolic fermentation of molasses alone, molasses in OPEFB hydrolysate and molasses in OPEFB hydrolysate with residual solid.

washed with distilled water until the pH reached 7.0 and then hydrolyzed by cellulase (Accellerase^{TM} 1500; Genencor, Finland), at 894 carboxymethyl cellulose (CMC) units (U)/g and 232 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 4.5 and incubating at 50 °C for 6 h. The OPEFB hydrolysate was then separated from the solid OPEFB residue by centrifugation.

2.4 Co-fermentation of cane molasses and OPEFB hydrolysate to ethanol

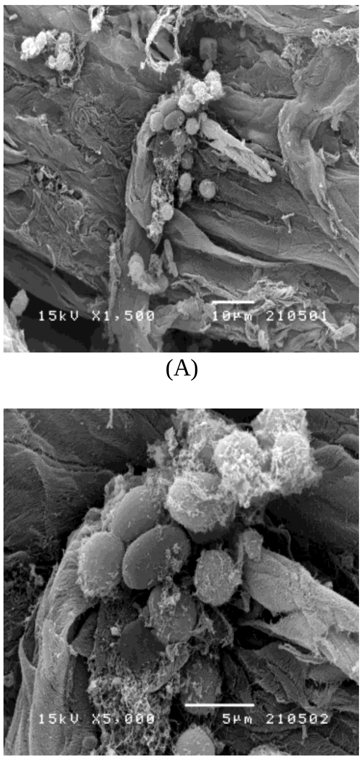
Cane molasses diluted to 22% (w/v) total sugar in 100 mM sodium-citrate buffer pH 4.5 was supplemented with 0.2% (w/v) $(NH_4)_2SO_4$, 0.6% (w/v) yeast extract, 0.9% (w/v) peptone; steriled and then fermented to ethanol by *K. marxianus* G2-16-1 (final 10^8 cells/mL) at 40 °C, 130 rpm under an oxygen limited condition for 96 h. The supernatant obtained after centrifugation was then analyzed for the ethanol by gas chromatography [7] and residual total sugar concentration by phenol sulfuric method [8]. The oxygen limited condition was performed by closing the airtight screw cap of the 100-mL fermentation (Duran) bottle tightly.

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.

3. Results and discussion

As shown in Figure 1, maximum ethanol concentration obtained from diluted molasses was 53.89 g/L (0.34 g/g sugar) at 72 h. Mixture of molasses and OPEFB hydrolysate which contained 22.21 g/l reducing sugar (data not shown) or molasses / OPEFB hydrolysate mixture gave a maximum ethanol concentration of 61.60 g/L (0.39 g/g sugar), also at 72 h. Since the ethanol production from the OPEFB hydrolysate alone was 8.09 g/L (data not shown), then the ethanol production from the molasses/OPEFB hydrolysate mixture was the cumulative sum of ethanol production from the molasses and the OPEFB hydrolysate (61.98 (53.89 + 8.09) *vs*. 61.6 g/L). The addition of the OPEFBS, as in the OPEFB hydrolysate plus residual fibers to the molasses, gave a maximum ethanol concentration of 68.77 g/L (0.44 g/g sugar) at 72 h. The ethanol produced was 1.13-fold higher than from 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 the fermentation [9].

When the solid residue of treated OPEFB fibers from the OPEFBS was examined by scanning electron microscopy after the fermentation, yeast cells were clearly observed to be adsorbed onto the solid residue of OPEFB (Figure 2).



(A)

(B)

Figure 2 Scanning electron micrographs of the solid pretreated OPEFB residue after ethanol fermentation at (A)

1,500 x and (B) 5,000 x magnification. Scale bars represent (A) 10 µm and (B) 5 µm.

This is consistent with previous reports, for example, ethanol production by *Candida shehatae* was increased in the presence of palm pressed fiber (PPF), a solid waste extracted from OPEFB through decortation, where the *C. shehatae* cells became immobilized on the PPF [10]. The pretreatment of the PPF by size reduction and delignification improved its immobilization support property. Moreover, other lignocellulosic waste such as corncobs and sugar beet pulp also has been reported as immobilization supporters [11].Therefore, The advantage of using cells immobilized to a natural immobilization support in ethanol fermentation are the ease of operation, less adverse effects to cells and the natural replacement of old cells with new active fermenting ones.

4. Conclusion

Ethanol production from molasses/OPEFB hydrolysate mixture was a cumulative sum of ethanol produced from each substrate. Including of solid residue of pretreated OPEFB in OPEFB hydrolysate increased the ethanol production from molasses/OPEFB hydrolysate mixture.

5. Acknowledgement

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