JIMMA UNIVERSITY
JIMMA INSTITUTE OF TECHNOLOGY
FACULTY OF CIVIL AND ENVIRONMENTAL ENGINEERING
ENVIRONMENTAL ENGINEERING CHAIR

BIO-ETHANOL PRODUCTION FROM BLENDS OF BANANA PEELS AND MACRO ALGAE

BY: FIKADU MULETA

A THESIS SUBMITTED TO JIMMA UNIVERSITY, JIMMA INSTITUTE OF TECHNOLOGY, FACULTY OF CIVIL AND ENVIRONMENTAL ENGINEERING, ENVIRONMENTAL ENGINEERING CHAIR IN PARTIAL FULFILLMENTS FOR THE REQUIREMENTS OF THE DEGREE OF MASTERS OF SCIENCE IN ENVIRONMENTAL ENGINEERING

OCTOBER, 2017
JIMMA, ETHIOPIA

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ADVISOR: DEJENE BEYENE (PHD)
CO-ADVISOR: DIDA ABERA (PHD CANDIDATE)

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DECLARATION

I, **Fikadu Muleta**, hereby declare that the thesis, entitled "***Bio-Ethanol production from blends of banana peels and macro algae*****''**, is entirely my original work done for the award of MSc. Degree in Environmental Engineering by the Jimma University. To the best of my knowledge, the work has not been presented for the award of MSc. degree or any other degree either in Jimma Institute of Technology or any other University. The work contains no material previously published by another person. Thorough acknowledgment has been given where reference has been made to the work of others.

FikaduMuleta

_____ _____ _____

Name (Researcher) Signature Date

This Thesis has been submitted for examination with my approval as University supervisor.

DejeneBeyene (PhD)

_____ _____ _____

Name (Main advisor) Signature Date

The Thesis has been submitted for examination with my approval as University co-supervisor.

DidaAbera

_____ _____ _____

Name (Co-advisor) Signature Date

As Chair Holder of Environmental Engineering I approved the submission of the Thesis for examination.

DejeneBeyene (PhD)

_____ _____ _____

Name (Chair Holder) Signature Date

ABSTRACT

Our lives are linked to energy use. We use energy to do work. Energy lights our cities. So, it has to be changed agricultural waste to sustainable energy and maintain our community from dangerous pollution and control climate change. The objectives of this research is to investigate Bio- ethanol production from blends of banana peels and macro algae waste using Saccharomyces cerevisiae yeast as fermentation agent. Fossil fuel depletion has become a great concern as the world population is increasing at an alarming rate. Current concerns such as global warming, depletion of fossil fuels and increasing price of petroleum-based fuels have forced the search for alternative and cost effective energy sources with lesser greenhouse gas emissions. The methodology of this research was the fruit peels were crushed in to 3-5 cm sizes for easy drying and grinding. Sample drying was carried out in oven (60 ^{o}C for 72hr) to obtain easily crushable material. After drying, each of the samples was milled separately. The maximum particle sizes of the ground mixed sample were 2 mm. Laboratory experiments of 16 run were conducted to produce bio-ethanol from those blends of banana peel and macro algae were pretreatment, hydrolysis, fermentation and distillation process respectively to produce bio-ethanol. The result of research was obtained in these study 75% banana peels and 25%micro algae had high potential application for bio-ethanol production. pH was adjusted at 4.5 for bio ethanol production. The optimum results were obtained at 0.75%v/v acid concentration, 115^{o}C temperature and 15min retention time. Under these condition 56% of bio-ethanol were obtained. Further researches have to be done to improve the production of high quality and quantity of fruit peel with blends different ratio to produced ethanol. The budget used for the research was 25,120 ETB and the duration of the research for six months.

Key words: *Ethanol, distillation, fermentation, fossil fuel, hydrolysis, petroleum, pretreatment and Saccharomyces cerevisiae yeast*

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Acronyms

AAiT	Addis Ababa institute of Technology
BEC	Blood Ethanol Concentrations
°C	Degree Celsius
CO_2	Carbon dioxide
CRD	Completely Randomize Design
C_2H_5OH	Bio-ethanol or ethyl alcohol
ERA	Ethiopia Road Authority
ETBE	Ethyl Tertiary Butyl Ether
FAO	Food Association Organization
H_2SO_4	Sulfuric Acid
MSW	Municipal Solid Wastes
JiT	Jimma institute of Technology
LRP	Lead Replaced Petrol
NaOH	Sodium Hydroxide
O_3	Ozone
RS	Rectified Spirit
SRWC	Short-Rotation Woody Crops
SSF	Simultaneous Saccharification and Fermentation
SPSS	Statistical Package for Social Studies
VOC	Volatile Organic Compounds
WWI	World Watch Institute

CHAPTER ONE
INTRODUCTION

1.1Back ground of the study

Bio-ethanol refers to ethyl alcohol (ethanol) produced by microbial fermentation processes as opposed to synthetically produced ethanol from petrochemical sources. It is produced through distillation of the ethanol wash emanating from fermentation of biomass-. It can be utilized as a liquid fuel in internal combustion engines, either pure or in blends with petroleum.

Fossil fuel depletion has become a great concern as the world population is increasing at an alarming rate. Current concerns such as global warming, depletion of fossil fuels and increasing price of petroleum-based fuels have forced the search for alternative and cost effective energy sources with lesser greenhouse gas emissions. Research into the development of renewable and sustainable fuels has recognized bio-ethanol as a viable alternative to fossil fuels, owing to its low toxicity, biodegradability and the ability to effectively blend with gasoline without any engine modifications (Harun*et al*., 2009, 2010a).

Ethanol can be produced from any feedstock that contains appreciable amounts of sugar or materials that can be converted into sugar such as starch or cellulose. It is typically made by fermentation in much the same way that beer is produced followed by distillation. Three feedstock can be considered for the production of ethanol: sugar crops such as sugar beet, sugar cane and sweet sorghum; starch crops such as corn, barley, rye, wheat, sorghum grain, potatoes and cassava and cellulosic feedstock such as forest residues, energy crops (e.g. fast growing trees and grasses), agricultural residues and solid wastes. The production of ethanol from these feed stocks involve fermentation of the sugars into ethanol; distillation to separate the aqueous ethanol (95%) from the fermented mash and dehydration to produce anhydrous ethanol (> 99.5%) suitable for blending with gasoline. Some sugars can be converted directly to ethanol, whereas starch and cellulose must first be hydrolyzed to sugar before conversion to ethanol (Hahn-Hagerdal*et al*., 2006).

The production of ethanol from starchy and cellulosic feed stocks requires acid or enzymatic hydrolysis in order to convert them to sugars that can be fermented into ethanol. Increased

ethanol concentration of microbial fermentation has been examined as a strategy to reduce energy cost in downstream distillation and waste treatment. Most of the polymeric raw materials are available at prices lower than refined sugars. Consequently, each country may preferably develop ethanol production based on the available raw material in that country (Philppidis *et al.*, 1993).

The species of *Saccharomyces cerevisiae* a traditional ethanol producer, yet it is sensitive to high concentration of ethanol. Ethanol diffuses freely across biological membrane in yeast cell allowing equalization of ethanol concentration between intracellular and extracellular pools. As a result, the increased ethanol concentration in a medium inhibits cell growth, damage cells viability and reduce ethanol yield. The microorganisms used to carry out the fermentation process are just as important as the substrate, and they have also been the target of much research. *Saccharomyces cerevisiae*, also known as baker's yeast, is the most widely used fermentation microbe in the baking and brewing industries. This is due to the fact that some species adopt different metabolic path ways by having special genes or special enzymes such as invertase genes and invertase enzymes, respectively for conversion of sugars to ethanol or other species (Fregonesi*et al*., 2007).

The alternative fuels are expected to satisfy several requirements including substantial reduction of greenhouse gas emission, worldwide availability of raw materials and capability of being produced from renewable feedstock (Hahn-Hagerdal*et al*., 2006).

Production of fuel ethanol from biomass seems to be an interesting alternative to traditional fossil fuel, which can be utilized as a sole fuel in cars with dedicated engines or in fuel blends. Ethanol is currently produced from sugars, starches and cellulosic materials. The first two groups of raw materials are currently the main resources for ethanol production, but concomitant growth in demand for human feed similar to energy could make them potentially less competitive and perhaps expensive feed stocks in the near future, leaving the cellulosic materials as the only potential feedstock for production of ethanol (Taherzadeh and Karimi, 2007).

Cellulosic materials obtained from wood and agricultural residuals, municipal solid wastes and energy crops represent the most abundant global source of biomass. These facts have motivated extensive research toward making an efficient conversion of lignocelluloses into sugar monomers for further fermentation to ethanol (Lin and Tanaka, 2006).

Bio-ethanol is being considered as a potential liquid fuel due to the limited amount of natural resources. It is cost-effective to blend bio-ethanol into gasoline in view of high crude oil prices. Bio-ethanol fuel is mainly produced by the sugar fermentation process, although it can also be manufactured by the chemical process of reacting ethylene with steam. The main sources of sugar required to produce bio-ethanol come from crops or energy crops. These crops are grown specifically for energy use and include corn, maize and wheat crops, waste straw, willow and popular trees, sawdust, reed canary grass, cord grasses, Jerusalem artichoke, my scan thus and sorghum plants (Louime and Uckelmann, 2008).

There is also ongoing research and development into the use of municipal solid wastes to produce bio-ethanol fuel. It was obvious that bio-ethanol production should be examined in economic terms such as: farm-gate price of the biomass, logistic cost (transport and storage of the biomass), direct economic value of the feed stocks taking into account the co-products, creation or maintain of employment, water requirements and water availability (Balat andBalat, 2009).

The excessive consumption of fossil fuels, particularly in large urban areas, has greatly contributed to generation of high levels of pollution. There is a need for environmentally sustainable energy sources to find a viable and long-term substitute for liquid petroleum. As a step to solve this problem, the use or addition of bio-fuels to gasoline, which reduces emission of carbon dioxide and unburned hydrocarbons that form smog, has widely been enforced in recent years. Converting a renewable non-fossil carbon, such as organic wastes and biomass consisting of all growing organic matter (plants, grasses, fruit wastes and algae) to fuel would assure a continual energy supply (Wyman, 1996). The economics of bio-ethanol production by fermentation are significantly influenced by the cost of the raw materials, which accounts for more than half of the production costs (Classen*et al.*, 1999).

Green macro algae exhibit several features of an ideal feedstock that can complement the increased global demand on energy and food production. The cultivation of this biomass does not require arable land, freshwater or fertilizer, circumventing adverse impacts on food supplies and resource availability. Large-scale cultivation of brown macro algae is already being practiced in several countries, yielding over 70 million metric tons per year in 2006 (Roesijadi, snowden, 2010). Because green blue macro algae do not contain lignin, simple bio-refinery

processes such as milling, leaching and extraction can separate the sugars for conversion into bio fuels Andre new able chemicals (Chapman, 1970). Additionally, valuable materials, such as protein meal for animal feed and potash fertilizer for crop production, can be separated to support sustainable food production (Chapman, 1970).

To achieve a lower production cost, the supply of cheap raw material is thus a necessity. Production of value added products from agro-industrial and food processing wastes is now a focusing area, as it reduces pollution in the environment in addition to energy generation. The annual availability of these wastes amounts to 1.05 billion tons (Anonymous, 2004). The major part of this is mostly discarded and it is the main source for increasing the pollution in environment on occasions and also, the discarding process become a very expensive step due to high transportation costs. Majority of fruit and vegetable wastes available from their processing industries are seasonal and they do not decompose rapidly.

The mechanical drying of these wastes (mango peel, banana peels, citrus peel, pineapple peel and tomato processing wastes) gave opportunity to store the substrate all over the year. The yeast *Saccharomyces cescerevisiae*and facultative bacterium Zymomonasmobilis are better candidates for industrial alcohol production. Z. mobilispossesses advantages over S. cerevisiae. However, ethanol is produced commercially by yeast because it ferments glucose to ethanol as a virtually sole product and it is known for its high ethanol tolerance, rapid fermentation rates and insensitivity to temperature and substrate concentration (Linden and Hahn-Hägerdal, 1989).

Bio-ethanol as an alternative source of energy has received special attention worldwide due to depletion of fossil fuels. In India, sugar cane molasses is the main raw material for ethanol production. But the short supply and increased cost is the main hindrance for its use. 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 such as fruit waste. Banana is one of major constitute the principal food resources in the world and occupy the fourth world rank of the most significant foodstuffs after rice, corn and milk (Benitez et al., 1983; Divanya*etal.*, 1992).

Most of the fruit peels/residues are dried, ground, pelletized, and sold to the feed manufacturers at a low price which is not considered a highly viable proposition (Mamma et al., 2008). As per the FAO statistics, India is the largest producer of banana in the world and accounts for nearly

30% of the total world production Simultaneous Saccharification and Fermentation (SSF) of banana peels to ethanol by co-cultures of AspergillusnigerandSaccharomyces cerevisiae was investigated at different temperatures (20°C to 50°C) and at different pH (4 to 7 Since banana peels contain lignin in low quantities (Hammond*et al*., 1996). It could serve as a good substrate for production of value-added products like ethanol. In order to make the fermentation method cost effective and to meet the great demand for ethanol, research studies are now being directed in two areas namely, the production of ethanol from cheaper raw materials and the study of new microorganisms or yeast strains efficient in ethanol production (Favela-Torres et al., 1986; Pandeyet al., 2000; Akin-Osanaiyeet al., 2008). In this respect, inexpensive raw materials such as agricultural wastes, cellulosic wastes, fruit wastes, vegetable wastes, municipal and industrial wastescan be used to produce ethanol cheaply (Park andBaratti, 1991; Schugerl, 1994; Joshi *et al.*, 2001; Akin-Osanaiye*et al*., 2008).

Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology. An ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, appropriate some tolerant, enhanced ethanol tolerance and good thermo tolerance (Benitez et al., 1983; Divanya*etal*., 1992). In most of these studies the preferred candidate for industrial production of ethanol has been *S.cerevisiae*. Yeast also has the ability to produce ethanol which is not contaminated by other products from the substrate(Jones *et al*., 1981).

Commercial amylases (frequently those produced by *Aspergillusspecies*) are used for liquefaction and saccharification of starch and represent a significant expense in the production of fuel alcohol from starchy materials. Fruits are highly perishable products, currently most of the perishable fruits are lost during their journey through the agro food chain, due to spillage, physiological decay, water loss, mechanical damage during harvesting, packaging and etc so recent years effort have been directed towards the utilization of cheap and renewable agricultural sources such as banana peels waste as an alternative substrate for production of alternative bio-fuel like ethanol. In this study an effort will be made to eliminate the enzymatic liquefaction and saccharification step by using symbiotic co- cultures of amylolytic and sugar-fermenting organisms and to evaluate a single-step system for the enhanced fermentation of banana peels to

ethanol by using symbiotic co cultures of AspergillusnigerandSaccharomycescerevisiae(Jones et al., 1981).

1.2Statement of the Problem

Climate change and energy security associated with the use of fossil fuel resources have become major concerns and many countries have agreed to reduce emissions of greenhouse gases. It has become increasingly obvious that continued reliance on fossil fuel energy resources is unsustainable, owing to both depleting world reserves and the greenhouse gas emissions associated with their use. Today, 86% of the world energy consumption and almost 100% of transportation energy is met by fossil fuels (Dorian *et al*., 2006).Finding sufficient supplies of clean energy is one of the most daunting challenges and is intimately linked with global stability, economic prosperity and quality of life. Our use of fossil fuels, primarily coal and oil, could warm the atmosphere enough to contribute to ever more destructive floods, serious and sustained droughts and relentless snowfalls. The increase of petroleum price and environmental pollution has triggered the search for renewable and potentially carbon neutral energy resources. One way to slow these trends is to develop and use clean and sustainable energy sources (Ebasa, 2014).As the carbon dioxide (CO_2) released when energy is generated from biomass is generally balanced by that absorbed during the feedstock production it is often regarded as a 'carbon neutral' process (Hammond*et al.*, 2008).

Bio-fuels such as bio-ethanol contribute little or no CO_2 to the buildup of greenhouse gas emissions. Because of their compatibility with the natural carbon cycle, bio-fuels offer the most beneficial alternative for reducing greenhouse gases (Ebasa, 2014).Ethanol which can be blended with gasoline at about 5 - 10% to yield gasohol (a gasoline-ethanol mix) has several advantages over petroleum gasoline. The presence of an oxygen atom in ethanol allows gasohol to burn "cleaner" than regular gasoline, with reduced emissions of carbon monoxide, nitrogen oxides and hydrocarbons. However, ethanol has lower energy content than gasoline. As a result a vehicle needs more gasohol to go the same distance as it could go on pure gasoline. Ethanol also helps to address concerns about greenhouse gas emissions mainly CO_2(Ebasa, 2014).Whereas burning fossil fuels increases atmospheric CO_2 concentrations, bio-fuel crops reabsorb the CO_2 emitted when bio-fuels are burned, creating a cycle that is carbon neutral(an activity that does not change the net atmospheric CO_2 concentration). In other words, the CO_2 released when ethanol is burned

is balanced by the uptake of CO_2 from the atmosphere by plants growing to produce bio-fuel. Thus, ethanol is more environmentally friendly fuel than gasoline. Bio-fuels (ethanol and biodiesel) are also less hazardous if spilled since they are bio-degrade able (Wyman, 1994).

However, alternate energy resources akin to first generation bio-fuels derived from terrestrial crops such as sugarcane, sugar beet, maize, wheat and other cereals place an enormous strain on world food markets, contribute to water shortages and the destroy biodiversity. Diverting food crops to fuel production may result in high food prices as it will significantly reduce the areas available for food production. The need for cleaner-burning fuel sources for environmental reasons does not fully justify the use of limited edible resources for energy production. Also the removal of forested lands for increased agriculture land for the production of energy crops will have negative impact on bio-diversity by devoting large tracts of land for mono-culture cultivation. In addition, the use of fossil fuels during the production and processing of bio-fuels do not meet the claimed environmental benefits of such fuels. Second generation bio-fuels derived from lignocellulosic agriculture and forest residues and non-food crop feed stocks address some of the problems. However, there is concern over competing land use or required land use changes. Therefore, based on current knowledge and technology projections, third generation bio-fuels specifically derived from algae are considered to be a technically viable alternative energy resource that is devoid of the major drawbacks associated with first and second generation bio-fuels.

Success in the conversion of cellulose into sugars and then into ethanol minimizes the potential conflicts between food and energy production and maximizes environmental benefits (Wyman, 1994).This enables us to use low-cost crops and forest residues, grasses, husks, wood process wastes and solid wastes as feed stocks for the production of ethanol. Poor collection and disposal of municipal and industrial solid waste creates a range of environmental problems. A considerable amount of wastes ends up in open dumps or drainage system, threatening both surface water and ground water quality and causing flooding, which provides a breeding ground for diseases-carrying pests. Open air burning of waste, spontaneous combustion in landfills and incinerating plants that lack effective treatment for gas emissions causes air pollution.

The aim of this research will be to investigate the possibility of using and transforming banana peel and macro algae to ethanol thereby contributing towards alternative energy supply as well as creating an employment opportunity (Wyman, 1994).

1.3Objectives of the study

1.3.1 General objective

The general objective of this study is to investigate Bio-ethanol production from blends of banana peels and macro algae using *Saccharomyces cerevisiae* as fermentation agent followed by dehydration.

1.3.2 Specific objectives

1. Production of Bio-ethanol from different blends of banana peels and macro algae (75%:25%, 25%:75%, and 50%:50%)
2. To determine the optimal acid concentration, temperature and time that gives the highest possible efficiency of converting banana peels and macro algae to ethanol
3. To characterize Bio-ethanol produced from blends of banana peels and macro-algae

1.4 Significance of the study

The study will be used for continuous monitoring of this solid waste of banana peels and we use as energy. So, the outcome of this study work would help to save society Concerns about the greenhouse effect and global warming, air pollution, and energy security have led to increasing interest and more development in renewable energy sources such as bio-fuel, solar, wind, geothermal, and hydrogen.

The aim of this thesis will be to investigate the possibility of using and transforming blends of banana peel waste and macro algae to something valuable, namely ethanol using the fungus *Saccharomyces cerevisiae* as ethanol producing organism. The result of this study will benefit the community in many way they are Energy is one of the most fundamental parts of our universe. We use energy to do work. Energy lights our cities. Energy powers our vehicles, trains, planes and rockets. Energy warms our homes, cooks our food. Energy powers machinery in factories and tractors on a farm.

1.5 Scope of the study

This study will be focused on Bio-Ethanol production from blends of banana peels waste and macro-algae using *Saccharomycescerevisiae* yeast as fermentation agent followed by dehydration.

CHAPTER TWO

LITRATURE REVIEW

2.1 Introduction

Bio-fuels are alcohols, ethers, esters and other chemicals made from cellulose-based biomass. This includes herbaceous and woody plant, agricultural and forestry residues and a large portion of municipal and industrial waste materials (Rainner and Dominik, 2007).Bio-fuels are renewable since they are produced from bio-mass organic matter, such as plants. They generate about the same amount of carbon dioxide (a greenhouse gas) from the tailpipe as fossil fuels, but the plants that are grown to produce the bio-fuels actually remove carbon dioxide from the atmosphere. Therefore, the net emission of carbon dioxide will be close to zero (RainnerJ and Dominik R, 2007). The bio-fuels industry has evolved from using first generation feedstock (typically food crops) to using second and third generation feed stocks, for both ethanol and biodiesel. While the term bio-fuels denote any fuel made from biological sources, for most practical uses, the term refers to either ethanol or biodiesel. The last few years have seen tremendous growth in bio-fuels (Cellulosic Ethanol, 2010).

The word alcohol derives from Arabic al-kuhul, which denotes a fine powder of antimony produced by distilling antimony and used as an eye makeup. Alcohol originally referred to any fine powder, but medieval alchemists later applied the term to the refined products of distillation, and this led to the current usage. Ethanol, the most widely used bio-fuel, is made in a process similar to brewing beer (Rainner J and Dominik R, 2007). Ethanol is a clean-burning, high-octane fuel that is produced from renewable sources. Because ethanol can be produced domestically in most countries, it helps reduce dependence upon foreign sources of energy for these countries. Ethanol is beginning to be used all around the world as transportation fuel, and it has some distinct advantages. Fuels that burn too quickly make the engine "knock", a characteristic rattling sound. The higher the octane rating, the slower the fuel burns, and the less likely the engine will knock. When ethanol is blended with gasoline, the octane rating of the petrol goes up by three full points, without using harmful additives. Similar to the case of biodiesel, adding ethanol to gasoline "oxygenates" the fuel. It adds oxygen to the fuel mixture so that it burns more completely and reduces polluting emissions such as carbon monoxide (Cellulosic Ethanol, 2010).

Transportation fuels: Today, applications in the transport sector are based on liquid fuels. The advantage of liquid fuels is that they are easy to store. Furthermore, today's infrastructure for transport is mainly based on liquid fuels. Gaseous fuels are less utilized in the transport sector. Even less applications exist for solid fuels. They were only used in the past e.g. for trains. However, today transport fuels are classified into two basically different categories: fossil fuels which are mainly based on crude oil and natural gas, and bio-fuels made from renewable resources. The use of bio-fuels largely depends on the potential of available feedstock sources. Bio-fuel policies on regional, national, European and global level largely influence the success of bio-fuel market penetration. In the EU several targets have been introduced to promote bio-fuels (Cellulosic Ethanol, 2010).

2.2 Bio-ethanol

Bio-ethanol or ethyl alcohol (C_2H_5OH) is a liquid obtained by distillation of fermented sugar. It can also be produced from various resources available domestically such as agriculture and forestry residue, organic portion of municipal solid waste, woody and herbaceous crops and dedicated starchy crops. Bio-ethanol can substitute petrol such as premium, super and lead replaced petrol (LRP).Bio-fuels (bio-ethanol) have attracted global attention due to concerns on climate change, energy security and dependency and import burden of petroleum products. They are increasingly considered as feasible to substitute the fossil fuel source in the transport sector (Dufey, 2006).

2.3 Bio-ethanol Feed stocks

The global fossil fuel energy sources are rapidly being depleted and becoming more expensive. Moreover, scientists attribute global warming mainly to the extensive use of fossil fuels and the resulting release of CO_2 (a greenhouse gas). So far, harnessing hydroelectric power, tidal power and wind power, solar energy and geo-thermal energy may become economically viable but such energy sources are unlikely to fill the global energy needs completely. Bio-ethanol can be extracted from every sort of carbohydrate material that can be divided into three main groups: sugary (sugar beets and sugar cane), starchy (corn, wheat, barley, rye and other cereals as well as sorghum grains, cassava and potatoes) and lignocelluloses biomass (agricultural residues, municipal solid waste, energy crops, waste paper, paper pulp)(Dufey, 2006).

Three main directions can be followed to achieve feedstock supplies to produce Bio-ethanol: cultivation of "***energy crops***", harvesting of natural vegetation and utilization of organic wastes.

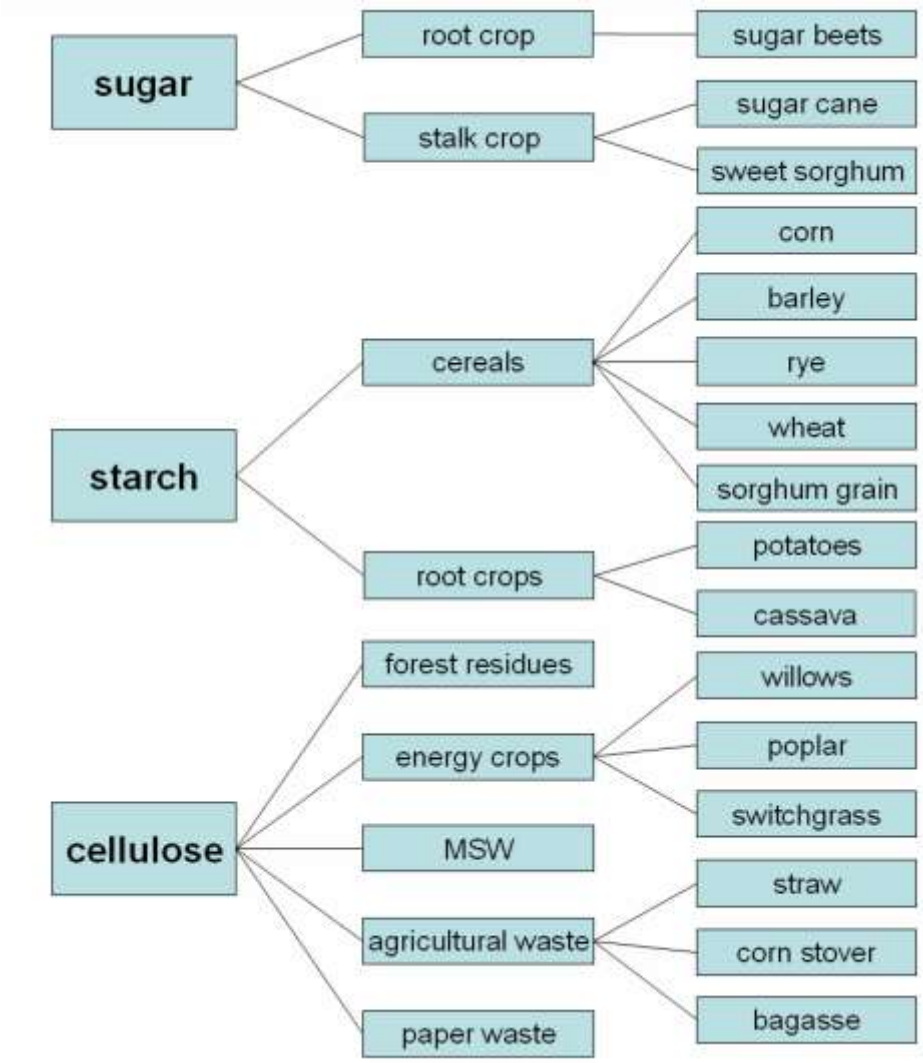


Fig. 2.1: Types of feedstock for ethanol production

2.3.1 First-generation feed stocks

First-generation feedstock for bio-ethanol production primarily refer to plant biomass sources that are also sources of human and animal nutrition: cereal starches and sugar crops. Since ethanol from sugar and starch source is readily available, these feedstock types are also called *first-generation feedstock*. First generation feedstock are characterized by the fact that only parts of the plants (starch, sugar, oil) are used for bio-fuel production (WWI 2006).Sugar based materials are predominantly derived from sugar cane (*Saccharum sp.),* sweet sorghum and sugar beet (*Beta vulgaris L*.) whilst starch-based materials are predominantly derived from cereal crops such as maize, wheat and other cereals. Sugar based feedstock for bio-ethanol production include sugar cane, sugar beet and sweet sorghum and these crops represent a readily fermentable sugar

source whilst cereal starches require pre-hydrolysis to obtain sugars that can be fermented by yeast. Thus, fermentation can be carried out without prior hydrolysis or other pre-treatments because the sugar is available in disaccharides which can be metabolized directly by enzymes present in yeast. For this reason, the conversion of Sugar based feedstock is the easiest and most efficient compared with other feedstock.

Fig.2.2: Harvested sugar beets (Germany)

2.3.1.2 Sugar cane

Sugar cane (*Saccharum*sp.) is a genus of 37 species of tall grasses and belongs to the family of the *Poaceae* and is native to warm temperate to tropical regions. All the species interbreed, and the major commercial cultivars are complex hybrids. Sugarcane is a grass originally from tropical Southeast Asia. The plants have stout, jointed fibrous stalks which are 2 to 6 meters tall and rich in a sugar bearing sap. Today about 107 countries grow sugarcane whereas Brazil is the world leading producer. Sugar cane is the most significant crop for bio-fuel production today, supplying more than 40 % of all fuel ethanol (WWI 2006 p.22).Besides the production of bio-ethanol, sugar cane is also used for the production of alimentary sugar, molasses, and rum.

Fig.2.3: Sugar cane plantation (WWI, 2006 p.22).

Sugarcane cultivation requires a tropical or subtropical climate, with a minimum of 600- 850 mm of annual moisture. It is one of the most efficient plants in photosynthesis which is able to convert up to 2 % of incident solar energy into biomass. In prime growing regions, sugarcane can produce up to 20 kg for each square meter exposed to the sun. Sugarcane is propagated from cuttings, rather than from seeds. Modern methods of stem cuttings have become the most common method of reproduction. Once planted, a stand of cane can be harvested several times as the cane continuously sends up new stalks. Usually, each successive harvest gives a smaller yield, and eventually the declining yields justify replanting (WWI 2006 p.22).

2.3.1.3 Sweet sorghum

Sweet sorghum (Sorghum bicolor) belongs to the Family of the Poaceae. The genus of Sorghum has many species of which sweet sorghum is one of the most grown plants. Similar to sugarcane it is cane-like plant with a high sugar content of the stalk. Besides the stalk, also its seeds can be used for several purposes. Farmers can harvest sweet sorghums a multi-used crop in separating the seeds at the top of the plant for food and the sugars in the stalk for fuels. In settings where land is particularly scarce, this co-harvesting of sorghum may be particularly efficient.

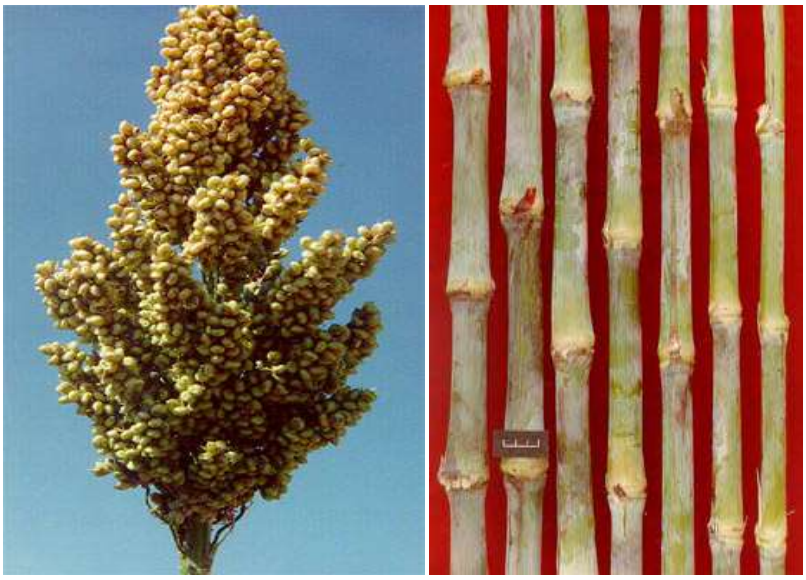


Fig.2.4: Seeds and stalks of sweet sorghum can be used as feedstock of bio-ethanol

Although currently not a significant ethanol feedstock, sweet sorghum has many promising advantages. For instance it grows under drier and warmer conditions than many other crops. It only needs 1/3 water of sugarcane and is tolerant to drought, heat, water logging and salt-alkali. But sweet sorghum can be grown in temperate areas as well. With its drought tolerance and ability to produce sugar, sweet sorghum could receive increasing attention as a feedstock for ethanol production (WWI 2006 p. 24).

2.3.2.2 Starch Crops

2.3.2.2.1 Cereals

Cereal crops are grasses which are cultivated originally for their edible grains or seeds(actually a fruit called a caryopsis). Worldwide cereal grains are grown in greater quantities and provide more food energy to the human race than any other type of crop. The most planted cereal crops are corn (maize)wheat and rice, which account for more than 80 % of all grain production worldwide(WWI 2006 p. 24).

Although each species has its specific characteristics, the cultivation of cereal crops is similar. In general, they are annual plants and consequently one planting yields one harvest. Nevertheless, in Europe, all cereals can be divided into cool-season and warm season types.

Wheat, rye, triticale, oats, barley, and spelt are the cool-season cereals. These are hardy plants that grow well in moderate weather and cease to grow in hot weather (approximately30 °C but

this varies by species and variety). Barley and rye are the hardiest cereals, able to overwinter in subarctic regions like Siberia. Wheat is the most popular cereal crop. Alcohols-season cereals are grown in the tropics as well, but only in the cool highlands, where it may be possible to grow multiple crops in a year. Cool-season cereals can be sub-divided into either winter or spring types. Winter varieties are sown in the autumn, germinate and grow vegetative, then become dormant during winter. They resume growing in the springtime and mature in late spring or early summer. This cultivation system makes optimal use of water and frees the land for another crop early in the growing season. Winter varieties do not flower until springtime because they require exposure to low temperature for a genetically determined length of time. Spring cereals are planted in early springtime and mature later that same summer. Spring cereals typically require more irrigation and yield less than winter cereals (WWI 2006 p. 24).

Warm-season cereals are tender and prefer hot weather. They are grown in tropical lowlands for the whole year and in temperate climates during the frost-free season. However, once the cereal plants have grown their seeds, they have completed their lifecycle. The plants die and become brown and dry. As soon as the parent plants and their seed kernels are reasonably dry, harvest can begin. In Europe, cereal crops are usually machine-harvested, typically using a combine harvester. It cuts, threshes, and winnows the grain during a single pass across the field (WWI 2006 p. 24).

Fig.2.5: Different types of starchy crops for ethanol production (wheat, barley, oat and corn)

2.3.2.2.2 Potatoes

The potato plant (*Solanumtuberosum*) is a perennial plant of the *Solanaceae*or nightshadefamily. It is commonly grown for its starchy tuber and is therefore the world's most widely grown tuber crop and the fourth largest crop after rice, wheat, and maize. The origin of thepotato plant is in South-America mainly in the Andes. Potatoes spread to the rest of the world after European contact with the Americas in the late 1400s and early 1500s (Liimatainen*et al*., 2004).

Fig.2.6: Blooming potato plants (left) and their starchy tubes

Potatoes are generally grown from the eyes of another potato and not from seed. They are planted as a row crop using seed tubers, young plants or micro tubers. In most cases three steps

of plowings including harrowing and rolling are necessary before the land is in suitable condition for planting potatoes. Commercial harvesting is typically done with large potato harvesters which also pre-clean the tubes. Further inspection and separation occurs when the potatoes are unloaded from the field vehicles and put into storage. Recently bio-ethanol is produced by using waste potatoes which are a co-product of the food industry. For instance waste potatoes are used as feedstock in Finland where Shaman Spirits Ltd in Tyrnävä (near Oulu) uses 1.5 million kilograms of waste potatoes per year for ethanol production (Liimatainen*et al*., 2004).

2.3.2.3 Second generation feedstock (cellulose-based)

The use of first generation feedstock to meet growing demands for future bio-fuel production is ultimately unsustainable and there are severe limitations to starch and sugar-based ethanol production. Non-food or second generations, feedstock for bio-ethanol are therefore the future due to abundance, ethical considerations and favorable economics. The second generation feedstock types provide the opportunity to use nearly the whole plant for bio-fuel production and not only parts of the plants (grains, tubes, stalks). In order to use second generation bio-fuels for ethanol production, advanced technologies are necessary (WWI 2006 p. 20). Second generation raw materials for bio-ethanol production typically refer to non-food biomass sources, mainly lignocelluloses biomass. This represents the most abundant form of carbon on Earth and encompasses two main categories of feed stocks. Since the technology for converting cellulosic feedstock into ethanol is not yet competitive, this feedstock is a second generation feedstock. Bio-ethanol production from cellulose is expected to significantly expand in the future, when technologies will improve (Liimatainen*et al*., 2004).

2.3.2.4Cellulosic wastes

Waste materials (straws, corn residues (stover, fibers and cobs), woody wastes/chippings, forestry residues, old paper/cardboard, bagasse, spent grains, municipal solid waste (MSW), agricultural residues (oilseed pulp, sugar beet pulp).Depending on their origin, cellulosic wasted can be divided into primary, secondary and tertiary wastes. **Primary cellulosic** wastes are produced during production and harvesting of food crops which include straw, corn stalks and leaves. Residues from forestry such as wood thinning from commercial forestry belong to primary cellulosic wastes. These types of biomass are typically available in the field or forest and must be collected to be available for further use. There are long-term economic and environmental concerns associated with the removal of large quantities of residues from

cropland. Removing residues can reduce soil quality, promote erosion and reduce soil carbon, which in turn lowers crop productivity and profitability (WWI, 2006).

Fig.2.7: Primary cellulosic wastes, such as forest waste (left) and agricultural residue (right)

Secondary cellulosic wastes are generated during the production of food products and biomass materials. It includes nut shells, sugar cane bagasse and saw dust, is available at industries for food and beverage production as well as at saw and paper mills. ***Tertiary cellulosic wastes*** become available after a biomass-derived commodity has been used. A large variety of different waste fractions is part of this category: organic part ofmunicipal solid waste (MSW), waste and demolition wood, sludge, paper, etc(Liimatainen*et al*., 2004).

2.3.2.5 Cellulosic energy crops

Feedstock from dedicated cellulosic energy corps is a promising source for ethanol production in the future. There are several advantages for the cultivation of cellulosic energy crops, such as perennial herbaceous plant species and short-rotation woody crops(SRWC). Firstly, the change of land from intensive annual crop production to perennial herbaceous species progressively increases the content of soil organic matter. Secondly, the roots of perennial crops protect soil from erosion. Thirdly, these crops generally require less fertilizer, pesticide and less energy input for crop management, especially since it is not necessary to plow the field each year. However, shifting land from natural cover to intensive annual crop production typically decreases soil organic matter steadily(Liimatainen*et al*., 2004).

Fig.2.8: Willow plantation (left) and poplar leaves (right)

Along with woody cellulosic energy crops also **perennial grass species** are a promising opportunity for future feedstock production. Miscanthus (*Miscanthussinensis, M.sacchariflorus, Miscanthus x giganteus*), switch grass (*Panicumvirgatum),* and reed canary grass (*Phalarisarundinacea)* are perennial crops that can be harvested every year. They have been the focus of considerable interest in Europe and North America where sugar bearing grasses such as sugar cane and sweet sorghum cannot be cultivated. It is expected that breeding could highly increase productivity and at least double energy grasses productivity. Such advances in breeding will be realizable much easier than breeding food crops since it is easier to breed for size rather than for particular quality, such as taste in fruits or vegetables (WWI 2006 p. 46f).

2.3. 3 Third Generation feed stocks

2.3.3.1 Algae

Algae belong to a large group of simple photosynthetic organisms. They are subdivided into two major categories based on their size. Microalgae are small free-living microorganisms that can be found in a variety of aquatic habitats. Algae, considered as the third generation biomass have proved to be superior to any other biomass due to its environmental and economic sustainability, security of supply, absence of lignin, high photosynthetic efficiency, fast-growing rate and role in reduction of greenhouse gas emissions. They have the potential to convert atmospheric carbon dioxide into useful biomass by growing even in wastewaters and can yield bio fuels without much harm to food supplies, biodiversity and agriculture. Algae can thus play a major role in the

treatment/utilization of wastewater and reduce the environmental impact and disposal problems (Melis A, Happer T, 2001).

They can be grown on saline/coastal sea water or artificial sea water and on non-agricultural lands (desert, arid and semi-arid land) (Packer M, 2009). Comparing in other advanced feedstock based on cellulose for bio-fuels production, algal genomics and basic research are more advanced and gaining in momentum (Melis A, Happer T, 2001).

Algae have high photon conversion efficiency and can synthesize and accumulate large quantities of carbohydrate biomass for bio-ethanol production, from in expensive raw materials (Harun R, Singh M, 2010). Aquatic algal cells are buoyant, avoiding the need for structural biopolymers such as hemicellulose and lignin that are essential forhigher plant growth in terrestrial environment. This simplifies the process of bioethanol production by eliminating the chemical and enzymatic pre-treatment steps (John RP,Anisha G,2011).Moreover, algal cells can be harvested within a short span of time compared to other feedstock and hence can meet the increasing demand of feedstock for ethanol production (Aresta M, Dibenedetto G,2005).

2.4 Bio-ethanol Production

Generally, ethanol can be produced either synthetically from petrochemical feedstock (petroleum) or by microbial fermentation which is applicable to bio-ethanol production. The process for production of fuel bioethanol from biomass can be broken down as follows (Aresta M, Dibenedetto G, 2005).

- Feedstock production: harvesting, reception, storage
- Physical pretreatment: milling
- Saccharification: conversion of starch and cellulose into sugar
- Chemical treatment: dilution of the sugars with water and addition of yeast or other organisms
- Fermentation: production of ethanol in solution with water along with waste and by-products
- Distillation: separation of ethanol
- Dehydration: Removal of the remaining water by molecular sieves (anhrourethanol)
- Co-product preparation: Drying of the alcohol free still age (mash) for high-value animal feed

These steps in the feedstock-to-ethanol conversion process largely depend on the type of feedstock.

2.4.1 Sugar-to-Ethanol Process

The simplest way to produce ethanol is the sugar-to-ethanol production. Thereby biomass is used that contains six-carbon sugars which can be fermented directly to ethanol. Examples for typical sugary feedstock types are sugar cane and sugar beets which contain substantial amounts of sugar. Although fungi, bacteria, and yeast microorganisms can be used for fermentation, the specific yeast *Saccharomyces cerevisiae* (Bakers' yeast) is frequently used to ferment glucose to ethanol. Traditional fermentation processes rely on yeasts that convert six carbon sugars (mainly glucose) to ethanol. Theoretically, 100 grams of glucose will produce 51.4 g of ethanol and 48.8 g of carbon dioxide (Badger 2002). In Brazil and in most tropical countries which produce ethanol, sugar cane is the most common feedstock for ethanol production. In these warm countries costs of ethanol production from sugar cane are among the lowest for any bio fuels. Since the climate for sugar cane is too cold in most parts of the European Union, sugar beets are used to produce ethanol.

2.4.2 Starch-to-Ethanol Process

Another potential ethanol feedstock is starch. In Europe and in the United states a large portion of bio-ethanol is produced from the starch component of grain crops, primarily corn and wheat in the US and wheat and barley in Europe. Starch molecules are made up of long chains of glucose molecules which have to be broken into simple glucose molecules (saccharification). Therefore, starchy materials require a reaction of starch with water (hydrolysis). Typically hydrolysis is performed by mixing the starch with water to form slurry which is then stirred and heated to rupture the cell walls. During the heating cycle, specific enzymes are added, which break the chemicalbonds (Badger 2002). Organisms and enzymes for starch conversion and glucose fermentation on a commercial scale are readily available. In conventional starch-to-ethanol processes, only the starchy part of the crop plant is used. The kernels of corn, barley or wheat represent a fairly small percentage of the total plant mass. The fibrous portion of these plants like seed husks and stalks remain. Current research on cellulosic ethanol production is focused on utilizing these waste cellulosic materials to create fermentable sugars. This leads to more efficient production of ethanol than from using just the sugars and starches directly available (OECD/IEA 2004).

2.4.3 Cellulose-to-Ethanol Process

Besides sugar and starch, also cellulose can be converted into ethanol, but the cellulosic biomass-to-ethanol production process is more complicated than the sugar- or starch-to ethanol process.

Cellulosic materials are comprised of lignin, hemicelluloses, and cellulose and thus are sometimes called lingo cellulosic materials. They have to be converted to five- and six carbon sugars, before they can be fermented and converted into ethanol. One of the primary functions of lignin is to provide structural support for the plant. Thus, in general, trees have higher lignin contents then grasses. Unfortunately, lignin which contains no sugars encloses the cellulose and hemicelluloses molecules, making them difficult to reach. Cellulose molecules consist of long chains of glucose molecules as do starch molecules, but have a different structural configuration .These structural characteristics plus the encapsulation by lignin makes cellulosic materials more difficult to hydrolyze than starchy materials (Badger, 2002).

Also hemicelluloses are comprised of long chains of sugar molecules. The exact sugar composition of hemicelluloses can vary depending on the type of plant .For complete fermentation of cellulosic materials special organisms are required (OECD/IEA, 2004). Bacteria have drawn special attention from researchers because of their speed of fermentation. In general, bacteria can ferment in minutes as compared to hours for yeasts (Badger, 2002). There are three basic process types for conversion of cellulose to ethanol: acid hydrolysis, enzymatic hydrolysis, and thermo-chemical process. The most common type is acid hydrolysis. Virtually any acid can be used. However, sulfuric acid is most commonly used since it is usually the least expensive (Badger, 2002).

2.5 Properties of Bio-ethanol

Bio-ethanol has many properties. Under ordinary conditions, ethanol is a volatile, flammable, clear, colorless liquid, miscible (i.e., mixes without separation) in both water and non-polar solvents and it is a monohydric primary alcohol. It melts at - 117.3°C and boils at 78.5°C. It is separated from water only with difficulty; ethanol that is completely free of water is called absolute ethanol. Ethanol vapor, like gasoline vapor, is denser than air and tends to settle in lower areas. However, ethanol vapor disperses rapidly (Ebasa, 2014).

Table 2.1: Properties of ethanol

S.NO	Property	Description
1	Density	0.789 g/cm^3
	Molecular mass	46.07 g/mol
2	Phase	Liquid
3	Solubility in water	Fully miscible
	Freezing point	-117 ^{o}C
	Flash point:	12.8 ^{o}C
4	Melting point	−114.3 °C (158.8 K)
5	Boiling point	78.4 °C
6	Acidity (pKa)	15.9 (H^+ from OH group)
7	Viscosity	1.200 cP at 20 °C
8	Dipole moment	1.69 D (gas)
	Ignition temp	425 ^{o}C
	Higher heating value (at 20^{o}C)	29,800 kJ/kg
	Octane number	99

Fuel ethanol mix with water, but at high enough concentration of water, the ethanol will separate from the gasoline. A fuel ethanol flame is less bright than a gasoline flame but is easily visible in day light. Pure bio-ethanol and bio-ethanol blends are heavier than gasoline. Bio-ethanol and bio-ethanol blends conduct electricity but, gasoline is an electrical insulator. Bio-ethanol is less toxic than gasoline or methanol. Carcinogenic compounds are not found in pure ethanol. At low temperature (32oC) E85 vapor is more flammable than gasoline vapor (Ebasa, 2014).

Use of Bio-ethanol for Transport bio-ethanol has become preferential, because of its potential of matching the convenient features of petroleum at competitive price (Wyman, 1996). It offers a number of benefits examples, high heat of vaporization that allows it to achieve higher engine efficiency. Its use reduces ozone (O3) and smog formation compared with the conventional gasoline due to its low volatility and photochemical reactivity. Its blended use reduce fossil fuel consumption and provide oxygen to promote more complete combustion that results less exhaust emission of carbon monoxide and unburned hydrocarbon (Wyman, 1996).

In addition to using the existing petroleum infrastructure, bio-ethanol can be blended with gasoline in any proportion up to 10per cent without the need for engine modification. Blends of 5

percent or 10 percent of bio-ethanol gasoline are denominated B5 and B10, respectively. In some cases they are denominated as E5 for 5% bio-ethanol blend (Bio-ethanol 5% and 95% gasoline) and E10 for10% (Dufey, 2006).

2.6 Energy Balance of Bio-ethanol

The energy balance of bio-ethanol depends on the energy input for processing bio-ethanol during the life cycle in comparison to the energy content of the final fuel. Typically, lifecycles of different bio-fuels can be very different and depend on feedstock type, agricultural practices, regional feedstock productivity, process technology, and final driving efficiency. Therefore attention has to be paid when using data about energy balances of ethanol. Generally they are valid only for dedicated cases which can vary considerably. Although there exist studies in which the energy balance of different methods of bio-ethanol production are analyzed and evaluated, in many of these studies assumptions and data on agricultural and industrial conversion technologies are not updated (Schmitz et al. 2005).More recent studies are based on new technologies. An extensive overview about these studies are given and compared by (Schmitz et al. 2005).

Table 2.2: Estimated fossil energy balances of selected fuel types (WWI, 2006)

Fuel type (feedstock)	**Estimates ofFossil EnergyBalance**
Ethanol (cellulose)	2 – 36
Ethanol (sugar cane)	~ 8
Ethanol (wheat)	~ 2
Ethanol (sugar beets)	~ 2
Ethanol (corn)	~ 1.5
Ethanol (sweet sorghum)	~ 1
Petrol (crude oil)	~ 0.8

The best energy balances are received for bio-ethanol from sugar cane. It is only exceeded by ethanol made from cellulosic feedstock, but the technology is not yet in commercial operation. Significantly, all types of bio-ethanol not only have better energy balances than fossil petrol, but even have energy balances larger than one (Schmitz*et al*., 2005).

2.7 Environmental Impact of Bio-ethanol

Bio-ethanol is harmless to the environment. The main environmental advantages of fuel-ethanol are its sustainability in using a renewable resource as a feedstock, thus promoting independence of fossil fuel, and maintaining the level of greenhouse gas (CO_2). Carbon dioxide in the atmosphere is assimilated through photosynthesis and metabolized to be a building block of plants. The energy of sunlight is used to make carbohydrates stored in crop sand in the whole plant body. While crops are useful as energy sources for human and animals, some crops like starch or oil containing crops can be converted to fuels or chemicals. Combustion of these fuels produces CO_2 gas which would be assimilated again by plants. In total, almost no net CO_2 is produced by using bio-fuels generated from biomass (Armstrong, 1999). The issues are mainly related to the net energy content in ethanol, and depend on the assumption of ethanol production routes. A number of life-cycle assessments have been studied, and show that a change from fossil fuel to bio-fuels could reduce CO_2 emission by factor of 1/2 to 1/5, depending on how significant the use of renewable fuels is at all stages in the process (Bernesson, 2006; Walter, 2006; Blottnitz and Curran, 2006; Kim and Dale, 2005; Rosillo-Calle *et al*., 2004).

In ground water and soil mixtures, ethanol can be rapidly degraded both aerobically (100 ml/L in 7 days) and an aerobically (100 mg/L in 3-25 days) (Armstrong, 1999). Bio-ethanol in surface water is also rapidly degraded and thus not harmful to the biotope as long as it is not present in concentrations directly toxic to microorganisms. The half-time of bio-ethanol in surface water is 6.5 to 26 hours. While bio-ethanol releases volatile organic compounds (VOC) due to its low vapor pressure, degradation of bio-ethanol in the atmosphere is also predicted to be rapid. Exposure of humans to bio-ethanol is harmless. The exposure may be carried out mostly by inhalation of bio-ethanol vapor as VOC, and by body contact or, rarely, ingestion from either blended fuel or denatured fuel. The occupation standard for bio-ethanol in the air is 1000 ppm (1900 mg/m3) on an eight-hour basis. Above this standard concentration, ethanol vapor causes eye and upper respiratory tract irritation, fatigue, headache and sleepiness (Massed *et al.*, 1985).

2.8 Toxic Exhaust Emissions

The major part of engine exhaust streams during ethanol combustion consists of the components nitrogen, carbon dioxide and water. All three components are non-toxic to human health.

However, about 1.4% of petrol engine exhaust emissions are composed of more or less harmful substances to human health (Mittelbach & Remschmidt 2004 p.185).

Apart from the above mentioned emissions, fuel combustion emits particulate matter (PM), volatile organic compounds (VOCs), nitrogen oxides (NOx), carbon monoxide (CO) and a variety of other toxic air pollutants. VOCs and NOx are precursors for troposphere ozone. Momentary weather conditions and local geographic characteristics influence the impact of these air pollutants. Ozone formation e.g. occurs more easily during hot weather. Also toxic air pollutants are more evident under hot weather conditions. They can be emitted either by the engine exhausts or by evaporation from fuel storage and fuel handling since ethanol has high volatility and generally increases evaporative emissions of gaseous hydrocarbons. As opposed to this, carbon monoxide is a larger problem in cold weather and at high altitudes.

To assess the environmental impact of substituting petrol with ethanol, both fuels have to be compared regarding their emissions. Therefore a detailed comparison between emissions of ethanol and petrol combustion will be done. Harmful engine exhaust emissions from combustion of ethanol are generally lower when compared to the tailpipe emissions of fossil petrol. Thus ethanol can reduce certain vehicle pollutant emissions which exacerbate air quality problems, particularly in urban areas (Macedo*et al*., 2003).

2.9 Greenhouse Gas Emissions

One of the major drivers of bio-fuel promotion worldwide is the concern about climate change and the potential of bio-fuels to reduce GHG emissions. Although it is incontestable that the use of bio-ethanol is able to reduce GHG emissions significantly when compared to fossil fuels, assessments of quantified GHG reductions are useful and necessary. However, the GHG balance for bio-ethanol is highly variable and includes emissions of cultivation, transport, conversion process and distribution (Macedo*et al*., 2003).

2.10 Sustainability of Bio-ethanol

Determining the direct environmental effects of ethanol production is complex. Environmental effects of using bio-ethanol vary, depending on the fuel itself, vehicle technology, vehicle tuning and driving procedure. Also agricultural production practices and the design of ethanol production plants differ largely. In this Research the main environmental problems of ethanol production and use are demonstrated.

2.10.1 Water Issues

Water issues are an important concern of both, ethanol processing and use of ethanol. In the first step, the water consumption of ethanol processing will be discussed. This will be followed by a discussion about water contaminating impacts of ethanol which is spilled and leaked unburned.

The water consumption for the production of bio-ethanol is considerably high. Thereby, much water is used for feedstock production. The amount of water used for agriculture depends on the humidity / aridity of the cultivated region and on the water demand of the feedstock type. But also for the conversion process much water is needed. The quantity of water needed for the ethanol production process depends on the design of the production plant. Modern technology and design can substantially reduce the amount of fresh water needed by a stand-alone ethanol plant. There is "zero discharge" plants in operation that recycle virtually all of the water used in production, limiting the need for large supplies. Most plants are designed with "in-house" water treatment systems for supply and discharge. However, there are always three water uses in a typical ethanol plant(Ulrich, 1999). The first water use in a typical ethanol plant is non-contact water, primarily used for cooling. The second use is for liquefaction of the feedstock. Water must be clean and treated so that there is no microbiological contamination in the fermentation process.

Thirdly, ethanol processing also results in large volumes of nutrient-rich waste water that, if not cleaned and recycled, can speed eutrophication of local rivers and streams by affecting the water dissolved oxygen content. In addition, sugar mills must be flushed every year, putting huge amounts of organic matter into local waterways (WWI 2006 p. 189).Apart from the water consumption during the ethanol production process, water contamination impacts of released ethanol are an important environmental issue as well. Since ethanol is a naturally occurring substance produced during the fermentation of organic matter it is expected to rapidly biodegrade in essentially all environments (Ulrich, 1999).

2.10.2 Land Use and Biodiversity

Bio-ethanol production can have positive and negative impacts on current land use and biodiversity. Thereby feedstock production has to be discussed in more detail, including both, quality and quantity of land use. The quality of land use practices strongly influences habitat and biodiversity aspects, as well as soil, water and air quality. The impacts depend on a variety of factors such as the choice of feedstock, what the feedstock replaces and how it is managed (WWI

2006 p.168).On the one hand, land use for ethanol production offers the potential to reduce the environmental load relative to conventional agriculture. Farming practices can be adjusted to maximize total energy yield rather than the oil, starch or sugar contents of crops. This can diversify plant varieties and reduce chemical inputs. Especially second generation feedstock, such as cellulose for ethanol production, can contribute to diversify current agriculture. On the other hand, feedstock production can cause severe environmental problems which can be the most environmentally disruptive stage of total ethanol production. For instance improper and massive use of pesticides and fertilizers could negatively affect ethanol production. This might be a problem especially in countries with low sustainability standards. Regarding the quantity of land use, considerable amounts of agricultural land have to be cultivated for feedstock production. The key factors in determining how much land is needed to produce bio-ethanol are crop yields and the resulting ethanol yields (OECD/IEA 2004 p.127).

2.10.3 Human Health

Ethanol is an important component of alcoholic beverages. It has been part of the human diet and the human environment for thousands of years. In low quantities and concentrations it is not harmful to human health, but pure or highly concentrated ethanol can permanently damage living tissue. Pure ethanol is a tasteless liquid with a strong and distinctive odor. It produces a characteristic heat-like sensation when brought into contact with the tongue or mucous membranes. Although low quantities of ethanol are not toxic to human health, concerns about the possible health consequences of using ethanol as transport fuel have been raised. These concerns mainly include the inhalation of ethanol vapors by using ethanol pure or blended in transport applications. Prediction of blood ethanol concentrations (BEC) following exposure to ethanol vapors must consider several factors: (a) the concentration of ethanol in air, (b) the duration of exposure, (c) breathing rate, (d) absorption of ethanol across the lungs, and (e) the body's elimination rate of ethanol. Nevertheless, it is highly unlikely that exposure to airborne ethanol associated with gasoline use could produce toxic effects. The reasons for this are the tiny doses that might be received and the body's rapid elimination of ethanol (Armstrong 1999).

2.11 Economy of Bio-ethanol

The economy of bio-ethanol mainly depends on its production costs and on policy frameworks, but it is also influenced by positive and negative external effects. These cost related issues will be discussed first and then followed by a market overview of bio-ethanol.

The ethanol costs for the production of bio-ethanol from starch and sugar crops vary considerably with the wide range of crop types, agricultural practices, land and labor costs, conversion plant sizes, processing technologies and policies in different regions and countries. Therefore Brazil is the lowest-cost producer of ethanol. This is a result of lower input costs, relatively large and efficient plants and the inherent advantages of using sugarcane as feedstock (OECD/IEA 2004 p.68).

2.12 Current Ethanol Production Policy of Ethiopia

Ethanol is manufactured from microbial conversion of biomass material through fermentation. The production process consists of conversion of biomass to fermentable sugars, fermentation of sugar to ethanol and the separation and purification of ethanol. Fermentation initially produces ethanol containing a substantial amount of water. Then this solution is distilled using distillation column the majority of water to yield up to 95 percent purity ethanol, the balance being water.

This mixture is called hydrous ethanol. If the remaining water is removed in further process, the ethanol is called anhydrous ethanol and suitable for blending with gasoline. Ethanol is "denatured "prior to leaving the plant to make it unfit for human consumption by addition of small amount of products such as gasoline (ESDA, 2005). The worldwide recent awareness for the use of ethanol to replace petroleum and generation of power along with sugar mill plants should have led to setting up of number of ethanol plants and co-generations. Ethiopia has several sugar real estate (Fincha, Metehara and WonjiShoa) industries which are run and administered by Sugar Development Agency.

Among molasses derived products ethanol takes the largest part, but its utilization must attract the attention of the government policy makers in order to utilize as a bio-ethanol. Bio-ethanol or bio-fuel is ethanol based products that can process into liquid fuels for transport purposes (ESDA, 2005).

Table 2.3: Projected annual ethanol production in million liters

Factory	Year				
	2009/10	**2010/11**	**2011/12**	**2012/13**	**2013/14**
Fincha	6.9	13.2	15.7	17.1	21.5
Methara	8.9	12.8	17.5	21.3	21.3
Wonji	-	-	10.6	10.6	10.6
Tendaho	-	15.9	30.1	43.6	55.4
Total	**15.8**	**41.9**	**73.9**	**92.6**	**108.8**

Source: Ethiopian Sugar Agency, 2009

2.13. Pre-treatment of Biomass

One of the main problems in application of lingo-cellulosic materials is their resistance against enzymatic depolymerization. Therefore, biomass pretreatment is a crucial step as it breaks down the crystalline structure of cellulose and releases the fermentable sugars, so that the hydrolysis of carbohydrate can be achieved more rapidly and with greater yields (Mosier *et al.,* 2005). The carbohydrate polymers in lingo-cellulose are tightly bound to lignin mainly by hydrogen bonds as well as by some covalent bonds which make it a recalcitrant substrate for hydrolysis and ethanol production. Thus, delignification is a crucial step prior to depolymerization and fermentation steps which can highly increase the rate of subsequent hydrolysis reaction. Delignification liberates cellulose and hemi-cellulose from their complex with lignin (Duff and Murray, 1996; Lin, 2006).

Citrus wastes are more susceptible to enzymatic hydrolysis, probably due to absence of lignin in their structure. However, presence of cellulose, hemi-cellulose and pectin polymers bound to each other in three dimensional structures make them relatively resistant materials for hydrolysis (Grohmann*et al*., 1995).

The objective of pretreatment is therefore to increase the surface area and porosity of the substrate, reduce the crystalline of cellulose and disrupt the heterogeneous structure of cellulosic materials. This process makes the carbohydrate polymers accessible ford polymerization. Pretreatment and hydrolysis of lingo-cellulosic materials result in a number of fermentable pentose and hexose sugars, leaving lignin as a by-product which can be used as fuel to produce heat or electricity. An appropriate pretreatment process can also prevent the information of inhibitors to the subsequent hydrolysis and fermentation (Sun and Cheng, 2002).

The main methods include physical treatment (such as milling and grinding), thermo-chemical pretreatment (such as steam explosion) and ammonia fiber explosion (Alvira*et al*., 2010).

2.13.1. Physical treatment

Physical pretreatment includes mechanical comminuting, steam explosion and micro-wave, radiation. Mechanical comminuting can be a combination of chipping, milling and grinding. It aims to reduce the particle size of the biomass to attain a larger surface area for enzyme access. The desired final particle size determines the appropriate technique to apply. For example, chipping is used when 10-30mm particle size is required whilst milling and grinding are for more fine particles (0.2-2mm). The higher energy cost of mechanical comminuting especially for large-scale applications makes it an unattractive approach for pretreatment (Elander, 2005).

It can result in significant changes in the physical characteristics of biomass, including smaller size, as well as a lesser degree of both crystalline and polymerization (Sun and Cheng, 2002).

Steam explosion microwave provides swift thermal expansion which opens up the structure of the target biomass. It is recognized as a suitable pretreatment method for hardwoods and agricultural residues, but less effective for softwoods. This kind of pretreatment is typically initiated at a temperature of 160-260 °C and the residence time varies from seconds to a few minutes (Sun and Cheng, 2002). The main factors affecting the results of steam explosion are residence time, temperature, particle size as well as moisture content (Duff, and Murray, 1996).

2.13.2 Chemical treatment

Chemical pretreatment, mainly employing chemical agents such as acids and alkalis can enhance hydrolysis and improve glucose recovery from cellulose because of the removal of hemicelluloses or lignin (Mosier *et al*., 2005).

However, anti-corrosion equipment and chemical recycling are required if such processes are scaled up. Hydrolysis of cellulosic materials includes the processing steps that convert the carbohydrate polymers e.g. cellulose and hemi-cellulose into monomeric sugars. Cleavage of these polymers can be catalyzed enzymatic ally by cellulose or chemically by acids such as sulfuric acid (Mosier et *al.*, 2005).

The factors that have been identified to affect the hydrolysis of cellulosic biomass include porosity or accessible surface area, cellulose fiber crystalline, and the content of lignin and hemicelluloses. Two main hydrolysis methods are widely used to produce monomeric sugar

constituents required for fermentation. These include acid hydrolysis (with dilute and concentrated acids) and enzymatic hydrolysis (Saha*et al*., 2005).

2.13.2.1. Dilute acid pre treatment

During the acid pre treatment, diluted H2SO4 is used for reducing the pH of the mixture which is shaken and heated by steam at high temperature (100°C). After certain hours, about 95%of the starch chains are transformed in to glucose. The acid pretreatment process dissolves the hemi-cellulosic component of the biomass and disassembles the cellulose into fermentable sugars which are accessible to enzymes (Wayman, 1996).

Acid hydrolysis can be performed with various types of acids including sulfuric, sulfurous, hydrochloric, phosphoric, nitric acid, etc. Acid hydrolysis is subdivided into as concentrated and dilute acid hydrolysis. Through the concentrated-acid hydrolysis, the biomass is treated with high concentration of acids at near ambient temperatures, which results in high yield of sugars. However, this process has drawbacks including high acid and energy consumption, equipment corrosion and longer reaction time. The use of concentrated acid is limited owing to higher cost, corrosion of containment material, and the formation of in habiting compounds (Galbe and Zacchi, 2002).

Dilute-acid hydrolysis, on the other hand, uses low-concentration acids e.g. 0.5-1% $H_2SO_4$and high temperatures. Dilute sulphuric acid is the most studied acid, and gives high hydrolysis yields. It can be applied at 180°C for a short period of time or at 120°C for 30-90min in different types of reactors such as plug flow, batch, shrinking-bed and counter-current reactors (Cheng *et al*., 2002). It was found that the highest bio-ethanol yield occurred with10g/L of microalgae, 3% (v/v) of sulphuric acid at 160°C for 15min. Despite low acid consumption and short reaction time in dilute-acid hydrolysis, application of high temperatures in this method accelerates the rate of sugar decomposition and increases equipment corrosion (Galbe and Zacchi, 2002; Taherzadeh, 2006).

The syrup obtained must be neutralized until reaching the pH for fermentation; this is achieved by adding NaOH. Mixture is filtered in order to separate the residues that can be used as fuel or fertilizer. On the other hand, the lingo-cellulosic material is shattered and crushed before being passed through the delignification process. NaOH is used in delignification process increasing the pH (Mosier*et al.*, 2005).

The main function of dilute acid pretreatment is to effectively remove the hemicelluloses heating over cellulose, while at the same time loosening the structure of lignin and decreasing the crystalline of digestible cellulose. During acid pretreatment, two reactions occur simultaneously relative to lignin: degradation and accumulation; therefore, the changes in lignin content greatly depend on which reaction is stronger. Although lignin removal is notsignificant in acid pretreatment, it has been stated that the structure of lignin is interrupted thus making the carbohydrates more accessible to enzymes (Wyman, 2004).

2.13.2.2 Enzymatic treatment

Enzymatic hydrolysis is the utilization of enzymes to release the fermentable sugars from the biomass. Hydrolysis of cellulosic materials can be catalyzed by a class of enzymes known as celluloses. The process cost of enzymatic hydrolysis is lower than acid hydrolysis as it avoids containment corrosion and occurs under mild temperatures and pH (Sanchez *et al*., 2004).

The enzymatic hydrolysis process can be accomplished using different strategies, of which the most important ones include separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). In SHF, hydrolysis and fermentation are carried out in separate vessels under their own optimal conditions; however end-produce inhibition of enzymes activity and contamination problems was associated with this process. In order to eliminate drawbacks of the SHF process, SSF that combines hydrolysis and fermentation in one vessel has been developed. Sugars produced during hydrolysis are immediately fermented into ethanol and thus, problems associated with sugar accumulation and enzyme inhibition as well as contamination can be avoided. The main drawback of SSF is the different optimum temperatures of the hydrolysis and fermentation processes. Most fermenting yeasts have an optimal temperature around 30-35oC while hydrolyzing enzymes show optimal activities around 50 ^{o}C (Galbe and Zacchi, 2004).

2.13.2.3 Alkali pretreatment

Alkali pretreatment is conducted under milder conditions at lower temperature and pressure compared with acid pretreatment. However, alkali pretreatment is much more time consuming and the reaction time greatly depends on the operation temperature selected (Mosier *et al*., 2005; Wyman *et al*., 2005). The major effect of alkali pretreatment is the saponification of intermolecular ester bonds which cross link lignin and carbohydrates, thus increasing porosity

and internal surface area of the biomass matrix as well as decreasing the degree of crystallinity of cellulose (Sun and Cheng 2002).

Lignin can also be disrupted and removed from the biomass matrix, resulting in improved susceptibility of the remaining polysaccharides to enzyme attack during hydrolysis. One limitation related to alkali pretreatment is the formation of unrecoverable salts within the biomass feedstock. Bases such as sodium hydroxide, potassium hydroxide, and ammonia can be used for biomass pretreatment. Base solutions cause swelling of biomass, which subsequently leads to decrease in the degree of polymerization, decrease in crystalline, disruption of the lignin structure, and separation of structural linkages between lignin and carbohydrates. Among the bases investigated, ammonia has the highest potential for use in commercial processes since it can be recovered and recycled due to its high volatility. Thus, It reduces chemical cost and waste treatment cost (Mosier *et al*., 2005).

2.13.3. Biological treatment

Biological pretreatment employs wood degrading microorganisms, including white, brown, and soft rot fungi, and bacteria to modify the chemical composition and/or structure of the lingo cellulosic biomass so that the modified biomass is more amenable to enzyme digestion. Most biological pretreatment so far has focused on the degradation of lignin in lingo cellulosic biomass. However, degradation of lignin usually accompanies the loss of cellulose and hemi-cellulose. In order to reduce and eliminate the sugar loss during biological pretreatment, the microbial strains should have low cellulase activity. White rot fungi are the most widely studied for biological pretreatment since they can degrade lignin more effectively and more specifically. Biological pretreatment appears to be a promising technique and has very clear advantages, including no chemical requirement, low energy input, mild environmental conditions, and an environmentally friendly working manner (Mosier *et al*., 2005).

2.14. Factors Affecting Bio-ethanol Production

2.14.1. Effect of temperature

Temperature plays a major role in the production of ethanol, since the rate of alcoholic fermentation increases with the increase in temperature. The optimum temperature of ethanol ranges between 25°C to 40°C which depends on room temperature. When temperature goes below the optimal range, their ability to catalyze the intended reaction slows down. On the other

hand, when the temperature increases, enzymes begin to denature or unfold and thus become inactive. Each enzyme will have a different temperature range where it becomes inactive. Even if one essential enzyme stops working, the organism fails to grow. Hence, the first essential enzyme that gets deactivated defines the maximal temperature at which that organism can grow. At the lower end, it gets more complicated. Usually, the enzymes are not inactivated but rather just slow down (Sanchez, 2007).

2.14.2. Effect of pH

pH value has significant influence on Bio-ethanol fermentation. pH of bio-ethanol produced from different fruit wastes were determined. The pH values of ethanol produced by the process of fermentation range from 4 to 6. Yeast survives in a slightly acidic environment that is with pH of between 4 and 6. Ethanol with high content of alcohol produced from different agro waste materials exhibited different pH value (Asli, 2010).

2.14.3 Effect of sugar concentration

With the increase in initial sugar concentration, the ethanol production increased significantly. Concentration is the measure of ethanol content present in the distillate. Concentration of ethanol was expressed in terms of percentage (Sanchez, 2007).

2.14.4. Effect of specific gravity

Specific gravity is used to measure the sugar content. As the fermentation progressed, the specific gravity considerably decreased. The decrease in specific gravity is clear indication of yeast fermenting the sugar resulting in ethanol production. The specific gravity reaching constant value after incubation period is the indication of end of fermentation (Asli, 2010).

2.15 Chemical Composition of the Substrates

2.15.1 Chemical component of banana peel

Banana peels contain about 7.8% protein, 16.69% polyphenol, 69.42% moisture content,15.20% dry mass, 6.50% lipid, 7.65% crude pectin, 1.70% starch and 7.89% ash. (Essien, *etal*., 2005).The main components of banana peels are fibers (31.7%), glucose (16%), sucrose (50%), ash (8.5%), lipids (1.7%), and proteins (0.9%) (Anhwange et al., 2009). Banana peels represent 30% of the fruit and total annual world production is estimated at 95.6 million tons of fruits (FAO, 2006).

Banana peels are often dumped in landfills, rivers, oceans and unregulated dumping grounds. Therefore, their reutilization would help to diminish the pollution problems caused by their

disposal. This kind of waste offers many possibilities of reuse, such as matrix for toxic compounds removal, feedstock for pectin, antioxidants, enzymes or bio-fuels. Little research has been done about bio-ethanol production from banana peel waste (Manikandanet al., 2008).Used five mutant strains of *S. cerevisiae* and evaluated the effect of temperature, pH and initial substrate concentration on ethanol yield. By pre treating banana peels with 2 ml/g of 67% sulphuric acid as a catalyst and steaming the slurry at 10% by weight for 60 min, the best ethanol-producing strain produced 9.8 g ethanol/L. The optimum temperature and pH for this strain were 33 °C an 4.5, respectively (Sharma*et al.*, 2007).

In conclusion, the fermentation parameters for bio-ethanol production from banana peels have already been optimized at lab scale. Further scaling-up to a pilot-scale is needed to make the process more cost effective.

Fig.2.12: Banana peels

2.17 Process of bio-ethanol production from macro algae

The simplified super structure flow sheet for the integrated production of bio-ethanol from algae was first, microalgae cultivation using sunlight energy is carried out in open or covered ponds or closed photo bioreactors, based on tubular or other designs. In the next step, the biomass needs to be concentrated by an initial factor of at least about thirty-fold, requiring very low-cost harvesting processes. The starch can be extracted from the cells with the mechanical tools (e.g.,

ultrasonic, explosive disintegration, mechanical shear, etc.) or by dissolution of cell walls using enzymes (John RP, Anisha G, 2011).

The starch is then separated by extraction with water or an organic solvent and used for fermentation to yield bio-ethanol. Both saccharification and fermentation processes can be simultaneously carried out in a single step if an amylase producing strain can be used for ethanol fermentation. Utilization of starch degrading ethanol producers can preclude the cost incurred for acid or enzymatic saccharification of starch. Fermentation of macro algal biomass involves minimum input of energy and the whole process is less complicated compared to biodiesel production. Recently (ArestaM, Dibennedatto A, 2005).

Macro-algae (the large sized algae) can also be utilized for ethanol fermentation (Horns, Aasen, 2000). Seaweeds are classified into three groups: green, brown, and red, and they contain various types of glucans which are polysaccharides composed of glucose, though the concentration of these glucans is known to be relatively low .The absolute absence or near absence of lignin makes the enzymatic hydrolysis of algal cellulose simple. Macro algal genera, such as, *Alaria, Saccorhiza* and *Laminari a*are belonging to brown algal group and grows up to meters and their main energy storage materials are laminarin and mannitol (Horns ,Aasen I ,2000).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Description of the Study area

This study was conducted in Jimma town at Jimma Institute of Technology (JiT) campus. Jimma town is found in Oromia national regional state, at 352 km southwest of the capital, Addis Ababa. The study area Jimma institute of Technology (JiT) campus lies between 7°4 1'17.805" N latitude and 36°49'16.888" E Longitude and with an elevation 1750 meters above sea level.

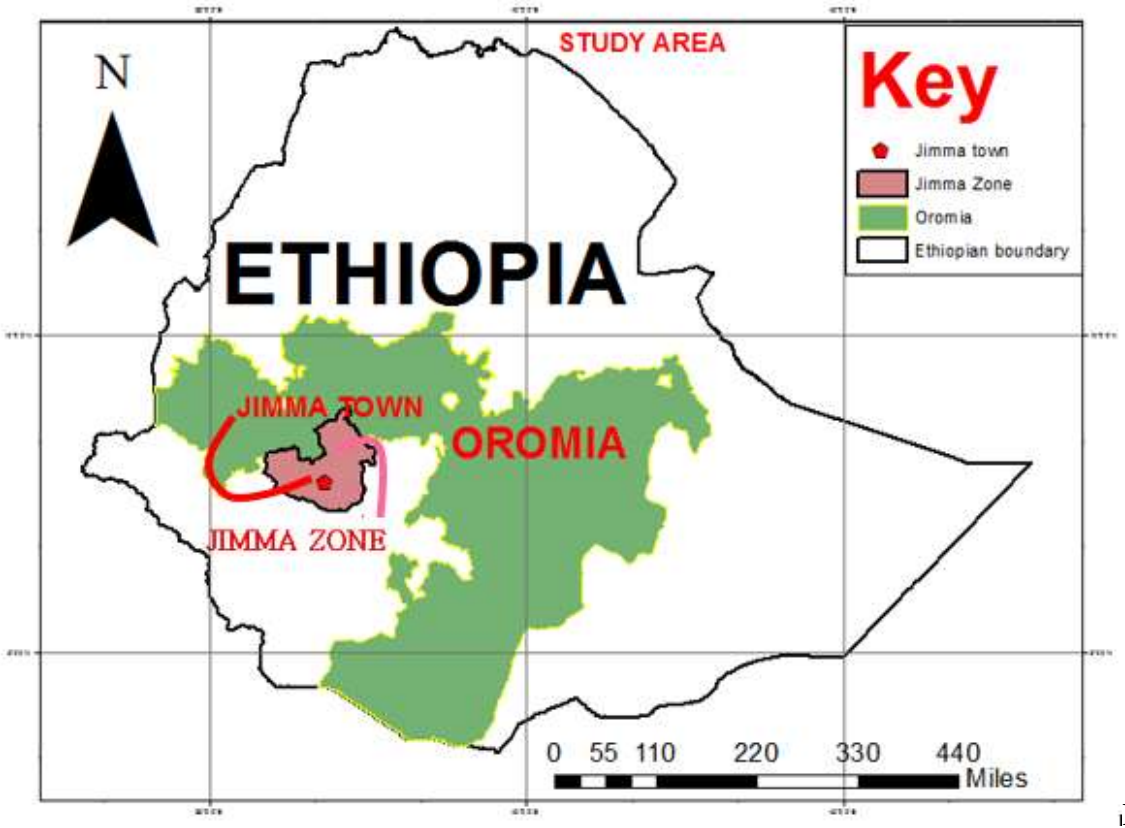


Fig: 3.1 Study Area

3.2. Materials Use for the Experiments

3.2.1. Equipments

The equipments I used throughout my works are:-Plastic bags: to collect and transport samples to the laboratory, Knife: for cutting the fruit wastes in to pieces, Digital and non-digital driers or ovens: to dry the sample, Crushers: to crush the dried sample, Sieves: to sieve the crushed sample to the particle size of 2mm, Balances: to weigh samples and yeast, Digital pH meter: to measure the pH of the hydrolyzate before fermentation, Thermostats: to control temperature of the sample under experiment (fermentation and distillation) isothermally at the set point, Vessels: - to hold samples and additives for hydrolysis, fermentation and distillation experiments. Centrifuge: - to separate the soluble liquid from non soluble part, Graduated cylinders of different volumes: - for volume measurement, Autoclave: - for sterilization and hydrolysis, Pycnometer: for density measurement, Shaker: to shake sample and its additive after hydrolysis and before fermentation and Fermentation and distillation set ups: to ferment and distillation respectively.

3.2.2. Chemicals

98% Sulfuric Acid (H_2SO_4): used as a pretreatment and hydrolysis fruit peel.

Sodium Hydroxide (NaOH): used to adjust the pH of soluble cellulose and hemicelluloses before fermentation, Yeast extracts (Agar): used as media preparation, Urea: - used as media preparation, Dextrose sugar: used as media preparation, Mg SO_4.7 H_2: used as media preparation Yeast (*Saccharomyces cerevisiae*).

3.3. Procedure of Experiment

The study was aimed at optimization of acid hydrolysis in the production of ethanol from banana peel and macro algae. Banana peels was collected from commercial area of vegetables fruits in Jimma town and macro algae was collected from JiT oxidation pond. Banana peels was collected in plastic bags and macro algae was collected in plastic pot and transported to the laboratory of Bio-chemical engineering AAiT for ethanol analysis. This section describes about the methodologies and approaches of how experiment was done in this research; it included all steps and procedures of the experiments.

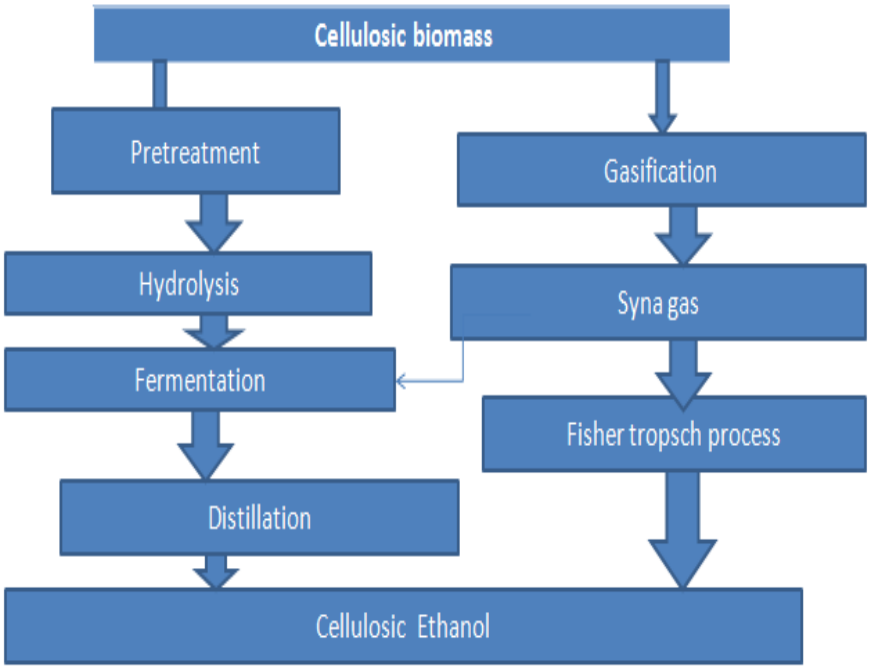


Fig: 3.2 Block flow diagram of ethanol plant

3.3.1 Sample Collection:

Samples algal sample and yeast, was Collected from JiT university oxidation pond, *Saccharomycescerevisiae* yeast was collected from Commercial market of Jimma town and Banana peels was collected from Commercial market of Jimma town.

3.3.2 Sample Preparation

The sample that was acquired had to be prepared and conditioned for pretreatment, hydrolyze, fermentation and distillation. Sample preparation process include: manual size reduction (Knife cutting), drying, grinding and sieving after the samples will be collected and mix to gather with macro algae. They were cut by knife into pieces of about 3-5 cm length for drying and grinding. Sample drying was carried out in oven 35 (60^{0} C for 72hr) to obtain easily crushable material. After drying, each of the samples was milled separately. The maximum particle size of the ground mixed sample was 2 mm. The sample of larger particle size than 2 mm was ground over and over again until all particle size became 2 mm. The sample was kept at low temperature until the next stage of experiment. Grinding of fruit peel into powder form increases the surface area of the sample which enhances the contact between hemicelluloses and cellulose with dilute acid to reduce cellulose crystallinity.

Fig.3.3: (a) Grinding machine (b) pretreatment of banana peels

3.4 Data quality assurance and Quality control

Proper quality assurance procedures and precaution was taken to ensure the reliability of the results. Sample was handled carefully and analysis within holding time to avoid physical, chemical and biological changes occur to them. For the sake of data quality assurance, data was assessed carefully and triple entry of data will be performed to assure quality of data.

3.5 Acid Hydrolysis

The cellulose molecules which are composed of long chains are broken down to simple sugar, before it is fermented for alcohol production. Even though there are many types of hydrolysis types, dilute acid hydrolysis is an easy and productive process and the amount of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis. Each sample had to pass through five primary experiments that were in series to get the final result ethanol, that is: size reduction, pretreatment, hydrolysis, fermentation and distillation. The three-parameter were applied to hydrolysis step of the experimentation. 100g of grinded banana peels and macro algae were used for each experiment and the factors for hydrolysis were time (5 to 25 minutes), hydrolysis temperature (100 to 132^{o}C), and acid concentration (0.5 to 1%).

Table3.1: minimum and maximum value of parameters

Factors	Minimum	Maximum
Hydrolysis time(minutes)	5	25
Hydrolysis temperature ^{0}C	100	132
Acid concentration(% by volume of distilled water)	0.5	1

Procedures for Acid Hydrolysis

Add 1 liter of 0.5% to 1% (v/v) diluted sulfuric acid to the non soluble component from pretreatment steps in the order of experimental design for all experiment and soak for 24hr.

- The fruit peels were then hydrolyze in the reactor between 100 and132 ^{o}C for 5 to 25min.
- After hydrolysis, neutralize with 10 M NaOH until the pH became around 7.
- Separate the solid particles from the liquid in the hydrolyzate by centrifugation (to remove the non fermentable lignin portion).
- After separate the solid part, wash the solid part with distilled water two times.

Fig: 3.4 (a) sample ready for centrifugal separation (b) centrifugal machine

3.6pH Adjustment

Before addition of any micro-organism to the above prepared samples, pH of these samples has to be adjusted. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 4.5 was maintained.

- **Procedures in pH adjustment**
- Mix pretreated and hydrolyzed solution, filtered, shaken substrate primarily checked for pH using a digital pH meter. The pH then adjusted to 4.5.

- Mix samples (pretreated and hydrolyzed) were acid hydrolyzed, so it needs highly basic solution to bring the pH in the range of 4.5.
- Sodium hydroxide solution was added drop wise to the other flask with constant stirring until the pH reaches to a range of 4.5.

3.7 Sterilization

The reactor and all the equipments that were used for fermentation purposes were sterilized (autoclaved). The sterilization was carried out at a temperature of 121 ^{o}C for 15 minutes.

Fig.3.5: sterilization equipment

3.8 Fermentation

The aim of the experiment was to measure the ethanol production by the fungus (*Saccharomyces cerevisiae*) using blends banana peels and micro algae hydrolyzate as energy and carbon source. The clear solutions then go to fermentation. The fermentation was carried out under anaerobic condition at a temperature of 30 ^{o}C, pH 4.5 with 200 rpm stirring condition for 4 days. Before conducting fermentation I had the preparation of media for the yeast. In order to prepare the media I should had the favorable condition for yeast growth or to supply the required amount of nutrients. Mix the following nutrients in there proportion.

Media Preparation

- For preparing 100 ml media, I add
- Sugar (Dextrose) = 10 gm
- Yeast extract = 0.2 gm

- Urea = 1.0gm
- Make up water = 100 ml
- Mg SO_4.7 H_2O = 1.0g

Fig.3.6: (a) chemicals for media and (b) media prepared

Procedures in Media Preparation

- To the above 100 ml media, 0.5 gm of yeast, *Saccharomyces cerevisiae* (instant premium) was added in a 250 ml conical flask.
- The conical flasks were properly covered with aluminum foil.
- The conical flask was then placed in a shaking incubator for 24 hours, a temperature of 30^{o} C and 200rpm.

Fig.3.7: (a) Shaker incubator (b) media after 24hr incubation

The Procedure for Fermentation

- The sample was conditioned to temperature of 30^{o}C before fermentation step was started. This was the temperature at which all fermentation experiments were carried out.
- The adapted media with the proportion of 1:10 to the soluble sample mix then placed in the shaking incubator at a temperature of 30^{o}C, 1 hour and 120 rpm.
- Set autoclavable reactor at 30^{o}C and 200 rpm and then mix the prepared sample with the media prepared into the autoclavable reactor using sterilized funnel.
- Base (2 M NaOH) added automatically by a pump into the bioreactor every time to drop pH 4.5.

Fig.3.8: (a) Fermentation setup and preparation for fermentation

3.9 Distillation

A distillation process is necessary for separation of ethanol from mixture and purification of ethanol after fermentation process. Process is performed simply with boiling ethanol-water mixture. Because of boiling point of water (100°C) is higher than boiling point of ethanol (78°C), ethanol vaporized before water (Kumar S, Singh N, Prasad R,2010).

Distillation was the last step in the production of ethanol from blends of banana peels and micro algae experiments. It is the purifications steps. Distillation is the method used to separate two liquid based on their different boiling points. However, to achieve high purification, several distillations are required. In this experiment separation were used by rotary evaporator at a temperature of 85 ^{o}C for 3hrs.

Components of experimental setup

- Distillation vessel
- Special top-fit of distillation vessel
- Condenser
- 90^{o} diverting glass that fits at the end of condenser and the top of harvesting vessel.
- Condenser tubing.
- Harvesting vessel.
- Stands and fixing screws.
- Beaker.
- Thermostat.
- The thermostat supporting flat metal bar.

All distillation experiments were carried out at a temperature of 85^{o} C and a distillation time of 3 hours.

Fig.3.9: Distillations (Rotary Evaporator)

3.10 Density Measurements

The ethanol concentrations of the samples collected every 3 hours intervals by rotary evaporator of fermented solution were measured by the following the procedure of (Geirwyr, 1995). The specific gravity of the produced alcohol was determined and alcohol concentration was got from the relationship between the specific gravity and the proportion of ethanol in alcohol solution at 20^{o}C and a distillation time of 3 hours. Weigh the Pycnometer (specific gravity bottle) with stopper after cleaning, drying and note the weight as X_1 at 20^{o}C. Filled the Pycnometer with distilled water and take the weight of the water at 20^{o}C and note as X_3. Make the Pycnometer empty, clean, dry and then filled with sample (alcohol) of the experimental result. Determine the weight of the sample at 20^{o}C and note as X_2. Calculate the net weight in grams of the alcoholic liquid in the Pycnometer by subtracting the weight of the empty specific gravity bottle or Pycnometer. Calculate specific gravity of sample according to the formula given.

Specific gravity of sample = $(X_2-X_1)/(X_3-X_1)$

Where: X_1 = weight (g) of empty Pycnometer

X_2- = weight (g) of Pycnometer + sample

X_3 =weight (g) of Pycnometer + water

Fig3.10: Pycnometer (Specific gravity bottle)

3.11 Estimation of bio-ethanol production

The amount of bio-ethanol Produced from blends of banana peels and micro algae was estimated by distillation of the filtrate at 780C using rotary evaporator. The quantitative analysis was carried out by using following formula (Amadi*et al.*, 2009).

$$\text{Density} = \frac{(WB + D) - WEB}{VD}$$

WB = Weight of the Bottle

D = Distillate

WEB = Weight of Empty Bottle

VD = Volume of Distillate

3.13 Data processing and analysis

The data obtained from laboratory experiment was analyzed and summarized in to tables and graphs by using Microsoft office excel spreadsheet and Statistical Package for Social Studies (SPSS) version 17.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Experimental Results

The process consists of four parts: pretreatment to remove lignin, reduce cellulose crystallinity, sterilize the banana peel and micro algae to increase the porosity of the materials, dilute acid hydrolysis and fermentation to produce ethanol, distillation to remove the ethanol. After following the above series of procedure, the experimental outcomes of those particular results are measured for their density using Pycnometer to know the yield of ethanol concentration. The results are indicated below, table 4.1 (using Pycnometer). Micro soft office excel 2007 software, gives a great tool to study the outcome (effect) of different variables in any process so that it also use this tool to discuss the result obtained from the experiment.

4.1.1 Bio-ethanol production for the ratio 25% and 75% macroalgae and banana peels*Table 4.1: Yield of ethanol (Pycnometer)*

S.NO.	Density (g/m^3)	Yield of ethanol (%)
1	0.91254	42.1
2	0.91157	42.2
3	0.92218	42.9
4	0.92875	40
5	0.91735	40.2
6	0.91014	48
7	0.92558	38.2
8	0.92117	42.1
9	0.92365	39
10	0.89912	55.4
11	0.89801	56
12	0.93057	31.5
13	0.90194	48.5
14	0.91007	47
15	0.91071	42.3
16	0.92687	35.1

As shown on the above table 4.1 the yield of ethanol concentration (%) Pycnometer is measured using the sensitive balance and produce five digit after point. Hence the discussions continue depend on the result from Pycnometer. From the result shown above (table 4.1) the maximum yields are run number: 11, 10, 13, 6 and 3 descending order. The minimum results were obtained at run number: 12, 16 and 9.

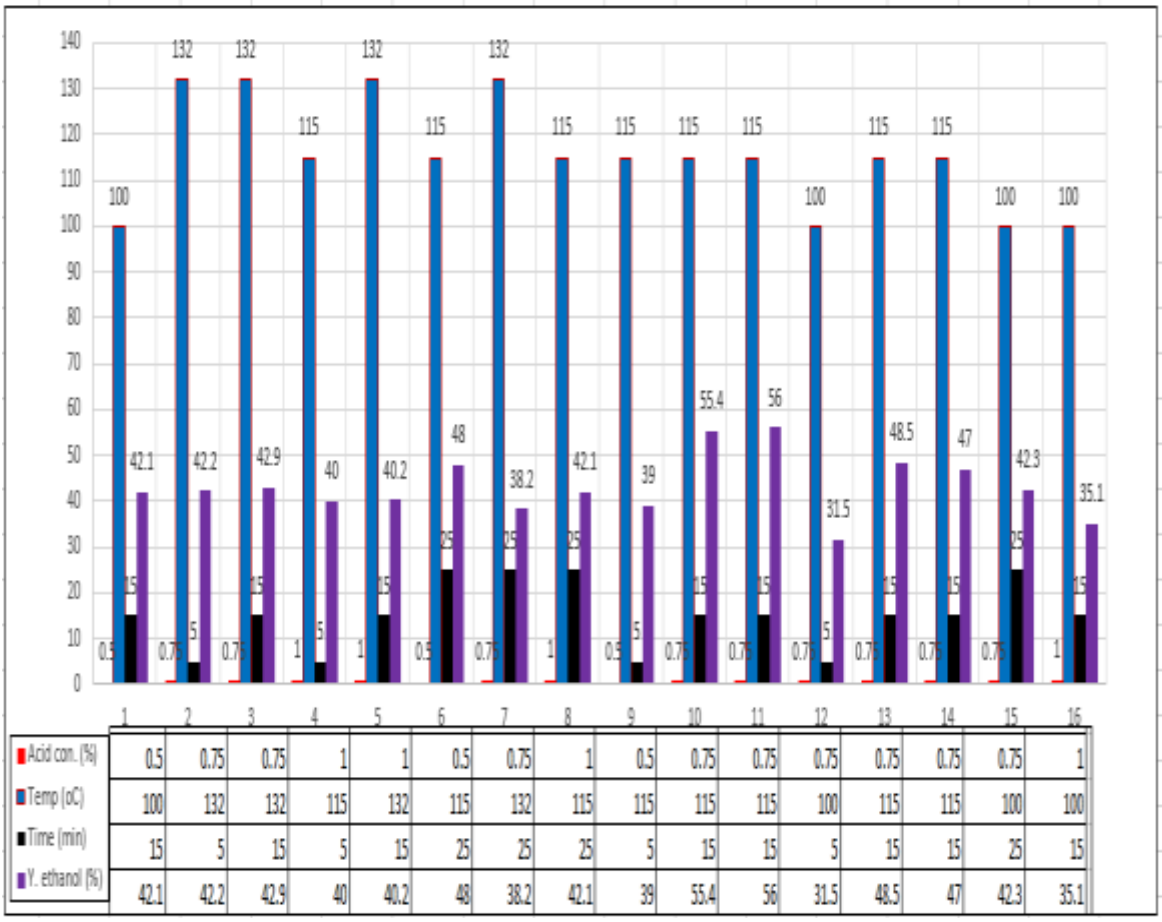


Figure 4.1 Ethanol yield and parameters condition

As we see from the above figure 4.1 high yield of ethanol were observed at 0.75% acid concentration, at 115 ^{0}C temperature and at the time of 15min.In this study experimental design techniques were used to determine the effects of the acid concentration, hydrolysis time and temperature on the efficiency of ethanol yield. A total of 16 experiments were carried out for optimization purpose where the effect of each factor was analyzed by using lower and higher values from optimized conditions. The ethanol yields 56obtained from experiments were used as a response parameter for optimization.

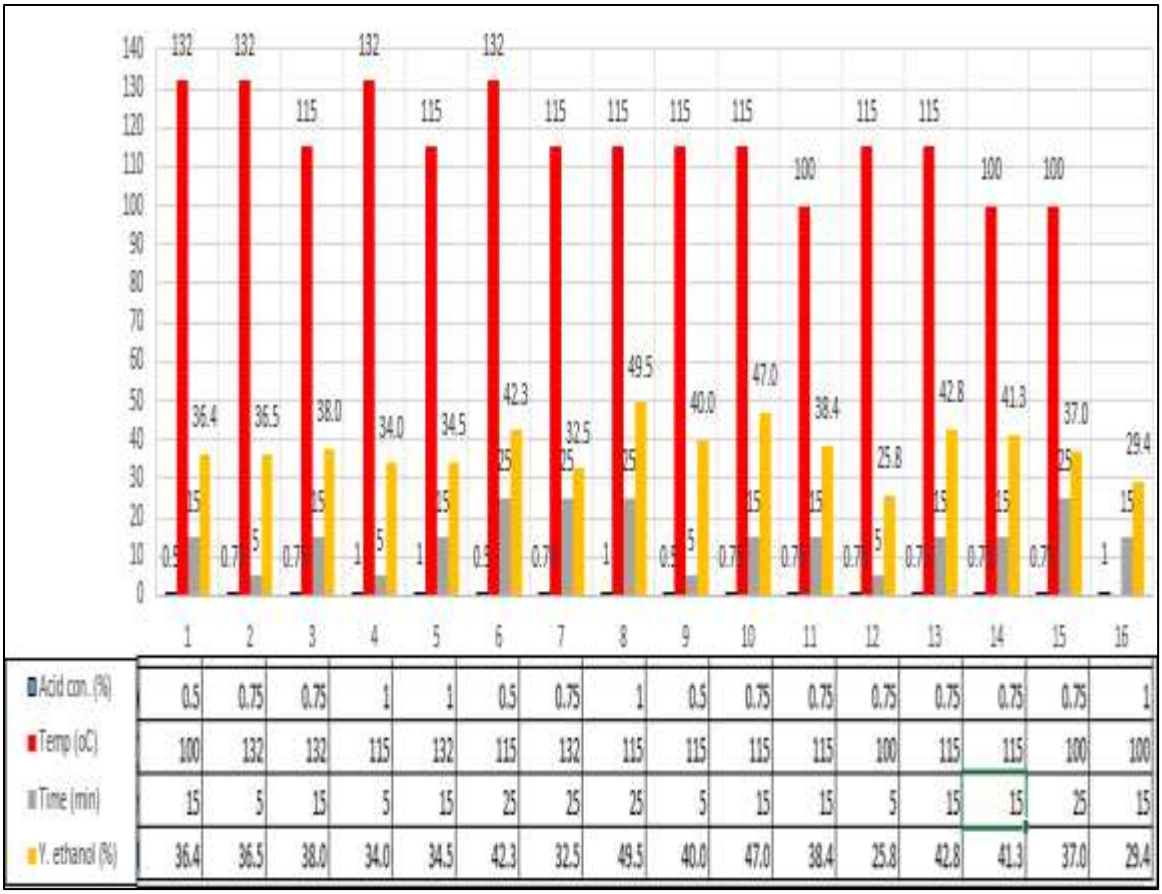
4.1.2 Bio-ethanol production for the ratio 50% and 50% macro algae and banana peel respectively

Table 4.2: Yield of ethanol (Pycnometer)

S.NO.	Density (g/ml)	Ethanol Yield (%)
1	0.87323	36.4
2	0.87226	36.5
3	0.88287	38.0
4	0.88944	34.0
5	0.87804	34.5
6	0.87083	42.3
7	0.88627	32.5
8	0.88186	49.5
9	0.88434	40.0
10	0.85981	47.0
11	0.85870	38.4
12	0.89126	25.8
13	0.86263	42.8
14	0.87076	41.3
15	0.87140	37.0
16	0.88756	29.4

As shown on the above table 4.2 the yield of ethanol concentration (%) Pycnometer is measured using the sensitive balance and produce five digit after point. Hence the discussions continue depend on the result from Pycnometer.

From the result shown above table 4.2 the maximum yields are run number: 8, 10, 13, and 6 descending order. The minimum results were obtained at run number: 12, 16 and 7.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acid con. (%)	0.5	0.75	0.75	1	1	0.5	0.75	1	0.5	0.75	0.75	0.75	0.75	0.75	0.75	1
Temp (oC)	100	132	132	115	132	115	132	115	115	115	115	100	115	115	100	100
Time (min)	15	5	15	5	15	25	25	25	5	15	15	5	15	15	25	15
Y. ethanol (%)	36.4	36.5	38.0	34.0	34.5	42.3	32.5	49.5	40.0	47.0	38.4	25.8	42.8	41.3	37.0	29.4

Figure 4.2 Ethanol yield and parameters condition

As we see from the above figure 4.1 high yield of ethanol were observed at 1% acid concentration, at 115 ^{0}C temperature and at the time of 25min.In this study experimental design techniques were used to determine the effects of the acid concentration, hydrolysis time and temperature on the efficiency of ethanol yield. A total of 16 experiments were carried out for optimization purpose where the effect of each factor was analyzed by using lower and higher values from optimized conditions. The ethanol yields 49.5obtained from experiments were used as a response parameter for optimization.

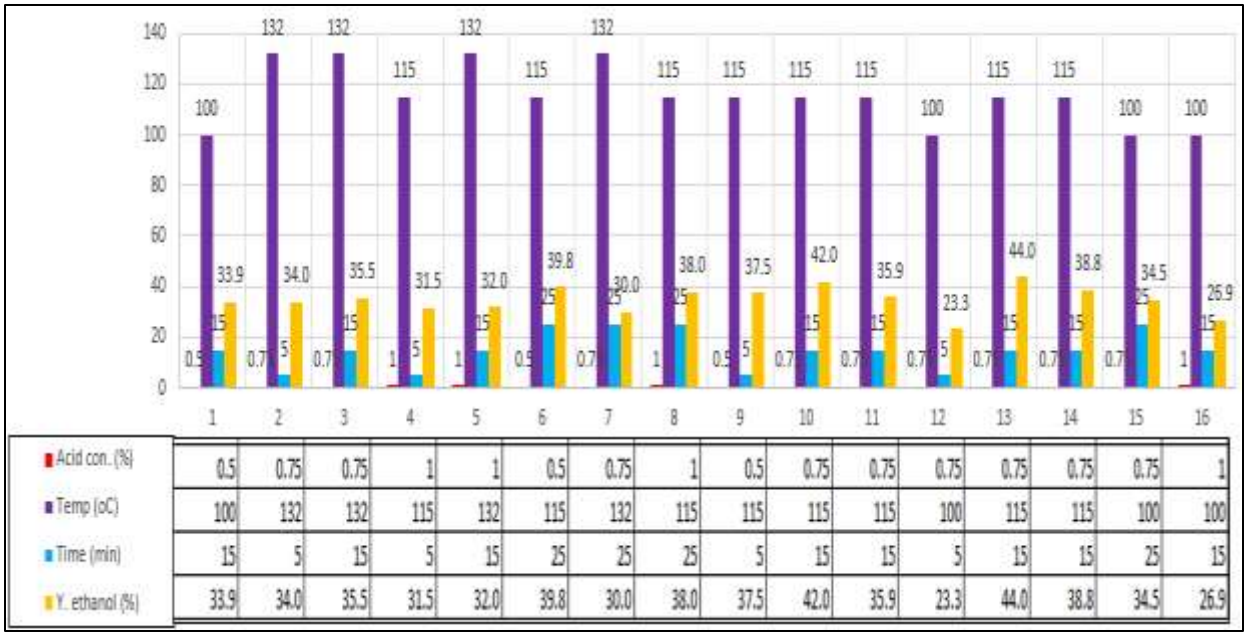
4.1.3 Bio-ethanol production for the ratio 75% and 25% macro algae and banana peel respectively

Table 4.3 Yield of ethanol (Pycnometer)

Run NO.	Density (g/ml)	Yield of ethanol (%)
1	0.86024	33.9
2	0.85927	34.0
3	0.86988	35.5
4	0.87645	31.5
5	0.86505	32.0
6	0.85784	39.8
7	0.87328	30.0
8	0.86887	38.0
9	0.87135	37.5
10	0.84682	42.0
11	0.84571	35.9
12	0.87827	23.3
13	0.84964	44.0
14	0.85777	38.8
15	0.85841	34.5
16	0.87457	26.9

As shown on the above table 4.3 the yield of ethanol concentration (%) Pycnometer is measured using the sensitive balance and produce five digit after point. Hence the discussions continue depend on the result from Pycnometer.

From the result shown above table 4.3 the maximum yields are run number: 13, 10, 6 and 8 descending order. The minimum result was obtained at run number: 12,16and7



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Acid con. (%)	0.5	0.75	0.75	1	1	0.5	0.75	1	0.5	0.75	0.75	0.75	0.75	0.75	0.75	1
Temp (oC)	100	132	132	115	132	115	132	115	115	115	115	100	115	115	100	100
Time (min)	15	5	15	5	15	25	25	25	5	15	15	5	15	15	25	15
Y. ethanol (%)	33.9	34.0	35.5	31.5	32.0	39.8	30.0	38.0	37.5	42.0	35.9	23.3	44.0	38.8	34.5	26.9

Figure 4.3 Ethanol yield and parameters condition

As we see from the above figure 4.1 high yield of ethanol were observed at 0.75% acid concentration, at 115 ^{0}C temperature and at the time of 15min.In this study experimental design techniques were used to determine the effects of the acid concentration, hydrolysis time and temperature on the efficiency of ethanol yield. A total of 16 experiments were carried out for optimization purpose where the effect of each factor was analyzed by using lower and higher values from optimized conditions. The ethanol yields 44 obtained from experiments were used as a response parameter for optimization.

4.1.4 Summarization of bioethanol production from blends of banana peels and macro algae.

Ethanol production was observed in all substrate concentration levels on fourth days of fermentation(Fig. 1).Many researchers (Ruchi*et al*., 2011; Nyachaka*et al*., 2013) reported the start of ethanol production on fourth days of fermentation. Though all substrate concentrations

resulted in ethanol production starting from the fourth day of fermentation, the blend ratio of 75% banana peels and 25%micro algae had high potential application for bio-ethanol production. The optimum results were obtained at 0.75%v/v acid concentration, 115^{0}C temperature and 15min retention time. Under these condition 56% of bio-ethanol were obtained.pH was adjusted at 4.5 for bio ethanol production.Therefore as 75g: 25g substrate is yielding the maximum amount of ethanol, 56% it can be considered as the optimum concentration.

4.2 Characterization of bio ethanol production from blends banana peels and macro algae

Table 4.4 Properties of bio ethanol produced

Properties of bio ethanol produced			
Properties	Units	Experimental Values	ASTM Standards
Moisture content	%	0.52	20
Density	g/cm^3	0.987	0.99
Refractive index	^{O}C	1.356	1.36
Flash point	^{O}C	19.2	18.6
Viscosity	^{O}C	1.34	1.2
Sulphur content	%	0.105	20
Ash content	%	0.5	30
Cloud point	^{O}C	19.76	23
Pour point	^{O}C	4.75	5.2
Specific gravity	oF	0.922	0.87

The fuel properties of the bioethanol produced was conducted and the results obtained

4.2.1 Determination of Refractive index

Refractive index of the produced bioethanol was determined and the results obtained as presented indicate that the refractive index of the produced is 1.37 which is a little higher than the set limit of 1.36. The essence of measuring the refractive index of fuels is to verify the purity of the fuels, it can be deduced from the results obtained that the bioethanol produced is pure.

4.2.2 Determination of flash point

Flash point is described as the lowest temperature at which the fuel will ignite when exposed to an ignition source. These parameters also provide information on the precautionary measures to be applied while handling such fuels. The results as presented indicate that the flash point of the produced bioethanol was 19.20 which are a bit higher compared to the set limit of 18.60, which implies that the bioethanol produced is slightly less flammable than the standard bioethanol fuel. The variation could be attributed to the nature of the feedstock used in this study.

4.3.3 Determination of Ash content

Ash content is another fuel properties tested for it gives quantity of metals present in the fuel. High ash content in fuel could result into injector plugging, post combustion residues or deposits and wear of the injection system of any engine. The ash content of ethanol produced is 0.5%. Hence the need to improve the quality of bioethanol produced so as to reduce the ash content.

4.4.4 Determination of Sulphur content

Sulphur content is associated with health and environmental pollution. Fuels with high sulphur content impact negatively on humans and the entire ecosystem at large. After characterization of the bioethanol sample it was observed that the sulphur content was 0.105%.

4.5.5 Determination of Distillation properties

Distillation properties of the bioethanol produced was determined and since bioethanol is not a carbon based fuel, it is only natural that its boiling temperature should be a single temperature value. The boiling temperature recorded in the distillation of the bioethanol sample was 78.3oC which in compare favorably with the set limit of 78oC.

4.6.6 Determination of Viscosity

Viscosity determines the ease of flow of fuels through pipes, orifices, nozzles hence viscosity is a property that determines fuels efficiency in an engine. Low viscosity depicts high flow rate and little or no accumulation in the engine. The results as presented in Table I indicate that the viscosity of the produced bioethanol is 1.34 which is high compared to the set limit of 1.20.

4.7.7 Determination of the specific gravity

The results as presented in Table I also indicate that the specific gravity of the produced bioethanol is 0.92 which is slightly higher than the recommended value of 0.87.

4.8.8 Determination of cloud point.

Cloud point which is described as the temperature at which a cloud of crystals will first appear in a liquid that is cooled under prescribed conditions is also an important properties of bioethanol tested for in this study. The results as presented indicate that the pour point of the produced bioethanol from blends of banana peels and micro algae is 19.76oC which is lower than the set limit of 23oC by the ASTM.

4.9.9 Determination of pour point

Pour point is an important characteristic of the bioethanol that gives the lowest operational temperature of the bioethanol. The pour point was determined according to ASTM D 97 and the value obtained as presented in Table 1 is 4.75oC which is also lower than the set limit of 5.20oC, which is an indication that the bioethanol produced can be used even in polar region where the atmospheric temperature is not less than 5oC. It can be inferred from the various analysis conducted on the bioethanol produced that the properties of the bio-ethanol produced compared favorably with some of the properties. The variation in some of the properties can be attributed to the nature of the feedstock blends of banana peels and micro algae used in this study.

4.3 Comparing the result obtained with previous study

4.3.1 Cumulative ethanol production from untreated and treated banana peels

Among the fermented pretreated substrates, banana peels showed the highest ethanolproduction (26.87% ± 0.045) in 100g of pretreated banana peel substrate with 1% of yeastsand the yeast biomass and reducing sugar were recorded as 2.78 ± 0.041 (OD) and 2.25 ±0.042 (g/ml) respectively at12th day of the fermentation time. The lowest ethanol yield (2.92% ±0.05) was recorded at the 4th day of the fermentation with 25g of untreated banana peel by 0.5% of yeast with cell density 0.62 ± 0.045 (OD)and reducing sugar 4.06 ± 0.03 (g/ml).

The cumulative ethanol production recorded up to 16th days of fermentation from for banana peel, the optimal bio-ethanol yield were 79.23%, with 100g oftreated substrate was fermented by 1% yeast. The minimum cumulative bio ethanol production was 21.67%, with 25g of untreated banana peel, by 0.5% yeast(Ebasa, 2014).

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From the experiment, it was proved that the bio-ethanol yield could be produced from blends banana peels and micro algae as the substrates. In all substrates maximum bio-ethanol was produced from high concentration of substrate by high inoculums concentration. Therefore, substrate concentration and inoculums concentration are directly proportional with ethanol production.

The fruit peels were crushed in to 3-5 cm sizes for easy drying and grinding. Sample drying was carried out in oven (60^0C for 72hr) to obtain easily crushable material. After drying, each of the samples was milled separately. The maximum particle sizes of the ground mixed sample were 2 mm. Laboratory experiments of 16 run were conducted to produce bio-ethanol from those blends of banana peel and macro algae were pretreatment, hydrolysis, fermentation and distillation process respectively to produce bio-ethanol. The ethanol concentrations of the samples collected every 3 hours intervals by rotary evaporator of fermented solution were measured by Pycnometer (specific gravity bottle). The specific gravity of the produced alcohol was determined and alcohol concentration was got from the relationship between the specific gravity and the proportion of ethanol in alcohol solution at 20^oC. The effects of acid concentration, temperature and time on dilute acid hydrolysis were investigated. The Microsoft office excel was applied to data discussion and analysis In these study 75% banana peels and 25%micro algae had high potential application for bio-ethanol production. pH was adjusted at 4.5 for bio ethanol production. The optimum results were obtained at 0.75%v/v acid concentration, 115^0C temperature and 15min retention time. Under these condition 56% of bio-ethanol were obtained.

5.2. Recommendations

The study revealed that it is possible to produce bio-ethanol from agro waste materials such as banana peels and micro algae. Further studies should be conducted on pretreatment of other agro waste materials to release high fermented sugars to increase ethanol yield from agro wastes.

- Further study is very important to describe how absolute bio-ethanol can be produced from agro wastes by using rotary evaporator, because it is difficult to make pure ethanol

since there are other chemicals that can evaporate below the boiling point of ethanol (78oC).

- Further researches have to be done to improve the production of high quality and quantity of fruit peel ethanol.
- Alternative extraction methods of ethanol such as enzymatic extraction have to be done in order to investigate the variation that could be arise on the quality and quantity of the ethanol yield as a result of using different extraction methods.
- To conclude the recommendation, there is an urgent need for proper collection; documentation and assessment of fruit peel yields of algae, orange, mango and banana as well as their seasonal variation in our country.

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