An Ecofriendly Approach to Utilize Water Hyacinth for Ethanol Production

Anurag Das & Pranjan Barman

Abstract— Water hyacinth is a free floating perennial aquatic plant, native to the amazon basin. It is considered as an ornamental plant in some European countries. However, in tropical countries like India, china, Bangladesh etc., due to its high replicating capability, invasive nature, and adaptability to a wide variety of fresh water aquatic ecosystem, it is considered as a noxious weed and poses a serious threat to the ecosystem. Because of its biological structure, low lignin content and high cellulose and hemi-cellulose content, it can be sustainably managed and utilized in highly promising biofuel production. The current study reports a method to utilize the cellulose and hemicellulose content of water hyacinth for ethanol production by strong acid hydrolysis followed by fermentation. With further studies, there are possibilities that the method can be made commercially viable.

Index Terms— Eichhornia crassipes Maritus, Aquatic plant, Biofuel, Ethanol, Hydrolysis, Fermentation

1 Introduction

Water Hyacinth (Eichhornia crassipes Maritus) is a fresh water, free floating, monocotyledonous perennial aquatic plant, which belongs to the pontederiaces family, related to the lily (liliaceae family) and native to the amazon basin. It is a renowned ornamental plant and can be found in various water garden all over the world. In South East Asia water hyacinth is a highly invasive aquatic plant found abundantly in water bodies. Although Water Hyacinth is considered as a ornamental plant, it can become a significant hazard by overgrowth [1]. Shoeb and Singh (2002) observed that under amiable circumstances it can achieve a growth rate of 17.5 metric ton per hectare per day [2]. It is highly invasive species, which invades fresh water habitats (Fig.-1) and is listed along with some worst weeds [3]. Many countries have placed this species in their quarantine zone and prohibited their sale or movement within their territory [4]. Water hyacinth is very laborious to exterminate by physical, chemical or biological methods and a significant amount of capital is utilized on its control globally per year [5].

On the other hand, Water hyacinth is an effective bioenergy crop due to its high cellulose content with low lignin content, easily degradable, resistant towards pests, insects and genetic pollution due to cross breeding etc. [6], [7]. In various developing countries, it is used as a traditional medicine and used to extract metallic toxicins from polluted water bodies [8].

The vast quantity of weed biomass can be successfully disposed of in an efficient and eco-friendly way through its utilization as a cheap feed stock in an innovative process of bioconversion into fuel ethanol. Here we report application of simple and reliable pre-treatment process with the hydrolysis of water hyacinth with concentrated mineral acid and consequently detoxifying with sodium hydroxide and terminally fermentation of acquired C-5 and C-6 sugar to yield bio ethanol which can be utilized for successful de-weeding as well as biofuel generation.

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Fig.1. Water Hyacinth causing clogging of drainage in India (Assam) in semi-urban areas.

2 Materials and Methods

2.1 Physical pretreatment

Fresh water hyacinths with long stem were collected from a natural pond. They were thoroughly washed several times with tap water to remove the adhering dirt. The roots were discarded, as they have been reported to absorb heavy metal pollutants from water bodies, and dried under the sun for about 48 hours. It was then chopped into small pieces of size 1-2 cm and dried again for 2 hours. It is further grinded to even smaller particles of size 2 mm.

2.1.1 Hydrolysis

The hydrolysis of cellulose to glucose is carried out by the action of concentrated sulphuric acid or hydrochloride acid on the polymeric chains of glucose, in which cellulose and hemicellulose is converted in to 5-carbon and 6-carbon sugar with little degradation. In the next step, for depolymerization of the cellulosic fraction the solid residue was dewatered and soaked in 30-40% sulphuric acid for 50min at 373K. The optimized hydrolysis condition was approximately 72% H_2SO_4, at 50 °C for 5 minutes. The entire procedure is summarized in fig. Further, the insoluble material principally the lignin portion of the biomass were separated by filtering and further processed into fuel or other beneficial use [9]. The remaining acid sugar solution was separated into its acid and sugar component by using commercially available ion exchange raisins to separate the component without diluting the sugar. The separated sulphuric acid was recirculated and re-concentrated to the level required by the de-crystallization and hydrolysis process. The entire process is summarized in Fig.-2

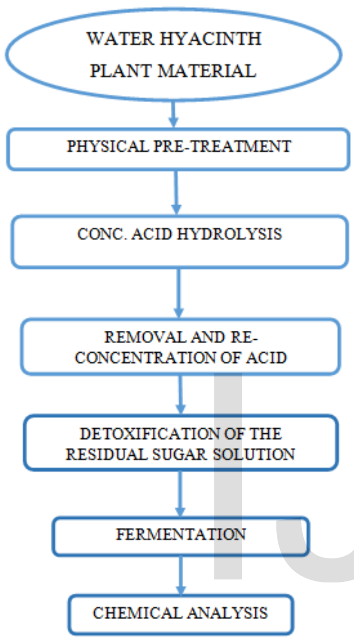

Fig.2. Work flow chart for extraction of sugars from dehydrated water hyacinth for fermentation.

2.1.2 Detoxification

Sugar solution from the previous step was first heated for exclusion of the volatile substances. Detoxification was carried out by the action of NaOH in the solution and it was adjusted to be approx. pH 6. The acquired processed sugar solution is concentrated by mild evaporation of water present in the solution.

2.1.3 Fermentation

Detoxified sugar solution was inoculated with *Zymomonas mobilis* for the fermentation of glucose and Pichia *stiptis* for the fermentation. The fermentation was carried out at 30 °C for 6 days. The broth was kept under constant agitation at 50 rpm. Samples were taken at regular intervals during fermentation to determine the concentration of cell mass, ethanol, and residual sugar in the broth. The oxidation test then detected the presence of ethanol. The solution was simply warmed with acidified potassium dichromate solution and the color changed from orange to green which indicated the presence of ethanol.

3 Results

Inflated petiole of Water Hyacinth was found to be most suitable for cellulose and Hemicellulose extraction. The root component was unsuitable due to its high lignin content and was kept separately for other purposes. The physical pre-treatment was necessary to break the crystalline structure of cellulose and hemicelluloses before it could be accessed by the acids during subsequent acid hydrolysis step.

3.1 Chemical composition of water hyacinth

Water Hyacinth leaves are considered to be a potential biomass source of cellulose and hemicellulose for conversion of to useful products. In this study, the average composition of Water Hyacinth leaves was total solid by 8.7%to 11.5% (5 by weight) moisture (%by weight), hemicellulose as (% of total solid) 32.69% cellulose (as of % of total solid) 19.2%, lignin (as % of total solid) 4.037%, crude protein 10.2% and starch about 4.16%. (table-1). It can be inferred from the study that leaf part of water hyacinth is predominantly rich in Cellulose and Hemicellulose [10].

Table- 1: Chemical composition of Water Hyacinth

Hemicellulose	32.69%
Cellulose	19.02%
Lignin	4.37%
Crude protein	10.02%

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3.2 Acid hydrolysis and Fermentation

Results of the oxidationtest confirmed presence of ethanol in the final product after fermentation. The extent of fermentation is summarized in table 2. The change of colors of the borth solution from after addition of Potassium dichromate solution indicated ethanol production. The intensity of color indicated extent of ethanol production.

Table-2: Conversion rate of sugars to ethanol. Per 1000 grams of dried powder made from Water Hyacinth petiole were subjected to acid hydrolysis and the quantities were measured biochemically.

Amount of dried water hyacinth material (grams)	Cellulose obtained (grams)	Glucose obtained (grams)	Ethanol fermented from glucose (ml)
1000	189.9	143.7	725.7

4 DISSCUSSION

Cellulose can be broken down chemically into its glucose units by treating it with concentrated acid or alkali solution. The reaction has three steps. Firstly, water molecule causes cellulose to limited swelling. Certain acid solutions or alkaline solutions now permeate into the crystalline region of cellulose. Thus, the acid or alkaline solution could cause cellulose unlimited swelling and results in cellulose decomposition. On the other hand, hemicellulose has hydrophilic property- which causes swelling of cell wall under water condition. The result indicates that water hyacinth leaves could be robust source of cellulose for bioconversion. Hemicellulose content of water hyacinth was found to be relatively higher, compared to that of cellulose which is a rare case among plant biomass.

The procedure for ethanol production from cellulose and hemicellulose was applied in three steps – Physical pretreatment, Acid hydrolysis, and fermentation. The procedure in laboratory condition was quite feasible and cost effective. The primary objective of the pre-treatment process was to break down the crystalline structure of cellulose, so that the acid can easily access and hydrolyse the cellulose.

Several methods have already been used to increase hydrolysis rate involving temperatures and pressures, acids (concentrated or dilute) or highly selective enzymes [11]. Mechanism of acid-catalysed hydrolysis is one of them where strong acids (H_2SO_4) are used in different concentration results in different degree of hydrolysis and yield [12]. In the present study, high concentration of acid was used which could be separated from the sugers solution by simple methods like filtration. Acid concentration affects degradation rate. Lower acid concentrations require more extreme conditions such as higher temperature and higher pressure and longer hydrolysis time for conversion of cellulose. Therefore, high acid concentration was used in the study to enhance hydrolysis. The significant objective of the study was to make the process economically viable, which can only be done when the acid used for hydrolysis is re-concentrated again for further cycles of acid hydrolysis. The acids could be reutilized for further cycles. The procedure followed in the present study indicates high suger yield after acid hydrolysis as shown in the table-2.

The hydrolysis rate of cellulose is also associated with the degree of the polymerization and the degree of crystallinity of cellulose. The study results show that the water hyacinth cellulose crystalline structure is suitable for normal acid hydrolysis and that the cellulose content could be used for fermentation for ethanol production. Sugar solution containing mainly cellulose and hemicelluloses (Table-1) was successfully used for ethanol production in normal laboratory condition using two species *Zymomonas mobilis* and *Pichia stiptis* isolated and maintained in the laboratory. In normal laboratory condition fermentation ferformance of the stains upon sugers extracted from water hyacinth was satisfactory as was indicated by the oxidation test. We need further study to establish the method for large scale application.

5 Conclusion

A procedure to utilize the sugar content of water hyacinth for ethanol production is demonstrated. The results reveal that the significant quantity of cellulose and hemi cellulose could be extracted from the inflated petiole of water hyacinth which could be converted to ethanol by fermentation. The procedure was demonstrated in laboratory conditions and further studies would evaluate the feasibility for industrial scale applications of the methods.

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