Bioethanol Purification from Fermentation of Suweg Starch Using Two Stage Distillation Method

H Hargono*a, B Josa, P Purwantoa, S Sumardionoa, MF Zakariaa

aDepartment of Chemical Engineering, Engineering Faculty, 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; mfahmizakaria@students.undip.ac.id)

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

Abstract
This study aims to employ two-stage distillation in the purification of fermented ethanol. Furthermore, 200g/L Suweg starch was fermented by Simultaneous Saccharification and Fermentation (SSF) method using Stargen™ 002 and Saccharomyces cerevisiae at a concentration of 1.5% (w/w) and 1.10% (w/w), respectively. Therefore, 99.52 g/L or 13.82% bioethanol was produced and further purified through a distillation method. Subsequently, the first stage was performed in a column without filling material, while the second was in a column with glass ball-shaped material (marbles). This two phase exposure was conducted to expand the contact between the bioethanol-water vapor mixture and the suffing material. In addition, the temperature was set at 78ºC, while the duration was varied at 45, 60, 75, and 90 minutes. Therefore, the samples were analyzed using high-performance liquid chromatography (HPLC) and the maximum yield of 94.6% was produced at 75 minutes.

1. Introduction
Global warming is caused by CO₂ emissions from fossil fuels. However, bioethanol is an alternative renewable and more environmental friendly energy source [1,2]. This is produced easily from diverse raw materials derived from agricultural products [3] comprising starch-based: cassava, gadung tubers, corn, wheat, sago [4], lignocellulose-based: rice straw, water hyacinth, sugarcane bagasse, corn stalk, grass, pineapple peel [5], and sugar-based: sugarcane (Saccharum officinarum), sugar beet (Beta vulgaris), molasses (beet molasses) [6]. Bioethanol combustion will not causes CO₂ emissions. Bioethanol is produced by fermentation.

The Simultaneous Saccharification and Fermentation (SSF) method of various feedstocks generally produces low levels of ethanol. Scordia et al. [7] performed SSF using yeast S. carlsbergensis, and a maximum ethanol yield of 15.9 g/L (2.21%) was generated at 48 hours. Furthermore, the research was continued [8] using experimental designs to optimize the output, using cellulases and yeast S. stipitis, and 18 g/L (2.5%) ethanol product. Silva et al. [9] also performed a similar study on giant reed, and an ethanol concentration of 39 g/L (5.41%) was generated at 72 hours.

Therefore, separation processes were used to purify the fermented crude ethanol [10], through distillation [11], adsorption [12], and pervaporation membrane [13]. Distillation is one of the separation processes which commonly used. Beside of this easy use, this method also has a low cost. So that, distillation will use in this study. This study aims to investigate the effect of fermentation time (SSF) on the ethanol concentration of 200 g/L Suweg concentration at 30°C, as well as the effect of 2-stage distillation time. The novelty of this study is using 2-stage distillation which the utilization as illustrated.
2. Materials and methods

2.1. Raw materials, chemicals, enzyme and microorganism

Materials
The raw material used was 10 months old Suweg tuber (Amorphophallus campanulatus B) from Sukorejo village, Gunung Pati District, Semarang, Central Java-Indonesia. These samples were then converted into starch.

Suweg starch making
The procedure for making Suweg starch was previously conducted by Hargono et al. [14]

Chemicals
Potassium sodium tartrate tetrahydrate and 3,5-Dinitrosalicylic acid (by Merck), NaOH (98%, Merck), Na2SO3 (98.5%, Merck), H2SO4 (98.5%, Merck), sodium acetate buffer (Merck), glucose (99.5 %, Merck) and ethanol (99.5%, Merck), (NH4) 2HPO4, MgSO4.7H2O and yeast extract were purchased from Sigma-Aldrich, Indonesia.

Enzyme
The enzyme used in the hydrolysis process includes GSHE as Stargen™ 002, commercially obtained from Genencor International (USA) [14]. This contains Aspergillus kawachii α-amylase, commonly reported in T. reesei and glucoamylase from T. reesei -Amylase, work synergistically to hydrolyze of starch granules into glucose. The enzyme activity was 570 GAU / g with pH ranging from 4.0 - 4.5, while the minimum for alpha-amylase and glucoamylase was 135 KNUg-1 and 270 GAUg-1 respectively. In addition, the amount of enzyme producing 1 g of reducing sugar every hour, measured as glucose from dissolvable starch substrate and under examined state is one glucoamylase unit (GAU).

Microorganism
The baker's yeast produced by PT. Pakmaya and obtained from Kabita Store, Semarang, Indonesia is Saccharomyces cereviceae. Furthermore, this is stored in a refrigerator before use and then scattered in clean water at room temperature at a concentration of 10 g/L (g dry pastry yeast / liter of DI water). Subsequently, 10 mL measurement was utilized as inoculum without further cultivation, and added to 90 mL of maturation medium to produce 10% (v/v) portion. The cup and medium were sanitized by operating the autoclave at 121 ° C for 0.5 h before vaccination, while the temperature and agitation speed was constant.

2.2 Enzymatic hydrolysis
The pre-hydrolysis step was performed to fulfill the SSF stage. Therefore, starch hydrolysis was carried out in 250 mL erlenmeyer, using Suweg and enzymes at concentrations of 200 g/L, and 1.5% (w/w), respectively, while the pH was maintained at 4 with 50 mM sodium acetate buffer. The mixture in the erlenmeyer was heated in water bath to reach 50°C for 30 hours at 100 rpm speed. Subsequently, the slurry was cooled to 30°C and incubated for 18 h, and then the filtrate was separated using a centrifuge, in order to determine the amount of glucose released.

2.3 Ethanol Fermentation
Enzymatic hydrolyzate fermentation was conducted using the concept of Simultaneous Saccharification and Fermentation (SSF). The pre-hydrolysis step ensured the increase in glucose level (from 200 g/L Suweg concentration). This was to generate sufficient substrate for yeast Saccharomyces cerevisiae activity in a reactor at 1 L volume, equipped with a pH control. The pH of the medium was maintained at 4.5, while “cultivation” was carefully controlled using 3 M NaOH. Furthermore, several nutrients were added to the
fermentation medium: (NH4)2HPO4 0.5 g L⁻¹, MgSO4, 7H2O 0.025 g L⁻¹ and yeast extract 1.0 g L⁻¹ for 15 hours in a shaker-incubator, and maintained at 37°C and 80 rpm. Also, 5 g L⁻¹ of dry yeast was added, and the experiment was performed for 78 hours. The samples were collected periodically: 6, 12, 18, and up to 78 hours. Therefore, the ethanol and glucose content were analyzed using high performance liquid chromatography (HPLC) Aminex HPX-87H column.

2.4 Bioethanol Purification

Figure 1 shows the purification of ethanol broth from SSF fermentation process, using 2-stage distillation. The first stage required heating the raw material in boiler-1 at 78°C to form an ethanol-water mixture steam. This vapor was then condensed for 15-90 minutes in a condenser column (A) filled with water as a coolant and equipped with a spiral pipe cooler. Therefore, samples were collected at a 15 mins time interval and the ethanol concentration was respectively analyzed using HPLC. The best yield obtained from stage one was placed into boiler-2 and heated to 78°C as feed for stage 2 distillation. This was performed in the condenser column (B) containing packing material for a time period, ranging from 6, 12, 18 to 78 hours. Furthermore, the ethanol concentration of the main output was analyzed using HPLC.

![Figure 1](image-url)

**Figure 1.** A. Condensor with cooling water, B. Column distillation containing packing material, C.01 Heater-1 for Boiler-1, C.02 Heater-2 for Boiler-2, T-01 Boiler-1, T-02 Boiler-2, Tr Thermometer.
3. Result and Discussion

3.1 The effect of fermentation time on glucose and ethanol concentration.

Table 1 shows the SSF results of glucose and ethanol concentrations, using 200 g/L Suweg, in a reactor set at 30°C and pH 4, over a 6 to 78 hours period. Furthermore, there was an increase in the glucose concentration formed at 6 to 12 hours from 38.22 g/L rose to 62.64 g/L. This elevation was due to the SSF process entering the pre-hydrolysis stage [9], where the enzyme activity during the hydrolysis of Suweg starch yielded the maximum glucose concentration. Hence, sufficient quantity was provided for conversion into ethanol. However, a further decline was observed during 18 to 78 hours and up to 54 hours (11.02 g/L). Subsequently, the value recorded remained stagnant at 6.38 g/L. Table 1 shows an increase in ethanol during SSF from 4.83 to 13.82% (v/v) between 6 and 78 hours. This phenomenon occurs because of the sufficient glucose available for fermentation by Saccharomyces cerevisiae yeast.

| Time, hour | Cglucose, g/L | Cethanol, g/L | Cethanol, % (v/v) |
|-----------|---------------|---------------|------------------|
| 0         | 0             | 0             | 0                |
| 6         | 38.22         | 34.80         | 4.83             |
| 12        | 62.64         | 53.55         | 7.43             |
| 18        | 56.8          | 65.73         | 9.12             |
| 24        | 23.24         | 77.18         | 10.72            |
| 30        | 16.28         | 87.62         | 12.16            |
| 36        | 15.63         | 94.75         | 13.15            |
| 42        | 14.11         | 98.46         | 13.67            |
| 48        | 11.15         | 99.49         | 13.81            |
| 54        | 11.02         | 99.52         | 13.82            |
| 60        | 6.38          | 99.55         | 13.82            |
| 66        | 6.38          | 99.57         | 13.82            |
| 72        | 6.38          | 99.57         | 13.82            |
| 78        | 6.38          | 99.57         | 13.82            |

Silva et al. [9] performed hydrolysis on giant reed (Arundo donax L.) before yeast was added to ferment SSF for 12 hours, thus resulting in 28 g/L glucose concentration. The final yield was used for SSF fermentation over a 78 hours period to produce ethanol 39 g/L. Hargono et al. [16] conducted a similar study on cassava starch prepared at a concentration of 200 g/L. Therefore, Stargen™ 002 (0.1% w/w) and yeast Saccharomyces cerevisiae was incorporated to produce ethanol 36.16 g/L. Kusmiyati, et al. [17] studied the hydrolysis of 10% Iles-iles flour (Amorphophalus campanulatus) to produce reducing sugars for use as a raw material in fermentation using Zymomonas mobilis. The optimum conditions were achieved at 4.5 pH, and 30 °C for 120 h, thus resulting in 10.33% (v/v) ethanol. Glucose decreased because of the reaction of fermentation which is glucose converted into ethanol. So that, the glucose concentration decreased and ethanol concentration increased.

3.2 Bioethanol Purification by Distillation Process

3.2.1 Effect of Distillation Time on Ethanol Concentration. Table 2 shows the yield of ethanol purification process achieved through distillation stage-1. This procedure was performed on the best SSF fermented crude ethanol (13.82%), produced at 54 hours. In addition, the ethanol concentration increased from 16.18% to 34.90% within the distillation time of 15-90 minutes, and a relatively constant value was recorded up to 105 minutes. This process is accompanied by condensation in column A (Figure 1) without the use of stuffing, thus subsequently increasing the ethanol concentration by up to a limited yield of 34.90%. This entire procedure is assumed to only consist of 1 stage, despite the increase in duration to 105 hours, as shown in Table 2.
Table 2. Effect of Stage-1 Distillation Time on Ethanol Concentration

| Time, minute | Ethanol concentration, % |
|--------------|--------------------------|
| 0            | 13.82                    |
| 15           | 16.18                    |
| 30           | 20.07                    |
| 45           | 22.84                    |
| 60           | 24.44                    |
| 75           | 30.20                    |
| 90           | 34.90                    |
| 105          | 34.94                    |

Distillation stage -2 is a method used to increase the ethanol concentration produced in stage-1 (34.90%). This 75 minutes process yielded a 94.60% product, as shown in Table 3.

Table 3. Effect of Stage-2 Distillation Time on Ethanol Concentration

| Time, minute | Ethanol concentration, % |
|--------------|--------------------------|
| 0            | 34.90                    |
| 60           | 84.83                    |
| 75           | 94.60                    |
| 90           | 93.25                    |
| 105          | 92.78                    |
| 120          | 92.30                    |

This distillation is a method used to separate the ethanol-water binary mixture. According to Huang et al. [18] this separation process is only able to produce a maximum ethanol concentration of 95.63% (w/w), with limitations to the azeotrope point. Adogbo and Ayodele [19] conducted a similar research using column with and without packing of 0.48 pore sizes. The ethanol yield obtained respectively was 87.5% and 62%. Vane et al. [20] conducted a study with 5% fermented ethanol broth and flow rate of 145 g/minute, using an integrated distillation with a membrane. The resulting ethanol concentration increased from 63.5 to 98.5%.

4. Conclusion
The performance of SSF process on Suweg starch 200 g/L for 72 hours resulted in glucose 6.38 g/L, and 13.82% ethanol broth. This was subsequently subjected to purification using distillation stage 1 and 2, which yielded 34.90% and 94.60% ethanol concentration at 90 and 75 minutes, respectively. However, the 2-stage distillation tool was designed to be suitable for the purification of fermentation products.

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