Indian Journal of Science and Technology, *Vol 9(6), DOI: 10.17485/ijst/2016/v9i6/82888, February 2016*
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Dilute Acid Induced Changes on Microscopic and Tomographic Structure of Water Hyacinth [*Eichhornia Crassipes* (Mart.) Solms] Biomass during Bioconversion Process to Xylitol

A. Bhattacharya[1], A. K. Sadhukhan[1], A. Ganguly[2] and P. K. Chatterjee[2*]

[1]Department of Chemical Engineering, National Institute of Technology, Durgapur – 713209, West Bengal, India; anamicab@gmail.com, t_sadhu@yahoo.com
[2]Thermal Engineering Division, CSIR Central Mechanical Engineering Research Institute, Durgapur – 713209, West Bengal, India; pradipcmeri@gmail.com, amitganguly022@gmail.com

Abstract

Background/Objectives: The present study was conducted on Water Hyacinth Biomass (WHB) to understand its anatomy, physiology, biochemistry, tomography and the response of dilute acid pretreatment under different parametric conditions on these aspects. **Methods/Statistical Analysis:** Fresh water hyacinth plants collected, Transverse Section (T.S) and Longitudinal section (L.S) of rhizome, root, stem/petiole and leaf of different ages (very young, middle and old plants) were made. Leaf and Stem collected were grinded to make fresh paste. T.S, L.S and fresh paste was soaked in 1, 3 and 5% of H_2SO_4 solution for 1, 2, 3 and 4 h under agitation of 130, 160 and 190 rpm at temperature of 30 °C, 40 °C, 50 °C and 60 °C and boiled for 15 and 30 min. Untreated and treated biomass were then dried and preserved. **Findings:** It has been observed under the microscope that there has been a prominent lysis in the cell wall, vascular bundles and several other tissues when the transverse section of young, middle and old aged root, stem and leaf are soaked in 1-5% acid with agitation of 130-160 rpm for 1-4 h at 30–60 °C. To justify the reason behind obtaining higher yield of xylose sugar by acid hydrolysis, fresh WHB paste, T.S and L.S was treated with dilute acid with same parametric conditions which gave higher xylose yield. Treated biomass was investigated under compound microscope and scanning electron microscope (SEM) and it was observed that the pretreatment alters the structural and chemical composition of complex structure of lignocellulose in WHB for rapid hydrolysis to fermentable sugars. **Applications/Improvements:** This paper represents the effect of hydrolysis on the WHB which is clearly evident from the anatomical studies in Compound microscope and SEM. The biomass can be efficiently utilized for bioconversion into value added Product as xylitol after acid hydrolysis.

Keywords: Anatomy, Hydrolysis, Lignocellulose, Physiology, Tomography, Water Hyacinth Biomass

1. Introduction

Water Hyacinth [*Eichhornia crassipes* Martius (Solms-Laubach)] is a prolific free floating aquatic macrophyte, which has proven to be a significant economic and ecological burden to many subtropical and tropical regions of the world[1]. It has established in most tropical and subtropical countries as well as in many warm-temperate regions between 40° N and 40° S. In the absence of natural enemies, Water Hyacinth forms large mats on still and slow-moving water bodies, where it severely degrades aquatic ecosystems and limits their utilization. It has been listed as one of the most productive plant on earth which has invaded freshwater system in over 50 countries on five continents, especially throughout Southeast Asia, the southeastern United States, Central and western Africa

*Author for correspondence

and Central America[2–4]. At present Water Hyacinth (*Eichhornia crassipses*) have been ranked as one of the world's worst invasive weeds causing problems to millions of users of water resources. It is known as "Blue Devil" or "Bengal terror" in India, "Florida devil" in South Africa, "German weed" in Bangladesh and "Water terror" by South Western Nigeria[5] with its disruptive impacts on aquatic ecosystem, agriculture, fisheries, production from electricity from hydral power plants, transportation, living conditions and social structures.

Water Hyacinth being the world's worst weed is also listed as one of the most productive plant on earth on the other hand. Along with its negative economic impacts worldwide which include clogging of irrigation channels, choking off navigational routes, loss of fishing area, depletion of oxygen, nutrients from water bodies, it has wide importance in waste water treatment, excellent source of biomass due to high productivity, used as fertilizers, ethanol, Natural gas, Methane etc. can be produced. Also some value added products such as Xylitol etc. can be produced as the biomass is rich in cellulose and hemicelluloses[6] containing hexose and pentose sugars. Besides cellulose, hemicelluloses and lignin, smaller amounts of pectin, protein, extractives and ash are also present. Cellulosic materials are renewable natural biological resources. Lignocellulosic structure is complex. Carbon is locked in lignocellulosic structure in the form different types of sugars. It has been reported that the biomass of Water Hyacinth has about 48% hemicellulose, 18% cellulose, 13.5% lignin[7], 18.4% cellulose, 49.2% hemicellulose and 3.55% lignin has been reported by Ashish Kumar et al. in 2009. 31.6% cellulose, 33.4% hemicellulose and 9.3% lignin have been reported by Kalhorinia et al. 2014. Though there is a significant amount of variability in composition reported by different labs, in general the biomass is considered to be rich in hemicellulose and with very less lignin content. The objective of this paper is microscopic study of the effects of acid hydrolysis on Water Hyacinth Biomass, different types of anatomical and morphological changes in the cell of WHB with changes in different parameters.

1.1 Origin

C. F. P. von Martius first described *E. crassipes* from Brazil in 1823. It is indigenous to the New World tropics and has its center of origin in Amazonia, Brazil[8] with anthropogenic spread to other areas such as Venezuela, parts of Central South America and the larger Caribbean islands[9]. The genus Eicchornia was established by C. S. Kunth, a German botanist in 1842 to design the members of family Pontederiaceae, with trilocular ovary, a numerous seeds per fruit. He named it in the honor of John Albert Friedrich Eicchorn, the Prussian Minister for Education, Culture and Medicine (1779–1856). The first authentic record of *E. crassipes* outside South America is from New Orleans in 1884[9]. Afterwards, *E. crassipes* plants spread around the USA and by the end of the 19th century were recorded in Egypt, India, Australia and Java[10]. Its distribution is now mainly pan tropical, but it also occurs in warm temperate regions of the world, limited to latitudes of 40° N and S[10]. Even though the first introduction of *E. crassipes* to the African continent was made in Egypt between 1879 and 1892, many invasions in Africa were first noticed only in the 1980s and it continues to invade many waterways. But its entry into Africa, Asia, Australia and North America was facilitated by human activities[11].

1.2 Morphological Description

A monocotyledonous plant, perennial, free-floating, except when stranded in the mud; mother plants and daughter plants attached by floating stolons, leaves formed in rosettes rise to three feet above the water; leaves entire, ovate, rounded, circular or broadly elliptic; thick, glossy, waxy green, waterproof; sides gently incurved and often undulate; leaf base hear shaped, squared or rounded; veins dense, numerous, fine, longitudinal, petioles (leaf stems) floating, creeping; inflated, bulbous, spongy, upto 12 inch long, inflorescence Spike, multiple (8 to 15) flowers in a single very showy, spike (spathe); spike at top of erect thick stalk, rising above the leaves; each flower in the flower-spike with six lavender-blue petals (perianths), petal tips slightly 2-lipped; uppermost petal somewhat larger, lavender, having a bright yellow, blue-bordered central oval splotch; stamens 6, stigmas 3, roots hanging submerged beneath floating leaves, dark purplish to black, feathery, tips with long root caps, fruit a capsule, 3-celled, with many seeds; seeds ribbed, formed in submerged, withered flower; fruit and seeds are rarely observed; seeds may produce many seedlings in moderate climates[12]. A flowering plant is shown in Figure 1.

The growth form of this plant is variable. Reproduction is both sexual and asexual. After pollination, the flower spike bends to position the seed capsule below the surface of the water, where the seeds are released. Each seed capsule contains up to 50 seeds, which sink and remain viable

(a) (b)

Figure 1. **(a)** Water Hyacinth plants growing inside CMERI campus pond. **(b)** Flowering plant with rhizome, root, shoot, leaf and flower.

in the sediment for 15 to 20 years. Seed germination occurs in moist sediments or in warm shallow water. However, the main mode of reproduction is asexual through the production of daughter plants or ramets, which are produced on stolons from the mother plant. *Eichhornia crassipes* populations increase rapidly, doubling every 11–18 days under suitable uncrowded conditions and in warm, tropical climates and eutrophic waters.

1.3 Genetics of Water Hyacinth

A global scale population genetic survey using amplified fragment length polymorphism markers of the world's most successful aquatic plant invader – Eichhornia crassipes (Water Hyacinth) was done. 1140 ramets from 54 populations from the native (South America) and introduced range (Asia, Africa, Europe, North America, Central America and the Caribbean) were investigated where 49 are clones and introduced populations exhibited very low genetic diversity and little differentiation compared with those from the native range and 80% of introduced populations were composed of a single clone. A widespread clone ('W') detected in two Peruvian populations accounted for 70.9% of the individuals sampled and dominated in 74.5% of the introduced populations. Nine of 47 introduced populations contained clonal diversity suggesting that sexual recruitment occurs in some invasive sites where environmental conditions favor seedling establishment. The global patterns of genetic diversity in *E. crassipes* likely result from severe genetic bottlenecks during colonization and prolific clonal propagation. The prevalence of the 'W' genotype throughout the invasive range may be explained by stochastic sampling or possibly because of pre-adaptation of the 'W' genotype to tolerate low temperatures[13].

1.4 Distribution

The center of origin of *Eichhornia crassipes* is the Amazon basin in Brazil and Peru. It has been spread to most of South America and the Caribbean islands and was first recorded in the United States in 1884 in New Orleans. By the end of the 19thcentury, the plant was recorded in Egypt, India, Australia, and Java[14]. The main mode of spread of Water Hyacinth throughout the world has been through anthropogenic means, via the horticultural and aquarium trades, due to the appeal of its attractive smooth, green foliage and beautiful purple flowers.

1.5 Utilization

There has been considerable research into the utilization of Water Hyacinth. Uses included in biogas and bioethanol production, animal fodder, fertilizer, mulch and water quality management. Water Hyacinth is an excellent and cheap source of lignocellulose. The main components of a lignocellulosic biomass are cellulose, hemi-cellulose and lignin. Cellulose is a biopolymer, composed of monomers of glucose, by β-(1-4) glycoside bonds[15]. Hemicellulose is a branched carbohydrate, made of both hexose and pentose sugar. Lignin is a macromolecule containing phenolic characteristics; its helical structure contains ether and carbon-carbon linkages. Different methods like chemical, physical, biological and thermal have been employed to utilize lignocellulosic biomass for fuel.

1.6 Effect

These plants grow in and dominate their freshwater habitat owing to their high vegetative propagation rate and to the robustness of their seeds. Today more than 50 countries suffer from the effects caused by the fast growth of the plant which include obstruction of shipping routes and reservoirs; losses of water in irrigation systems by evaporation; high consumption of dissolved oxygen by spoiled plant material; nesting area for Anopheles and other harmful organisms[10,16].

2. Materials and Methods

2.1 Collection of Water Hyacinth Plant

Fresh Water Hyacinth plants of different ages (very young, middle and old) were collected along with rhizome, root, shoot and leaf, from the pond inside CSIR-CMERI Campus. The plant was washed with running tap water.

Different parts of the plants were separated and kept in a beaker containing water for further investigation.

2.2 Preparation of Slides for Microscopic Study

Transverse and Longitudinal Section (TS and LS) of rhizome, root, stem and leaf of different ages (young, middle, old plants) were made and soaked in 1, 3, 5 % of sulphuric acid for 1.2.3 and 4 h. After the soaking period is over, the sections were mounted in slide and placed under compound microscope (Magnus LED Microscope Model MLX-B) for anatomical study.

2.3 Preparation of the Biomass

Leaves and Stems (petioles) of the plants were taken and cut into small pieces and grinded to make a fresh paste. The paste is evenly squeezed, so that excess water comes out from it. Then the moisture content was measured using a moisture meter. 8 gms of paste is soaked in 1, 3 and 5% of acid solution for 1, 2, 3 and 4 h under agitation of 130,160 and 190 rpm in different soaking temperature as 30 °C, 40 °C, 50 °C and 60 °C and boiled for 15 and 30 min. Untreated and treated (1, 3, 5% H_2SO_4) biomass was then dried and preserved for SEM.

2.4 Analytical Method

The content of cellulose, hemicellulose, lignin and ashes were determined by the detergent extraction method[17]. Reducing sugars were determined by the method of 3.5-Dinitrosalicylic Acid (DNS)[18]. Xylose sugar was determined using the Phloroglucinol assay[19,20] with the hydrolysate obtained from acid hydrolysis.

2.5 Scanning Electron Microscopy (SEM)

The treated and untreated biomass was then examined under Scanning Electron Microscope (SEM) which uses a focused beam of high-energy electrons to generate a variety of signals at the surface of solid specimens. The signals that are generated from electron-sample interactions gives information about the sample including external morphology (texture), chemical composition and crystalline structure and orientation of materials present in the biomass to clearly distinguish between treated and untreated biomass.

3. Results and Discussion

3.1 Characterisation of Water Hyacinth Biomass

Plant biomass/Lignocellulosic feedstock is composed primarily of cellulose, hemicelluloses and lignin and smaller amounts of pectin, protein, extractives and ash. The composition of cellulose and hemicelluloses content of water hyacinth reported by other researchers are compared with our results (Table 1 and Table 2). Eichhornia crassipes was found to be rich in flavonoids, amino acids, crude protein, cyanide, phosphate, organic matter and organic carbon[21]. Fresh plant contains 95.5% moisture, 0.04% N, 1.0% ash, 0.06% P_2O_5, 0.20% K_2O, 3.5% organic matter. On a zero-moisture basis, it is 75.8% organic matter, 1.5% N and 24.2% ash. The ash contains 28.7% K_2O, 1.8% Na_2O, 12.8% CaO, 21.0% Cl and 7.0% P_2O_5. The CP contains, per 100 g, 0.72 g methionine, 4.72 g phenylalanine, 4.32 g threonine, 5.34 g lysine, 4.32 g isoleucine, 0.27 g valine and 7.2 g leucine[22]. The physico-chemical composition was also studied and compared which is well represented through Table 3.

3.2 Anatomical Study of Water Hyacinth Plant

3.2.1 Root of Water Hyacinth Plant

Transverse section of treated and untreated root was kept under microscope for anatomical study of different tissue,

Table 1. Comparative analysis of lignocellulosic characterization of WHB as reported by other researchers

Plant Material	Nigam, 2002 [7]	Kumar et al. , 2009 [24]	Deuk Joo Ahn. et al., 2012 [25]	J. R. –Alfaro et al., 2013 [26]	Kalhorinia et al., 2014 [6]	Present study (Uniform mix of leaf and stem)
Cellulose	18.2	18.4	34.19	31.6	31.6	32.5
Hemicellulose	48.7	49.2	17.66	27.3	33.4	38.1
Lignin	3.5	3.6	12.22	3.9	9.3	11
Final product	Bioethanol	Bioethanol	Bioethanol	Fermentable sugar	Xylitol	Xylitol

Table 2. Lignocellulosic characterisation in leaf, stem and root of WHB

	Leaf of WHB (%)	Stem of WHB (%)	Root of WHB (%)	Uniform mix of Leaf and Stem (%)
Cellulose	33.4	29.6	9	32.5
Hemicellulose	29.1	36.2	20	38.1
Lignin	12.4	14.1	31.5	11.3

Table 3. Physico-chemical characterisation of WHB

Parameters (%)	Nigam, 2002 [7]	Kumr et al. 2009 [24]	J.R. Alfaro et al. 2013 [26]	Present study
Moisture	92.8–95	92.6–95	93.10	95.2
Ash			1.6	1.86
Crude protein	13.3	12.6	12.7	14.2
Crude Fibre			19.18	

changes in their structural orientation with the change in pretreatment parameters. An untreated root has an epidermis which consists of rectangular, single layered cells, which have a cuticle on the outer side. Hypodermis is composed of multi layered thick-walled cells. The outer cortex contains 3–4 layered parenchymateous cells, inner cortex consists of 8–10 layers of parenchymateous cells. Sclerenechyma cells are absent in the cortex. Each air space has trabeculae or partitions of parenchyma cells. The stele is present which has a single layered endodermis where casparian strips are inconspicuous. Pericycle is present below the endodermis which is single layered. Xylem bundles are present alternating with phloem bundles which are 6–8 layered, which consists of metaxylem vessel surrounded by smaller vessels. Sclerified parenchymatous cells are present in the root center[23].

After acid hydrolysis with 1, 3 and 5% of sulphuric acid for 1, 2, 3 and 4 h soaking time, it was found that the cells get ruptured, the thick cuticle is removed after 2-3 h of soaking in 3 and 5% acid solution. The trabeculae is broken, the cells in the cortex are hydrolysed and broken as shown in Figure 2 (a-c). Most of the cells get affected after 2 h soaking in 3 and 5% acid. After 4 h of soaking all the cells are deformed and loses identity. The effect of dilute acid hydrolysis on the anatomical changes in water hyacinth substrate is shown in Table 4, Table 5 and Table 6.

(a) **(b)** **(c)**

Figure 2. TS of root of young Water Hyacinth Plant (Eichhornia crassipes) **(a)** TS of a root treated with 1% H_2SO_4 for 3 h. **(b)** TS of a root soaked in 3% H_2SO_4 for 3 h. **(c)** TS of a root treated with 5 % H_2SO_4 for 3 h.

3.2.2 *Stalk/Petiole of Water Hyacinth Plant*

A distinct epidermal layer is present in the transverse section of petiole which is composed of single layered parenchymatous cells where cuticle is not present. Layers of parenchymatous cells are present where vascular bundles are distinct. Sclerenechymatous cells are present in vascular bundles. Aerenchymatous cells or air spaces are present which are hexagonal and surrounded by single layered parenchyma cells. Vascular bundle comprises of xylem tissue containing tracheids, vessels, parenchymatous cells and fibers. Sieve tubes and companion cells are present in Phloem. Aerenchyma cells are present which contains sclereids. Transverse and longitudinal sections of young, middle and old stem of Water Hyacinth after soaking in different acid concentration (1, 3, 5% of sulphuric acid) are shown in Figure 3 (a - l).

3.2.3 *Leaf of Water Hyacinth Plant*

Transverse section of leaf lamina contains a distinct epidermis which comprises of single layered rectangular cells. A thin cuticle is present on the epidermal cell wall. Palisade and spongy mesophyll tissue can be easily differentiated. The upper epidermis has 5-6 layers and the lower epidermis has 2-3 layers. A large number of aerenchymatous cells are present surrounded by thin walls of chloroplast. Sclereids are observed in the air spaces. Small and large vascular bundles are present. Smaller vascular bundles are found in both upper and lower side of epidermal layer, in contact with the epidermis. Vascular bundles are collateral with xylem towards the lower epidermal side and phloem towards the upper epidermal side. Tracheary elements are present which consist of tracheids, vessels and parenchyma cells. Sieve tubes and companion cells are present in phloem. Bundle sheath extensions are observed. Each vascular bundle is surrounded by a bundle

Table 4. Description of the effects in different types of cells of root of a WHB plant

Sl. No.	Type of cells	Effects after dilute acid treatment in root			
		Untreated (Control)	1% H_2SO_4, 2 h	3% H_2SO_4, 2 h	5 % H_2SO_4, 2 h
1	Cuticular cells	Present	Removed	Removed	Removed
2	Epidermis	Do	No change	Structure is deformed	Epidermal line is rupture
3	Hypodermis	Do	No change	Partially breaks	Cells are ruptured
4	Outer cortex	Do	Partially breaks	Cells are ruptured	Removed
5	Parenchyma	Do	Partially breaks	Cell wall is ruptured	Most Cells are removed and some are deformed
6	Air space	Do	Trabeculae is ruptured	Trabecular wall broken	All the cells get dissolved
7	Endodermis	Do	Structured is deformed	Lysis in endodermal cells	Cells broken
8	Xylem vessels	Do	No change	Ruptured	Lysis is vascular bundle
9	Phloem cells	Do	No change	Ruptured	Lysis is vascular bundle
10	Pith	Do	Partially breaks	Ruptured	Removed and hollow is found

Table 5. Description of the effects in different types of cells of stem of a WHB plant

Sl. No.	Type of cells	Effects after dilute acid treatment of stem			
		Untreated (Control)	1% H_2SO_4, 2 h	3% H_2SO_4, 2 h	5 % H_2SO_4, 2 h
1	Epidermis	Present	No change noticed	Structure is deformed in young stem, middle & old stem is not effected	Epidermal line is ruptured in young, middle and old stem.
2	Hypodermis	Do	Lysis in few cells	Partially breaks in young, middle and old is unaltered	Cells are ruptured in young, cell wall broken in middle and old
3	Aerenchyma (Air cavity)	Do	The air chamber is distinct in middle and old stem, cavity enlarged in young stem	Parenchymatous cells are broken so the cavities are deformed	Air cavities enlarged and deformed
4	Parenchyma	Do	Partially breaks in young, unaltered in middle and old	Cell wall is ruptured in young and middle. Old cells are partially broken	Most Cells are removed in middle and old and some are deformed
5	Trachieds	Do	Vascular bundle is ruptured in young	Lysis in the xylem fibre, vessel is ruptured in middle and old	Tracheary cells are damaged in young, partially broken in old and middle.
6	Xylem vessels	Do	No change	Ruptured	Vessels are deformed in young, ruptured in
7	Phloem cells	Do	No change	Ruptured	Lysis is vascular bundle
8	Sclereids	Do	Partially breaks	Walls are Ruptured in young	Broken in young, ruptured in middle and old

Table 6. Description of the effects in different types of cells of leaves of a WHB plant

Sl. No.	Type of cells	Effects after dilute acid treatment in leaf			
		Untreated (Control)	1% H_2SO_4, 2 h	3% H_2SO_4, 2 h	5 % H_2SO_4, 2 h
1	Epidermal cell	Do	No change	Partiall breakage	damaged
2	Parenchyma cell	Do	Some cells broken	Lines are disturbed	breaks
3	Air cavity	Do	Air cavity is enlarged	enlarged	Other adjacent cells are damaged so hollow is formed
4	Palisade cells	Do	Layers are disrupted	Cells are deformed	Cell wall s broken
5	Bundle sheath cells	Do	Not effected	disturbed	distorted
6	Xylem	Do	Not effected	effected	Xylem fibre is damaged
7	Phloem cells	Do	Not effected	effected	Cells are disrupted

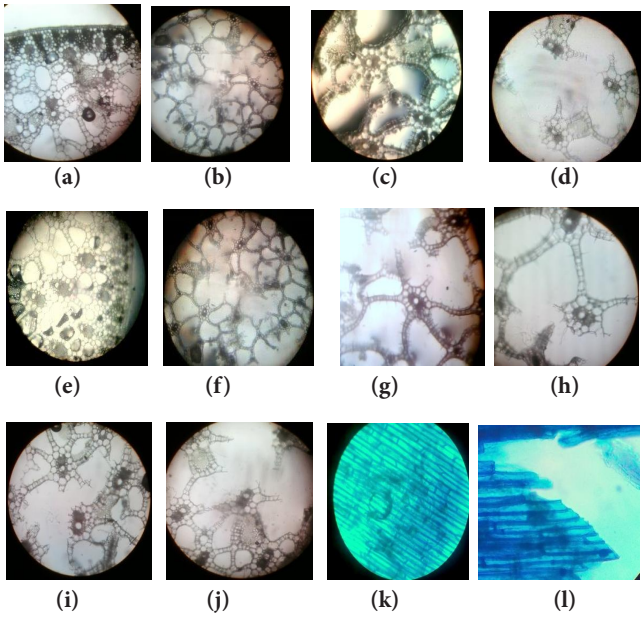


Figure 3. TS and LS of young, middle and old Stem (Petiole) of Water Hyacinth Plant (*Eichhornia crassipes)* **(a)** TS of untreated young stem of a young. **(b)** TS of young stem treated with 1% H_2SO_4 for 3 h. **(c)** TS of young stem soaked in 3% H_2SO_4 for 3 h. **(d)** TS of young stem soaked in 5% H_2SO_4 for 3 h. **(e)** TS of middle aged stem soaked in 1% H_2SO_4 for 3 h. **(f)** TS of middle aged stem soaked in 3% H_2SO_4 for 2 h. **(g)** TS of middle aged stem soaked in 5% H_2SO_4 for 2 h. **(h)** TS of old stem soaked in 1% H_2SO_4 for 3 h. **(i)** TS of old stem soaked in 3% H_2SO_4 for 3 h. **(j)** TS of old stem soaked in 5% H_2SO_4 for 3 h. **(k)** LS of young stem (untreated). L. LS of young stem in 3% H_2SO_4 for 3 h.

sheath of parenchyma cells. Sclereids are present inside air spaces. Transverse and longitudinal sections of young, middle and old leaves of Water Hyacinth were studied under microscope after hydrolysis at different conditions are shown in Figure 4 (a-d).

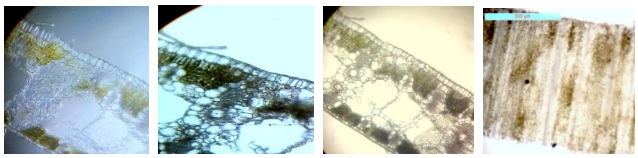
3.3 Scanning Electron Microscope (SEM)

The treated and untreated biomass was further analysed by Scanning Electron Microscopy (SEM).

It has been noticed that the untreated biomass is generally homogenous, less pore sizes, cylindrical shape; layers are not prominent (Figure 5a). The treated biomass has more pores (Figure 5b). The heterogeneous distribution of the pores and rough texture on the surface structure confirm the pronounced effect of pretreatment on the Water Hyacinth Biomass with heterogeneous distribution of pores and rough texture on the surface. The pore openings at 500X magnification shows that pore of the surface bubbled out, which exhibits higher surface area for hydrolysis with acid. At higher magnification (x1000, x 1500 and x 2000) pore opening is very clear that provide accessibility to internal layer for reaction (Figure 5c). At low magnification (x 500) surface morphology can be studied where rigid and highly ordered fibrils are present. Fibers of 1%, 3% and 5%, 160 rpm and 2 h soaked sample appear to be distorted as shown in Figure 5 (d-e).

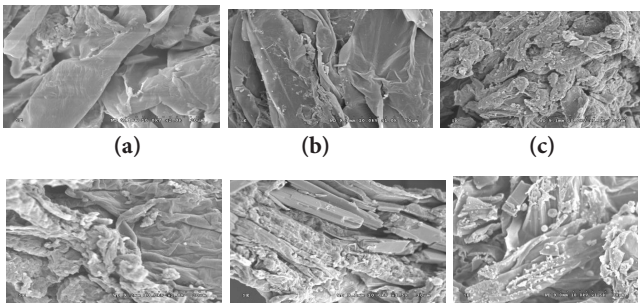
At a 4 times higher magnification i.e. x2000, fibrillar arrangement is very clear with delignified portions with

light color, thus increasing the external surface area and the porosity with more accessibility to sugar polymers. Presence of different elements were detected in specific points of the heterogenous biomass which showed present of elements like Mg, Ca, Cl, K, S, F, O, Pb and Nb and represented in Figure 6 and Table 7.



(a) (b) (c) (d)

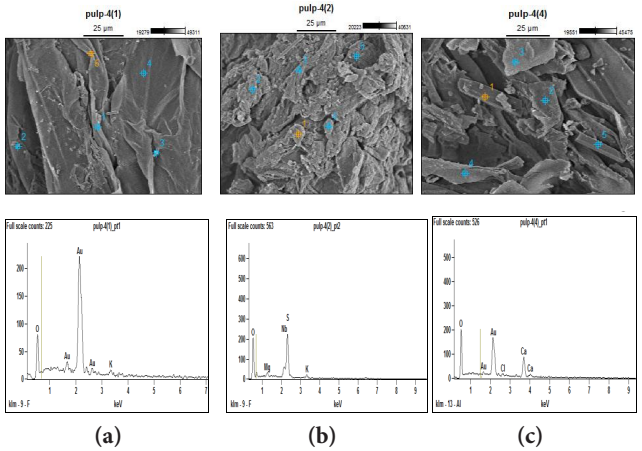
Figure 4. **(a)** TS of young leaf (untreated). **(b)** Middle aged leaf treated with 3% H_2SO_4 for 2 h. **(c)** Old leaf treated with 3 % for 3 h. **(d)** LS of young leaf (untreated).



(a) (b) (c)

(d) (e) (f)

Figure 5. SEM Pictures. **(a)** Untreated biomass. **(b)** Biomass boiled in water for 30 mins. **(c)** 1% H_2SO_4, 160 rpm, 50 °C, 2 h ST, 15 min boil. **(d)** 3% H_2SO_4, 160 rpm, 50 °C, 2 h, ST, 15 min boil. **(e)** 5% H_2SO_4, 160 rpm, 50 °C, 2 h ST, 15 min boil. **(f)** 3% H_2SO_4, 160 rpm, 50 °C, 2 h soaking time, 30 min boil.



(a) (b) (c)

Figure 6. SEM pictures and Scanning Electron Micrograph with analysis of different elements present in treated and untreated WHB in specific points **(a)** Untreated biomass, **(b)** Treated with 3 % acid, **(c)** Treated with 5 % acid.

Table 7. Atomic wt% of different elements present in the biomass

Atomic wt. %	O	F	Mg	S	K	Nb	Pb	Ca	Cl
Untreated (A)	12.38	-	-	-	2.64	-	-	-	-
Treated with 3 % acid (B)	52.08	5.61	1.68	33.28	8.12	17.51	18.62	-	-
Treated with 5 % acid (C)	53.13	-	0.92	-	10.25	-	-	27.22	12.73

4. Conclusion

Water Hyacinth is one of the common weed abundantly available worldwide, causing major problems to aquatic ecosystem due to invasive growth. Being a non edible, abundantly available, cheap renewable source, with high percentage of hemicellulose and cellulose, it can be an excellent biomass for bioconversion. The shoot portion contains high percentage of hemicellulose (38%) and cellulose (33%) which can be hydrolysed to obtain maximum amount of monomeric sugars for bioconversion to desired product. The hemicelluloses present in WHB can be hydrolysed to obtain xylan sugar that is fermented to xylitol. Many studies were performed earlier to examine chemical and physico- chemical characteristics of water hyacinth biomass, but this paper focus on the effect of acid hydrolysis on WHB with detail anatomical, tomographical and microscopic studies for analysis of structural changes which justify the higher xylose yield from WHB. The effects of acid hydrolysis are clearly evident from the anatomical studies in microscope and SEM. Thus WHB can be utilised for bioconversion of value added product xylitol, after dilute acid hydrolysis of fresh uniform paste made from leaves and stems/petioles of young and middle aged plants which extends further scope of studies on optimisation of process parameters for acid hydrolysis for higher xylose yield and fermentation to obtain xylitol.

5. Acknowledgement

The authors express their gratitude to the Director CSIR-CMERI and Director, NITD for his kind support to carry out the research work.

6. Reference

1. Jafari N. Ecological and socio-economic utilization of water hyacinth (Eichhornia crassipes Mart. Solms). Journal Applied Science Environmental Management. 2010 Jun; 14(2):43–9.
2. Bartodziej W, Weymouth G. Water bird abundance and activity on Water Hyacinth and Egeria in the St-Marks River, Florida. J Aquat Plant Manage. 1995; 33:19–22.
3. Brendock L. The impact of Water Hyacinth (Eichhornia crassipes) in a eutrphic subtropical impoundment (Lake Chivero, Zimbabwe). II. Species diversity. Arch Hydrobiology. 2003; 158(3):389–405.
4. Lu J, Wu JG, Fu ZH, Zhu L. Water Hyacinth in China: A sustainability science based management framework. Environment Management. 2007 Dec; 40(6):823–30.
5. Bolorunduro PL. Water Hyacinth inundation: Nuisance or nugget. Proceedings of the International Conference on Water Hyacinth; 2002. p. 111–21.
6. Kalhorinia S, Naseeruddin S, Yadav KS, Goli JK, Rao LV. Optimization of acid and enzymatic saccharification of lignocellulosic substrate Water Hyacinth (Eichhornia crassipes). ISRJ. 2013 Oct; 3(9):1–10.
7. Nigam JN. Bioconversion of Water Hyacinth (Eichhornia crassipes) hemicellulose acid hydrolysate to motor fuel ethanol by xylose-fermenting yeast. Journal of Biotechnology. 2002 Aug; 97(2):107–16.
8. Barrett SCH. Sexual reproduction in Eichhornia crassipes (Water Hyacinth) II. Seed production in natural populations. Journal of Applied Ecology. 1980 Apr; 17(1):113–24.
9. Penfound WMT, Earle TT. The biology of the Water Hyacinth. Ecological Monographs. 1948 Oct; 18(4):447–72.
10. Gopal B. Aquatic Plant Studies 1. Water Hyacinth. New York: Elsevier Publishing; 1987.
11. Dagno K, Lahlali R, Diourte M, Haissam J. Fungi occurring on Water Hyacinth (Eichhornia crassipes [Martius] Solms-Laubach) in Niger River in Mali and their evaluation as Mycoherbicides. Journal of Aquatic Plant Management. 2012; 50:25–32.
12. Hill MP. The impact and control of alien aquatic vegetation in South African aquatic ecosystems. African Journal of Aquatic Science. 2003 Jan; 28(1):19–24.
13. Zhang YYE, Zhang DY, Barrett SCH. Genetic uniformity characterizes the invasive spread of Water Hyacinth (Eichhornia crassipes), a clonal aquatic plant. Molecular Ecology. 2010 May; 19(9):1774–86.
14. Wilson JR, Rees M, Holst N, Thomas MB, Hill G. Water Hyacinth population dynamics. Biological and Integrated Control of Water Hyacinth. (Eichhornia crassipes). ACIAR. 2001; 96–104.
15. Khanna S, Santos M, Ustin S, Haverkamp P. An integrated approach to a biophysiologically based classification of floating aquatic macrophytes. International Journal of Remote Sensing. 2011; 32(4):1067–94.
16. Patel S. Threats, management and envisaged utilizations of aquatic weed Eichhornia crassipes: An overview. Reviews in Environmental Science Biotechnology. 2012 Sep; 11(3):249–59.
17. Robertson JB, Van Soest PJ. The detergent system of analysis and its application to human food. James, W.P.T., Theander, O. (Editors). The Analysis of Dietary Fiber in Food. NY: Marcel Dekker, Inc.; 1981.
18. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry. 1959; 31(3):426–28.
19. Elberts TJ, Sample RH, Glick MR, Ellis GH. A simplified, colorimetric micro method for xylose in serum or urine with phloroglucinol. Clinical Chemistry. 1979 Aug; 25(8):1440–3.
20. Johnson SL, Bliss M, Mayersohn KA. Phloroglucinol-based colorimetry of xylose in plasma and urine compared with a specific gas-chromatographic procedure. Clinical Chemistry. 1984 Sep; 30(9):1571–4.
21. Nyananyo BL, Gijo AH, Ogamba EN. The physico-chemistry and distribution of Water Hyacinth (Eichhornia cressipes) on the river Nun in the Niger Nelta. Journal of Applied Science Environmental Management. 2007 Sep; 11(3):133–7.
22. Matai S, Bagchi DK. Water Hyacinth: A plant with profile bioproductivity and photosynthesis. Applications of Solar Energy (Editor A. Gnanam et al.) Proc Internat Symp on Biol. India, Madras: MacMillan Co; 1980. p. 144–8.
23. Mahmood Q, Zheng P, Siddiqi MR, et al. Anatomical studies on Water Hyacinth (Eichhornia crassipes (Mart.) Solms) under the influence of textile wastewater. Journal of Zhejiang University Science. 2005 Oct; 6(10):991–8.
24. Kumar P, Barrett DM, Delwiche MJ, Stroeve P. Methods for pretreament of lignocellulosic biomass for efficient hydrolysis and biofuel production. Industrial Engineering Chemistry Research. 2009; 48(8):3713–29.
25. Ahn DJ, Kim SK, Yun HS. Optimization of pretreatment and saccharification for the production of bioethanol from Water Hyacinth by Saccharomyces cerevisiae. Bioprocess and Biosystems Engineering. 2012 Jan; 35(1-2):35–41.
26. Reales-Alfaro JG, Trujillo LT, Arzuaga G, Castaño H, Polo A. Acid hydrolysis of Water Hyacinth to obtain fermentable sugars. CT and F - Ciencia, Tecnología y Futuro. 2013 Jan-Jun; 5(2):101–12.