Biosynthesis for the Production of Bio Ethanol from Rice water.

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Abstract - **Ethanol is a commonly used chemical compound that is used in different sectors such as industry, foods, and beverages. Ethanol has good liquid fuel properties. The cost of fuel can be reduced if ethanol is mixed with liquid fuel. For this, it is very important to produce ethanol in a cost-effective manner. Ethanol can be manufactured from various agricultural raw materials like potato waste, molasses, banana waste, waste food grain, etc. Rice water waste is a rich source of starch, which can be easily broken down into reducing sugars, and thus can be used to improve the process of bioethanol production. Amylases have a central role in the bio-conversion of starch to bioethanol. Rice water waste, in the form of liquefied gel, can be easily attacked by amylases, which is an advantage, as it is found in nature as insoluble, non-dispersible, crystalline granules resistant to enzyme breakdown. Rice water can be a good source for bioethanol production, as it contains high levels of carbohydrates like glucose and starch. Bioethanol produced from rice water can be used as an alternative fuel source in various industries. For example, it can be used as a gasoline additive to reduce emissions from vehicles, or as a fuel to run machinery and generators. Additionally, bioethanol is a renewable and sustainable energy source, as it is produced from renewable plant material rather than fossil fuels. In conclusion, rice water can be an excellent source for bioethanol production, as it is a readily available and abundant source of carbohydrates. Bioethanol produced from rice water is a sustainable and eco-friendly alternative to traditional fossil fuels and has the potential to play a significant role in the future of renewable energy. Currently, most of the rice water from granaries is treated and disposed of. This can be made to good use by fermenting it into the wort and distilling out the ethanol from it.**

Index Terms- **Molasses, Enzyme, Bioconversion, Amylase, Enzymatic hydrolysis, Fermentation, Distillation, wort.**

I. INTRODUCTION

Ethanol, also known as ethyl alcohol, drinking alcohol, or grain alcohol, is a flammable, colorless, mildly toxic chemical compound, and is best known as the alcohol found in alcoholic beverages. It is a simple alcohol with the chemical formula C_2H_5OH. Natural energy resources such as petroleum and coal have been consumed at high rates over the last decades. The heavy reliance of the modern economy on these fuels is bound to end, due to their environmental impact and the fact that they might eventually run out. Therefore, alternative resources such as ethanol are becoming more important (Kulkarni et al., 2015).

Ethanol is a volatile, flammable, clear, colorless liquid. Ethanol is a good solvent. It is also used as a germicide, beverage, antifreeze, fuel, depressant, and chemical intermediate. Acting as the fourth main crop behind rice, wheat, and maize, starch plays an important role in the human diet all over the world. With the deepening of starch processing industries, complete utilization of the raw material shows more and more importance for the urgent demand for reducing feedstock waste and releasing the environmental pressure from rice residue. Therefore the whole production is replacing traditional starch extraction, which accelerates the staple food strategy of starch (Borglum, 1980).

The production of bioethanol from rice water is a multi-stage process that involves several key steps, including:

(1) Rice water collection: Rice water, also known as rice starch wastewater, is a byproduct of rice processing. It is collected from rice mills and factories where rice is washed, soaked, and milled to remove the husk.

(2) Pre-treatment: The collected rice water undergoes pre-treatment to remove impurities and solid particles. This is done through a series of filtration and sedimentation processes.

(3) Enzymatic hydrolysis: In this step, enzymes are added to the treated rice water to break down the complex starch molecules into simple sugars like glucose and maltose (Axelsson, 2011).

(4) Fermentation: The sugar-rich liquid is then fermented using specialized yeast strains that convert the sugars into ethanol and carbon dioxide. This process takes several days to complete, depending on the desired ethanol concentration(Neves et al., 2006).

(5) Distillation: Once fermentation is complete, the liquid is distilled to separate the ethanol from the rest of the mixture. This is done by heating the liquid to evaporate the ethanol, which is then condensed and collected as bioethanol.

The resulting bioethanol can be used as a clean, renewable fuel source in a wide range of applications, including transportation, heating, and electricity generation. The production of bioethanol from rice water not only reduces waste but also cuts down on greenhouse gas emissions and promotes sustainability. Moreover, it opens up new opportunities for economic growth and job creation in rice-producing regions around the world (Duruyurek et al., 2015).

According to the current energy demand, environmental status, depletion of fossil fuels, and hike in oil prices, there is an urgent need to search for alternative fuels which are renewable, cost-effective, eco-friendly, and have fewer greenhouse gases emission. Bioethanol is one of the most promising alternatives to fossil fuels that can ensure energy security and address environmental pollution problems. Bioethanol is produced from agricultural residues, lignocellulosic and starchy biomass. Lignocellulosic biomass is cheaper and available in plenty but its conversion to bioethanol involves many steps and is expensive. The primary motive for producing fuel ethanol is to reduce the foreign exchange burden of oil imports (Lugani et al., 2020).

I.I RICE WATER

The usual way of preparing rice is boiling, a thermal process that gives it a lower digestibility as compared to instantiation, extrusion or expandation. In view of this fact, the possibility of biotechnologically improving boiled rice digestibility was investigated in a laboratory study. In this respect, boiled rice was solid-state fermented using a strain of Saccharomycopsis fibuligera, an amylase-producing yeast originating from ragi. Fermented rice was then analyzed from the point of view of its content in easily assimilable sugars, protein, amino acids, phosphorus, and vitamins from the B group (Duhan et al., 2013). Biochemical analyses revealed that fermented rice has a ten times higher content of reducing sugars than boiled rice, due to starch hydrolysis, while chromatographic studies proved that the fermented rice contains glucose, maltose, maltotriose and maltotetraose that are easily assimilable carbohydrates. Fermented rice has a protein content that is two times higher than that of boiled rice because it contains the yeast biomass, and is enriched in vitamins from B groups (B1, B2, and B6) that are synthesized by the yeast. Inorganic phosphorus present in rice doubles its concentration in fermented rice, which means that phosphorus bioavailability is increased. The sensorial profile of boiled rice is also improved by fermentation. This study proves the possibility to have a processing method that is relatively cheap, and practical, and of which the resulting product has good nutritive qualities and does not pose safety problems due to pure culture utilization as a starter.

II. MATERIALS AND METHOD

II.I Preparation of Rice Water

About 1 litre of rice water was collected from local hotels using sterile containers and subjected to enzymatic hydrolysis using Separate Hydrolysis and Fermentation (SHF) method. The collected rice water is filtered using filter paper and roughly 500 ml of the sample is measured for enzymatic hydrolysis as shown in Figure 1.

Fig.1. Rice Water

Fig.2. Hydrolysis of rice water

II.II Enzymatic hydrolysis

The contents of the beaker were heated to 80 C for 30 minutes by continuously stirring in a heater. Once the temperature dropped to 60 C, the rice water was allowed to cool before alpha-amylase (3 gram) (S.V Biotech Pvt Ltd, Kacheripady) was added and the beaker was kept warm until it reached room temperature. A thermometer was used to measure the temperature (Figure. 2).

The degree of hydrolysis of native starch in rice water depends on factors such as substrate concentration, type and concentration of the enzyme used, and the process conditions such as pH, temperature, etc. The higher enzyme concentration leads to higher fermentable reducing sugar content. Obviously, the same conversions could be achieved with lower enzyme concentration, although requiring longer times. The longer exposure of the enzyme to high temperatures needed for gelatinization of the starch granules and to achieve a good susceptibility to enzyme action could lead to slight enzyme deactivation.

II.III Fermentation

Starch hydrolysate obtained by enzymatic hydrolysis was subjected to ethanol fermentation. The prepared three samples with substrate concentrations 5%, 10%, and 15% (w/v) were taken in three conical flasks respectively. 2gm of Baker's yeast (obtained from local marker) measured using an electronic balance was added to each sample, mixed thoroughly, and cotton plugged. The mixture was allowed to ferment for 48 hours at room temperature in a dark place as shown in Figure. 3. Fermentation of sugars was carried out by *Saccharomyces cerevisiae* (Baker's yeast) at pH 4.5 – 7, 30 c, and 72 hours. The wort was distilled and the specific gravity and concentration of the distillate were plotted

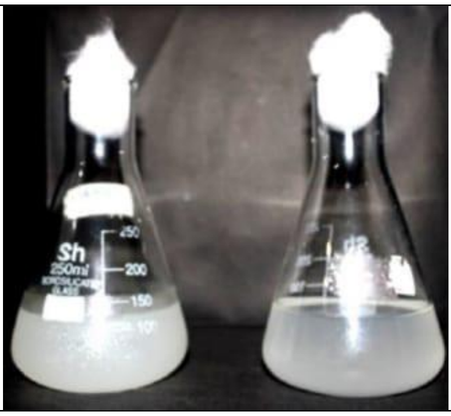

Fig. 3 Fermentation at room temperature.

Fig. 4 Distillation

II.IV Distillation

After 48 hours, the fermented wort samples were taken out from the conical flask and filtered out into a beaker using filter paper and a funnel. The filtrate was fed into a simple distillation column. The boiling temperature of ethanol is 78°C hence distillation was carried out around that temperature to facilitate the evaporation of ethanol. The vapor was collected and got condensed by means of the circulation of cold water around the column. The distillate having ethanol was recovered in a beaker at the other end of the column as shown in Figure. 4

III. RESULTS AND DISCUSSION

III.I Effect of pH

Yeast is found to be capable of fermenting glucose best in an acidic environment. The maximum yield was observed at a pH of 5.5. The yield was lower at an acidic pH range of 2 to 3 and even lower when the fermentation was carried out at a basic pH range of 7 to 8. At an acidic pH, there is always a chance of the death of yeast cells. This indicates that glucose metabolism is affected by the changes in pH which eventually affects the ethanol production

III.II Effect of Particle Size

As the particle size decreases the surface area available for the hydrolysis reaction is more, producing maximum glucose units that are possible eventually it is all fermented to ethanol. In this study, the ethanol yield was observed higher using an intermediate particle size (0.157mm). Using a bigger particle size reduces the degree of gelatinization as a result the substrate is not fully available for hydrolysis by the enzymes.

III.III Liquid State Fermentation

A substrate concentration of 10%(W/V) with a moisture content of 75% gave a better yield in both rice and water.

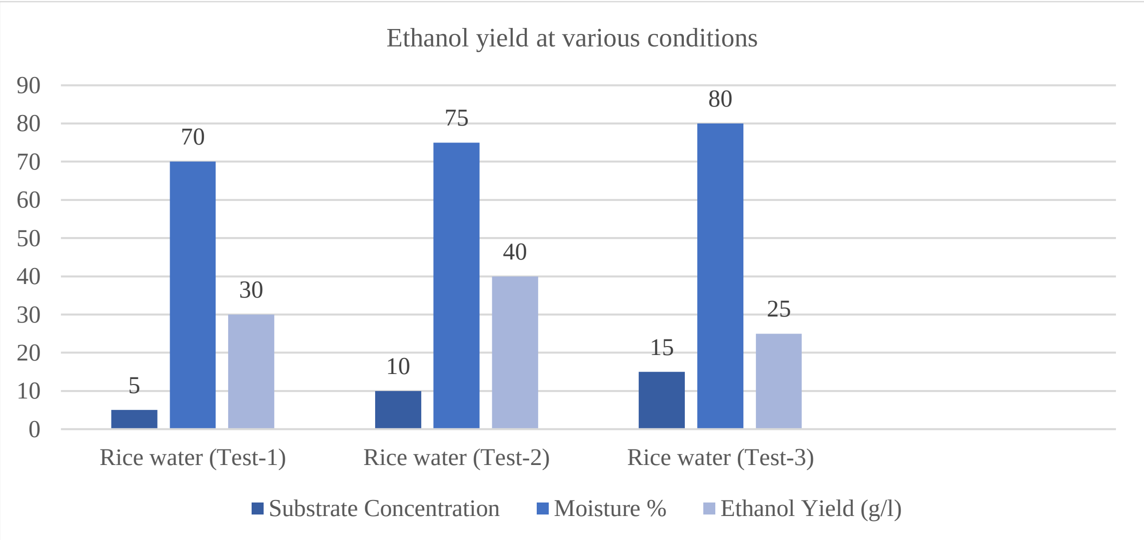

Fig. 5 Ethanol yield at various moisture conditions

The results indicate that the critical parameters for ethanol production from rice water were the enzyme combination, the dose, and the residence time of hydrolysis. After saccharification, fermentation by *S. cerevisiae* converted sugars to high yields of ethanol.

III.IV Specific Gravity Determination

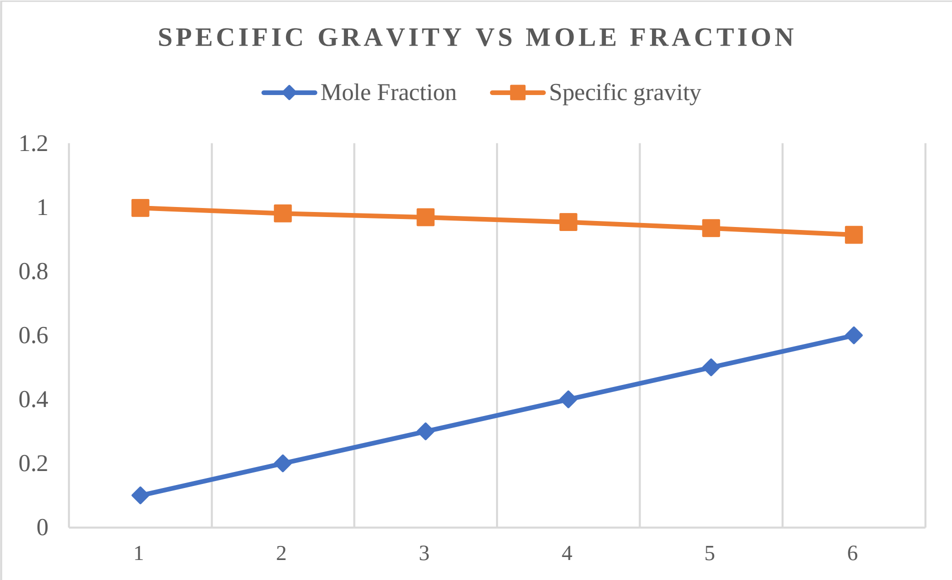

Fig 6. Specific gravity vs mole fraction graph

The above values are the specific gravities of ethanol used for comparison. Ethanol water mixtures are prepared in different concentrations such as 0.1 -1.0 etc.
The Bioethanol obtained from the fermentation of rice water was used to determine the specific gravity in which 10 ml of sample was diluted with 90 ml water and measured using a specific gravity bottle.

IV. CONCLUSION

Ethanol production from rice water is a relatively new topic and limited research has been conducted about the utilization of rice water for ethanol production. This study aimed to explore some of the optimal conditions of biofuel (bioethanol) production by Saccharomyces cerevisiae yeast (baker's yeast) from very cheap carbon source rice water. The impacts of fermentation periods and yeast extract concentrations were evaluated. The bioethanol was successfully produced . The optimum conditions for fermentation was 35C , Ph 5-7 and fermentation time of 72 hours in anaerobic condition. High yield of Bioethanol (25ml) was produced from 200 g of rice water.

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