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Waste Dried Leaves (Neem and Moringa) in the Production of Bioethanol using Saccharomyces Cerevisiae.

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Abstract:- Bioethanol is one of the most promising biofuel nowadays, considered it is feasible, sustainable and it is alternative to fossil fuels today. Bioethanol is a volatile and flammable liquid produced through microbial fermentation process. Ethanol is an important solvent and used in chemical feed stock, food and Pharmaceutical industries. Bioethanol reduce the greenhouse gas emissions from transport and other industries. The use of edible plants for biofuels production has recently been of great concern because they compete with food materials. This is an attempt to investigate the viability of sourcing bioethanol from a readily available neem and drumstick leaves. The population of human being is increasing on the average worldwide, hence the demand for energy source increases. In order to avoid these foreseen worrisome, lignocelluloses biomass should be utilized in the production of bio-ethanol and biofuels in general. It is apparent that current fuel bioethanol production from grain-based feedstock is not favorable as it may lead to food shortage to the teaming world populace. This showed that the neem tree leaves and drumstick leaves, made of lignocellulose materials, contain some appreciable percentage of sugar that can be fermented to produce ethanol. Immobilizing the yeast with cheap supporting materials is another strategy to optimize the production process in less cost manner. . Since the large quantities of agro waste are available from plantations, their disposal can be a problem. Therefore, lignocellulose pretreatment and the yeast fermentation technology are still an area of research interest for the second generation fuel production. This showed that the neem tree leaves and drumstick leaves, made of lignocellulose materials, contain some appreciable percentage of sugar that can be fermented to produce ethanol.

Keywords:- *Bioenergy, biofuel, sustainable, renewable, biomass, yield, bioethanol, microbes, yeast, enzyme, fermentation, sugar.*

I. INTRODUCTION

However, ethanol produced from renewable energy sources is one of the most promising biofuels for the future. Although bio ethanol fuels can be manufactured using the chemical reaction between ethylene and stream, it is mainly produced through fermentation of sugars derived from crops containing starch, such as corn, wheat, sugar cane, sorghum plants, etc. It is currently used in the fuel industry as an additive for petrol. It is a high octane fuel and has replaced lead as an octane enhancer in petrol. Blending ethanol with petrol oxygenates the fuel mixture so that it burns completely and reduces harmful emissions. The most common blend is 90% petrol and 10% ethanol. Bioethanol is entirely comprised of biological products, and hence the combustion of bioethanol results in cleaner emissions (carbon dioxide, steam and heat(Yusuf Muhammad et al.,2016). Carbon dioxide is absorbed by plants and processed via photosynthesis to help the plant grow. This cycle of creation and energy combustion means bioethanol could potentially be a carbon neutral fuel source. . Recently, as to address the uncertain fuel supply, in an efforts to reduce carbon dioxide emissions and to provide feasible alternative to fossil transport fuel all over the world bioethanol production has attracted the attention of the world. Bioethanol can be produced from various sources, like starch crops, sugar crops, household waste, agricultural waste, fruit juices, fruit wastes etc. Among these sources, non-edible source seems to be the best options

II. AIM

This study is to investigate the production of bioethanol from waste things like dried neem and drumstick leaves using Saccharomyces cerevisiae and to check the presence of bioethanol from these followings.

Sample Collection and Sample Preparation.

Pre-treatment of the sample with Acid.

Reducing sugar determination.

Fermentation process.

III. MATERIALS AND METHOD

A. Sample Collection And Sample Preparation

Dried leaves of neem and moringa leaves were collected in polythene bags and taken to the analysis at Karunya institute of technology and sciences, Coimbatore. The Saccharomyces cerevisiae used(dried baker's yeast). Dried neem and the moringa leaves were grounded to powder followed by sieved.

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B. Pre-Treatment of The Sample With Acid And Fermentation Process

Thirty (30g) of neem and moringa powdered sample leaves were taken into three conical flasks and to it 300ml of H_2SO_4 was added. Then the flasks were cotton plugged and wrapped with aluminium foil sheet and it was autoclaved at 121ºC for 15 minutes. The mixture changes its colour from light green to red brown after autoclave at 121°C. Mixtureis been filtered. Then the pH is adjusted to 4.5. 3g of *S. cereviciae* is taken into the flasks and then it incubated at 37°C for five days. After that the fermented broth was distillated with the round bottom distillation coloumn enclosed with a running tap water and the temperature was set upto 78.3°C. After this, the distillate was collected in the other end of the distillation coloumn.

C. Reducing Sugar Determination

For reducing sugar determination glucose as standard were prepared at the rate of(0.5,1.0,1.5,2.0,2.5)in standard. Then the samples were treated with 300ml of dinitrosalicylic acid (DNS) reagent as 3ml in each test tube including test and blank. Distilled water is added to (3ml in blank)(2.5 , 2.0 , 1.5, 1.0, 0.5)(1.5)in 3 test samples. Then the optical density is been tested (Miller G.L., et al 1972). Absorbance of this mixture portion from each test tube was measured using UV-Visible spectrophotometer at 540nm. The corresponding concentrations of the samples were extrapolated with the standard glucose curve.

D. Qualitative And Quantitative Test For Ethanol

2drops of acidified (0.1ml $K_2cr_2o_7$) is added to the2mlof distillate which is collected from the round bottom flask of distillate. After 30min of waterbath, the appearance green colour determines the presence of ethanol. The quantity of ethanol produced was determined using potassium dichromate VI an oxidizing reagent in whereby the ethanol will be oxidized to ethanoic acid. This was carried out using UV-VIS quantitative analysis. 1cm3 of absolute ethanol (98% v/v) was to prepare series of standard solution. The content of each test tube was then heated in water bath for five (5) minutes, for the colourto develope. The absorbance of each concentration was measured at 585nm using UV-VIS Spectrophotometer and the reading was used to develop standard ethanol curve. Consequently, Five (5cm3) of each of the sample were taken in the test tubes, and then 2ml of Dichromate reagent was added to each. The content of each test tube was then heated in water bath for five (5) minutes, for the colour development. The absorbance of each concentration was measured at 585nm, using U-V Visible spectrophotometer.

IV. FTIR SPECTROSCOPY

The samples of bioethanol produced were analyzed using MB 3000 FTIR Spectroscopy machine to determine the vibrational frequencies of the bioethanol produced. The results in Table1 indicated that 5% acid hydrolysis gives the highest reducing sugar (3.20g/kg), followed by 2% (1.80g/kg) and 10% (1.23g/kg) which gives the lowest concentration. The significant variation was observed ($P< 0.05$), hence using 5% acid hydrolysis is the optimum amount required for the break down the complex sugar contained in the neem sample. However, no significant ($P>0.05$) variation was observed using either 2% or 10%, however there is numerical differences. The highest yield bioethanol obtained that is using 5% acid hydrolysis were subjected to FTIR Spectroscopy analysis, the samples shown strong broad peak at 3450 - 2850cm-1, therefore indicating -CH2- and -CH3 stretching vibrations and well resolved Peak around 3416cm-1 can be assigned to alcoholic -OH vibrations. These values are in agreement with the values obtained According to Spectra (2014) free O-H stretching normally occurs at 3550 – 3200 cm-1, while C-H stretch occurs at 3000 – 2840 cm-1. Therefore, the production of ethanol was successful. The results obtained from the present study can be concluded that 5% H2SO4 acid hydrolysis gave highest concentration of ethanol production. This indicates that, Baker's yeast (*Saccharomyces cerevisiae*) is a suitable fermenting agent in the production of bioethanol using neem leaves sample. Production of ethanol from ligno-cellulosic materials has received extensive interest due to their availability, abundance and relatively low-cost. Neem leaves (*Azadirachtaindica*) is therefore an abundant and sustainable biomass and non-food material that could be exploited for bio-ethanol production especially in the northern part of the Nigeria. Neem leaves (*Azadirachtaindica*) however could serve this purpose since from the study it is indicated that with proper pretreatment and appropriate method bio-ethanol could be obtained.

V. RESULTS

The result showsto FTIR Spectroscopy analysis, the samples shown strong broad peak at 3450 - 2850cm-1, therefore indicating -CH2- and -CH3 stretching vibrations and well resolved Peak around 3416cm-1 can be assigned to alcoholic -OH vibrations. These values are in agreement with the values obtained According to Spectra (2014) free O-H stretching normally occurs at 3550 – 3200 cm-1, while C-H stretch occurs at 3000 – 2840 cm-1. Therefore, the production of ethanol was successful. Appearance of green colour indicates the presence of ethanol in quantitative and qualitative test. hence, the amylase treated Moringa leaves the maximum ethanol yield were noticed as 1% and from neem the ethanol yield was found to be 5% respectively . .Carbohydrates are rich in powdered leaf of both neem and moringa so the test on reducing sugar was made shows it shows that the moringa and neem and moringa mixture gives the concentration at the 540nm absorbance in uv spectroscopy. Since these test determines that the bioethanol is present in the test samples

VI. CONCLUSION

Due to the increase in population, the demand for fuel increases. The fuel used should be environmental friendly. So using these type of waste dried leaves can be used in the production of promising biofuels.

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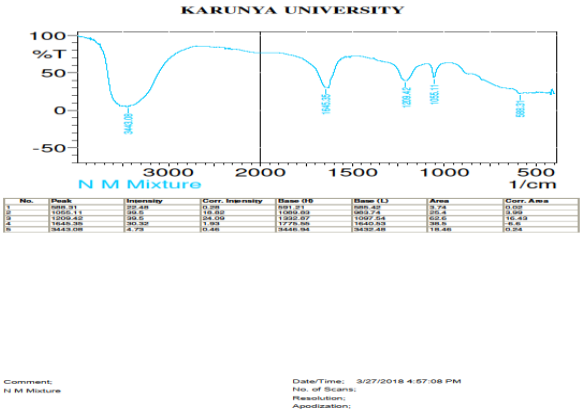
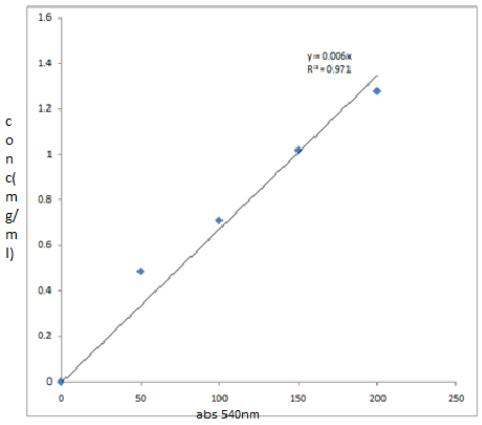


Ftir Test for Neem and Moringa Mixture.



Reducing Sugar Test

Quantitative and Qualitative Test