SYNOPSIS

1. Bio-chemical conversion of Water Hyacinth (*EichhorniaCrassipes*) to bio-ethanol.(37S_B_BE_005)
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4. Keywords: Water Hyacinth (EichhorniaCrassipes), Bioethanol.
5. **introduction**

Bioenergy is a renewable energy and is produced by using various biological organisms. Bioenergy is expected to solve the global warming problem by decreasing the carbon dioxide levels in the atmosphere. Bio-ethanol is one of the most promising replacements for fossil fuel since it is renewable and emits 85% less green-house gases compared to gasoline.Water bodies are complex ecosystems and may include a variety of water plants that contribute to water quality and environmental health. Water hyacinth is one of the world's worst aquatic weeds. It infests rivers, dams, lakes and irrigation channels on every continent except Antarctica.

6. **objectives**
   The main objective of the study is to extract the bio-ethanol from Eichhorniacrassipes (Water Hyacinth).

SPECIFIC OBJECTIVES:
To characterize Eichhorniacrassipesforphysico-chemical and biological parameters.

To carry out the experimental studies of hydrolysis and production of ethanol by baker's yeast.

To find out the efficiency the bio-ethanol extracted by characterizing for different physico-chemical parameters.

7. Fresh water hyacinth was collected from Dantaramakki Lake, Chikmagalur. and these chopped pieces were taken in different forms.
8. The preparation of water hyacinth is as follows:

- The water hyacinth with leaves and stalks was thoroughly washed several times with tap water to remove adhering dirt, chopped into small pieces (1 to 2 cm)
- This sample was divided into 2 forms: one is sun dried and other is grinded into fine paste. Then again fine paste is dried in 3 forms that is sundried, oven dried and auto claved.
- After drying, add 1 - 5% sulfuric acid, after adding heat the sample at 121˚c for 15 mins, later cool at room temperature, after cooling filter it, hydrolysate is obtained.
- The pH of hydrolysate should be adjusted using 10M NaOH, then add 0.2gm yeast for 6 days at 30 ˚c, finally determine quantity and presence of alcohol.

The above procedure shows the production of ethanol. Water hyacinth was divided into four samples and they were subjected to hydrolysis, detoxification and neutralization followed by fermentation using baker's yeast. Fresh Water Hyacinth with long stems was collected from Lake Dantaramakki of Chikmagalur. The Water Hyacinth was thoroughly washed several times with tap water to remove adhering dirt, chopped into small pieces (~1 to 2 cm) and

- One half was sun dried for two weeks and powdered. The powdered material was stored at room temperature until use.
- The other half was ground into fine paste.
- One portion was sun dried

Another was placed in hot air oven at 70^0C for 6 hrs

And the last portion was autoclaved for a period of 6 hours at 105^0C

- **Acid Pretreatment**

1gm of the sample (dried Water Hyacinth) is mixed with 1,2,3,4 and 5% of sulfuric acid solution to a final volume of 100 ml. The samples will be poured to a Pyrex bottle. After that the mixture will be autoclaved at 130°C for 15 min and further cooled down to room temperature. The hydrolysatewillbe filtered to remove the solid parts (not digested) of the material. The filtrate will be collected and subjected to analysis of sugar content.

- **Sugar Estimation**

Total reducing sugar is estimated by using dinitrosalicylic acid (DNS) reagent. 3ml of dinitrosalicylic acid (DNSA) reagent is added to 3ml of hydrolyzed sample in a test tube. The mixture is heated at 90ºC for 5 to 15minutes to develop the red brown color. Further 1ml of 40% sodium tartarate is added to stabilize the colour. After cooling at room temperature in cold water bath, record the absorbance with spectrophotometer at 540 nm.

- **Detoxification of Hemicellulose Acid Hydrolysate**

Detoxification is done by adding calcium hydroxide and it will be then overlimed with solid NaOHto neutralize the solution.

- **Alcohol fermentation**

About 0.2gm of baker's yeast is used for inoculation of the concentrated hemicellulose acid hydrolysate. Fermentations are carried out the petridish and the media is sterilized by autoclaving at 120°C for 20min. The pH is maintained at 6.0 with 1N HCl and 1N NaOH.The fermentation temperature was kept at a constant value of 30°C by a temperature control incubator.

- **Extraction of ethanol**

The sample was distilled and the distillate was tested for its purity.

TEST FOR ETHANOL

Add 10 drops of the distillate into a test-tube.

1 Add 25 drops of iodine solution.

2 Add 10 drops of sodium hydroxide solution.

3 Gently swirl the test-tube a few times. The dark colour of the iodine should start to fade.

The solution in the test-tube becomes cloudy and a yellow precipitate of triidomethane (iodoform) is seen in case there is presence of ethanol. It has a distinct 'antiseptic' smell. In case of presence of methanol solution remains clear.

9. **Results and conclusions**.
    - From the analysis of the sundried sample we observed that maximum absorbance is observed at 4% concentration of sulfuric acid and minimum at 1% and 2% concentration of sulfuric acid.
    - From the analysis of the oven-dried sample we observed that maximum absorbance is observed at 5% concentration of sulfuric acid and minimum at 1% concentration of sulfuric acid.
    - From the analysis of the sundried sample we observed that maximum absorbance is observed at 4% concentration of sulfuric acid and minimum at 1% and 2% concentration of sulfuric acid.
    - From the analysis of the autoclaved sample we observed that maximum absorbance is observed at 2% concentration of sulfuric acid, minimum at 5% concentration and at 3% there is no absorbance of sulfuric acid.
    - From the analysis of the finely powdered sample we observed that maximum absorbance is observed at 2% concentration of sulfuric acid, minimum at 4% concentration and at 2% there is no absorbance of sulfuric acid.
    - Finally from this studies we concluded that maximum reducing fermentable sugars was formed from oven-dried samples and also it gives most convincing result in extraction of bio-ethanol.

10. **Scope of future work**

    As more percentage of sulfuric acid used for hydrolysis, formation of reducing fermentable sugar will be more and hence extraction of bio-ethanol will be in favorable amount.
        - We can speed up the reaction by using certain type of enzymes. Fluctuations of pH can be prevented by using peptone water as buffer.
        - If the quantity of extracted ethanol is in a larger quantity, the efficiency of the blend can be analysed.