Utilization of Oil Palm Empty Fruit Bunch Hydrolysate for Ethanol Production by Baker’s Yeast and Loog-Pang

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Abstract

Oil palm empty fruit bunch is a potential source of glucose which can serve as a promising raw material for production of ethanol. Ethanol production was investigated by using separation hydrolysis and fermentation process (SHF), in which yeast from loog-pang (a Thai traditional fermentation starter culture) and baker’s yeast were used as biocatalyst. After alkali pretreatment with 15\% w/v NaOH for 40 min at 130°C, Cellulose was analyzed to be 61.94\% (on dry weight basis). Glucose was 33.45 g/L after cellulose was hydrolyzed by 7\% v/v H\textsubscript{2}SO\textsubscript{4} at 119 °C for 110 min. The resulting aqueous phase rich in glucose was fermented by yeast from baker’s yeast to produce ethanol with the concentration of 8.49 g/L with is 56 times higher than loog-pang as yeast. Therefore, implementation of oil palm empty fruit bunch for the development of alternative source of energy could be an effective approach and key enable to the billion-ton biofuel vision.

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1. Introduction

Ethanol is a renewable energy resource producing through fermentation of simple sugars by yeasts. Ethanol is widely used as a partial gasoline replacement in US and other parts of the world such as Canada. It can also be used in a variety of cooking, heating, and lighting appliances. Fuel ethanol that is produced from corn has been used in gasohol or oxygenated fuels since 1980. Ethanol that is blended directly with gasoline in a mix of 10\% ethanol and 90\% gasoline is called gasohol. The benefit of develop

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biomass-to-ethanol technology are: increased national energy security, reduction in greenhouse gas emissions, use of renewable resources, foundation of a carbohydrate-based chemical process industry, macroeconomic benefits for rural communities and society at large. The present major biomaterials in Thailand are sugarcane molasses and starch from cassava. These are food resources. Therefore, ethanol production from non-food resources such as oil palm empty fruit bunch might be one of new alternative sources. Oil palm empty fruit bunch are agricultural residues and lignocellulosic material (OPEFB) which can used for production of ethanol. Lignocellulosic material is a less expensive source of carbon [1].

Ethanol production from lignocellulosic materials comprises the following steps: degradation of the lignocellulosic structure to a fermentable substrate followed by fermentation and distillation of the fermentation broth to obtain 95% ethanol. The ethanol yield in the process is an important parameter with regard to economy both because the cost of the raw material constitutes a major part of the total production cost and also because the processing costs are typically associated with the amount of material passing through the process and not the amount of product made.

Pretreatment is an important tool for practical cellulose conversion processes, and is the subject of this article. Pretreatment is required to alter the structure of cellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars.

The process step in which ethanol is actually produced. The main problems encountered in the efficient conversion of the lignocellulosic hydrolysates to ethanol are twofold. Firstly after pretreatment, the hydrolysate contains not only fermentable sugars, but also a broad range of compounds having inhibitory effects on the microorganisms used for fermentation. The composition of these compounds depends upon the type of lignocellulosic material used and the chemistry and nature of the pretreatment process. Secondly, the hemicellulose hydrolysates contain not only hexoses but also pentoses. Hexoses can easily be fermented by *Saccharomyces cerevisiae*. Baker's yeast is the common name for the strains of yeast commonly used as a leavening agent in baking bread and bakery products, where it converts the fermentable sugars present in the dough into carbon dioxide and ethanol. Baker's yeast is of the species *Saccharomyces cerevisiae*, which is the same species commonly used in alcoholic fermentation. Loog-pang, commonly is known as Chinese yeast cake to the Western people, is a Thai term for dry form of fermentation starter for production of traditional fermented products from starchy raw materials. These starters are apparently mixed cultures of molds, yeasts and bacteria grown on rice or other cereals. In certain localities native herbs were added. *Saccharomycopsis fibuligera* is a common yeast species found in many starter cakes including loog-pang. While *Pichia anomala* is presented in *bubod*, loog-pang and *murcha*. *Candida* spp. are the common yeast in *ragi*. *Saccharomyces* spp. has been found only in some samples of *bubod*, loog-pang and *ragi*.

The target of the present investigation was to study feasibility of ethanol production by baker’s yeast and loog-pang and to determine ethanol with fermentation of different yeast in order to reduce investment cost for ethanol production from lignocellulosic biomass using yeast from folk wisdom, loog-pang.

2. Materials and methods

2.1. Oil palm empty fruit bunch

Oil palm empty fruit bunch (OPEFB) fiber was collected from local palm oil, sun-dried and ground to a particle size <1 mm [2].

2.2. Alkali pretreatment

OPEFB was soaked in NaOH (15% w/v) with a OPEFB:NaOH ratio of 1:10 (w/v). The alkali treated OPEFB was heated at 130°C for 40 min; filtered and neutralized by extensive washing [3]. The
neutralized sample of OPEFB was dried in oven at 60-70°C for 24 h. The dried product of OPEFB was then determined by the AOAC method [4].

2.3. Acid hydrolysis

Acid hydrolysis of OPEFB biomass was carried out in 125 mL Erlenmeyer flasks by 7% v/v of H₂SO₄ at 119 °C for 110 min [5]. The filtrate was then analyzed for determination of glucose. Glucose in the acid hydrolysate was analyzed by High performance Liquid Chromatography (HPLC, 1100, Hewlett Packard, Germany) using Luna NH₂ column size 250x4.6 mm. and RI detector. Aqueous acetonitrile (75%) was used as mobile phase with flow rate of 0.5 mL/min and oven temperature was maintained at 35 °C.

2.4. Fermentation

The ethanol production from the hydrolysate obtained after acid hydrolysis was investigated. The fermentation was carried out in 125 mL Erlenmeyer flasks containing 15 ml of fermentation media incubated at 30°C in a rotary shaker at 150 rpm for 5 days. Fermentation was inoculated with different yeast (loog-pang and baker’s yeast) for ethanol production at different levels varying from 1-2 g/flask. Aliquots were withdrawn every 24 h for the analysis of sugar and ethanol determination. Ethanol was estimated by gas chromatography (GC) with an HP-FFAP polyethylene glycol column (30 m X 0.25 mm) at 120°C, flame ionization detectors (FID) at 250°C and injector at 230°C. The carrier gas was helium; flow rate of 2 mL/min.

3. Result and discussion

3.1 Composition of OPEFB

The composition of OPEFB was analyzed according to the AOAC method. The results of an analysis were based on a 105 °C, dry weight of the composition of OPEFB. The composition of OPEFB was determined to be 52.6% cellulose, 37.3% hemicellulose, 9.5% lignin and 0.6% other. The results agree well with the recent analysis data reported by Umikalsom et al. [2]. The first part of this research focused on a study of Alkali pretreatment. Cellulose in OPEFB was 61.94 %, which was increased after pretreated by sodium hydroxide solution compared with cellulose composed in OPEFB which were not pretreated by chemical. This indicated that sodium hydroxide solution can be dissolved the compositions of oil palm empty fruit bunch. However, during alkali pretreatment cellulose and hemicellulose dissolve in liquid phase, the finding implied that the saponification reaction was inferred to occur during pretreatment. Such a reaction damaged the ester bond linkage between lignin and carbohydrate and released cellulose from the encapsulation of lignin, making more cellulose exposed [6]. Lignin slightly decreased resulting from sodium hydroxide solution which is not able to remove lignin directly, but through the removal of soluble hemicelluloses which is because of lignocellulose material structure (lignin and hemicellulose bonding).

3.2 Sugar formation

The cellulose was converted to reducing sugar by hydrolysis. The hydrolysate contained glucose of 33.45 g/L. The results from recent analysis data reported by Rahman et al. [7] show hydrolysis of untreated OPEFB under optimized conditions and the best results of glucose obtained were 7.61 g/L when hydrolyzing acid used was 6% under operating temperature of 130 °C and time period of reaction was 90 min. From this investigation indicated that alkali pretreated OPEFB gave higher amount of glucose than untreated OPEFB.
3.3 Ethanol production

The experimental results in Fig. 1 shows clearly that ethanol obtained by baker’s yeast is higher than by loog-pang about 56 times, Ethanol obtained by baker’s yeast is in the range of 6.23 to 8.49 g/L, while Ethanol obtained by loog-pang in the range of 0.05 to 0.21 g/L because 1.5 g of loog-pang has several types of yeast for the fermentation of ethanol, as a result in the minimum ethanol. In loog-pang, there are both yeast and mold, Genus of mold such as *Mucor sp.*, *Amylomyces rouxii* and *Rhizopus oryae*, there are fungus that are more helpful than harmful. Fungus officiate to produce Amylase enzyme from degrade starch to sugar. Yeast such as *Saccharomyces sp.*, It was used to ferment sugar into alcohol. However, it is rare yeast in loog-pang.

![Fig.1. Ethanol production from baker’s yeast and loog-pang](image)

The fermentation by baker’s yeast has higher total ethanol than Loog-Pang. Therefore, the effect of fermentation time and yeast loading were investigated by adding baker’s yeast 1.0, 1.5 and 2 g for fermentation. As shown in Fig. 2, varying the amount of yeast for fermentation from 1.0, 1.5 to 2.0 g, the results show that 1.5 g yeast can produce the highest ethanol of 8.4 g/L. And the fermentation time of 5 days gave the highest amount of ethanol for all case. However, it was slightly different compared to the 3 days fermentation. Thus, the 3 days fermentation is a suitable time according to economics aspects. Initial yeast loading, fermentation time, pH, temperature, initial fermentable sugar and byproduct in hydrolysate occurred during acid hydrolysis, all factors effect to ethanol production.

The effect fermentation time to ethanol production as shown in Fig. 2, ethanol is gradually increased during the 1st to 3rd day due to the fermentable sugar is sufficient for the growth of yeast to digest sugars into ethanol. When the fermentation time increased, after 3 days, all fermentable sugar was exhausted to the growth of yeast. Ethanol after 3 days was relatively stabled or slightly decreased. In theory, 100 g of glucose produce ethanol 51.4 g. Ethanol 10.28 grams should be produced from initial glucose 20 g in this investigation causing that ethanol is less than the theoretical value because of the inhibitor occurring during acid hydrolysis. Aziz et al. [8] stated that during the hydrolysis reaction. There are inhibitors such as acetic acid, phenolic compounds, furfural and HMF (5-Hydroxymethyl Furfural) reducing rate of ethanol production in the fermentation process. Initial yeast of 50 percent have survived and other death, these are destroyed by inhibitors.
4. Conclusion

Ethanol productions from OPEFB using separation hydrolysis and fermentation process (SHF) were investigated. After alkali pretreatment with 15% w/v NaOH for 40 min, cellulose was hydrolyzed by 7% v/v H₂SO₄ at 119 °C for 110 min. Optimum fermentation condition was for 3 days fermentation by 1.5 g of baker’s yeast. Ethanol production was obtained 8.49 g/L. In hydrolysis process can be use enzyme to convert cellulose into sugar for higher ethanol yield and environmentally friendly. Loog-pang needs to isolate in order to using suitable isolated yeast to improve yield of ethanol and save cost of yeast because loog-pang is cheaper than baker’s yeast or specific yeast for ethanol production.

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References


