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Research article

Bioethanol Production from Avocado Seed Wastes Using *Saccharomyces Cerevisiae*

Abebe Reda Woldu[1, 2, *], Yenework Nigussie Ashagrie[1, 2], Yeshitila Asteraye Tsigie[1, 2]

[1]Department of Chemistry, Bahir Dar University, P.O. Box 79, Ethiopia

[2]Energy Research Center, Bahir Dar University, P.O. Box 79, Ethiopia

Phone: +251 922 744123, Fax: +251 582 202025

[*]Corresponding Author: abebe.reda2@gmail.com

Abstract

This study employs powdered avocado seed wastes as a raw material for the production of Bioethanol. Ethanol content from hydrolysate of powdered avocado seed wastes was analyzed, and the different parameters of fermentation conditions were optimized. The effects of fermentation time, fermentation temperature, and pH on the bioethanol yield were studied. The optimum bioethanol yield was achieved at 3 days fermentation time, pH 5.5, and 30^{o}C fermentation temperature with maximum of 6.365±0.372% (w/v) under the optimized conditions.

Keywords: Bioethanol, Avocado seed wastes, fermentation, renewable energy, *Saccharomyces* cerevisiae

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Introduction

Society has been searching for better sources of sustainable energy for many years. Because of limited access to petroleum-based fuels, rising of fossil fuel costs and its consequences on emission of greenhouse gases, the struggle to maintain a green planet has become more challenging over time. In recent years, energy consumption and global carbon intensity have increased worldwide, reinvigorating worries about potential depletion of fossil fuel (Martinelli and Filoso, 2008) and scarcity of petroleum reserves (Eltawil and Belal, 2009). Such increase, accompanied by increment of geopolitical consequences in oil-producing regions, has instigated many countries to search for alternative forms of energy. However, the burning of fossil fuel and anthropogenic activities results in increasing atmospheric CO_2 concentration in the atmosphere, irritated hazardous changes in the climatic system of the Earth. These problems have enforced some defect to the types of alternative energy that can be used and conditions on how this energy is achieved (Martinelli and Filoso, 2008; Haile, 2003).

The oil reservoirs are diminishing rapidly and as a result gases from the greenhouse are released daily. Global warming significantly increases due to the use of fossil fuels in the form of oil, natural gas and coal, on which modern society depends for energy. Fuel ethanol which is produced from renewable sources can be utilized as an alternative to these fuels. This ethanol fuel is environmentally friendly and helps in saving national energy and in improvement of economy as well (Albalasmeh et al, 2013).

Demand of the energy increases with the increase of the world population and urbanization and thus, development of bioenergy as an alternative energy might help to reduce these problems. Bioenergy can be defined as energy obtained from Biomass, which is the biodegradable fraction of products, waste and residues from agriculture like vegetables and animal origin, forestry and related industries and also, from the biodegradable fraction of industrial and municipal waste. Different forms of bioenergy can be produced from a wide range of Biomass sources, for example, agricultural residues (Hossain et al, 2011; Hossain and Fazliny, 2010).

Biofuel is a promising source of energy because it is generated by the process of photosynthesis, where energy from the sun is captured and transformed into Biomass that can be combusted to produce energy. Moreover, the price of production is relatively low (Martinelli and Filoso, 2008; Oliveira et al, 2005).

Because of the gradual decrement in fossil fuels, bioethanol has got the interest of numerous researchers to utilize it as alternative source of energy throughout the world. Bioethanol is formed from the fermentation of renewable feedstocks for fuel purposes. Besides, bioethanol is considered to be produced from Biomass based materials, other than toxic methyl tert-butyl ether (MTBE) and tert-amyl methyl ether (TAMES). Bioethanol is by now utilizing as additive of gasoline in various parts of the world (Khan et al, 2012).

In this study, the hydrolysates of the reduced sugars obtained from our previous work on hydrolysis of Avocado seed wastes was the major raw materials for the production of bioethanol in an environmentally friendly and cost effective way. The researchers believe that the seed wastes does not disturb human's food chain and it is massive potential source of waste in anywhere as a residues in cities and towns, causing in environmental pollution and unpleasant odor.

Experimental Part

Materials and Chemicals

Drying oven (GALLENKAMP), electrical grinder (ZAIBA super blender), thermometer, Electrical Balance (ae ADAM, PW 124), UV-Vis spectrometer (NV203 spectrophotometer), Spectrum 65 (PerkinElmer), fermentation and distillation set up, Pycnometer (50 mL, KW 14/23), hydrometer (Araometer nach Dichte fur schwefelsaure Temp. 20^oC), digital pH meter (pH meter 3310, JENWAY), ICP-OES (ICP-spectroscopy, ULTMA-2), yeast (*Saccharomyces cerevisiae*), methylene blue indicator, Fehling A (prepared by dissolving 34.6 g of copper (II) sulfate pentahydrate in 500 mL) and Fehling B (prepared by dissolving 125 g of potassium hydroxide and 173 g of potassium sodium tartrate tetrahydrate in 500 mL of distilled water), sulfuric acid, D (+) – glucose as standard (PANREAC, MONTPLET & ESTEBAN SA, Barcelona. Madrid), and calcium hydroxide are the major materials and chemicals used in this study.

Sample Collection

The hydrolysates of the reduced sugars collected from our previous work on hydrolysis of avocado seed wastes at optimized conditions were the major raw materials for the production of bioethanol.

Fermentation

The fermentation studies were carried out using *Saccharomyces cerevisiae* in the hydrolysates obtained from previous study on hydrolyzed of avocado seed samples. The hydrolysates were filtered and specific gravity was measured by Hydrometer before fermentation. A separate set of fermentation experiment with triplicate was carried out in a similar manner using the hydrolysates (Faga et al, 2010). The pH of the fermentation medium was adjusted to 4, 4.5, 5, 5.5, and 6 by adding required amount of 4 M NaOH and 2.5 M HCl, fermentation incubation time was conducted at 2, 3, 5, 7 and 9 days, and the temperatures 25, 30, 32, 35 and 40 ^oC to investigate the optimized condition at optimized yeast extraction of 4g/L (Hadeel et al, 2011). The ethanol concentration was determined based on the density of alcohol distillate at 20 ^oC and expressed in weight % (w/v) by Hydrometer and Pycnometer (50 mL, KW 14/23) measurement (Igwe et al, 2012; Park, 2000). 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 by K_2CrO_4 were carried out to ascertain that the distillate was actually ethanol (Khan et al, 2012). After fermentation separation was made using distillation set up at a temperature of 85 ^oC for 3 hrs (Faga et al, 2010). Consequently, the yield was calculated using both Hydrometer and Pycnometer readings using Equns. 1 and 2, respectively (Hadeel et al, 2011; Igwe et al, 2012; Park, 2000).

$$\text{Ethanol \% (w/v)} = 126.582 \left(\frac{OSG - FSG}{OSG}\right) \quad (1)$$

Where; 126.58 is from; (Specific gravity of water / Specific gravity of pure ethanol), OSG is original specific gravity (specific gravity before fermentation) and FSG is Final specific gravity (specific gravity after fermentation).

$$\text{Specific gravity of sample} = \frac{(x_2 - x_1)}{(x_3 - x_1)} \qquad (2)$$

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

Metal Analysis and IR Characterization of Major Functional Groups

A series of standard concentration of selected metals such as Fe, Mg, Ca, Pb, and Cr were prepared to determine their concentration in ethanol yield of powdered avocado seeds. This helps to ascertain the produced bioethanol from powdered avocado seed is environmentally friendly, and has no effect on engine performance (Rocha et al, 2010). Determination of each metal concentration was performed by using Inductively Coupled Plasma Optical Emission Spectrometry (ICP- Spectroscopy, ULTIMA-2) (Hossain et al, 2011; Rocha et al, 2010). Besides, the major functional groups of the produced bioethanol have been identified using Spectrum 65 (PerkinElmer) to understand it is a real ethanol.

Data Analysis

An OriginPro8 software and Microsoft excel 2007 were used to determine the effect of operating variables of the fermentation in ethanol production from avocado seed wastes.

Results and Discussion

In this study, the bioethanol yield was investigated through fermentation process. The ethanol potential from powdered avocado seed wastes at optimized hydrolysis from the previous work; and effect of fermentation pH, fermentation time, and fermentation temperature on bioethanol production were investigated.

Determination of Bioethanol Content

After hydrolysis, the hydrolysates at optimized conditions from the previous study were used for fermentation using *Saccharomyces* cerevisiae. The effects of fermentation time, fermentation temperature, and pH on the ethanol yield of the fermentation product of powdered avocado seed wastes was studied and the results are discussed below.

Effect of pH on Ethanol Yield

pH is one of the important factors that affect the performance of SHF (separate hydrolysis and fermentation). The rate of ethanol production by yeast cells is highly affected by the pH of the fermentation medium. The acidic condition hinders the growth of harmful bacteria and enhances yeast grows (Tahir et al, 2010; Tahir and Sarwar, 2012). However, more acidic and basic conditions retard the yeast metabolic pathways and the growth of the cells (Tahir et al, 2010). So, optimum pH is required for growth and ethanol yield by the yeast. Hence, the pH of the hydrolysates was adjusted to 4, 4.5, 5, 5.5, and 6.0 using 4 M NaOH and 2.5 M HCl solution to optimize the pH value of the solution.

Table 1: Yield of Ethanol at 25^{o}C, 4 g/L yeast extract, 3 days, and different pH fermentation

pH	Amount of ethanol yield V (%)	
	Hydrometer	Pycnometer
4	4.792±0.673	4.981±0.820
4.5	4.925±0.125	5.086±0.257
5	4. 973±0.243	5.258±0.513
5.5	5.428±0.214	5.835±0.913
6	4.835±0.523	5.014±0.437

Increasing pH from 4 to 5.5 increases the concentration of ethanol from 4.792±0.673 to 5.428±0.214% and 4.981±0.820 to 5.835±0.913% using Hydrometer and Pycnometer measurement, respectively. However, it starts to decrease at pH 6.0 (4.835±0.523 and 5.014±0.437% using Hydrometer and Pycnometer measurement, respectively. As a result, pH 5.5 is the optimized pH for maximum ethanol yield for powdered avocado seed (Table 1).

Previous studies showed that high ethanol production was obtained using pH 5.0 to 6.0. It was also shown that no ethanol production exists lower than pH 4.0 (Fadel, 2000), and hence this study is in good agreement with the previously reported works.

Effect of Fermentation Time on Ethanol Yield

The fermentation was carried out under anaerobic condition at an optimized pH (5.5), 4 g/L, and room temperature (25^{o}C) for 2, 3, 5, 7 and 9 days to optimize the fermentation time. The flasks were covered using aluminium foil and incubated for 2, 3, 5, 7 and 9 days at room temperature.

Table 2: Yield of Ethanol at 25^{o}C, 4 g/L yeast extract, pH 5.5, and different fermentation time

Fermentation time	Amount of ethanol yield V (%)	
	Hydrometer	Pycnometer
2	4.857±0.371	5.083±0.490
3	5.428±0.214	5.835±0.913

5	5.064±0.000	5.429±0.153
7	3.797±0.317	4.203±0.462
9	3.791±0.520	4.024±0.215

As shown in Table 2, the maximum and minimum ethanol yield was obtained at 3, and 9 days fermentation time i.e. 5.428±0.214 and 5.835±0.913%, 3.791±0.520 and 4.0244±0.215 % using Hydrometer and Pycnometer, respectively. From these, 3 days fermentation time is the optimized fermentation time for maximum ethanol production from powdered avocado seeds.

Previous works revealed that, the ethanol yield increased gradually by increasing the incubation time and reaching its maximum after 60-72 hrs and dramatically decreased with further extension of time (Faga et al, 2010; Suryawati et al, 2008). In our study, ethanol production at 3 days (72 hrs) fermentation time of powdered avocado seeds reached a maximum of 5.428±0.214% through Hydrometer measurement. This has been confirmed with Pycnometer measurement at 3 days of fermentation time with maximum of 5.835±0.913%.

Effect of pH on Fermentation and Ethanol Yield

When the temperature is too high the yeast destroys, on the other hand, yeast activity decrease at lower temperature (Tsigie et al, 2013). 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). Therefore, it is must to optimize the reaction temperature, and hence the fermentation was conducted at temperature range of 25-40°C to optimize the temperature at which it yields highest concentration of ethanol. As the temperature increases from 25 to 30^{o}C , the ethanol yield increases from 5.428±0.214 and 5.835±0.913% to 5.909±0.102% and 6.365±0.372% using Hydrometer and Pycnometer reading, respectively. However, as the temperature further increase the ethanol content decreases significantly (Table 3). The best ethanol yield was achieved at 30^{o}C with 5.909±0.102% and 6.365±0.372% with Hydrometer and Pycnometer reading, respectively.

Table 3: Yield of Ethanol at optimized conditions of fermentation time (3 days), pH 5.5, and at 4 g/L yeast extract, and different reaction temperature.

Temperature (^{o}C)	Amount of ethanol yield V (%)	
	Hydrometer	Pycnometer
25	5.428±0.214	5.835±0.913
30	5.909±0.102	6.365±0.372
32	5.684±0.310	6.064±0.259
35	5.430±0.251	5.791±0.572

40	4.604±0.271	4.978±0.148

The effect of temperature on bioethanol production was studied by Duhan et al., 2013 and obtained the maximum bioethanol production at 35^oC. Temperature between 30-35^oC has been usually employed for culturing of yeast and temperature above 35^oC has been found inhibitory to ethanol fermentation due to yeast growth inhibition at higher temperatures (Tahir et al, 2010). Therefore, those works are almost similar with the present work which yields maximum ethanol at 30^oC.

In general, the current result revealed that ethanol obtained from avocado seeds is a promising substituent for other agricultural products such as avocado and mango juices, cassava and corn, etc. Avocado seed is non-edible material and can not affect the food chain of mankind**.**

IR Characterization of the Produced Bioethanol

Alcohols have characteristic IR absorptions associated with the O-H, C-O and the C-H stretching vibrations. When run as a liquid film the region 3500-3200 cm^{-1} with a very intense and broad band indicated the O-H stretch of alcohols, while the region 1260-1050 cm^{-1} confirms the C–O stretch. The bands at around 2880 and 2930 cm^{-1} were assigned as the symmetric stretching modes of the $–CH_2$ and$–CH_3$ groups, respectively (Coates and Meyers, 2000; Yu et al, 2007). This ascertains that the product obtained from powdered avocado seeds is definitely ethanol due to the confirmation of these regions (Figure 1).

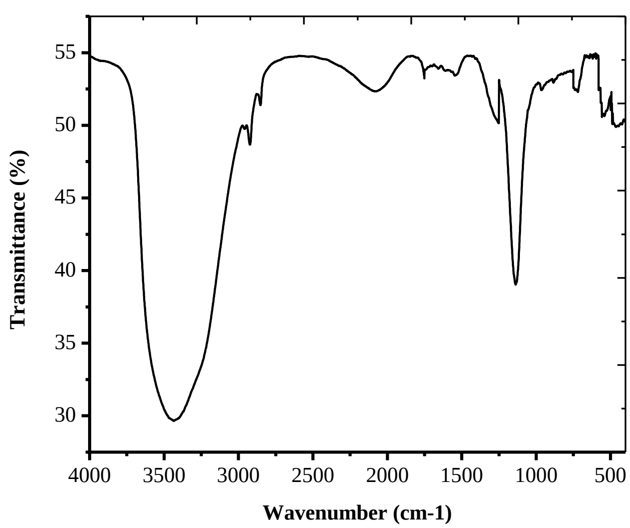

Figure 1: Infrared spectra of the produced bioethanol from powdered avocado seed wastes in the region of 4000-400 cm^1.

Metal analysis

The concentration of the metals in the produced bioethanol from avocado seed wastes were 0.00, 0.79, 16.07, 23.80, and 10.70 mg/L for Cr, Pb, Mg, Fe, and Ca, respectively. Therefore, the smaller concentration of Lead and zero concentration of chromium results in environmentally friendly and fit for engine use. In general, all of the elements followed the ASTM standard that is better for engine use (Hossain et al, 2011; Rocha et al, 2010).

Conclusion

Bioethanol production from avocado seed wastes and optimization of different factors in the fermentation process were investigated. The optimized conditions for the fermentation process were 3 days, pH 5.5, and 30 ^oC fermentation conditions. Comparable results have been achieved using both Hydrometer and Pycnometer in the fermentation step. The bioethanol obtained by dilute acid hydrolysis of avocado seed wastes (6.365%) was highly satisfactory and hence, it is promising feedstock for bioethanol production. Besides, the elemental analysis of this work was performed with no detection of chromium and smaller concentration of lead (0.79 mg/L) as compare to the others. The IR characterization of our sample has been done and the intense and broader band at 3500-3200 cm^{-1} and 1260-1050 cm^{-1} has appeared due to the O-H and C-O stretching, respectively.

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