The effect of dry yeast *Saccharomyces Cereviceae* concentration on fermentation process for bioethanol production from palm oil empty fruit bunches

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Received 8 October 2012; accepted 22 October 2012

Abstract

Bioethanol production are now being developed rapidly worldwide due to the predictions of oil depletion in the near future, and global warming claims. The raw material of bioethanol can be derived from starch, sugar, and lignocellulosic biomass. Oil palm empty fruit bunches (EFB) is one of lignocellulosic biomass. The conversion of EFBs to bioethanol consist of pretreatment process for removing of lignin and reducing the crystalline of cellulosic material, hydrolysis process for converting cellulose into fermentable sugar using cellulase enzyme and β-glucosidase enzyme and fermentation process for converting fermentable sugar into bioethanol. The only component in the fermentation process that produces bioethanol is yeast. The present study was undertaken to investigate the effects of dry yeast *Saccharomyces cereviceae* concentration on fermentation process. Five different concentration of commercial dry yeast (0.5%, 1%, 2%, 3% and 5% w/v) were added to five appropriately labelled erlenmeyer already containing 25 ml of EFBs hydrolyzate. The EFBs hydrolyzate was produced by hydrolysis process. The hydrolysis process was conducted in two litre volume and contained of 20% w/v EFBs-treated substrate, 15 FPU of cellulase enzyme and β-glucosidase enzyme. The enzyme ratio of β-glucosidase to cellulase is 1:5. The hydrolysis process was carried out at 32°C, pH 4.8, at 300-400 rpm for 72 hours. The fermentation process was carried out at 32°C with agitation at 150 rpm for 72 hours. Glucose and bioethanol were analysed using High Performance Liquid Chromatography (HPLC). The yeast growth was analysed using a haemocytometer and a microscope. The experiments resulted that the addition of 0.5% and 1% w/v concentration of yeast produced the highest concentration of bioethanol were 6.36% and 6.49% w/w at 48 hours fermentation. While the addition of 2%, 3% and 5% w/v concentration of yeast produced the highest concentration of bioethanol were 6.86%, 6.93% and 6.99% w/w at 24 hours fermentation. The yeast cell number increased from 24 hours to 72 hours fermentation with the addition of 0.5% w/v concentration yeast. While the addition of 1%, 2%, 3% and 5% w/v yeast concentration increased yeast cell number from 24 to 48 hours and then decreased at 72 hours fermentation process.

Keywords : fermentation; saccharomyces cereviceae; bioethanol; palm oil empty fruit bunches.

Selection and/or peer-review under responsibility of Research Centre for Electrical Power and Mechatronics, Indonesian Institute of Sciences

1. Introduction

Renewable energy are now being developed rapidly worldwide related to the prediction of fuel depletion and global warming issues. One of renewable energy that can replace gasoline is bioethanol. Bioethanol production is now being scaled up rapidly worldwide due to the substantial rise in oil prices, predictions of probable oil depletion in the near future, and of global warming claims [1]. The raw material of bioethanol can be derived from starch, sugar, lignocellulose biomass and algae. In recent years, there is a growing interest in research and development to convert lignocellulosic biomass into bioethanol through fermentation as a second generation bioethanol. Lignocellulosic biomass is typically nonedible plant material composed primarily of the polysaccharides cellulose and hemicellulose. The third major component is lignin, a phenolic polymer that provides structural strength to the plant. The minor components in biomass can include protein, ash, organic acid and other nonstructural materials [2].

The production of bioethanol from lignocellulosic biomass waste will furthermore contribute to the countries environmentally, and socio-economically. Indonesia is rich in biomass so that the utilization of biomass as an energy source potential to be developed. Being abundant lignocellulosic waste is derived from agricultural wastes (grass, rice hulls, rice husk, wheat straw, waste paper,
Based on these data, the current total EFBs production in Indonesia are approximately 18.72 million tons. Yeast cell number is one of the important parameters in the fermentation process. Inoculation rates in the fuel alcohol industry are arbitrarily set, but in general adhere to the following rules of thumb with initial rates in the fuel alcohol industry being set 250 to 450 grams per 1,000 liters [7].

The conversion of EFBs to bioethanol consists of pre-treatment process for removing of lignin and reducing the crystalline of cellulosic material, hydrolysis process for converting cellulose into fermentable sugar using cellulase enzyme and β-glucosidase enzyme, and fermentation process for converting fermentable sugar into bioethanol. The only component in the fermentation process that produces bioethanol is yeast. Yeast cell number is one of the important parameters in fermentation process. Inoculation rates in the fuel alcohol industry are arbitrarily set, but in general adhere to the following rules of thumb with initial rates in the fuel alcohol industry being set 250 to 450 grams per 1,000 liters [7].

Commercially produced yeast is widely used for bioethanol production to the present time. Commercial processing of yeasts for use in bioethanol production are liquid yeast, cream yeast, stabilised liquid yeast, crumbled yeast, and commercial dry yeast [8]. The present study was undertaken to investigate the effects of dry yeast Saccharomyces cerevisiae concentration on fermentation process for bioethanol production from oil palm empty fruit bunches. The type of dry yeast is instant dry yeast (IDY). IDY is a type of active dry yeast that was originally created for baking so it could be directly added to the flour without prior rehydration [8]. Effects of dry yeast concentration on fermentation of EFBs for bioethanol production and yeast growth were evaluated.

2. Materials and methods

2.1. Raw material

Oil palm empty fruit bunches (EFBs) fiber was collected from an Oil Palm Plantation belongs to PT Perkebunan Nusantara VIII, in Pandeglang, Banten, Indonesia. Furthermore, EFBs fiber was dried and milled. Later, EFB was treated with NaOH pretreatment. The ratio of NaOH solutions 10% b/b and EFB is 5:1. Pretreatment process was carried out at temperatures 150°C and 4-7 kg/cm² of pressure for 30 minutes. The compositions of EFBs-treated were determined by using standard Biomass Analytical Procedures provided by National Renewable Energy Laboratory (NREL). EFBs-treated was hydrolysed by enzyme. The hydrolysis process was conducted in two litre volume and contained of 20% w/v EFBs-treated substrate, 15 FPU of cellulase enzyme and β-glucosidase enzyme. The enzyme ratio of β-glucosidase to cellulase is 1:5. The hydrolysis process was carried out at 32°C, pH 4.8, at 300-400 rpm for 72 hours. The compositions of EFBs hydrolyzate were analysed using HPLC. EFBs hydrolyzate and commercial dry yeast Saccharomyces cerevisiae will be used in the fermentation process.

2.2. Fermentation process

Five different concentration of commercial dry yeast Saccharomyces cerevisiae (0.5%, 1%, 2%, 3% and 5% w/v) were added to five appropriately labelled erlenmeyer already containing 25 ml the EFBs hydrolyzate. The fermentation process was carried out at 32°C with agitation at 150 rpm for 72 hours. Fermentation process performed in two replicate for each variable concentrations of yeast.

2.3. Analytical methods

Glucose and bioethanol were analysed using High Performance Liquid Chromatography (HPLC). The HPLC (Waters, USA) system was equipped with AMINEX HPX 87H column, and a guard column, an automated sampler, a gradient pump, and a refractive index detector. The mobile phase was 5 mM H₂SO₄ at flow rate of 0.6 ml min⁻¹ and oven temperature was maintained at 65°C. Prior to HPLC injection, all samples filtered through 0.2μm syringe filters.

The yeast growth was analysed using a haemocytometer and a microscope. In observing the yeast by haemocytometer, it was needed a microscope with a 40x objective, some pipettes, and glassware for dilutions. Fermentation broth was shaken until become homogeneous suspension, then taken a little with the sterilized pipet and diluted by distilled water until yeast suspention became translucent. Conditioning is set in the microscope until each section in haemocytometer have below 5 ~ 6 cells. Amount of yeast is counted within 5 sections.

3. Results and discussion

3.1. Raw material

Oil palm empty fruit bunches (EFBs) used in this study was treated with NaOH pretreatment. The compositions of EFBs-treated were determined by using standard Biomass Analytical Procedures provided by National Renewable Energy Laboratory (NREL). The composition of EFBs-treated consist of 77.50% cellulose, 6.83% xylose, 10.32% lignin and 1.22% ash. Later, EFBs-treated was hydrolysed by cellulase enzyme and β-glucosidase enzyme for 72 hours. The EFBs hydrolyzate was analyzed by HPLC-R1 to get the data of glucose and xylose concentration. The composition of EFBs hydrolyzate consist of 10.39% w/w of glucose and 2.37% w/w of xylose.
3.2. Effect of yeast concentration in fermentation process

Yeast Growth

The yeast strain used in this study was commercial instant dry yeast Saccharomyces cerevisiae as shown in Fig. 1a. Instant dry yeast have 3-4 years shelf life, 3.5-5.5% moisture content and appearance cylindrical strands/threads [8].

The variations of dry yeast concentration in fermentation process were 0.5%, 1%, 2%, 3% and 5% w/v. The initial cell numbers were analysed using a haemocytometer and a microscope. The results of initial cell number analysis for 0.5%, 1%, 2%, 3% and 5% w/v dry yeast concentration were $2 \times 10^9$, $6 \times 10^9$, $6 \times 10^9$, $1.16 \times 10^{10}$ and $2.16 \times 10^{10}$ cell/ml. Observation of the yeast growth during fermentation process also done every 24 hours. See Fig. 2.

Fig. 2 shown that the rate of yeast growth increases rapidly with increasing the amount of yeast added. The yeast cell number increased from 24 hours to 72 hours fermentation with the addition of 0.5% w/v dry yeast concentration. While the addition of 1%, 2%, 3% and 5% w/v dry yeast concentration increased yeast cell number from 24 to 48 hours and then decreased at 72 hours fermentation process. From the beginning of fermentation process, yeast cells are in the lag phase. During this time, yeast cells are adapting to the mash by optimising internal enzyme systems. Very little glucose was consumed at this time [9].

The lag phase at the addition 1%, 2%, 3% and 5% w/v dry yeast concentration was for 24 hours, meanwhile the lag phase for the addition of 0.5% w/v occurred during 48 hours. That was because of the initial cell number for the addition of 0.5% w/v was fewer than the other variation of the dry yeast concentration in the fermentation process with the same amount of glucose. If too much glucose is delivered during the early stage of fermentation, one of two things can occur: yeast growth can be inhibited by the osmotic effects of high levels of glucose, or the cell will grow rapidly in the early stages and stop prematurely [9].

So at the addition of 0.5% w/v dry yeast concentration, yeast growth was inhibited due to too much glucose compared with its initial cell number. At 24 to 48 hours fermentation process, yeast growth occurs rapidly for addition variation of 1%, 2%, 3% and 5% w/v dry yeast concentration, while for addition of 0.5% yeast growth occurs rapidly started at 48 hours. This phase of yeast growth is the exponential phase. During this phase, yeast population is growing and budding occurs at a high rate. During this rapid growth phase, there is a high demand for glucose as the glucose source for growth. If the glucose delivery by the glucoamylase cannot keep up with the growth, glucose starvation may occur. Once yeast cell have been subjected to glucose limitations, they lose their ability to ferment efficiently. This is most likely caused by a rapid depletion in cellular energy (ATP)[10]. If there is energy depletion, the yeast cells will stop budding and the production rate of bioethanol will decrease [9]. After 48 hour fermentation process, the stage of yeast cell growth in stationary phase, caused by one or more growth nutrients becoming limiting. During this phase, the yeast stops budding and the rate of alcohol production per cell decreases [9].

Bioethanol production

The effects of dry yeast concentration on fermentation process of EFBs for bioethanol production shown in Fig 3. Fig 3a. Shown that the addition of 0.5% and 1% w/v concentration of yeast produced the highest concentration of bioethanol were 6.36% and 6.49% w/w at 48 hours fermentation. While the addition of 2%, 3% and 5% w/v concentration of yeast produced the highest concentration of bioethanol were 6.86%, 6.93% and 6.99% w/w at 24 hours fermentation as shown in Fig. 3b.

At the addition of 0.5% and 1% w/v bioethanol concentration decreased after 48 hours, while at addition of 2%, 3% and 5% bioethanol decreased after 24 hours. It caused by glucose depletion, the yeast cells will stop budding and the production rate of bioethanol will decrease. The yeast does not make ethanol 100% of the theoretical yield. That is because of the cell’s objective is to make daughter cells that will survive in the medium. These uses up a good percentage of the 7-10% of the sugar that does not end up in ethanol.

The yeast also makes a number of other end products in lesser amount, including glycerol, organic acids, ester, aldehydes and higher alcohol [11]. At addition of 2%, 3% and 5% w/v dry yeast concentration shown that produced bioethanol is not much different, so it can be concluded that the optimum addition of yeast optimum concentration is
2% by 24 hours the fermentation process. At the optimum condition we got the fermentation process cost saving compared to the addition of yeast concentration at 1%, 3% or 5%.

4. Conclusion

The addition variation of dry yeast concentration on fermentation process affects the production of bioethanol, optimum fermentation time and yeast growth. The optimum condition of dry yeast addition is at 2% w/v of concentration and 24 hours of fermentation time. At this combined condition, we can choose the best setting in order to saving the cost of production of bioethanol with avoid using higher dry yeast concentration, 3% w/v of concentration or even 5% and also for fermentation time. By using 2% w/v of concentration we can stop the process of fermentation in 24 hours running. It is enough to achieve the optimum result. More than 24 hours the process will take decreasing of bioethanol instead. This research hopefully can be a thought in development of fermentation process in industry scale to coverage national production of bioethanol.

Acknowledgement

The authors are grateful to “Peningkatan Kemampuan Peneliti dan Perekayasa (PKPP)” project for research grant on 2012 which fully supported in this research.

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