

Potential of Bioethanol Production and Optimization Test from Agricultural Waste: The Case of Wet Coffee Processing Waste (Pulp)

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Abstract-The objective of this study was to evaluate the feasibility and to find the suitable condition of bioethanol production from coffee pulp by using commercial bakery yeast, i.e., *S. cerevisiae*. To conduct this study, fermentation was held at temperature 30 °C and pH 5. Coffee pulp was hydrolyzed by refluxing, a solid to liquid ratio of 1:10, using dilute sulphuric acid (1, 2 and 4 % H₂SO₄ (v/v)) and distilled water at hydrolysis time of 1, 2, 4, 6 and 10 hours keeping boiling temperature. 90 % maximum total sugar concentration was obtained at 4 hours acid free hydrolysis. Based on these hydrolysis results, fermentation process was performed.

In the process, it was observed that ethanol concentration decreased with an increase in acid concentration, hydrolysis time and fermentation time. The maximum result was obtained with distilled water hydrolysis for 4 hours and 24 hours fermentation. Under these conditions maximum ethanol concentration production was 7.4 gram per litre (g/L). The result indicated that being available in plentiful amounts and non-edible material, coffee pulp will be potential feedstock for bioethanol production in Ethiopia.

Key words- Coffee pulp, hydrolysis, fermentation

1. Introduction

In the 20th century, the world economy has been dominated by technologies that depend on fossil energy, such as petroleum, coal, or natural gas to produce fuels, chemicals, materials and power [1]. As concern about global warming due to dependence on fossil fuels grows, increase in the price of petroleum and crude oil products, the search for renewable energy sources has become a matter of widespread attention [2]. Replacing petroleum with biofuel can reduce air pollution, improve rural economies by creating job opportunities and raising farm incomes, diversify energy portfolios, minimize dependence on foreign oil and improve trade balances in oil-importing nations. To reduce the net contribution of GHGs to the atmosphere, bioethanol has been recognized as a potential alternative to petroleum-derived transportation fuels and cooking fuels [3].

Biomass is a potential renewable energy source that could replace fossil energy for transportation [4]. The use of food crops (like corn, maize, sorghum) for biofuel production may cause inflation of cost of these crops leading to food insecurity. To alleviate such problems, alternative and non-edible agricultural products must be investigated.

Coffee pulp represents the most abundant and non-edible agricultural waste obtained by wet process from red cherries of coffee. An availability of 525,000 tonnes per year of coffee residue has been estimated in Ethiopia, obtained from wet coffee milling [5]. Use of coffee pulp and other by-products has become a priority in coffee producing countries for economic, ecological and social reasons. Sugar contents in coffee pulp hydrolysates are xylose (0.08-3.23), arabinose (0.23-11.26), fructose (0.9-3), glucose (1.30-6.31), sucrose (0.08-3.96), and maltose (0.01-3.50), expressed in gram per

litre (g/L) [6]. The above literature showed that a wet coffee residue will be desirable and very beneficial when it is used as raw material for bioethanol production.

Therefore, the aim of this work was to study the suitable hydrolysis condition of coffee pulp with diluted sulphuric acid and distilled water, and determining the influence of acid concentration and retention times. Also to evaluate the feasibility of ethanol production by fermentation of coffee pulp by using commercial bakery yeast such as *Saccharomyces cerevisiae*.

2. Methodology and Materials

2.1. Sample Preparation

Wet coffee residue (coffee pulp) of *Coffea arabica* was collected in icebox from a pulping center, Jimma Zone, Manna district, Doyokebele and then taken to Addis Ababa University, Chemistry Department laboratory for analysis. The pulp was oven-dried at 60 °C for 48 hours (to moisture content of 15 %), grinded by coffee grinder and sieved [6]. The samples were stored in hermetically closed plastic containers at room temperature, until required for treatments.

2.2. Hydrolysis

Coffee pulp was hydrolyzed with dilute sulphuric acid (H₂SO₄) at different concentrations (1, 2 and 4 % H₂SO₄ (v/v)). In order to break down the cellulose and hemicelluloses into simple sugar the ground coffee pulp sample was maintained at solid to liquid ratio of 1:10, in 250 mL round bottom flask, and refluxed, retaining samples of 1, 2, 4, 6 and 10 hours for subsequent fermentation experiments. Similarly, the hydrolysis experiment was repeated with distilled water without using dilute sulphuric acid. After hydrolysis the liquid fraction of the hydrolysate samples were cooled, filtered, collected, and adjusted to pH 5 by adding concentrated sulphuric acid and 2N Sodium hydroxide, and the solutions were prepared for fermentation.

2.3. Fermentation

Microorganism

The yeast *S. cerevisiae*, purchased from local market in Addis Ababa, Ethiopia, was used in all experiments throughout this work.

Fermentation process

After hydrolysis, the flasks containing the hydrolyzed samples were covered with cotton wool, wrapped in aluminium foil, autoclaved for 15 minutes at 121 °C and allowed to cool at room temperature. Fermentation was carried out in 250 mL Erlenmeyer flask with 3 g/L of yeast (*S. cerevisiae*) at incubation temperature of 30 °C [7]. Ethanol concentration was analyzed by gas chromatography

at different fermentation times (12, 24, and 48 hours). Samples were withdrawn every 12 hours and the fermentation was carried out for 48 hours.

2.4. Analytical Methods

All the fermented solutions were centrifuged at 10,000 rpm for 5 minutes to separate supernatant. After centrifugation, the supernatant was filtered and then analyzed for ethanol concentration by gas chromatography.

2.4.1. Determination of Sugar Content

The amount of sugar in the hydrolyzed samples was determined by Fehling method. 50 millilitre (mL) of hydrolyzed sample solution was dissolved in 10 mL of distilled water and 2 mL of concentrated HCl (hydrochloric acid) was added and boiled. The obtained sample was neutralized with NaOH (sodium hydroxide) and the solution was made up to a volume of 300 mL and taken into the burette. The 5 mL of Fehling A and 5 mL of Fehling B were taken and mixed with 90 mL of distilled water in 250 mL Erlenmeyer flask and Methylene blue indicator was added. The solution in the flask was titrated with burette solution in boiling conditions until disappearance of blue colour and the volume at which brick red colour observed were recorded. For each sample the sugar content was calculated by using the formula given below [8]:

$$\text{Sugar Content(\%)} = \frac{300 \text{ mL} \times f}{V} \times 100$$

Where: *f* - Fehling factor (0.051); *V* - volume used in the titration (titrate value) (mL).

2.4.2. Gas Chromatographic Determination of Bioethanol

The ethanol concentration was determined by gas chromatography. Gas chromatograph (DANI GC 1000) equipped with flame ionization detector (FID) was employed for the separation and quantification of ethanol. A fused silica capillary column (30m 0.32mm) coated with 95 % methylpolysiloxane (stationary phase) was fitted into the instrument to provide on column injection. The injector and detector temperature were maintained at 210 and 250 °C, respectively. The oven starting temperature was 50 °C, one minute hold time with heating rate of 30 °C per minute to 155 °C. Nitrogen was used as carrier gas at a flow rate of 0.5 bars and for Hydrogen at 0.65 bars was adjusted. The concentration of ethanol in the samples was determined using isopropanol as internal standard.

2.4.2.1. Standard Solution of Ethanol

Internal standard concentration spiking solution was prepared, using reagent grade isopropanol (99%). The internal standard spiking solution was added in the same proportion to every standard and sample. The internal

standard concentration was 0.9 g/L universally throughout the experiment.

Standard solutions of ethanol was prepared containing 2, 3, 5 and 7.5% (v/v) of ethanol (96%) in distilled water and all containing the internal standard of 0.9 g/L isopropanol as shown in Figure 1 below.

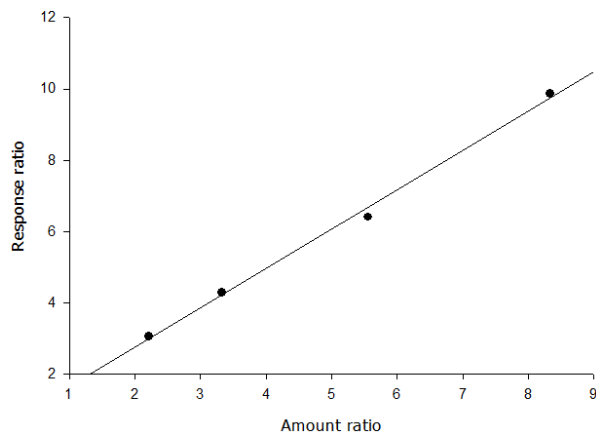


Fig. 1. Calibration curve of ethanol standard solution

Where: Response ratio = Area peak of ethanol/Area peak of isopropanol; Amount ratio = concentration of ethanol/concentration of isopropanol; $y = 1.102x - 0.540$, $R^2 = 0.996$ (y - response ratio and x - ethanol concentration).

The area under peak was determined for samples and by comparing with standard curve the concentration of ethanol (g/L) was calculated. From standard solutions a micro litre of solution was injected and chromatogram was recorded.

3. Results and Discussion

3.1. Effect of Acid Concentration on Sugar Content

In this section, the results of the experiment carried out on coffee pulp for bioethanol potential through distilled water and acid hydrolysis, the effect of acid concentration on the amount of glucose formed was investigated and discussed here under. The sugar content of the coffee pulp hydrolysates was presented in Table 1 below.

Table 1. The sugar content of coffee pulp in different acid concentration hydrolysates

Types of hydrolysates	Amount of sugar content (%)
Distilled water	90
1% H ₂ SO ₄	75
2% H ₂ SO ₄	66.5
4% H ₂ SO ₄	50

Table 1 shows that the maximum reducing sugar concentration of 90% was produced from distilled water hydrolysate of coffee pulp followed by the production of 75, 66.5 and 50% of sugar from 1, 2, and 4% dilute sulfuric acid treated hydrolysate, respectively. This shows that distilled water hydrolysis is more effective in simple sugar production than dilute sulfuric acid of different concentrations (1, 2, and 4%) hydrolysis. The result showed that the amount of sugar

obtained decreases as the acid concentration increases and reaches maximum in acid free hydrolysis.

The decrease of sugar content in acid treated samples with increasing of acid concentration may be due to degradation of monomeric sugars (xylose, glucose) to furfural and HMF (hydroxymethylfurfural) or may be derived from dehydrating or oxidizing by sulfuric acid on glucose or it could be attributed from the conversion of glucose to levulinic and formic acid which leads to decrease in glucose yield. These substances are toxic substances for yeast and can inhibit the yeast growth [9].

3.2. Effects of Acid Concentration and Hydrolysis Time on Bioethanol Concentration

3.2.1. Optimization of acid concentration for maximum amount of bioethanol production

The experiment was conducted for 1 hour hydrolysis, using acid concentrations (0, 1, 2 and 4% H₂SO₄) and 24 hours fermentation, as shown in Figure 2 below.

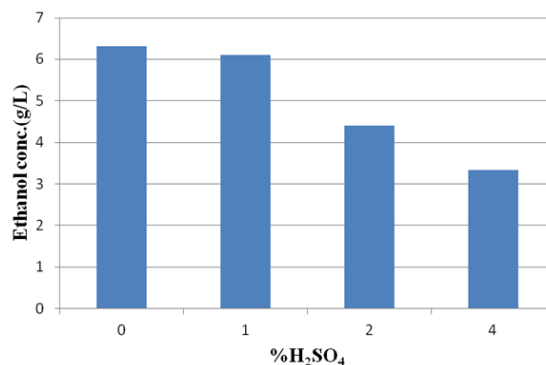


Fig. 2. The effect of acid concentration on bioethanol concentration

Figure 2 showed that increasing the acid concentration decreased the production of ethanol. Acid concentration of 1, 2 and 4% H₂SO₄ for 1 hour hydrolysis, resulted the ethanol concentration of 6.097, 4.395 and 3.323 g/L, respectively. Dilute acid hydrolysis resulted low ethanol production when compared with distilled water hydrolysis. The maximum ethanol concentration of 6.315 g/L was obtained from coffee pulp hydrolyzed with distilled water, which is an optimum condition. This decrease in bioethanol concentration may account for the further sugar degradation that occurred under the severe acidity. Overall, these results indicate that extreme acidity had an unfavorable effect on sugar conversion of coffee pulp [9].

3.2.2. Optimization of Hydrolysis Time for Maximum Amount of Bioethanol Production

Based on the acid optimization for bioethanol production, distilled water hydrolysis was selected as an optimum condition and employed for the second series of the experiment. The effect of hydrolysis times (1, 2, 4, 6, and 10 hours) on bioethanol yield was investigated under the constant conditions of distilled water and 24 hours

fermentation (Fig. 3). The effect of hydrolysis time of coffee pulp on the bioethanol yield was significantly different from that of the acid concentration. Slightly prolonging the hydrolysis time significantly increased bioethanol concentration and then started to decline after 4 hours hydrolysis.

As shown in Figure 3 below, hydrolysis time optimization was performed in distilled water and 24 hours fermentation.

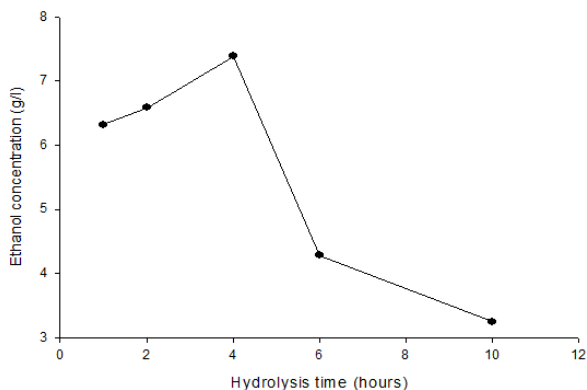


Fig. 3. The effect of hydrolysis time on bioethanol concentration

Figure 3 showed that at 1, 2, 4, 6 and 10 hours hydrolysis of coffee pulp, 6.315, 6.582, 7.380, 4.279 and 3.250 g/L bioethanol concentration was obtained, respectively. The maximum bioethanol concentration of 7.380 g/L was achieved at 4 hours hydrolysis time. However, as hydrolysis time increased from 4 hours it resulted in decreasing concentration of bioethanol. The reason for this could be that longer residence time makes the sugars degraded to form inhibitors (furfural and HMF) [9]. Therefore, distilled water hydrolysis and 4 hours residence time were selected as the optimum conditions for hydrolysis of coffee pulp for bioethanol production.

3.3. Effects of Fermentation time on bioethanol concentration

3.3.1. Optimization of fermentation time for maximum amount of bioethanol production

Figure 4 shows the production of bioethanol from coffee pulp at different fermentation time under fixed hydrolysis time and with distilled water. The hydrolysates obtained from 4 hours hydrolysis of the coffee pulp substrates with distilled water was collected and used for the subsequent fermentation experiments.

This experiment was done based on the above optimization conditions for optimum bioethanol production with distilled water as shown in Figure 4 below.

The concentration of bioethanol increased with increasing fermentation time and decreased at the end of fermentation time. Maximum ethanol concentration, 7.4 g/L was obtained at 24 hours and the result started to decrease after 24 hours of fermentation time (Fig. 4). The figure also indicated that the lowest concentration of bioethanol

production of 7.339, 7.114 and 7.028 g/L was obtained at fermentation time of 16, 36 and 48 hours, respectively. From the optimization experiment, the highest concentration of ethanol was achieved at 24 hour of fermentation and started to level off.

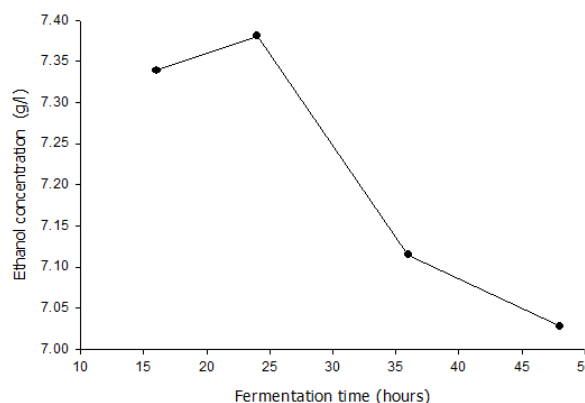


Fig. 4. The effect of fermentation time on bioethanol production

The bioethanol production decreased as the fermentation time increased beyond 24 hours, this might be due to the consumption of sugar by the microorganisms for ethanol production or the hydrolyzate does contain significant levels of metabolic inhibitors (e.g., furfural and HMF) that can interfere with fermentation [10].

Based on this finding, acid free distilled water was employed to investigate the fermentation time dependency of the ethanol concentration. At this point it is worthwhile to mention that the concentration of ethanol obtained by the hydrolysis of the coffee pulp using distilled water, which is about 7.4 g/L was highly satisfactory compared to the maximum amount of ethanol obtained from the enzymatic fermentation of Barley straw (10 g/L) [11], the maximum ethanol concentration obtained by the batch fermentation of acid hydrolyzate of coffee husk using *S. cerevisiae* (13.6 g/L) [7], 11 g/L of ethanol formed from Wheat stillage hydrolyzate [12], 59 g/l from cassava starch hydrolyzate [13], 16.8 g/L of ethanol from Corn stover [14] and wheat straw (18.1 g/L) and sweet sorghum bagasse (16.2 g/L) [15]. The result is much higher than the maximum amount of ethanol from Corn stalks (5 g/L) [11].

A comparison of ethanol production with literature data is can be observed that production of ethanol by fermentation of coffee pulp was quite satisfactory in comparison to literature data for other residues, given all residues that provided higher ethanol production were either supplemented with sugar or underwent hydrolysis/saccharification. Furthermore, there are many possibilities for improving ethanol production based on this type of residue, including the addition of pre-treatment steps (enzymatic saccharification), use of other microorganisms, simultaneous saccharification and fermentation, and others.

4. Conclusion

Coffee pulp is promising lignocellulosic feedstocks for bioethanol production. One of the most important factors in

the acid treatment of lignocellulose is the determination of optimal conditions required to provide the maximum yield of fermentable sugars and the least amount of inhibitors. In this study, the feasibility of ethanol production from coffee pulp by means of dilute acid and distilled water hydrolysis techniques and ethanol fermentation time by *S. cerevisiae* was investigated. Dilute acid and distilled water hydrolysis was applied to produce simple sugars from coffee pulp which followed by fermentation for production of bioethanol. The bioethanol production from coffee pulp and optimization test have shown that distilled water is preferable than dilute acid hydrolysis. The optimization study showed that the highest bioethanol concentration of 7.4 g/L was observed under the optimum conditions of with distilled water hydrolysis for 4 hours by keeping boiling temperature with reflux, and fermentation time of 24 hours held at 30 °C with backer yeasts, which is appreciable.

The results obtained in this study have demonstrated that efficient ethanol production from coffee pulp is possible. Coffee pulp hydrolysis without the addition of dilute sulphuric acid proved more efficient ethanol yield than with dilute sulphuric acid. The production of ethanol from coffee pulp is a significant finding that can constitute a valuable way of using derivative products from coffee beans at farm level.

Expanding ethanol production could entail diverting valuable cropland from producing cereal crops needed to feed people to producing cereal crops for ethanol factories.

Based on these facts, compared to food crops coffee pulp which is an agricultural waste is a promising alternative feedstock for bioethanol production. The use of this waste as an alternative can also reduce the environmental impacts arising from dumping of the waste directly to the nearby rivers and could also contribute to the solution of fossil fuel replacement in Ethiopia. Considering the remarkable potential of ethanol that can be produced from coffee pulp further improvement is still needed for maximum results especially in the fermentation processes.

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