

UTILIZATION OF MANGO WASTE FOR BIOETHANOL PRODUCTION USING *ASPERGILLUS NIGER* AND *SACCHAROMYCETES CEREVISIAE*: A PILOT-SCALE STUDY

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ABSTRACT

*The modern life is highly dependent on energy including for fuel, electricity, and industry. Fossil fuels are the main of energy source is used. However, negative environmental effects are needed to be considered. Biomass energy using waste or plant matter produces a lower level of greenhouse gas emissions than fossil fuels. Through this study, we attempt to use mango waste to produce bioethanol. This source is cellulosic material which is abundant in traditional markets, especially when the peak of harvest season comes. We treated the mango waste in pilot-scale experiment with three different ways using alcohol percentage as an indicator of the alcohol production. Monoculture fermentation of *Saccharomycetes cerevisiae* produced the highest bio- ethanol percentage (83% v/v). A slightly higher in alcohol percentage (79% v/v) was achieved by coculture fermentation of *Aspergillus niger* and *S. cerevisiae*. The lowest result was obtained in fermentation of *A. niger* was followed by the addition of *S. cerevisiae* (70% v/v). These results indicate that monoculture fermentation of mango waste gives the best results. This research may be useful in production of bio-ethanol for industrial scale.*

Keywords: bioethanol, mango waste, saccharification, fermentation

ABSTRAK

Kehidupan modern sangat bergantung pada energi termasuk untuk bahan bakar, listrik, dan industri. Bahan bakar fosil merupakan sumber energi utama yang digunakan. Namun, efek lingkungan negatif perlu dipertimbangkan. Energi biomassa yang menggunakan limbah atau bahan tanaman menghasilkan tingkat emisi gas rumah kaca yang lebih rendah daripada bahan bakar fosil. Melalui studi ini, kami berupaya memanfaatkan limbah buah mangga untuk menghasilkan bioetanol. Sumber ini merupakan bahan selulosa yang melimpah di pasar tradisional, terutama pada saat puncak musim panen tiba. Kami mengolah limbah buah mangga dalam percobaan skala percontohan dengan tiga cara berbeda menggunakan persentase alkohol sebagai indikator produksi alkohol. Fermentasi monokultur *Saccharomycetes cerevisiae* menghasilkan persentase bioetanol tertinggi (83% v/v). Persentase alkohol yang sedikit lebih tinggi (79% v/v) dicapai dengan fermentasi kokultur *Aspergillus niger* dan *S. cerevisiae*. Hasil terendah diperoleh pada fermentasi *A. niger* diikuti dengan penambahan *S. cerevisiae* (70% v/v).

Hasil tersebut menunjukkan bahwa fermentasi monokultur limbah buah mangga memberikan hasil yang terbaik. Penelitian ini semoga bermanfaat dalam produksi bioetanol untuk skala industri.

Kata kunci: bioetanol, limbah buah mangga, sakarifikasi, fermentasi

INTRODUCTION

National energy consumption increases with increasing population growth. It is being used for industry, household needs, electricity, vehicle, and other goods. According to Ministry of Energy and Mineral Resources of Republic of Indonesia (2016) productions of fossil fuels Indonesia continue to shrink in the last 10 years from 4.3 billion barrels to 3.9 billion barrels. Therefore, we need to find alternative energy to secure energy supplies. Indonesia has great potential in energy sources, especially in renewable energy including wind, water, sun, geothermal heat, and biomass. Recently, Biomass is attractive topic for bioethanol production since this material is one of the most abundant renewable resources (Kim & Dale, 2004) and also present the most likely used as biofuel for motor vehicles (Demirbas, 2008). It is due to bioethanol has some advantages including higher oxygen content that implies a less amount of required additive and increasing percentage of oxygen allows a better oxidation of the gasoline hydrocarbons with the consequent reduction in emission of carbon monoxide (CO) and aromatic compounds (Sánchez & Cardona, 2008).

The main bioethanol producers in the world are Brazil and USA, approximately 62% of the world production (Kim & Dale, 2004). Bioethanol produces from waste, residue, or from plant matters which contains sugar and starch materials. Currently, bioethanol is produced based on substances that human and animal meals (corn, cassava, and grains). Using waste will avoid competition of food versus fuel. Moreover, it reduces cost and helps waste disposal management.

In Indonesia, the quantity of agricultural wastes or agro-industrial by products such as straw, bagasse, bran, peel, seeds, and fruits is often becoming waste. Over ripe fruits would not be consumed by human so that becomes waste. Mango is one of the fruits that are becoming waste in traditional markets, particularly when the peak of harvest season comes. According to Arumugam & Manikandan (2011) the proximate of mango fruit was 81.26% moisture, 7.96%

protein, 1.48% lipid, 13.08% ash, and 0.507% starch. The dietary fiber and polyphenol content were 73.04% and 54.45%, respectively. The ripening process would degrade starch and sucrose into reducing sugars such as fructose and glucose. The huge amount of reducing sugars would generate bioethanol (Saifuddin, 2014). Indonesia is a tropical country and great with season for fruits. One of the most famous fruit from Indonesia is mango. The season for mango is around August to November. On the peak of season, this fruit will overflow on market and so often becoming waste. To reduce mango waste from environmental and make this waste more useful, converting mango waste become bioethanol is a good opportunity. On this study, we would like to know the opportunity of the mango waste to become bioenergy such as bioethanol.

Biotechnological production of bioethanol from simultaneous saccharification and fermentation using *Aspergillus niger* and *Saccharomyces cerevisiae* has reported by some scientists (Ohta et al., 1993; Zakpa et al., 2009; Abouzied & Reddy, 1986). Ohta et al. (1993) used *A. niger* to produce secondary metabolism of inulase, while Zakpaa et al. (2009) utilized *A. niger* to produce cellulose enzyme. Then, the liquid culture of enzymes produced and together with *S. cerevisiae* was used for simultaneous saccharification and fermentation. On the other hand, Abouzied & Reddy (1986) applied inoculums of *A. niger* directly for saccharification. The results showed that using coculture of *A. niger* and *S. cerevisiae* increased several-fold ethanol production compared to monoculture. Therefore, the purpose of this study was to evaluate the feasibility of the utilization of mango waste to produce bioethanol via saccharification and fermentation by using culture inoculum of *A. niger* and *S. cerevisiae*.

METHODS

Materials

The fungus of *A. niger* was obtained from food microbiology laboratory of Gajah Mada University and was maintained on potato dextrose agar slant. The pre-inoculum was obtained by incubating the fungus on potato dextrose agar (PDA) at 30°C for 7 days. *S. cerevisiae* was used commercial baker's yeast with brand Fermipan. PDA was used from oxoid, and potato dextrose broth (PDB) was made from extract potato.

Preparation of culture Media for inocula production

The growth medium used for preparing the *A. niger* inoculum on potato dextrose broth

(PDB) contained, per 1 L: 400 g of potato; 15 g of dextrose; 1 L of water. Potato cut into pieces the size of dice and then added water. Cooked the potato to a boil then allowed to 1 hour with small flame. The potato was filtered and added dextrose. The PDB was sterilized for 2 hours using pressure cooker. When the temperature of media was 30°C, each flask was inoculated with triple plugs (1 cm x 1 cm) from 1-week-old cultures grown on PDA and incubated without shaking for 10 days at 30°C.

Yeast inoculums preparation contained, per 1 L: 100 g of sucrose and 1 L of water, sterilized for 2 hours using pressure cooker. When the temperature of media around 30-33°C, yeast was added 50 g and then incubated for 8 hours at 30°C (Azizah et al. 2012).

Substrates

Mango waste was collected from around traditional market in Balikpapan. The mango waste was seeded off manually. The fruit including peel and flesh was cut off into small pieces and mashed by using kitchen blender to form a pulp. Each 20 L of mango pulp was added with 20 L of water and pasteurized for 1 hour at 70°C.

Saccharification and Fermentation

Saccharification and fermentation proceeded with 3 different methods and carried out in 60 L bucket with lid. In the middle of the bucket lid was given one-hole with 4 mm in diameter and connected with aquarium pipe. The first method proceeded simultaneously within one bucket. The mango pulp (40 L) dispensed into bucket and supplemented 800 mL (2%) of inoculums of *A. niger* and 4 L (10%) of inoculum of yeast cells from the inoculum culture as described above and was incubated at room temperature (28-30°C) for 7 days. The second method was conducted 40 L of mango pulp and supplemented with 4 L (10%) of 800 mL (2%) of inoculums of *A. niger* and incubated at room temperature for 3 days. On the 3th day, 4 L (10%) of inoculum of yeast cells was added to the medium and continued to be incubated until the 7th day. The third method was used 40 L of mango pulp and supplemented with 4 L (10%) of inoculum of yeast cells and incubated at room temperature for 7 days. All methods were also supplemented with 65 g of urea and 14 g of nitrogen, phosphorus, and potassium (NPK). For each method was carried out in triplicate.

Determination of Ethanol Concentration

Ethanol produced was separated by evaporation using evaporator with 12 inch in diameter

witch connected to distillation column 8 inch in diameter and 2 m in height. The ethanol concentration was measured using alcoholmeter.

RESULTS AND DISCUSSION

The study of mango waste converts into bioethanol can be useful in waste disposal management. Our study has proved the feasibility of producing bioethanol from mango waste as shown at Fig. 1. The concentration of bioethanol was highest produced at 3th method (83%v/v), followed by 1st method (79% v/v), and the lowest was at 2nd method (70% v/v). However, according to statistically data 3th method was not significantly different with the 1st method (> 0.05). Investigation of direct fermentation unhydrolyzed potato starch using monocultures of an amylolytic fungus, *A. niger*, and cocultures of *A. niger* and *S. cerevisiae* has been reported by Abouzied and Reddy (1986). They found that in cocultures ethanol yield increased several fold compared to monocultures. It is due to *A. niger* and *S. cerevisiae* in starch medium would prevent accumulation of inhibitory concentrations of reducing sugar and that this would result in enhancement of the amylolytic activity, the amount of starch metabolized, and the total ethanol yield. However, our experiment results conflict with the results shown by Abouzied and Reddy (1986). Using cocultures *A. niger* and *S. cerevisiae* in 1st and 2nd method decreased yield of concentration bioethanol 4-folds and 13-folds, respectively compared to monocultures of *S. cerevisiae*. The reason of using of *A. niger* in this study due to the mango pulp contained high amount of starch which need to hydrolyze into sugar. The more glucose produced would generate more bioethanol. However, the presence of *A. niger* has inhibited performance of *S. cerevisiae* to convert glucose into bioethanol. It may be due to the excess of oxygen during fermentation to increase biomass yield of *A. niger*. Moreover, the increasing this biomass has inhibited growth of *S. cerevisiae*. As shown at Table 1, mycelia mat of *A. niger* was the thickest on 2nd method (+++) compared to the others where the *A. niger* has grown for 3 days and then followed addition of *S. cerevisiae* (see materials and methods for a description of the terms). According to Abouzied and Reddy (1986) that fermentation under aerobic condition resulted in the least amount of ethanol production but gave the highest biomass yield compared to the anaerobic and anaerobic- N_2 conditions. The effect of oxygen has also reported by Kosaric et al. (1983) that excess oxygen in the fermentation medium would promote respiration and cell growth resulting in lower productivity of ethanol. Moreover, Richana (2011) reported that *S. cerevisiae* could grow well in

aerobe conditions and hydrolyzed sugar into water and CO₂. To produce bioethanol, *S. cerevisiae* required strictly anaerobe conditions.

In this study we also measured pH of the media after saccharification and fermentation using pH paper or litmus paper. The results showed that the pH in the range 5 to 6. This range of pH is common in most experiment (Abouzied and Reddy, 1986). Masiel et al. (2008).

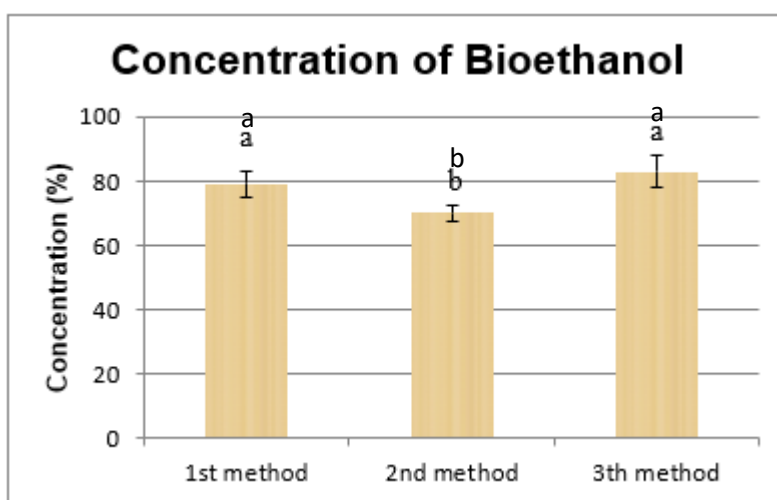


Fig 1. Bioethanol concentration was determined at different methods. The 1st method was simultaneously saccharification and fermentation by *A. niger* and *S. cerevisiae*; 2nd method was saccharification by *A. niger* followed by fermentation by *S. cerevisiae*; 3th method was fermentation by *S. cerevisiae*. The data are means of three replicates and the values in the same letter are not significantly different (One way Anova > 0.05).

Table 1. Mycelial mat of *A. niger* was recorded at the end of fermentation

Experiment	Mycelial mat of <i>A. niger</i> ^a
1st method	++
2nd method	+++
3th method	-

^a +++ : thick, ++ : medium, + : thin, - : no growth

The volume of bioethanol produced was measured using measuring cylinder and the result has reported at Fig. 2. The highest volume has collected from 1st method (520 mL), followed by 3th method (492 mL), and the last was 2nd method (357 mL). These volume values were not

significantly different within the methods (> 0.05). It may be obtained through a small volume to pilot scale, however, this could be the consistency of the media wastothick.

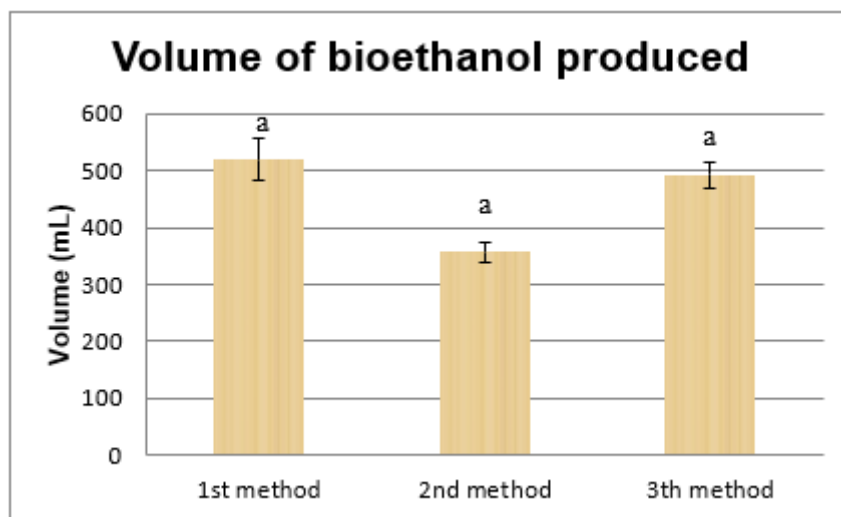


Fig 2. Volume produced during bioethanol production. The data are means of three replicates and the values in the same letter are not significantly different (One way Anova > 0.05).

CONCLUSION

This study showed that mango waste could be converted to bioethanol with great value of alcohol was 85%. The used of the 1st and 3th methods were not significantly different on this study. However using *A. niger* simultaneously with *S.cerevisiae* was better result than *A. niger* followed *S.cerevisiae*.

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