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A Comparison of the Bioethanol Production from *Suweg* (*Amorphophallus campanulatus*) through Separate Hydrolysis and Fermentation as well as Simultaneous Saccharification and Fermentation Using *Saccharomyces cerevisiae*

H Hargono^{1*}, B Jos¹, P Purwanto¹, S Sumardiono¹, MF Zakaria¹

¹Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Semarang, Central Java, 50275, Indonesia. (Email: hargono@che.undip.ac.id; baktijos10@gmail.com; purwanto@live.undip.ac.id; siswo.sumardiono@che.undip.ac.id; mahmizakaria@students.undip.ac.id)

*Corresponding author

H Hargono, Department of Chemical Engineering, Faculty of Engineering, Diponegoro University, Semarang, Central Java, 50275, Indonesia. (Email: hargono@che.undip.ac.id)

Abstract. *Suweg* has the potential to develop bioethanol because of the high starch content. This is a form of ethanol produced from living organisms. The processes used include separate hydrolysis and fermentation (SHF) as well as simultaneous saccharification and fermentation (SSF). Furthermore, the components utilised in this study include StargenTM 002 (1.5%, w/w) for hydrolysis, *Saccharomyces cerevisiae* for fermentation at a temperature, and PH of 30°C and 4.5 respectively, with varying starch concentrations (150-250 gL⁻¹). The results showed the highest ethanol yield of 89.57 and 99.52 gL⁻¹ from SHF and SSF, respectively from a *suweg* concentration of 200 gL⁻¹ treated for 54 h. Meanwhile, 250 gL⁻¹ samples attained 62.22; 99.57; and 101.56 gL⁻¹, respectively. Therefore, SSF method provided a more efficient process for producing bioethanol using the 250 gL⁻¹ concentration, although the increase from 200 gL⁻¹ to 250 gL⁻¹ only produced a 1.96% higher yield.

1. Introduction

The product ethanol has a high potential for use as a solvent, fuel, chemical reagent, and sterilizing agent [1]. Furthermore, concentrations ranging from 42.6% (w/w) are effective in preventing the SARS coronavirus, MERS coronavirus, ebolavirus, and influenza A virus within 30 seconds [2]. Meanwhile, almost pure concentrations are used as amore environmentally friendly alternativeto fossil fuels [3].

Bioethanol is a form of ethanol derived from biomass products, including; glucose, sucrose, lignocellulose, and starch, usually processed through hydrolysis and fermentation processes [4]. The function of hydrolysis is to break down polymer compounds into monomers using water [5]. Meanwhile, the Enzymatic form is considered capable of producing more specific products and suppressing by-products [6]. The purpose of fermentation is to convert reducing sugar into ethanol using a yeast referred to as *Saccharomyces cerevisiae*. This is due to the high ethanol profitability and capacity of the microorganism [7].



Starch, in this case, carbohydrates, is a source of energy for humans, and a vital product because of the huge market demand, broad industrial applications, and several derivative products including maltose, dextrin, maltodextrin, glucose, and fructose [8]. Furthermore, *Suweg* (*Amorphophallus campanulatus* B) is a type of tuber, widely found in Indonesia, especially in Sumatra and Java [9], used as a raw material for bioethanol due to the high starch content, of about 77% [10].

Conventional bioethanol production is initiated with the processes of hydrolysis and continued with fermentation. This method is termed Separate Hydrolysis and Fermentation (SHF), involving the use of enzymatic activity at a temperature of 80°C. The product is subsequently fermented using yeast at 37°C [4]. Furthermore, another technique used is Simultaneous Saccharification and Fermentation (SSF), where hydrolysis and fermentation are carried out simultaneously in the same reactor with the addition of enzyme and yeast, hence glucose is quickly converted to ethanol [11]. According to a report by Hargono *et al.* [12], the production of bioethanol from bitter cassava by the SSF method using Stargen™ 002 2% (w / w) and *Saccharomyces cerevisiae* decreased the sugar concentrations to 4.5 gL⁻¹ after 48 hours. Meanwhile, the resulting yield after a 60 hours process was 68.65 gL⁻¹.

The purpose of this study was to compare the SHF and SSF methods in the production of bioethanol from *Suweg* starch. Furthermore, the enzymes used were 1.5% (w/w) Stargen™ 002 and *Saccharomyces cerevisiae* yeast. The initial concentration of *suweg* starch used was varied in the range of 150 - 250 gL⁻¹. Also, the experiment was carried out in batches.

2. Experimental

The experiments were carried out using 10-month old *Suweg* tubers obtained from Sukorejo Village, Gunungpati District, Semarang City, Central Java, Indonesia. Therefore, the starch extraction method adopted in this study was similar to the technique previously used by Hargono [12].

2.1. Separate Hydrolysis and Fermentation Method.

2.1.1. Hydrolysis. Various sample starch concentrations (150-250 gL⁻¹) were dissolved into water, in a 500 mL erlenmeyer. Therefore, the slurry was adjusted to a pH of 4.5, using 50 mM sodium acetate and heated at 62°C for 1 hour, before adding Stargen™ 002 1.5% (w/w). This mixture was further stirred and heated to attain 80 ± 1°C, at a speed of 100 rpm for 15 minutes, before cooling to 30 ± 1°C and incubated for 18 hours. The filtrate was then separated using a centrifuge at a speed of 100 rpm, and 40°C temperature for 10 minutes, and glucose concentration was analysed. [4].

2.1.2. Fermentation. This process was carried out in a 1.2 L reactor equipped with temperature and pH controllers. The hydrolysis sample was further adjusted to pH 4.5 using NaOH 3 M solution. Subsequently, some nutrients including, (NH₄)₂HPO₄ 0,5 g L⁻¹, MgSO₄·7H₂O 0,025 gL⁻¹, and yeast extract of 1gL⁻¹ were added to the medium and incubated for 15 hours in a shaker at 30°C with a speed of 80 rpm. Furthermore, 5 gL⁻¹ of dry yeast *Saccharomyces cerevisiae* was added and the experiment was performed for 78 hours. The samples were then collected every 6 hours and analysis for glucose and ethanol concentrations were conducted [4].

2.2. Simultaneous Saccharification and Fermentation Method

2.2.1. Hydrolysis and Fermentation. These processes are carried out in a 1.2 L reactor through a device equipped with temperature control, pH control, and heater. Furthermore, various starch concentrations (150 - 250 gL⁻¹) were dissolved in distilled water, and the pH adjusted to 4.5 using NaOH 3M solution. The reactor was then sterilized using an autoclave at a temperature of 121°C for 30 minutes, and the fermentation medium was equipped with the SHF process, which served as a nutrient source. This experiment was initiated by adding the Stargen™ 002 enzyme (1.5% w / w) at 50°C for 24 hours. The heat was subsequently lowered to 30°C, before 5 gL⁻¹ of *Saccharomyces cerevisiae* was added and

counted from time 0. This experiment was sustained for 78 hours, and samples were collected every 6 hours in order to analyze the glucose and ethanol concentrations. [11].

2.3. Analysis Method

2.3.1. Glucose Analysis. The glucose analysis method used was similar to Hargono *et al.* [13], while fermented ethanol was estimated using the Aminex HPX-87H column of High-Performance Liquid Chromatography (HPLC). This tool was selected to identify more specific compounds, including glucose.

2.3.2. Ethanol Analysis. This method was similar to Hargono *et al.* [13], while the fermented ethanol was estimated using the Aminex HPX-87H column of High-Performance Liquid Chromatography (HPLC). This technique was also used for sample-line analysis because of the relatively high water content.

3. Results and Discussion

3.1. The Effect of Fermentation Method towards Ethanol Concentration

The data obtained from fermentation using the SHF and SSF methods are shown in Figures 1a and 1b, respectively. Furthermore, the experiments were conducted using an initial *Suweg* starch concentration of 200 gL^{-1} , while the temperature was sustained at 30°C for 78 hours. This process was performed in batches and the pH maintained at 4.5, using the StargenTM 002 enzyme and *Saccharomyces cerevisiae* yeast.

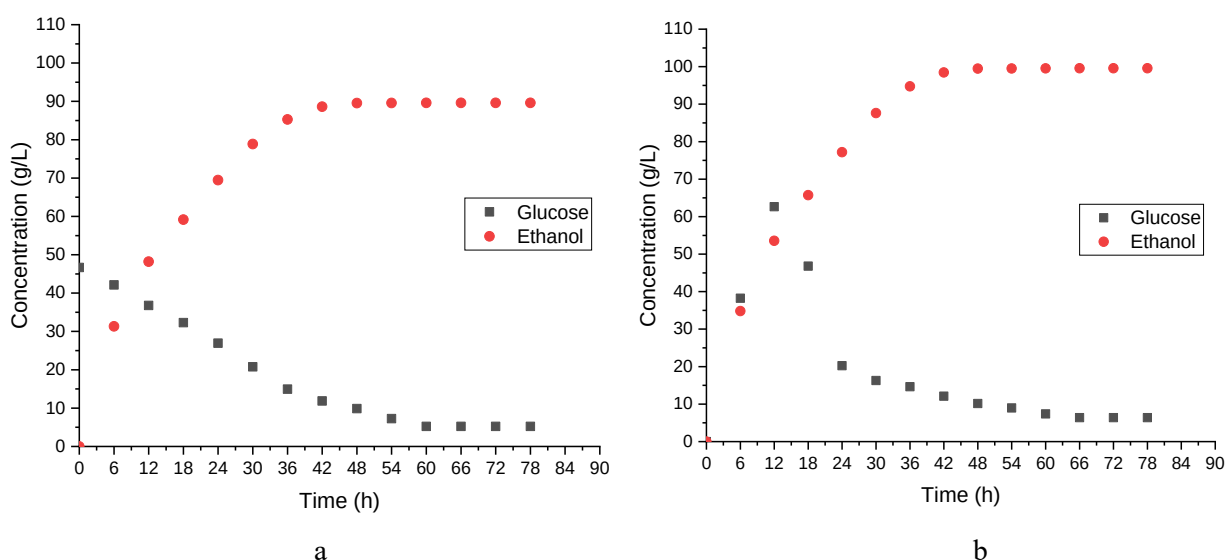


Figure 1. Distribution of glucose and ethanol concentrations in the SHF (a) and SSF (b) methods

There was a decrease in glucose concentration from the initial concentration of 46.68 gL^{-1} during the SHF treatment. This was accompanied by a decline in the fermentation process, although the concentration value was constant and 5.24 gL^{-1} between 60 and 78 hours. Meanwhile, the ethanol concentration increased along with the fermentation process and showed a tendency to be constant at 48 hours, while 89.61 gL^{-1} was reported at 78 hours.

The initial glucose concentration of 0 gL^{-1} resulted from the delayed hydrolysis reaction. This increased to 62.64 gL^{-1} after 12 hours, and subsequently reduced significantly after 66 hours, marked by a constant value of 6.38 gL^{-1} . Meanwhile, the ethanol concentration increased along with fermentation and showed a tendency to be constant at a concentration of 99.57 gL^{-1} after 54 hours.

In contrast, the 78 hours fermentation process using SSF produced higher ethanol yield compared to the SHF method. According to a report by Dahnum *et al.* [14] the use of the SSF was greater in the optimization of bioethanol production process using the Empty Fruit Bunch (EFB). This method operates at low temperatures, and therefore saves energy. This is in line with Rana *et al.* [15], where ethanol production was substantially faster, indicating a lower enzyme inhibition by glucose. Also, Mithra *et al.* [16] emphasized on higher yield generated using method in a batch system. In addition, enzyme inhibition by glucose was prevented because of the simultaneous ethanol conversion, and the process was conducted in 1 reactor to save cost.

3.2. The Effect of Suweg Starch Initial Concentration on the Ethanol yield

The experiments were carried out using the SSF method with a batch system, and the initial *Suweg starch* concentrations used were 150, 200, and 250 gL⁻¹. This process ensued within the operating conditions at a temperature of 30°C and pH 4.5 for 78 hours. Subsequently, data were obtained every 6 hours and analyzed using HPLC as presented in Table 1.

Table 1. Data on ethanol concentration at various initial concentrations of *suweg* starch (150, 200, 250 gL⁻¹) for 78 hours

Time (h)	Ethanol Concentration (gL ⁻¹)		
	150 gL ⁻¹	200 gL ⁻¹	250 gL ⁻¹
0	0	0	0
6	21,75	34,80	35,50
12	33,47	53,55	54,62
18	41,08	65,73	67,04
24	48,24	77,18	78,73
30	54,76	87,62	89,37
36	59,22	94,75	96,65
42	61,54	98,46	100,43
48	62,18	99,49	101,48
54	62,2	99,52	101,51
60	62,22	99,55	101,54
66	62,23	99,57	101,56
72	62,23	99,57	101,56
78	62,23	99,57	101,56

Based on the data in Table 1, an elevation in the initial concentration of *Suweg* starch at 150, 200, and 250 gL⁻¹, resulted in a rise of the ethanol yield, by 62.23; 99.57; 101.56 gL⁻¹ respectively, with a constant state attained at 48 hours. Furthermore, only 1.96% increase was observed with the the 250 gL⁻¹starch sample.

This finding was congruent with a report by Hargono *et al.* [17], particularly with the yield of reducing sugars. The ethanol conversion was greater due to an increase in substrate concentration, which enhances the glucose content [18]. Consequently, ethanol production is inhibited because of the elevation in viscosity as reported by Triwahyuni *et al.* [19]. This increase lead to the formation of a nonhomogeneous media, and therefore, prolonged production duration of ethanol from yeast [19]. Furthermore, there is also an accumulation in the reactor along side poisonous yeast cells, and a significant decline in the production rate [20].

4. Conclusion

The bioethanol production process from *Suweg* starch was conducted at a temperature of 30°C for 78 hours. Furthermore, a batch system was used under conditions higher ethanol yield for SSF method, compared to the SHF, at a value of 99.57 and 89.61 gL⁻¹, respectively. Also, more significant amounts of product were generated with greater starch concentration through SSF processes, although only a 1.96% increase was recognized with the 250 gL⁻¹ sample starch, Hence, a 200 gL⁻¹ starch concentration was considered to be more efficient.

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