

Bioethanol Production from Papaya Waste

Dissertation submitted in partial fulfilment of the requirement for the degree of

BACHELOR OF TECHNOLOGY

IN

BIOTECHNOLOGY

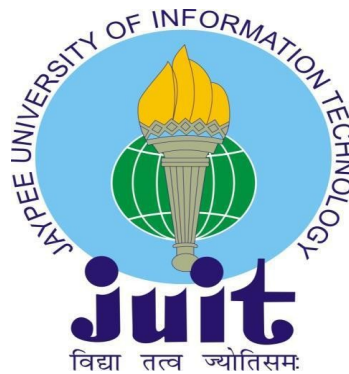
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May 2022

DECLARATION

I decree hereby that the work presented in this report entitled “**Bioethanol Production from Papaya Waste**” in partial fulfillment of the requirements for the award of the degree of Bachelor of Technology in Biotechnology submitted in the department of Biotechnology, Jaypee University of Information Technology, Waknaghat is a real record of my own work carried out over a period from August 2021 to May 2022 under the supervision of Dr Garlapati Vijay Kumar.

The matter embodied in the report has not been submitted for the award of any other degree or diploma.

(Kannu Priya, 181814)

SUPERVISOR'S CERTIFICATE

This is to certify that the work reported in the B. Tech. thesis entitled “Bioethanol Production from Papaya Waste”, submitted by Kannu Priya (181814) at Jaypee University of Information Technology, Waknaghat, India, and is a bonafide record of their original work carried out under my supervision. This work has not been submitted elsewhere for any other degree or diploma.

(Signature of Supervisor)

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(Kannu Priya, 181814)

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ABSTRACT

Food waste or fruit waste is increasing day by day. The accumulation of such waste materials is constantly growing due to overproduction, exploitation, over-buying, and other individual reasons. A sustainable approach is a necessary need of the world for such serious issues that the world is facing today, as food waste is causing many severe damages. Conversion of food waste/fruit waste to bioenergy and biofuel is a possible approach to deal with such problems. The generation of biofuel from fruit waste will help the world face the fuel crisis or energy crisis that the world will face in the future as overexploitation of the natural energy resources has led to their depletion of them, which has increased in price and demand of natural resources. Such approaches help to cause less contamination to the environment and decrease the dependency on natural energy resources. The best sustainable approach as it cause no harm to the environment and is cost-effective. In the present study, papaya waste has been utilized for bioethanol production by studying the reducing sugar content and pH with yeast fermentation.

Keywords: Papaya Waste; Bioethanol; Reducing sugar; pH

CHAPTER 1

Introduction

Biomass may be handled in a tenable form to produce bio-amount for a circular green saving. Data composed from the Internet, in India, the household foodstuff waste supposed is about 50 kg per person occurring, or 68,760,163 tons a year. A supposed 931 heap tones of cuisine were emaciated everywhere in 2019, enough to circle the Earth on seven occasions, in accordance with a UN report that pronounced that the foodstuff waste generated in household activities in the India is approximately 68.7 heap tons in a year. Utilization of the foodstuff waste for an era of many possessions like biofuel etc. may be a large step towards tenable happening. Biomass is used to generate biogas out of it, and pellets are drained of long fiber bio-manure and reused in water. It is imaginary that one of the results of the bio-located crop, feed, and drug pieces, fine, concentration and platform chemical compound, polymers, alongside biofuel and bioenergy from biomass is obtainable to attain overall sustainability each substitute of fossil properties. Sustainable resourcing and consumptions of biomass are essential because technique does not unfavorably influence the ecosystem and the atmosphere across worth chains. An increase in Foodstuff waste amount had a connection with an increase in community and living standard bettering is developed into harsh tangible question in two together expanding and advanced countries. Food Waste from canteens, cafeterias, local markets, and likewise from the extraction of miscellaneous enzymes and added beneficial matters that hold an extreme content of surely referring to practices or policies that do not negatively affect the environment natural resources, is judged as excellent bioenergy beginning as it is not any more resistant for devouring on account of differing negative effects.

Foodstuff Waste holds abundant amounts of mysterious natural resources and extreme microscopic compounds to a degree lipid, sugars, starches, and proteins, present as postponed mass. The bulk of foodstuff waste is either thrown away at landfills, incinerated, or produces. While land filling and burning do not savor good public agreement, make ready to bear does. This is generally on account of the era of a material that may be secondhand in land frameworks either to help plant development or enhance soil character. In light of the discovery of rising contaminations, in the way that Per- and Polyfluoroalkyl Substances in foodstuff waste, more states achieving basic waste bans, more accurate tangible managing, and public pressure challenging a better approach to management

foodstuff waste, it is more immediate than always before those scientists, regulators, and expert endure contribute to addressing these challenges. On the other hand, creative and out-of-the-box methods need to be expected projected and proven to revolutionize cuisine waste into merchandise that is valuable. Only when foodstuff waste is promoted in definite habits, medicated as a source alternatively litter, can this waste and the questions guide allure conclusion be really concluded.

The use of waste to produce potential and get back additional minerals not only enhances the industrial circumstances of a country but likewise aids the tenable happening of humans. The current use of nonrenewable fuel sources and the impact of greenhouse gases on the surroundings is forceful research into energy from undeletable sources resulting from biowaste. Treating this biowaste accompanying miscellaneous biowaste-to bioenergy sciences is a reasonable approach to both considering waste and producing power. Various organic designs (anaerobic digesting, esterification, effervescence, and electro fuel containers) and physicochemical orders (pyrolysis, gasification, burning, and landfills) are skilled to convert waste into resource. Interest in energy from undepletable source origins has currently developed on account of fossil source reduction, developing potential demand, and worldwide atmospheric changes. Biomass has existed as a spotlight in the Search for alternate inceptions of carbon skeletons to displace chemical compounds and matters originated fossil possessions Biomass is tempting energy from the undepletable source that may be convinced into miscellaneous bioproducts by means of biorefinery methods. Biomass feedstock secondhand in consideration of bioenergy results are detached into three categories; carbohydrate-based, starch-based, and lignocellulosic-based resources. Among sustainable element frame resources, chitin is second only to organic compounds composed of carbon as the ultimate abundant biopolymer on the planet. Accompanying this expanding demand for reasonable fuels and feedstock, waste biomass from technical foodstuff manufacture and ordinary existence has improved a definitely better choice as an origin for alternate feedstock than waste biomass from agriculture in addition most lignocelluloses for biofuel change, because it is can regulate an extreme yield of biofuel outside some compromise to feed supplying and additional normal beginnings.

Apparently, exploiting food trash as a natural resource in consideration of bioenergy change includes a better advantage than utilizing it as mammal feed. Additionally, foodstuff production waste is greater engaging than waste from added beginnings by way of allure cheap and determined use. Before this time, starch and oil, that are common parts from food, are the greatest tempting feedstock, usual for adaptation to bioenergy. Without correct management, food processing wastes

construct smell and cleanliness concerns and cause negative natural impacts. Large amounts of hard foodstuff transform wastes are dumped in landfills, practiced to the land, or charred on-ground. Solid biological wastes from slaughterhouses can include various classes of microorganisms, containing potential pathogens. Mammals concede the possible increase in miscellaneous metals, drugs, and added chemical compounds amounted to their feed for nutritional and drug purposes. Fatty wastes are an important beginning of contamination, and land filling of oily wastes is not adequate in a few advanced lands.

CHAPTER 2

Review of Literature

2.1 Food Waste

Food waste is generating severe material and public difficulties across the planet. Foodstuff waste or municipal continuous waste is disposed away regularly, out of all 40% of that is composing of putrescible. Such putrescible consists biological dump or trash that is acknowledged to constitute smell upon decay. Almost, 90% of putrescible are foodstuff wastes. Foodstuff waste possibly raw, cooked, eatable, and indigestible parts produce all along manufacture, storehouse handling, and consumption of foods. Two third of the foodstuff waste is mainly acquired from households; inasmuch as, one-third of foodstuff waste originated economic and technical origins.

Foodstuff waste is a known inconsumable origin of phosphates, carbohydrates, amino acids, and lipids etc., Data from diversified researches discloses that confectionery and combined food wastes consist of a large amount of carbohydrates and lipids. It depends upon the source of the foodstuff waste; the overall lipid content was approximately 30% and the overall sugar content was approximately 50%, in an average. A number of categories of foodstuff wastes can be hydrolyzed by using enzyme for production of lipids and hydrolysate. The hydrolysate obtained from the food is enriched with carbohydrate rich and is recycled or used for the manufacture of Bioethanol; inasmuch as, the lipid content obtained can be possibly transformed into biodiesel.

2.2 Bioenergy

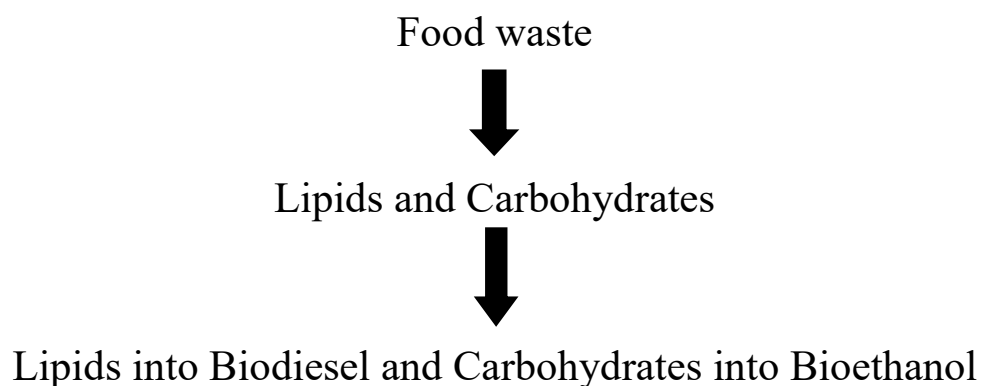
Bioenergy can offer inexhaustible, reduced-carbon energy systems, sequestering climatic element in addition to offer abundant environmental and socioeconomic benefits and so upholding worldwide atmosphere change aims and more off-course environmental, friendly, financial, and sustainable targets. There is experimental evidence of the benefits of bioenergy, but results are frequently liable to be subjected variation and uncertainty. Additionally, it is main to contemplate miscellaneous sustainable phases of bioenergy structures further element. Treating bioenergy only as few the strength areas will abandon to guarantee: tenable biomass result and sourcing, clean requests accompanying reduced fitness impacts, and fair and inexpensive strength headings. Ensuring that bioenergy offers the necessary whole issuance decline, circumstances, particular and complete approaches are inevitable to accept cooperation and tradeoff of the bioenergy and accompanying land and silviculture structures. Assessing the environmental and more expansive tenable impacts of

bioenergy, brimming supply chains in addition to direct and unintended collaborators, their chauffeurs, benefits and challenges needs expected deliberate. With these, we should evaluate and judge bioenergy and allure impacts in the framework of the distinguishing structure it is part off and allure direct and more expansive impacts on atmosphere, frugality, and humankind.

2.3 Biofuel

Biofuel manufacture is expeditiously expanding as the planet encounters deterioration difficulties because of the combustion of fossil fuels and other fuels made up of coal. Additionally, oil resources are definite source and most of the fossil fuel sources are situated in politically changeable lands. This proves that the alternative source for fuels is essential for the future world or upcoming generation. Many countries are developing or researching upon the power approaches in order to manufacture of energy using undepletable source. Presently, biofuels such as Bioethanol and biodiesel are manufactured from different eatable plant oils that are obtained from canola, soybean, etc. are used for manufacturing biodiesel. When in fact, Bioethanol possibly composed from a type of feedstock like wheat, sugarcane bagasse, stover, sugar beet, corn, cereal, molasses, barley and other sources enriched in carbohydrates are widely formed from eatable foodstuff components.

Biogas is used as a power source. Instead, foodstuff wastes possibly valorized for the manufacturing energy by utilizing diverse approaches such as composting, converting, and burning. Even though, such type of processes is suitable modifying foodstuff dump into fuels and worth products advancement of sustainable and more progressive sciences is needed. Development of biofuels from eatable food, and components are assigned to the reason for foodstuff insufficiency and the foodstuff vs. fuel debate is before stormy. Instead, nonedible feedstock possibly recycled for the manufacture of biofuels.



(Flowchart of two types of Biofuels)

2.3.1 Biodiesel- Synthetic the biodiesel manufacture process is termed transesterification. All along transesterification, the tri-, di-and monoglycerides react accompanying methanol in the appearance of a catalyst to produce biodiesel. Biodiesel is a newfangled inexhaustible fuel composed by reacting edible oils, refined oils, and animal fats, including triglycerides and free greasy acids as the main components, accompanying methanol. The three main reactions steps in transesterification of triglyceride accompanying methanol are triglyceride (T), diglyceride (D), and monoglyceride (M) reactions among methanol (CH₃OH) to form D, M, and glycerol (G) particularly in addition methyl oleate (MeOl), or more interminable attached methyl ester – depending on glyceride chain length. Fatty acid methyl esters specific as MeOl is acknowledged as biodiesel. [2]

2.3.2 Bioethanol- The result of the Bioethanol process includes pretreatment, synthetic hydrolysis, fermentation, and purification steps. By fermentation substrate is converted into products such as Bioethanol by microbes, fungi, yeast and more microorganisms commonly present in the gut. The chemical equation of Bioethanol manufacture from glucose fermentation is like 1 mol glucose is disintegrated into 2 mol ethanol and 2 mol carbon dioxide.

Bioethanol was individual of the up-and-coming biofuels to solve the greatest issue of energy crisis, and accompanying the extensive investments invested for the manufacturing of Bioethanol, the amount of Bioethanol produced from lignocellulose and carbohydrate raised fast that not only satisfied the demand of fuel, but also raised the difficulty for the use of Bioethanol. So, the change of Bioethanol to hydrocarbons, oxygenated chemical compound, and other low molecule weight compounds is conferred, containing the change methods, application of the adaptation produce, in addition to the benefits and troubles of the change process. In addition, few approaches are deal with the further incident of these projected conversions. It is trusted that Bioethanol is hopeful expected both as an adjustable program particle for the result of differing chemical compound, that is a possible habit to resolve the reduced business-related adeptness of Bioethanol result.

2.4 Advantages of Biofuels and Bioenergy

Biofuels have huge benefits that surely override some hurts that can survive. Ultimate prevalent biofuels are intoxicating and biodiesel. They are harmonized accompanying fuel to create alternative fuels.

Sustainable, Tenable Fuel - Biofuel creation includes waste material, natural resources or fertilizer, crop waste, plant waste, etc. So, the feedstock for biofuels itself is sustainable, making biofuels sustainable. Different established fuels, biofuels are speedy and smooth to produce. Additionally, they defeat element issuances.

Cost-Effective - Biofuels use crop/ plant waste – used cooking oil, organic matter, and plants etc. as natural resources. All these beginnings of result are environmental and smooth to accumulate besides being inexpensive also. Biofuels are non-harmful and environment-friendly. In entirety, biofuel production power gives the impression a high-priced employment, but the financial cost is in the negative. From now on, it is necessary expected inferior as the demand shoots up. An increase in biofuel demand will defeat the cost of result. Also, the tangible impact of fossil fuels is large when distinguished to biofuels. Biofuels are environmentally companionable.

The durability of the vehicle's motor - Biofuels maybe surely mixed to adapt existent transformer designs. It has a taller cetane number and lubricating competencies. Exemplification, accompanying biodiesel as a flammable fuel, the persistence of the tool increases. It raises the unending capacity of turbines at reduced perpetuation. Too, it keeps contamination and element issuances under check. Additionally, biodiesel is tenable and much more incendiary as distinguished to oil engine.

Influence our Economy - Most nations believe lubricate imports to meet their fuel needs. Still, a piecemeal shift to utilizing biofuels will help encourage our country's business-related protection. Additionally, individual can weaken the extreme reliance on fossil fuels. Advancing biofuel allows the creation of enrollment convenience and boosts the tumor of local land commerce. More, the cost of biofuels is inferior than fossil fuels. Accompanying raised task potential, country financial publicity, and discounted fuel costing, a country can better guarantee allure financial safety.

Decrease Dependency - All the nations deliver fossil fuels to meet their strength needs. These fuels are not usable in the country but foreign from an external land. Biofuels are a substitute for not only the usual fossil fuels but further remove the need for mean lubricate. Biofuels help fuel supply and create the supply tenable. Because it is tenable, the reliance on established fossil fuels and imports can climax completely from now on.

Environmental Effectiveness - Fossil fuels are restricted and have a damaging affect the surroundings. In addition, biofuels are tenable/ inexhaustible and decrease tangible shame. Biofuels can decrease until 80% of element diffusions. Still, the transfer process of natural resources/ feedstock secondhand for biofuels is alleviated out through reusing and talks over again. Biofuels are referring to practices or policies that do not negatively affect the environment that afterward humble soil and secret water adulteration. They assist in protecting the air and considerably lower the dirtiness level.

Less Contamination - Fossil fuel explosion releases hothouse vapor that is carbon dioxide. This leads to all-encompassing baking. Biofuels are an excellent alternative to defeat element issuances

and worldwide heating. These are also known as green fuels, as they do not increase the colorless odorless gas burden.

Community is awakening to the financial, friendly, and incidental benefits of biofuels. The management is communicable steps and active towards carrying out a greener saving. Diversified boards/ partnerships are being started for effective administration of natural resources so that produce biofuels. Still, we are still certainly from a complete change.

Stimulated a piece view of lowering our dependency on unrefined petroleum, we found that if composed and treated capably, used cooking lubricate has huge potential to realize green fuel use in the country.

India, accompanying allure large use of cooking oil, has an overwhelming likelihood of appropriate liberated in the result of biodiesel. This accomplishment will more supplement the custom of electric automobiles. While biodiesel can support public transport, electric cars can capably protect the services section.

2.5 Disadvantages of Biofuel and Bioenergy

Skilled are forever two hands to a coin. Bioenergy actually is an alternative value taking everything in mind replacement fossil fuels. Nevertheless, it likewise creates few disadvantages that you need expected aware of. While few troubles come into being fuel usage, others are from its manufacture process or application

Bioenergy is not as clean as it is recognized to be- Element neutrality is actually natural for bioenergy. Nevertheless, when it is scorched, element is not the only injurious outgrowth. This can influence polluting the atmosphere, though not as extreme as fossil fuels. Still, it is disaster polluter between sustainable possessions. The polluting facet of bioenergy is a monstrous disappointment to allure claim a suggestion of choice to fossil fuels.

Bioenergy ancestry includes extreme costs. - Though less high-priced when distinguished to fossil fuels, between added renewable energy beginnings, bioenergy is far from the most inexpensive alternative. When different opportunities like cosmic, wind, or water strength are possible alternatives, biomass is not of value the finance. The cost of bioenergy involves the cost complicated in claiming biomass possessions and replenishing the biomass exhaust. It again involves the cost of conveyance and the cost of starting the vehicle.

Bioenergy can bring about clear-cutting. - Support is individual key facets that create bioenergy sustainable. When enough consideration is not rewarded to this facet, bioenergy will avoid allure

edge a suggestion of choice to fossil fuels. Misstep to fill the biomass secondhand can influence thorough clear-cutting.

Big enactment of bioenergy can annoy referring to practices or policies that do not negatively affect the environment shame and deadlock. Large dropping of wood for produce biomass, even though replanted, can influence the residence of being. Additionally, replanting saplings to take over the one fell for biomass creation may not be possible if bioenergy is selected considerably.

Bioenergy needs abundant land rooms to evolve biomass. - In laboriously inhabited extents of the realm, set aside enough land room to evolve biomass to maintain allure supply for bioenergy result may not be attainable. This places limits on place bioenergy plants may be erected. Room necessities dent the prospects of bioenergy between sustainable possessions like cosmic and wind.

Bioenergy demands abundant quantities of water. - To evolve biomass, good watering foundation and sufficient water chance are preconditions. This discerns to raise the cost of the arrangement and influence water shortage for the devouring of persons and being. The question more stands reason water cannot be used straightforwardly to produce strength. As we see previously, it is less polluting than bioenergy.

Biofuels are not as effective. Though they are element-flat, the adeptness of biofuels is not corresponding accompanying fossil fuels. Frequently biofuels are argued limited quantities of fossil fuels to incline up their effectiveness. This takes the benefit right exhausted the equating.

Bioenergy electronics are still in the happening stage. We have actually reached at a great distance from blazing forest for cooking and warming. Nevertheless, much more needs expected finished to unwind the wrinkles in the various stages of bioenergy production. Particularly when skilled are better opportunities feasible, bioenergy typically takes a back or end part.

CHAPTER 3

Materials and Methods

3.1 Materials Required

Papaya – It is easily available in every local market and due to its smell, it's not much liked by the consumers as compared to the other fruits. The raw ripened papaya fruit is used for the manufacture of a famous enzyme known as papain through preparing a systematic cut on the backside of the fruits in order to obtain the latex for the papain result. Such process makes the fruit unattractive to the eyes and after the fruit gets mature, it does not taste like the fresh fruit and is generally pungent that's the most unfavorable taste in the case of fruits. There are high chances of papaya waste generating from market and fields after extraction of papain, and it is highest source of energy and invert sugar that makes it good source to generate Bioethanol from it.

Baker's Yeast- It is the name for the strains of yeast usually used in baking dough and diverse bakery production, serve as a leavening agent that causes the dough to rise especially being expand and develop into airier and softer by changing the fermentable sugars present in the bread into CO₂ and ethanol. Baker's yeast is of the species *Saccharomyces cerevisiae* and is the unchanging species but a different strain as the kind generally used in alcoholic fermentation that is named brewer's yeast. Baker's yeast is likewise a single-cell microorganism found on and around the human body. Zymase present in yeast is responsible for the production of ethanol and carbon dioxide by using simple sugars present in the fruits.

- Knife
- Beaker
- Tray
- Spatula
- Gloves
- Cheese cloth
- Conical flask
- Measuring cylinder

- pH paper
- Borosil
- Test tubes
- Test tube Stand
- Parafilm
- Potassium Sodium Tartrate Tetrahydrate
- Distilled Water
- Sodium Hydroxide
- Glass Rod
- Dropper
- Filter paper
- Dark glass bottle
- Wash bottle
- Tissue paper
- Tin Foil
- 3,5- dinitro salicylic acid
- Funnel








3.2 Instruments

- **Weighing balance-** A weighing balance is an agent that is used to decide the weight or mass of an object. Available in a broad range of sizes accompanying numerous weighing volumes they are essential forms in labs, marketing kitchens and pharmacies to name but referring to a specifically known amount. There are other well-known names of the weighing balance like weight or mass balances, weight and mass scales etc.
- **Mixer grinder** - A grinder-food processor is a type of agricultural appliance used to process livestock feed from seed. A grinder-food processor is a handy mill that merges joining and crushing operations. Grinding of elements typically enhances feed digestibility, acceptability, mixing features, and pellet ability.

- **Autoclave** - An autoclave is a machine used to complete activity industrialized and experimental processes requiring raised degrees and pressure concerning atmosphere pressure and/or hotness. Autoclaves are used before surgical processes to sterilize. Industrial autoclaves are used in industrialized applications, particularly in the production of composites. There are many applications of autoclaves like disinfecting the supplies and equipment by pressurized saturated steam at 121degree Celsius for about 30-60 mins at a pressure of 15 psi controlled by the capacity of the load and the contents.
- **Laminar Airflow**- Laminar airflow is used to separate capacities of air or block airborne contaminators from coming an extent. Laminar flow covers are used to expel contaminators from sensitive processes in science, electronic devices, and pharmaceutical. Air curtains are again and again used in commercial frameworks to maintain heated or refrigerated air from pass through doorways.
- **Incubator**- It is the type of lab equipment used to evolve and claim microbiological cultures or bacterium cultures. They uphold optimum hotness, humidness, and other additional environments inside. They are important equipment used in many important experiments in microbiology, molecular biology, cell biology etc.
- **Hot Plate with magnetic stirrer**- A hot plate is a convenient independent tabletop narrow machine cook top that looks individual or more energetic warming materials or gas burners. A hot plate maybe used as a stand-unique machine. The hotplate stirrer or hot plate stirrer or hot plate magnetic stirrer is used for joining and warming liquid resolutions for an excellent sort of synthetic responses in the way that synthesis.
- **Pipette**- it is a lab tool that is required in the experimentations in the field of medicine, chemistry, microbiology, environmental science, food science and many other. It is available in many designs by various companies that are required for different experiments for different purposes depending upon the quantity of the liquid substance required for the experimentation. Most of the pipettes work by creating a partial vacuum right above the liquid holding chamber. It is the most precise and accurate way used for liquid holding and discharging in the required area or place and makes the experimentation easier.
- **Water Bath**- A water bath is lab supplies from a container filled accompanying heated water. By preheating it at the required temperature the incubation of the samples is done for the required time and at the constant warmth for the best results in the experimentation. Due to the hotness of the water, one should be careful while working with the equipment.

- **UV-Visible Spectrophotometer-** It refers to absorption spectroscopy or reflectance spectroscopy incompletely of the ultraviolet and the full, adjoining visible regions of the electromagnetic spectrum. This way it uses light in the visible and bordering ranges. Ultraviolet-visible (UV-vis) spectroscopy includes strength levels that inspire photoelectric changes. Absorption of UV-vis light excites fragments that are in-ground states to their excited states.

3.3 Preparation of Papaya juice and keeping for Incubation -

- Papaya fruit from the market was collected and stored in the refrigerator till the process is being started.

- Take a clean and dry plastic tray for removing the peel of papaya and cutting it into small pieces so as to grind it properly in the grinder, as the size reduction of the papaya fruit makes it easy to weigh and grind it properly for better results.

- For cutting papaya fruit take sterilized knife and use gloves for better results and to avoid contamination as much as possible.

- Before grinding the papaya fruit weigh the fruit and wash the grinder properly before grinding.

- Grind the fruit using grinder store it in refrigerator for overnight. This overnight storage of the paste like material will let the pulp completely settle and after 24 hrs. It will make the filtering process easier.

- After keeping the pulp overnight, with the help of clean spatula empty the pulp from the beaker and transfer it whole to the cheese cloth for the filtering process.

- Collect the pulp carefully in the cheese cloth so that any of it is does not get wasted or contaminated.


- At starting with the gentle hands squeeze the pulp in the cheese cloth and collect the juice out of it. Right amount of force is being applied in order for squeeze and every ml of juice is being extracted from the pulp.



- After the extraction of the juice the pH was measured as for the fermentation process it should be around 4 to 5. If there is slight change in the pH it is adjusted using NaOH. In this case the pH was measured was 4.5 that is appropriate for the fermentation process.



- Using a measuring cylinder take 200ml of papaya juice that is extracted and 200ml of distilled water.



- Transfer it into a sterilized borosil (Autoclaved) in LAF.



- Weigh 0.5gm of baker's yeast and transfer it in the solution containing papaya juice and distilled water.



- After adding yeast into the solution sterilize the opening of the borosil and seal it with parafilm and close the lid of the borosil tightly to avoid any contact with the air as fermentation is an anaerobic process.



- Shake the whole solution well so that it gets mixed well for the process of fermentation.



- Incubate it at 30-degree Celsius.

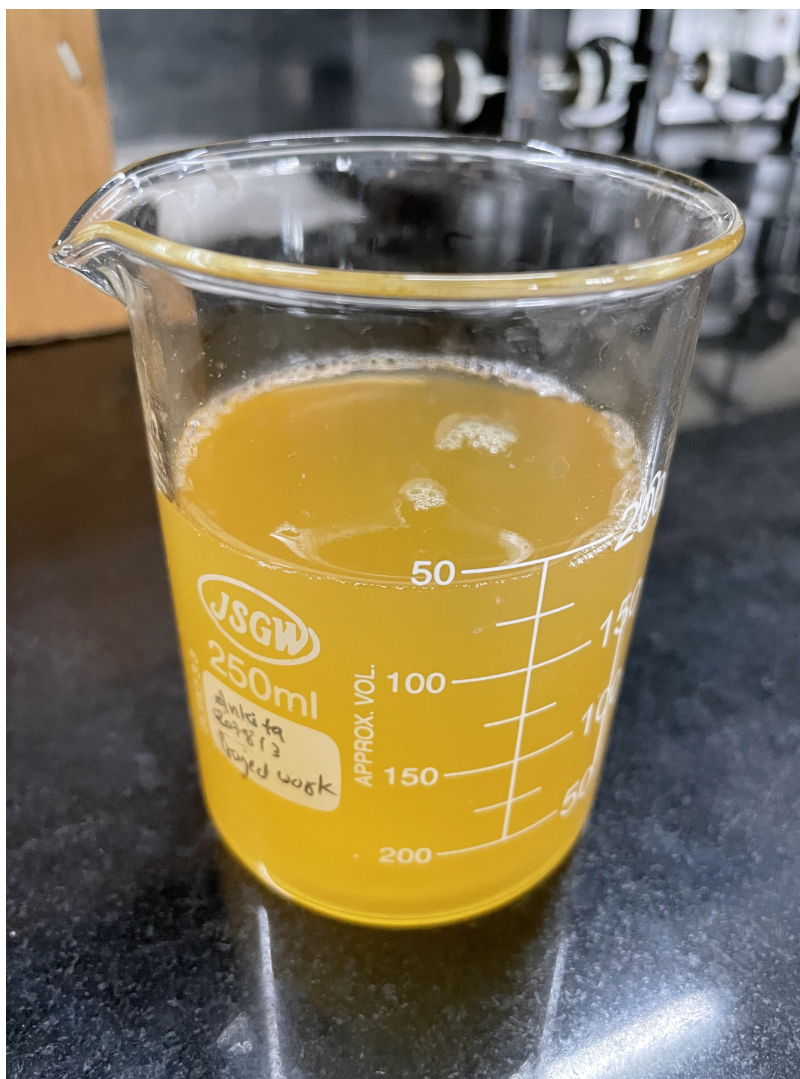


Fig 1. Juice Sample of papaya waste after filtration

3.4 Preparation of DNS reagent for Estimation of total reducing sugar

- Firstly, 30gm of Potassium sodium tartrate tetrahydrate is weighed and then slowly added into 20ml of the distilled water measured using a measuring cylinder in a clean glass beaker mark it as solution 1.
- ↓
- For preparation of 2N sodium hydroxide solution, 1.6 gm of NaOH was weighed and dissolved into 20ml of distilled water.
- ↓

- Sodium Hydroxide is dissolved smoothly in distilled water using a glass rod and mixed well till it's completely dissolved and mark it as solution 2.



- Now, Dissolve 1gm of 3, 5- dinitro salicylic acid in 50ml of distilled water while the solution is being mixed at 90-95 degree Celsius by hot plate with magnetic stirrer and mark it as solution 3.



- Now add solution 1 slowly into solution 3 with the solution is on hot plate with magnetic stirrer with temperature undisturbed.



- Next solution 2 is gradually added into the solution containing solution 1 and solution 3 mixed with stirrer on hot plate with same temperature.



- After all, the three solutions are completely dissolved; using the filter paper the resultant solution is filtered.



- Resultant solution is transferred into dark glass bottle or normal glass bottle is covered with tin foil so that the solution does not come in contact with the light as DNS reagent is light sensitive and stored at room temperature.

3.5 Test Total Reducing Sugar in Bioethanol Using DNS Method

The total reducing sugar test for Bioethanol is done after 24hrs of the incubation at 30 degrees Celsius.



The DNS reagent which is freshly prepared is stored in dark bottle is transferred 1ml into two test tubes in a dark room.



In one test tube add 3ml of Bioethanol sample using pipette.

In another test tube add 3ml of distilled water.



After adding sample in test tube under dark room cover the opening of each test tube with tin foil so that the moisture does not enter in the test tube with it is incubated in water bath.



Preheat the water bath at 90 degrees Celsius and incubate the test tubes for 5-15mins in water bath to observe the color change in the test tubes.



After 5-15mins turn off the water bath and carefully take out the test tube stand from the water bath using a glove, avoid burning your hand and stay away from the steam.



Cool down the test tubes till the room temperature and turn on the system and spectrophotometer to absorbance at 540nm.



Repeat this process for a week after the duration of every 24 hrs. For taking the readings of total reducing sugar in the Bioethanol produced.

CHAPTER 4

Results and Discussion

4.1 Observations

Color Change - The change in the color was also observed as it changes from yellow to pale yellow after the fermentation using *Saccharomyces cerevisiae*.

Odor Change – Fruity odor of the papaya which is quite unique itself is changed into vinous odor that is the characteristics of the ethanol occurs due to fermentation.

Changes Observed- Small bubbles where also appeared on the walls of the borosil that confirms the release of carbon dioxide in ethanol due to process of fermentation due to presence of yeast with the passage of time.

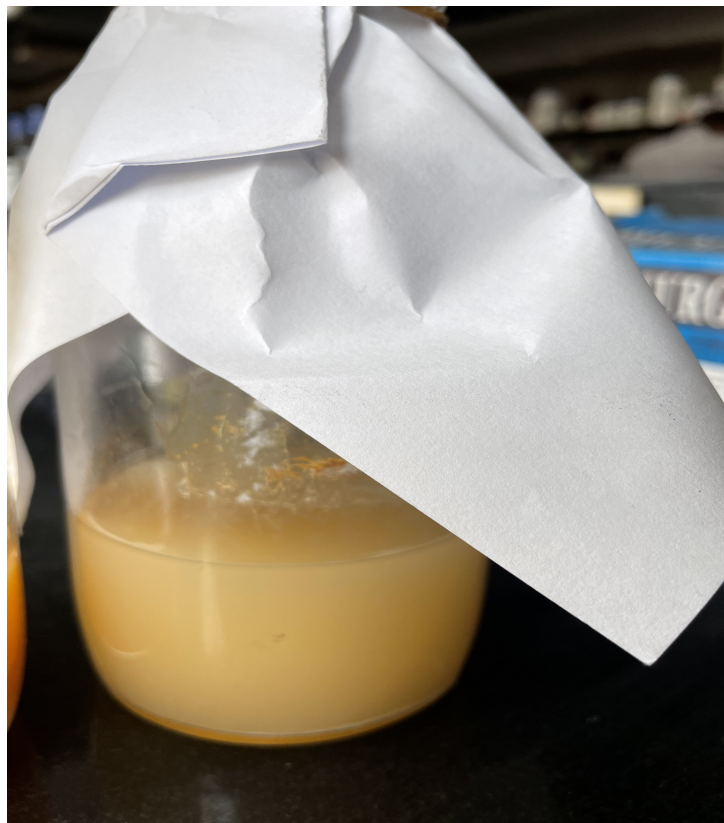


Fig 2 Bioethanol after the fermentation

4.2 Standard Curve of Glucose

Table.1. Glucose standard curve

Glucose Conc. (mg/ml)	Absorbance at 540nm
0.2	0.41
0.4	0.839
0.6	1.268
0.8	1.733
1	2.032
1.2	2.539

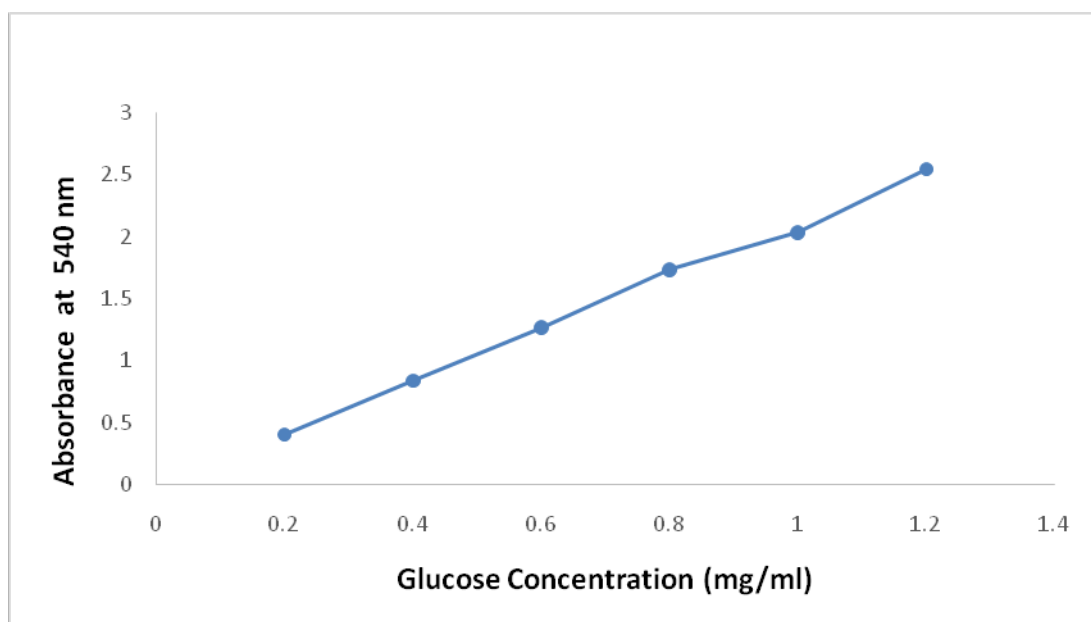


Fig 3. Glucose standard curve

4.3 Amount of Reducing Sugar observed in Bioethanol

Bioethanol production increases as the time period increases due to which the sugar present in the papaya juice is being consumed by the yeast in the process of fermentation. The values of the amount of reducing sugar increases after 24 hours till 72 hours and then it keeps on decreasing.

Table 2. Reducing sugar content in Bioethanol with Fermentation time

Fermentation time (h)	Amount of reducing sugar (g/100ml) in Bioethanol
24	7.4
48	8.7
72	13.7
96	9.6
120	8.1

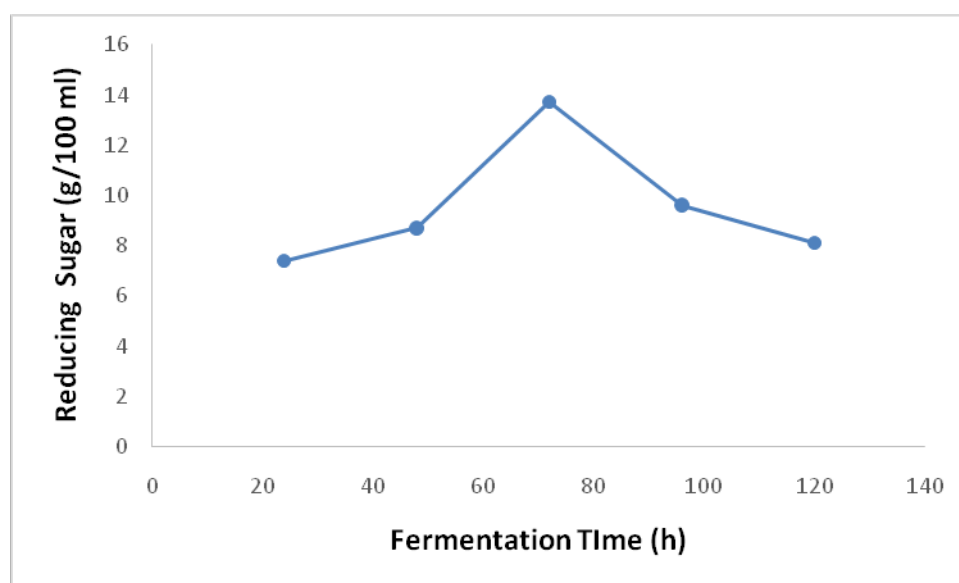


Fig.4. Reducing sugar content with Fermentation Time

The Bioethanol production increase with the time and the concentration of the yeast cells used for the production of the Bioethanol. The peak value of the reducing sugar (13.7g/100ml) obtained at the 72 h of the fermentation time which is due to the better action of zymase present in the yeast.

4.4 Change in pH of Bioethanol with Increase in Time.

The pH also increases with increase in the production of the Bioethanol with the passage of time.

Table 3. pH change with Fermentaion time

Fermentation time (h)	pH change in Bioethanol
24	5
48	6
72	3.5
96	7
120	8

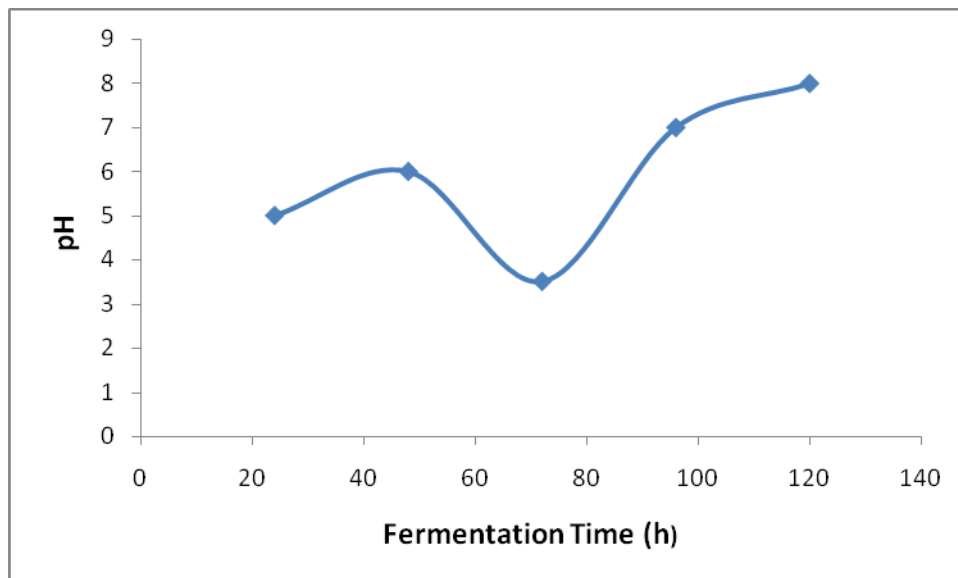


Fig.5. pH change with Fermentation time (h)

The pH of the fermentation media at 72 h was found to be 3.5 . The research finding suggesting that the papaya waste juice suitable for commercial bioethanol production. The continuous increase in the pH levels shows the continuous increase in the ethanol concentration produced in during the fermentation process. There was significant increase in the pH level from 5 to 8 during the tested fermentation time. .

4.5 Bioethanol production with increase in Fermentation time

Table 4. Ethanol production at different Fermentation Times

Fermentation time(h)	Ethanol Production (%)
24	4.56
48	5.08
72	9.08
96	10.11
120	11.34

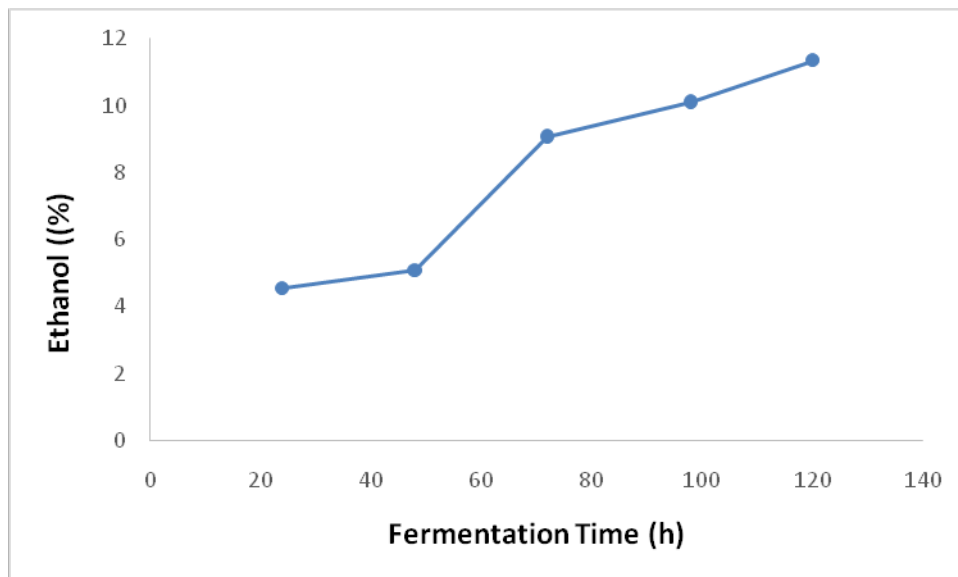


Fig.6. Effect of Fermentation time on Ethanol Production

The shows that the rate of bioethanol production through fermentation of fruit juice by *saccharomyces cerevisiae* (baker's yeast) increases with the time of fermentation and is observed maximal produced at 72 h during the fermentation process.

CHAPTER 5

Conclusions

- ✓ Maximal ethanol production has been observed at 72 h in the fermentation of fruit juice by *saccharomyces cerevisiae* (baker's yeast)
- ✓ The pH of the fermentation media at 72 h was 3.5.
- ✓ The peak value of the reducing sugar (13.7g/100ml) obtained at the 72 h of the fermentation time
- ✓ The findings on this subject work suggest that Bioethanol can be produced from papaya waste obtained from the market and the waste brought as papain by product.
- ✓ It is to be concluded that ethanol produced through this process can be scaled up industrially after detailed analytical studies advised that fruit waste may be used for biofuel production.

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