

## Bioethanol from water weed-Water Hyacinth

Nishu

University Institute of Biotechnology, Chandigarh University, Gharuan

nishu.uibt@cumail.in

### Abstract

*Water hyacinth, an aquatic weed is one of the budding substrate intended for the production of bioethanol. Bioconversion of biomass is an attractive method for production of sustainable fuel. Water hyacinth encompassing of cellulose, hemicelluloses and lignin is pretreated and then fermented to yield bioethanol. Selection of pretreatment method, microbe for fermentation, production conditions like pH, temperature, substrate concentration and microbe concentration affects the yield of bioethanol.*

**Keywords:** *Water hyacinth, bioethanol, aquatic weed, bioconversion*

### Introduction

Water hyacinth, one of the largest growing water weed is one of the major concern of the society. It reproduces very swiftly, forming large carpet which blankets the surface of the water bodies. This weed blocks the sunlight from reaching other aquatic animal and plants; consume the dissolved oxygen in the water, which kills aquatic animals. The efforts are being made to control the growth of this weed. Eradication by way of utilization is the best approach. One of the approaches is utilization of water hyacinth in the bioethanol production.

Bioethanol is a clean resource of potential alternative fuel (Rezania et al., 2015). Bio-ethanol is produced from sugar-rich crops and starch but lignocellulose is considered better option as of it costs less and easy availability (Zhao and Xia, 2010; Das et al., 2015). Utilization and bioconversion of aquatic weed (biomass) to clean fuel is a promising alternative. Water hyacinth has low content of lignin and high contents of hemicellulose and cellulose (Gressel 2008; Poddar et al., 1991). These lignin and cellulose can be biologically converted with the help of enzymes to fermentable sugar, as a result consequentially converting in an exploitable biomass for bioethanol production.

The biological alteration of complex lignocellulosic biomass intended for modified bioethanol production necessitate proficient pretreatment for delignification to release hemicellulose and cellulose, effective saccharification of the cellulose and hemicellulose to generate free sugars and fermentation of 6 carbon and 5 carbon sugars. The dried biomass of water hyacinth have been reported to contain mainly 7–26% lignin, 18–31% cellulose and 18–43% hemicelluloses, which is capable of being simply hydrolyzed to reducing sugars and after that fermented to yield bioethanol via viable yeasts (Bergier et al., 2012). On the other hand, there are numerous problems preventing the effectual hydrolysis, one of these tribulations is the seal of lignin that forestall breaching by enzymes responsible for degradation. Thus, numerous scientists endeavored to look for compelling pretreatment strategies to fracture the lignin seal (Ma et al., 2010; Forrest et al., 2010; Gao et al., 2013). Further tailback is the feedback inhibition of cellobiose on fermentation procedure following hydrolysis amid bio-ethanol generation (Ha et al., 2013; Guan et al.,

2013). The chief efficient technique to unravel the feedback inhibition setback is synchronized and instantaneous saccharification and fermentation, a procedure which involves enzymes and hydrolyzes lignocellulosic components to sugars and further ferments to bioethanol simultaneously (Soares and Gouveia, 201; Huang et al., 2013).

## **Conversion of Water Hyacinth to Bioethanol**

### **Water Hyacinth Preparation**

Water hyacinth with elongated stalk ought to be chosen and should be cleansed thoroughly numerous times, for few minutes through normal water to expel sticking soil particles, and then, chopped into diminutive sections (approx. 2 cm in size), mix together to small particles (approx. 5 mm), and then dehydrated in a hot air oven at 100–105 degree celcius for 5–6 hours. The dried water hyacinths were stored at room temperature.

### **Pretreatment**

The pretreatment method should be performed to step up the saccharification of cellulosic biomass and then their biological conversion to ethanol. The pretreatments attempt to (1) reduce the sugar loss, (2) minimize energy consumption, (3) advances the enzymatic digestibility, (4) decrease the amount of fermentation derivatives and inhibitors, and (5) trim down the cost involved.

### **Acid Hydrolysis**

Water hyacinth should be hydrolyzed utilizing distinctive corrosive agents to create glucose, arabinose, xylose and acetic acid via breakage of the acetyl groups and  $\beta$ -1, 4 connections of xylose or glucose monomers. In general fermentable sugar accessible by hydrolysis through acid might be 90% (Lavarack et al., 2002; Frederick et al., 2008). Continuous mild acid process has to be carried out at temperature range of 120–200° C and pressure maintained from 15 psi to 75 psi for 30 min to 2 hours (Badger et al., 2002; Kim et al., 2002). Intended for making elevated levels of sugar, the concentrated acid protocols may be followed. The process involve the utilization of 60–90% sulphuric acid, tender temperatures, and moderate pressures formed by impelling equipments from one container to another container for effectual hydrolysis. The principal leading point of the concentrated acid process is the elevated of levels recovery and efficiency, for both monosaccharide xylose and six carbon sugar i.e. glucose (Badger et al., 2002). Whereas, hydrolysis processes utilizing acid have some drawbacks like configuration of noxious fermentation inhibiting compounds, such as furfural, acetic acid, levulinic acid, hydroxyl-methyl furfural, formic acid, etc. Exclusion of these compounds adds to additional costs. Employing lime for neutralizing acid has the drawback that the significant amount of sugar is lost in the form of gypsum. Conversely, such procedures may perhaps be substituted by very reasonable chromatographic separation techniques with recycling of the acid as well.

### **Alkali pretreatment**

Outcome of basic pretreatment is dependent on the amount of lignin present in the resources. For prior treatment of water hyacinth, some of the bases might be used. The method of basic hydrolysis involves saponification of intermolecular ester bonds crosslinking xylan hemicelluloses along with additional gears, for eg, hemicellulose and lignin. Removal of the crosslinks from the lignocellulose amplifies the porosity of

lignocellulosic resources boosts with the dil. NaOH (0.5%) treatment of lignocellulosic resources roots to swelling, leading to an enlarged internal surface area, a decline in the degree of polymerization, decline in crystallinity, division of structural linkages between carbohydrates and lignin, and disruption of the structure of the lignin. Ammonia was also used for the pretreatment to eliminate lignin. The effectiveness of delignification was reported to be 50–70% for water hyacinth.

### Biological Treatment

The treatment includes, utilization of either entire organisms or enzymes in water hyacinth's pretreatment. For the treatment of water hyacinth either of bacteria or fungi may be used but fungal pretreatment of lignocellulose is better and improved alternative method for digestion. White-rot, soft-rot and brown-rot fungi are commonly employed for degradation of hemicellulose and lignin in water hyacinth. White-rot fungi are one of the chief effectual basidiomycetes for biological pretreatment of lignocellulosic resources. It has been reported that fungi like *Aspergillus terreus*, *Trichoderma sp.*, *Cyathus stercoreus*, *Lentinus squarrosulus* and *Penicillium camemberti* (Keller et al., 2003; Shide, Wuyep & Nok, 2004; Taseli, 2008) grown-up at temperature range of 25–35° C for 3–22 days consequenced to 45–75% of holocellulose and 65–80% of lignin.

Lignocellulosic bacterial pretreatment involves both aerobic and anaerobic frameworks. Anaerobic degradation employs chiefly mesophilic, bacteria derived from rumen. Three key clusters of cellulases are occupied in the hydrolysis procedure: (1) endoglucanase, assaults districts of small crystallinity in the fibers of cellulose, generating free ends of chains; (2) exoglucanase or cellobiohydrolase (CBH), degrades the molecular fragment additionally by eliminating units of cellobiose from the free ends of chains and (3)  $\beta$ -glucosidase hydrolyzes cellobiose to form 6 carbon sugar. Various supplementary enzymes assault hemicellulose, such as glucuronidase, acetylerase glucomannanase, xylanase,  $\beta$ -xylosidase, galactomannanase and feruloylsterase. Ligninolytic enzymes are essentially included within breakage of lignin during oxidative reactions.

**Preparation of hemicellulose acid hydrolysate:** 100 gms of dehydrated water hyacinth is assorted by 1% to 10% of sulfuric acid and final volume is maintained upto 1000 mL. The assortment is autoclaved at for 15 min at temperature 121° C, pressure 103 kPa and chilled down to normal temperature. The hydrolysate, subsequently be filtered to eliminate the untreated matter. The filtrate will be lastly collected and analyze for monosaccharide xylose content.

**Detoxification of hemicellulose hydrolysate:** The hemicellulose corrosive hydrolysate should be warmed to 60° C and afterward pH to 9.0–9.5 was increased with NaOH  $\text{Ca}(\text{OH})_2$  would be supplemented to the solution to detoxify dangerous materials that are available in the hydrolysate. After evacuation of inexplicable remains, the supernatant ought to be gathered for additional utilization as fermentable sugars.

### Lignin separation

**Preparation of the lignocellulosic hydrolysate:** The pretreated matter in the wake of being cleaned with water might be legitimately sifted and the filtrate may possibly then be gathered for sugar investigation.

**Detoxification procedure:** The process for detoxification by enzymatic action incorporates treated samples; treatment involves 1M laccase, 1M lignin peroxidase, mixture of 1M laccase and 1M lignin peroxidase, and water as control. The sample desires to be tested for its fermentability capabilities by means of GC–MS and phenolic content can be evaluated by gel-permeation chromatography. The pH of the hydrolysate may then be acquainted to 5.3 utilizing NaOH and for tests to be utilized for controls and for treatment with laccase, to pH 3.2 for treatment with lignin peroxidase, and to pH 4.5 for consolidated treatment with laccase and lignin peroxidase. Laccase, lignin peroxidase or water might be included as requisite. Every example may be pursued by brooding at 30° C for 12 h in a rotational shaker (90 rpm). 0.2 M Hydrogen peroxide ought to be enhanced every hour to the examples including peroxidase and towards the finishing up of the examination; catalase (0.04 mg/mL) may be incorporated.

### **Fermentation**

**Use of *Pichia stipitis* and *C. shehatae*:** The microbes proficient to mature monosaccharides there and likewiswe, oppose budding inhibitors in the hydrolysates. *S. cerevisiae*, regularly used ethanol maker, can't mature 5-carbon sugar, which might be involved up to 40% of the unrefined material. Amidst the xylose aging yeasts *P. stipitis* has shown ensure for mechanical pertinence, since it ages xylose quickly with raised yields of ethanol and appears to deliver no xylitol and is capable to age a wide exhibit of sugars than *C. shehatae*. The capacity of *P. stipitis* and *C. shehatae* yeasts to age xylose capably to ethanol has become unlimited thought and incited various research investigations which use the lignocellulosic hydrolysate as fermentable substrate. By and by, there is monstrous degree of research toward this way with different microorganisms to develop a standard course of action for development of the biomass entailing less time.

### **Conclusion**

Water hyacinth, one of the fastest and uncontrollable growing aquatic weed is one of the major concern for society. Using different methods to eradicate and utilizing this biomass for the bioconversion to bioethanol is one of the promising approaches. Though bioconversion is time consuming process but is environment friendly. Water hyacinth can prove to be one of the cheapest substrate for bioethanol conversion.

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