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# UTILIZATION OF EICHHORNIA CRASSIPES IN BIOFUEL PRODUCTION

Biological Science

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## ABSTRACT

The world's worst, troublesome and fastest growing aquatic weed Eichhornia crassipes (Water hyacinth) can be potentially beneficial in the production of six carbon compound glucose - Biofuel of living beings. The concept of eradication through utilization is being attempted to overcome the problems caused by the weed. A potent gram positive species Staphylococcus makes use of branched polymer cellulose and degrades it into simple glucose units which can be further processed for bioethanol production. The present study is to identify the maximum yield of glucose that can be measured quantitatively using appropriate method (3, 5 dinitrosalicylic method for glucose estimation) with varying substrate concentration (0.5, 1.0, 1.5, 2.0, 2.5, 3.0). We observed maximum amount at increased substrate concentration that obeys Michalis menton and Line weaver Burk plot.

## KEYWORDS:

Aquatic weed, Staphylococcus, glucose, cellulose.

## 1. INTRODUCTION

Increased energy demands and limited resources are the consequences of industrial development and population growth. The world-wide energy consumption has increased 17 fold in the last century [1]. The conventional energy resources, like fossil fuels cannot meet the increasing energy demand. All these effects have a considerable negative environment e.g. increased greenhouse gas emissions. Therefore, one of the challenges for the society is to meet the growing demand for energy [2]. These problems make it urgent to develop alternative energy resources that are both renewable and environmentally friendly. Our energy systems will need to be renewable and sustainable, efficient and cost-effective, convenient and safe.

The aquatic weed, Eichhornia crassipes commonly referred to as Water hyacinth is a native plant of Brazil but has been naturalized in many tropical/temperate countries. Its rate of proliferation under certain circumstances is extremely rapid and it can spread to cause infestations over large areas of water causing a variety of problems. Examples are the destruction of ecosystems, irrigation problems and increase in mosquito populations [3]. These negative effects therefore, on one hand, attempts have been geared towards the use of biological, chemical and mechanical approaches for preventing the spread of, or eradication of, water hyacinth.

Water hyacinth has been identified by the International Union for Conservation of Nature (IUCN) as one of the hundred most aggressive invasive species [4] and recognized as one of the top ten worst weeds in the world [5]. Besides this water hyacinth contains 20% cellulose , 10% lignin, and 33% hemicelluloses [4]. So this high content of cellulose makes it favorable for sugar production. In our study, we compared both Gram positive (Staphylococcus, Bacillus) and Gram negative species (Pseudomonas) [6]. Depending on the experimental setup subjected to desired pH 6, room temperature, plant extract and CMC (Carboxy Methyl Cellulose) as control, Staphylococcus was found to be more potent in degrading cellulose than compared to the other two species. Thus we produced fermentable sugar using appropriate method which is the initial step to produce bioethanol.

## 2. MATERIALS AND METHODS

### 2.1. Sample collection

Fresh Water hyacinth plant was collected from Ukkadam lake, Coimbatore. Leaves were washed to remove dirt, blotted using filter paper and chopped into small pieces.

### 2.2. Sample processing

#### Drying

Leaves were dried in hot air oven at 105°C for one and half hours [7]. The dried leaves are then blended in a mixer to obtain powdered form. The net weight of the plant powder is found to be 11.330g.

#### Sample Extraction

Cellulose is a huge polysaccharide polymer and because of its high molecular weight and cyrstalline structure it is insoluble in water and has poor ability to absorb water. The combination of ethanol and toluene (1:2) is found to more significant solvent for removing cellulose from plant cell wall [8]. 10% of water hyacinth extract is prepared in this mixture which is used as a substrate to be utilized as carbon source by microorganisms and also used for the enzyme assay.

### 2.3 Microbial selection

Three bacterial species- Bacillus, Pseudomonas, Staphylococcus were collected from Sri Ramakrishna hospital, Coimbatore and made to grow in CMC agar medium. CMC agar medium is prepared according to standardized procedure as included by ATTC. This agar medium is used as control to compare with the test medium containing plant extract. The pH of the medium is adjusted to 6.

#### Inoculation

After sterilization process, the three species is inoculated and kept at room temperature for 2-3 days. After 3 days it is checked for the clear zone which is a result of enzymatic hydrolysis. This is compared with the control and the organism showing maximum hydrolytic activity is selected for further assay.

#### Congo red clearing zone assay

This staining procedure is done to view zone of hydrolysis clearly which enhances quick and easy comparison. 0.1% Congo red solution is spread on media and left for 15 minutes with intermittent shaking. It is then destained with 1M sodium chloride solution. Thus, clear zone is visible.

### 2.4 Enzyme extraction

Prepare CMC broth (control) and the broth containing plant extract (test), autoclave for 20 minutes and the pH of the medium is adjusted before inoculating the organism. After inoculation, the broth is incubated at 37°C and kept overnight. Turbidity can be seen as a result of microbial growth after the incubation of 24 hours. It was then centrifuged at 1000 rpm for 25 minutes and the supernatant was

collected which contains the enzyme cellulase.

### 2.5. Quantitative estimation

3,5- Dinitrosalicylic acid is the best method employed for glucose estimation [9]. The amount of cellulose present in the plant sample is identified quantitatively by increasing the substrate concentration and measuring the activity of enzyme in response to increased substrate concentration.

## 3. RESULTS AND INTERPRETATION

Combination of biological and chemical methods is used for preventing the spread of Water hyacinth [10]. Cellulose biomass is being investigated as a potential substrate for bioethanol production [11].

### Comparative study of cellulose degrading bacteria

**Fig1: Bacteria showing maximum Cellulolytic activity**

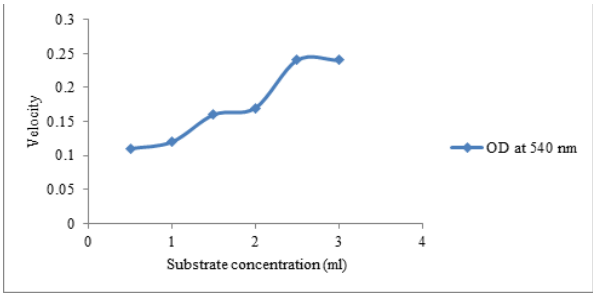
Cellulose present in Water hyacinth is used as carbon source which is utilized by the bacteria and clear zone can be seen as a result of cellulose breakdown. Three bacterial species namely Bacillus, Pseudomonas, Staphylococcus are compared among which Staphylococcus a gram positive species showed maximum zone of hydrolysis (Fig 1). CMC agar is used as control.

### DNS assay for cellulose quantification

**Table 1: DNS Method for cellulose estimation**

| Volume of substrate(ml) | OD at 540 nm |
|---|---|
| 0.5 | 0.11 |
| 1.0 | 0.12 |
| 1.5 | 0.16 |
| 2.0 | 0.17 |
| 2.5 | 0.24 |
| 3.0 | 0.24 |

From the above data obtained, it can be observed that as the concentration of plant sample is increased, the rate of enzyme catalyzed reaction also increases with time which enhances maximum product formation. This follows first order reaction and therefore straight curve is obtained. After certain point of time the increase in substrate concentration dose not enhance the increase in reaction rate, thus Vmax is achieved indicating the saturation point. This follows zero order reaction and curve obtained is hyperbola which is shown below:




**Graph 1: Effect of substrate concentration on the activity of Cellulase**

The above figure shows the enzyme kinetics of Cellulase isolated from Staphylococcus. Cellulase from Staphylococcus was used for the Michalis-Menton kinetics. The enzyme specific activity of isolated enzyme was determined at various concentrations of substrates and the km value was determined as 1.1*10-2 M. Based on the activity of enzyme the amount of substrate present in the plant sample can be calculated, as the product formed is directly proportional to enzyme-substrate concentration.

This quantitative test is mainly carried out to evalulate the conditions for the saccharification process with microbial enzymes[12].

## 4. CONCLUSION

Tremendous progress has been made technologically in the last few years in the area of biofuel production, fuelled by ever increasing price and shortage of fossil fuel. There are also concerns about global climate change and severe food shortage. Biomass is the least expensive and most globally available resource. Therefore, priority should be shifted towards utilizing biomass, leaving aside food for human consumption.

New methodologies of fermentation and hydrolysis of biomass have become available, along with development of transgenic varieties amenable for biofuel production. In our study, we observed staphylococcus as the potent cellulose degrading bacteria which is the unique work done. The substrate concentration 1.1*10-2 M which was evaluated using DNS method is found to be efficient for bioethanol production.

5. APPENDIX
No. of Tables: 1
No. of Figures: 1
No. of Charts: 1
No. of Words: 1772
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