Improving the Value of Spent Coffee Ground by Converting Carbohydrates into Sugars by *Saccharomyces cerevisiae* to Produce Bioethanol

Syahruddin Syahruddin[1(✉)], Hayyun Lisdiana[2], and Erdawati Erdawati[2]

[1] Department of Mechanical Engineering, Balikpapan State Polytechnic, Balikpapan 76129, Indonesia
syahruddin@poltekba.ac.id

[2] Department of Chemistry, State University of Jakarta, Jakarta 13220, Indonesia

Abstract. Spent coffee grounds contained 45% carbohydrate, 26% lignin, 13% lipid, and 13% protein. To improve the value of spent coffee grounds in a sustainable way, a study was done on how to convert carbohydrates into fermentable sugars which could be used to make value-added products. To accomplish this, a pre-treatment process, followed by acid-promoted hydrolysis, fermentation using *Saccharomyces cerevisiae*, and a distillation process, were performed to obtain bioethanol. The purpose was to find out the effect of various sulphuric acid concentrations and periods of fermentation on bioethanol yield. Analysis of the bioethanol content was performed using Chromatography gas. The result showed that a slight increase in sulphuric acid concentration, triggered higher glucose production, reaching optimum level at 0.1 M, then lowered gradually. Furthermore, as fermentation time increased, bioethanol yield from glucose conversion also increased, reaching a peak at day 4, then decreasing gradually on subsequent days. This study revealed that 10 g of spent coffee ground, subjected to hydrolysis using 200 ml of 0.1 M sulphuric acid, run at 55 °C for 60 min, and fermentation over 4 days period, produced 40% bioethanol.

Keywords: Spent Coffee Ground · Hydrolysis · Fermentation · Bioethanol

1 Introduction

Bioethanol is a promising alternative source of green energy. Its utilization is the potential in reducing worldwide reliance on conventional non-renewable fossil fuels. A number of unused materials have been investigated by scientists for the production of bioethanol, for example from food left-over [1], vegetable or fruit waste [2], expired cookies [3], microalgae [4, 5], grain residues such as coffee [6] and lignocellulose biomass such as rice straw [7].

Bioethanol production comprised five steps, namely: pretreatment, hydrolysis, fermentation, distillation, analysis, and biofuel testing. Pretreatment is a lignocellulosic bioconversion, aiming at lignin-segment detachment from the lignocellulose materials.

N. I. Inda et al. (Eds.): ISST 2022, APR 7, pp. 208–214, 2023.
https://doi.org/10.2991/978-94-6463-228-6_23

This process lowered the degree of polymerization and cellulose-crystal characteristics, and improved hydrolysis efficiency [8]. Pre-treatment methods commonly used biological or chemical agents and physical approaches.

Hydrolysis involved adding water in breaking down large molecules into smaller segments, upon which its monomers, are still part of the original substances. This polymer breakdown process could be assisted by the presence of chemical agents and/or enzymes. Acid-promoted hydrolysis using sulphuric acid and hydrochloric acid [9], base-promoted hydrolysis using sodium hydroxide [10], as well as enzymatic hydrolysis [11] have been applied in bioethanol production.

The fermentation step converted glucose into crude bioethanol, which was later purified by the distillation method. Bioethanol content analysis from fermentation relied on the resource availability of the applied technology. The selection of the microorganism for the fermentation step was influenced by biomass feedstock. For instance, to produce alcohol from glucose, fructose, and sucrose, *Zymomonas mobilis*, a gram-negative, rod-shaped bacterium was selected because of its well-tolerant characteristic towards high sugar concentration, capable of producing a great amount of alcohol. *Saccharomyces cerevisiae* yeast was applied, due to its high glucose-ethanol conversion rate and its prominent catalytic feature in transforming hydroxymethyl furfural and furfural into non-inhibitory substances during fermentation [12]. The optimum conditions for this fermenting organism were at 30 °C and pH levels ranging from 4 to 5.5 [13].

Spent coffee grounds as carbohydrate-rich feedstock is the potential for generating bioethanol [14]. In this study, bioethanol was produced from spent coffee grounds, subjected to pre-treatment followed by sulphuric acid-promoted hydrolysis, and then fermentation with *Saccharomyces cerevisiae.* The effect of various sulphuric acid concentrations and periods of fermentation toward bioethanol yield were evaluated.

2 Material and Methods

2.1 Material and Apparatus

Material: spent coffee grounds, distilled water, sulphuric acid, yeast *S. cerevisiae.* Apparatus: autoclave, analytical balance, glassware, stirring rod, PH meter, erlenmeyer, rotary shaker, distillation unit, GC (HP 5890 series II).

2.2 Pre-treatment

Spent coffee ground collected from a cafe in Balikpapan, was dried under adequate sunlight for 3 days, followed by sieving using 80 mesh sieve. Next, 25 g of sieved spent coffee ground was soaked into glassware containing 250 mL distilled water. After being covered by an aluminum foil layer, the mixture was put into the autoclave, operating at 121 °C for 15 min. Filtration was then carried out; the filtrate (product A) was secured for the subsequent step, whilst the pre-treated spent coffee ground sediment was used for hydrolysis.

2.3 Hydrolysis with Sulphuric Acid

10 g of pre-treated spent coffee ground sediment was added into 200 mL sulphuric acid of various concentrations (0.01 M - 0.2 M), and stirred well using a glass rod until homogenous. Then, using Erlenmeyer covered with an aluminum foil layer, the mixture was heated at 55 °C for 60 min. Filtration was then carried out; the filtrate (product B) was preserved for further use.

2.4 Fermentation

Products A and B were poured into 500 ml Erlenmeyer, then 0.1 M sodium hydroxide was dripped until the pH solution reached 5.5. After that, 15 g *S. cerevisiae* yeast was added into the solution. Next, Erlenmeyer containing the solution was covered by tissue and kept on a rotary shaker at 30 °C, 100 rpm, and PH level around 4–5 for 2 up to 10 days. This fermented solution was then filtrated using Whatman filter paper; the filtrate is known as crude bioethanol (product C).

2.5 Distillation

The crude bioethanol (product C) was distilled at 78 °C using a set of distillation units to obtain the final bioethanol (product D).

2.6 Bioethanol Content Analysis

Gas chromatography (GC) is equipped with a flame ionization detector (FID), column temperature of 170 °C, and injector temperature of 250 °C. The inert N_2 gas was flowed at a constant temperature, and pressure of 0.5 bars. The final bioethanol to be assessed (Product Ds) was injected into the GC column through a heated injection port, operating at optimized conditions. The resulting chromatograms illustrated time retention and surface area which were plotted into calibrated curves against standard ethanol concentration. Bioethanol content was determined by plotting the peak area, being compared to standard ethanol concentration.

3 Results and Discussion

3.1 Pre-treatment

The result showed the average water content of spent coffee grounds was 2.88%. This relatively low water content discouraged grows of microorganisms, allowing longer storage time. The sieving method using 80 mesh sieves was conducted to decrease the particle size of the spent coffee ground. Reducing particle size contributed to a shorter chain/branch of polymer involved in the sample-reactant interaction, assisting the lignin removal process [15]. Smaller particle sizes also increased the surface area of treated spent coffee grounds, enhancing the hydrolysis process [16].

According to Farrukh et al. [17], the pre-treatment process led to porous enhancement, lignin cell degradation, and cellulose-crystal reduction. It also enhanced sugar formation by preventing carbohydrate loss, and elicited better yield, thus reducing production costs. The dark color of the pre-treated filtrate (product A) was evident due to the lignin fraction decrease in the solution.

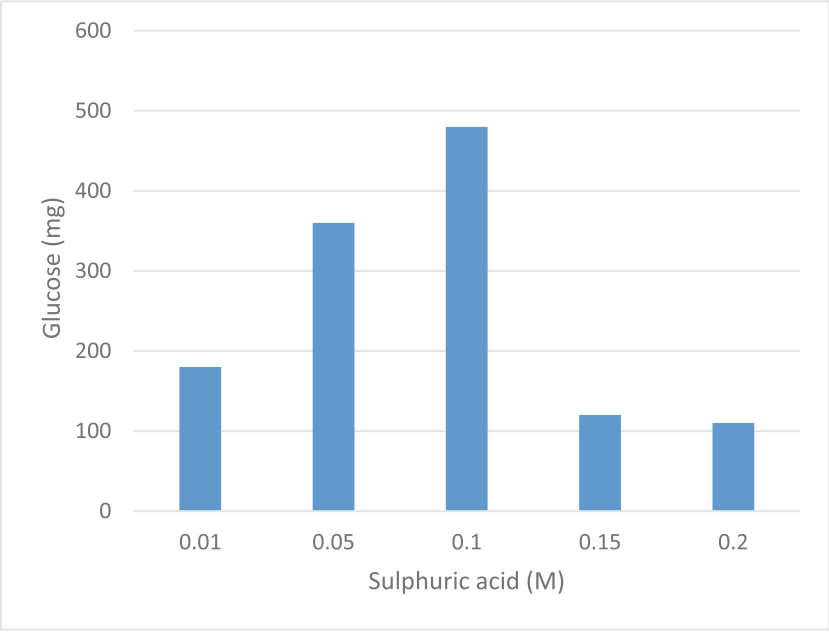

Fig. 1. The effect of sulphuric acid concentration on glucose content

3.2 Hydrolysis with Sulphuric Acid

Figure 1 showed the effect of sulphuric acid concentration on glucose content. It was found that an optimal glucose concentration of around 480 ppm was generated on hydrolysis using 0.1 M sulphuric acid. A slight increase in sulphuric acid concentration initially led to higher glucose production. This occurred because a higher concentration of sulphuric acid triggered cellulose and hemicellulose degradation into glucose. Yet, further increase in acid concentration decreased glucose levels as glucose and its derivative further degraded, forming furfural and hydroxymethylfurfural [18]. Hydrolysis is targeted at breaking cellulose, hemicellulose, and carbohydrate into simpler sugar known as glucose. Sulphuric acid was applied as a solvent in this study because as a strong acid, it acted as a proton donor in an aqueous solution. After losing its hydrogen ions, sulphuric acid turned into acid conjugate, causing unstable conformation such that C-O bonds stretched and broken down. Dissociation of water into H^+ ions and OH^- ions, triggering OH^- ions to interact and then attach to carbonium ions, giving off glucose and proton. The dissociated protons would attach to the oxygen part of the glycoside on the two units of a glucose molecule. This chemical reaction continued, until cellulose fully hydrolyzed into glucose [19].

3.3 Fermentation Period

Figure 2 showed the effect of fermentation time on bioethanol yield. It showed that there has been a fluctuation in bioethanol yield within 2–10 days. There was an increase in the

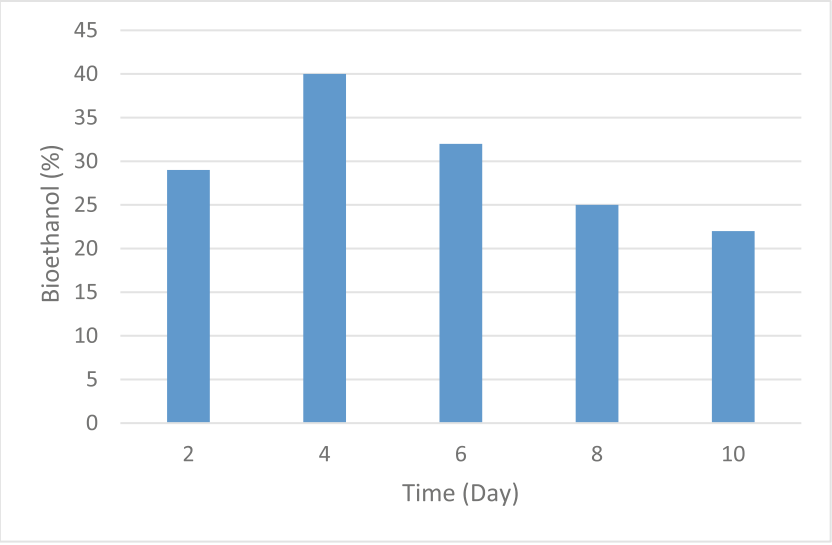

Fig. 2. The effect of fermentation time on bioethanol yield

bioethanol yield on day 2, reaching the peak on day 4, then followed by a gradual decrease in the bioethanol yield on subsequent days (days 6, 8, and 10). In this study, an optimal bioethanol yield of approximately 40% occurred on day 4 of the fermentation period. This phenomenon was in accordance with the fact that a longer period of fermentation time resulted in an increased amount of the fermenting organism, facilitating more glucose conversion into bioethanol. This glucose-bioethanol conversion would continue to rise, up to the level at which it was intolerable for the yeast to carry out this process. A fermentation period beyond 4 days might generate other compounds such as ester, decreasing the bioethanol yield [20]. The yeast also consumed glucose to survive, as subjected to a longer incubation period, thus further lowering the yield of bioethanol.

4 Conclusion

High carbohydrate contents in spent coffee grounds make it possible for bioethanol production. The yield of the produced bioethanol was influenced by hydrolysis and the fermentation process. This study showed that hydrolysis using 0.1 M sulphuric acid, produced around 480 ppm of glucose, and fermentation using *S. cerevisiae* for 4 days yielded approximately 40% bioethanol.

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