International Journal of Emerging Technology and Advanced Engineering
Volume 3, Special Issue 3: ICERTSD 2013, Feb 2013, pages 42-46
An ISO 9001:2008 certified Int. Journal, ISSN 2250-2459, available online at www.ijetae.com

BIOETHANOL PRODUCTION THROUGH WATER HYACINTH, EICHHORNIA CRASSIPES VIA OPTIMIZATION OF THE PRETREATMENT CONDITIONS

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ABSTRACT
Water Hyacinth (Eichhornia crassipes Martius) is a monocotyledonous freshwater aquatic plant, belonging to the family Pontederiaceae, related to the lily family (Liliaceae) and is a native of Brazil and Equador region. The aquatic weed collected from Harikepattan, Amritsar is found to be problematic for many reasons which include increasing evapo-transpiration, providing a refuge for disease-carrying vectors, causing silting of channels, slowing down of the water flow and flooding. Utilization of this weed for energy production seems to be highly beneficial. However the biofuel production through water hyacinth needs some processing before the further conversion to ethanol. Pretreatment process does require chemicals which make the process costlier. In this paper, comparisons are made between acid and alkaline hydrolysis as pretreatment method for the biomass. The hydrolysis optimization was attempted to decrease the time of fermentation in selected biomass. This paper summarizes fermentation mechanism in water hyacinth. The carbohydrate analysis enables the possibility towards ethanol production. The results show the potential of water hyacinth as feedstock for ethanol production however the high moisture content and other chemical analysis also favors the methane production.

Keywords: Water hyacinth, Pretreatment, Bioethanol, lignocellulosic biomass.

1. INTRODUCTION
Energy consumption has increased steadily over the last century as the world population has grown and more countries have become industrialized [1]. Bioenergy is 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 [2]. A considerable amount of research is currently being conducted on the production of bioenergy due to the increasing demand for fossil fuel and its limited quantities in reserve. Bioethanol is one of the most promising replacements for fossil fuel since it is renewable and emits 85% less green-house gases compared to gasoline [3]. Recently, more research has focused on using non-edible biomass as raw materials including lignocelluloses, celluloses, and marine algae rather than the first generation biomass such as starch and sugar biomass [4]. Lignocellulosic feedstock is considered as an attractive raw material because of its availability in large quantities at low cost [5] not only for the liquid transportation fuel but also for the production of chemicals and materials, i.e. the development of carbohydrate-based biorefineries [6]. Besides terrestrial plants, aquatic plants are also promising renewable resource.

Water bodies are complex ecosystems and may include a variety of water plants that contribute to water quality and environmental health. In a healthy ecosystem there is usually a complex mix of plant species but sometimes the natural balances are disturbed. For example, increased nutrient loads entering water bodies, low water flows and increased temperatures often cause an excessive growth of some plants to the point where they become a problem. Water hyacinth is one of the world's worst aquatic weeds. It infests rivers, dams, lakes and irrigation channels on every continent except Antarctica [7].

Fig 1: Water Hyacinth Source [12]

ISSN 2250-2459, *Volume 3, Special Issue 3: ICERTSD 2013, Feb 2013, pages 42-46*

The figure 1 shows water hyacinth, *Eichhornia crassipes*, is a tropical species belonging to the pickerelweed family (Pontederiaceae). The water hyacinth plant is a free-floating aquatic plant originating from the Amazon River basin in South America and has spread to more than 50 countries on five continents. The plant tolerates extremes in water level fluctuations, seasonal variations in flow velocity, nutrient availability, pH, temperature and toxic substances [8]. It can even grow at salinity levels up to 0.24% as was shown in Indonesia [9]. Extremely high growth rates of up to 100–140 ton dry material Ha^{-1} $year^{-1}$ [10] were reported, depending on the location and time of the year [11]. The coverage of waterways by water hyacinth has created various problems. It devastates aquatic environments and costs billions of dollars every year in control costs and economic losses [7].

Singh et al. (1984) reported that the daily average productivity of water hyacinth was 0.26 ton of dry biomass per hectare in all seasons. It also grows from seed which can remain viable for 20 years or longer. This enormous reproductive capacity causes annual reinfestation from seed and rapid coverage of previously treated areas, making ongoing control necessary. Currently management of this weed includes preparation of fish and livestock feeds, bio-gas production, charcoal briquetting, wastewater treatment for domestic and industrial use [13].

Bio-ethanol feed stocks can be divided into three major groups: (1) sucrose-containing feed stocks (e.g. sugar cane, sugar beet, sweet sorghum and fruits), (2) starchy materials (e.g. corn, wheat, rice, potatoes, cassava, sweet potatoes and barley), and (3) lignocellulosic biomass (e.g. wood, straw, and grasses). Biofuel/Bioethanol production from agricultural products has been in practice for the past 80 years which has created a direct competition between food and energy sector. Limited fraction of the biomass can be converted with known enzymatic technology today for production of cellulosic ethanol.

Anderson et al. (2005) reviewed the structural and chemical properties of grasses in relation to biofuels conversion and observed that carbohydrate bioconversion is limited by associated lignins and low molecular weight phenolic acids. Production of ethanol provides several advantages over gasoline: first, utilization of abundant and inexpensive sources of renewable resources; second, reduction in greenhouse gas emission and toxic substances; third, macroeconomic benefits for rural community and social aspect of sustainability; and fourth, pertaining in national energy security [15].

An ideal biomass pretreatment process should meet the following requirements:

• High rates of hydrolysis and high yields of fermentable sugars
• Minimal degradation of the carbohydrate fractions
• No production of compounds that are inhibitory to microorganisms used in the subsequent fermentation.
• Inexpensive materials of construction
• Mild process conditions to reduce capital costs
• Recycle of chemicals to reduce operating costs
• Minimal wastes

Table1. Average biomass composition of water hyacinth [10].

Components	% composition
1. Lignin	10
2. Cellulose	25
3. Hemicellulose	35
4. Ash	20
5. Nitrogen	03

Table 1 shows the main composition of water hyacinth which is lignin, cellulose and hemicellulose. The carbohydrate polymers in the lignocellulosic material needs to be converted to simple sugars before fermentation, through a process called hydrolysis. There are several possible methods to hydrolyze lignocelluloses. The most commonly applied methods can be classified in two groups: Chemical hydrolysis and enzymatic hydrolysis [16]. Among chemicals acid and alkali are used as pretreatment methods. Acids are predominantly applied in chemical hydrolysis. Acid hydrolysis can be divided into two groups: (a) Concentrated acid hydrolysis and (b) Dilute acid hydrolysis. Concentrated acid process can be operated at low temperature (eg 40°C) however concentration of acid is very high (eg: 30-70 %). Dilute acid hydrolysis is commonly applied. It can be used either as a pretreatment preceding enzymatic hydrolysis or as the actual method of hydrolyzing lignocelluloses to sugars as shown in figure 2 [17]. But one drawback in this is the formation of undesirable products as furfural, formic acid, acetic acid, uronic acid etc [18, 16].

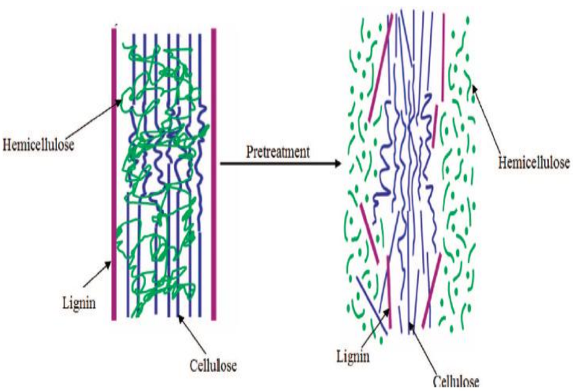

Fig 2: Schematic of the role of pretreatment in the conversion of biomass to fuel. [3]

ISSN 2250-2459, *Volume 3, Special Issue 3: ICERTSD 2013, Feb 2013, pages 42-46*

This paper discusses the pretreatment methods during the utilization of water hyacinth for ethanol production.

2. MATERIAL AND METHODS

(i) Feedstock processing/ Preparation of the water hyacinth powder

Whole, fresh Water Hyacinth plants were collected from back waters in Amritsar district in Punjab was washed with tap water and leaves and stalks were dried in sunlight along with roots. Many cuts were made in the stalks to dry the stalks. Dried plant was grinded in a mixer and grinder thereby reducing the particle size to few mm and stored in air tight containers at room temperature until used as shown in figure 3.

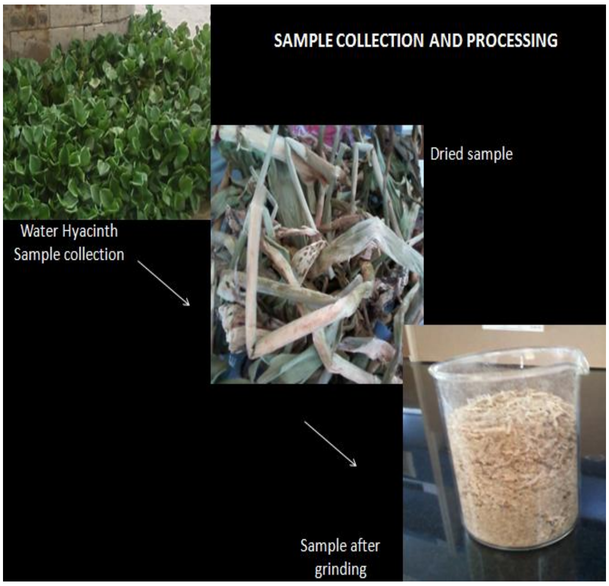

Fig 3: Water Hyacinth Sample collection and processing

(ii) Pretreatment optimization

1. Pretreatment with Acid

Pretreatment was carried out in Erlenmeyer flasks (250 ml) by mixing 3g of the dried water hyacinth with different acids i.e. HCl/H_2SO_4 /HCOOH(2% v/v) .

Fig 4: Samples after treatment with different acids

The mixture was autoclaved at 121 °C, 15 lbs for 15 min and further cooled down to room temperature (Figure 4). The hydrolysate was filtered using Whatman filter paper No. 1 to remove the unhydrolysed material. The filtrate was collected and analyzed for the reducing sugar content by using DNS test.

2. Pretreatment with Alkali

Following the similar procedure the dried water hyacinth was treated with alkalis ie. NaOH and KOH which also had the concentration of 3% (v/v) (Figure 5).

Fig 5: Samples after treatment with different alkalis.

3. Pretreatment with H_2SO_4 at different Concentrations

On estimation of the reducing sugars content in different acids/alkalis maximum concentration was obtained using 2% H_2SO_4. Therefore, dried water hyacinth was subjected to H_2SO_4 treatment at different concentrations ie. 1%,2%,3% and 4% (v/v) to obtain a concentration of H_2SO_4 that yielded maximum sugar (Figure 6).

Fig 6: Samples after treatment with different acids and alkalis.

(iii) Sugar estimation

Total reducing sugar was estimated by using dinitrosalicylic acid (DNS) reagent [19]. 3ml of DNSA reagent was added to 3ml of hydrolyzed sample in a test tube. The mixture was heated at 90ºC for 5-15min to develop the red brown color. Further 1ml of 40%

ISSN 2250-2459, *Volume 3, Special Issue 3: ICERTSD 2013, Feb 2013, pages 42-46*

Potassium tartarate (Rochelle salt) solution to stabilize the color. After cooling at room temperature in a cold water bath, record the absorbance with a spectrophotometer at 575 nm.

4. RESULTS AND DISCUSSION

Pretreatment results

Water Hyacinth sample were pretreated using different acids (Hydrochloric acid, Sulphuric acid, Formic acid) and alkalis (Sodium hydroxide, Potassium hydroxide). Among acids 2% (v/v) Sulphuric acid gave best results and among alkalis 3% (v/v) Sodium hydroxide was most effective in pretreatment.

Table 2: Sugar concentration achieved after pretreatment.

Reagents used	Absorbance at 575nm	Sugar conc. (μg/ml)	Sugar conc. (mg)Per 100ml of filtrate	Sugar conc. (mg) Per gram of sample
2% H_2SO_4 *	**0.4007**	**352.64**	**35.264**	**11.755**
2% HCl	0.3219	296.37	29.637	9.879
3% HCOOH	0.1078	143.425	14.342	4.78
3% NaOH	0.1476	171.855	17.185	5.728
3% KOH	0.1428	168.45	16.845	5.615

Fig 7: Comparative yield of sugars using different reagents.

The highest amount of sugars i.e. 11.755 mg per gram of water hyacinth taken was obtained by treating sample with H_2SO_4 as shown in table 2 and figure 7. Further test was done by pretreating water hyacinth with varying concentration of H_2SO_4 to calculate the optimum concentration.

Table 3: Sugar concentration achieved after pretreatment with varying concentration of Sulphuric acid

Conc. Of Sulphuric acid used	Absorbance at 575nm	Sugar conc. (μg/ml)	Sugar conc. (mg)Per 100ml of filtrate	Sugar conc. (mg) Per gram of sample
1% H_2SO_4	0.3399	309.21	30.921	10.31
2% H_2SO_4	0.387	342.855	34.285	11.43
3% H_2SO_4	0.4092	358.714	35.871	11.96
4% H_2SO_4	**0.4375**	**378.925**	**37.892**	**12.63**
5% H_2SO_4	0.3091	287.214	28.721	9.57

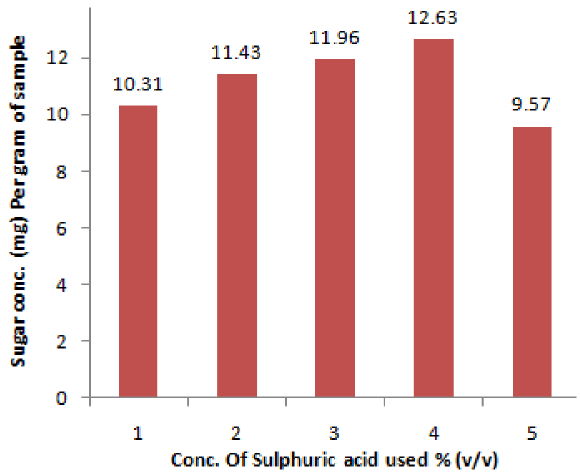

Fig 8: Sugar yield by using different concentrations of sulphuric acid.

The maximum sugar yield of 12.63 mg per gram of water hyacinth was obtained when sample was treated with 4% (v/v) of H_2SO_4 as shown in table 3 and figure 8.

5. CONCLUSIONS

Lignocellulose-to-ethanol bioconversion holds great potential as the substrate is abundant and relatively of low cost. However, the integration of low cost pretreatments with advanced ethanol-producing microorganisms may play a crucial role in lowering the cost of biomass bioconversion processes.

Sulphuric acid gave best results for the yield of sugars as compared to other acids and alkalis which was 12.63 mg of sugar per gram of water hyacinth sample when treated with 4% (v/v) of H_2SO_4.

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