Ethanol production from agricultural residues using Loog-Pang Kao Mhark

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Abstract – Ethanol production from the weak skin of jelly seeds of the Palmyra Palm mixed with the orange peel, agricultural residues, was studied under non-sterilized fermentation by using organisms from Loog-Pang Kao Mhark. The significant parameters that were firstly investigated were the material mixture (the weak skin to orange peel) ratios in the range of 5:1 to 5:4. Then pretreatment and fermentation processes were carried out. For pretreatment, boiling, at boiling temperatures in the range of 75-90 °C, boiling time between 15-60 min and water amounts of 20-40 ml were done to find an optimum condition. It was the mixture ratio of 5:3 at 80 °C for 45 min with 40 ml water amount. It provided 6.01 g/L reducing sugar content. Important parameter for hydrolysis following by fermentation under Loog-Pang Kao Mhark amounts of 1-10%wt for 1-10 days with an initial pH 5 at ambient air temperature were studied. The optimum condition was 3%wt Loog-Pang Kao Mhark for 9 days at a room temperature. It achieved 3.39%v ethanol product that could be reached to 95%v using a rotary vacuum evaporator.

Keyword: Palmyra Palm, Ethanol, Loog-Pang Kao Mhark
1. Introduction

The increase in petroleum prices and decrease in worldwide petroleum amount are significance that force on many countries take an interest in alterative energy. Ethanol is bio-fuel that can be used instead of petroleum fuels. Even though most of ethanol is currently produced by the catalytic conversion of ethylene, many researches have been focused on fermentative ethanol production from various biomass resources [1-3]. Ethanol can be used as transport fuels in many forms such as anhydrous ethanol (100% ethanol), hydrous ethanol (95% ethanol and 5% water), anhydrous ethanol-gasoline blends (10-20% ethanol in gasoline) and as octane booster to gasoline for ethyl tertiary butyl ether (ETBE) instead of methyl tertiary butyl ether (MTBE).

Many materials were used for ethanol production via fermentation process that can be classified into three main groups. Firstly, sugar that can be converted directly into ethanol such as sugarcane and molasses. Secondly, carbohydrate that have to be hydrolyzed to fermentable sugar by acid or enzymes such as rice, cassava and grain plants. Thirdly, cellulose is converted to fermentable sugar by acid, base and enzymes. However, the main production cost of the ethanol is high-cost feed stocks, which are usually consumable agriculture products. They are sugary and starchy materials that can be transformed into ethanol easily. A way of reducing ethanol cost is to use low cost materials such as agricultural residues and cellulosic materials. With feedstock having high cellulose, ethanol production is processed in three steps. They are pretreatment, hydrolysis and fermentation steps [4].

The ethanol production for shredded cassava using Chinese yeast cake was carried out under fermentation time for 31 days and using 8 g yeast. It provided 9.32% ethanol content [5]. The ethanol production for cassava starch using the selected fungi from Tan-Koji (Loog-Pang) and Saccharomyces cerevisiae were investigated. The highest ethanol content was obtained at 14.36 g/l after 24 h of saccharification process (SFF) and using 6% Cassava starch [6]. The high fiber sugarcane was pretreated by dilute ammonia pretreated following by enzymatic hydrolysis to remove lignin and hemicelluloses, and then fermented with Saccharomyces cerevisiae. The ethanol yielded 23 g ethanol per 100 g of dry biomass [7]. The mild alkali and stream pretreatment of corn fiber followed by fermentation (SSF) by Trichoderma reesei provided 5.5 g ethanol per 100 g of corn fiber [8]. The white-rot fungal, Phanerochaete chrysosporium, treatment of cotton stalk gave 2.7 g ethanol per 100 g of lignocellulosic substrate [9].

Loog-Pang Kao Mhark is a traditional starter culture of alcoholic food and drink production for industries that has been widely used in Asia [10]. Loog-Pang Kao Mhark is a microorganism source that was effective to convert starch to sugar and then convert the sugar to ethanol. Yeast in Loog-Pang Kao Mhark is isolated and characterized by its morphological, genetic, physiological and fermentation properties. The main microorganisms were found in Loog-Pang Kao Mhark such as Amylomyces rouxii, Amylomyces offic. rouxii, Rhizopus oligosporus, Rhizopus oryzae, Saccharomyces cerevisiae, Candida glabrala and Pichia anomala [11].

The aim of this work was to investigate the potential of agricultural residues for ethanol fermentation by Loog-Pang Kao Mhark and to find the optimum condition of pretreatment and hydrolysis combined with fermentation.

2. Materials and Methods

2.1. Materials and Organisms

The weak skin of edible jelly seeds of Palmyra palm was obtained from agriculturist group that transforms the Palmyra Palm product in Sathing Phra and the orange peel was obtained from fruit refreshment stall in Hat-Yai, Songkhla province, Thailand. Loog-Pang was bought from a local market in Narathiwat province, Thailand. The seed skin and orange peel component are given in table 1.

2.2. Ratios of raw materials

The ratios of material mixture, agricultural residues, were studied firstly. The seed skin and orange peel were mixed at weight ratios of 5:1 to 5:4. After that they were crushed to be about 1 mm particle size. The material mixture was boiling at 85 °C for 60 min and then fermented with 5%wt of Loog-Pang Kao Mhark (weight of Loog-Pang to weight of materials) for 5 days at a room temperature. Then the liquid phase product was centrifuged to obtain the clear liquid phase at 5000 rpm for 5 min before analyzing ethanol content by gas chromatography (GC).

2.3. Pretreatment

The seed skin and arrange peel were mixed in an optimum ratio that gotten from section 2.2. Then the material mixture was crushed. The 60 g of the materials and 20-40 ml of clean water, were mixed into 250 ml flasks. The flasks were immersed into an oil bath at a designed temperature in the range of 75-90 °C and a boiling time of 15-60 min at a constant shaking...
rate of 80 rpm. After that they were cooled down until they reached to a room temperature.

2.4. Hydrolysis and Fermentation

Hydrolysis process together with fermentation process was carried out at the same time by using Loog-Pang Kao Mhark that was a co-culture. The pretreated products were mixed with Loog-Pang followed by feeding the nitrogen gas for anaerobic process in 250 ml air-locked flasks. The important factors, namely Loog-Pang amount of 1-10%wt, fermentation time of 1-10 days at a room temperature with an initial pH of 5. After fermentation, the product mixture was separated into liquid and solid phases using a fabric filter. Then the liquid phase was centrifuged at 5000 rpm for 15 min to obtain a clear liquid product before its composition analysis by GC.

2.5. Analytical methods

Reducing sugars were estimated with 3,5-dinitrosalicylic acid (DNS), using glucose as the standard sugar [12]. Method using a double beam UV-Vis Spectrophotometer by UV-Visible ChemStation Software (Model: HP 8453).

A standard curve of glucose solution (reducing sugar) was prepared. One gram of the pure glucose was dissolved in distilled water to obtain the 100 ml solution. This stock solution (10.0 g/l) was used to make seven appropriate glucose dilutions from 0.5 – 10.0 g/l that DNS reagent was added into each tube at 1:1 volume ratio. The blank solution was prepared similarly to stock solution by using distilled water. The solutions in the tubes were boiled at 85 °C for 30 min. Then they were cooled until reach to a room temperature and diluted to 10.0 ml with distilled water. The transmittance value was measured at 570 nm on a spectrophotometer.

Ethanol was quantified by gas chromatography (GC 6890 frame ionization detector, Hewlett Packanol, USA). The HP-INNOWAX column was 30 m length, 0.32 mm ID. The column oven was operated isothermally at 150 °C, and the detector and injection were kept at 250 °C. Nitrogen carrier gas was fed at 20 ml/min. The combustion gas was a mixture of hydrogen and air.

3. Results and Discussion

3.1. Components of materials

Table 1 shows the components of the weak skin of jelly seeds of Palmyra Palm and orange peel. The moisture content that is the major component of the weak skin and orange peel are 92.78% and 81.75%. The moisture is significant for the organism living and fermentation process and another major component is carbohydrate that can be transformed to ethanol. These show that these agricultural residues are appropriate feedstock for ethanol production.

![Fig. 1. The effect of raw material ratio on ethanol content.](image)

Fig.1 shows the effect of raw material ratio on ethanol content. The ethanol content was increased with increase in orange peel amount. Because the orange peel composes more carbohydrate content than the weak skin. The optimum conversion was achieved at the weight ratio of 5:3 of the weak skin to the orange peel. Further increase of ratio did not significantly increase the amount of ethanol. That might be the effect of pretreatment step, because raw materials...
amount was not balance to water amount that was effect to heat distribution.

3.3. Pretreatment

Boiling was a physically heating pretreatment which could hydrolyze the partial of carbohydrate and fiber contents in the raw materials into the fermentable sugars or reducing sugars. The reducing sugars could be assayed in a term of glucose.

3.3.1 Effect of boiling temperature and time

![Graph showing effect of boiling temperature and time on reducing sugar content.]

Fig. 2. The effect of boiling temperature and boiling time on reducing sugar content.

Effect of boiling temperature and time on reducing sugar content are shown in Fig. 2. It was found that the optimum boiling temperature was 80 °C as it gave the maximum amount of reducing sugar. In addition, the heating pretreatment time of 45 min was enough to activate the carbohydrate and fiber molecules for hydrolysis in the next step. The active molecules that could be easily transformed into fermentable sugar, could be achieved from an optimum pretreatment condition. It was to provide the pretreated products that reached the gelatinization and liquefaction points.

3.3.2 Effect of water amount

![Graph showing effect of water amount on reducing sugar content.]

Fig. 3. The effect of water amount on reducing sugar content for 15, 30, 40 and 60 min boiling time at 80 °C.

For all water amount the reducing sugar content was increased with boiling time from 15 to 60 min (Fig. 3). However, the conversion rates were not much changed in the range of 45-60 min. The highest reducing sugar content was achieved with 40 ml water amount at a 80 °C boiling temperature for 45 min boiling time because water amount have an effect on heat distribution. That 40 ml water amount was high efficient for heat distribution so it was more feasible to break down starch molecule.

3.4. Hydrolysis and Fermentation

The pretreated product was converted to ethanol by yeast in Loog-Pang Kao Mhark.

3.4.1 Effect of fermentation time

![Graph showing effect of fermentation time on ethanol content with Loog-Pang Kao Mhark amount.]

Fig. 4. The effect of hydrolysis and fermentation time on ethanol content with Loog-Pang amount at 1-10%wt at a room temperature.

Fig. 4 shows the effect of time on ethanol content with Loog-Pang amount of 1, 3, 5, 7, 9 and 10%wt for 1-10 days. For 3-10%wt, the rapid fermentation of ethanol was observed. It can be seen that 9 and 10 days provide the highest ethanol content for this process. Because Loog-Pang Kao Mhark is mixed cultures of mould and yeast, so they need more time to produce fermentable sugar and then fermented to ethanol.

3.4.2 Effect of Loog-Pang Kao Mhark amount
Ethanol content of fermentation in relation with Loog-Pang amount for 9 and 10 days is shown in fig. 5. For both days, the rapid fermentation of ethanol was obtained by using 3%wt Loog-Pang amount. The result shows that 3%wt of co-culture, mould and yeast sources, was enough to provide 3.39 and 3.20%v ethanol product for both 9 and 10 days.

4. Conclusion

The ethanol production from the weak skin of jelly seeds of Palmyra Palm mixed with orange peel, agricultural residues (low cost biomass), using organisms from Loog-Pang Kao Mhark had been evaluated. The optimum condition for heating pretreatment of a 5:3 ratio of the weak skin to orange peel was a boiling temperature of 80 °C for 45 min. For hydrolysis combined with fermentation, the optimum condition was 3%wt Loog-Pang amount with initial pH 5 for 9 days at a room temperature. It could provide 3.39%v ethanol product.

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References