ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIO-ENGINEERING

ENVIRONMENTAL ENGINEERING STREAM

OPTIMIZATION OF PARAMETERS AND PRODUCTION OF BIO-ETHANOL FROM RAW COFFEE WET PROCESSING WASTE

By: Asrat G/Mariam W/Senbet

Addis Ababa University

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Approval of Examination Committee

This is to certify that the thesis prepared by Asrat G/Mariam W/Senbet, entitled: "*Optimization of Parameters and Production of Bio-Ethanol from Raw Coffee Wet Processing Waste"* and submitted in fulfillment of requirements for the Degree of Doctor of Philosophy in Chemical (Environmental) Engineering complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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Abstract

A large amount of coffee waste water is generated from coffee processing plants. These residues possess serious environmental problems following the direct discharge in to the nearby water bodies and cause serious environmental and health problems. This study aimed to: (i) review wet coffee processing waste management practice in Ethiopia, (ii) characterize wet coffee processing waste and determine its total reducing sugar potential, (iii) quantify wet coffee processing waste and estimate its bio-ethanol potential and also evaluate the feasibility of bio-ethanol production and (iv) optimize the parameters and produce bio ethanol from wet coffee processing waste. The review of wet coffee processing waste management practice in Ethiopia revealed that there are about 1026 operational wet coffee processing industries and many more industries are under construction. In 2012, the estimated amount of wet coffee processing waste from operational industries in Ethiopia was about 291,600 tones / year. The study also examined the characteristics of wet coffee waste. The volatile solid of the Pulp juice and Mucilage were determined and showed that the wastes have high organic component, i.e. Pulp juice, 66.5% and Mucilage, 90.2%. The study shows the waste (pulp juice and mucilage) is acidic with pH 4.75 and 3.67, respectively. Pulp juice and Mucilage had very high BOD and COD Concentration, i.e. BOD = 25,600 mg/L and COD = 45,000 mg/L for pulp juice and BOD = 19,810 mg/L, COD = 33,600 mg/L for Mucilage. The COD: BOD ratio is less than 2:1, which shows the wastes are bio-degradable. This study shows that the coffee wastes are potential environmental problems and cause water pollution due to high organic component and acidic nature. The waste was hydrolyzed by dilute H_2SO_4 (1, 2, 3 and 4%) and distilled water. Total sugar content of the sample was determined titrimetrically and using refractometer.

Maximum total sugar content (90%) was obtained from hydrolysis by 3% H_2SO_4. The results obtained at hydrolysis of 4, 2, 1% H_2SO_4 and distilled water are 72.86, 76.50, 63.75 and 56.66%, respectively. Ethanol production was monitored by gas chromatography. The maximum ethanol yield of 78% was obtained from coffee waste hydrolyzed by 0.4 M H_2SO_4 for 1 hour hydrolysis, temperature of 100 ^{0}C and fermentation for 24 hours and initial pH of 4.5. Based on the data, it was concluded that coffee may be considered as one of the most valuable primary products in world trade, crucial to the economies and politics of many developing countries. As a consequence of big market, the reuse of the main coffee industry wastes is of significant importance from environmental and economical view points. In conclusion, this study has proposed to utilize the wet coffee processing waste to produce bio-ethanol which provides the alternative energy source from waste biomass and solves the environmental waste disposal as well as human health problem.

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Abbreviations

GHG	Green house gas
CW	Coffee waste
WW	Waste water
Lab	Laboratory
TS	Total solid
VS	Volatile solid
GC	Gas chromatography
CDM	Clean development mechanism
MoA	Ministry of Agriculture

1. Introduction

1.1. Background

The world's economy is highly dependent on various fossil energy sources such as oil, coal, natural gas, etc. These are being used for the production of fuel, electricity and other goods (N. Sarkar et al. 2012). Primary energy sources can be divided into non-renewable and renewable. The reality shows that the energy availability from the non-renewable sources is limited, and beyond that, the exploration, the processing and the use of energy impose considerable impacts on the environment (A. Demirbas et al. 2011). Excessive consumption of fossil fuels, particularly in large urban areas, has resulted in generation of high levels of pollution during the last few decades. The level of greenhouse gasses in the earth's atmosphere has drastically increased. With the expansion of human population and increase of industrial prosperity, global energy consumption also has increased gradually. Import of transport fuel is affected by limited reserves of fossil fuel. Annual global oil production will begin to decline within the near future (N. Sarkar et al. 2012).

Transportation sector is responsible for 60% of the world oil consumption and accounts for more than 70% of global carbon monoxide (CO) emissions and 19% of global carbon dioxide (CO_2) emissions. CO_2 emissions from a gallon of gasoline are about 8 kg (M. Balat. 2011). Rising fuel prices, concerns about environmental impact and supply instability are among the main factors concerning world today (W. H. van Zyl, A. F. A. Chimphango et al. 2011).

An alternative fuel must be technically feasible, economically competitive, environmentally acceptable, and readily available. Numerous potential alternative fuels have been proposed, including bio-ethanol, biodiesel, methanol, hydrogen, boron, natural gas, liquefied petroleum gas (LPG), Fischer‑Tropsch fuel, p-series, electricity, and solar fuels. Biomass-based fuels, also known as biofuels offer many advantages over petroleum-based fuels : (1) biofuels are easily available from common biomass sources, they are represent a CO_2-cycle in combustion, biofuels have a considerable environmentally friendly potential, there are many benefits on the environment, economy

and consumers in using biofuels, and they are biodegradable and contribute to sustainability (M. Balat. 2011).

Africa still remains a large consumer of traditional sources of energy, mainly fuel wood, and a greater proportion of its population faces energy insecurity. The availability and accessibility of socially and environmentally acceptable sources of energy are still very low and disproportionate between rural and urban areas. With the exception of fuel wood, other energy sources (coal, crude oil and more recently bio-fuels) have been the major sources of power driving the transport and industry sectors (W. H. van Zyl, A. F. A. Chimphango et al. 2011).

Biofuels are made from bio-based materials through thermo-chemical processes such as pyrolysis, gasification, liquefaction, supercritical fluid extraction, supercritical water liquefaction and biochemical. Thermo-chemical reforming of biomass concerns the processes of catalytic and non-catalytic pyrolysis as well as the gasification, which aims at the maximization of the production of energetically exploitable liquid and gaseous products.

Biofuels include bio-ethanol, bio-methanol, vegetable oils, biodiesel, biogas, biosynthetic gas, bio-oil, bio-char, Fischer‑Tropsch liquids, and bio-hydrogen. The term biofuels can refer to fuels for direct combustion for electricity production, but is generally used for liquid fuels for transportation sector. Renewable liquid biofuels for transportation have recently attracted huge attention in different countries all over the world because of its renewability, sustainability, common availability, regional development, rural manufacturing jobs, reduction of GHG emissions, and its biodegradability (M. Balat. 2011).

Bioethanol is by far the most widely used bio-fuel for transportation worldwide. Bioethanol and bio-ethanol/gasoline blends have a long history as alternative transportation fuels. It has been used in Germany and France as early as 1894 by the then

incipient industry of internal combustion engines (ICEs). Brazil has utilized bio-ethanol as a fuel since 1925 (M. Balat. 2011).

Depending on the level of advancement of agricultural technology, Africa will have the largest potential for bio-energy production by 2050 in the world (W. H. van Zyl, A. F. A. Chimphango et al. 2011). For Africa to realize its potential for bio-energy production, advanced agricultural technologies and practices must be employed in a sustainable way to serve the needs of rural and urban communities, foster development of the industrial sector, reduce greenhouse gas (GHG) emissions, develop agricultural infrastructure and lead to land restoration and ecologically healthy landscapes.

Owing to the wide range of commercial opportunities for second-generation bio-fuel production, in particular the opportunity for integration of production of bio-fuels with existing biomass, both biological and thermo-chemical conversion processes should be considered. Although biochemical and thermal processes for lingo-cellulose conversion have comparable efficiencies and economics, the selection of a preferred technology on the basis of the particular industrial scenario for commercialization is still required (W. H. van Zyl, A. F. A. Chimphango et al. 2011).

Agricultural wastes are renewable, less costly and abundantly available in nature. Agricultural wastes do not demand separate land, water, and energy requirements. They do not have food value as well (N. Sarkar et al. 2012). Lignocellulosic material constitutes the world's largest bio-ethanol renewable resource. In the U.S. alone the production of biomass from lignocellulosic materials is estimated to be nearly 1.4 billion dry tons per year, 30% originating from forest biomass (A. Limayem et al. 2012).

Lignocellulose is globally recognized as the preferred biomass for the production of a variety of fuels and chemicals that may result in the creation of a sustainable chemicals and fuels industry, with significant benefits in agricultural development. It represents the most widespread and abundant source of carbon in nature and is the only source that could provide a sufficient amount of feedstock to satisfy the world's energy and chemicals needs in a renewable manner (W. H. van Zyl, A. F. A. Chimphango et al. 2011). It has great potentials for the production of affordable fuel ethanol because it is less expensive than starch (e.g. corn) and sucrose (e.g. sugarcane) producing crops and available in large quantities (Yi Zheng, Zhongli Pan et al. 2009).

Lignocellulosic materials could produce up to 442 billion liters per year of bio-ethanol. Lignocelluloses consist mainly of cellulose, hemicellulose and lignin; these components build up about 90% of dry matter in lignocelluloses, with the rest consisting of e.g. extractive and ash. The basic structure of all woody biomass consists of three basic polymers: cellulose $(C_6H_{10}O_5)_x$, hemicelluloses such as xylan $(C_5H_8O_4)$m, and lignin $[C_9H_{10}O_3(OCH_3)_{0.9\text{ - }1.7}]_n$ (M. Balat. 2011). Biochemical conversion of lignocellulosic materials through saccharification and fermentation is a major pathway for bio-ethanol production from biomass (M. Balat. 2011).

Coffee by-products of the wet method of processing such as pulp and mucilage constitute around 40% of the wet weight of the fresh fruit. Wet processing uses up to 15 m^3 of water to produce one ton of clean bean (A. Kivaisi, B.Assefa et al. 2010). These are commonly disposed by dumping in to the natural water systems or to nearby agricultural or grazing land in Ethiopia. Such ways of residue disposal have been the major health challenges to

coffee farmers living in the vicinity of coffee processing plants. Moreover, such unsafe way of waste disposal has greatly affected terrestrial and aquatic biota. Hence, alternative ways of residue disposal that are safe to the environment and public health are highly needed in Ethiopia (D.P. Navia, D.J. Reinaldo et al. 2011).

Coffee waste contains high amounts of organic substrates for bioconversion into value added bio-products (A. Kivaisi, B.Assefa et al. 2010). Coffee pulp waste is generated in large quantities during wet method of coffee cherry processing, which is known to contain 23-27% fermentable sugars on dry weight basis. Most of the coffee pulp waste remains underutilized in many countries (S. Nayak, M.J. Harshitha et al. 2012), (M. Adams and A.E. Ghaly 2007). Therefore, large amount of unutilized biomass (waste) should be changed in to value added bio-products in order to increase use of alternative energy sources and improve environmental problems related to waste disposal.

1.2. Statement of the problem

Due to the increase in the price of petroleum and environmental concerns associated with the fuel combustion emissions and improper waste disposal, the search for alternative fuels has become vital. The use of food crops (like maize) for bio-fuel production may cause inflation of cost of these crops leading to food insecurity. To alleviate such problems, alternative and non-edible agricultural products should be investigated.

Environmental consequences due to coffee processing are broad and considerable. These consequences can range from water pollution (surface and underground water), bad odors and concentration of toxic elements in soils, which results in decreased land productivity and increased use of chemicals in addition to causing climate change.

Developing countries, like Ethiopia, are facing a serious problem in properly disposing of the waste produced by coffee processing plants. Wet coffee processing requires a high degree of processing knowledge and produces large amounts of processing effluents which have the potential to damage the environment.

There are more than 1026 functional wet coffee processing industries in Ethiopia generating waste and are not familiar with the waste treatment (management) systems Therefore, it is very essential to mitigate the problems associated to wastes generated from wet coffee processing industries by making use of the wastes to produce value added products like, bio-ethanol.

1.3. Objectives

1.3.1. *General objective*

- Utilization of wet coffee waste to produce value added product (bio-ethanol) and optimizing the production parameters.

1.3.2. *Specific* objectives

- Review wet coffee processing waste management practice in Ethiopia
- Characterizing wet coffee processing wastes.
- Theoretical estimation of Bio-ethanol potential of wet coffee processing waste.
- Optimizing parameters and producing bio-ethanol from coffee waste.
- Characterization of the sludge.

2. Literature Review

2.1. Overview

Biomass exploitation has great raw material availability challenges, particularly in the technological scheme of fuel bio-ethanol. Bio-ethanol is an environmentally friendly and directly exploitable fuel for substitution of petrochemicals, which today are used for the 97% of the transportation needs (T. Tsoutsos and D. Bethanis 2011).

Bio-fuels provide a valuable route to resolving environmental issues from reliance on petroleum and keeping the price of energy affordable. Currently, most bio-fuel is in the form of bio-ethanol, which is produced from starch or sugar. To meet the increasing requirement of energy, one promising way is engineering more advanced microbes to utilize more abundant feeding stock, such as cellulosic biomass. Another potential way is increasing the ethanol productivity through optimal control of fermentation process based on current industrial microbial process utilizing cellulosic feed stock (Z. Li , A. Dewan et al. 2012).

Ethanol has been valuable industrial solvent, germicide, antifreeze and is a major feedstock for a number of chemical derivatives, polymers, esters etc. The shortage of crude oil coupled with environmental problems associated with its use has led to extensive research for alternative energy sources. Ethanol, in spite of its lower heating value may become a partial replacement of fossil fuels, particularly for automotive use. It is a natural fuel, which burns cleaner than petrol and causes less environmental problems (B.K. Highina, Hashima et al. 2011).

If bio-ethanol is used as fuel, the net emission of carbon dioxide is zero due to its role in photosynthesis (J. Nörgård 2007). It is a readily available liquid that can be produced and utilized with existing technologies. The most economic way of manufacturing ethanol from variety of renewable sources involves fermentation by yeasts. Approximately 70% of the current production process for ethanol is through a batch-wise fermentation, which disadvantages it because of high capital costs, labor intensity and difficult process and product quality control. An obvious method to minimize the difficulties is to use a continuous production process (B.K. Highina, Hashima et al. 2011)

Today almost all bio-ethanol is produced in Brazil, USA and Canada. The vast majority of bio-ethanol is used in the transport sector as an oxygenated fuel additive. The raw material used is sugar-based or starch-based. In Brazil sugar canes are used, from which the sugar is simply extracted and then fermented. When using starch based raw material, it first has to be saccharified before being fermented. The hydrolysis can be achieved with acids or enzymes or a combination of both. The reason for trying to produce ethanol from lignocellulosic material is the vast quantities of potential raw material in places (J. Nörgård 2007).

Ethanol is produced by fermentation of microorganisms such as yeasts and bacteria. They convert sugar or carbohydrate to ethanol and carbon dioxide via the glycolysis pathway under anaerobic condition. Theoretically, the yield is 0.511 for ethanol and 0.489 for carbon dioxide on the basis of 1 g of metabolized glucose. The initial sugar concentration in the fermentation medium directly relates to ethanol concentration produced. In normal gravity fermentation, the initial sugar concentration of 150 to 200 g/L achieves ethanol

concentration of only 7.5 to 10% (v/v). To increase ethanol concentration, higher initial sugar concentrations of above 200 g/L are required but high ethanol concentration produced can cause an increase in the stress to yeast cells, resulting in stuck fermentation (O. Deesuth, P. Laopaiboon et al. 2012).

Under appropriate environmental and nutritional conditions, *Saccharomyces cerevisiae* can tolerate high ethanol concentrations. Very high gravity fermentation is a process improvement aimed at increasing both the rate of fermentation and ethanol concentration, which reduce capital costs, energy costs per liter of alcohol and the risk of bacterial contamination.

The ability of yeast to produce ethanol depends on many factors such as strains, macro and micronutrients and environmental factors. One of the most environmental factors affecting yeast growth and ethanol production efficiency is temperature. Temperature had many effects on yeast such as: Growth rate, Viability, Rate of fermentation, Length of lag phase, Activity of enzyme and Membrane function

Carbon and nitrogen are essential nutrients in fermentation media. Nitrogen is necessary for yeast growth and influences the rate of ethanol production and ethanol tolerance. Yeast extract, a complex nutrient, is widely used as a nitrogen source for yeast growth as well as a nutrient supplement for ethanol production and lactic acid production. Trace elements are also important factors for promoting cell growth and ethanol fermentation. Zinc (Zn^{2+}), Magnesium (Mg^{2+}) and Manganese (Mn^{2+}) were reported as the trace elements for yeast growth and ethanol fermentation. Zn^{2+} affects both cell growth and yeast metabolism. Mg^{2+} involves in physiological function, growth, metabolism and

enzyme activity of yeast. Regarding Mn^{2+}, it is important in the metabolism of *S. cerevisiae* as a part of some enzymes relating to ethanol fermentation such as pyruvate carboxylase. Mn^{2+} addition can enhance cell growth and ethanol concentration (O. Deesuth, P. Laopaiboon et al. 2012).

Currently practiced technologies in fuel ethanol industry are primarily based on the fermentation of sugars derived from starch and sugar crops and are quite mature with little possibility of process improvements. However, the conversion of starch and sugar crops to ethanol also has concerns since it draws its feedstock from a food stream (Y. Zheng and Z. Pan 2009).

Lignocellulosic biomass is a domestic feedstock that has potential to produce considerable quantities of bio-ethanol and other bio-energy and bio-based products. Processing of lignocellulosic biomass to ethanol consists of four major operations:

1. Pretreatment,
2. Enzymatic / Acid hydrolysis,
3. Fermentation and
4. Ethanol separation/ purification

To implement the bio-ethanol production process successfully, the first drawback that must be solved is the efficient removal of lignin and hemicellulose through pretreatment process (T. L. Arenas, P. Rathi et al. 2010). An ideal pretreatment is needed to reduce the lignin content and crystallinity of the cellulose, and increase the surface area of these materials (V. Sindhu, C. N. Kanchana et al. 2012).

Recently it has been demonstrated that the dilute acid pre-hydrolysis can achieve high reaction rates in short time and significantly improve cellulose hydrolysis. Most of the techno-economic studies based on process simulations for bio-ethanol production from lignocellulosic biomass take into account the hydrolysis reactions of hemicellulose to produce mainly sugar monomers and acid-soluble lignin. The main factors affecting the acid pretreatment are the type of biomass, the type of acid, the feed acid concentration, the reaction time and the reaction temperature (T. L. Arenas, P. Rathi et al. 2010).

2.2. Energy

The increasing industrialization and motorization of the world has led to a steep rise for the demand of petroleum-based fuels. Today fossil fuels take up 80% of the primary energy consumed in the world, of which 58% is consumed by the transport sector. The sources of these fossil fuels are becoming exhausted and found major contribution in greenhouse gas (GHG) emissions by consumption of fossil fuels to fulfill the energy demand, which leads to many negative effects including climate change, retreating of glaciers, rise in sea level, loss of biodiversity, etc (P. S. Nigam and Singh 2011).

The use of bio-energy ranges from traditional energy in rural populations to the use of liquid biofuels in the transport sector and 98% of current bio-fuel production involves the production of ethanol from sugars and biodiesel from oil seeds (A. Campbell and Doswald 2009).

2.3. Renewable Energy

Renewable energy attracts attention for the protection of the environment and supplies our energy needs by reducing dependence on petroleum and non-renewable energy sources (G. Izmirlioglu and A. Demirci 2012).

Petroleum will undoubtedly be depleted since it is a non-renewable fossil fuel source. It is estimated that crude oil production will decline worldwide and become drastically reduced by 2050 (G. P. da Silva, E. F. de Araújo et al. 2005).

Bio-ethanol, which is one of the energy sources, is known to be a potential alternative to petroleum-derived fuels and has the potential to meet the increasing demand for energy for industrial processes, heating and transportation (G. Izmirlioglu and A. Demirci 2012). Increase prices of molasses and its limited availability is another reason to divert the attention toward alternate resource (Z. Anwar, M. Gulfraz et al. 2012).

At present, the transportation sector is almost entirely dependent on petroleum-based fuel, it is being responsible for around 60% of the world oil consumption. Nowadays, industrial bio-ethanol production is mainly focused on corn, wheat and sugarcane, as well as on highly abundant agricultural wastes. The use of residual biomass for bio-ethanol productions has the added advantage of transforming a waste material into a valorized product. The increase in the prices of fuel and possibility of shortfalls has led to an extensive evaluation of alternative sources of energy to meet the global energy demand (S.Geetha, A.Kumar et al. 2013).

In 2009, 19,534.99 millions of gallon of ethanol were produced worldwide . The U.S. and Brazil are the two major countries and produced 10,600.00 and 6,577.89 million gallon of ethanol in 2009, respectively. Sugar cane, as a raw material, is used for 60% of global ethanol production, however; corn is the main raw material of ethanol production in the United States (90%). These carbon sources are high value products as a food source (G. Izmirlioglu and A. Demirci 2012).

Alcohol fuel presents several advantages over gasoline: alcohol combustion does not cause an increase in atmospheric CO_2 concentration, and is less polluting than gasoline because alcohol produces less toxic substances and less gaseous emissions. Ethanol is a pure substance of known composition, whereas gasoline is a mixture of different compounds. Alcohol is also a more secure energy source because it is renewable and can be produced any wherein the world from biomass (G. P. da Silva, E. F. de Araújo et al. 2005).

Ethanol can be blended up to 20% with diesel or petrol. At present ethanol is produced from molasses, which is byproduct of sugar industries. The cost of production increases as the demand for molasses has increased. Hence, it is absolutely necessary to search for alternate source for ethanol production. So far, common crops recognized are sugarcane, sweet sorghum, beet and potato. The agricultural crop residues such as paddy straw, wheat straw and bagasse are abundantly available having rich source of sugars (A. K. Singh, S.Rath et al. 2014).

2.4. Bio-Ethanol

Bio-ethanol is the leading renewable alternative liquid bio-fuel reported to be environmentally-friendly compared to other energy sources. Cellulosic livestock from agricultural residues is an attractive alternative feedstock that can be fermented to ethanol after appropriate pretreatment without impacting the food and feed supply (E.I. Riyantia and P.L. Rogers 2009).

Traditionally, ethanol has been produced by fermentation of the sugar products from starch and sugar crops. The continued use of first-generation ethanol is problematic for socio-economic reasons such as competition with food crops causing food shortages and increasing food prices, and for environmental reasons (G. A. Stanley and G. J. Dumsday 2010).

Mixing ethanol and gasoline increases the octane number of the mixture, reducing the need for toxic, octane-enhancing additives. Ethanol also provides oxygen for the fuel, which will lead to the reduced emission of carbon monoxide and non-combusted hydrocarbons (M. Galbe and G. Zacchi 2002).

A substantial development of bio-fuels is forecasted in the medium term, and to achieve it, it is necessary to set resources aside for improving the existing technologies, and to research and develop second generation bio-fuels at a commercial level (D.P. Navia, D.J. Reinaldo et al. 2011).

It is important to use biomass energy as a means of providing modern energy. It would complement solar, wind, and other intermittent energy sources in the renewable energy

mix of the future. One of the most immediate and important applications of biomass energy systems could be in the fermentation of ethanol from biomass (Y. Lin and Tanaka 2006), (L.S. Oliveira and A.S.Franca 2009).

Bioethanol production requires biomass with significant starch or sugars, which is fermented through enzymatic biological processes to generate liquid bio-fuel. The current major feedstock in the production of bio-fuels in the world is starchy biomass, which accounts for nearly 53% of all bio-ethanol production. Maize, wheat, sorghum and other starchy materials are the main starchy feed stocks used in bio-ethanol production. The second method uses sugarcane and sugar beet biomass, the feedstock that is already in sugar form, and the rest of the processes are the same as in starchy biomass; the last method uses biomass from cellulosic materials such as bagasse, straw and wood biomass.

Technology associated with starchy biomass and sugarcane is available and can be replicated, maize and other starchy biomass feed stocks have a very important role in food security in sub-Saharan Africa. To some extent, the use of these feed stocks (maize included) in the promotion of bio-fuel production makes it less attractive for most parts of Africa. On the other hand, secondary products from, for example, processing of sugar from sugarcane generate co-products like bagasse, molasses and fiber, which can be used to generate electricity and provide additional revenue if exported (W. H. van Zyl, A. F. A. Chimphango et al. 2011). Bio-fuel potential and type of biomass used in some African countries are shown in Table 1.

Table 1. Bio-fuels potential in selected African countries in mega liters (ML)

Country	Raw material	Bio diesel (ML)	Ethanol (ML)
Benin	Cassava	-	20
Burkina Faso	Sugarcane	-	20
Ivory cost	Molasses	-	20
Ghana	Jatropha	50	-
Guinea	Cashew	-	10
Mali	Molasses	-	20
Malawi	Molasses	-	146
Kenya	Molasses	-	413
Ethiopia	Molasses	-	80
Niger	Jatropha	10	-
Nigeria	Sugarcane	-	70
Sudan	Molasses	-	408
Swaziland	Molasses	-	480
Senegal	Molasses	-	15
Tanzania	Molasses	-	254
Togo	Jatropha	10	-
Uganda	Molasses	-	119

(W. H. van Zyl, A. F. A. Chimphango et al. 2011)

The constraints related to the availability of additional land suggest that second-generation bio-fuel industries should focus on currently available feedstock sources in the

initial phase of the industry. Agricultural and forestry residues form a readily available source of biomass can provide feedstock from current agricultural and forestry activities without need for additional land cultivation (D.P. Navia, D.J. Reinaldo et al. 2011).

2.5. Lignocellulosic substances

Cellulose is the abundant biopolymer on the earth, and is considered as best renewable energy resource. The major limitations exist for the production of ethanol from agri-cultural are compact physical and chemical associations between lignin and polysaccharides of plant cell wall along with cellulose crystallinity (Z. Anwar, M. Gulfraz et al. 2012).

The cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is therefore expensive. Under such circumstances a novel approach is essential to use renewable substrates (A. K. Singh, S.Rath et al. 2014).

Since the price of feedstock contributes more than 55% to the production cost, inexpensive feed stocks such as lignocellulosic biomass and agri-food wastes, are being considered to make bio-ethanol competitive in the open market. The production of ethanol from comparatively cheaper source of raw materials using efficient fermentative microorganism is the only possible way to meet the great demand for ethanol in the present situation of energy crisis (R. Arumugam and M. Manikandan 2011). The key or economical important step for the production of ethanol depends on efficient conversion of cellulose to their monomeric sugars (Z. Anwar, M. Gulfraz et al. 2012). The constituent of Lignocellulosic Substances is shown in Figure 1.

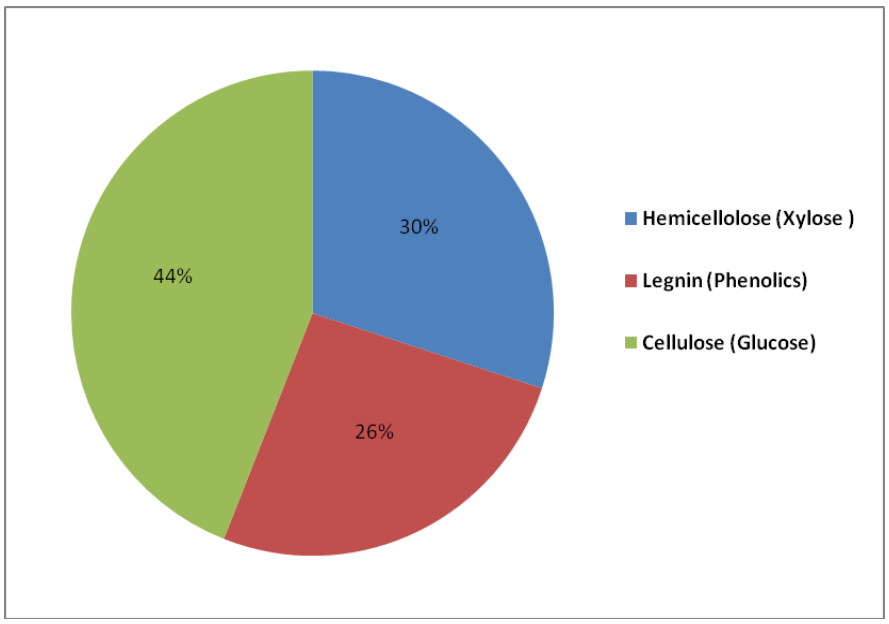


Fig. 1. Constituents of Lignocellulosic Substances (http://bioenergy.ornl.gov/main.aspxand GMIS, Oak Ridge National Laboratory)

2.6. Hydrolysis

Hydrolysis is a process of breaking down starch (amylopectin and amylose) into fermentable sugars and is needed before the fermentation. Hydrolysis is carried out at high temperature (90–110 ^{0}C); however, at low temperatures, it is possible and can contribute to energy savings. To convert starch into the fermentable sugars, either acid hydrolysis or enzymatic hydrolysis needs to be performed. Each has their own set of advantages and disadvantages for use. The limitations of acid hydrolysis can be by-products inhibition on growth of yeast (such as 5-hydroxymethylfurfural (5-HMF)), neutralization before fermentation and expensive constructional material due to corrosion risks. On the other hand, high prices of enzymes play a crucial role when feasibility is of concern. Enzyme hydrolysis is chosen even though high cost of enzymes and initial

investment because of high conversion yield of glucose (G. Izmirlioglu and A. Demirci 2012).

The hydrolysis of lignocellulosic material into fermentable sugars is a crucial stage, which mainly determines the overall process efficiency. Various methods are available for the generation of sugars from lignocellulosic biomass, of which the chemical and enzymatic methods have been proved to be more successful (P. Binod, K. U. Janu et al. 2011).

2.6.1. Acid hydrolysis

The concentrated acid process for producing sugars from lignocellulosic biomass has a long history. The ability to dissolve and hydrolyze native cellulose in cotton using concentrated sulfuric acid followed by dilution with water was reported in the literature as early as 1883. The concentrated acid disrupts the hydrogen bonding between cellulose chains, converting it to a completely amorphous state. Once the cellulose has been de-crystallized, it forms a homogeneous gelatin with the acid. The cellulose is extremely susceptible to hydrolysis at this point. Thus, dilution with water at modest temperatures provides complete and rapid hydrolysis to glucose, with little degradation (P. Binod, K. U. Janu et al. 2011). Dilute acid hydrolysis has been successfully developed for pretreatment of lignocellulosic materials. The dilute sulfuric acid pretreatment can achieve high reaction rates and significantly improve cellulose hydrolysis. At moderate temperature, direct saccharification suffered from low yields because of sugar decomposition. High temperature in dilute acid treatment is favorable for cellulose hydrolysis (Y. Sun and J. Cheng 2002).

2.7. Fermentation

Fermentation of sugars by microbes is the most common method for converting sugars inherent within biomass feed stocks into liquid fuels such as ethanol (J. Houghton, S. Weatherwax et al. 2006).

Alcoholic fermentation is one of the most important examples. As a consequence, ethanol is the most promising liquid fuel since it can be readily produced from various agriculture-based renewable materials, like sugarcane juice, molasses, potatoes, corn and barley etc. Currently, yeast *Saccharomyces cerevisiae* is used as the major ethanol producing microorganism worldwide (S.Geetha, A.Kumar et al. 2013). The most well-known and commercially significant yeasts that been primarily used for bio-ethanol production are the related species and strains of *Saccharomyces cerevisiae* (M. Fakruddin, M. A. Islam et al. 2013).

Fermentation efficiency of *S. cerevisiae* at high temperatures (above 35 ^{0}C) is low because of increased fluidity in membranes, which changes the fatty acid composition. In addition, increase in growth temperature, usually results in the biosynthesis of heat shock proteins that are implicated in conferring thermal and ethanol cross-tolerance in various organisms (M.I. Rajoka, M. Ferhan et al. 2005).

Current ethanol production organisms are primarily the natural occurring *Saccharomyces* strains of yeast. They have long been used for brewing and fermentation of distilled products. Today they are almost exclusively used for large scale industrial production (J. R. Hettenhaus 1998).

2.8. Evaluation of feedstock

Lignocellulosic biomass is the most promising feedstock considering its great availability and low cost, but the large-scale commercial production of fuel ethanol from lignocellulosic materials has still not been implemented. For designing fuel ethanol production processes, the assessment of the utilization of different feed stocks is required considering the big share of raw materials in ethanol costs.

2.8.1. Biomass

Biomass is seen as an interesting energy source for several reasons. The main reasons are:

- Bio-energy can contribute to sustainable development (SD)
- Resources are often locally available
- Conversion into secondary energy is feasible without high capital investments and
- Biomass energy can play an important role in reducing GHG emissions (Y. Lin and Tanaka 2006).

Plant biomass is therefore the only foreseeable renewable feedstock for sustainable production of renewable transport fuels (W. H. van Zyl, A. F. A. Chimphango et al. 2011). Among the main types of raw materials, cellulose materials represent the most abundant global source of biomass and have been largely unutilized (Y. Lin and Tanaka 2006).

2.8.2. Lignocellulosic biomass

For countries where the cultivation of energy crops is difficult, lignocellulosic materials are an attractive option for the production of bio-fuels. The main challenge in the conversion of biomass into ethanol is the pretreatment step.

Due to the complex structure of the lignocellulose, the pretreatment is required for its degradation, the removal of lignin, the partial or total hydrolysis of the hemicellulose, and the decrease in the fraction of crystalline cellulose related to the amorphous cellulose, the most suitable form for the subsequent hydrolysis step. In this step, the cellulose undergoes hydrolysis in order to obtain glucose that is transformed into ethanol by process microorganisms.

Process synthesis for conversion of lignocellulosic biomass to ethanol is oriented to the generation of different process configurations that could become viable alternatives for the production of a given product.

Considering that the biomass-to-ethanol conversion technologies are relatively immature and are not completely developed compared to cane ethanol or ethanol from starch, process synthesis methodologies can offer invaluable tools for the design of more cost-effective configurations with improved techno-economic and environmental indicators (C. A. Cardona and O. J.Sa´nchez 2007).

2.8.3. Conversion technology for cellulosic ethanol production

Production of ethanol from lignocellulosic biomass contains three major processes: pretreatment, hydrolysis, and fermentation. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its submicroscopic structural and chemical composition to facilitate rapid and efficient hydrolysis of carbohydrates to fermentable sugars. Hydrolysis refers to the processes that convert the polysaccharides into monomeric sugars. The fermentable sugars obtained from hydrolysis process could be fermented into ethanol by ethanol producing microorganisms, which can be either naturally occurred or genetically modified. Cellulose in lignocellulosic biomass is usually organized into microfibrils, each measuring about 3 to 6 nm in diameter and containing up to 36 glucan chains having thousands of glucose residues. process flow diagram of production of ethanol from cellulosic material is shown in Figure 2. According to the degree of crystallinity, cellulose is classified into crystalline and Para-crystalline (amorphous) cellulose.(Y. Zheng and Z. Pan 2009)

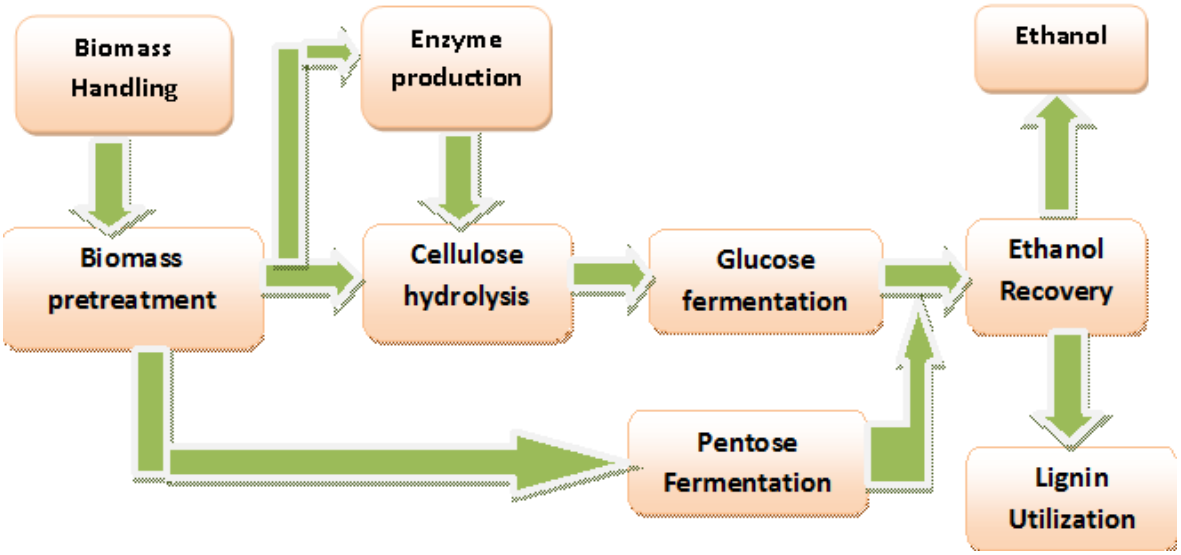


Fig. 2. Production of ethanol from cellulosic biomass

2.8.4. Lignocellulose to bio-ethanol

The production of lignocellulosic sugars is perhaps the most important stage in the ethanol production process, because good quality solutions favor the efficient conversion to ethanol. However the process is not complete, because the lignin-hemicellulose complex inhibits the penetration of the hydrolytic means, while due to its crystallinity the chemical breakdown of the cellulose is difficult.

Generally, three hydrolysis alternatives exist after the pre-treatment process:

1. Enzymatic hydrolysis
2. Concentrated acid hydrolysis
3. Dilute acid hydrolysis

Dilute acid hydrolysis is selected for the production of fermentable sugars than those in the case of concentrated acid. During this process dilute acid concentration is used at temperatures of 80 - 130 ^{0}C. Several acids can be used, such as HCl, H_2SO_4, H_3PO_4 and HNO_3. During pre-hydrolysis the lignin-hemicellulose complex is broken down, facilitating the hydrolysis of hemicellulose and the production of sugar, mainly xylose. Conversion of lignocellulosic materials to ethanol is shown in Figure 3. However, at increased temperature xylose is broken down and undesirable byproducts are formed.

The two stage process has several advantages such as:

- Allow the production of useful by-products such as xylitol and arabitol
- Increases the cellulose breakdown during hydrolysis and consequently the sugar yield
- Is more economical than the concentrated acid reaction because it requires cheaper equipment
- Environmental problems related to the use of strong acids are avoided and management is less complicated than that of enzymatic hydrolysis (C. A. Cardona and O. J.Sa´nchez 2007).

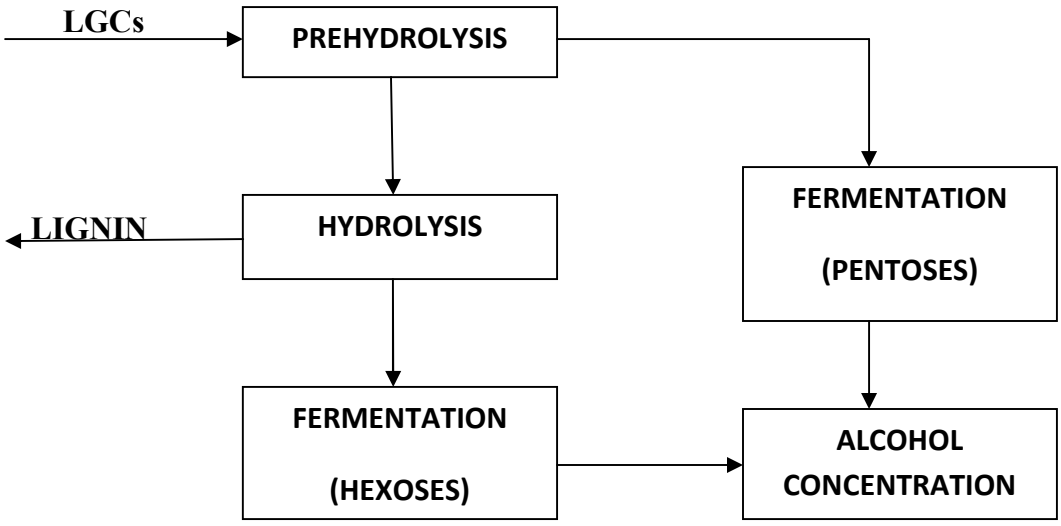


Fig. 3. Lignocellulose to ethanol (T. Tsoutsos and D. Bethanis 2011)

It is noted that the operating conditions need to be carefully selected in order to avoid high concentrations of byproducts with significant inhibitory effect during the fermentation. Also, before the fermentation process, the hydrolyzates' pH should be regulated, in order not to suspend the metabolism of the fermentation microbial cultures.

The use of combined microorganisms, which can simultaneously ferment hexoses and pentoses, is also reported (T. Tsoutsos and D. Bethanis 2011).

The development of cost-effective technologies for fuel ethanol production is a priority for many research centers, universities and private firms, and even for different governments. Due to the large amount of existing and not completely developed technologies for the production of ethanol the application of process engineering tools is required. The development of environmentally friendly technologies for bio-ethanol production can be carried out utilizing different design approaches.

Process optimization is another crucial tool employed within the framework of process design. Optimization plays a decisive role not only during the experimentation, but also during the design steps.

One of the most important approaches for the design of more intensive and cost-effective process configurations is process integration. Process integration looks for the integration of all operations involved in the production of fuel ethanol. This can be achieved through the development of integrated bioprocesses that combine different steps into one single unit. Thus, reaction separation integration by removing ethanol from the zone where the biotransformation takes place, offers several opportunities for increasing product yield and consequently reducing product costs.

Other forms of integration may significantly decrease energetic costs of specific flow sheet configurations for ethanol production. Process integration is gaining more interest due to the advantages related to its application in the case of ethanol production:

reduction of energy costs, decrease in the size and number of process units, intensification of the biological and downstream processes.

2.8.5. Trends in process design for fuel ethanol production

The design of cost-effective processes for fuel ethanol production implies the selection of the most appropriate feed stocks, and the selection and definition of a suitable process configuration.

The task of defining a proper configuration of the process requires the generation and assessment of many process flow sheets for finding those ones with improved performance indicators. This step of process design is called process synthesis. During process analysis, the structure of the synthesized flow sheets is established in order to improve the process through a more detailed insight of it (C. A. Cardona and O. J.Sa´nchez 2007).

It is expected that further expansion of the fuel ethanol market will depend upon conversion of lignocellulose, which includes agricultural residues. These feed stocks have advantages over traditional feed stocks in that they could have a much lower carbon footprint and a higher net energy return.

Despite the lower carbon footprint, the amount of biomass that will be needed to produce bio-fuels is immense. Thus, the logistics of harvesting, storing, and transporting the unprecedented quantities of biomass needed to produce sufficient volumes of bio-fuels is a great challenge (M. F. Digman, K. J. Shinners et al. 2010).

2.8.6. Ethanol and the environment

Ethanol represents closed carbon dioxide cycle because during both fermentation of biomass to ethanol and combustion of ethanol, the released carbon dioxide is recycled back into plant material because plants use CO_2 to synthesize cellulose during photosynthesis cycle. Ethanol production process only uses energy from renewable energy sources; no net carbon dioxide is added to the atmosphere, making ethanol an environmentally beneficial energy source.

In addition, the toxicity of the exhaust emissions from ethanol is lower than that of petroleum sources. Ethanol derived from biomass is the only liquid transportation fuel that does not contribute to the GHG effect. As energy demand increases the global supply of fossil fuels cause harm to human health and contributes to the GHG emission. The reduction of GHG pollution is the main advantage of utilizing biomass conversion into ethanol.

2.9. Coffee

Coffee is one of the most important commodities in the world economy, next to oils South America, South East Asia and Africa took the first three positions accordingly (Ethiopian Commodity Exchange Authority 2008).

Coffee has been consumed for over 1,000 years and today it is the most consumed drink in the world (more than 400 billion cups yearly) (I.M. Solange, M. S. M. Ercília et al. 2011)

The production of this commodity varies across regions. Coffee has been the Ethiopia most important cash crop and largest export commodity, which account 90% of exports and 80% of total employment. By its very nature, coffee is highly labor-intensive production activities. Thus very significant part of the population derives its livelihood from coffee. Coffee thus has a significant impact on the socio-economy life of the people and economic development of the country (Ethiopian Commodity Exchange Authority 2008).

Ethiopia has become particular interest to the world for its inherent quality and coffee production potential due to its arabica coffee, an indigenous variety. Coffee arabica has been grown in the wild forests of the southwestern massive highlands of the Kaffa districts of the country (Ethiopian Commodity Exchange Authority 2008).

2.9.1. Coffee beans processing

Coffee is processed by two widely known methods (wet and dry methods) (Ethiopian Commodity Exchange Authority 2008). Coffee cherries are the raw fruit of the coffee plant, which are composed of two coffee beans covered by a thin parchment like hull and further surrounded by pulp. These cherries are usually harvested after 5 years of coffee trees plantation and when the bear fruit turns red. The processing of coffee initiates with the conversion of coffee cherries into green coffee beans, and starts with the removal of both the pulp and hull using either a wet or dry method. Depending on the method of coffee cherries processing, i.e., wet or dry process, the solid residues obtained have different terminologies: pulp or husk, respectively. The dry method, commonly used for

Robusta, is technologically simpler comparing with the wet method, which is generally used for Arabica coffee beans (I.M. Solange, M. S. M. Ercília et al. 2011).

2.9.1.1. Wet method

Approximately half of the world coffee harvest is processed by the wet method in which the coffee berry is subjected to mechanical and biological operation in order to separate the bean or seed from the exocarp (skin), mesocarp (mucilaginous pulp) and the endocarp (parchment). The fraction represents about 40% of the weight of the fresh fruit and presently is underutilized, causing pollution problems. In wet method, the pulping involves the removal of the outer red skin (exocarp) and the white fleshy pulp (mesocarp) and the separation of the pulp and beans. Immature cherries are hard and green and very difficult to pulp. If the coffee is to be wet processed, correct harvesting is essential. For small-scale units, the cherries can be pulped in a pestle and mortar, and is very labor intensive (R. Padmapriya, J. A. Tharian et al. 2013).

2.9.1.2. Dry method

In dry method, the coffee cherries are dried immediately after harvest. This is usually sun drying on a clean dry floor or on mats. The bed depth is less than 40 mm and the cherries are raked frequently to prevent fermentation or discoloration. However, there are problems associated with this method. The most serious problem is dust and dirt blown onto the produce. Another problem is rainstorms often appear (even in the dry season) with very little warning. This can soak the produce very quickly. Finally, labor has to be employed to prevent damage or theft. Sun drying is therefore not recommended. Alternatively solar drying is done where the solar cabinet drier and the excel solar drier are used. In this way the coffee is placed in the trays in the solar drier. The layer of the

crop is deeper than one inch (3 cm) and the whole tray area is covered. The drier will be made ready as early in the day as possible so that all possible sunlight hours are used. The coffee is stirred regularly so that a uniform coloration is formed. At night, the crop should be placed in a cool dry room. In the wet season solar drying of produce is difficult. Rain is very unpredictable and frequent. Solar driers will prevent the coffee getting wet (R. Padmapriya, J. A. Tharian et al. 2013).

2.9.2. Coffee world production

World coffee production has grown more than 100% from 1950 to 1960, and there was a prediction to grow more 0.5–1.9% by 2010. Coffee is nowadays produced in a large number of countries worldwide. Nevertheless, the ten largest coffee producing countries are responsible for approximately 80% of the world production. Of this percentage, South America participates with around 43%, Asia with 24%, Central America 18%, and Africa with 16%. Brazil, Vietnam, Colombia, and Indonesia are respectively the first, second, and third largest world producers, responsible for more than half of the world supply of coffee.

The world consumption of coffee in 2007, estimated by the International Coffee Organization, has been around 124,636 million bags of 60 kg, representing an increase of 2.88% regarding the 121,150 million sacks consumed in 2006. Despite the financial crisis, the world consumption of coffee in 2008 was around 128 million bags. According to ICO, the consumption of coffee was not affected by the crisis. The consumers will not stop drinking coffee, but instead of drinking high quality coffee, people will start to take coffee of middle quality (I.M. Solange, M. S. M. Ercília et al. 2011).

The agro-industrial and the food sectors produce large quantities of waste, both liquid and solid. Due to the great demand of coffee, coffee industries are responsible for the generation of large amount of residues, which are toxic and represent serious environmental problems (I.M. Solange, M. S. M. Ercília et al. 2011). Average Coffee production of coffee producing countries in Africa is shown in Table 2.

Table 2. Africa: Coffee production (Average in thousand bags of 60 kg each)

Year	1980 - 89	1990 - 99	2000 - 09	2010 -12
Total Africa	19888	16078	15372	15712
Ethiopia	3128	2973	4904	6450
Uganda	2724	2811	2924	3002
Cote d'ivoire	4338	3448	2692	1291
Cameroon	1771	1022	821	845
Tanzania	875	779	796	686
Congo, D.R.	1610	1019	383	681
Kenya	1726	1377	766	669
Madagascar	1092	780	490	566
Others	2625	1868	1597	1522

The wastewater generated from coffee processing plant contains organic matter like pectin, proteins, and sugars (R. B. Mendoza and M. F. C. Rivera 1998). Coffee pulp, one of the principal by-products of wet processed coffee constitutes almost 40% of the wet weight of the coffee berry, is rich in carbohydrates, proteins, amino acids, poly-phenols, minerals, and appreciable quantities of tannins, caffeine and potassium. The poly-phenols

and caffeine are reported to be the anti-physiological factors on animal feed. Hence, coffee pulp has to follow a preliminary treatment before it is used (R. Sebastianos, P. G. Isabelle et al. 1998.). Coffee pulp is generated to the extent of 40% in the fermentation of coffee berries poses many problems in the coffee producing countries. Its disposal in nature, without any treatment, causes severe environmental pollution due to putrefaction of organic matter.

2.9.3. Coffee waste

Ethiopia is the third largest Arabica coffee (*Coffee arabica* L.) producer in the world and coffee is still a major growing household in Ethiopia (D.P. Navia, D.J. Reinaldo et al. 2011).

Coffee processing byproducts constitute a source of severe contamination and a serious environmental problem in Ethiopia and other coffee producing countries of the world (D.P. Navia, D.J. Reinaldo et al. 2011).

Wet and dry methods discard away 99% of the biomass generated by the coffee plants at different stages. This includes cherry wastes, coffee parchment husks, sliver skin, coffee spent grounds, coffee leaves, coffee pulp and wastewater (A. Kivaisi, B.Assefa et al. 2010).

Organic waste products, such as mucilage and pulp represent a major source of environmental pollution and their disposal is usually done in the water resources closest to the processing sites, such as rivers and lakes. Pulp and mucilage consume the oxygen in water, resulting in the death of plants and animals due to the lack of oxygen or the

increased acidity. The idea of using these products came from the need to minimize their negative environmental impacts, to give them added value, and to satisfy the demand for resources suitable for ethanol production (D.P. Navia, D.J. Reinaldo et al. 2011).

2.9.4. Residues generated in the coffee industry

The generation of residues and by-products is inherent in any productive sector. The agro-industrial and the food sectors produce large quantities of waste, both liquid and solid. Coffee is the second largest traded commodity in the world, after petroleum, and therefore, the coffee industry is responsible for the generation of large amount of residues. In the last decade, the use of such wastes has been subject of several studies, but this concern did not exist in past decades (1930 to 1943) when 77 million bags of green coffee were simply burned and released to the sea and in landfills (I.M. Solange, M. S. M. Ercília et al. 2011).

2.9.5. Wet coffee processing waste management practice In Ethiopia

Coffee is the most important and strategic cash crop and largest export commodity, which account 90% of exports and 80% of total employment in Ethiopia. Ethiopia had been the origin of coffee since coffee plant was initially found and cultivated in the Kaffa province (Bonga, Makira) of Ethiopia (ITC (International Trade Centre) UNCTAD/WTO 2002.) Coffee in Ethiopia contributes 41% of the country's total foreign exchange earnings and about 10% of the gross domestic product. Over 25% of the populations of Ethiopia are dependent on coffee for their livelihoods. There are four types of coffee production system in Ethiopia: forest coffee (10%), semi-forest coffee (35%), garden coffee (35%),

and plantation coffee (20%) (5% government,15% private). Coffee contains over 1500 chemical substances, 850 volatile and 700 soluble, and involves 13 independent chemical and physical variables. When coffee is extracted in water, most of the hydrophobic compounds, including oils, lipids, triglycerides, and fatty acids remain in the grounds, as do insoluble carbohydrates like cellulose and various indigestible sugars. Structural lignin, protective phenolics and the wonderful aroma-producing essential oils are also present in coffee. It is a major plantation crop grown worldwide and is one of the most popular beverages consumed throughout the world. There are three common species of coffee: robusta, arabica and liberica. 75-80% of the coffee produced worldwide is Arabica and 20% is Robusta.

2.9.6. Coffee industry residues applications

There is great political and social pressure to reduce the pollution arising from industrial activities. Almost all developed and underdeveloped countries are trying to adapt to this reality by modifying their processes so that their residues can be recycled. Consequently, most large companies no longer consider residues as waste, but as a raw material for other processes (S. I. Mussatto, G. Dragone et al. 2006).

The presence of organic material and its demand of great quantities of oxygen to degrade confer a toxic nature. Despite the negative characteristic and the large amounts that they are generated, there are few studies focusing on their use in different and profitable applications. Besides to add value to these unused materials, finding alternative forms to use them would be useful to decrease their impact to the environment (I.M. Solange, M. S. M. Ercília et al. 2011)

2.9.7. Proposed utilization of coffee industrial residues

Chemical composition of coffee waste, based on cellulose, hemicellulose, and protein, opens up possibilities for application of the residues in the production of different value-added compounds. Cellulose, for example, is a linear homo polymer of repeated glucose units extensively used for the pulp and paper production. Besides this potential application, cellulose can be converted to sugars such as polysaccharides, oligosaccharides, and mono saccharides by different treatment processes using acids or enzymes as catalysts. The conversion of cellulose to glucose is the first step in the large-scale chemical utilization of cellulose since this sugar may be subsequently converted to several products of interest such as ethanol (I.M. Solange, M. S. M. Ercília et al. 2011).

Hemicelluloses are heteropolymers constituted by 5-carbon sugars such as xylose and arabinose, and 6-carbon sugars including mannose, galactose, and others. Also glucose can be released from cellulose, these pentose and hexose sugars may also be released from the hemicellulose structure by means of some chemical or enzymatic pretreatment. Furfural once served as the raw material for nylon until displaced by butadiene, a chemical currently derived from petroleum (I.M. Solange, M. S. M. Ercília et al. 2011).

2.10. Chemical kinetics of Fermentation Process

Due to the difficulty in finding a strict mechanism for hydrolysis reactions, it is common to use simplified models to determine the kinetics of the hydrolysis of lignocellulosic materials. The simplest and widely used model involves a series of pseudo-homogeneous irreversible first-order reactions from solid polymer to aqueous monomer and then onto

decomposition products, Equation (2.1a), where the kinetic rate constants, k_i, is given according to Equation (2.1b):

$$A_{\text{polymer (biomass)}} \xrightarrow{k_1} B_{\text{monomer}} \xrightarrow{k_2} D_{\text{decomposition products}} \quad (2.1a)$$

$$K_i = C^n_{\text{acid}} A_i e^{-Ei/RT} \quad (2.1b)$$

where A_i is the pre-exponential constant for reaction i, C_{acid} is the sulfuric acid concentration (% w/w of liquid), E_i is the activation energy for reaction i, n is the order of reaction with respect to acid concentration, T is the temperature.

Acetic acid does not follow the mechanism of Eq. (2.1a). Its kinetic mechanism is according Eq. (2.1b), which depends slightly on the temperature and on the acid concentration (T. L. Arenas, P. Rathi et al. 2010).

$$A_{\text{Acetic group}} \xrightarrow{k_1} B_{\text{Acetic acid}} \quad (2.2)$$

Several factors affect the production rate of ethanol by fermentation, and a suitable mathematical description of the fermentation process has been developed. This helps in interpreting fermentation measurements with a view to early detection of poor fermentation performance, the ability to predict future fermentation behavior and application to design and advanced control of fermentation and optimization. For cell concentration, X, the logistic model was derived as follows;

$$\frac{dx}{dt} = \mu_m X \left(1 - \frac{x}{x_m}\right) \quad (2.3)$$

Where, μm is the maximum specific growth rate with respect to the fermentation conditions as the form of the Monod relationship with the following boundary conditions:

t = 0, X = Xo, S = So, P = 0

By integrating Eq. (2.3), the kinetic model can be formulated. The biomass production rate yields the following equation:

$$X = \frac{X_o \; X_m \; e^{\mu_m t}}{X_m - X_o + X_o e^{\mu_m t}} \tag{2.4}$$

Eq. (2.4) shows the relationship between biomass and fermentation time, which is used to fit experimental data of biomass concentration.

A delay of ethanol production was found compared with the cell growth, and little ethanol was produced during the yeast lag phase. Therefore, a parameter of the lag time, Δt, was introduced to describe the delay of ethanol production to cell growth, and the equation of ethanol production rate was modified as equation (2.5)

$$\frac{dp}{dt} = y_{p/x} \frac{dx}{d(t-\Delta t)} \tag{2.5}$$

Equation (2.5) can be integrated using two estimated parameters from equation (2.4) μm and Xm, and the Model is described by equation (2.6).

$$P = y_{p/x} \left[\frac{X_o \; X_m \; e^{\mu_m (t-\Delta t)}}{X_m - X_o + X_o e^{\mu_m (t-\Delta t)}} - \frac{X_o \; X_m \; e^{-\mu_m \Delta t}}{X_m - X_o + X_o e^{-\mu_m \Delta t}} \right] \tag{2.6}$$

The equation describing the substrate consumption rate takes into account two aspects, the sugar consumption in the formation of biomass and the maintenance of biomass. (G.A.Uduak and A. A. Adamu 2008) described the consumption rate of sugar as:

$$-\frac{ds}{dt} = \frac{1}{Y_{x/s}}.\frac{dx}{dt} + m.x \qquad (2.7)$$

Combining Eqs. (2.3), (2.5) and estimated parameters, Eq. (2.7) can be integrated and the sugar consumption equation can be represented by equation (2.8):

$$S = S_0 - \frac{1}{Y_{X/S}}\pi r^2\left[\frac{x_0\ x_m\ e^{\mu_m i}}{x_m - x_0 + x_0\ e^{\mu_m i}} - x_0\right] - \frac{x_m\ m}{\mu_m}\ln\frac{x_m - x_0 + x_0 e^{\mu_m t}}{x_m} \qquad (2.8)$$

Table 3 : Definition of terms and symbols use in equations

Term/Symbol	Definition (Units)
X	Biomass concentration (g/L)
X_m	Maximum biomass concentration (g/L)
X_0	Initial biomass concentration (g/L)
M_s	Maintenance coefficient (g sugar/g biomass h)
Δt	Lag time (hour)
T	Time (hour)
P	Produced ethanol concentration (g/L)
S	Fermentable sugar concentration (g/L)
S_0	Initial fermentation sugar concentration (g/L)
$Y_{p/x}$	Yield coefficient of ethanol on biomass (g ethanol/ g biomass)
$Y_{x/s}$	Yield coefficient of ethanol on biomass (g biomass / g sugar)
μ	Specific growth rate ($hour^{-1}$)
μ_m	Maximum Specific growth rate ($hour^{-1}$)

Source: (G.A.Uduak and A. A. Adamu 2008)

3. Materials and Methods

3.1. Study site selection and sample collection

The wet coffee waste sample (mucilage and pulp juice) in triplicate was collected from Bonga, Teppi, Goma II and Limu Kosa. All the processing industries from which the sample collected are private owned. Sample sites were selected based on some stated criteria, such as, the size of discharge to the rivers, the amount of water used for the process, the capacity of the processing industry and the location of coffee processing industry. The pulp juice was collected by pressing the pulp without physical damage and crashing. The juice was filtered using mesh and collected in the flasks. The Collected samples were put in ice box and transported to Addis Ababa Institute of Technology, School of Chemical and Bio-Engineering and kept in Environmental Engineering and Bio Innovative laboratory for further pretreatment, preparation and characterization process.

Table 4. Description of study area.

Study area	Country	Region/Zone	Coordinate	Elevation above sea level	Population (2007)
Bonga	Ethiopia	SNNPR/Kaffa	7°16′N 36°14′E / 7.267°N 36.233°E	1,714 m	20,858
Teppi	Ethiopia	SNNPR/Sheka	7°12′N 35°27′E / 7.200°N 35.450°E	1,097 m	134,519
Goma II	Ethiopia	Oromia/Jimma	6° 1' 0" N, 37° 1' 0 E	1,680 m	213,023
Limu Kosa	Ethiopia	Oromia/Jimma	7°50’N 36°44’E/ 8°36’N, and 37°29’E	3020 m	164,629

3.2. Materials and chemicals

Different analytical reagents were used for the analysis of wet coffee waste samples. Sulfuric acid (98%, sd fine-chem. limited, Mumbai, India), sodium hydroxide (Abron Chemicals, India), hydrochloric acid (Abron Chemicals, India), methylene blue, fehling solution (A and B), *Pichia anomala* (M_4) (1% glucose, 0.5% peptone, 3% malt extract, and 2% agar-agar), yeast (*S.cervisiae*), filter paper, crucible, Erlenmeyer flask, round bottom flask, pH meter (3505-JENWAY, UK), balance (HCB1002-ADAM), stove (seven star, Germany), oven (202-OA, Germany), centrifuge, refractometer, gas chromatography (DANI, model GC-1000, Italy) and furnace (SX-2.5-12, box type resistance furnace, China) were used throughout the research work.

3.3. Methods

3.3.1. Wet coffee processing waste management practice in Ethiopia

Formal and informal discussions with concerned individuals were conducted while studying coffee waste management practice in Ethiopia. The methodology also employed reviewing literatures related to waste management practice in Africa as well as in Ethiopia. Sectoral policy and strategy documents, proclamations, regulations, relevant guidelines and official reports were also assessed. The sources of the policy information documents are Ministry of Agriculture, Ethiopian Commodity Exchange and different archives. Interviews were also conducted with knowledgeable individuals in search of information about waste generation and its management practice.

3.3.2. Characterization of wet coffee waste

a. Determination of moisture content

The sample was oven dried at 105 ^{0}C for 24 hours and the moisture content was determined in Environmental Engineering laboratory at AAiT using equation 3.1:

$$\%\ Moisture\ content = \frac{W_1 - W_2}{W_1} * 100 \qquad (3.1)$$

where, W_1 = weight of the sample before oven drying, W_2 = weight of the sample after oven drying.

b. Hydrolysis of waste

Cellulosic and hemicelluosic materials of the sample were broken down in to fermentable sugars by hydrolyzing the sample with dilute sulfuric acid at different concentrations (0.2, 0.4, 0.6, 0.8 and 1 M) and with distilled water and the hydrolysate samples was allowed to cool. The hydrolysate was filtered and determined for sugar composition by fehling method and the pH was adjusted to pH =5 using concentrated sulfuric acid and concentrated sodium hydroxide.

c. Determination of sugar content

Solution 1 was prepared by: (i) dissolving 50 mL of hydrolyzed sample solution in 10 mL of distilled water, (ii) adding 2 mL of concentrated HCl to the solution and boiling, (iii) neutralizing the sample with NaOH, and (iv) making the volume of solution up to a volume of 300 mL and taking into the burette.

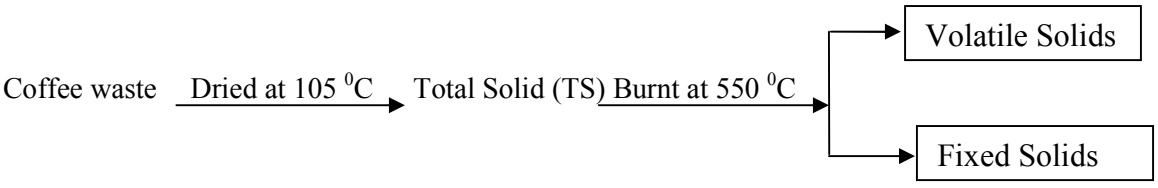
Solution 2 was prepared by: (i) mixing 5 mL of Fehling A and 5 mL of Fehling B with 90 mL of distilled water in 250 mL Erlenmeyer flask, (ii) adding two drops Methyl blue indicator. The sugar content of the sample was determined by: (a) titrating solution in the flask (solution 2) with the solution in the burette (solution 1) in boiling conditions until blue color disappear, (b) recording the volume at which brick red color observed, and (c) calculating the sugar content using the formula given below (S.V. Periyasamy, S. Venkatachalams et al. 2009).

$$Sugar\ Content\ (\%) = \frac{300\ ml\ x\ f}{v}\ x\ 100 \qquad (3.2)$$

where: f – Fehling factor (0.051); v–volume used in the titration (titrate value) (mL).

d. Determination of total solid (TS) and volatile solid (VS)

Total solid and Volatile solid of wet coffee processing waste was determined at the School of Chemical and Bio Engineering, Environmental Engineering laboratory according to the following process diagram.



3.3.3. Theoretical estimation of bio-ethanol potential of wet coffee processing waste

The study involved semi structured interviews, field visits and document review. Formal and informal discussions were also conducted. The methodology also employed reviewing bio-fuel development related sectoral and cross-sectoral policy and strategy documents, proclamations, regulations, relevant guidelines and official reports. The sources of the policy information documents are Ministry of Agriculture (MoA), Ministry of Water and Energy (MoWE), Ethiopian Commodity Exchange (ECX) and different archives. Interviews were also conducted with knowledgeable individuals in search of policy, strategy and legal information and materials. Field visits were conducted in various woreda (districts) within the regions (SNNPR and Oromia) where wet coffee processing is going on. The quantity of waste generated from wet coffee processing industries and the bio ethanol potential of coffee waste was estimated.

3.3.4. Optimization of Parameters

To avoid microbial contamination, coffee waste was sterilized at 120 ^{0}C for 15 min, cooled to room temperature and kept in the fridge at 4 ^{0}C before use.

a. Organism and culture media

The *Pichia anomala* (M_4), non-saccharomyces yeast was used for ethanol production optimization study. The yeast strain was maintained on agar slants (1% glucose, 0.5% peptone, 3% malt extract, and 2% agar-agar) and used to inoculate pre-fermenting media containing 2% sugar and 3g/L yeast extract and incubated for 12 hours before use in the

optimization studies following procedures as developed by (C.A.VIEGAS, I. S. CORREIA et al. 1985).

b. Optimization of initial pH

pH is very essential in fermentation since it affects the ionic state of mineral components and cellular surface of fermenting yeast. Eight sets of pH values were used to provide a wide range of selection of an optimum pH condition for ethanol production. 100 mL of coffee waste was poured into 250 mL conical flasks and pH was adjusted using HCl and NaOH to 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5 using digital pH meter.

The flasks were sterilized at 120 ^{0}C for 15 minutes. Upon cooling; the pre-fermentative yeast measuring 5 mL was inoculated to the sterilized media. Every 12 hours, 1.5 mL of the fermenting media was withdrawn to tubes according to the procedure described by (S.V. Periyasamy, S. Venkatachalams et al. 2009). The tubes were centrifuged at 13000 rpm for 4 minutes to get supernatant for ethanol analysis. The sample was analyzed using refractometer.

c. Optimization of hydrolysis time

A series of experiments was performed for different hydrolysis times (30, 60, 90, and 120 min). In each experiment the hydrolysis time was kept constant as the fermentation times were varied from 12 to 48 hours and the results were recorded.

d. Optimization of hydrolysis temperature

Suitable temperature for maximum production of ethanol was studied. Coffee waste was hydrolyzed with various concentrations of sulfuric acid (0.2, 0.4, 0.6, 0.8 and 1 M) and distilled water in 500 mL Erlenmeyer flask and separately heated at 85, 100 and 115 ^{0}C for 1 hour using oil bath thermostat. After hydrolysis the liquid fraction was cooled, filtered and determined for glucose concentration using titration method. The acid hydrolysates were adjusted to pH 5-6 using concentrated sulfuric acid and 2 N sodium hydroxide, and the solutions were filtered and made ready for fermentation (L. Dawson and R. Boopaty 2008) and after fermentation, quality of ethanol was analyzed at JIJE LABOGLASS Pvt. Limited Company.

e. Optimization of fermentation time

The hydrolysate obtained from hydrolysis of the coffee waste was collected and divided into four samples and used for the subsequent fermentation experiments. The fermentation was done at 12, 24, 36 and 48 hours, respectively withdrawing samples after 12 hours respectively and the sample is analyzed using Refractometer.

3.3.5. Bio-ethanol production from wet coffee processing waste

a. Dilute acid hydrolysis

Dilute sulfuric acid is used to hydrolyze the components of the mucilage and coffee pulp juice that may resist fermentation. In the first stage different concentrations (0.2, 0.4, 0.6, 0.8 and 1 M) of sulfuric acid and distilled water were used to hydrolyze the materials.

The solution of sample and different concentrations of sulfuric acid was heated at 100 ^{0}C for 1 hour. The liquid hydrolysate was then be neutralized and recovered from the process.

b. Fermentation process

Yeast (*S.cervisiae*) was added to the solution (hydrolysate). The yeast contains an enzyme called invertase, which acts as a catalyst and helps to convert the sucrose sugars into glucose and fructose (both $C_6H_{12}O_6$). The fermentation process was carried using different fermentation bottles in the Incubator and ethanol concentration was analyzed by GC (DANI, model GC-1000, Italy).

c. Ethanol analysis

Samples analyzed for ethanol production were taken at the end of fermentation after 12, 24, 36 and 48 hours of incubation. Ethanol analysis was done by using gas chromatography (GC) (DANI, model GC-1000, Italy) in which the sample is injected manually. Flame Ionization Detector (FID) was set at 280 ^{0}C.

Separation was effected in a 30 m, 0.25 mm and 1 μm column type CP- SIL 8 CB, with the temperature maintained at 45- 55 ^{0}C at a rate 2 ^{0}C/min. for 5 min then at 10 ^{0}C/min to 200 ^{0}C. Column flow was employed at 1.5 mL/min with nitrogen as a carrier gas and hydrogen and compressed air as a combustion gases.

d. Distillation process

The ethanol, produced from the fermentation process, contain a significant quantity of water, which must be removed. This is achieved by using the fractional distillation process by boiling the water and ethanol mixture. Since ethanol has a lower boiling point (78.3 ^{0}C) compared to that of water (100 ^{0}C), the ethanol turns into the vapor state before the water and it can be condensed and separated. The distillate (Ethanol) was analyzed by density method using Pickno-meter and further checked at National Alcohol and Liquor Factory so as to compare the density of ethanol from the sample with that of standard ethanol.

3.3.6. Characterization of the Residue

Nitrogen and trace elements are essential elements in the production of ethanol by yeasts. Thus, their presence in the fermentation media was determined according to the procedure described by (A.L. Allen and C.D. Roche 1989). The minerals of the coffee waste were obtained by dry ashing method. About 5 g of Coffee waste was placed in a porcelain crucible and dried in an oven at 105 ^{0}C until a constant weight was obtained. The dried Coffee waste was then placed in a muffle furnace and was ignited at 550 ^{0}C for 2 hours to get the ash. The obtained ash was used for analysis of the minerals following the procedure described by (A.L. Allen and C.D. Roche 1989)..

Sludge characterization includes physical parameter, pH and chemical properties of total nitrogen, total phosphorus and total potassium. The sample was analyzed for the following parameters: total nitrogen, total phosphorus and total potassium using hach

method (Hach Company 2007). Final analysis was conducted at JIJE LABOGLASS Pvt. Limited Company.

4. Results and Discussion

4.1. Wet coffee processing waste management practice in Ethiopia

According to the data collected and information gathered from different sources, in Ethiopia there are more than 1026 functional wet coffee processing industries generating 291,600 tons of waste per year. Presently, the coffee processing industries are not familiar with the waste treatment (management) systems and the wastes are discharged to the nearby streams in which the local community uses for different purposes of their daily life

Wet coffee processing requires high degree of processing knowledge. Effluents from the industries have potential to damage the environment. The local community is facing a serious problem in properly disposing of the waste produced by processing of coffee. The photo evidence about practice of coffee waste disposal of some coffee processing industries in Ethiopia is attached as an Annex.

4.2. Characterization of wet coffee waste

The characteristics of wet coffee waste (pulp juice and mucilage) obtained in this study together with that reported in the literature is given in Table 6. From the Table, it can be seen that the data for physico- chemical characteristics of coffee waste obtained from the literature (India and Vietnam) is not complete, but this study tried to generate all the necessary data and compare with the literature.

Table 6. Physico-chemical characteristics of pulp juice and mucilage

Country	Source	Sample	pH	Moisture (%)	Total solid (%)	Volatile solid (%) of TS	Fixed solid (%) of TS	BOD_5 (mg/L)	COD (mg/L)
Ethiopia	Coffee waste from Ethiopia (this study)	Pulp juice	4.75	90.0	10.0	66.5	33.5	25,620	45,000
		Mucilage	3.67	96.9	3.1	90.2	9.8	19,810	33,600
India	Coffee waste from India	(Pulp juice + mucilage)	3.58-4.21					3,800-4,780	6,420-8,480
Vietnam	Coffee waste from Vietnam	Pulp juice	----	81.8	----	98.5	1.5	---	---
		Mucilage	----	84.2	----	99.3	0.7	---	---

As it can be seen from Table 6, Ethiopian coffee waste is acidic (pH = 4.75 and 3.67), therefore, discharging the waste directly to the water causes pollution. Volatile solids (organic matter or degradable components) are 66.5% and 90.2% for pulp juice and mucilage, respectively. The values show that the wastes have high potential to pollute water bodies since the organic components are degradable. The organic components provide food for microbes, and potential vectors, therefore, increases potential odors and consequently increase attraction of vectors. The fixed solid (FS) is 33.5% and 9.8% for pulp juice and mucilage, respectively, and it constitute the residual inorganic compounds (N, P, K, Ca, Cu, Zn, Fe, etc.) in dissolved state.

The Table also shows that BOD_5 of pulp juice is 25,620 and that of mucilage is 19,810 mg/L. The result shows that the waste is high oxygen-demanding. It is a measure of the strength of effluent and its pollution potential. The COD of pulp juice and mucilage is 45,000 and 33,600 mg/L respectively. The COD: BOD_5 ratio is frequently used as an

indicator of biological degradability: ratios less than 2:1 indicate high digestibility. Therefore, the result shows that the ratio of COD: BOD_5 is less than 2:1, which indicates the biological degradability of the waste.

The data obtained during the study and presented in Table 6 is in agreement with the literature, i.e. the values BOD_5 and COD for Pulp juice and Mucilage (25620 , 45000 and 19810 , 33600) are within the interval (M. Selvamurugan, P. Doraisamy et al. 2010) and the values of moisture content and volatile solid are in agreement with the values reported in the literature (J.C.V. Enden and K.C.Calvert 2010). The total sugar of the waste was determined by titration method (Association of Official Analytical Chemists AOAC 1995) and the data obtained are shown in Table 7.

Table 7. Percentage of total sugar of the coffee waste (Wort) hydrolyzed at different concentrations of H_2SO_4.

Concentration of acid used for hydrolysis	% sugar from mixture of Bonga and Teppi waste		% sugar from Bonga waste		% sugar from Teppi waste	
	Pulp	Mucilage	Pulp	Mucilage	Pulp	Mucilage
0 % (distilled water)	56.66 ± 1.05	45.00 ± 1.32	58.85 ± 2.27	52.75 ± 0.91	54.64 ± 0.98	51.00 ± 1.70
1%	63.75 ± 2.66	51.00 ± 0.85	66.52 ± 1.45	58.85 ± 2.27	61.20 ±2.46	56.66 ± 1.06
2%	76.50 ± 1.92	54.64 ± 1.95	80.52 ± 2.12	63.75 ± 1.34	66.52 ± 1.45	63.75 ± 2.66
3%	85.00 ± 2.37	61.20 ± 1.22	90.00 ± 2.65	69.54 ± 1.58	72.85 ± 1.74	69.54 ± 1.58
4%	72.86 ± 1.74	52.75 ± 1.82	76.50 ± 1.92	61.20 ± 1.22	63.75 ± 1.34	61.20 ± 2.45

Table 7 shows that total reducing sugar content increases with increase in acid concentration up to 3% acid used for hydrolysis and then decreases. The maximum

amount of total reducing sugar (90.00%, pulp juice from Bonga) is obtained at 3% H_2SO_4 and minimum amount of total reducing sugar (45.00%, mucilage, mixture of Bonga and Teppi) is obtained at hydrolysis of 0% (distilled water). The increase in total reducing sugar content that resulted from the acid hydrolysis is similar to the one reported by (D. P.Navia, D. J.Reinaldo et al. 2011) for the waste but the yield decreases after optimum point. As can be seen from the Table, waste from Bonga contains relatively higher sugar content than waste from Teppi, this might be because Teppi is low land compared to Bonga and some of the sugar from the waste may be fermented. Furthermore, the table also shows that pulp juice contains more reducing sugar than Mucilage. One can observe that, the result obtained from the mixture of waste from Bonga and Teppi is an average between the results obtained from the wastes of Bonga and Teppi.

Total reducing sugar in the waste increases with increase in concentration of acid used for hydrolysis and decreases after the optimum point (3% H_2SO_4). The discharge of effluents (coffee waste) into receiving water bodies invariably result in the presence of high concentrations of pollutant in the water. The pollutants have been shown to be present in higher enough concentrations, which is toxic to different organisms. The effluents also have considerable negative effects on the water quality of the receiving water bodies and as such, they are rendered not good for human use. It is therefore recommended that the careless disposal of the wastes without pretreatment should be discouraged.

4.3. Theoretical estimation of bio-ethanol potential of wet coffee processing waste

The contribution of Ethiopia to world green coffee production is increasing year to year. According to (International Coffee Organization (ICO) 2014), MoA), the contribution of Ethiopia increased from 5.12% (2007 G.C.) to 5.60% (2012 G.C.)

There are about 1026 operational wet coffee processing industries in Ethiopia which are particularly situated in Oromiya, SNNPR and Gambela regional states. In addition, numbers of new wet coffee processing industries are under construction. It is possible to see that there are considerable numbers of industries with different processing capacity which can generate huge waste in the country. It can be projected that the amount of waste that will be generated will increase considerably when the industries under construction become operational.

Since the number of processing industries and the amount of green coffee production shows dramatic increase, so does the amount of waste generated from the processing industries.

The amount of waste generated from wet coffee processing industries in Ethiopia for consecutive six years is estimated and tabulated as follows:

Table 5. Estimated amount of waste generated from wet coffee processing in Ethiopia

Year	Amount of waste (tons)
2007	214,812
2008	178,164
2009	249,516
2010	270,000
2011	244,728
2012	291,600

From Table 5, it can be seen that the amount of waste generated from the wet coffee processing industries in Ethiopia is generally increasing from year to year. Since a number of processing industries are under construction and becoming operational very soon, much more waste will add up to the current amount.

Lignocellulosic biomass has a higher bio-ethanol yield per ton feedstock (L/t) than most of the commercialized bio-ethanol feedstock. However, improvement had to be made on the conversion efficiency to obtained higher ethanol yield to make it more comparable with the sugar containing and starchy material. The composition of substance that can be converted to glucose played a big influence on the ethanol yield per ton feedstock. With the large amount of glucose convertible material and abundant availability, these lignocellulosic biomasses are potential feedstock for bio-ethanol production. In both instances, ethanol use can improve urban air quality. Enough waste materials can be made available to produce sufficient ethanol to replace all gasoline. Estimated amount of waste generated from wet coffee processing in 2012 in Ethiopia is around 291,600 tons.

There are more processing industries under construction which will be operational soon, indicating huge amount of waste to add up to the existing amount.

The potential ethanol supply from biomass is substantial, and large scale application would thereby reduce the strategic vulnerability to disruption in oil supply, while substantially improving the balance of trade deficit for imported oil. Technologies have advanced significantly for the conversion of lignocellulosic biomass in to ethanol, so that the price of ethanol from lignocellulosic biomass is competitive with ethanol derived from corn. Ethanol production from lignocellulosic biomass offers the added advantage in that no net accumulation of atmospheric CO_2.

4.4. Optimization of Parameters

a) Initial pH optimization

The natural pH of coffee waste in Ethiopia was observed to vary between 3.5 and 5.5. The results from this study show that the concentration of ethanol produced differed significantly with the variation in initial pH values.

The lowest ethanol concentration of 2.04% (v/v) was found in pH 3 but increased with increase in pH to a maximum concentration of 4.7% (v/v) at pH 4.5, beyond which it started to show a slight decreasing trend. According to these results, pH 4.5 provides optimal condition for ethanol production. However, ethanol concentrations above pH 4.5 were almost equally high, suggesting that the natural pH of coffee waste (pH 5.5) can support yeast growth and ethanol production at appreciable levels. Thus coffee waste at its natural pH can be used in the production of bio-ethanol with little or no cost related to

pH adjustments. Similar studies by (S.Geetha, A.Kumar et al. 2013) reported significant increase in ethanol yield from pH of 4.5 to 5.5, beyond which the levels did not increase much. Slightly lower optimal pH values (pH 4.25) have been reported by (K. Pramanik 2003) However, at lower pH (3-4) the production of ethanol was slightly lower compared to pH 4.5. The optimum pH value is shown in Table 8 and Figure 4 below.

Table 8. Ethanol yield at different pH

pH	Ethanol yield (% v/v)
3	2.04
3.5	2.41
4	3.62
4.5	**4.70**
5	4.63
5.5	4.57
6	4.55
6.5	4.50

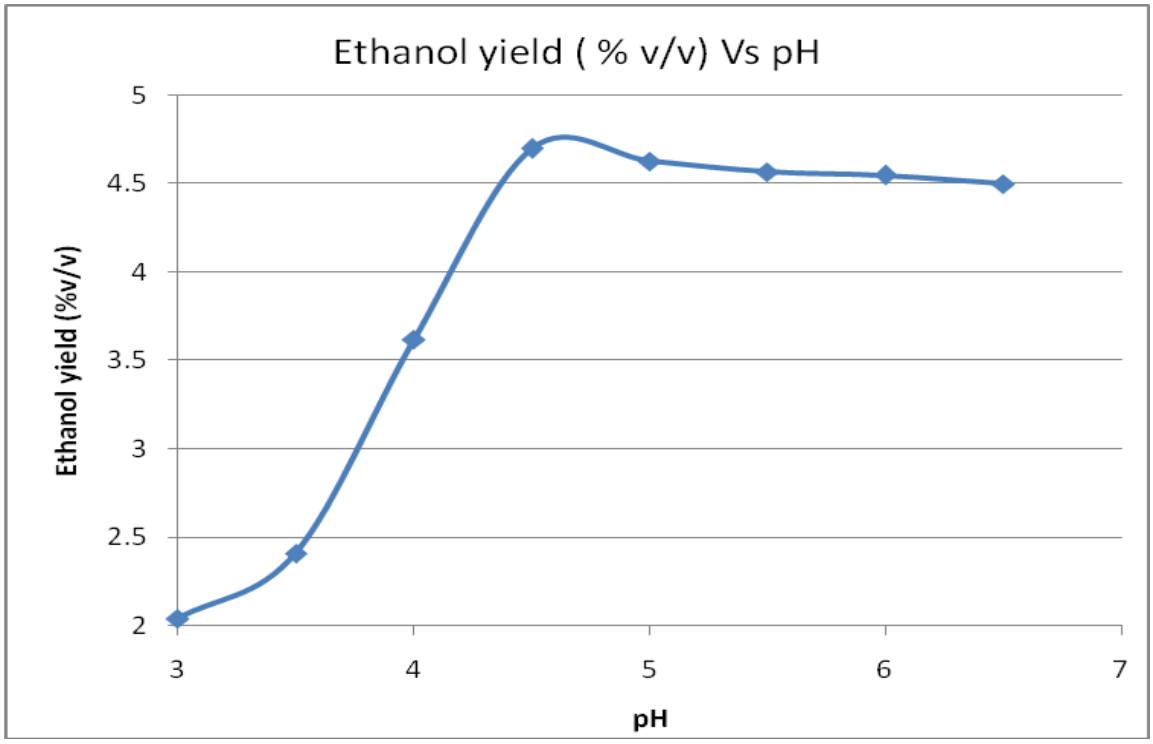


Fig. 4 . Effect of pH on ethanol concentration

b) Hydrolysis time

It was observed that sequentially prolonging the hydrolysis time significantly increased bio-ethanol concentration and then started to decline after 60 min hydrolysis time. The result is shown in the Figure 5.

Table 9. Ethanol yield at different residence (hydrolysis) times at constant fermentation time (24 hours)

Sample	Hydrolysis time (hour)	Ethanol yield (g/L)
1	0.5	5.95
2	1	6.12
3	1.5	5.76
4	2	5.43

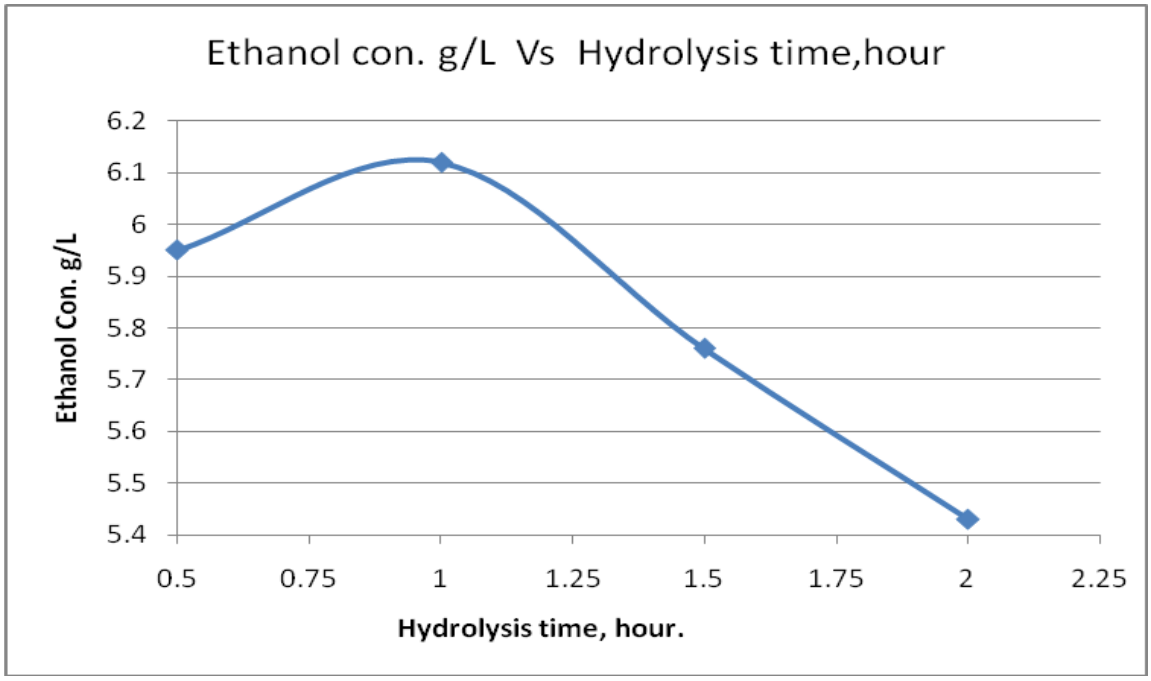


Fig. 5. Effect of hydrolysis time on ethanol concentration

Figure 5 show that at (30, 60, 90 and 120 min hours) hydrolysis time and constant fermentation time of 24 hour; 5.95, 6.12, 5.76 and 5.43 g/L bio-ethanol concentration was obtained, respectively. The maximum bio-ethanol concentration of 6.12 g/L was achieved at 60 min hydrolysis time. However, as hydrolysis time increases above the optimum

point, concentration of bio-ethanol decreases. This could have resulted because the longer residence (hydrolysis) time makes the sugars to degrade and form inhibitors.

c) Hydrolysis temperature

A series of experiments were performed for different hydrolysis temperature (85, 100 and 115 ^{0}C) since the hydrolysis process is carried out under heating condition. In each experiment the hydrolysis temperature was kept constant as the fermentations times were varied from 12 hours to 48 hours. It was observed that bio-ethanol concentration increases with increase in temperature and then started to decreases after 100 ^{0}C. The results were recorded and maximum yield was obtained at 100 ^{0}C. The results are tabulated and plotted in the Figure 6.

Table 10. Ethanol yield at different residence (hydrolysis) temperature at constant fermentation time (24 hours)

Sample no	Hydrolysis temperature (^{0}C)	Ethanol yield (g/L)
1	85	5.23
2	100	6.12
3	115	5.41

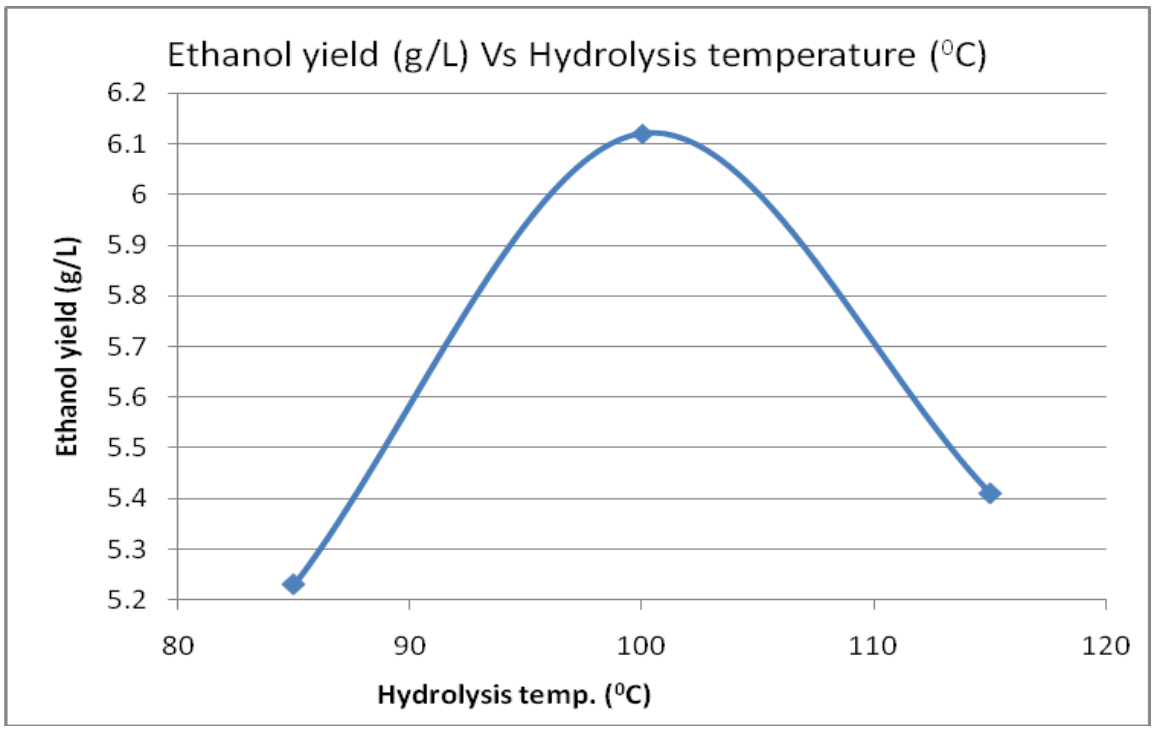


Fig. 6. Effect of hydrolysis temperature on ethanol concentration

Figure 6 show that at (85, 100 and 115 ^{0}C) hydrolysis temperature and constant fermentation time of 24 hour; 5.23, 6.12 and 5.41 g/L ethanol concentration was obtained, respectively. The maximum bio-ethanol concentration of 6.12 g/L was achieved at 100 ^{0}C hydrolysis temperature. However, concentration of ethanol decreases after 100 ^{0}C.

d) **Fermentation time**

This experiment was done based on optimized conditions (optimum hydrolysis time and hydrolysis temperature). The fermentation was allowed for two days withdrawing samples after each 12 hours interval. The results obtained are tabulated in the table below and a graphical representation is also made as shown in Figure 7.

Table 11. Ethanol yield at different fermentation times at constant hydrolysis time and temperature (1 hour, 100 ^{0}C)

Sample	Fermentation time (hour)	Ethanol yield (g/L)
1	12	6.09
2	24	6.12
3	36	5.90
4	48	5.84

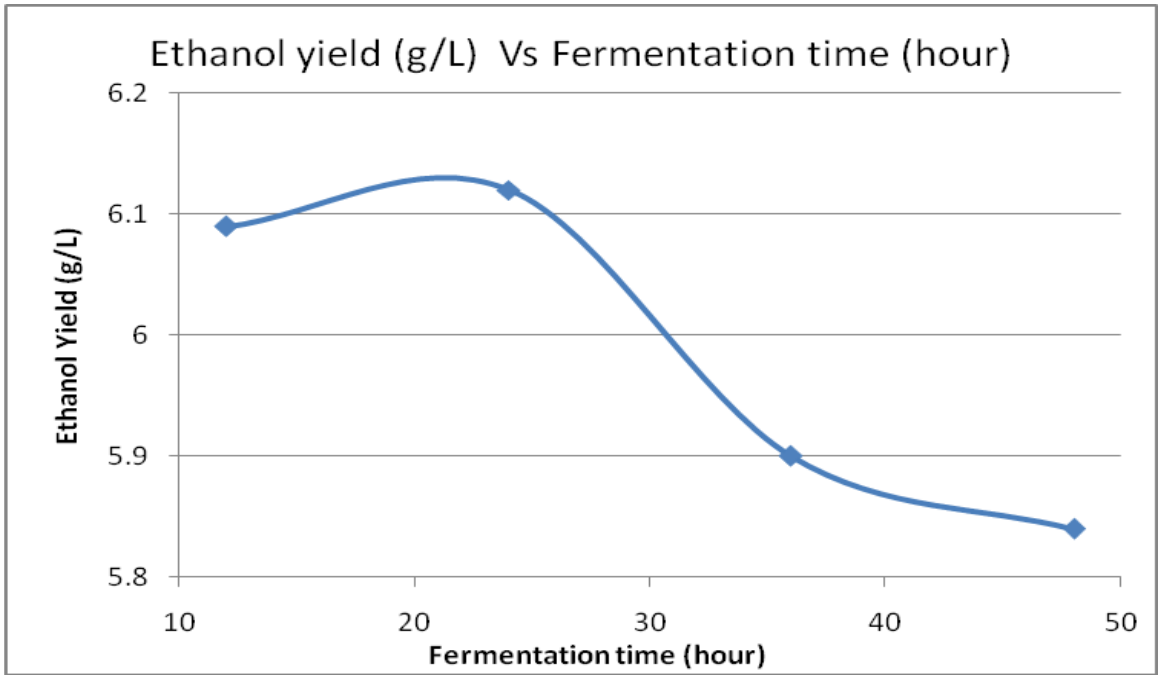


Fig. 7. Effect of fermentation time on the concentration of ethanol

The concentration of ethanol increased with increasing fermentation time and decreased at the end of fermentation time. Maximum ethanol concentration, 6.12 g/L was obtained after fermentation for 24 hours and the result started to decrease after 24 hour of fermentation time. The figure also indicated that the lowest concentration of ethanol

production of 6.09, 5.90 and 5.84 g/L was obtained at fermentation time of 12, 36 and 48 hours, respectively. From the optimization experiment, the highest concentration of ethanol was achieved at 24 hours of fermentation and started to level off.

The decrease in concentration of bio-ethanol after 24 hours might be due to the consumption of sugar by the microorganisms or the hydrolysate does contain significant levels of metabolic inhibitors that have accumulated and interfere the fermentation process.

4.5. Bio Ethanol production from wet coffee processing waste under optimized conditions

a. Gas chromatogram (GC) results

Standard ethanol (99.4%)

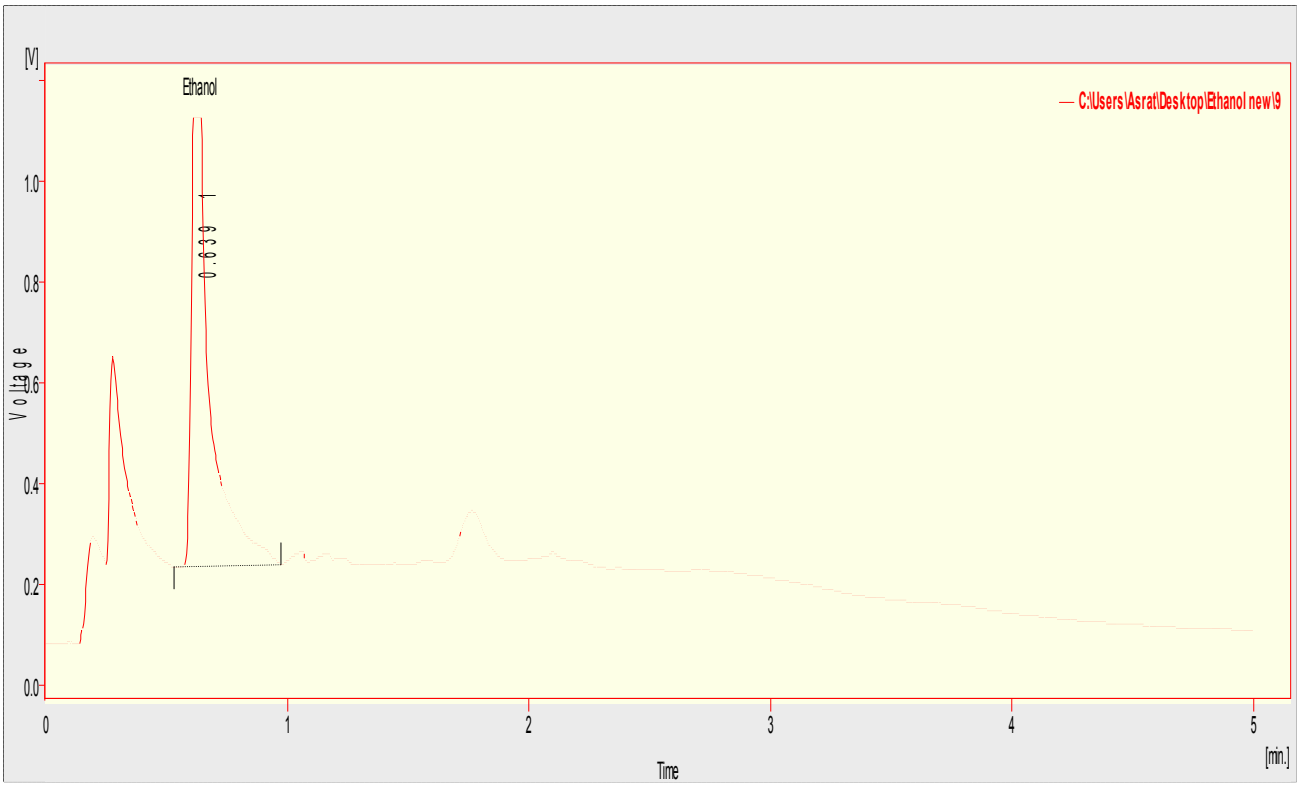


Table 12: Summary of chromatogram of pure ethanol (99.4%)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05[min]
1	0.639	5069.207	892.917	100.0	100.0	0.07
	Total	5069.207	892.917	100.0	100.0	

The above chromatogram and Table 12 show that ethanol is detected by gas chromatogram (GC) at the retention time of 0.639 min and its area [%] is 100%.

0.2 M H_2SO_4 hydrolyzed sample after 24 hours fermentation

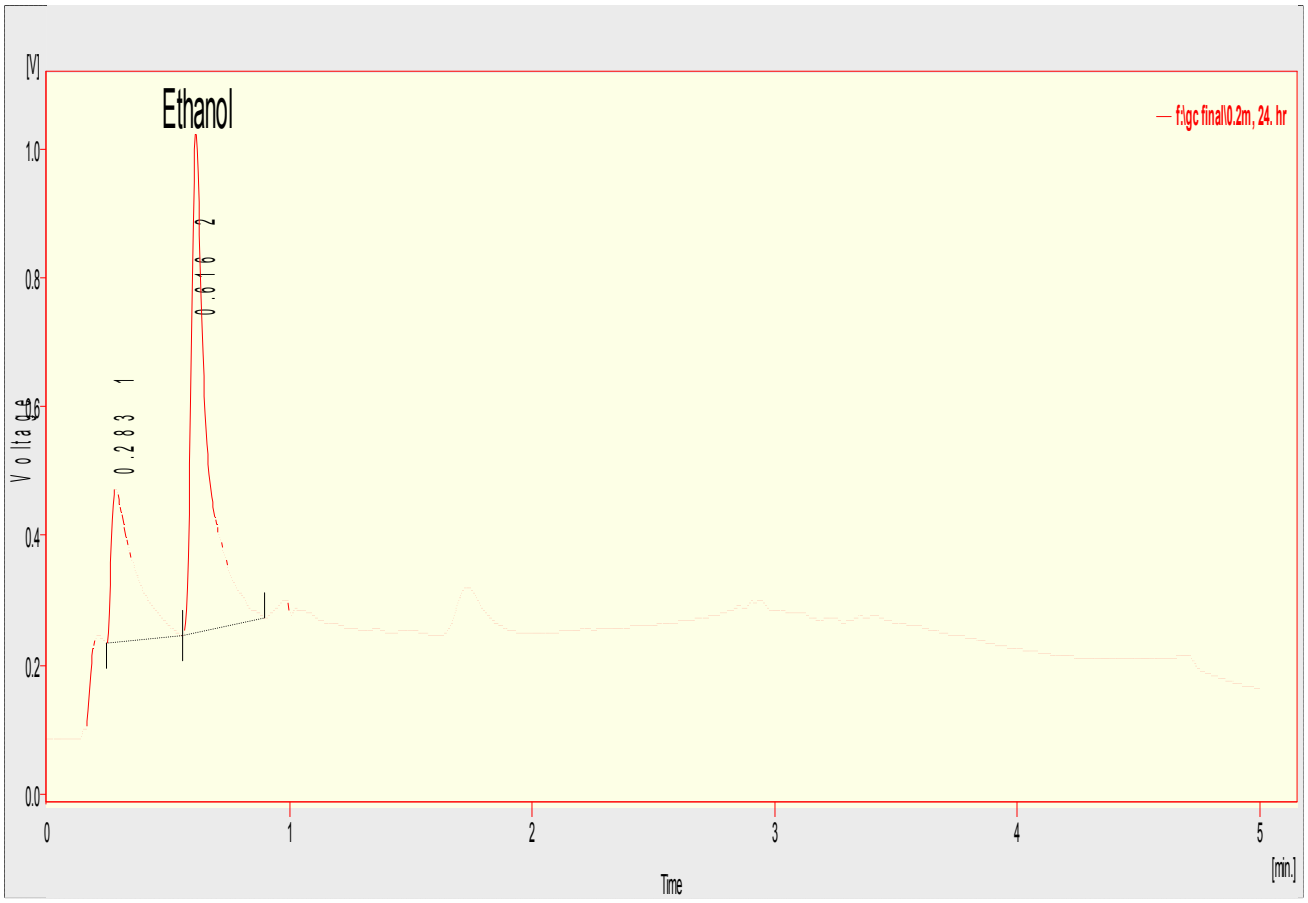


Table 13: Summary of chromatogram of sample hydrolyzed by 0.2 M H_2SO_4 and 24 hour fermentation

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.283	1623.075	240.600	31.3	23.7	0.10
2	0.616	3565.409	773.660	68.7	76.3	0.06
	Total	5188.484	1014.260	100.0	100.0	

The result shows that the peak is observed at relatively the same retention time with the retention time of the peak for standard ethanol but the intensity of the peak for the sample is 68.7%. From which, it could be possible to say that the peak in the sample represents ethanol, but it needs further approval.

0.2 M H_2SO_4 hydrolyzed sample after 24 hours fermentation on which 2 mL of pure ethanol is added.

Table 14: Summary of chromatogram of sample hydrolyzed by 0.2 M H_2SO_4 and 24 hours fermentation on which 2 mL of pure ethanol is added.

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.276	2147.998	401.939	24.4	31.4	0.06
2	0.639	6665.344	879.251	75.6	68.6	0.08
	Total	8813.342	1281.191	100.0	100.0	

As it can be seen from the above two chromatograms and tables 13 & 14, on addition of 2 mL of pure ethanol to the sample hydrolyzed by 0.2 M H_2SO_4, the intensity of the chromatogram of the sample increases from 68.7% to 75.6%. It can also be noticed that the retention time is almost similar but very small variation might be because the sample is injected manually. Therefore, The chromatogram observed around the retention time of 0.616 min for the sample hydrolyzed by 0.2 M H_2SO_4 and fermented for 24 hours indicates the presence of ethanol in the sample.

Sample ID: Sample hydrolyzed by 0.2M H_2SO_4 and fermented for 24 hours

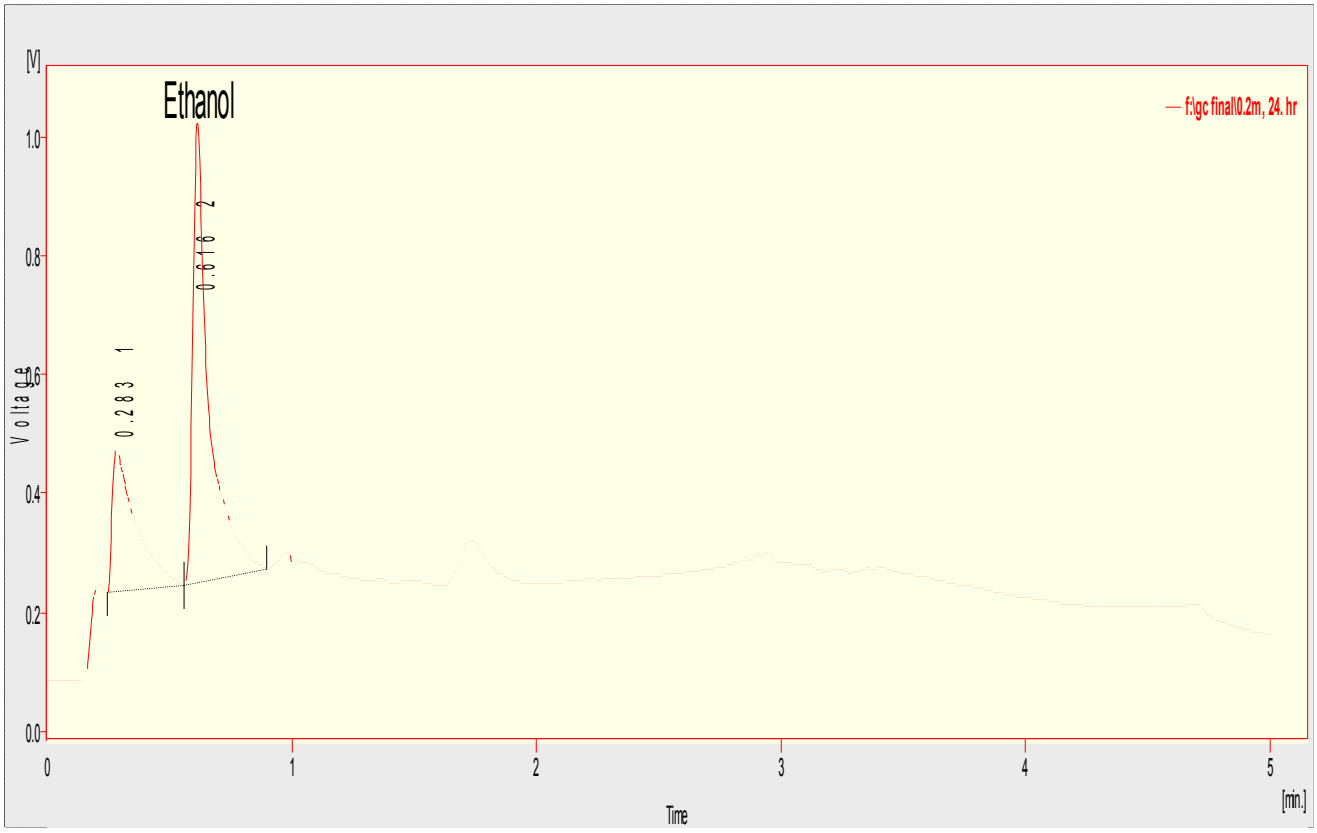


Table 15: Summary of chromatogram of sample hydrolyzed by 0.2 M H_2SO_4 and 24 hour fermentations

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.283	1623.075	240.600	31.3	23.7	0.10
2	0.616	3565.409	773.660	68.7	76.3	0.06
	Total	5188.484	1014.260	100.0	100.0	

Sample ID: Sample hydrolyzed by 0.4M H_2SO_4 and fermented for 24 hours

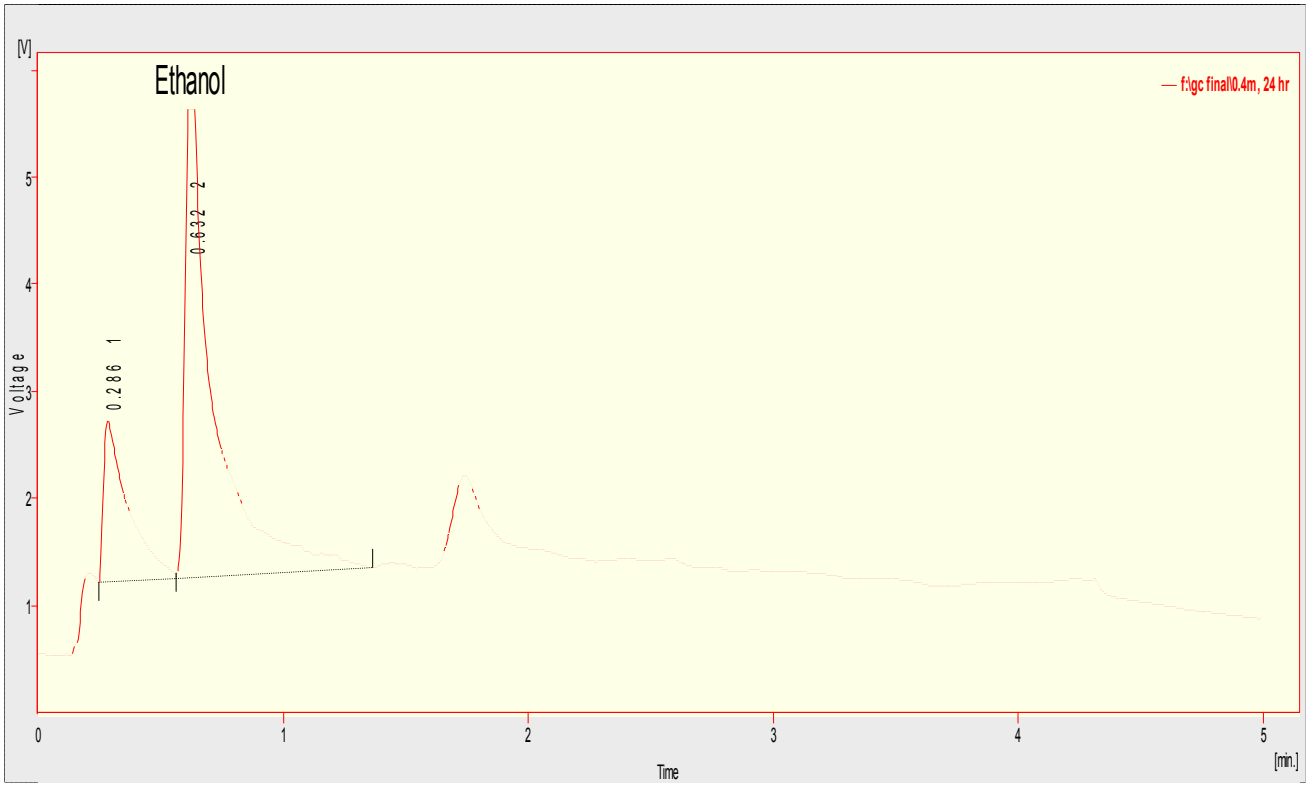


Table 16: Summary of chromatogram of sample hydrolyzed by 0.4 M H_2SO_4 and 24 hours fermentation

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.286	2100.887	299.959	22.0	25.5	0.10
2	0.632	7450.375	875.197	78.0	74.5	0.09
	Total	9551.262	1175.156	100.0	100.0	

Table 16 shows that the chromatogram is observed at relatively the same retention time indicating the presence of ethanol in the sample. The intensity of the peak for the sample hydrolyzed by 0.4 M H_2SO_4 is relatively greater than that of the sample hydrolyzed by 0.2 M H_2SO_4 which in turn shows the concentration of ethanol increases with increase in concentration of H_2SO_4 used for hydrolysis.

Sample ID: Sample hydrolyzed by 0.6 M H_2SO_4 and fermented for 24 hours

Table 17: Summary of chromatogram of sample hydrolyzed by 0.6 M H_2SO_4 and 24 hours fermentation

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.283	2528.233	314.605	24.5	26.2	0.11
2	0.609	7803.649	884.811	75.5	73.8	0.07
	Total	10331.882	1199.416	100.0	100.0	

The result still shows the presence of ethanol in the sample but the intensity of the peak (Concentration of ethanol) decreases compared to 0.4 M H_2SO_4 hydrolyzed sample.

Sample ID: Sample hydrolyzed by 0.8 M H_2SO_4 and fermented for 24 hours

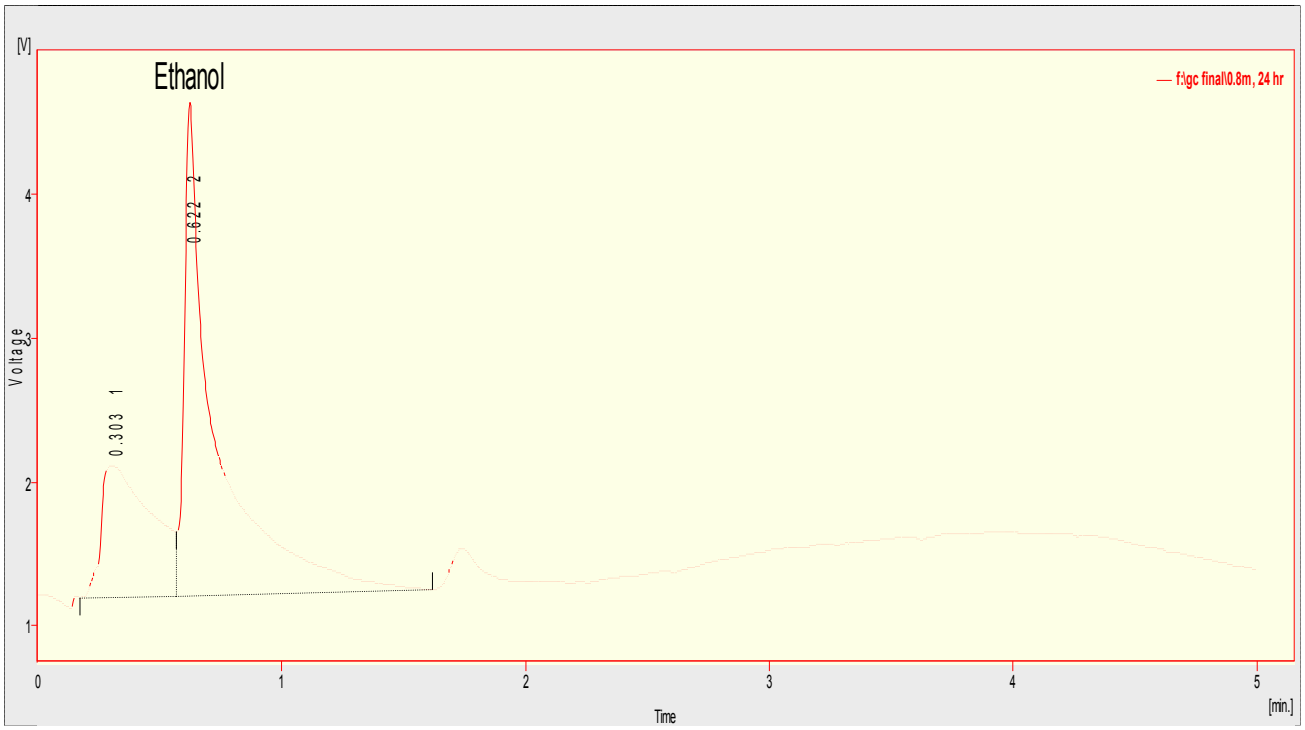


Table 18: Summary of chromatogram of sample hydrolyzed by 0.8 M H_2SO_4 and 24 hours fermentation

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.303	2623.786	183.148	29.6	21.0	0.31
2	0.622	6233.147	687.505	70.4	79.0	0.08
	Total	8856.933	870.653	100.0	100.0	

Table 18 shows the presence of ethanol in the sample with reference to GC result for the standard ethanol.

Sample ID: Sample hydrolyzed by 1 M H_2SO_4 and fermented for 24 hours

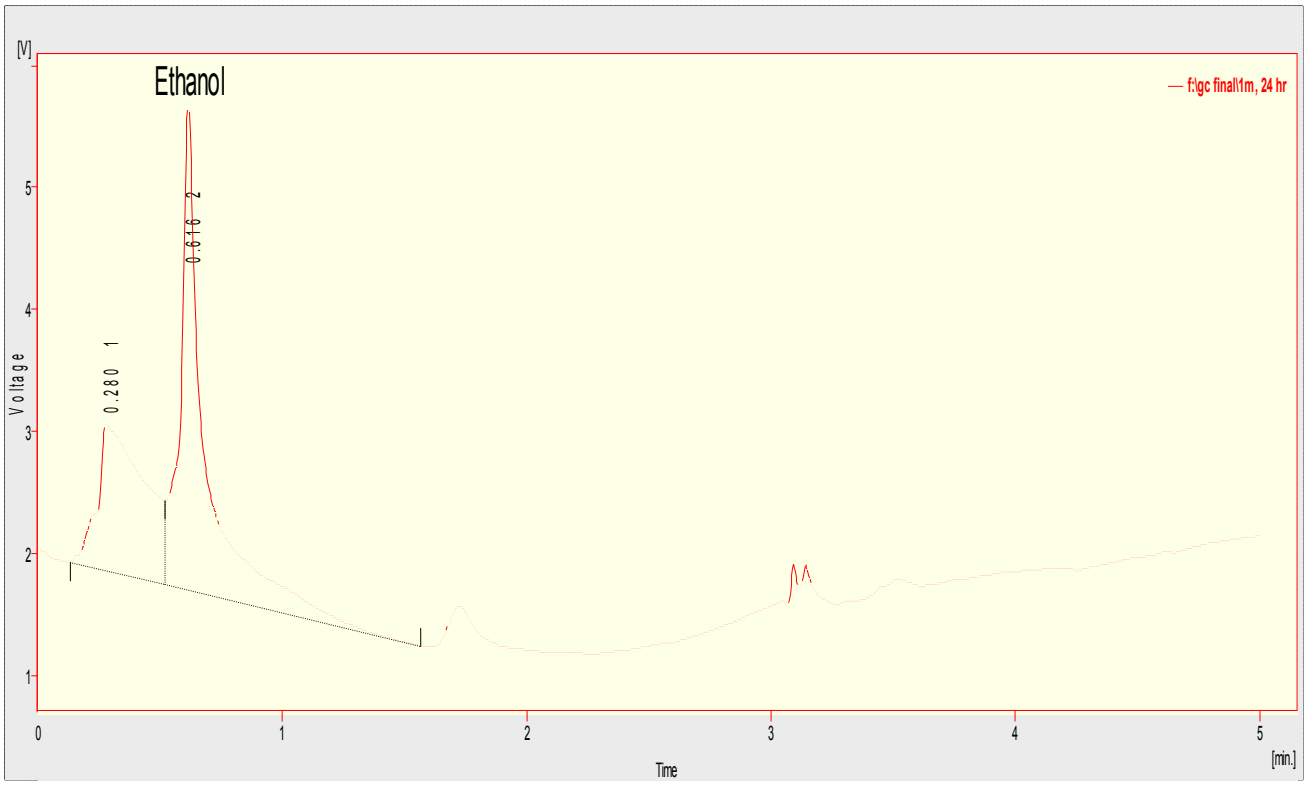


Table 19: Summary of chromatogram of sample hydrolyzed by 1 M H_2SO_4 and 24 hours fermentation

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.280	3296.158	240.551	36.5	23.4	0.25
2	0.616	5734.934	789.477	63.5	76.6	0.06
	Total	9031.093	1030.028	100.0	100.0	

Table 19 shows that at relatively high concentration of H_2SO_4 (1 M) used for hydrolysis, the concentration of ethanol produced goes on decreasing after 0.4 M H_2SO_4.

Sample ID: Sample hydrolyzed by distilled water and fermented for 24 hours

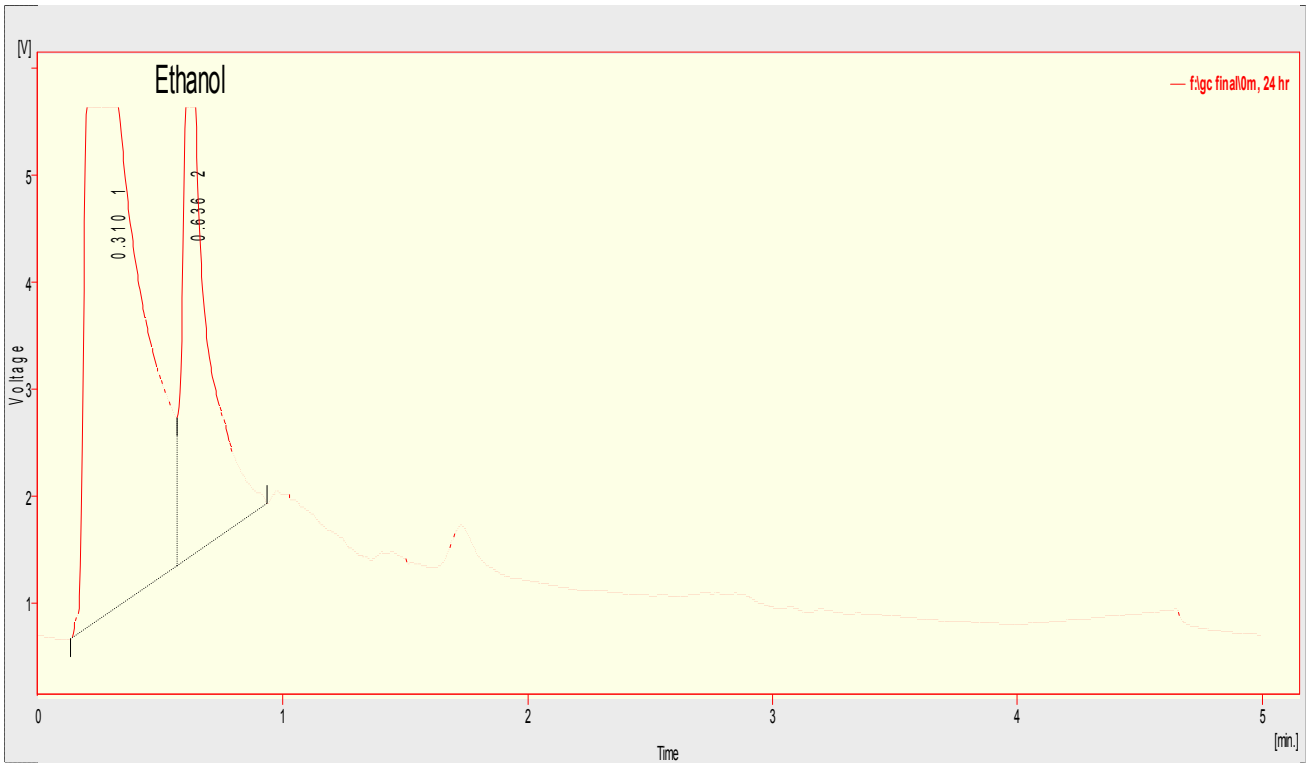


Table 20: Summary of chromatogram of sample hydrolyzed by distiled water and 24 hours fermentation

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.310	15723.570	936.511	70.2	52.9	0.29
2	0.636	6680.112	833.100	29.8	47.1	0.10
	Total	22403.682	1769.612	100.0	100.0	

Table 20 shows that the concentration of ethanol is minimum in the sample hydrolyzed by distilled water compared to the sample hydrolyzed by different concentrations of H_2SO_4.

Table 21: Compiled gas chromatogram (GC) result

Fermentation hour	0.2M H_2SO_4	0.4M H_2SO_4	0.6M H_2SO_4	0.8M H_2SO_4	1M H_2SO_4	Distiled water
12 hr	50	58.5	56.3	54.2	45.8	29.7
24 hr	68.7	78.0	75.5	70.4	63.5	29.8
36 hr	67.1	74.4	67.4	64.4	63.5	27.8
48 hr	53.1	71.3	65.3	61.7		

Note: The result is obtained at optimized temperature, time and initial pH (100 ^{0}C, 1 hour and pH of 4.5)

Table 21 presents the result for ethanol concentration of wet coffee waste hydrolyzed by different concentrations of sulfuric acid & distilled water and allowed to ferment for different hours of fermentation (12, 24, 36, 48 hours) at optimized temperature initial pH. It can be observed that maximum yield 78.0 % is obtained from the sample hydrolyzed by 0.4 M H_2SO_4 after fermentation for 24 hours.

The comparison of bio ethanol potential of coffee waste with different agricultural and agro industrial wastes is shown bellow.

Table 22: Bio ethanol production potential of different agricultural wastes

Feed stock	Ethanol production (g/l)	Reference
Banana peels	9.8	(Manikandan et al. 2008)
Sugarcane bagasse	10.2	(Raghavendra et al 2006)
Poultry manure	5	(A. G. Woldesenbet et al 2013)
Coffee waste	6.12	This study

It is worthwhile to mention that the concentration of ethanol obtained (6.12 g/L) by the hydrolysis of the wet coffee waste is quite satisfactory compared to the maximum amount of ethanol obtained from the enzymatic fermentation of Banana peels (9.8 g/L) and Sugar bagasse (10.2 g/L).

The results obtained in this study show that coffee waste from wet coffee process has potential for bio-ethanol production. Wet coffee waste has not been utilized in Ethiopia as a source of renewable energy. This has been the case despite the fact that Ethiopia is one of the most coffee growing countries.

Furthermore, coffee production residues can be analyzed under the concept of bio-refinery, in order to devise a more proper way of addressing the issue of adequate waste disposal and recovery, and environmental protection. The process of renewable energy from coffee waste can create a self-sustaining wet coffee milling process. Large scale wet coffee processing can produce large amounts of wet coffee waste. If the coffee waste is used in ethanol production it can result into sufficient amounts of ethanol. The ethanol can be blended up to 20% with petrol or diesel.

4.6. Characterization of the Residue

Coffee waste contained appreciable amount of minerals including nitrogen which are important in supporting growth of yeasts. As a result, no additional elements were used in the coffee waste during fermentation. The mineral content of coffee waste is shown in Table 23.

Table 23: Mineral content of coffee waste residue

Mineral	Amount
Manganese	1.74 mg/kg
Magnesium	136.5 mg/kg
Zinc	5.28 mg/kg
Iron	45.52 mg/kg
Copper	4.02 mg/kg
Nitrogen	0.56%
Phosphorus	0.15%
Potassium	0.50%

Coffee waste is a rich source of nutrients: 0.56% nitrogen; 0.15% phosphorus, and 0.5% potassium. It can be treated and used as organic fertilizer. Usually the coffee pulp is placed on piles and left to compost for about 3 to 4 months. During that time, it turns into black humus excellent for composting. Using organic fertilizers improves soil conditions and increases agricultural yield.

5. Conclusion and Recommendations

5.1. Conclusion

Coffee waste is a good feedstock for bio-ethanol production when used in substantial quantities. The feasibility of ethanol production from coffee waste by means of dilute sulfuric acid hydrolysis was investigated. Distilled water and different concentrations of sulfuric acid was used in the hydrolysis for comparison and to ensure maximum ethanol yield. The optimization study showed that the highest ethanol concentration of 6.12 g/L was observed under the optimum conditions of hydrolysis with 0.4 M Sulfuric acid for 1 hours by keeping boiling temperature at 100 ^{0}C, and fermentation time of 24 hours with commercial baker‘s yeast. From the results, it is thus possible to derive ethanol from wet coffee waste. The utilization of wet coffee waste as an alternative energy production reduces the environmental pollution and dependence on oil and petroleum in Ethiopia. It also provides alternative energy solutions for small-scale holders. From the experiment, it is thus possible to state that there is potential for bio-ethanol production from wet coffee waste. A theoretical integrated model for joint production of biogas and bio-ethanol from wet coffee waste could also be formulated on a more sophisticated scale that is beyond the scope of this study.

Presently, many coffee waste systems are not operating efficiently. They could reduce on their losses if they employed qualified staff, adopted modern management techniques and maintained good relations with credit institutions. Unless they improve their profitability, they will consider demands to take on additional investments, e.g. for environment as a burden.

Many by-products from wet coffee processing industries offer additional sources of revenue, employment and new enterprises. It is therefore high time that coffee waste started putting a price.

Great strides have been made in the development of second-generation technologies for cellulose conversion to bio-ethanol and other biofuels. New opportunities using agricultural residues, energy crops or invasive plant species for bio-fuel production potentially allow for the sustainable production of biofuels in significant quantities, while at the same time stabilizing food production by providing alternative markets to farmers, as well as addressing human development specifically in rural communities. First-generation bio-fuels production has been modest in Africa compared with the huge potential of Africa to produce biomass.

Although the geographical potential of Africa to produce bio-fuels is at least as large as any other continent, recent developments in second-generation technologies have not had much impact in Africa. The limited resources of many African economies, together with fragmented trade and economic policies, apparently limit the abilities of governments to provide financial incentives for second-generation biofuels production.

However, the positive considerations of second generation bio-fuels production, such as GHG emission reductions, renewability, absence of competition with food/feed, negligible land-use impacts and sustainability, should translate into a greater willingness by governments and consumers to accept higher prices for second-generation biofuels, compared with those produced by first-generation technologies. Policies to enable such price differentials in the market place have not been implemented in Africa. Fossil fuels

remain generally cheap, and the development of a sufficient policy environment, which can provide attractive economic returns for second-generation biofuels through incentives, subsidies and carbon taxes, mandated blending, carbon emissions legislation and the financial benefits of carbon trading, is lacking in the African context. To actively take a step in the right direction, actions need to be considered to ensure that Africa benefits along the full value chain of bio-fuel production and utilization. Some worthy actions could include:

i. Proper analysis, understanding and consensus on the potential of bio-energy, including biofuels production with second-generation technologies to realize a sustainable Africa;
ii. African scalable demonstration projects using second-generation technologies for learning perspectives, e.g. training to strengthen local manpower; this could also show best practices in energy efficiency and resource protection in transport, electricity supply, cooking and other household needs; and
iii. Alignment of international, regional and local policies on trade, aid, land tenure and development, needed to facilitate integrated value chains of agriculture and forestry for food and bio-energy in Africa.

Bio-fuels present one of the most cost-effective solutions for a global sustainable low-carbon-energy future. This future demands sustainable agriculture and forestry in Ethiopia to supply food and bio-energy in support of Africa and the world..

5.2. Recommendations

With regard to the findings in this study the following recommendations are forwarded.

1. There should be an economic feasibility analysis on a large-scale basis of the overall conversion of wet coffee waste to green/ bio-ethanol for the purpose of commercialization.
2. There should be a joint attempt in the implementation of waste utilization of coffee waste by relevant parties such as local authorities (Ministry of Agriculture), stakeholders, students, farmer groups and village.
3. More research should be undertaken to determine the proper conditions for process optimization of the production of bio-ethanol.
4. Further investigations should be carried out on the possibility of implementation of the integrated model for joint utilization of coffee waste pulp and wastewater for bio-ethanol and biogas production.

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Annex

Bio Ethanol production from wet coffee processing waste under optimized conditions of Hydrolysis time, temperature and initial pH

Pure Ethanol (99.4%)

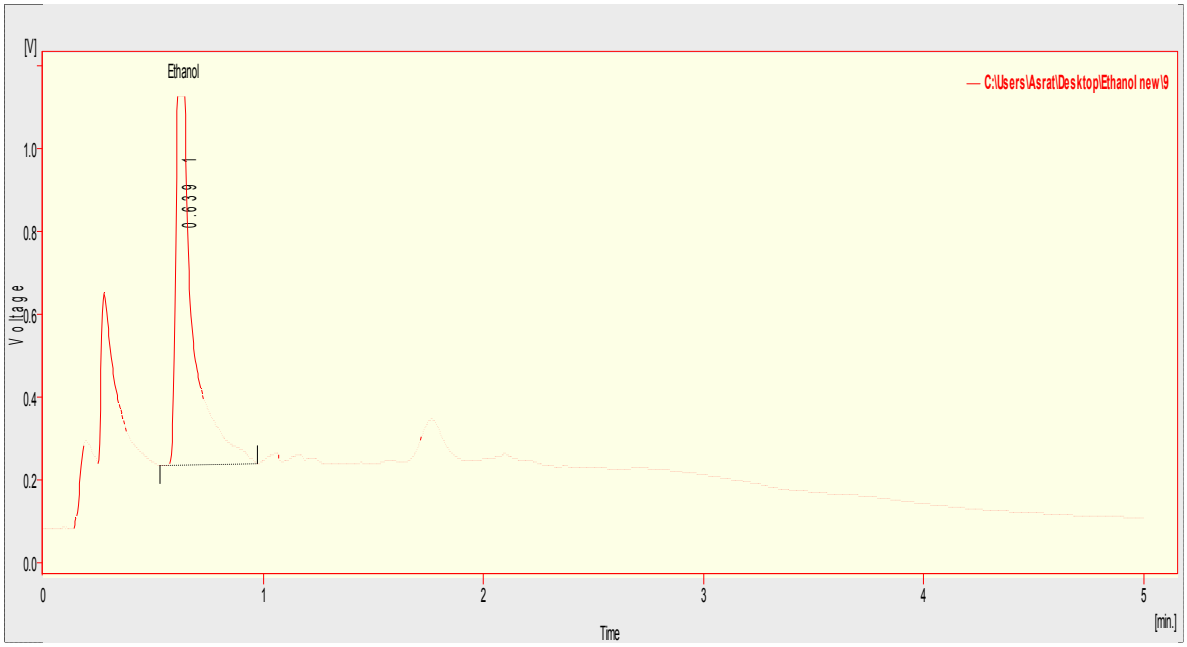
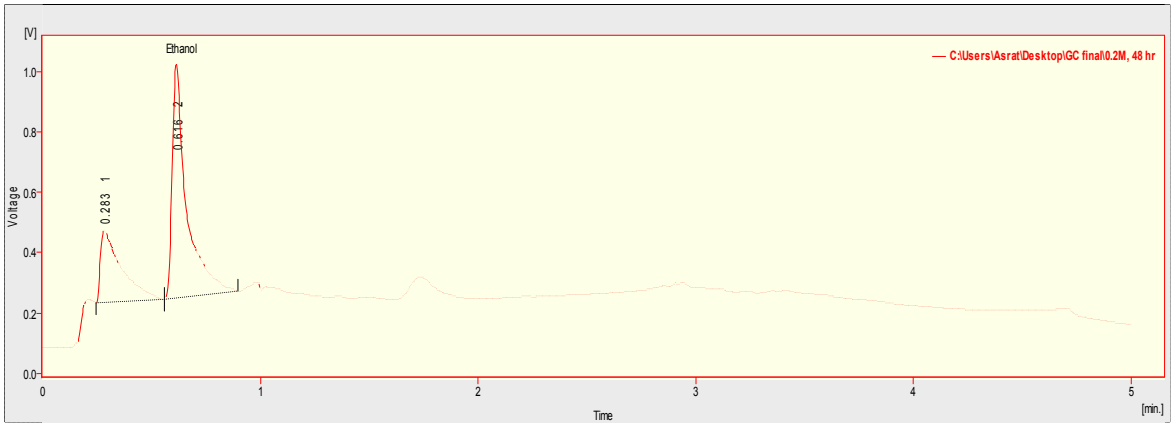


Table 16: Summary of chromatogram of pure ethanol (99.4%)

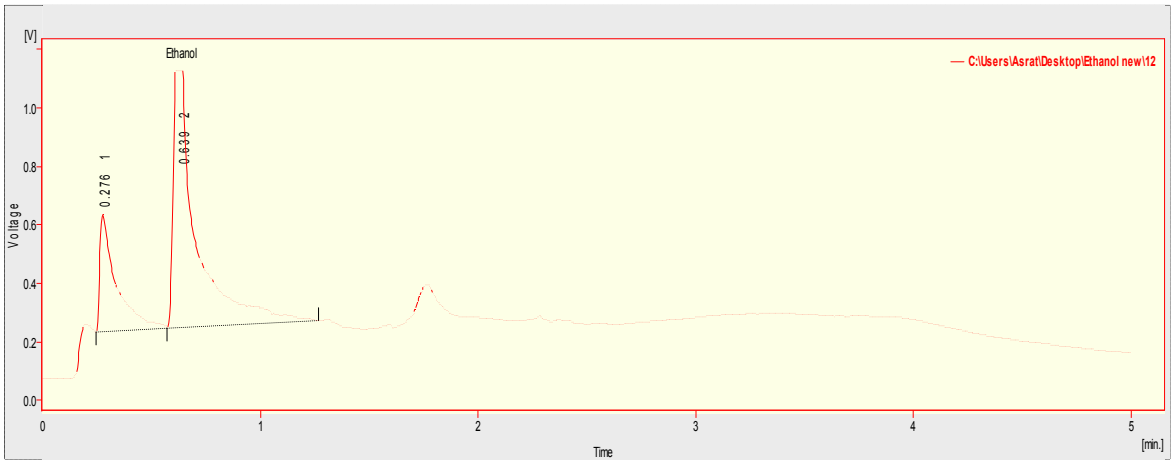
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05[min]
1	0.639	5069.207	892.917	100.0	100.0	0.07
	Total	5069.207	892.917	100.0	100.0	

0.2 M H_2SO_4 hydrolyzed sample after 24 hr fermentation



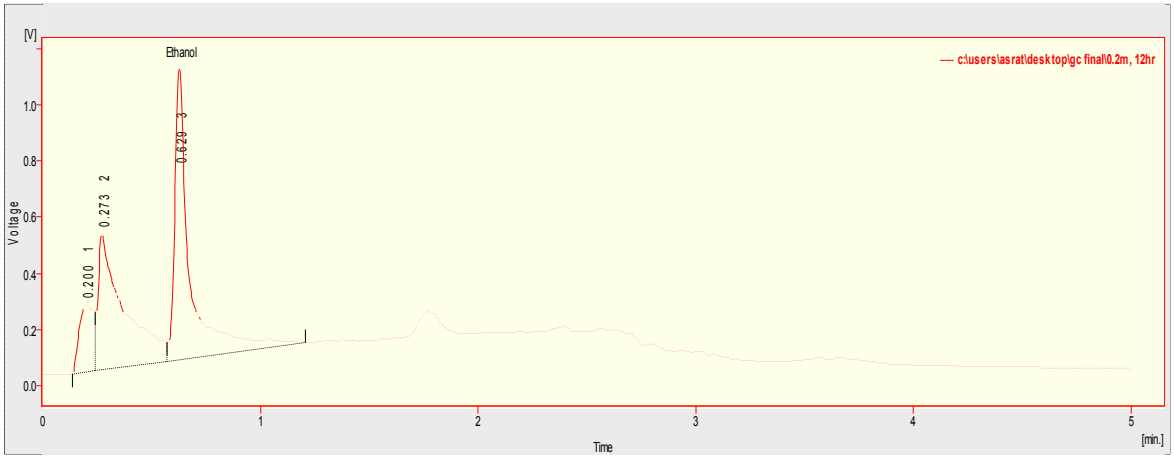
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.283	1623.075	240.600	31.3	23.7	0.10
2	0.616	3565.409	773.660	**68.7**	76.3	0.06
	Total	5188.484	1014.260	100.0	100.0	

0.2 M H_2SO_4 hydrolyzed sample after 24 hr fermentation on which 2 ml of pure ethanol is added.



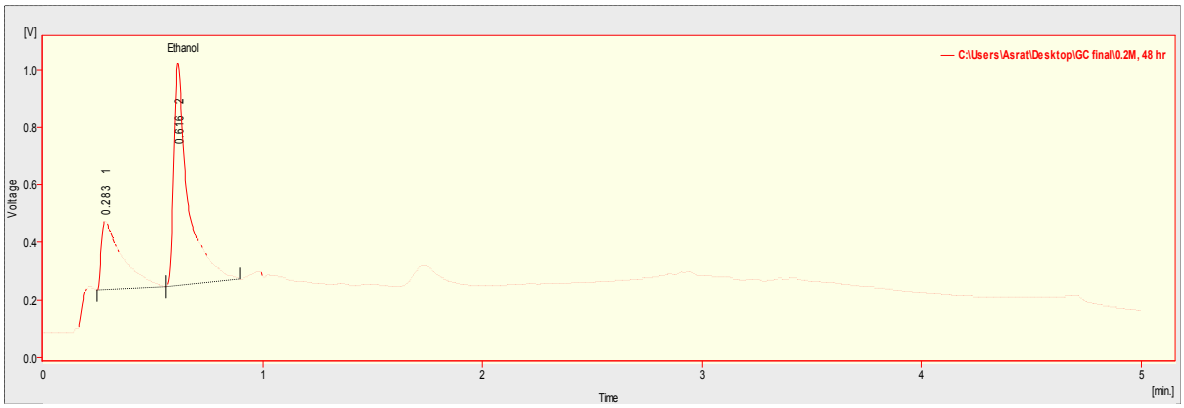
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05[min]
1	0.276	2147.998	401.939	24.4	31.4	0.06
2	0.639	6665.344	879.251	**75.6**	68.6	0.08
	Total	8813.342	1281.191	100.0	100.0	

Sample ID: 0.2M, 12 hr



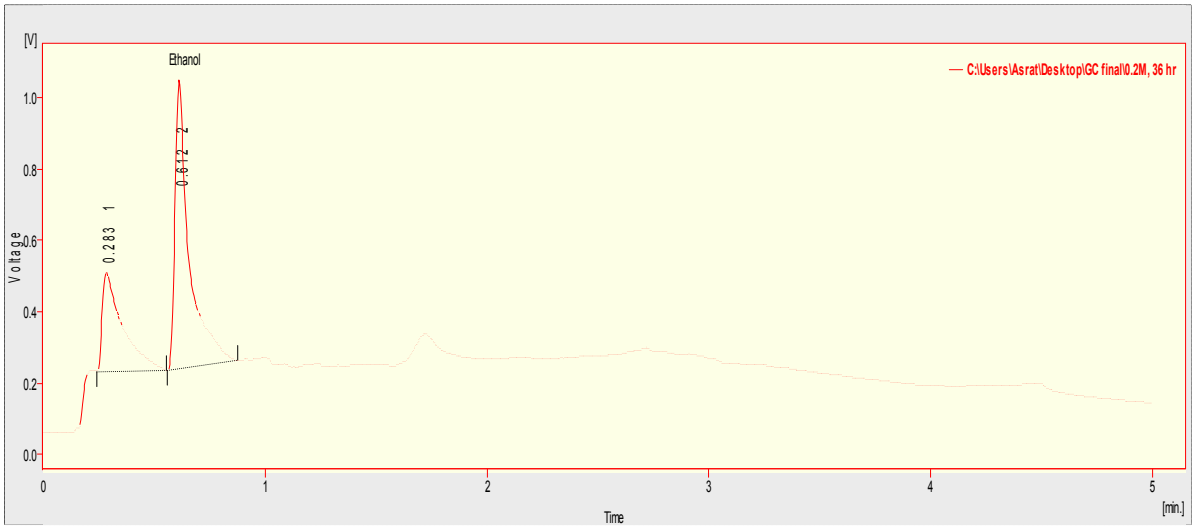
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.200	1081.141	235.838	10.6	13.5	0.08
2	0.273	3996.607	483.193	39.3	27.6	0.10
3	0.629	5087.627	1033.709	**50.0**	59.0	0.05
	Total	10165.375	1752.739	100.0	100.0	

Sample ID: 0.2M, 24 hr



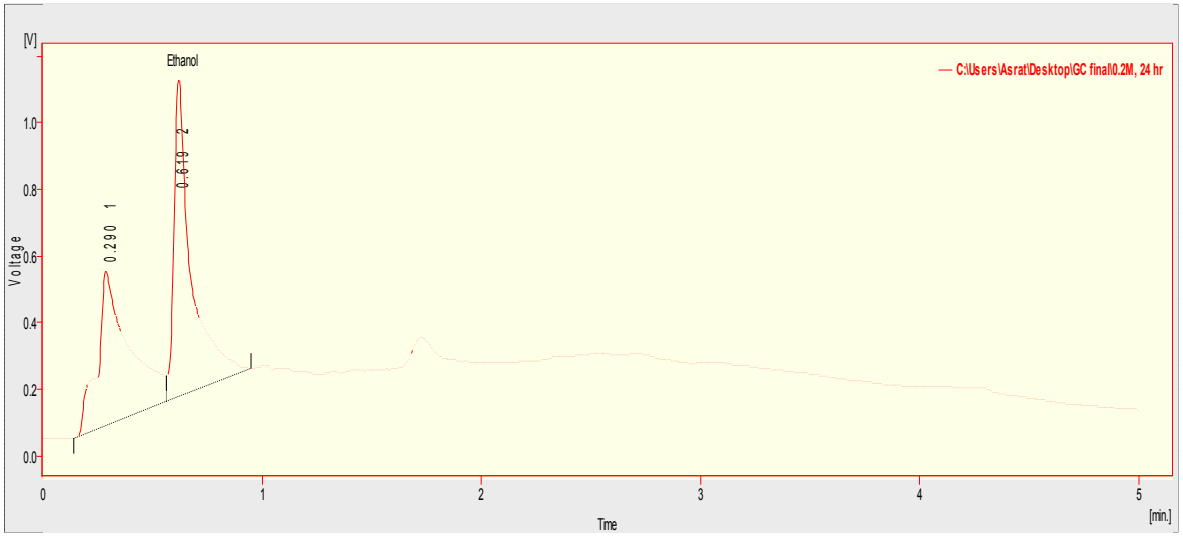
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.283	1623.075	240.600	31.3	23.7	0.10
2	0.616	3565.409	773.660	**68.7**	76.3	0.06
	Total	5188.484	1014.260	100.0	100.0	

Sample ID: 0.2M, 36 hr



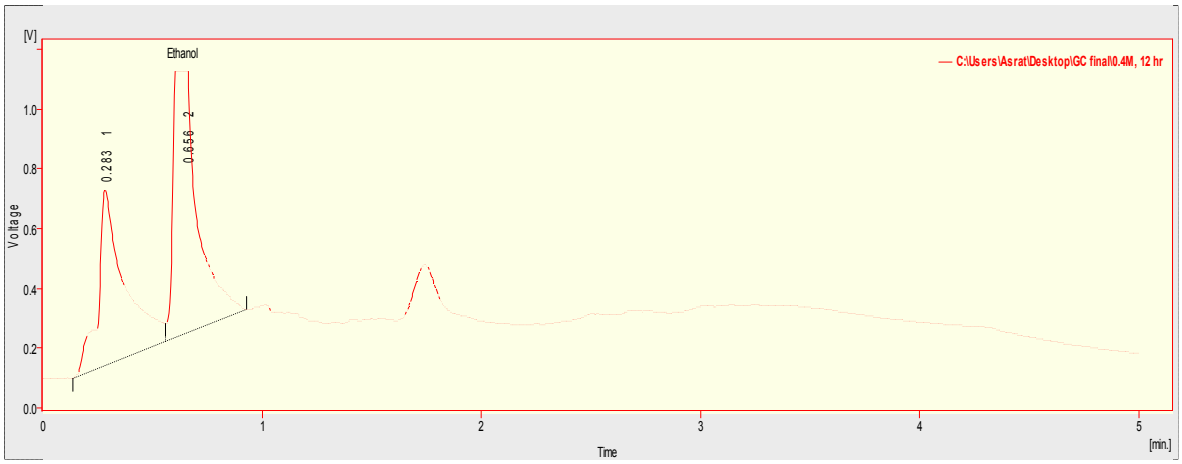
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.283	1754.335	276.615	32.7	25.5	0.09
2	0.612	3606.722	808.442	**67.3**	74.5	0.06
	Total	5361.058	1085.057	100.0	100.0	

Sample ID: 0.2M, 48 hr



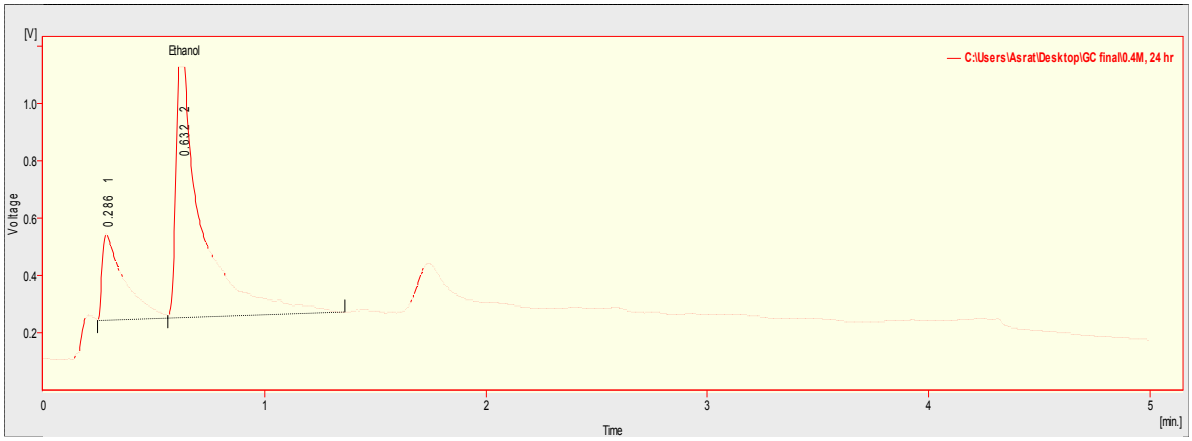
Reten. Time [min]		Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.290	4598.905	460.563	46.9	32.7	0.14
2	0.619	5213.897	949.194	**53.1**	67.3	0.06
	Total	9812.802	1409.757	100.0	100.0	

Sample ID: 0.4M, 12 hr



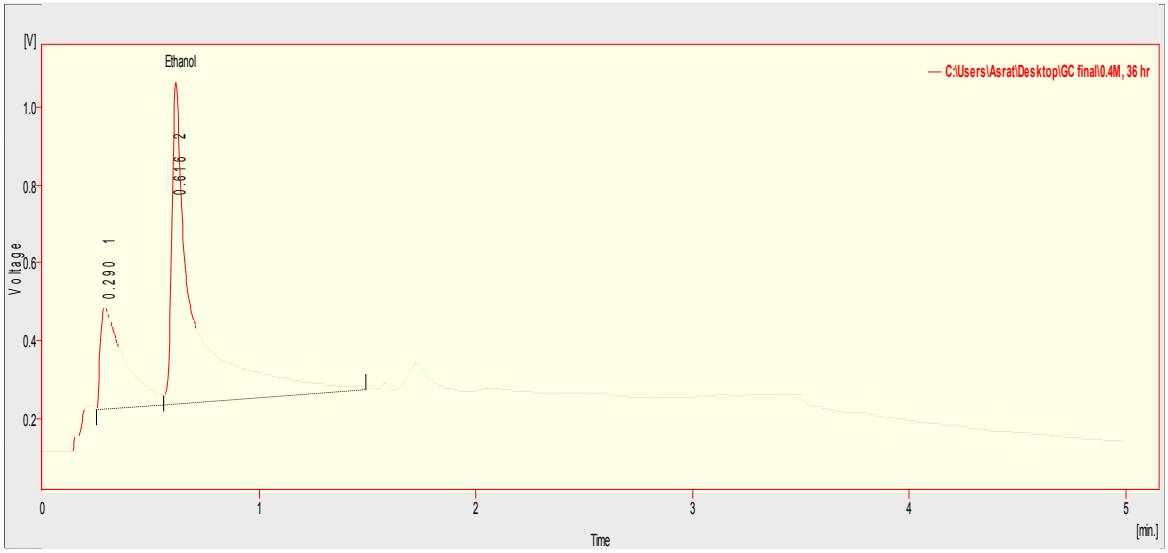
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.283	4927.156	587.734	41.5	40.1	0.10
2	0.656	6953.180	878.047	**58.5**	59.9	0.10
	Total	11880.336	1465.781	100.0	100.0	

Sample ID: 0.4M, 24 hr



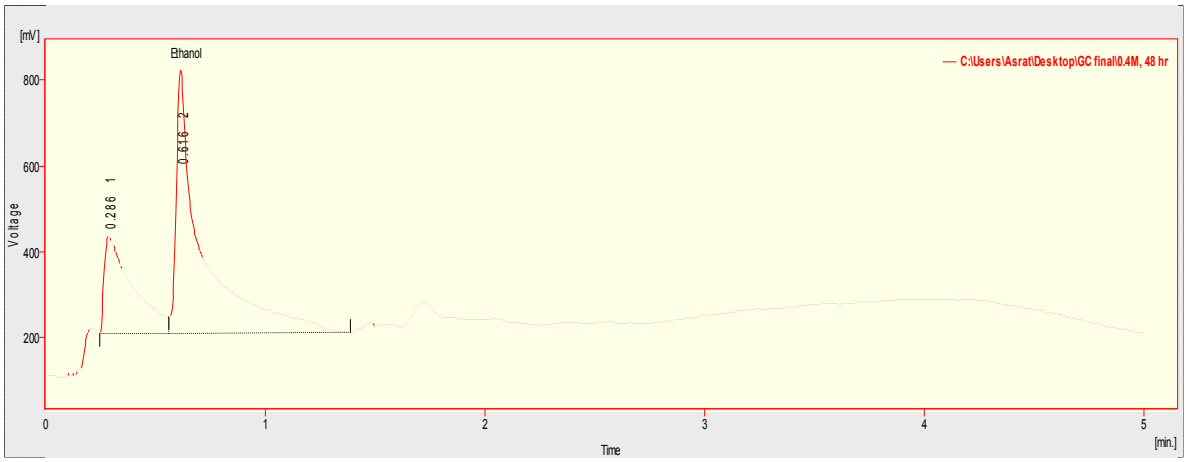
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.286	2100.887	299.959	22.0	25.5	0.10
2	0.632	7450.375	875.197	**78.0**	74.5	0.09
	Total	9551.262	1175.156	100.0	100.0	

Sample ID: 0.4M, 36 hr



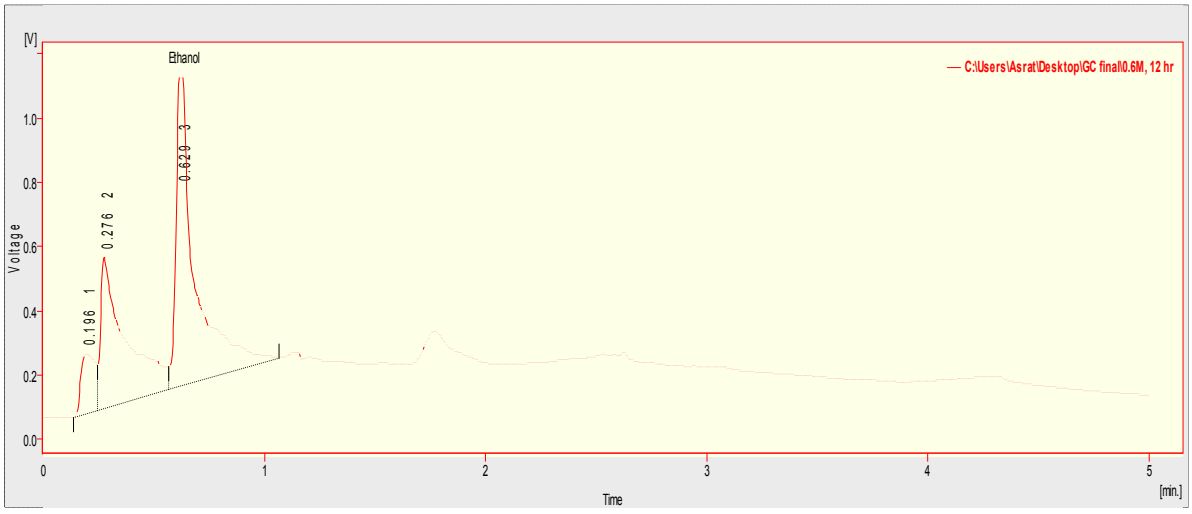
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.290	2053.486	260.706	25.6	24.0	0.11
2	0.616	5982.619	824.169	**74.4**	76.0	0.06
	Total	8036.106	1084.875	100.0	100.0	

Sample ID: 0.4M, 48 hr



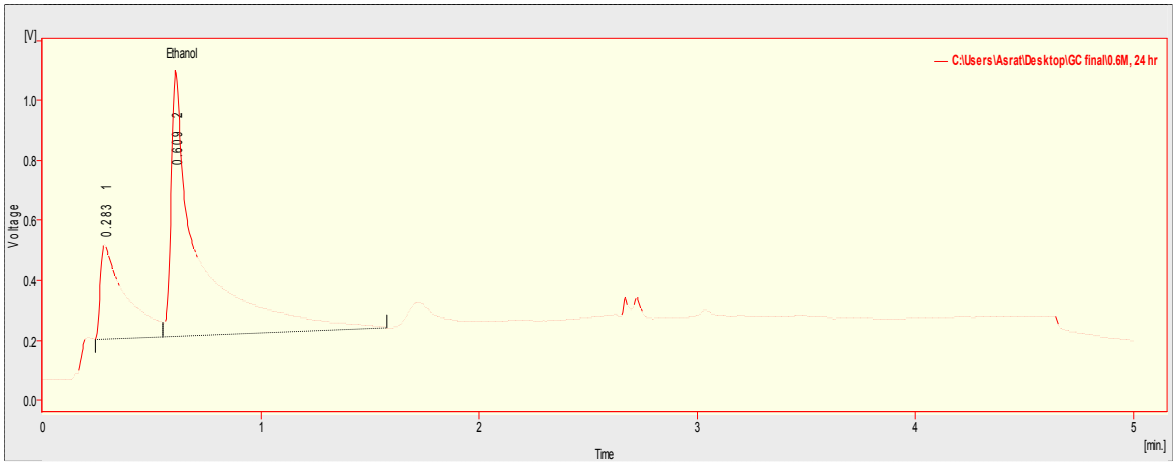
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.286	2038.848	226.764	28.7	27.0	0.13
2	0.616	5053.700	611.845	**71.3**	73.0	0.07
	Total	7092.548	838.609	100.0	100.0	

Sample ID: 0.6M, 12 hr



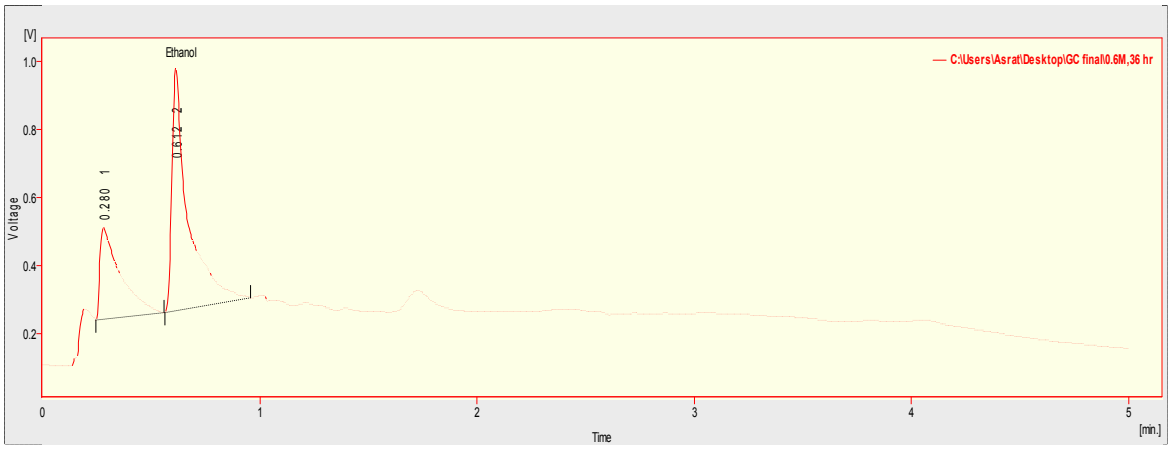
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.196	813.862	186.174	7.9	11.5	0.08
2	0.276	3690.169	470.251	35.8	29.1	0.10
3	0.629	5813.432	959.274	**56.3**	59.4	0.06
	Total	10317.462	1615.699	100.0	100.0	

Sample ID: 0.6M, 24 hr



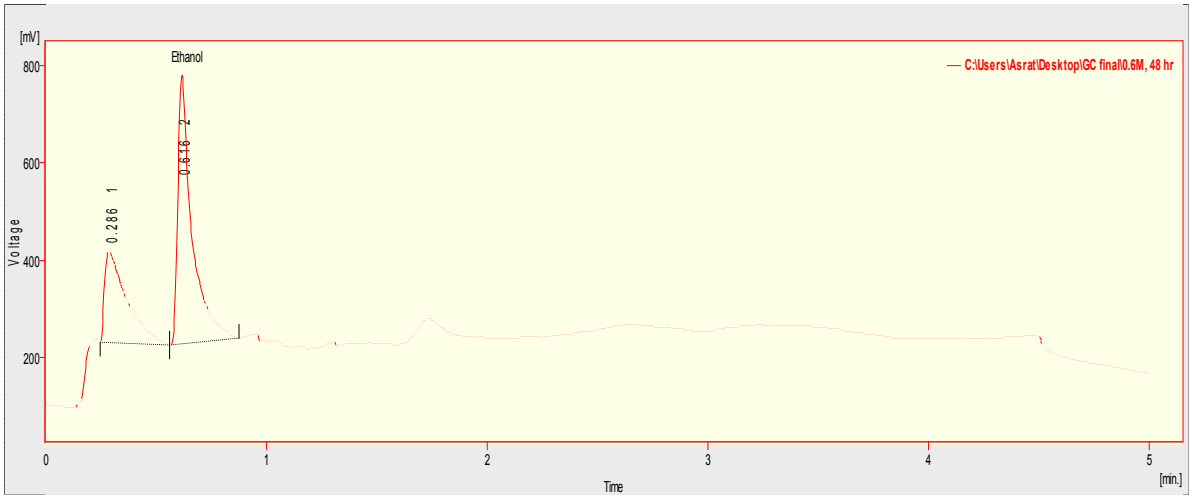
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.283	2528.233	314.605	24.5	26.2	0.11
2	0.609	7803.649	884.811	**75.5**	73.8	0.07
	Total	10331.882	1199.416	100.0	100.0	

Sample ID: 0.6M, 36 hr



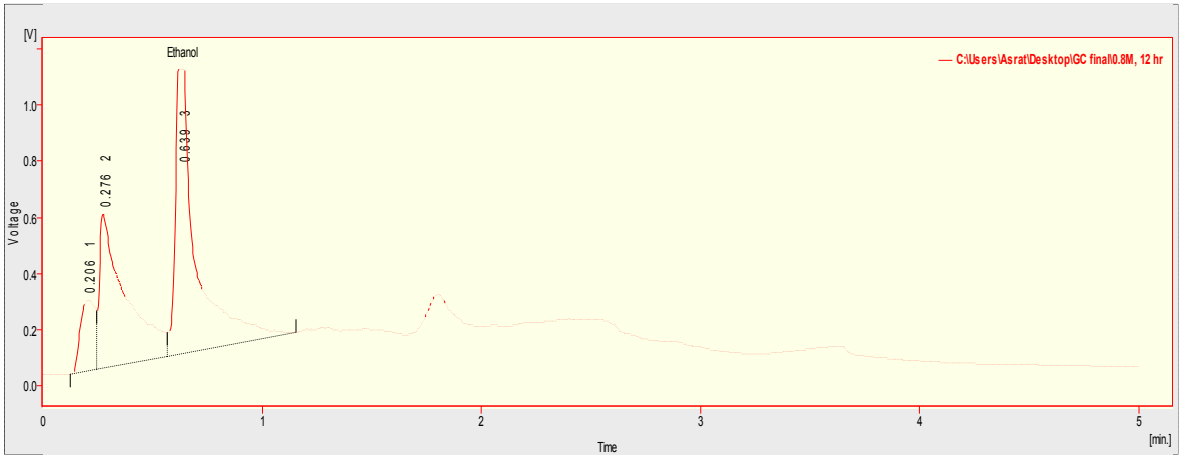
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.280	1798.607	270.334	32.6	27.6	0.10
2	0.612	3713.150	709.876	**67.4**	72.4	0.06
	Total	5511.757	980.210	100.0	100.0	

Sample ID: 0.6M, 48 hr



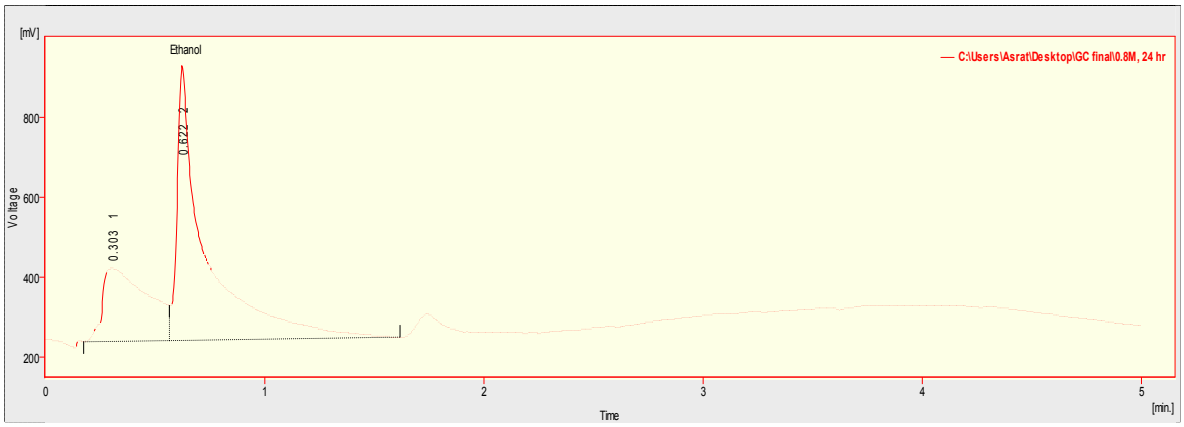
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.286	1318.532	188.477	34.7	25.5	0.10
2	0.616	2478.365	552.055	**65.3**	74.5	0.06
	Total	3796.897	740.532	100.0	100.0	

Sample ID: 0.8M, 12 hr



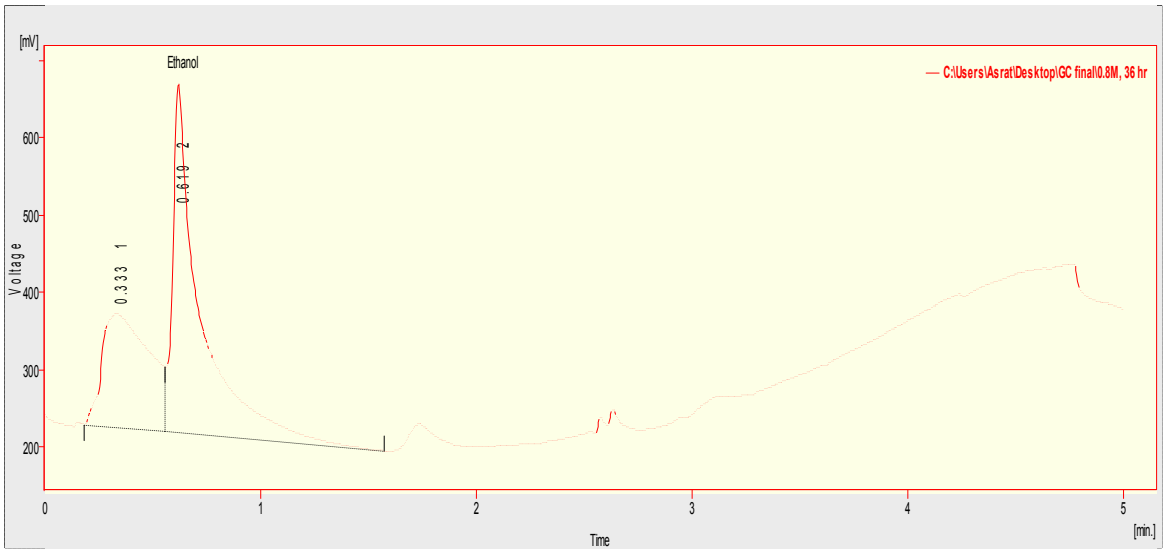
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.206	1144.094	251.226	9.2	13.9	0.08
2	0.276	4564.411	547.759	36.6	30.3	0.11
3	0.639	6752.340	1011.369	**54.2**	55.9	0.07
	Total	12460.846	1810.355	100.0	100.0	

Sample ID: 0.8M, 24 hr



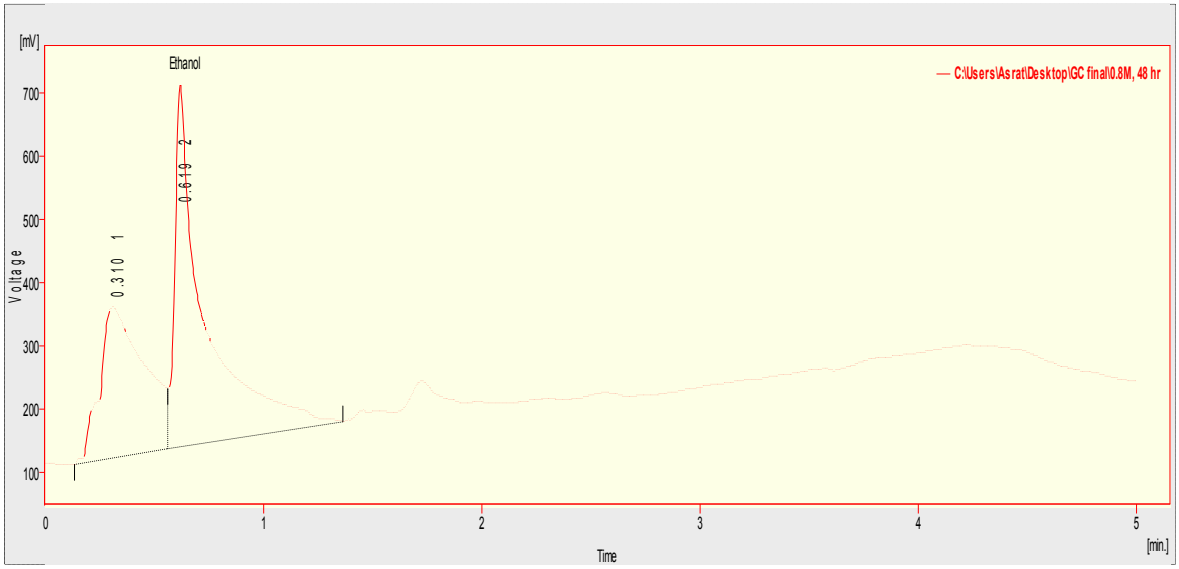
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.303	2623.786	183.148	29.6	21.0	0.31
2	0.622	6233.147	687.505	**70.4**	79.0	0.08
	Total	8856.933	870.653	100.0	100.0	

Sample ID: 0.8M, 36 hr



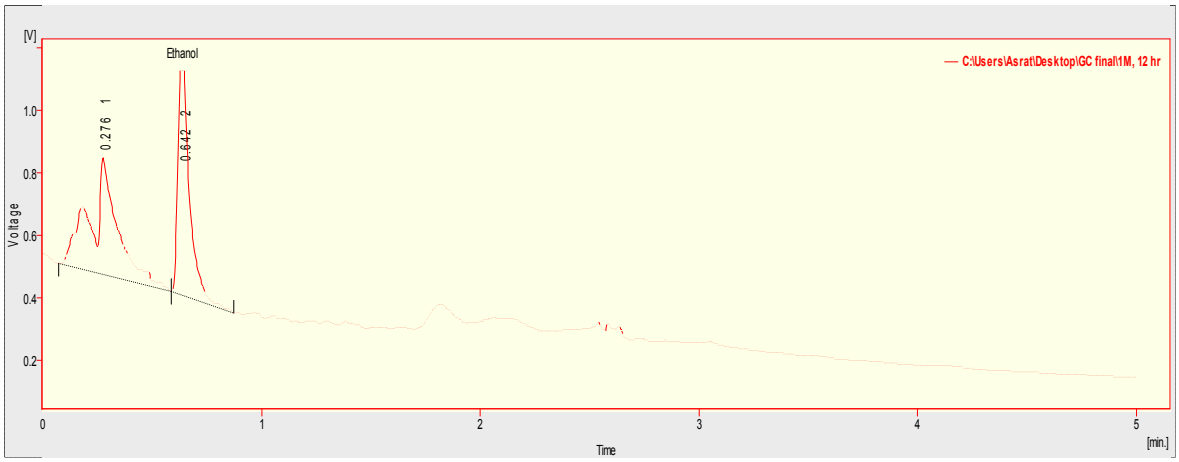
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.333	2225.407	147.451	35.6	24.6	0.30
2	0.619	4026.659	452.198	**64.4**	75.4	0.09
	Total	6252.066	599.650	100.0	100.0	

Sample ID: 0.8M, 48 hr



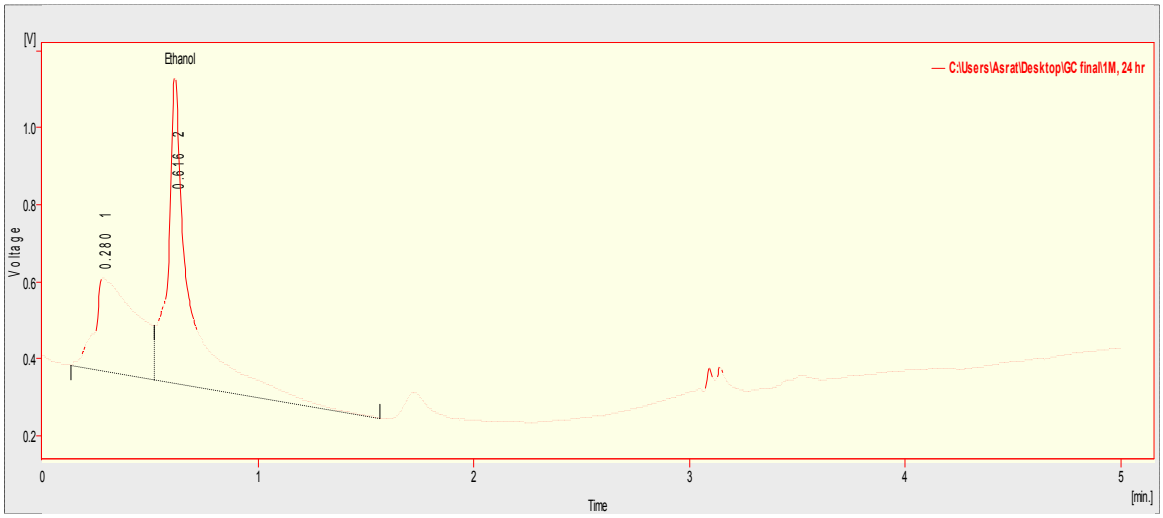
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.310	3387.149	238.650	38.3	29.4	0.27
2	0.619	5468.128	572.188	**61.7**	70.6	0.08
	Total	8855.277	810.838	100.0	100.0	

Sample ID: 1M, 12 hr



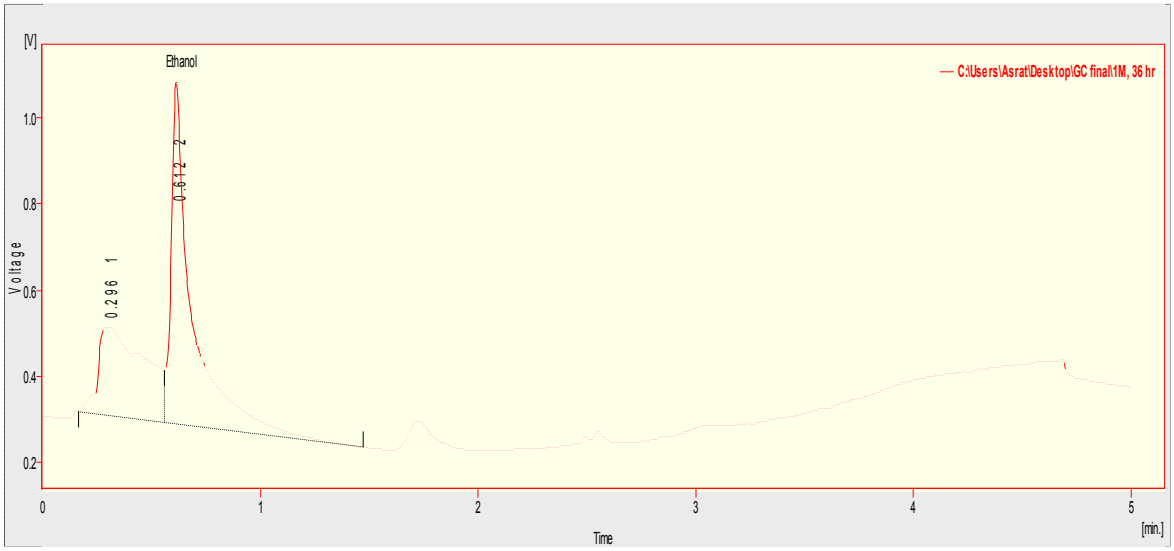
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.276	3256.604	372.494	54.2	34.1	0.07
2	0.642	2755.527	718.562	**45.8**	65.9	0.06
	Total	6012.131	1091.056	100.0	100.0	

Sample ID: 1M, 24 hr



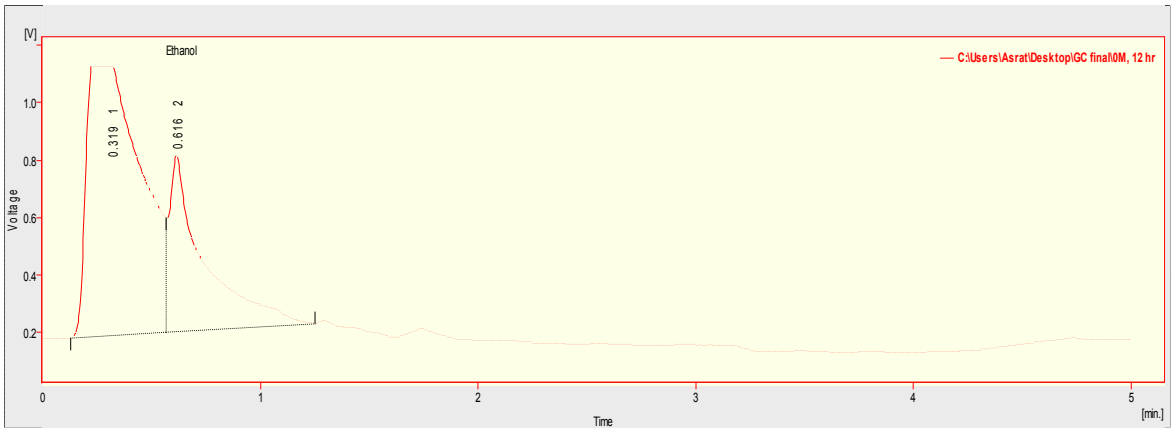
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.280	3296.158	240.551	36.5	23.4	0.25
2	0.616	5734.934	789.477	**63.5**	76.6	0.06
	Total	9031.093	1030.028	100.0	100.0	

Sample ID: 1M, 36 hr



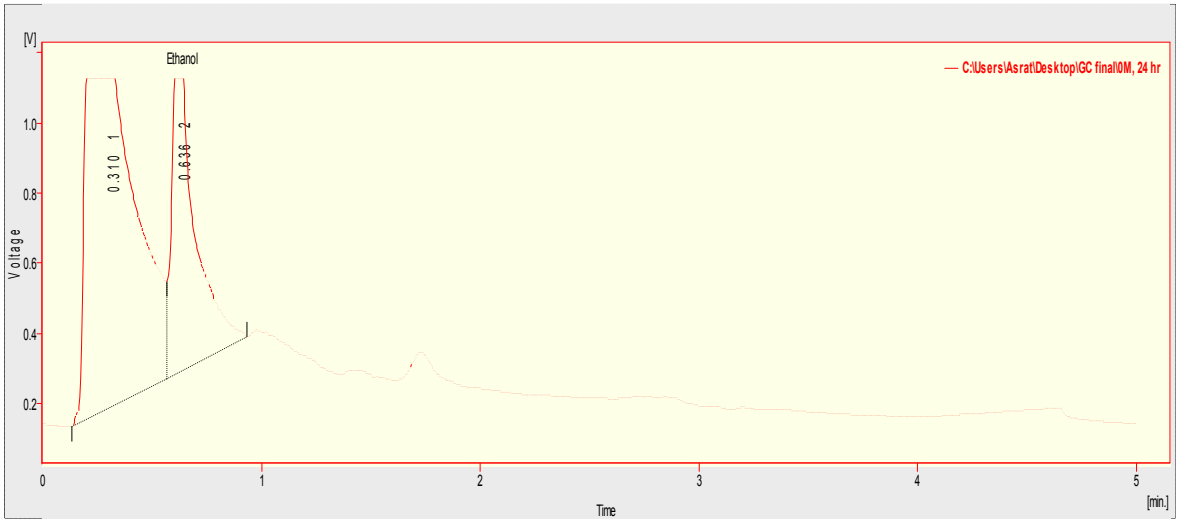
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.296	3049.922	204.170	36.5	20.5	0.31
2	0.612	5300.871	789.867	**63.5**	79.5	0.07
	Total	8350.793	994.037	100.0	100.0	

Sample ID: 0M, 12 hr (Distilled water)



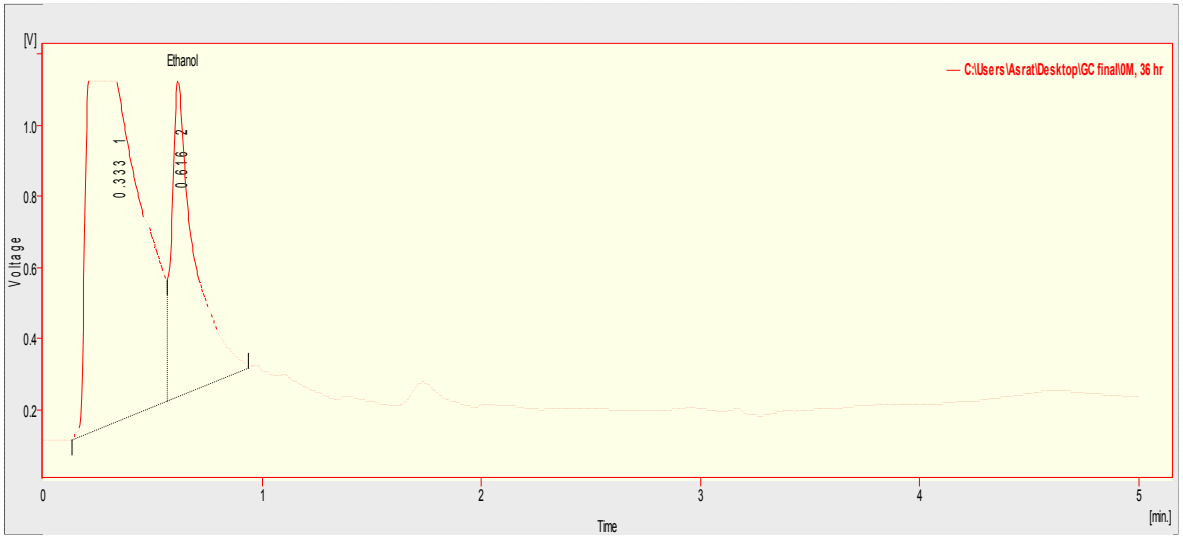
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.319	16405.327	935.838	70.3	60.4	0.33
2	0.616	6939.391	613.752	**29.7**	39.6	0.13
	Total	23344.718	1549.590	100.0	100.0	

Sample ID: 0M, 24 hr (Distilled water)



	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.310	15723.570	936.511	70.2	52.9	0.29
2	0.636	6680.112	833.100	**29.8**	47.1	0.10
	Total	22403.682	1769.612	100.0	100.0	

Sample ID: 0M, 36 hr (Distilled water)



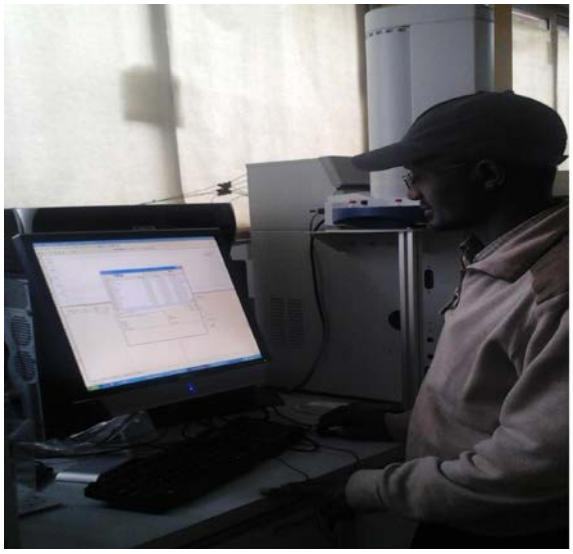
	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	0.333	16998.367	961.270	72.2	51.9	0.34
2	0.616	6543.028	889.190	**27.8**	48.1	0.09
	Total	23541.395	1850.460	100.0	100.0	






1'-E
2'-E
3'-E

supertek
500ml
400
300
200

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