Bioethanol production from finger millet (*Eleusine coracana*) straw

Teshager Mekonnen Tekaligne, Abebe Reda Woldu, Yeshitila Asteraye Tsigie*

Bahir Dar University, College of Science, Department of Chemistry, P.O. Box 79, Bahir Dar, Ethiopia

ABSTRACT

The possibility of producing bioethanol from the biomass of finger millet straw was studied. The effects of temperature, acid concentration, hydrolysis time, and substrate concentration were investigated. The result showed that a maximum sugar content of 79.04 and 82.01 %w/w was achieved using phenol-sulfuric acid and Fehling method, respectively, from hydrolysis of 10 % biomass concentration at 2 % sulfuric acid, 35°C reaction temperature, and 4 days of hydrolysis time. The optimized hydrolyzate sample was fermented at optimized pH 6.0, 4 g/L yeast concentration, 32.5 °C reaction temperature, 4 days of fermentation time, and maximum of 7.28 %w/v of ethanol content was obtained using Pycnometer measurement. In general, the bioethanol achieved from FMS (7.28 %) at optimized conditions were highly promising and hence, it can be employed as an alternative lignocellulosic feedstock for bioethanol production rather than using food crops such as corn, sugarcane, etc.

Keywords: Acid hydrolysis, bioethanol, finger millet straw, fermentation, *Saccharomyces cerevisiae*.
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INTRODUCTION

Ethanol production through biotechnological methods has acquired considerable interest due to possible utilization of bioethanol as an alternative fuel. The rises in prices and environmental problems caused by fossil fuels have contributed to this recent interest of alternative energy sources. Consequently, research efforts have become more focused on low-cost lignocellulosic materials derived from agricultural and forest residues along with herbaceous materials and municipal wastes (Yeshitila Asteraye *et al*., 2013).

Utilization of bioethanol can reduce the world's dependence on fossil fuels, in addition to decreasing net emissions of greenhouse gases. Burning fossil fuels such as coal and oil release CO_2, which is a major cause of global warming (Erdei *et al*., 2010), while bioethanol is clean, safe, environmentally friendly. Besides, the short round of growing plants, burning fuel made from them does not contribute CO_2 to the atmosphere (Kumar *et al*., 2009; Zhao and Xia, 2010). Ethanol contains 35% oxygen that facilitates total combustion of fuel and hence decreases particulate emission that causes health problem to living things (Ali *et al*., 2011).

About 6,000 varieties of millet with different colors like pale yellow, gray, white, and red are found in the world. Originally, the millet varieties were originated from both Africa and Asia. Finger millet was taken to India and Europe about 3,000 years ago and at the beginning of the Christian era, respectively (Molla Fentie, 2012).

Finger millet can be grown in marginal lands beneath low input system and in a broad range of altitudes. The seeds can be stored for a long period

*Corresponding author: yeshiasteraye@yahoo.com

and it has a high malting quality. It is least prone to insect pests and diseases. In Ethiopia, finger millet is the 6th important crops after teff, wheat, maize, sorghum and barley. It comprises about 5 percent of the total land devoted to cereals. It is mainly grown in North Gondar, West Gojam, West Wollega, some parts of Tigray, and Benishangul Gumuz regional states of Ethiopia (Singh and Raghuvanshi, 2012).

Straw is one of the plentiful lignocellulosic waste feedstocks in the world. It is a byproduct of cereal crop production and a great bioresource (Singh and Raghuvanshi, 2012). The study utilized straws of finger millet from Pawe Woreda agricultural research center and farmer farm lands, Ethiopia, as a major raw material for the production of bioethanol using S*accharomyces cerevisiae*.

EXPERIMENTAL

Materials

Drying oven (GALLENKAMP), electrical grinder (ZAIBA super blender), electrical balance (ae ADAM, PW 124), UV-Vis spectrometer (NV203 spectrophotometer), fermentation and distillation set up, pycnometer (50 mL, KW 14/23), hydrometer (Araometer nach Dichte fur schwefelsaure Temp. 20^{o}C), digital pH meter (pH meter 3310, JENWAY), ICP-OES (ICP-spectrometer, ULTMA-2), and heat mantle (Labmaster, isopad, type LMUL/ER/1L with 220/240 volts), were the equipment used in this work.

Chemicals

Methylene blue indicator, Fehling A and Fehling B (the detail is mentioned in Fehling method below), sulfuric acid, D (+) – glucose as standard (PANREAC, MONTPLET and ESTEBAN SA, Barcelona. Madrid), and calcium hydroxide were the major chemicals used in this study. Moreover yeast (*Saccharomyces cerevisiae*) was the biological material that was used in this study to facilitate the fermentation process.

Sample Collection and Preparation

Finger millet straw (13 kg) was collected in polyethene bags from Pawe Woreda Agricultural Research Center (Benshangul Gumz, Ethiopia) and farmer farm lands. It was cut by sickle into pieces of about 3-5 cm length for drying and grinding. The sample was dried through direct sunlight to obtain an easily crushable material. After drying, the sample was ground with electrical grinder. The maximum ground particle size of 1-2 mm.

Pretreatment

100 mL of a 0.5% sulfuric acid was added to 50 g of the sample to remove lignin, reduce cellulose crystallinity and increase the porosity of the materials. The mixture was heated to 125 - 130 ^{o}C under a pressure of 25 psi for 1 hr. The pretreated sample was collected and used in the subsequent step. Confirmatory test by iodine (test for the presence of starch) was carried out. Appearance of helically coiled blue complex ascertained that the pretreated material was actually free of lignin (Mishra *et al*, 2011).

Hydrolysis of the Pretreated Samples

50 g of pretreated FMS was used for the triplicate experiments of hydrolysis and the factors investigated for hydrolysis were time (1-5 days), temperature (25, 30, 35, 40 and 45 ^{o}C), acid concentration (0 - 4 %), and biomass concentration (6.25, 7.14, 8.33, 10.00, 12.5, and 16.61 % w/v). The effect of substrate (biomass) concentration was studied by adding 50 g sample in 800, 700, 600, 500, 400 and 300 mL of distilled water containing 2 % of sulfuric acid.The mixture was transferred to glass bottles and sealed to avoid vaporization of acid due to heat. The liquid fraction of the hydrolysate sample was filtered and its sugar content was determined by Fehling and phenol-sulfuric acid methods.

Fehling method

The Fehling method was conducted as described elsewhere (Periyasamy *et al*., 2009; Adane Muche and Sahu, 2014; Sahu, 2014). The filtered hydrolyzed sample solution (50 mL) was neutralized with the required amount of 4 M NaOH and 2.5 M HCl and the solution was made up to a volume of 300 mL and taken into the burette. Then, 5 mL of Fehling A (prepared by dissolving 34.6 g of copper (II) sulfate pentahydrate in 500 mL distilled water) and 5 mL of Fehling B solutions (prepared by dissolving 125 g of potassium hydroxide and 173 g of potassium sodium tartrate tetrahydrate in 500 mL of distilled water) were taken and mixed with 90 mL of distilled water in 250 mL Erlenmeyer flask to which methylene blue indicator was added. The solution in the flask was titrated with solution in the burette under boiling situation until departure of blue color and the volume of the titrate causing brick red color was noted. For each sample the sugar content was calculated by using Eqn. (1).

$$\text{Sugar content (\%)} = \frac{300 mL.f}{V} \text{x100\%} \qquad (1)$$

Where: *f* is Fehling factor (0.051), V is the volume of titrant used.

Phenol-sulfuric acid method

Glucose dehydrates to furfural derivative (hydroxymethylfurfural) in hot acidic medium, when this derivative reacts with phenol, it develops detectible color (Dubois, 1956) and has 490 nm absorption maxima. Phenol-sulfuric acid method is a widely used colorimetric method for determination of carbohydrate concentration in aqueous solutions. The total sugar concentration was determined by using UV-visible spectrophotometer at 490 nm wavelength of glucose absorbance. Calibration curve was obtained for series of standard glucose solutions and the regression equation was used to calculate the total sugar concentration in the sample. The standard procedure of this method is as follows. A 1 mL aliquot of a sample solution was mixed with 1 mL of 5% aqueous solution of phenol in a test tube. Subsequently, 5 mL of concentrated sulfuric acid was added rapidly to the mixture. The test tubes were kept al room temperature for 10 min at room temperature, and then placed in a water bath for 20 min for color development. Its absorption at 490 nm was recorded. Blank solution was prepared in the same way as above, except that the 1 mL aliquot of a sample solution was replaced by distilled water (Albalasmeh *et al*., 2013).

Preparation of Standard and Reagent Solutions

Stock glucose solution was made by dissolving 4 g of glucose in 100 mL of distilled water. To prepare the standard working solutions, 1, 2, 3, 4 and 5 mL aliquots of the stock glucose solution were separately pipetted out into different 100 mL volumetric flasks and subsequently diluted with distilled water to the mark resulting working standard glucose solutions of 0.04, 0.08, 0.12, 0.16, and 0.2 g/mL, respectively.

To determine the calibration curve for standard glucose, 1 mL of each of the standard solutions were pipetted out and taken into a separate test tube. 1 mL of 5% aqueous solution of phenol reagent and 5 mL of 96% sulfuric acid were added. Then, the amount of total reduced sugar content present in the sample solution was calculated using the calibration curve and expressed as gram glucose equivalents (GE) per 50 g of sample (Miliauskas, 2004; Albalasmeh *et al*., 2013).

Fermentation

The fermentation studies were carried out using *Saccharomyces cerevisiae* in the hydrolysates obtained from pretreated and acid hydrolyzed FMS. The specific gravity of the filtered hydrolysates was measured through hydrometer and a separate set of

fermentation experiment was carried out using the pretreated hydrolysates (Galbe and Zacchi, 2002). The pH of the fermentation medium was varied to 4, 4.5, 5, 5.5, 6.0, and 6.5 by adding required amount of 4 M NaOH and 2.5 M HCl. Besides, the yeast was added at a concentration of 2, 3, 4, 5 and 6 g/L, fermentation incubation time was conducted at 2, 3, 4, 5, 6 and 7 days and the temperature was set at 25, 27.5, 30, 32.5, 37.5 and 40 ^{o}C to investigate the optimum conditions. Based on the density of alcohol distillate at 20 ^{o}C, the ethanol yield was determined and expressed in weight % (w/v) by Hydrometer and Pycnometer (Park, 2000; Igwe *et al*., 2012).

The mouths of the flasks were tightly sealed with aluminum foil to maintain anaerobic condition and an outlet was provided to release CO_2. The other end of the outlet was dipped in lime water to confirm the release of CO_2 as it turns lime water milky. Confirmatory tests were carried out to ascertain that the distillate was actually bioethanol as it changes to blue green color in the presence of Jones reagent ($K_2CrO_4 + H_2SO_4$) (Mandal and Kathale, 2012). After fermentation, separation was made using distillation set up at a temperature of 85 ^{o}C for 3 hrs (Faga *et al.,* 2010). Consequently, the yield was calculated using both Hydrometer and Pycnometer measurements using Eqns. 2 and 3, respectively (Park, 2000; Hadeel *et al*., 2011; Igwe *et al.*, 2012).

$$\text{Ethanol \%(w/v)} = 126.58\left(\frac{OSG - FSG}{OSG}\right) \qquad (2)$$

Where: 126.58 is obtained from (Specific gravity of water / Specific gravity of pure ethanol) multiplied by 100%, OSG and FSG are original specific gravity (specific gravity before fermentation) and final specific gravity (specific gravity after fermentation), respectively.

$$\text{Specific gravity of sample} = \frac{(x_2 - x_1)}{(x_3 - x_1)} \qquad (3)$$

Where: x_1, x_2 and x_3 are weight (g) of empty pycnometer, weight (g) of pycnometer + sample and weight (g) of pycnometer + water, respectively.

Metal Analysis

Since the quality of the fuel used affects the engine life and the degree of pollution of the environment, the concentration of metals such as Fe, Mg, Ca, Pb, and Cr in the biofuel was determined using the Inductively Coupled Plasma-Optical Emission (ICP-OES) (Rocha *et al.*, 2010; Hossain *et al*., 2011).

Data Analysis

An Origin Pro8 software and Microsoft excel 2007 were used for the analysis of data collected.

RESULTS AND DISCUSSION

Effects of Different Parameters on Hydrolysis

The Effect of biomass concentration on Hydrolysis

The effect of substrate concentration was investigated at 30 ^{o}C for 48 hrs. The highest sugar content (about 67.04 and 67.52 %w/w by phenol-sulfuric acid and Fehling method, respectively) was obtained at 10% biomass concentration. As presented in Table 1, the sugar content increased with increasing substrate concentration.

Effect of acid concentration on Hydrolysis

From Table 2, the maximum sugar content of 68.72 and 70.65% by phenol-sulfuric acid and Fehling method, respectively, was produced using 2% acid hydrolysate of FMS with minimum yield at 0% acid concentration (27.64%).

This shows that 2 % sulfuric acid hydrolysis is more effective in simple sugar production as compared to 1, 3, and 4 % sulfuric acid hydrolysis. The result showed that the amount of sugar obtained increases as the acid concentration increases from 0-2 % and decreases as the acid concentration increases from 2-4 %.

Table 1. Total sugar content (% w/w) determined at various biomass concentration, 2 % H_2SO_4, 2 days and 30 °C.

Substrate concentration (% w/v)	Amount of sugar content (% w/w)	
	Phenol-sulfuric acid method	Fehling method
6.25	38.53±0.00*	41.70±1.20*
7.14	43.53±1.92*	45.02±1.32*
8.33	56.99±2.90*	56.71±2.10*
10.00	67.04±2.93*	67.52±2.59*
12.50	Out of range	Very high (V**<15 mL)
16.61	Out of range	Very high (V**<15 mL)

*= Standard deviation

Where; V** volume used in the titration (titrate value) (mL).

Table 2. Total sugar content (%w/w) determination of FMS using the two methods at different acid concentration (H_2SO_4) hydrolysates of 10 %w/v biomass concentration, two days, and 25 °C.

Acid concentration (% v/v)	Amount of sugar content (% w/w)	
	Phenol-sulfuric acid method	Fehling method
0	25.14±0.00*	27.64±0.22*
1	58.66±2.70*	59.63±1.23*
2	68.72±2.91*	70.65±1.91*
3	53.93±2.70*	55.317±1.17*
4	35.20±0.00*	36.72±0.51*

*= Standard deviation

The decrement in reduced sugar content with increasing acid concentration from 2-4 % may be due to degradation of monomeric sugars (xylose, glucose) to furfural and HMF. Besides, it 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. But, further analysis needs to be undertaken to confirm the formation and concentration of those expected products.

According to Fadel (2000), when acid concentration is higher than 6 %, not only lower glucose concentration was obtained but an increment in inhibitor concentration was seen. 5-HMF (5-hydroxymethylfurfural) was not detected when using 2 % sulphuric acid. At 6% acid concentration, the concentrations of inhibitors (5-HMF and furfural) were observed. When acid concentration was raised to 10 %, the concentration of HMF and furfural became higher (Fadel, 2000). In this study the maximum reduced sugar from finger millet straw was achieved at 2 % sulfuric acid, which is an optimum condition in acid concentration.

Effect of Hydrolysis Temperature

Temperature is one of the major constraints that determine the sugar yield because temperature exerts a profound effect on conversion of cellulose or hemicellulose to simple sugars. To know the optimum temperature for sugar production, the hydrolysis media were kept at 25, 30, 35, 40 and 45^{o}C (Table 3).

The sugar yield increase due to increasing in temperature from 25^{o}C to 35^{o}C and maximum at 35^{o}C. Beyond this temperature the sugar content was decreased significantly. The maximum sugar yield at 35^{o} C was 68.72 and 70.65 % by phenol-sulfuric acid and Fehling method, respectively. Because, at low temperatures (25^{o}C), the reaction was reduced and it slows the rate of conversion of substrate into reduced sugar. A reason behind significant lower production of sugar at high temperature is degradation of sugar in to unwanted materials. Overall, these results indicate that extreme temperature had an unfavorable effect on sugar conversion of FMS due to formation of 5-HMS and furfural that are toxic for *S. cereviciae* in fermentation (Nutawan *et al*., 2010; Yeshitila Asteraye *et al*., 2013; Sahu, 2014).

Table 3. Determination of sugar content (% w/w) of FMS hydrolysate using the two methods at different temperature of 10 % w/v biomass concentration, two days and 2 % H_2SO_4.

Temperature (^{o}C)	Amount of total sugar content (% w/w)	
	Phenol-sulfuric acid method	Fehling method
25	57.01±2.71*	58.12±1.15*
30	62.01±2.91*	62.05±1.34*
35	68.72±2.91*	70.65 ±1.74*
40	58.66±2.90*	58.85±0.00*
45	51.93±0.00*	52.17±0.93*

*= Standard deviation

Table 4. Total sugar content (%w/w) determination using the two methods at different hydrolysis time of 10 % w/v biomass concentration, 2% H_2SO_4 and 35°C reaction temperature.

Time (days)	Amount of sugar content (%w/w)	
	Phenol-sulfuric acid method	Fehling method
1	48.57±0.00*	49.90±0.91*
2	61.89±2.89*	62.05±1.47*
3	72.08±2.91*	73.98±2.10*
4	79.04±2.92*	82.01±2.31*
5	67.04±2.91*	67.53±1.74*

*= Standard deviation

Effect of Hydrolysis Time

Based on the above optimizations for hydrolysis, 10 % biomass concentration, 2 % acid concentration and 35°C were selected as optimized conditions for hydrolysis.

Prolonging the hydrolysis time significantly increased sugar concentration and then started to decline after 4 days hydrolysis. Table 4 showed that at 1, 2, 3, 4, and 5 days hydrolysis of FMS, 48.56 and 49.9, 61.89 and 62.05, 72.08 and 73.98, 79.04 and 82.01, 67.04 and 67.53 % of sugar content were obtained by phenol-sulfuric acid and Fehling method, respectively. The maximum sugar content, 79.04 and 82.01 %, were achieved at 4 days hydrolysis time for both phenol-sulfuric acid and Fehling methods, respectively. However, as hydrolysis time goes beyond 4 days it resulted in decreasing sugar content. The reason for this could be that longer residence time makes the sugars degraded to form inhibitors (furfural and HMF) (Nutawan *et al*., 2010). FMS has a maximum reduced sugar of 79.04-82.01% which is more comparable as compared to coffee husk (Sahu, 2014), olive-tree biomass (Kumar *et al*., 2009), sweet potato (Kumar *et al*., 2014), etc having 90%, 83%, and 78.19%, respectively.

Therefore, 2% sulfuric acid, 10% biomass concentration, 35 °C, and 4 days residence time were selected as the optimum conditions in hydrolysis of FMS for bioethanol production.

Effects of Different Parameters on Fermentation

There are many parameters which should be considered in fermentation process such as; temperature, reaction time, amount of yeast added, and pH. The effects of those parameters on bioethanol production are important for the successful progress of the fermentation. Considering all the mentioned parameters, the experimental outcomes of those particular results were measured their density using Hydrometer and Pycnometer to determine alcoholic content of the produced bioethanol.

Effect of pH on Fermentation

The effect of pH on ethanol production was studied by conducting from pH 4.0 to 6.5 for yeast strains (*S. cerevisiae*) by keeping initial substrate concentration (4 g/L), initial temperature (30 °C) and 3 days of fermentation period (Table 5).

Table 5. Yield of ethanol at 30 ^{0}C, 4 g/L yeast concentration and for 3 days, but at various pH

pH	Specific Gravity			Yield of ethanol (%w/v)	
	Hydrometer reading				
	Before fermentation	After fermentation	Pycnometer Reading	Hydrometer value	Pycnometer value
4.0	1.030±0.016*	0.996±0.010	0.9935±0.0004*	4.18	4.54
4.5	1.032±0.010*	0.995±0.020*	0.9929±0.0007*	4.54	4.99
5.0	1.038±0.017*	0.995±0.032*	0.9921±0.0007*	5.24	5.59
5.5	1.039±0.051*	0.994±0.026*	0.9916±0.0005*	5.48	5.97
6.0	1.040±0.037*	0.990±0.036*	0.9908±0.0003*	6.08	6.58
6.5	1.037±0.045*	0.996±0.044*	0.9923±0.0004*	5.00	5.44

*= Standard deviation

As shown in Table 5 the maximum ethanol concentration 6.08 and 6.58% by Hydrometer and Pycnometer measurement was achieved, respectively using *S. cerevisiae* culture grown at pH 6.0 and then decreased marginally above this value. Control of pH during ethanol fermentation is important for two reasons: (1) the growth of harmful bacteria is retarded by acidic solution. (2) Yeast grows well in acidic conditions (Tahir *et al*., 2010; Tahir and Sarwar, 2012).

The ethanol yield increased significantly from pH 4.0 to 6.0 beyond this level there is a decrement in ethanol yield. High ethanol production was achieved by using initial pH 5.0 to 6.0 (Fadel, 2000). Osman *et al*., (2011) tested wide initial pH range and confirmed that at pH 3.0 no growth was observed and no ethanol was produced, while pH 6.0 was the optimum for ethanol production. Similar results were obtained when *Ziziphus mauritiana* fruit pulp, potato (*Kufri Bahar*), and mahula (*Madhuca latifolia* L.) were used as a substrate (Akponah and Akpomie, 2011; Duhan *et al*., 2013), respectively.

Effect of Yeast on Ethanol Production

Effect of yeast extract was studied by varying its concentration from 2 to 6 g/L keeping rest of the parameters at their optimal conditions. The effect of yeast extract at different concentration is shown in Table 6.

Table 6 indicates that as the concentration of yeast extract increased from 2 to 4 g/L, ethanol production was also increased from 3.92 and 4.02 to 6.26 and 6.66% by hydrometer and Pycnometer measurement, respectively, however, above this concentration, ethanol production was decreased.

Table 6. Ethanol yield at different amount of yeast extract, 30 °C, 3 days and pH, 6.

Yeast extract (g/L)	Specific Gravity			Yield of ethanol (% w/v)	
	Hydrometer reading		Pycnometer Reading	Hydrometer Value	Pycnometer Value
	Before fermentation	After fermentation			
2	1.031±0.004*	0.999±0.000*	0.9942±0.0006*	3.92	4.02
3	1.031±0.004*	0.991±0.003*	0.9928±0.0007*	4.91	5.06
4	1.031±0.004*	0.980±0.008*	0.9907±0.0011*	6.26	6.66
5	1.031±0.004*	0.994±0.004*	0.9918±0.0007*	5.48	5.82
6	1.031±0.004*	0.998±0.002*	0.9936±0.0005*	4.05	4.47

*= Standard deviation

Many scholars have studied the effect of yeast extract concentrations on sugar consumption for ethanol production and maximum ethanol was achieved from sweet sorghum juice at 9.0 g/L of yeast extract (*S. cerevisiae* NP 01) (Nuanpeng *et al*., 2012), at 2.0 g/L yeast for *S. cerevisiae* MTCC-170 when potato (*Kufri Bahar*) was used as a substrates (Duhan *et al*., 2013).

Effect of Temperature on Ethanol production

Too high temperature destroys yeast, and yeast activity slows down at lower temperature (Yeshitila Asteraye *et al*., 2013). Thus, keeping a specific range of temperature is required. In this study ethanol fermentation was conducted at temperature range between 25-40 °C for optimizations.

From Table 7, the ethanol yield increases as the temperature increases from 25 to 32.5 °C. Beyond this level the ethanol content decreases significantly. The maximum ethanol yield was achieved at 32.5 °C with 6.70 and 7.12% by Hydrometer and Pycnometer measurements, respectively.

At low temperatures, the yeast activity suppresses and the yield slows down. Further, the increasing temperature reduced the percentage of ethanol production and it is mainly due to denaturation of the yeast cells (Periyasamy *et al*., 2009). Duhan *et al*. (2013) studied the effects of temperature on bioethanol yield and observed that maximum bioethanol was produced at 35 °C. Temperatures between 30-35 °C have been usually employed for culturing of yeast and temperature above 35 °C has been found inhibitory to ethanol fermentation due to yeast growth inhibition at higher temperatures (Tahir *et al*., 2010). This study is in good agreement with previously reported works.

The maximum ethanol content was recorded at 30 °C (Rani *et al*., 2010), 32 °C (Asli, 2010), and 28-30 °C (Osman *et al*., 2011) temperatures. Therefore, those observations are almost similar to present work which yields maximum ethanol at 32.5 °C.

Effect of Fermentation Time on Ethanol production

The fermentation was carried out at different time periods (2, 3, 4, 5, 6 and 7 days) and the results are shown in Table 8.

From Table 8, maximum ethanol production was observed after 4 days fermentation (6.92 and 7.28 %

Table 7. Ethanol yield at pH 6, 3 days, 4 g/L yeast extract and different fermentation temperature.

Temperature (°C)	Specific Gravity			Yield of ethanol (%w/v)	
	Hydrometer reading		Pycnometer Reading	Hydrometer Value	Pycnometer Value
	Before fermentation	After fermentation			
25	1.031±0.007*	0.997±0.002*	0.9938±0.001*	4.17	4.32
27.5	1.033±0.005*	0.995±0.005*	0.9928±0.001*	4.65	5.06
30	1.037±0.007*	0.988±0.007*	0.9911±0.002*	5.98	6.35
32.5	1.039±0.010*	0.984±0.004*	0.9907±0.004*	6.70	7.12
35	1.038±0.008*	0.992±0.011*	0.9916±0.001*	5.60	5.97
37.5	1.032±0.015*	0.993±0.003*	0.9929±0.003*	4.53	4.99
40	1.030±0.009*	0.998±0.001*	0.9939±0.001*	3.93	4.24

*= Standard deviation

from Hydrometer and Pycnometer measurements, respectively). Further increase in time period resulted in decreasing of ethanol production. The difference of alcoholic content measured using Hydrometer and Pycnometer are not much significant.

The concentration of bioethanol increased with increasing fermentation time, and decreased in farther increment of fermentation time. From Table 8, the lowest concentration of bioethanol production (4.67 and 4.84 %) through Hydrometer and Pycnometer measurments were obtained at fermentation time of 7 days, respectively. Beyond 4 days of fermentation time the bioethanol yield started to level off. This might be due to the consumption of sugar by the microorganisms for ethanol production or the hydrolysate does contain significant levels of metabolic inhibitors (e.g., furfural and HMF) that can interfere with fermentation (Weil *et al*., 2012).

At this point it is worthwhile to mention that the concentration of ethanol obtained by the hydroly-

Table 8. Ethanol yield at 32.5°C, pH 6.0, 4 g/L yeast extract, and different fermentation time

Time (day)	Specific Gravity			Yield of ethanol (%w/v)	
	Hydrometer reading		Pycnometer Reading	Hydrometer value	Pycnometer Value
	Before fermentation	After fermentation			
2	1.035±0.005*	0.996±0.005*	0.9930±0.000*	4.76	4.91
3	1.036±0.006*	0.995±0.001*	0.9925±0.0013*	5.00	5.29
4	1.042±0.011*	0.985±0.012*	0.9899±0.0017*	6.92	7.28
5	1.040±0.009*	0.989±0.006*	0.9910±0.0011*	6.21	6.43
6	1.039±0.006*	0.992±0.010*	0.9916±0.0015*	5.72	5.97
7	1.033±0.014*	0.995±0.005*	0.9931±0.0018*	4.65	4.84

*= Standard deviation

sis of the FMS using optimum conditions (6.92 and 7.28% from Hydrometer and Pycnometer measurements, respectively) was highly satisfactory compared to the maximum amount of ethanol obtained from acid hydrolysis of groundnut hulls (6.2%) and 5.5% from rise husks (Ali *et al*., 2011), the enzymatic fermentation of mango juice (7-8.5%) depending on the type of mango species) (Reddy, 2007).

The result revealed that ethanol obtained from FMS is a promising substituent for other agricultural products such as mango juice, cassava and corn. FMS is not edible material by human being and hence it can avert food crisis by doing away with food crops for bioethanol production.

Metal Analysis

The concentration of the metals in finger millet straw was obtained as 0.32, 0.82, 1.2, 0.38, and 0.45 mg/L for Cr, Fe, Mg, Pb, and Ca, respectively. From the results of the elemental analysis, the concentration of metals in the produced bioethanol from FMS ranges from 0.32 to 1.2 mg/L. Bioethanol obtained from FMS had smaller value of chromium and lead (0.32 and 0.38 mg/L, respectively) as compared to the other metal concentrations. Therefore, the produced bioethanol is good for engine use and it is an environmentally friendly energy source. Generally, most of the element concentrations followed the ASTM standard that is better for engine use (Iqbal *et al*., 2010; Rocha *et al*., 2010; Hossain *et al*., 2011).

CONCLUSION

The optimizations showed the highest bioethanol concentration was observed at dilute sulfuric acid hydrolysis and fermentation time of 4 days held at 32.5 ^{o}C with *S. cerevisiae*. Both phenol-sulfuric acid and Fehling method in the hydrolysis step, and the Hydrometer and Pycnometer in the fermentation have comparable values. The bioethanol obtained by dilute acid hydrolysis of FMS (7.28%) was highly satisfactory and hence, it is promising lignocellulosic feedstock for bioethanol production as compared to food crops such as corn, sugarcane, etc as it is not disturb the food chain of mankind.

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