Production of Bioethanol from Mango Peel Wastein Small Scale (Lab.)

Sagni Bedassa Miressa
Lecturer
Department of Agricultural Engineering, Agricultural Machinery Engineering(M.Sc.)
Ambo UniversityInstitution of Technology, Ambo,Ethiopia

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Abstract – Bioethanol fuel is mainly produced by the sugar fermentation process. Ethanol or ethyl alcohol (C2H5OH) is a clear, colourless biodegradable liquid and is less toxic and causes less environmental pollution. It is a high-octane fuel and has replaced lead as octane enhancer in petrol. Mango peel waste collection and disposal creates a range of environmental problems in our environment. A considerable amount of waste 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 are causing air pollution. Waste disposal has become one of the major concerns for our city juice house, Ambo, Addis Ababa etc. The objective of this study Production of bioethanol from mango peels using Saccharomyces cerevisiae and to determine the properties of bioethanol. The mango peels were crushed in to 3-5 cm sizes for easy drying and grinding. Sample drying was carried out in oven (600C 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 mango peel wastes. The mill samples of 100gm sample were taken and mixed, then passed through steam pretreatment, hydrolysis, and fermentation and distillation process respectively to produce bio-ethanol. The present study was done with objectives to produce bioethanol from mango peel which solves the waste disposal problem. In a country like Ethiopia, it is very hard to do proper disposal of wastes and thus generation of infectious diseases is rapid here. So, using these wastes not only provide a use of those wastes but also help to be beneficial economically. We recommended that government or other investor's to recover this very valuable product as well as to contribute to the country in reducing the highly rising quantity of wastes. To conclude the recommendation, there is an urgent need for proper collection, documentation and assessment of fruit peel yields of mango well as their seasonal variation in our country.

Keywords: Mango, Bio-ethanol, Production, Fermentation

1. INTRODUCTION

1.1. Background

Bioethanol fuel is mainly produced by the sugar fermentation process. Ethanol or ethyl alcohol (C2H5OH) is a clear, colorless biodegradable liquid and is less toxic and causes less environmental pollution. It is a high-octane fuel and has replaced lead as octane enhancer in petrol. The economics of ethanol production by fermentation is significantly influenced by the cost of the raw materials. It accounts for more than half of the production costs. 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 an area of focus because it reduces pollution in the environment in addition to the energy generation. The major part of this is mostly discarded and it becomes the main source for increasing the pollution in environment. Above all, the discarding process becomes a very expensive step due to high transportation costs. Majority of fruit and vegetable wastes available from their processing industries are seasonal and do not decompose rapidly. The principle fuel used as a petrol substitute is bioethanol. Bioethanol fuel is mainly produced by the sugar fermentation process.

Mango (Mangifera indica) of the family Anacardiaceous is a tropical, subtropical and frost-free fruit (Bally et al., 2009). It is the fifth largest fruit crop produced worldwide after banana, mango, apples and oranges. It is the second most important tropical fruit with 27 million tons being produced annually worldwide (Bally et al., 2009). It originated from the foothills of the Himalayas of India and Burma and has been cultivated in that region for at least 4,000 years. In Kenya, it has been the third most important fruit in terms of area and production for the last ten years after banana and pine apple (HCDA, 2010). The hectares under mango production, production output

(ton) and the revenue earned have continued increasing over years. In 2010, hectares increased from 36,304 to 59,260 (Ha)

Fig.1- Fresh Mango

Biomass provides self-sufficiency in the field of energy generation across the globe. The energy in biomass can be accessed by turning the raw materials, or feed stocks, into a usable form. Several compelling issues have driven an international effort to develop and improve technology to make biofuels. The dependence on petroleum for fueling the transportation sector threatens energy security, affects the environment and weakens the economy. Developing the technology to produce and use biofuels will establish safe, clean, sustainable alternatives to petroleum and will promote self-dependence in the field of energy production. The principle fuel used as a petrol substitute is bioethanol.

The production of ethanol from lignocellulosic biomass has received considerable attention because of the potential of producing large quantities of ethanol for use as a transportation fuel. Hemicellulose and cellulosic components of lignocellulosic biomass are hydrolysed to their component sugars for subsequent conversion to ethanol by a fermentative process. Hemicellulose and cellulose are usually hydrolysed with a chemical process (acid) or biological (enzyme) attack. The economic success of ethanol production will depend on efficient conversion of cellulose and hemicellulose to their monomeric sugars and the efficiency of fermenting those sugars to ethanol, while also reducing capital and operating costs (Schell et al., 1992).
The mechanical drying of these wastes (mango peel, citrus peel, pineapple peel and tomato processing wastes) gives opportunity to store these substrates over long periods of time. The yeast, Saccharomyces cerevisiae, and facultative bacterium, Zymomonas mobilisare best suited candidates for industrial alcohol production. However, ethanol is produced commercially by yeast because it ferments glucose to produce ethanol.

1.2. The Statement of the Problem

Mango peel waste collection and disposal creates a range of environmental problems in our environment. A considerable amount of waste 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 are causing air pollution. The situation is exacerbated in slums where households cannot make use of garbage collection containers.

Lack of the most basic mango peel waste services in crowded, low-income neighbours are a major contributor to the high morbidity and mortality among the urban poor. The adverse effects of inadequate solid waste service on productivity and economic development of the city are significant. Solid waste such as fruit peels largely obtained as a by-product from hotels, restaurants and juice processing houses in our country. These wastes can entail serious environmental problems unless they change or convert in to some useful products or disposed properly. The aim of this thesis was to investigate the possibility of using and transforming mango peel waste to something valuable product, namely ethanol there by contributing towards alternative energy supply as well as creating an employment opportunity.

2. Objective of the study

To Production ofbioethanol from mango peels using Saccharomyces cerevisiae.
To determine the properties of bioethanol
To determine mango peel gives ethanol yield from fermentation
To know the factors were significant variables for the yield of ethanol

3. Material andMethods

The experiment of production of ethanol from mango peel is carryout in the laboratory of Chemistry Department at the main campus, Ambo University.

3.1. Materials Use for the Experiments

3.1.1. Equipment's

- 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 hydrolyzates 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 and Hydrometer: - for density measurement.
- Shaker: -to shake sample and its additives after hydrolysis and before fermentation.
- Fermentation and distillation set ups: - to ferment and distill respectively

3.1.2. 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.
- $Mg\ SO_4.7\ H_2O$: - used as media preparation.
- Yeast (Saccharomyces cerevisiae).

3.2. Procedure of Experiment

The study is aim at optimization of acid hydrolysis in the production of ethanol from mango peel. Mango peels were collected from juice house in Ambo. They are collected in plastic bags and transport to the laboratory of chemistry for ethanol analysis. The following methods are following. This section describes about the methodologies and approaches of how experiments were done in this research; it includes all steps and procedures of the experiments. The followings are basic steps for the production of ethanol alcohol, these processes are: -

- Sample collection.
- A pre- treatment phase (size reduction) to make mango peel agreeable to hydrolysis.
- Hydrolysis to break down the molecules of cellulose and hemicelluloses into simple sugars.
- Yeast fermentation of the resulting (sugar) solution.
- Distillation to produce alcohol.

3.2.1. Sample Preparation

The sample that is acquired has to be prepared and condition forpre-treatment, hydrolyze fermentation and distillation. Sample preparation process include: manual size reduction (Knife cutting), drying, grinding and sieving after the samples is collect.

Fig3. 1-preparation of mango peels

Fig3. 2-ovendry mango peel

Waste peel of mango 3kg is use for the sample preparation.

They are cut by knife into pieces of about 3-5 cm length for drying and grin (60^0 C for 72hr) to obtain easily crushable material. After drying, each of the samples is mill separately. The maximum particle sizes of the ground mix sample were 2 mm. The sample of larger particle size than 2 mm is 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 mango peel into powder form increases the surface area of the sample which enhances the contact between hemicellulose and cellulose with dilute acid to reduce cellulose crystallinity.

Figure 3.3- mango powder

3.2.2. Pre –Treatment of Fruit Peel Powder

The purpose of the pretreatment is to remove lignin, reduce cellulose crystallinity and increase the porosity of the materials. Pretreatment must meet the following requirements: improve the formation of sugar, avoid the degradation or loss of carbohydrate, avoid the formation of by-product inhibitors and must be cost effective.

Steam Pretreatment: The powders of mango peel aretreating inside autoclave; every different sample is treating separately. Steam pretreatment uses steam at 121^{o}C temperatures. Flow through processes steam at temperature of 121^{o}C through the hemicelluloses and cellulosic material. First, the mango peel powders are treating and it feed as batches, every batch contains 100 g of screened mango peel powder with 10:1(v/w) ratio of water to the sample. The temperature was applying at 121°C; then release the pressure until the pressure became 0 bars. The retention time for every batch was 15min. Finally, the samples was kept in autoclave for the given pretreatment time and temperature and allow to cool.

Figure3. 4- Mango powder weight

Procedures in Steam Pretreatments

- ✓ Add 100 g of grind mango peel in to 2000 ml conical flasks
- ✓ Add 1000ml of distill water.
- ✓ The conical flasks capped with the help of rubber plugs.
- ✓ Autoclave at a temperature of 121^{o}C for 15 minutes
- ✓ After finishing the given pretreatment time and temperature the sample in autoclave and allow to cool and separate soluble from the non-soluble portion. The non-soluble portion is hydrolyzing in the next steps and put the soluble solution in another 2000ml conical flask.

4)
5)
6)
7)
8)
9)
10)
11)
12)

b)

Figure 3.5**(**a**)** Steam Pretreatment of mango peels powder in autoclave (b) Sample ready for pretreatment

3.2.3. Acid Hydrolysis

The cellulose molecules which are composing of long chains are broken down to simple sugar, before it is ferment 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 produce in case of acid hydrolysis is more than that of alkaline hydrolysis. Sample is to pass through five primary experiments that are in series to get the final result ethanol, that is: size reduction, pre-treatment, hydrolysis, fermentation and distillation.

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 mangospeels are then hydrolyzing 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 hydrolyzates by centrifugation (to remove the non-fermentable lignin portion).
- After separate the solid part, wash the solid part with distilled water two times. The washing is performing in order to extract all soluble sugars from the solid mango peel material.
- To obtain the original sugar concentration in the hydrolyzates, the liquid parts were boiled until the liquid weight become 1.0 kg. Then solid and liquid parts place in the freezer until use.
- Then mix the soluble component with the previously filter solution from the pretreatment step for the next procedure.
-

3.2.3.1. PH Adjustment

Before addition of any micro-organism to the above prepares samples, pH of these samples is to be adjusting. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 5.0 -5.5 is maintain.

Figure 3.6 pH meter

Procedures in pH adjustment

- ✓ Mix pretreat and hydrolyze solution, filter, shaken substrate primarily checks for pH using a digital pH meter. The pH then adjusts to 5.0-5.5.
- ✓ Mix samples (pretreat and hydrolyze) are acid hydrolyze, so it needs highly basic solution to bring the pH in the range of 5.0-5.5.
- ✓ Sodium hydroxide solution is add drop wise to the other flask with constant stirring until the pH reaches to a range of 5.0-5.5.

- ✓ If suppose the pH goes beyond 5.0-5.5, concentrate sulfuric or hydrochloric acid is add drop wise to maintain the pH in the range.
- ✓

3.2.4. Sterilization

The reactor and all the equipment that is use for fermentation purposes is sterilize (autoclave). The sterilization is carrying out at a temperature of 121°C for 15 minutes.

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Figure 3.7 Sterilization equipment

3.2.5. Fermentation

The aim of the experiment is to measure the ethanol production by the yeast (Saccharomyces cerevisiae) using mango peel hydrolyzates as energy and carbon source. The clear solutions then go to fermentation. The fermentation is carryingout under anaerobic condition at a temperature of 30°C, pH 5.5 with 200 rpm stirring condition for 3 days. Before conducting fermentation, we are the preparation of media for the yeast. In order to prepare the media, we will the favorable condition for yeast growth or to supply the require amount of nutrients. Mix the following nutrients in their proportion.

Media Preparation

- Sugar (Dextrose) = 10 gm.
- For preparing 100 ml media, we add
- Yeast extract = 0.2 gm.
- Urea = 1.0gm
- Make up water = 100 ml
- Mg SO_4.7 H_2O = 1.0g
-

Procedures in Media Preparation

- ➢ To the above 100 ml media, 0.5 gm. of yeast, Saccharomyces cerevisiae (instant premium) is add in a 250 ml conical flask.
- ➢ The conical flasks are properly covered with aluminum foil.
- ➢ The conical flask is then place in a shaking incubator for 24 hours, a temperature of 30° C and 200r

(a) (b)

Figure 3.8(a) Shaker incubator (b) media after 24hr incubationand 200rpm.

The Procedure for Fermentation

- The sample is condition to temperature of 30°C before fermentation step is start. This is the temperature at which all fermentation experiments are carriesout.
- The adapt media with the proportion of 1:10 to the soluble sample mix then place in the shaking incubator at a temperature of 30°C, 1 hour and 120 rpm.
- Set autoclavable reactor at 30°C and 200 rpm and then mix the prepare sample with the media prepare into the autoclavable reactor using sterilized funnel. The parameters of fermentation i.e. fermentation time, yeast concentration (yeast proportion) and fermentation temperature are set to be at 72 hours, 10% (with the proportion of 1:10 that is the prepare media and sample respectively) and 30° C respectively. And after 72 hours of fermentation, the samples are taken out and distill.
- Base (2 M NaOH) add automatically by a pump into the bioreactor every time to drop pH below 5.5.

Figure 3.9 Samples ready for fermentation

3.2.6. Distillation

Distillation is the last step in the production of ethanol from mango peel experiments. It is the purifications steps. Distillation is the method use to separate two liquid based on their different boiling points. However, to achieve high purification, several distillations are requiring. In this experiment separation is use 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 bars
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Figure 3.10 Distillations (Rotary Evaporator)C and a distillation time of 3 hours.

4. Result and Discussion

4.1. Statistical Analysis of the Experimental Results

The process consists of four parts: pre-treatment to remove lignin, reduce cellulose crystallinity, sterilize the mango peel and 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 hydrometer.

4.2. Extraction of sugars from dried mango peel

From the aqueous extraction, yield of ethanol concentration low amount of sugars wasobtained; only 20% (w/v). Mango peel treated with crude pectinase yielded higher levels of solubilization and reducing sugars (Tables4.1). The optimum incubation period for solubilization of the maximum sugars was found to be 24 h (Figure4.1). The results also indicated by the sugars released from the mango peel. Another significant observation made during this study was decrease in the initial pH from 5 to 4.5 at the end.

Table.4.1. Composition of fresh and dried mango peel used in this study.

Content	Fresh mango peel	Dried mango peel
Moisture	70 ± 5	10 ± 1.2
Total solids	25.6 ± 4.6	70.5 ± 2.7
Reducing sugars	7.0 ± 1.8	30 ± 2.5
Non-reducing sugars	5.9 ± 0.4 4.3 ± 0.5	
Protein	3.5 ± 0.5	4.0 ± 0.8
Cellulose and lignin	25.2 ± 2.0	23 ± 1.2

4.3. Pretreatment of mango Peel

The yields of reducing sugar from the mango peel by different pretreatment methods are shown in Figure4. 1. The reducing sugar and cellulose content when pretreated in autoclave for 15 minutes is higher than with other pretreatments.

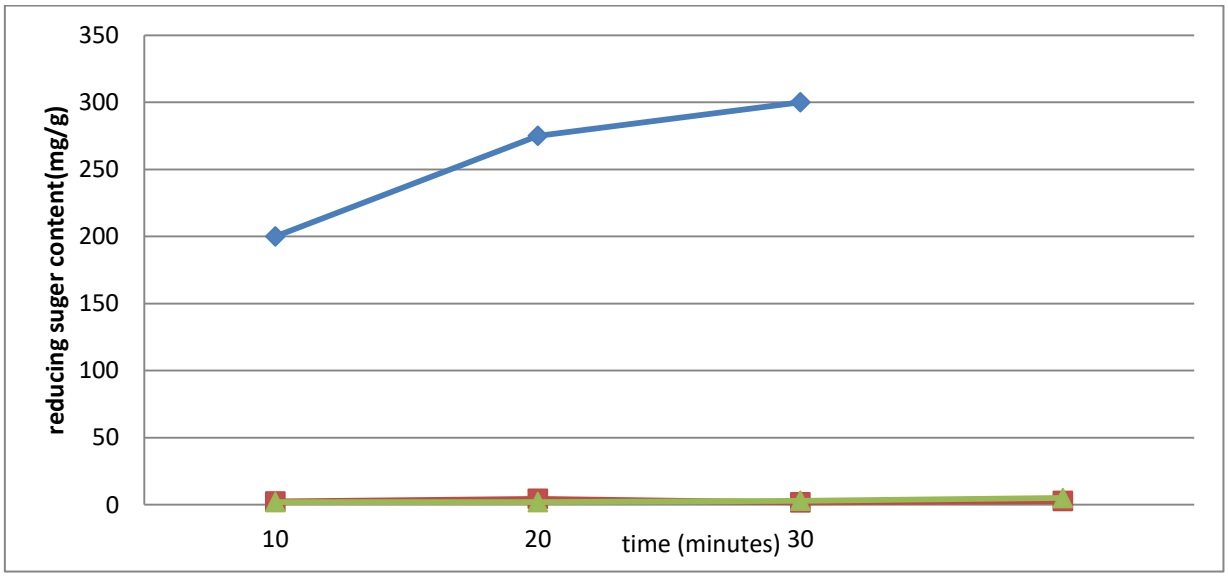

Fig4. 1 Effects of pretreatment methods of mango peel to ethanol fermentation

The results showed that the sugar yield was affected significantly by pretreatment methods. Acid pretreatment method was optimal for better yield of fermentable sugars from mango peels. The initial pretreatment, in case of fibrous peel residues, is needed to breakdown its structure to make it more susceptible to an enzymatic attack, whereas for pulp residues, it is required to avoid the necessary for reducing the weight of the biomass and to inhibit fermentative microorganisms which degrade compounds.

4.3.1. Bioethanol Simultaneous Saccharification and Fermentation from mango Peel

1) Influence of Fermentation Time: Fermentation time is an important factor affecting the bioethanol fermentation process. If the time is too short, fermentation does not occur completely, ethanol is produced few. If the time is too long, the fermentation environment will be infected other microorganisms, and if there is the presence of oxygen, the alcohol is oxidized to acetic acid, which reduces the strength and cause alcohol damage. Usually the determination of the end of fermentation process bases on the moment which the total soluble solids values do not change for at least 6 consecutive hours. At the end of fermentation process, yeast cell no longer converts sugar into ethanol, because amount of formed ethanol sufficient inhibits to fermenting. Therefore, the determination of appropriate fermentation time is very important.

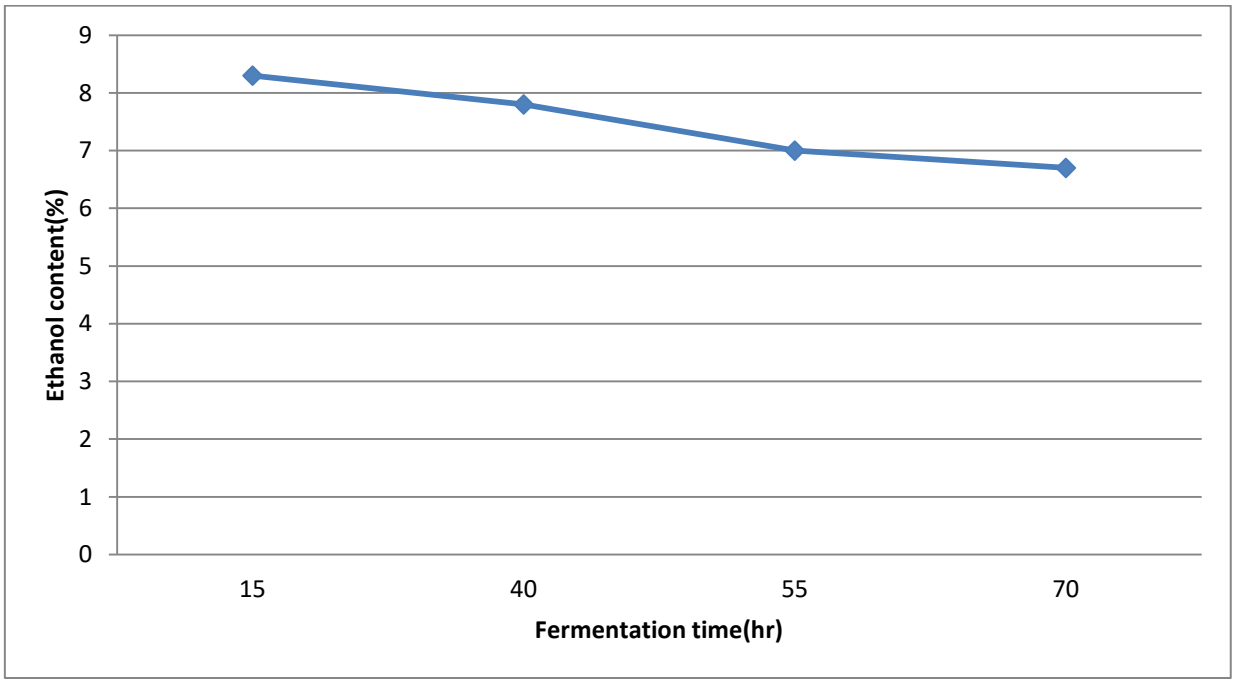

Fig.4. 2 Influence of fermentation time to the bioethanol fermentation from mango peel.

The influence of fermentation time is presented in Fig.4.2. As soon as yeast cells is inoculated into fermentation medium, because a large amount of oxygen is available, yeast cells consumed sugar and other soluble nutrient molecules for cell division and development, ethanol has not been produced yet.

When oxygen became exhausted, yeast cells converted substrate to ethanol molecules in anaerobic condition. Sugar and nutrient molecules were metabolized much at this stage, the rapid fermentation occurred in the period from 15 to 69 hrs. Almost all of substrate molecules were converted into ethanol and CO2 and some byproducts such as organic acids, higher alcohol, aldehydes. Thus, medium pH decreased from 5.5 to 4.5. The moment of 24 hour is the end of bioethanol fermentation from mango peel. After 24 hours, the reducing sugar content continued to increase, but the ethanol content decrease. This result is similar to the results which were reported in some previous researches. The fermentation time of mango waste is 3 days. The maximum ethanol content was obtained after 75hrs for fermentation process.

2) Influence of Temperature: The temperature of fermentation can affect the growth of Saccharomyces cerevisiae. The yield of ethanol is related to medium temperature. Temperature factor can impact the sensitivity of yeasts in ethanol production, growth rate, rate of fermentation, viability, length of lag phase, enzyme and membrane function. Many yeast strains behave differently in their response towards temperature;therefore their common optimum temperature could not be easily established.

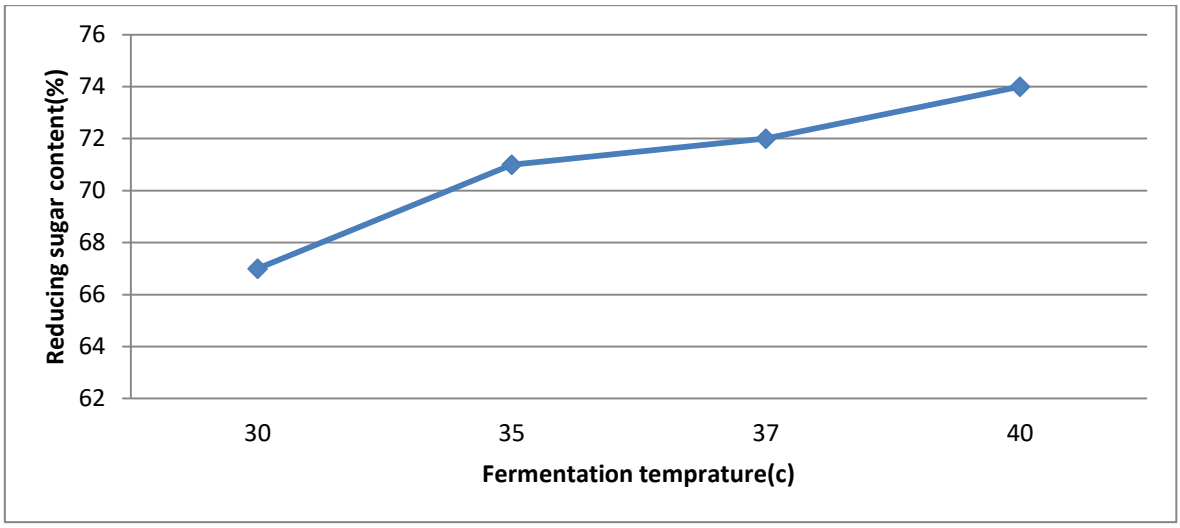

Fig.4. 3 Influence of temperature to the bioethanol fermentation from mango peel

The results on the variation of ethanol percentage against temperature are represented in Fig4.3. The temperature was varied between 25°C and 37°C. The pH of sample was maintained at 4.5 and fermentation period was kept for 24 hours. The ethanol concentration reached the highest level at 37oC and decreased not only with the increase in temperature up to 40°C but also with the decrease in temperature to 35°C. The yield of reducing sugar also reached the highest level as yeast cell lived 37oC. Yeast does not grow at temperature over 40oC and below 30°C its growth slows down dramatically. Besides, as cellulose activity of commercial enzyme is most appropriate at 40-50oC, 37oC is appropriate to activity of yeast cell and enzyme. Similar to this result, the content of ethanol from mango peel was optimum at 30-31oC. The bioethanol production from mango was maximum at 37oC.

3) Influence of Initial pH: The initial pH factor has significant influence on bioethanol fermentation. To observe the influence of pH on the quantity of ethanol, the experiments were carried out fermentation period of 24 hour at 37oC. The pH value of mango peel samples was varied 4.0-6.0 with an increment of 0.5. Fig.4 shows the variation of ethanol content (%) produced with PH. The ethanol content was least of at pH~4.5 and increased to reach the highest value at pH~4.5. At this pH, the pH different is lowest (ΔPH=0.5). However, keep increasing pH to 6.0, the ethanol content significant decreased. The important reason is the optimum initial pH for the growth of yeast is about 4.5-6.0, and the most appropriated pH for cellulose activity is about 4.5-5.5. The lower activities of yeast towards bioethanol fermentation are shown at a pH of 4 because these pH values are too low to activate the of yeast to run their functions.

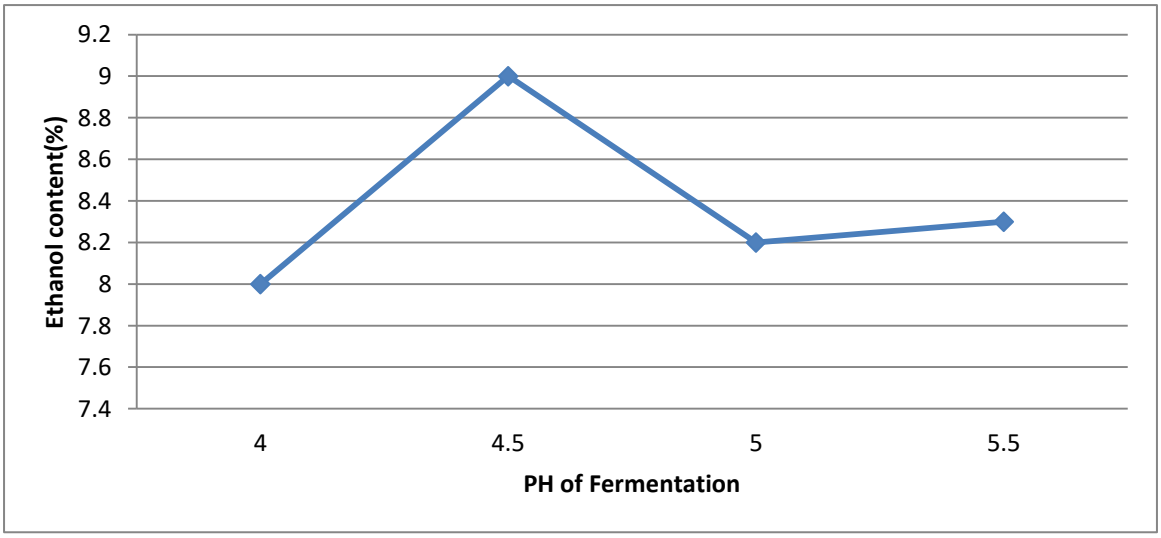

Fig.4.3. Influence of initial pH to the bioethanol fermentation from mango peel

4.4.Property of production (bio-ethanol)

I need to compere our product with standard bio ethanol as table below:

Table 4.2 Property of bioethanol which I produce in lab from mango peel

Items	Our product	Standard
Density(kg/m3)	0.80	0.79
Viscosity(mm2/s)	-	1.5
Flash point(C)	<21	<21
Boiling point(c)	79.5-85	78.5-85
PH meter	4.9-5.6	5-5.5
Color	Clear Colourless liquid	Clear Colourless liquid
Fire test	Blue flame	Blue flame

Source: Glucose, Alcohol, CO_2, Cell Mass Levels during Fermentation (Source: N. Mosier and M. Ladisch).

5. CONCLUSION

Production of Bioethanol, a high-octane fuel, therefore may be a good replacement. As bioethanol can be prepared with the help of fermentation, it does not need huge number of ingredients. The present study was done with objectives to produce bioethanol from mangopeel which solves the waste disposal problem. In a country like Ethiopia, it is very hard to do proper disposal of wastes and thus generation of infectious diseases is rapid here. So, using these wastes not only provide a use of those wastes but also help to be beneficial economically. The bioethanol was found to be equally comparable to the other.

- Very high and low acid concentration, temperature and retention time have negative effect on the yield of ethanol.
- Production of ethanol from mango peels is feasible from the economic point of view in that its internal rate of return provides a return greater than the current rate of return.
- When we compered our product with standard Property of bio ethanol almost all has the same property.

Finally, it can be concluded that the produced mango peel- Bioethanol is economically and environmentally viable. And can be a good substitute ofPetrol. Although some more research works in different states and different. Environments should be done to find out any better result. Productivity andeconomic viability are also some fields to be taken care of to have a wonderful Energy source.

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