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PRODUCTION OF BIOETHANOL FROM SUGARCANE BAGASSE THROUGH HYDROLYSIS AND FERMENTATION: THE EFFECT OF FE^{2+} AND CA^{2+} IONS ADDITION ON BIOETHANOL CONCENTRATION

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ABSTRACT

Fossil energy sources are one of the driving factors in the economic development and prosperity of the nation. The availability of fossil energy sources is currently shrinking and is expected to run out quickly, so an alternative is needed to replace it. Sugarcane bagasse is a lignocellulosic biomass which can be utilized for bioethanol production as an alternative energy source. Lignocellulosic hydrolysis with the addition of H2SO4 catalyst was carried out to obtain glucose. The addition of Fe2+ and Ca2+ ions was performed to increase the glucose concentration. Commonly, Saccharomyces cerevisiae is used to convert glucose into bioethanol using fermentation process. The objective of this study was to investigate the effect of Fe2+ and Ca2+ ions on reducing sugar concentration, and the concentration of distillate bioethanol. The effect of yeast type on the concentration of bioethanol was also discussed in this paper. The result shows that the addition of Fe2+ and Ca2+ ions in the hydrolysis process can increase the concentration of glucose produced. This is because the higher concentration of cellulose, the more substrate is converted into reducing sugars. The use of Fe2+ and Ca2+ ions in the fermentation process will increase ethanol concentration compares with fermentation without metal ions. This is because Ca2+ ions have an important role in the flocculation process of bioethanol fermentation and Fe2+ ions are important nutrients for eukaryotes, which play a role in cell oxidation-reduction reactions. The type of yeast that can produce higher concentrations of ethanol is tapai yeast. This is because tapai's yeast has three supporting ingredients for the fermentation process, whereas baker's yeast only contains two microorganisms.

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KEYWORDS: Sugarcane bagasse; Fe^{2+} and Ca^{2+} ion; hydrolysis; fermentation; bioethanol

1. INTRODUCTION

Like most other developing countries, Indonesia is also facing challenges due to rapid population growth and industrial expansion, namely increasing demand for energy. Various negative impacts arising from fossil energy have raised ambitions to switch to renewable energy, which has the potential to meet energy supply and demand challenges [1]. Biofuel is one of the alternative energy potentials that can be developed. The most common used liquid biofuels are biodiesel and bioethanol [2]. Compare with conventional energy fuels, bioethanol is cleaner, pure, and renewable. The implementation of bioethanol can minimize fossil fuels consumption and reduce carbon emissions.

One of the most efficient raw materials for bioethanol production is lignocellulosic biomass [3][4]. Lignocellulosic biomass contains cellulose, which can be converted into glucose, and also it can be converted into bioethanol. One lignocellulosic biomass is sugarcane (*Saccharum officinarum L*. The main product of sugar cane is sucrose, which accumulates in the internodes. This sucrose can be extracted and refined in the special cane sugar industry. The residue remaining after extraction is sugarcane bagasse, which is a valuable raw material because it is a by-product of the sugar industry [4].

Bagasse is a lignocellulosic compound consisting of cellulose, hemicellulose, lignin, and other plants. It contains about 25-30% hemicellulose, 45-50% cellulose, 2.4-9%, and ash 25% lignin, [5]. Hemicellulose is a macromolecular polysaccharide in plant biomass that contains sugars such as glucose, galactose, mannose, xylose, and arabinose. Lignin is a very complex molecule formed from phenylpropane, methoxy, and non-carbohydrate polyphenolic units connected in a three-dimensional structure [6]. Meanwhile, cellulose is a structural material formed by plants from glucose and polysaccharides. Cellulose can be reduced to simple sugars (glucose) through a hydrolysis process and can be converted into ethanol through a fermentation process.

Hydrolysis is the process of breaking molecules with water [7]. The hydrolysis process is an important step in the manufacture of bioethanol, which determines the amount of glucose to be converted into bioethanol through fermentation. The hydrolysis process requires a catalyst so that the reaction takes place quickly. Usually, acid catalyst is often employed in fermentation process, because it is cheaper and feasible compare with enzyme catalyst. Ethanol fermentation takes a place by converting glucose into ethanol (C_2H_5OH) and carbon dioxide (CO_2). Ethanol fermentation converts sugars (glucose, fructose and sucrose) into energy (ATP), while ethanol and carbon dioxide are by-products [8].

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The objective of this study was to investigate the effect of Fe^{2+} and Ca^{2+} ions on reducing sugar concentration, and the concentration of distillate bioethanol. The effect of yeast type on the concentration of bioethanol was also discussed in this paper.

2. MATERIALS AND METHODS

2.1. Materials

Sugarcane (*Saccharum officinarum*) baggasse was purchased from local street food seller in Semarang, Indonesia. Baker's yeast and tapai's yeast were supplied from local small enterprises in Semarang, Indonesia. Urea, monopotassium phospate, magnesium sulfate, potassium hydroxide, acetic acid, sulfuric acid (as a catalyst) was purchased from CV. Indrasari (Semarang, Indonesia).

The materials used are, sugarcane bagasse from the seller of sugarcane ice in front of Tembalang Elementary School, baker's yeast and tapaiyeast, urea, KH_2PO_4, $MgSO_4$, NaOH, CH_3COOH, H_2SO_4 as a catalyst, glucose, fehling A and fehling B, indicator MB, aquadest, $FeCl_2$, and CaO.

2.2. Methods

Sugarcane bagasse pretreatment

Pretreatment of sugarcane bagasse starts by drying the bagasse in the sunlight, then grinding the dried bagasse with a grinder until it becomes a powder and sieving it.

Starter Produce

Starters made for breeding of the Saccharomyces cerevisiae. The steps start by extracting sugarcane juice. Then 60 ml of sugarcane juice should be sterilized by boiling. After the sugarcane juice is cooling down to room temperature (27ºC), 4 g of KH_2PO_4, 4 g of $MgSO_4$, and 4 g of urea are added as nutrients. The pH of solution is adjusted to 5 then add 5 g of yeast to the solution and then cover it for two days. This step is repeated twice for different yeasts.

Hydrolysis with Fe^{2+} and Ca^{2+} Addition

The hydrolysis process starts by mixing 20 g of bagasse powder into 250 mL of water in a three-neck flask. Then, add 2 mL of $FeCl_2$ and CaO solution for each concentration variable, which is 0; 0.3; 0.6; 0.9; and 1.2 g/L, 3 ml of 0.02 N H_2SO_4 as a catalyst. The hydrolysis process was conducted by heating process at 70°C for 1 hour.

Analysis of Reducing Sugar with the Lane-Eynon Method

Analysis of reducing sugars using the Lane-Eynon method was done to investigate the effect of Fe^{2+} and Ca^{2+} on the levels of reducing sugars resulting from hydrolysis. Determination of reducing sugar levels

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using this method begin with standard glucose preparation and standardization. Standard glucose was prepared by dissolving 1.25 g of glucose anhydrite in 500 ml of distilled water. Standardization of glucose levels was carried out by diluting 5 ml of standard glucose to 100 ml and then taking 5 ml of neutralized pH. Then 5 ml of fehling A and 5 ml of fehling B were added to the solution. After that, the solution was heated to a temperature of 60-70ºC while titrated with standard glucose until the blue color almost disappeared and then add 2 drops of methylene blue indicator. The solution was titrated again using standard glucose while heating in the temperature range of 60-70ºC until the color of the solution changed to brick red. Record the titrant volume (F = titrant volume).

The next steps were to dilute 5 ml of the hydrolyzed solution using a 100 ml volumetric flask. The resultant dilution solution was taken as much as 5 ml and its pH got neutralized. Then 5 ml of fehling A and B, and 5 ml of diluted standard glucose were added into the hydrolized solution. After that, the solution heated in temperature of 60-70ºC. The solution was titrated with standard glucose in hot condition until the color of the solution became blue, then add 2 drops of methylene blue indicator. The solution is titrated again using standard glucose in a temperature range of 60-70ºC until the color of the solution changes to brick red. Record the need for titrant to calculate the level of glucose produced (M = titrant volume with sample within it).

$$X=\frac{(F\text{-}M)\times\frac{V\ total}{V\ titration}\times\frac{V\ dilution}{V\ taken}}{V\ total\times\rho}\times 0{,}0025\times 100\%$$

Fermentation Using Baker's Yeast and Tapai's Yeast with Fe^{2+} and Ca^{2+}

The products from hydrolysis solution in the form of reducing sugar are fermented to produce bioethanol. The fermentation process of the solution starts by preparing an Erlenmeyer as a place for fermentation. As much as 100 ml of hydrolysis results were cooled and then filtered until no dregs were filtered. The results of hydrolysis solution was added to 60 ml of starter with baker's' yeast, tapai's yeast, Fe^{2+} and Ca^{2+} ions (concentrations 0.3, 0.6, 0.9, and 1.2 g/L). Mixed solution covered using aluminum foil. Let the fermentation process lasts for 5 days.

Distilation of Bioethanol

The fermented solution (bioethanol), which is not pure at a low concentration, need to be purified using distillation process. The distillation process starts by heating 160 ml of fermented solution with variable ions Fe^{2+} and Ca^{2+} (concentrations 0.3, 0.6, 0.9, and 1.2 g/L) at 80ºC for 3 h. The samples were analyzed using HPLC to determine the ethanol concentration.

3. RESULTS AND DISCUSSIONS

3.1. Effect of Fe^{2+} and Ca^{2+} Ions on Reducing Sugar Concentrations

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Fe^{2+} and Ca^{2+} metal ions at concentrations of 0, 0.3, 0.6, 0.9, and 1.2 g/L are used in the hydrolysis process. The effect of Fe^{2+} and Ca^{2+} ions on the concentration of reducing sugars produced can be seen in Figure 1.

Effect of Fe^{2+} and Ca^{2+} ions on reducing sugar concentration during hydrolysis process for 1 h. The concentration of reducing sugar produced increases with a higher value in the concentration of Fe^{2+} and Ca^{2+} ions. The optimum reducing sugar concentration is achieved with Fe^{2+} and Ca^{2+} ions at a concentration of 1.2 g/L. It is due to the formation of H^+ ions as a catalyst resulting from the addition of $FeCl_2$ and CaO to water. An increasing in the concentration of $FeCl_2$ and CaO results in a greater formation of H^+ ions [10]. H^+ ions act as catalyst in the hydrolysis process, leading to the cleavage of more C-O bonds in cellulose and its consequent instability. As a result, there is an increase in the amount of reducing sugars released during the hydrolysis process. Fe^{2+} ions can form intermediate complexes by attracting the reducing sugar oxygen atoms on cellulose, which subsequently lowers the activation energy, leading to an increase in the efficiency of the hydrolysis process [11].

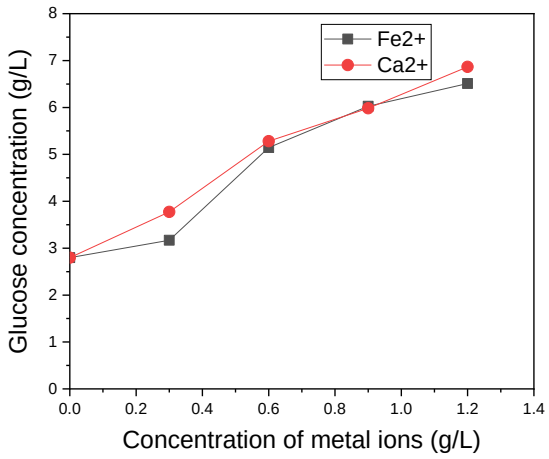

Figure 1 Effect of Fe^{2+} and Ca^{2+} ions on reducing sugar concentration during hydrolysis process for 1 h

3.2. Effect of Fe^{2+} and Ca^{2+} Ions on Bioethanol Concentration with Baker's Yeast

The study employed Fe^{2+} and Ca^{2+} metal ions at concentrations of 0, 0.3, 0.6, 0.9, and 1.2 g/L for fermentation using Baker's yeast. The impact of metal ion on bioethanol concentration is depicted in Figure 2.

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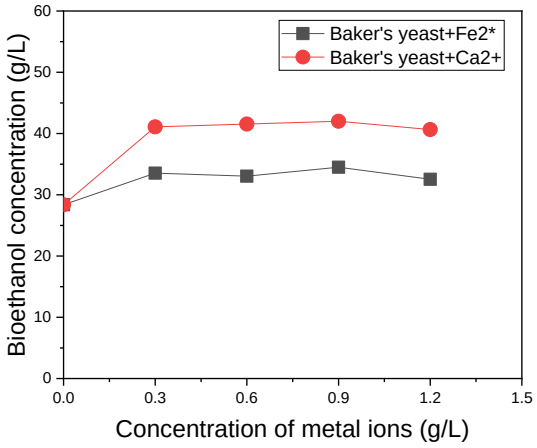

Figure 2 Effect of fermentation using Fe^{2+} and Ca^{2+} ions with baker's yeast on the concentration of distillate bioethanol.

Figure 2 indicates that the optimal Ca^{2+} requirement is at a concentration of 0.3 g/L, whereas higher ion concentrations yield a constant ethanol concentration. This can be attributed to the fact that the requirement of Ca^{2+} metal ion for yeast growth is low [12]. Nevertheless, these ions play a significant role in the flocculation process in bioethanol fermentation. Similarly, the optimal concentration of Fe^{2+} ion is0.3 g/L, further high-level ion concentrations will lead to a constant bioethanol concentration. Fe^{2+} ion is a critical nutrient for eukaryotic cells as it plays a vital role in various cell oxidation-reduction reactions such as lipid biosynthesis, cell respiration, oxygen transportation, DNA replication and repair [13]. Therefore, excess Fe^{2+} metal ion in growth media can be toxic to yeast cells [14].

3.3. Effect of Fe^{2+} and Ca^{2+} Ions on Bioethanol Concentration with Tapai's Yeast

The study employed Fe^{2+} and Ca^{2+} metal ions at concentrations of 0, 0.3, 0.6, 0.9, and 1.2 g/L for fermentation using Tapai''s yeast. The impact of metal ions on bioethanol conversion is shown in Figure 3.

Figure 3 shows that the utilization of Fe^{2+} and Ca^{2+} ions during the fermentation process led to a higher bioethanol concentration than control without any metal ion. In addition, when Ca^{2+} metal ion is included during the fermentation process, they lead to higher bioethanol concentration than Fe^{2+} metal ions. This is due to the crucial role of Ca^{2+} ions in the flocculation process that occurs during bioethanol fermentation. In contrast, Fe^{2+} metal ion is considered crucial nutrients for eukaryotic cells as they take part in different cell oxidation-reduction reactions, including those involved in lipid biosynthesis, oxygen transportation, cell respiration, DNA replication and repair. Despite being toxic under certain conditions [13], they play

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a significant role in these processes.

The addition of Ca^{2+} metal ion in bioethanol, will reach a peak at a metal ion concentration of 0.3 g/L. However, at higher ion concentrations, the bioethanol concentration remains constant. Similar to Ca^{2+} metal ions, higher level of Fe^{2+} metal ions concentration will increase the bioethanol concentration. Concentration of bioethanol reached its peak at a metal ion concentration of 0.3 g/L. However, at higher ion concentrations, bioethanol concentration remained constant. The reason behind the decrease in the rate of bioethanol production, when excessive metal ions are added, is because yeast cells' ability to adapt to environmental stress decreases [15].

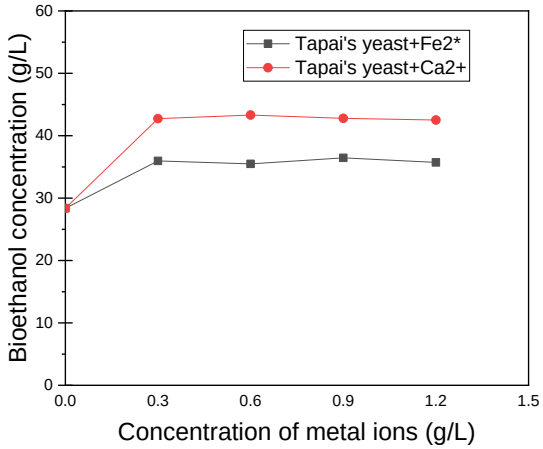

Figure 3 Effect of fermentation using Fe^{2+} and Ca^{2+} ions with Tapai's yeast on the concentration of distillated bioethanol.

3.4. Effect of Yeast Type on the Concentration of Bioethanol

The same quantity of baker's yeast and tape yeast was used in this fermentation process study. The impact of yeast types on the concentration of bioethanol is shown in Figure 4.

The impact of yeast type on bioethanol concentration is depicted in Figure 4. Yeast plays a crucial role in the production of bioethanol, facilitate the fermentation of sugar into bioethanol. The selection of two different yeast types aimed to establish the superior yeast strain for bioethanol production. Bioethanol process production without metal ions exhibit tapai's yeast (30.54%) as more effective than baker's yeast (28.37%) for bioethanol production.

With the addition of the same type of metal ions (both Ca^{2+} and Fe^{2+}), tapai's yeast produce bioethanol higher than baker's yeast'. This phenomenon is caused by baker's yeast only contains two

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microorganisms, namely *Saccharomyces cerevisiae* and *Sorbitan monostearate* [16]. In contrast, tapai's yeast contains three supporting ingredients for the fermentation process, namely mold (*Amylomyces rouxii, Aspergillus sp, Rhizopus sp.* and *Mucor sp*), yeast (*Saccharomycopsis malanga, Saccharomycopsis fibuligera, Saccharomyces cerevisiae, Pichia burtonii*, and *Candida utilis*), and bacteria (*Bacillus sp, Acetobacter sp, and Pediococcus sp*.) [17]. Based on experiment, it can be seen that tapai's yeast has a more complex population than baker's yeast, which affects the performance of producing bioethanol in the fermentation process [18]. According to Hargono et al. (2020) [19], distillation stage -2 is a method used to increase the bioethanol concentration produced in stage-1 (34.90%) and in stage-2 process yielded of bioethanol 94.60% [19].

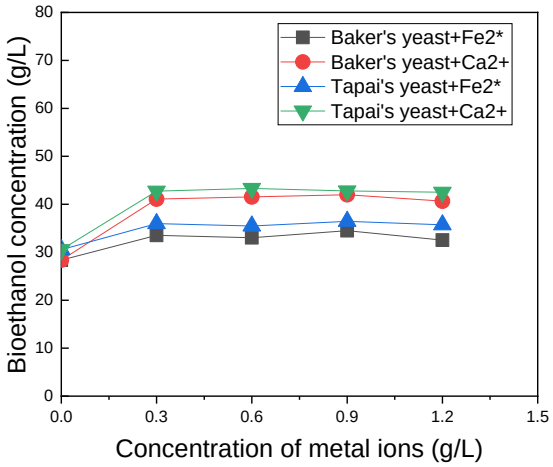

Figure 4. Effect of yeast type on the concentration of bioethanol

4. CONCLUSION

The inclusion of Fe^{2+} and Ca^{2+} ions during the hydrolysis process can increase the concentration of reduced sugar produced. The concentration of reducing sugar produced is directly proportional to the concentration of Fe^{2+} and Ca^{2+} ions present. The addition of Fe^{2+} and Ca^{2+} ions during fermentation lead to higher bioethanol concentrations compared to the absence of metal ions. The addition of Fe^{2+} and Ca^{2+} metal ions will increase bioethanol concentration with a peak at a metal ion concentration of 0.3 g/L. However, at higher ion concentrations, the bioethanol concentration remained constant. The addition of the same type of metal ions, both the addition of Ca^{2+} and Fe^{2+}, the type of yeast that produces a higher concentration of bioethanol is tapai's yeast.

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