

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF BANANA (MUSA ACUMINATA) PEEL FOR BIOETHANOL PRODUCTION

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Abstract: -

Banana (*Musa acuminata*) peel is discarded after the fruit processing process. Banana peel biomass can be used as raw material to produce bioethanol. In this research, banana peel was fermented by simultaneous saccharification and fermentation using *Saccharomyces cerevisiae*. Production of bioethanol was determined and the effects of various operation conditions which included different parameters in fermentation process: fermentation time, temperature, medium pH, concentration of yeast, enzyme loading, and shaking period. peel was cut, pretreated with H₂SO₄ 2% and simultaneous hydrolyzed and fermented. Ethanol production was greatest (over 10,5%) when the initial pH of fermentation medium was adjusted to 4.5, 5% yeast supplement (8×10^7 cfu/ml), 7% cellulase (100 IU, v/v) loading, 24 hours and 37°C incubating. The results illustrated that the simultaneous saccharification and fermentation (SSF) of banana peel with *S.cerevisiae* has potential for industrial application.

Keywords: - Banana peel, *Musa acuminata*, bioethanol, cellulase, fermentation



I. INTRODUCTION

Urban pollution, global warming and reserves depletion of fossil fuels have been the driving forces for current research on alternative energy sources, especially which derived from fruit waste. Bioethanol produced from different renewable fruit waste contributes an alternative fuel for engines.

Using bioethanol as a fuel decreases fossil fuel consumption and provides energy supply security. It can be used purified or mixed with gasoline. Besides, bioethanol is considered biodegradable and sulphur free. Carbon content of bioethanol has a vegetable origin and as a consequence, when it is released during the combustion process, it does not contribute to the increase of CO₂ content in the atmosphere, reducing environmental pollution [1].

The production of bioethanol by fermentation faces competition with bioethanol industry from petroleum. But with the increasing value of these petrochemical feedstocks, bioethanol fermentation is more interest and more attention [2]. In this regard, use of fruit waste will be more economical, because they are cheap and easily available.

Many countries over the world use fruit waste as option rather than use food supply for energy production, like Zimbabwe and Australia. In Zimbabwe, many projects have been performed on energy industry from crop residues. The total energy consumption was over 44% in Zimbabwe which came from agro-waste [3].

Banana (*Musa acuminata*) is one of the tropical fruits that belong to *Musaceae*. In Vietnam, the total area of banana is over 100,000ha. The peel is waste materials resulting from the banana confectionery processing and disable fruits, they are normally discarded. These discarded peels may cause the environmental pollution, particularly water pollution. Thus, in addition to making fertilizer, the peels can be used in the production of bioethanol, which would then increase the potential producing and value for the banana.

Most of the researches which produce bioethanol were conducted with separated hydrolysis and fermentation method. Currently, due to the SSF process has many favorable advantages (reduced equipment costs, enzyme costs, ethanol formed during the process to facilitate the continuous production...), the bioethanol production plants changes to research to bioethanol produce by SSF method.

The objectives of this work were to investigate the effect of operating condition to the ethanol simultaneous saccharification and fermentation from banana peel on a laboratory scale including fermentation time, temperature, addition of yeast, addition of enzyme, pH of medium and shaking.

II. MATERIALS AND METHODS

A. Raw materials

Banana (*Musa acuminata*) peel were harvested at confectionery processing factories in Ho Chi Minh city, Vietnam. The grounded peel was then dried in a dryer oven at 60°C for 24h to reach the moisture content of $10 \pm 1\%$. Subsequently, the dehydrated peels were grounded to powder by food processor (Phillip, Holland) and packed polyethylene bags, sealed and stored at 5°C. These treated peels were used for bioethanol fermentation.

Cellulase was provided from Brenntag Company, Viscozym Cassava C.

The yeast strain *Saccharomyces cerevisiae* ATCC was obtained from American Collection for Type Culture (ATCC, America) and activated in 10ml of Sabouraud Dextrose Broth (SDB) which consisted of glucose 30g/l, peptone 10 g/l. The yeast inoculums was placed in a 250ml erlene containing 100ml autoclaved SDB for 48 hour at room temperature.

B. Methods

1) Chemical Analysis:

Research some chemical composition of banana peel which includes: 1) moisture content (was determined by infrared dryer Mettler Toledo, at 108°C), 2) total soluble solids (was determined by Atago digital refractometer, Japan), 3) pH (was determined by Hanna instrument), 4) reducing sugar content (using DNS reagent, as Miller method) [5], 6) cellulose content [4] and 7) lignin content (as Klason method), 8) Alcohol percentage in all fermentations were determined by using HPLC.

2) Pretreatment of Dried Banana Peel:

In this study, some pretreatments method were applied to treating the dried banana peel as follow: - H₂SO₄: 2, 3, 4, 5% during 5 days

- NaOH: 0.25, 0.5, 0.75, 1.0% during 5 days

- Pressing: 1 atm, 121°C during 10, 20, 30 minutes

3) Bioethanol Simultaneous Saccharification and Fermentation:

SSF were conducted using a Viscozyme cassava C (Brenntag) and *Saccharomyces cerevisiae*. SSF also conducted 250 mL flask, enzyme was added at the beginning and the biomass was hydrolyzed at 50°C, pH 5.0, static culture.

10g dried banana peel was weighed into 250ml erlene and 100ml distilled water was added. This was autoclaved at 121°C, 1 atm, 15 minutes. The fermentation was then monitored from 15 hour to 96 hour. Different concentrations of the yeast were used and the pH of sample was also adjusted with NaOH 0.5N, pH~5.0. The inoculated media were incubated in anaerobic condition. At the end of the fermentation, the fermented sample was centrifuged, 5000 round per min (rpm), 5 minute. Measuring the reducing sugar and ethanol content.

Based on bioethanol procedure, the influences of six factors were investigated. The experiment plant is represented in Table 1. The optimum result of previous experiment would be applied in the next experiment. All experiments were carried out in triplicate.

TABLE I
EXPERIMENT PLANT OF FERMENTATION CONDITIONS OF DRIED BANANA PEEL

Factor/ Method	Fermentation time (h)	Fermentation temperature (°C)	pH of medium	Yeast adding (%v/v)	Enzyme adding (% v/v)	Shaking (rpm)
1	0-69	t ^o _{room}	4.7-5.0	5		non
2-4		35, 37, 40, t ^o _{room}	4.7-5.0	5		non
5-9			4.0, 4.5, 5.0, 5.5, 6.0	5		non
10-13				2.5, 5.0, 7.5, 10.0		non
14-17					3, 4, 5, 6,	non
18-20						50, 100, 150

III. RESULTS AND DISCUSSES

A. Chemical Composition of Banana Peel

The chemical composition of banana peel is presented in Table 2. The data indicated that the banana peel is a rich source of sugars, total acidity and total soluble solids content. The dried banana peel contained high amount of reducing sugars (up to 89.43mg/g) and the results are similar to those of previous reports ([6],[7]). The high cellulose content of banana peel could serve as the important source for bioethanol fermentation use microbial culture.

TABLE II
CHEMICAL COMPOSITION OF BANANA PEEL

Composition	Content	Composition	Content
Moisture (%)	82 ± 2.16	Pectin (%)	1.33 ± 1.1
Ash (%)	1.37 ± 0.1	Total acidity (%)	0.14 ± 0.001
Cellulose (%)	9.5 ± 0.01	Reducing sugar (mg/g)	1.08 ± 0.017
Lignin (%)	3.12 ± 0.5	Peel content (g/100g)	15.23 ± 2.13

B. Pretreatment of Banana Peel

The yields of reducing sugar from the banana peel by different pretreatment methods are shown in Figure 1. The reducing sugar and cellulose content when pretreated with H₂SO₄ is higher than with other pretreatments. The glucose content increased when the H₂SO₄ concentration increased but decreased after reaching the highest level. This implies that hydrolysis process depend on acid which shows that by increasing concentrations of H₂SO₄, it doesn't enhance the hydrolysis performance. However, when the treating process was increased from 4 to 5 days, the glucose production had decreased. Perhaps, by increasing the duration to 5 days, it could play as an inhibitor of improving higher reducing sugar yield.

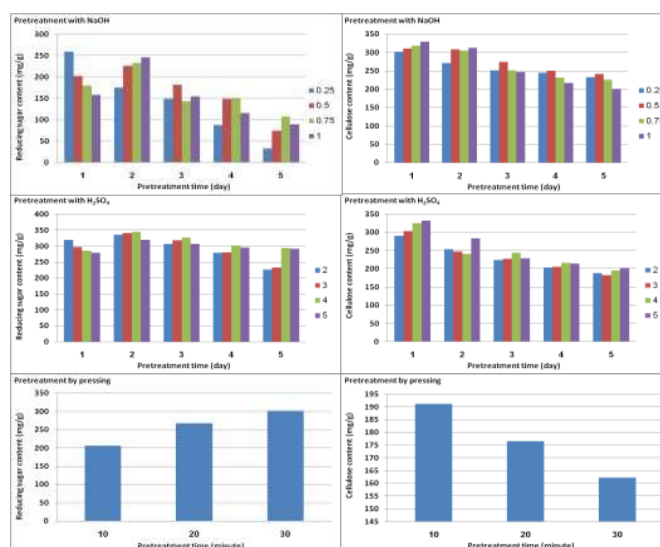


Fig. 1 Effects of pretreatment methods of banana peel to ethanol fermentation

The results showed that the sugar yield was affected significantly ($p < 0.05$) by pretreatment methods. The sugar released 301.5 mg/g. As Sirkar et al [8], acid pretreatment method was optimal for better yield of fermentable sugars from fruit

peels. Acid, alkaline pretreatment of biomass has been extensively studied and in an experiment using fruit wastes, Patle and Lal [9] has researched reducing sugar yield of 49.84 g/L and bioethanol production of 23.32 g L in the HCL pretreated to fruit and vegetable residues and the enzymatic hydrolyzate reached 36.123 g/L of reducing sugars and 11.54 g/L of ethanol. The initial pretreatment, in case of fibrous peel residues, is needed to breakdown its structure to make it more susceptible to an enzymatic attack, where as for pulp residues, it is required to avoid the necessary for reducing the weight of the biomass and to inhibit fermentative microorganisms which degrade compounds.

C. Bioethanol Simultaneous Saccharification and Fermentation from Banana Peel 1) Influence of Fermentation Time:

Fermentation time is an important factor affecting the bioethanol fermentation process. If the time is too short, fermentation does not occur completely, ethanol is produced few. If the time is too long, the fermentation environment will be infected other microorganisms, and if there is the presence of oxygen, the alcohol is oxidized to acetic acid, which reduces the strength and cause alcohol damage. Usually the determination of the end of fermentation process bases on the moment which the total soluble solids value do not change for at least 6 consecutive hours. At the end of fermentation process, yeast cell no longer converts sugar into ethanol, because amount of formed ethanol sufficient inhibits to fermenting. Therefore the determination of appropriate fermentation time is very important.

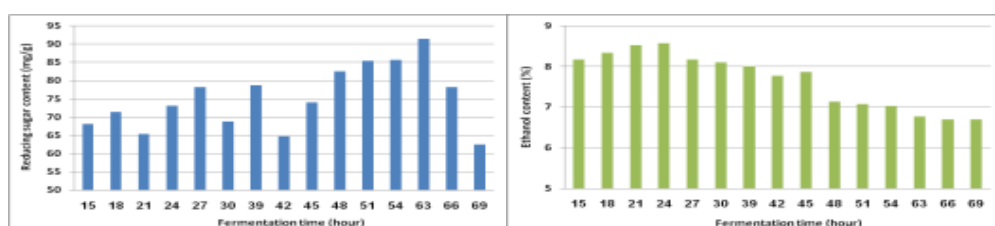


Fig. 2 Influence of fermentation time to the bioethanol fermentation from banana peel

The influence of fermentation time is presented in Fig.3. As soon as yeast cells is inoculated into fermentation medium, because a large amount of oxygen is available, yeast cells consumed sugar and other soluble nutrient molecules for cell division and development, ethanol has not been produced yet. When oxygen became exhausted, yeast cells converted substrate to ethanol molecules in anaerobic condition. Sugar and nutrient molecules were metabolized much at this stage, the rapid fermentation occurred in the period from 15 to 69 hrs. Almost all of substrate molecules were converted into ethanol and CO₂ and some byproducts such as organic acids, higher alcohol, aldehydes... Thus, medium pH decreased from 4.50 to 3.97. The alcohol content increased to 8.57% and the yield of converting reducing sugar increased to 77.62%. The moment of 24 hour is the end of bioethanol fermentation from banana peel. After 24 hour, the reducing sugar content continued to increase, but the ethanol content decrease. The reason is the ethanol concentration 8.53% inhibited ethanol fermentation and ethanol molecules were converted tho other compounds . This result is similar to the results which were reported in some previous researches. The fermentation time of pawpaw fruit waste is 3 days [11]. Bioethanol was produced from *Hylocereus polyrhizus*'s pericarps for 75hrs [12]. The maximum ethanol content was obtained after 75hrs for fermentation process (over 60 g/l) [13].

2) Influence of Temperature:

The temperature of fermentation can affect the growth of *Saccharomyces cerevisiae*. The yield of ethanol is related to medium temperature. Temperature factor can impact the sensitivity of yeasts in ethanol production, growth rate, rate of fermentation, viability, length of lag phase, enzyme and membrane function... Many yeast strains behave differently in their response towards temperature, therefore their common optimum temperature could not be easily established.

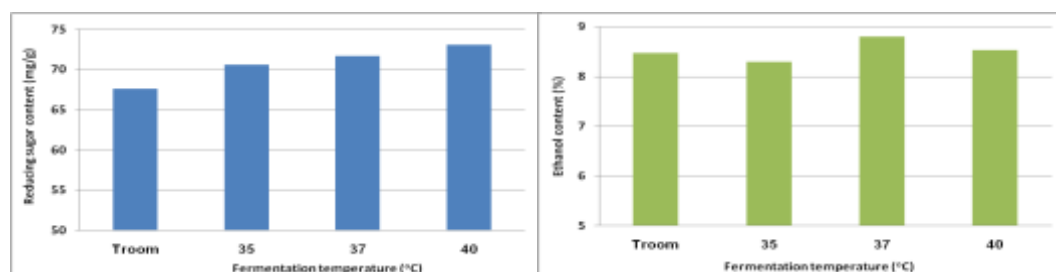


Fig. 3 Influence of temperature to the bioethanol fermentation from banana peel

The results on the variation of ethanol percentage against temperature are represented in Fig.3. The temperature was varied between 25°C and 37°C. The pH of sample was maintained at 4.5 and fermentation period was kept for 24 hour. The ethanol concentration reached the highest level at 37°C (8.80%) and decreased not only with the increase in temperature up to 40°C (8.53%) but also with the decrease in temperature to 35°C (8.30%). The yield of reducing sugar also reached the highest level as yeast cell lived 37°C. Yeast does not grow at temperature over 40°C and below 30°C its growth slows down dramatically. The enzymes activity that catalyzes the biochemical reactions in the yeast cell to function at those separate temperatures. Enzymes have a special optimal temperature degree. When temperature of

medium goes below the optimal range, catalytic activity of enzyme slows down. On the contrary, when the temperature increases, enzymes will be denatured or unfolded and thus become inactivated situation. Even if only one essential enzyme stops working, the organism will fail to grow [14]. Beside, as cellulase activity of commercial enzyme is most appropriate at 40-50°C, 37°C is appropriate to activity of yeast cell and enzyme. Similar to this result, the content of ethanol from mango (*Mangifera indica* L.) peel was optimum at 30-31°C [13]. The bioethanol production from banana was maximum at 37°C (8.8%) [11].

3) Influence of Initial pH:

The initial pH factor has significant influence on bioethanol fermentation. To observe the influence of pH on the quantity of ethanol, the experiments were carried out fermentation period of 24 hour at 37°C. The pH value of banana peel samples was varied 4.0-6.0 with an increment of 0.5. This pH range is selected according to Morris and Sarad [15] and Adams and Flynn [16]. Fig.4 shows the variation of ethanol content (%) produced with pH. The ethanol content was least of 7.97% at pH~4.5 and increased to reach the highest value 8.95% at pH~4.5. At this pH, the pH different is lowest ($\Delta pH=0.5$). However, keep increasing pH to 6.0, the ethanol content significant decreased (8.21%). The important reason is the optimum initial pH for the growth of yeast is about 4.5-6.0, and the most appropriated pH for cellulase activity is about 4.5-5.5. The lower activities of yeast towards bioethanol fermentation are shown at a pH of 4 because these pH values are too low to activate the enzymes (of yeast) to run their functions .

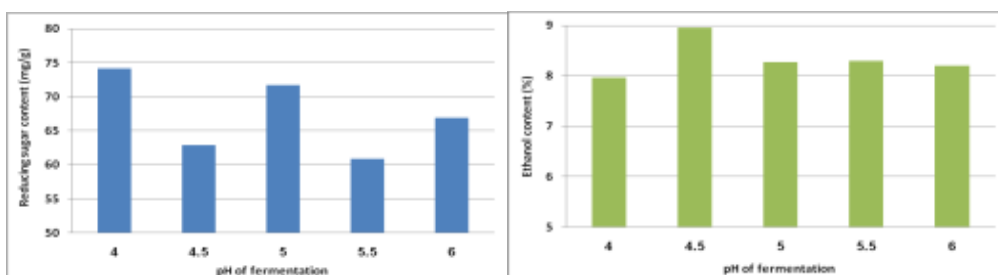


Fig.4. Influence of initial pH to the bioethanol fermentation from banana peel

4) Influence of Adding of Yeast Culture and Enzyme :

The yeast culture (10^8 cfu/ml) and cellulase (100 IU/ml) was added to fermentation flask with different ratio (v/v). Generally, irrespective of the pH, optimum fermentation period was 24 hour. It was also researched that there was increase in ethanol concentration with increase of yeast concentration (2.5-10%), enzyme concentration (1-7%) and reached the yield (9.37%) when added 5.0% yeast culture broth. Yield of converting reducing sugar increased as increased volume of yeast culture. Reducing sugar was converted optimally as carried out the fermentation added 5% culture (9.37%) (Fig.5). The ethanol yield continue to increase to 9.73% when add 7% enzyme (Fig.6). The reason is when increasing enzyme supplement, the reducing sugar content increased. Beside, with high density of yeast, bioethanol fermentation ability also increased. However, the price of cellulase is high, adding so much enzyme will reduce economic efficiency of bioethanol SSF process.

This observation is consistent with the report of Morris and Sarad [15], which indicated that the amount of yeast influenced ethanol production. As Akin [11], there was increase in bioethanol yield with increase in yeast concentration up to 10%.

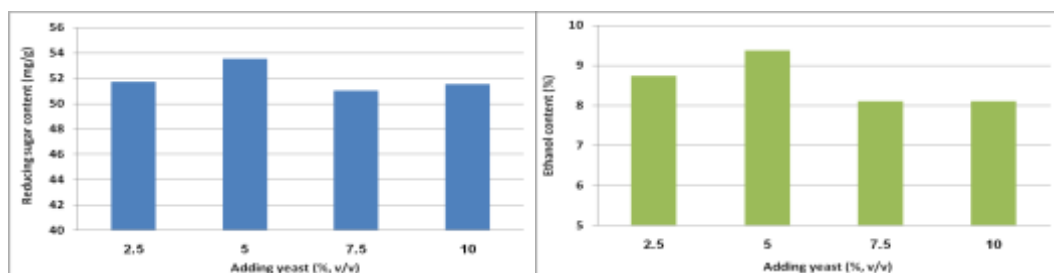


Fig.5 Influence of adding yeast culture to the bioethanol fermentation from banana peel

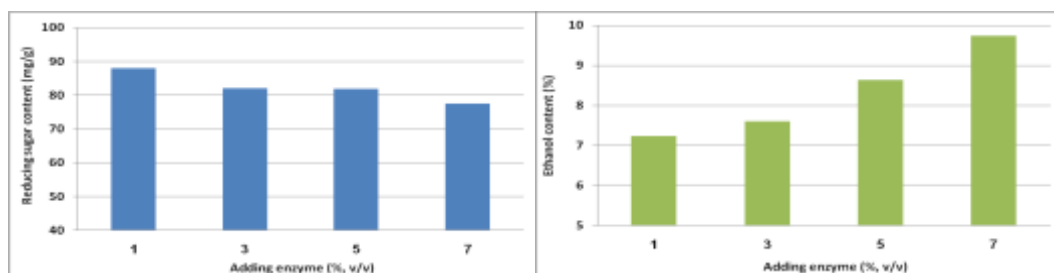


Fig.6 Influence of enzyme addition to the bioethanol fermentation from banana peel

5) Influence of Shaking Rate:

The concentration of bioethanol at different shaking rate was shown in Figure 7. The fermented banana peel mash that have been shaken for a long time produced higher bioethanol with 10.67%. Based on the data, the reducing sugar content of fermented banana peel were lower than before fermentation. From the pH results, measurements of the pH before fermentation (4.5) were higher than after fermentation (4.10). The pH of the fermented banana peel mash without shaking exhibited the higher value than those that have been shake 50rpm. Shaking make a clear medium, yeast cells approach and take nutrient molecules better, so yeast cells grew and actived better, the fementation rate was also faster. However, the shaking rate was increased faster (100 rpm) would affect and inhibit the activity of yeast cell.

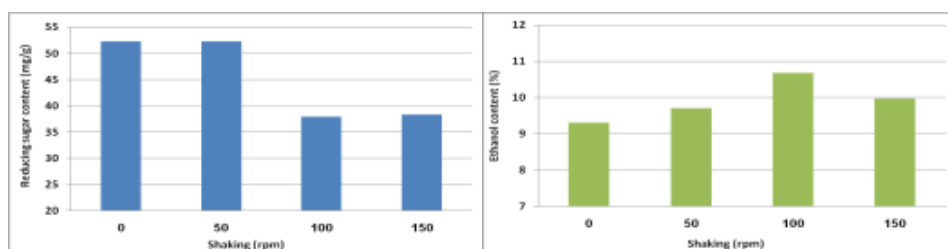


Fig.7 Influence of shaking to the bioethanol fermentation from banana peel

IV. CONCLUSION

In this study, banana peel was proved as one of the potential raw material for bioethanol production by simultaneous saccharification and fermentation. Studies on peel hydrolysis using commercial enzyme will make the process economically. Optimization of fermentation conditions includes fermentation time, temperature, initial pH, adding yeast culture, adding enzyme, shaking enhanced the fermentation rate and final ethanol concentration. The final ethanol concentration of SSF from banana peel is 10.67%, this concentration is higher than separated hydrolysis and fermentation form banana peel (8.91%, control experiment) and is much higher than bioethanol production with banana peel which is not pretreatment (5.11%, control experiment).

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