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PRODUCTION OF BIOETHANOL BY SIMPLE BATCH FERMENTATION OF ACACIA ETBAICA (SERAW): A POTENTIAL BIOMASS RESOURCE FOR ERITREA

Ramesh Babu Aremanda[1]
Sinit Abraham
Soliana Tesfaldet

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A B S T R A C T

Socio-economic progress of a specific nation determined by the amended energy facilities. Electricity produced from Eritrea majorly relies on imported fossil fuels. In the global scenario, recently bioethanol derived from lignocellulosic biomass sources has become a significant renewable energy form as a blended fuel for the transportation sector.Woodland forest covers totally 60% of the total land in Eritrea. Acacia etbaica, a bushy wood plant native to Eritrea was selected as a feedstock for bioethanol production. Two stage fermentation of aerobic followed by anaerobic in the presence of yeast made potential to generate 3.9% (v/v) bioethanol from Acacia etbaica (Seraw) via multistage dilute H_2SO_4 pretreatment. Bioethanol was purified by a simple batch distillation and the first stage distillate was identified with 13 % of ethanol by UV spectrophotometer. With a dedication of50,000 ha of land, Eritrea can harvest 45000 ton/year of Acacia etbaica biomass and subsequently produce 15620 lit/year of bioethanol for a 5 year crop rotation.

1. INTRODUCTION

Potential of energy production and consumption determines the socio-economic development of a country. In Africa, the main source of energy is fossil fuels with 79.96 percent of the total energy produced in 2017 whereas for the world as a whole, fossil fuels are also the main energy source with 65.81 percent (Report on Energy mix of Eritrea). Despite of the rapid depletion of fossil fuel reserves, excessive use of fossil fuels is posing serious environmental issues. In pursuit of alternative fuels, today renewable energy resources are paid of great interest in the world. Amongst several renewable energy options such as solar power, wind energy, hydroelectricity and biomass etc., conversion of biomass to bioethanol has become a prodigious substitute of liquid fuels as blended fuels to contribute significantly in the transportation fuel sector with least environmental issues.

Eritrea is a northeast African country with its greatest share of electricity generated from fossil fuels. In 2019, 94 % of Eritrea's electricity produced from non-renewables, the rest in the form of solar power and it has a target of achieving at least 70 % of electricity production from renewables by 2030 (IRENA Report on Energy Profile of Eritrea). Whilst electrification with

[1] Corresponding author: Ramesh Babu Aremanda
Email: ramesh.nitrkl@gmail.com

renewable options is a viable option for road transport, the optimal pathway has yet to become clear in the global aspect. Since Eritrea depends completely on imported liquid fuels for transportation, though the amount of energy consumed in transportation is negligible, transport plays a vital role by facilitating the movement of people and global trade. Besides the concern of fossil fuel reliance with economic and political crisis, burning of petroleum-based fuels causes the increase of CO2 level in the environment which is directly responsible for global warming (Naik et al, 2010).

Table 1. Composition of different Lignocellulosic feedstocks for bioethanol production

Feedstock	Cellulose	Hemicellulose	Lignin	Reference
Acacia etbaica	48.87± 1.01	21.37± 0.86	29.77± 1.04	(Naser and Aref, 2012)
Acacia tortilis	46.92± 0.56	21.10± 1.26	31.81± 1.68	(Naser and Aref, 2012)
Eucalypt wood	47.2	69.9	22.9	(Chun et al. 2012)
Hardwoods	45 ± 2	30 ± 5	20 ± 4	(Reddy and Yang, 2005; Saini et al., 2015)
Softwoods	42 ± 2	27 ± 2	28 ± 3	(Reddy and Yang, 2005; Saini et al., 2015)
Sorghum straw	32	24	13	(Reddy and Yang, 2005; Saini et al., 2015)
Wheat straw	33-38	26-32	17-19	(Reddy and Yang, 2005; Saini et al., 2015)
Grasses	25-40	25-50	10-30	(Reddy and Yang, 2005; Saini et al., 2015)

Bioethanol is considered as an important renewable fuel to partly replace fossil-derived fuels. The global bioethanol industry has witnessed high growth primarily because of the mandatory usage of bioethanol fuel blends in many countries, especially in the transportation vehicles. Bioethanol production in the world has increased to 29.03 billion gallons in 2019 from 13.12 billion gallon in 2007. Together, the United States and Brazil produce 84% of the world's ethanol, mostly using corn or sugarcane (DOE, US). The global bioethanol market size is projected to grow from USD 33.7 billion in 2020 to USD 64.8 billion by 2025, at a Compound Annual Growth Rate (CAGR) of 14.0% (GNW report). In developing economies, food-related feedstock is preferably replaced by nonfood raw materials. The use of common biomass could significantly increase the bioethanol production, and lignocellulose-based bioethanol is therefore an important matter today (Kang et al, 2014).

Available Lignocellulosic biomasses can be classified into primary sources, produced as either crop or key product; secondary sources, as residues from the production processes; and tertiary sources, as residues produced during and after application end. The abundant wood biomass is renewable and very suitable to serve as feedstock for second-generation cellulosic bioethanol. Lignocellulosic materials could provide up to 50 billion tons of dry matter on a global scale (Alfaro et al., 2009). Approximately 2 million tons per year of biomass are consumed in Eritrea, primarily in the household sector. About 80% of the energy use in Eritrea is from biomass resources such as fuel wood, dung and crop residues. Burning or disposal of biomass materials in the form of wastes or as a fuel creates more carbon dioxide emissions and other health and environmental problems.

In Eritrea, by 2015 the woodland forest cover 7,132,000 ha, approximately 60 % of the total land (GFRA). The green belt zone on the Eastern Escarpment is one of the natural woody forests in upland Eritrea dominated by Acacia etbaica (Seraw), a bushy wood plant used in this study to survey its potential for bioethanol production (Elias, 2001). The category “bush” is the dominant vegetation in Eritrea covering 63% of the total area. Acacia etbaica has a wide occurrence, from semi-desert scrub to wooded grassland at altitudes that extend from 1200 to 2000 m (Masresha, 2003). Acacia etbaica is a tree or shrub (min. 2m) 2.5-12 m tall, survive with a mean annual rainfall of 200 -1400mm, and mean annual temperature of 22.3 ºC and native to Eritrea, Ethiopia, Kenya, Somalia, Sudan, Tanzania and Uganda (Orwa, 2009). Tree biomass of Acacia etbaica was estimated approximately as 4.5 ton ha-1yr-1 by using allometry equations with tree height and canopy area as parameters in structure from motion (SfM) method (Sakai et al., 2019).

A comparative study of chemical compositions of potential lignocellulosic biomass for the production of bioethanol in Eritrea listed in the Table.1 describes their suitability for the selection as feedstocks. Biomasses are mainly composed of cellulose, hemicellulose and lignin polymers. Cellulose, generic formula (C6H10O5)n, is the main structural constituent of plant cell walls. High cellulose content in lignocellulosic material is a promising condition for biofuel production. Hemicellulose, generic formula (C5H8O4)n is the second most abundant polymer of plant cell walls and is mainly composed of xylan and mannan. As hemicellulose is wrapped around the cellulose fibrils, it needs to be removed in order to increase the cellulose digestibility. Thus, increase in hemicellulose removal increases the accessibility of the cellulose and its hydrolysis rate. Lignin is a three–dimensional polymer of 4-propenyl phenol, 4-propenyl-2- methoxy phenol and 4-propenyl-2.5-dimethoxyl phenol, and it is the third most abundant constituent of lignocellulosic biomass. High lignin content results in low digestibility of the biomass therefore; increasing the lignin removal increases the biomass digestibility.

Acacia etbaica has a composition of 48.87 % cellulose, 21.37 % hemicellulose, 29.77 % lignin, and 2.35 % of ash contents and possessing a heating value of 19.14 MJ/kg (Naser & Aref, 2012) which are prominent features of the plant in the selection as a feed stock for bioethanol production.

The production of ethanol from lignocelluloses biomass consists basically of four steps; pretreatment, enzymatic hydrolysis, fermentation, and distillation. Pretreatment constitutes the most challenging step, since it has to cope with many factors limiting enzymatic hydrolysis, such as the cellulose crystallinity, lignin and hemicellulose barrier, and fiber accessibility. This study was done to assess the conversion of acacia etbaica plant wood substance into bioethanol by Separate Hydrolysis and Fermentation (SHF) process using simple batch fermentation method. The process included several steps such as pretreatment cum acid hydrolysis followed by fermentation, filtration and simple distillation. Physical characterization of the feedstock was performed to determine the amount of fixed carbon and composition analysis of the product was investigated to confirm the quantity of ethanol by using UV spectrophotometer. Fermentation was done using instant yeast (*Saccharomyces cerevisiae*) in two stages as aerated fermentation for 60 hrs followed by 72 hrs under anaerobic conditions. Filtration along with centrifugation applied to separate clear fermented solution from the solids and then distilled by simple distillation process to obtain the rectified ethanol.

2. METHOD AND MATERIALS

Biomass Feedstocks: Acacia etbaica wood trunks were collected from the premises of Mai-Nefhi College of Engineering and Technology (MCOET), Mai-Nefhi, Eritrea.

Chemicals used: Dilute H_2SO_4 of 1.5 %, NaOH solution, Instant Yeast and $K_2Cr_2O_7$ Solution were used from Chemical reaction engineering lab, Department of Chemical Engineering, Mai-Nefhi College of Engineering and Technology (MCOET), Eritrea.

2.1 Methods

Separate hydrolysis and fermentation (SHF) process was employed through several steps as described in Figure 1.

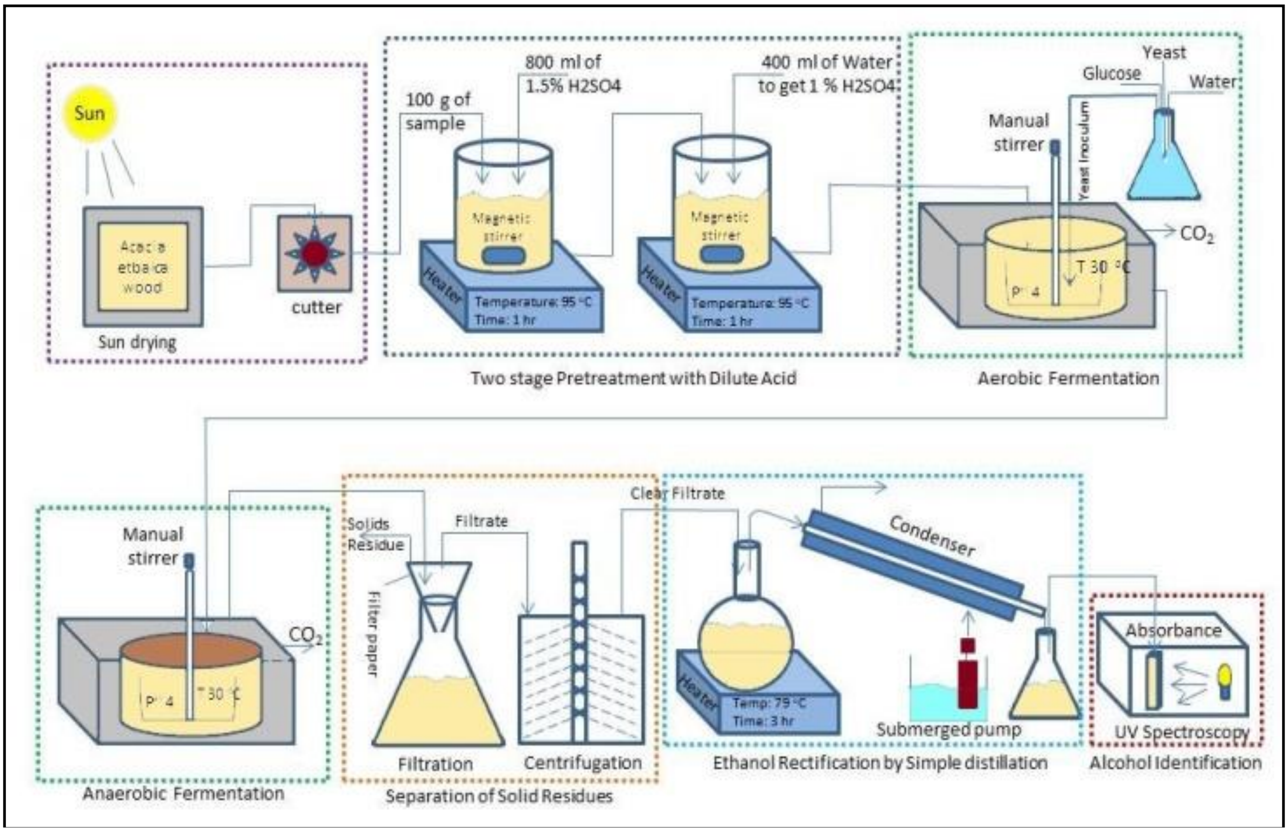

Figure 1. Schematic diagram for experimental process of bioethanol production from Acacia etbaica wood

2.1.1 Sample Preparation

½ m to 1 m long wood trunks of the acacia etbaica were cut and kept for sun-drying about 2 weeks, subsequently they were debarked and reduced to the size of ½ to 1 in. later, 100 g of the prepared sample was measured and taken.

Proximate Analysis: Samples were analysed to determine the moisture, volatile organic matter, ash content according to IS 1350-Part I-1984 method and Fixed carbon was estimated as:

$$Fixed\ carbon(FC) = 100 - (VM + ASH - MOIST)$$

2.1.2 Pretreatment cum Acid Hydrolysis

Pretreatment together with dilute acid hydrolysis was performed in two stages; in the primary stage, 800 ml of 1.5 % H_2SO_4 solution was gradually mixed with 100 g of the sample initially at 95 °C for 1 hr in a glass vessel of 2 liter capacity provided with continuous stirring. During second stage sulfuric acid concentration was diluted to 1 % by adding 400 ml of distilled water and heated for one more hour with continuous stirring as depicted by Figure 2.

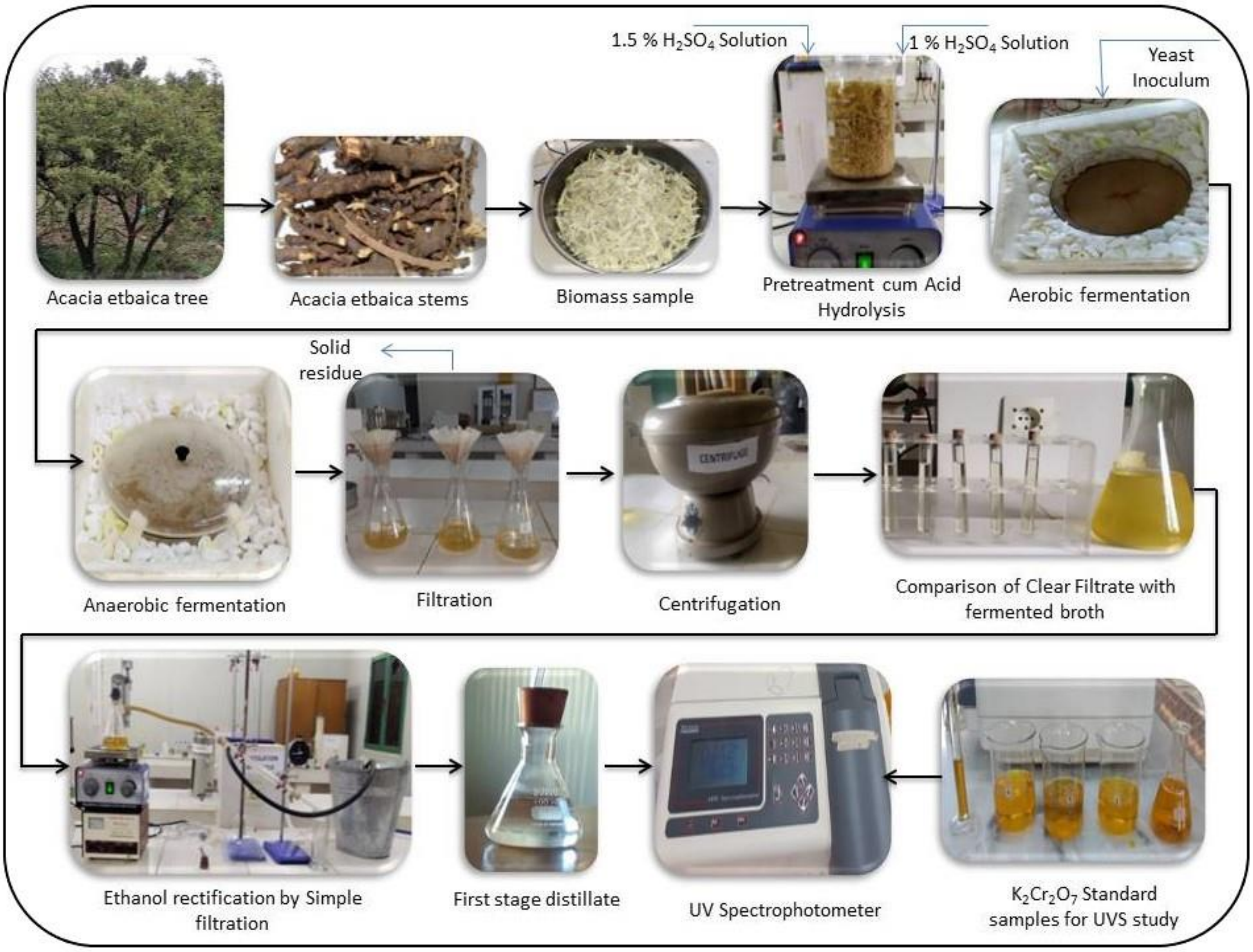

Figure 2. Experimental steps for the generation bioethanol from acacia etbaica biomass

2.1.3 Yeast Inoculation and Fermentation

5 g of instant yeast was added to a pre-sterilized conical flask, and it was mixed with 5 g of glucose diluted in 100 ml of distilled water at 35 °C. Afterward immediately it was inoculated into fermentation reactor. A conventional laboratory scale fermenter was made with available facilities which comprised of a glass vessel protected with a wooden box and filled with polystyrene balls to retain the consistent temperature. It was periodically heated under sunlight during day.

Fermentation was conducted in two phases; in the prior phase, it was facilitated for aerobic conditions at 35-37 °C with a P^H of 4.5. P^H was slightly adjusted with NaOH solution when it was low. Aerobic fermentation was allowed for 60 hr, later it was arranged for anaerobic conditions with proper sealing of the fermenter for another 72 hr provided with a periodic manual stirring. A vent was provided for CO_2 gases to react with lime water.

2.1.4 Separation of Solids

The fermented broth from the fermentation tank was filtered through whatman filter paper to separate coarse solid residues. From the relatively clear solution that obtained from filtration process, 80 ml of solution was taken as a batch into test tubes of 10 ml capacity and they were placed into cartridge of centrifuge that was available in CRE laboratory. The solution was centrifuged at 2500 rpm for 1 hr and the top clear solution was collected to proceed for simple distillation.

2.1.5 Ethanol Rectification by Simple Distillation

150 ml of clear filtrate obtained by centrifugal filtration was taken into a 250 ml conical flask and arranged to simple distillation setup as shown in fig. and it was connected with a condenser provided with cooling water circulation by a submerged pump. Heating provision was provided with hot plate facilitated with a temperature control to maintain a constant temperature of 79 ^oC. Collected 50 ml of first distillate within 3 hr of operation and proceeded to repeat the similar cycle to distillate the remaining filtrate.

2.1.6 Identification of Ethanol

Jenway, 6400 Spectrophotometer, available at Environmental Engineering Laboratory, Department of Civil Engineering, MCoET was used to identify the concentration of Ethyl alcohol in the first distillate. Ethanol can be identified directly at 290 nm of wavelength with the help of UV spectroscopy. But the existing instrument has a tungsten halogen lamp which can detect the components that are having the wavelength in the range of 320 to 1100 nm. Hence and indirect approach followed by using $K_2Cr_2O_7$ (Potassium Dichromate) Solution to standardize the reference data. Initially the standard solutions of 96 % (v/v) ethanol were prepared with 0, 1, 2, 3, 4, 8, 10, 12, and 15 ml in to 100 ml beakers separately. Later added 4 ml of $K_2Cr_2O_7$ solution along with distilled water to make up and mixed it well. Afterward it kept in an incubator at 55 ^oC for 10 min. Even the unknown sample (distillate) was also taken into 100 ml beaker and added with 4 ml of Potassium dichromate solution and kept in incubator along with other samples. UV spectrophotometer has set into 'Photometrics' mode tomeasure the absorbance values of all the samples at 600 nm wavelength.A plot of concentration verses absorbance was built to determine the unknown sample concentration with the absorbance values noted.

3. RESULTS & DISCUSSIONS

3.1 Proximate Analysis

Acacia etbaica (Seraw) trees grown in Mai-Nefhi were taken as lignocellulosic feedstock for the production of bioethanol by simple batch fermentation method. The characterization of biomass samples were done to determine fixed carbon and ash contents. Proximate analysis of Acacia etbaica biomass has revealed that it has the ash content of 2.45 % against 12-16 %, 0.80% and 0.50 in agricultural residues, hardwood and softwoods respectively [Alya et al].

Table 2. Proximate analysis results of Acacia etbaica biomass

Content	Weight %
Moisture	10.9%
Volatile Organic Matter	84.83%
Ash Content	2.45%
Fixed Carbon	23.62%

3.2 Material and energy balance for experimental scale

100 g of the prepared biomass sample was taken as the experimental basis for the production of bioethanol. Pre-treatment was carried out in two stages with 1.5% and 1 % H_2SO_4 solutions respectively. The consumption of pure (distilled) water and H_2SO_4 (98 %) were shown in the table. And 7.75% of yeast inoculum was added for fermentation which was carried out in multi stages, aerobic and anaerobic respectively. Approximately 3 % (by weight) of CO_2 was produced during entire fermentation process.

Filtration and centrifugation processes for the separation of solids were followed by simple distillation process maintained 3 hr as cycle time to collect 30.33 % (by weight) of distillate. This distillate was tested for the identification of ethanol by UV Spectrophotometer. Electrical energy consumed during energy intensive steps such as pre-treatment and simple distillation was recorded in Table 3.

Table 3. Energy consumption in experimental process

Pre-treatment cum Acid Hydrolysis	Simple Distillation
1.5 MJ	0.9 MJ

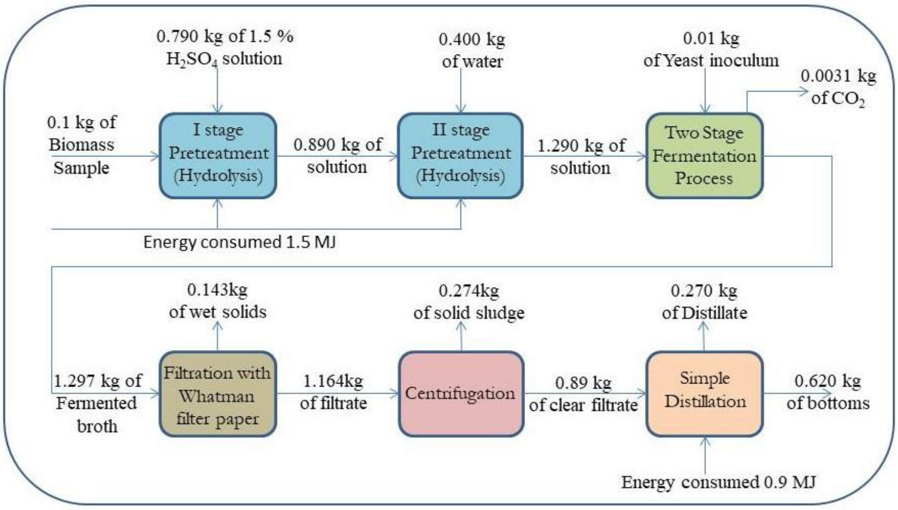

Figure 3.Block diagram for material and energy balance of experimental work

Table 4. Material balance on pre-treatment and fermentation for experimental scale process

Pre-treatment cum Acid Hydrolysis			**Fermentation**			
I Stage		II Stage	Inputs		Outputs	
Amount of H_2SO_4	Water	Water	Feed	Yeast Inoculum	Fermented broth	CO_2
0.012 kg	0.778 kg	0.40 kg	1.29 kg	0.010 kg	1.297 kg	0.0031 kg

Table 5. Material balance on filtration, centrifugation and distillation for experimental process

Filtration		**Centrifugation**		**Simple Distillation**		
Filtrate	Solids	Clear Filtrate	Solid Sludge	Feed	Distillate	Stillage
1.164 kg	0.143 kg	0.89 kg	0.274 kg	0.890 kg	0.270 kg	0.620 kg

Table 6.Standard solutions and their UVS-absorbance

Ethanol % (v/v) in Aqueous samples	**1 %**	**2 %**	**3 %**	**4 %**	**8 %**	**10%**	**12 %**	**15%**	**Unknown Sample**
UVS-Absorbance	0.004	0.008	0.012	0.016	0.032	0.04	0.048	0.062	0.052

Table 7.Potential of lignocellulosic bioethanol production from acacia etbaica in Eritrea

Species	**Plant spacing (m)**	**Rotation (year)**	**Harvest mass (ton/ha/year)**	**Annual harvest area (ha)**	**Harvest mass (ton/yr)**	**Ethanol production (lit/yr)**
Acacia etbaica	1 * 1	5	4.5	10000	45000	15620

3.3 Quantification of Ethanol

Content of ethanol in the distillate was measured by using UV Spectrophotometer through an indirect method while oxidation of ethanol occurs with potassium dichromate solution to produce ethanoic acid at 600 nm of wavelength. Absorbance of standard solutions of known compositions 1, 2, 3, 4, 8, 10, 12 and 15% ethanol were first recorded as Table.6 and developed a plot shown in Figure.4 to determine the ethanol in distillate. Absorbance of unknown distillate sample was noted as 0.052 which corresponds to 13 % of ethyl alcohol as shown in the Figure 4.

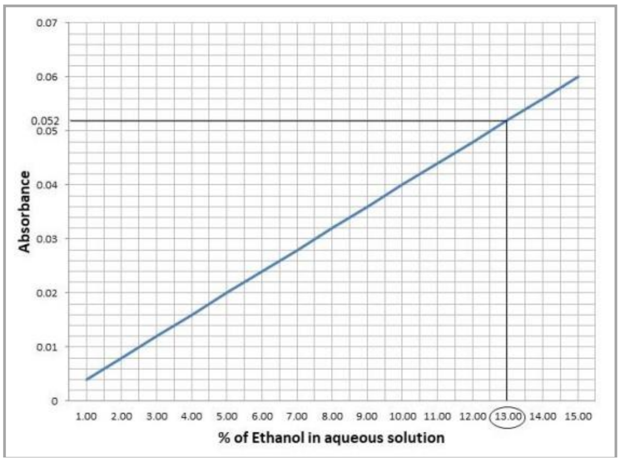

Figure 4. Ethanol % (v/v) in aqueous solutions against their absorbance measured by UVS

3.4 Yield of Ethanol

Yield of Ethanol was calculated as 3.9 % (v/v) based on the total filtrate taken as the feed to the distillation and 34.71 ml of ethanol was produced per 100 g of acacia etbaica biomass sample.

$$Yield\ of\ Ethanol = \frac{volume\ of\ ethanol}{volume\ of total\ filtrate} \times 100$$
$$= \frac{34.71\ ml}{890\ ml} \times 100 = 3.9\ \%$$

3.5 Potential of lignocellulosic bioethanol production from Acacia etbaica in Eritrea

The growth rate of acacia etbaica in Ethiopia was estimated as 4.5 ton $ha^{-1}year^{-1}$ (Sakai et al., 2019). Based on geophysical climatic conditions, it can be assumed that a similar growth rate in Eritrea to determine the potential of bioethanol production from hardwood plantations such as acacia etbaica, assuming availability of atleast 50,000 ha of plantation as shown in table.7 By taking 5 years, crop rotation time, sowing 10000 ha every year and harvesting it subsequently after 5 years, Eritrea can produce 15620 lit of bioethanol per annum. Biomass productions could also be increased by improved silviculture practices and tree clone breeding. Although acacia grows slower comparatively with eucalyptus, these plants have could have other benefits of afforestation, such as carbon sequestration, water and soil conservation, biodiversity promotion, etc. On the other hand, residues after fermentation could also be compacted to produce pellets for additional fuel supply (Wang et al.,2012; Eriksson and Kjellström, 2010).

4. CONCLUSIONS

Bioethanol could be a better substitute for liquid fuels in the form of a blended fuel for Eritrea. Bioethanol can be produced from the lignocellulosic biomasses such as hardwoods of acacia etbaica by separated hydrolysis and fermentation [SHF] process through simple batch fermentation with yeast. The two-stage pre-treatment with dilute acid hydrolysis made possible to generate ethanol from lignocellulosic biomass through fermentation. Energy consumption in pre-treatment and in distillation processes can be further optimized by energy intensification studies towards reducing hydrolysis time and cycle time for simple distillation. If Eritrea dedicates 50,000 ha of land for Acacia etbaica plantation, it can generate 15620 lit per annum of bioethanol that may contribute as a blended fuel for transportation. Further studies on size reduction in biomass sample, conversions of sugars, and effect of mixing and validation of by-products will definitely optimize the applied process.

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Ramesh Babu Aremanda
Department of Chemical Engineering,
Mai Nefhi College of Engineering &
Technology(MCoET),
Asmara,
Eritrea
ramesh.nitrkl@gmail.com

Sinit Abraham
Department of Chemical Engineering,
Mai Nefhi College of Engineering &
Technology(MCoET),
Asmara,
Eritrea
sinitabraham12@gmail.com

Soliana Tesfaldet
Department of Chemical Engineering,
Mai Nefhi College of Engineering &
Technology(MCoET),
Asmara,
Eritrea
solianatesfaldet@yahoo.com