

PRODUCTION OF BIOETHANOL FROM OIL PALM EMPTY FRUIT BUNCH

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Abstract

Several methods have been identified to produce bioethanol. Bioethanol can be synthesized from cellulose and hemicelluloses that originate from the many sources of biomass. Current studies focused on the production of bioethanol from oil palm waste using *saccharomyces cerevisiae* as fermentation agent. The main objective of this study was to determine the yield of bioethanol produced from oil palm empty fruit bunch (EFB), a biomass waste. The studies also extended to cover the effects of glucose and *saccharomyces cerevisiae* concentration towards the yield of bioethanol. The bioethanol in this experimental work was synthesized from two-stage production method; in the first stage, the sodium hydroxide-pretreated sample was hydrolyzed with sulfuric acid. Then, the EFB was hydrolyzed with different set of acid concentration. In the second stage ensued, fermentation with *saccharomyces cerevisiae* was carried out in anaerobic condition. Different concentration of yeast was employed in this stage. The results demonstrated that the inoculum concentration did not show significant effect on the final ethanol concentration. Nonetheless, the duration of fermentation decreased with the increase of the yeast concentration. Results obtained also indicated that, as the concentration of glucose increased, the ethanol concentration also increased. Highest ethanol yield obtained in this work with a concentration of 15 mg/ml of glucose was 13.8 % (w/w).

Keywords: Bioethanol; Glucose; Biomass; Fermentation

Introduction

Bioethanol is one form of renewable energy source that is fast gaining foothold as potential fuel to power automotive engine. Contrary to gasoline which is refined through distilling crude fossil fuel, bioethanol can be synthesized from the starchy parts of natural plants. Microscopic yeast cells break down the starch and water, creating the so called bioethanol and carbon dioxide as end products. Bioethanol burns to produce carbon dioxide and water in complete combustion, a process akin to gasoline. It also possesses a high octane fuel, subsequently has replaced lead as an octane enhancer in petrol [1].

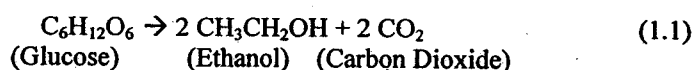
Bioethanol can be produced via traditional methods, such as fermentation, and it can be distributed using the same petrol forecourts and transportation systems as before. Two reactions are key ingredients in converting cellulosic biomass into bioethanol, *viz.* acid hydrolysis followed by fermentation. Hydrolysis converts complex polysaccharides in the raw feedstock to simple sugars. In the biomass-to-bioethanol process, acids and enzymes are used to catalyze this reaction. On the other hand, fermentation is a series of bio-catalyzed reactions that convert simple sugars to ethanol. The fermentation reaction is aided by yeast or bacteria, which fed on simple sugars. Bioethanol and carbon dioxide are produced as the sugar is consumed.

Conversion of bioethanol from biomass, especially from empty fruit bunch (EFB) offers a simple yet effective treatment of waste from palm industry. By using EFB, the competition with food-based raw materials can be alleviated, thus preserving the price of food commodity. The abundance sources of oil palm-based biomass provide an impetus for sustainable generation of bioethanol as oil palm production constitutes major agricultural industry in Malaysia. In total, it contributes approximately US\$7.3 billion in export earnings each year, mostly from the export of palm oil. Currently, there are more than three million hectares of oil palm plantations [2] which generating an approximately 90 million MT of renewable biomass (*i.e.* trunks, fronds, shells, palm press fiber and the EFB) on each and every single year.

Of particular interest, this work was carried out to evaluate the conversion of EFB into bioethanol. EFB represents about 9% of the total 90 million MT of renewable biomass generated from palm industry [2]. They are often the residue left after the fruit bunches are pressed to extract oil at oil mills. In the past, it was often used as fuel to generate steam at the mills [3]. From this, liquid wastes are generated. The solid residues, mainly EFB, constitute more than 20% of the fresh fruit weight [4]. More than 500 kg (around 0.5 m³) of liquid wastes, mainly in the form of palm oil mill effluent (POME), are discharged during the processing of 1.0 MT of fresh fruit bunches [5]. This discharge of POME in return has inflicted severe impact towards the protection of environment.

EFB is fibrous in nature, and as such EFB mainly comprised of cellulose and hemi-cellulose. These cellulose and hemi-cellulose compounds are to be converted into sugar prior to fermentation process. To produce sugars, it should be pre-treated with acids or enzymes to effect feedstock size reduction. The cellulose and the hemi cellulose portions are broken down (hydrolyzed) by enzymes or dilute acids into sucrose sugar that can be fermented into bioethanol.

The hydrolysis using dilute acid hydrolysis process remains one of the oldest, simplest yet most efficient methods for producing ethanol from biomass. Dilute sulfuric acid (0.4% ~ 0.7%) is used to hydrolyze the biomass to liquid hydrolytes, typically in high temperature. The hydrolysis process converts the cellulite of biomass into sugar solutions that can then be fermented into ethanol. Yeast is added to the solution, which is then heated. The yeast contains an enzyme called invertase, which acts as a catalyst and helps to convert the sucrose sugars into simple sugars ($C_6H_{12}O_6$). The sugar formed in the hydrolysis is fermented into ethanol. The simplified fermentation reaction for the 6-carbon sugar, glucose, is written as follow:



The most common microorganism used for this purpose is *Saccharomyces cerevisiae*, ordinary baking yeast [6]. Various species of *Saccharomyces* are examined for ethanol production processes because they are very efficient in converting sugars into ethanol [7]. Besides glucose, *Saccharomyces cerevisiae* has the ability to ferment mannose as well.

Experimental

Acid Hydrolysis

The EFB was collected from Felda Lepar Hilir 2, Kuantan, Pahang, a local oil palm plantation company. It was cut into smaller pieces. The cut pieces were then washed thoroughly using deionized water to remove dirt and debris [8]. The washed samples were oven-dried at 80°C for three consecutive days. The dried samples were then grounded to approximately 0.5 ~ 10 mm particle using a fiber grinder (IKA-WERKED-79219). The determination of particle size was done using a sieve.

The biomass preparation procedure adopted in this work followed laboratory techniques reported by previous researcher [9]. EFB was delignified through pretreatment with solution of 1% NaOH (Merck) for two hours at 100°C. The ratio of sample to NaOH solution was controlled at a setting of 1:50 (w/v). The pretreated samples were then thoroughly washed and dried to constant weight.

In the step followed, a small amount (0.25 - 1.0 g) of sample was treated with a solution of 75 wt% sulfuric acid (Merck) for one hour at 50°C. The ratio of acid to the sample weight was 15:1 (w/w). This ratio works well on fibers of the oil palm fruit, bagasse and rice husk [9]. The sample was then treated with dilute solution of sulfuric acid in a water bath at 80°C for two hours. The above experiment was repeated with the last procedure being carried out using different concentration of sulfuric acid (3.0% - 6.0%). The solution with the solids was filtered through a Whatman® 12 mm filter paper and neutralized with a solution of 2.5 M NaOH before analyzed for the glucose content using UV-Visible Spectrophotometer.

Glucose Fermentation

Before fermentation process was carried out, stock cultures were prepared. Seed growth media was grown in a media comprised of a mixture of six gram Saboured Dextrose Broth (SDB) dissolved in 300 ml of distilled water. The broth media was sterilized at 121°C for 15 minutes using autoclave. The stock culture of the microorganisms was transferred to the broth media placed in a laminar flow cabinet using inoculating loop for the preparation of seed culture. Pre-cultures were grown in a minimal medium and incubated in orbital shakers at 30°C and 150 rpm.

After that, stock culture was prepared to provide nutrients for an extended period of time. Inoculating loop was flamed with a Bunsen burner until red hot to sterilize it. Then, small portion of pure culture was taken out using the inoculating loop and inserted into the test tube that contained agar (solution mixture of DI water and Saboured Dextrose Agar). Same procedures were repeated for the next test tubes. The test tubes were then incubated in the Double Stack Shaking Incubator at 30°C and 150 rpm for two days. The test tubes were then kept in the refrigerator at -4°C. Acetate buffer solution with a pH 4.8 was prepared from mixing aqueous sodium acetate anhydrous with glacial acetic acid.

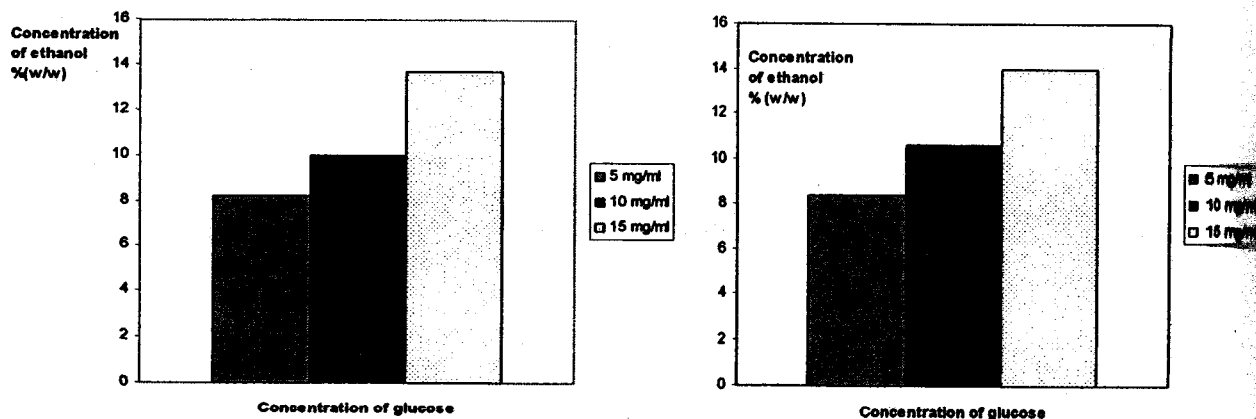
Batch fermentation was the chosen mode for the ensuing fermentation process. A 2-L Biotron Fermenter (LiFlux Gx) was employed in this experimental work. The fermenter together with fermentation medium was autoclaved at 121°C for 15 min. for sterilization. Then, it was left to cool. As soon as the temperature of autoclave dropped to 50°C, the fermenter was carried out and let cooled to the room temperature. Inoculums was then transferred to the fermenter. Fermentation was carried out at 100 rpm and 30°C. The absorbance of the medium was analyzed every six hours for the bioethanol content

using refractometer (METTLER TOLEDO Refracto 30 PX). The same procedures were repeated using different concentration of inoculums (10% and 15%).

Results and Discussion

Effect of Substrate Concentration on Ethanol Yield

The investigation of substrate concentration effects on ethanol yield was carried out at two different concentrations of inoculums, 10% and 15% respectively. Figure 1 shows the ethanol concentration % (w/w) at different glucose concentration within 48 hours of fermentation.



(a) Inoculums at 10%

(b) Inoculums at 15%

Figure 1 Effect of Substrate Concentration on Ethanol Yield

For both cases, higher glucose concentration resulted in higher yield of bioethanol. This shows that the carbon source from the glucose is indeed the nutrient for yeast fermentation process. In anaerobic fermentation, a large fraction of substrate carbon is converted to bioethanol. Therefore, the production of bioethanol depends on the carbon source. The maximum amount of bioethanol that can be produced from this procedure was approximately 14 wt%, obtained at a glucose concentration of 15 mg/ml. It also has been shown from the same figure that the inoculums concentration did not have pronounced effect on the final bioethanol concentration. This is because in this fermentation, glucose was growth-limiting factor, therefore the inoculums concentration did not play a major part.

Effect of Inoculums Concentration on Fermentation Period

As being pointed out in previous discussion, inoculums concentration did not influence the final amount of bioethanol produced. However, it affects the duration of fermentation process. Figure 2 shows that at glucose concentration of 5 mg/ml, fermentation was accomplished after approximately 18 hours at inoculums concentration of 15%, in comparison with 24 hours for 10% inoculums concentration. This signaled a faster fermentation rate. The same behavior was observed for 15 mg/mL glucose fermentation. This situation happened because the growth of yeast was different for different concentration of inoculums. It was best described by a phenomenon known as *diauxic growth* which is caused by a shift in metabolic pathway in the middle of growth cycle. The process to consume the nutrients became shorter since the growth of yeast become dominant in higher concentration of inoculums as glucose was growth-limiting factor in the medium.

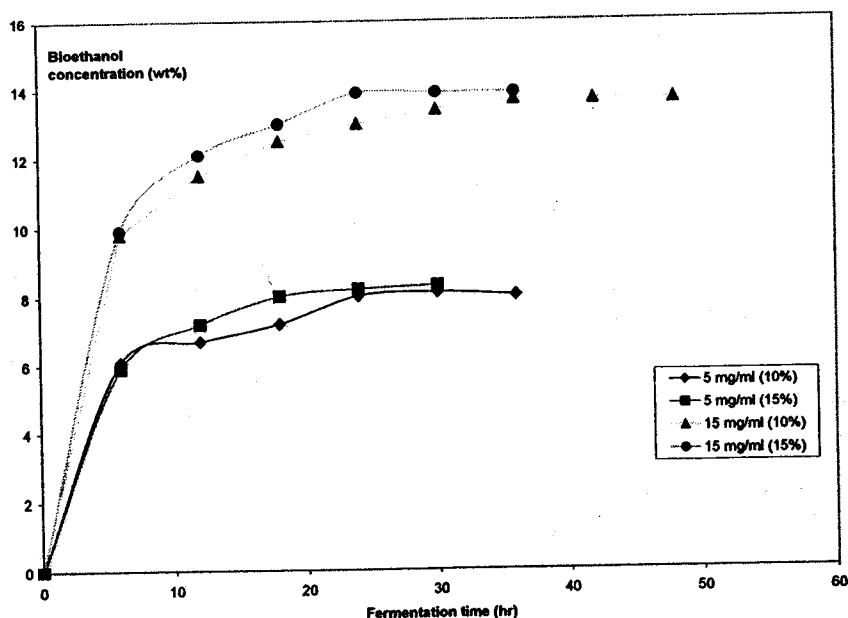


Figure 2 Effect of Inoculum Concentration on Fermentation Duration

Conclusion

Bioethanol was successfully produced from EFB using two-stage processing method, viz. acid hydrolysis followed by fermentation. Fermentation with *Saccharomyces cerevisiae* was done in anaerobic condition. Different concentrations of inoculums were used in this work. Results showed that increasing the inoculum's concentration from 10-15% would reduce the fermentation time. It was also shown that as the concentration of glucose was increased, the concentration of bioethanol would also increase accordingly. The highest concentration of bioethanol obtained from this experimental work was 13.8% (w/w) using 15 mg/ml of glucose. Although the concentration of bioethanol produced from EFB is quite low, it is still an interesting process since it only consumes several chemicals and uses biomass as raw material. Further work will be carried out to evaluate the economic potential of this process.

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