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WATER HYACINTH AS A POTENTIAL BIOFUEL CROP

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ABSTRACT

Water hyacinth was introduced as an ornamental crop species in many countries more than a century ago, because of their attractive blue, lilac to purplish flowers and round to oval leaves. They were supplied to many gardeners and horticulture institutes and were supposed to be a prized species. Soon, it was realized to be an invasive species due to their adaptability to a wide type of fresh water ecosystems and interference with human activities. Thus a huge amount of money, and efforts have been invested since then for their management. However, it was recently realized that they could be sustainably managed in their natural ecosystem and used in biofuel production, generating ample avenues of research, development and marketing of their end product (i.e. bioethanol and biogas). As the search for alternatives to fossil fuel intensifies in this age of modernization and industrialization, fuelled by increasing energy costs, water hyacinth holds a strong promise in the 21st century biofuel industry.

KEYWORDS

Water hyacinth, ornamental, low lignin, biofuel crop.

INTRODUCTION

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. It is also a well known ornamental plants found in water gardens and aquariums, bears beautiful blue to lilac colored flowers along with their round to oblong curved leaves and waxy coated petioles. It grows from a few inches to about a meter in height. The stem and leaves contain air filled sacs, which help them to stay afloat in water.

Other useful sources of floriculture related data for ornamentals include national governments, the International Association of Horticultural Producers, the United Nation Comtrade statistics publications and various online trade journals. The value of additional aspects of floriculture related products are even more difficult to determine (Chandler and Lu 2005; Chandler and Tanaka 2007). Precise data on the various aspects of the water hyacinth as an ornamental crop is not available for different countries of the world, as considerable amount of unrecorded economic activities take place in local markets.

In the developing world, it is used in traditional medicine and even used to remove toxic elements from polluted water bodies. They reproduce both asexually through stolens and sexually through seeds, which remain viable for up to 20 years and therefore are difficult to control (Center et al. 1999). Thus, it is also considered as a noxious weed in many parts of the world as it grows very fast and deplete nutrient and oxygen rapidly from water bodies, adversely affecting flora and fauna. There have been instances of complete blockage of waterways by water hyacinth making fishing and recreation very difficult. Shoeb and Singh (2000) reported that under favorable conditions water hyacinth can achieve a growth rate of 17.5 metric tons per hectare per day. There is a great discrepancy among policy makers, environmental agencies and research scientists on the way to control this invasive species and the practical benefits that can be obtained (Lu et al. 2008). There is a need for sustainability and a new perspective when it comes to managing this species and understanding and implementing their marketability as an ornamental or in their alternative products or as a newly found biofuel crop.

Water hyacinth is an invasive species, which invades fresh water habitats and is listed along with some of the worst weeds (Center et al. 1999). Some countries have even placed this species in their quarantine list and banned their sale or movement within their sphere of influence. Water hyacinth is very difficult to eradicate by physical, chemical, and biological means, and a substantial amount is spent on their control annually throughout the world. It is also a very sturdy species. It cause blockage of irrigation channels affecting the flow of water to fields, get entangled with motorboat rotors, making fishing difficult, and almost makes any place inhabitable and inaccessible. They may block hydroelectric turbines causing enormous damage, which are vital for economy and green environment. They out-compete almost all other species growing in their vicinity thereby decreasing biodiversity (Crafter et al. 1992). They destroy the beauty of a given place, and sometimes can be a breeding ground for disease causing insects and pests. They also can accelerate the process of evaporation from water bodies. They tend to absorb nutrients quickly thus making the ecosystems less fertile. This may have a large impact on the life of marginal farmers, increasing poverty in the less developed world.

2 Biological Attributes of Water Hyacinth for An Efficient Bioenergy Crop

Attributes of an ideal biofuel crop are:

1. Naturally grown vegetation, preferably perennials.
2. High cellulose with low lignin content per unit volume of dry matter.
3. Easily degradable.
4. Should not compete with arable crop plants for space, light and nutrients.

5. Resists pests, insects and disease.
6. Not prone to genetic pollution by cross breeding with cultivated food crops.

Water hyacinth is low in lignin content (10%) and contains high amounts of cellulose (20%) and hemicellulose (33%) (Bolenz et al. 1990, Poddar et al. 1991, Gressel 2008). Detailed composition of water hyacinth is described in Table 1. A typical biomass from land plants can have 30-50% cellulose, 20-40% hemicellulose and 15-30% lignins. In plants, lignin (composed of phenylpropanoid groups) acts as a polymer around the hemicellulose microfibrils, binding the cellulose molecules together and protecting them against chemical degradation. Lignin cannot be converted into sugars. Thus, it is not practical in biofuel production. Their degradation is a high-energy process. Water hyacinth has low lignin, which means the cellulose and hemicellulose are more easily converted to fermentable sugar thus resulting in enormous amount of utilizable biomass for the biofuel industry (Fig. 1a). Masami et al. (2008) suggested a new method of extracting ethanol by saccharification with diluted sulfuric acid, and hastening the process by using yeast.

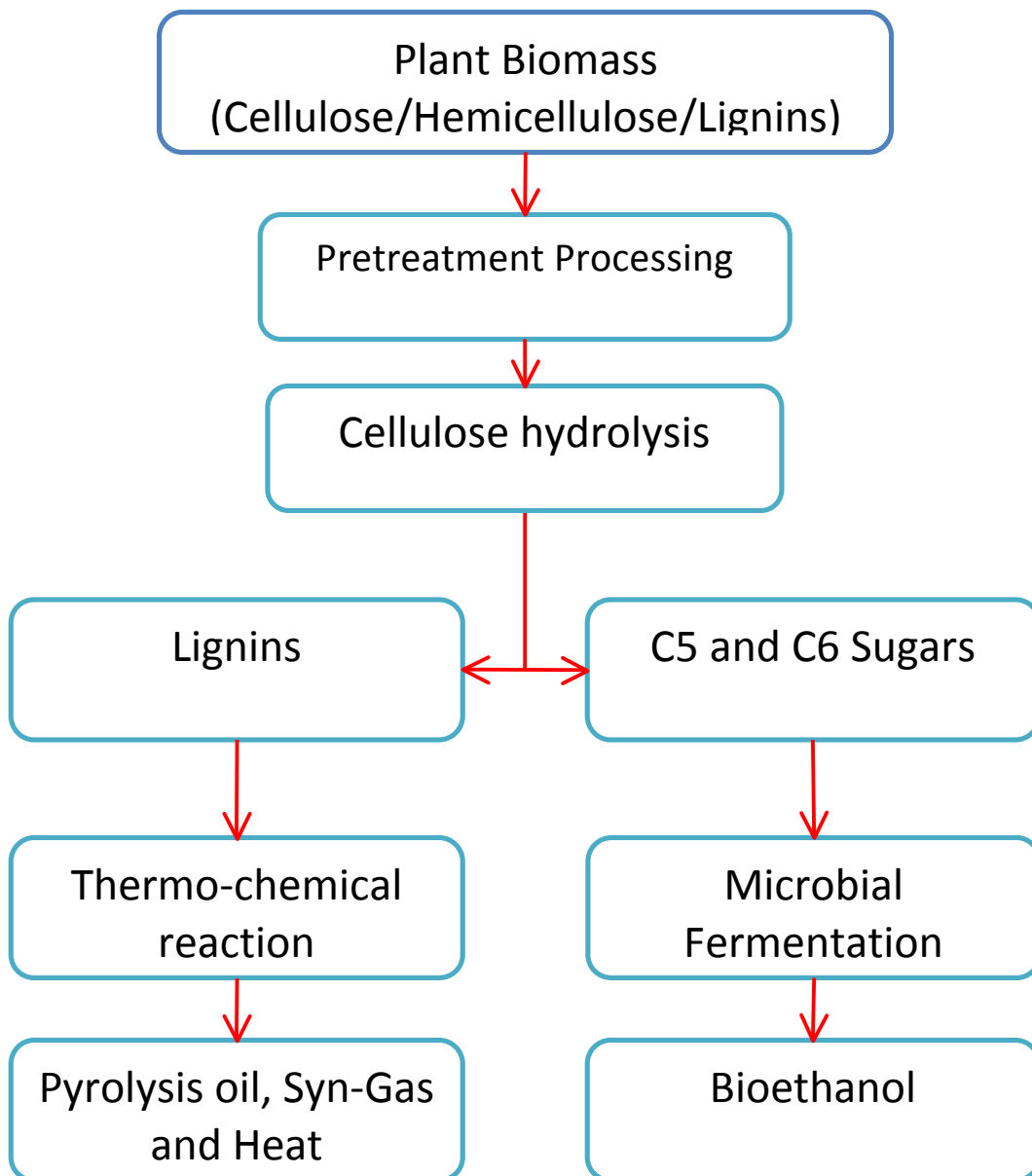
Further, water hyacinth grows at a very rapid pace and contains very high nitrogen content. The mixture of cowdung and water hyacinth slurry has proven to produce more biogas than when used alone (El-Shinnawi et al. 1989). The left-over slurry or sludge can be transported as liquid fertilizer. They can practically grow in any habitat and requires little to no maintenance, but they prefer to grow in warm climate. Further, they can be used to purify water bodies containing high amounts of heavy metal contamination. The biomass can be used to produce biogas and the byproducts can be used as organic manure or for producing bioethanol by further decomposition of fermentable saccharides. However, there are no exact figures available for bioethanol production from water hyacinth (Nigram 2002).

In addition, aquatic plants do not compete with land resources used in arable food crop cultivation and thus are an incentive factor when it comes to biofuel production. For the past few years, there have been reports of genetic engineering of microorganisms, which can increase ethanol production from hemicellulose by fermenting it into oligosaccharides (Dien et al. 2003; Mishima et al. 2008). Mishima et al. (2008) also found that bioethanol generating capacity of water hyacinth can be compared to that obtained from agricultural waste, thus is a potential new crop for biofuel production and an employment generating industry.

Table 1. Average biomass composition of water hyacinth. Adapted from Poddar et al (1991); Gunnarsson and Petersen (2007).

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

a.



b.

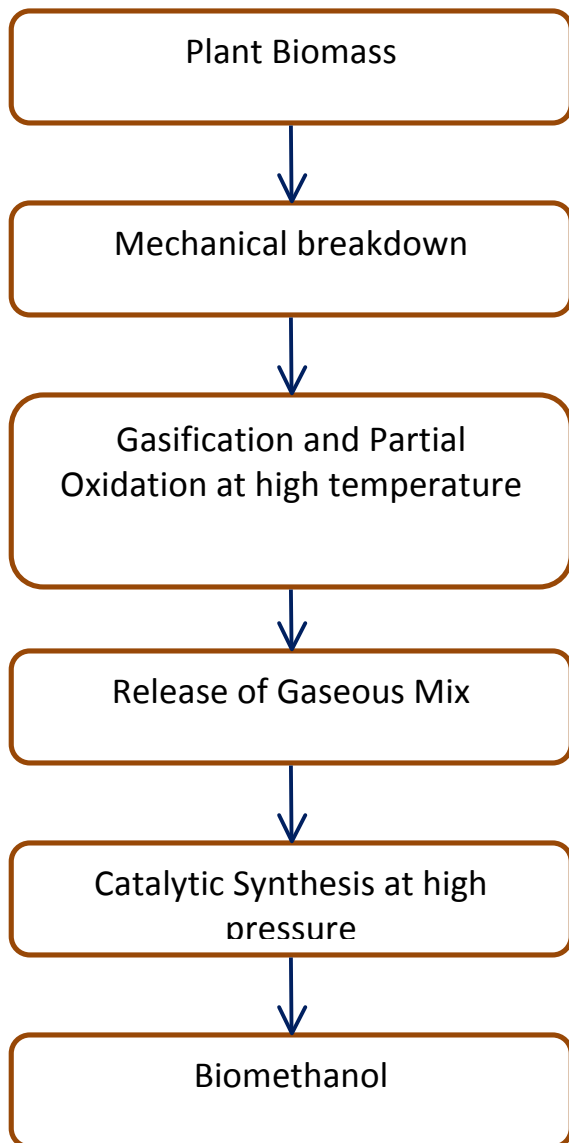


Figure 1. Flowchart showing step wise procedure involved in the production of a. Bioethanol and Biogas
b. Biomethanol from water hyacinth.

3 Genetic Manipulation of Possible Biosynthetic Pathways to Enhance Biofuel Production

Prerequisite of using crops in efficient biofuel production is to design the most effective way to break down complex polysaccharides into fermentable sugars. These production costs could be cut down by bioengineering crop plants in such a way that the necessary cell wall degrading enzymes, such as cellulase and hemicellulase, are produced within the plant system, thereby reducing the dependency on microbial bioreactors for enzymes. Further cost reduction could also be achieved by genetically engineering the lignin biosynthetic pathway in plants so that there is a minimal need for expensive pre-treatment and any crop plant which is low in lignin will hasten our objective. Another

strategy is to up-regulate the cellulose biosynthesis pathway enzymes for increased polysaccharide production, which in turn will increase the yield of cellulosic biofuel (Sticklen 2008).

Ralph et al. (2006) found that flexible polymerization is possible in plants, whereby monolignins can be actively substituted by polyphenols. This in turn may not affect the development process of plants, but will facilitate the extraction of saccharides needed for biofuel production from the plants. This same strategy can be applied to water hyacinth, which is naturally low in lignin and thus it will be more effective to extract fermentable saccharides. Vanden Wymelenberg et al. (2006) reported a large number of genes involved in breakdown of lignin from the fungus *Phanerochaete chrysosporium* genome. Thus, there is also the possibility of expressing such genes in water hyacinth by a transgenic approach which may further reduce the cost of biofuel production. The use of transgenes could also underpin conventional breeding to modify biofuel production, in view of growing public concern over potential environmental damage and health hazards by the use of fossil fuel, and the limited availability of suitable genes to modify synthesis of sugars, lignin and lipids in plants, by adopting classical breeding approaches.

The first step will be to find an effective protocol for tissue culture and transformation of different cultivars of water hyacinth. Tissue culture refers to the collection of strategies available for growing a large number of cells in an axenic and controlled environment. The basic principle involved in tissue culture is cell totipotency (the ability to regenerate intact, usually fertile plants), which is based on the expression of developmental genes. The latter are usually heritable, in response to the tissue culture environment. Thus, several combinations of PGRs (plant growth regulators) have to be evaluated in order to establish conditions for plant regeneration or indirect organogenesis (Hassanein and Soltan 2000). This is a crucial pre-requisite for the use of tissue culture as a baseline for genetic transformation. It is also a key component in plant transformation using *Agrobacterium tumefaciens*-mediation and biolistics gene delivery (Bhatia et al. 2004). A major application of tissue culture is micropropagation, mass multiplication of plants. It offers a new means of obtaining specific pathogen-free plants from meristem culture, genetic manipulation, and the production of haploid plants. Micropropagation has been extensively applied in the propagation of numerous plant species. The success of a tissue culture system depends upon the plant species, the axenic culture conditions, availability of utilisable carbon, callus induction response, synergistic interaction between growth regulators and, crucially, composition of the growth media. The latter includes organic compounds, plant growth regulators, carbon source, trace elements and inorganic salts (Slater et al. 2003).

The public acceptance of GM technology is low especially in Europe, as it is considered to generate unacceptable risks (Uzogara 2000). This is not the case for other developed and developing countries. This concern is primarily seen as a potential risk of the flow of transgenes to wild species. Terminator gene technology as reviewed by Kuvshinov et al. (2001), can prevent the flow of transgenes to wild species or to other crops, thus reducing the anticipated risks.

4 Amenability to Physical and Chemical Methods of Extracting Fermented Sugar

The potential to harvest biofuel from aquatic plants should be emphasized. Aquatic plants like water hyacinth are naturally low in lignin content and they grow at a rapid pace (Ripley et al. 2006; Gressel 2008). Aquatic plants have waxy coatings on their surface (which are complex alcohols), which are modified fatty acid deposition and helps in increasing the yield of biofuels. Gunnarsson and Petersen (2007) estimated the presence of lignin in aquatic plants at less than 10%. This would enable them to be processed efficiently into biofuels. The major hurdle to producing biofuel is the high cost of conversion of cellulose and hemicellulose into ethanol. The major share of the cost in biofuel production is the expense incurred in the production of cellulase enzymes in microbial

bioreactors. Secondly, the cost of pre-treating lignocellulosic matter for its breakdown to the intermediate components, and subsequent removal of the lignin, is very high. The technique of digesting lignin is still in infancy and the costs associated with it far outweigh the beneficial effects of adopting this technology on a large scale. With the recent advent of increasing prices of food products and their shortage, the first generation of biofuel crops was discouraged. Instead research was more concentrated on emerging biofuel crops like water hyacinth, low in naturally occurring lignin, that do not compromise the food needs of mankind. This strategy could lead to potential sources of employment, and at the same time help in land reclamation with little or no subsequent maintenance.

The area of ethanol production showing the most promise, both in terms of potential energy savings and in terms of national security interests, is ethanol derived from cellulose. It has several advantages, such as it will be sustainably available in the future and it is considered to be environmentally friendly, since it has low net release of carbon dioxide and sulphur content. Complex carbohydrates like cellulose and hemicellulose are first converted into their component sugars through a hydrolysis process and then the sugars are anaerobically fermented into biofuel such as bio-ethanol. At present only the cellulose and hemicellulose components of plant biomass can be converted into biofuels by the action of anaerobic microbes. The major obstacle in increased biofuel production is to find an efficient way to break down complex plant polymers into simpler derivatives. Biomass first is ground into smaller particles or chips. These chips are then pre-treated often with sulfuric acid to break down the hemicellulose into simple 5- and 6- carbon sugars to unlock the cellulose. Then this treated biomass is separated into glucose and the various pentoses from the hemicellulose. Glucoses are fermented by yeasts and/or bacteria.

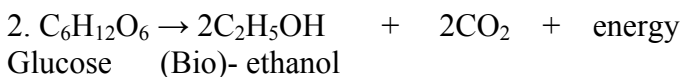
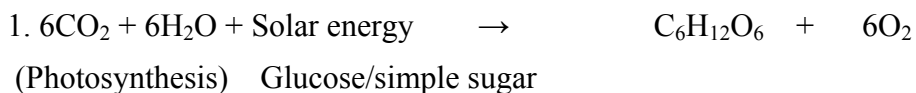
Unlocking the cellulose from plant cell walls and efficiently converting it to ethanol is the key to making ethanol an universal, inexpensive form of fuel for the future. Now, the emphasis is also on bio-hydrogen which can be extracted from wood residues when acted upon by microbes to degrade the complex cellulosic material. Biogas can be produced from sewage plants and industrial wastes. Generally methane, hydrogen and carbon-monoxide are produced. The end product of using cellulosic biomass may one of the following:

a. Bioalcohol

The simple and complex polysaccharide plant food reserves are broken down into simple sugars, and fermented to produce bioalcohol. They are specifically designated as Bioethanol and Biobutanol.

a.1 Bioethanol

Photosynthetic processes in plants produce simple and complex sugars, which can be decomposed by fermentation in the presence of microorganisms such as *Escherichia coli*, *Klebsiella oxytoca*, *Saccharomyces cerevisiae*, and *Zymomonas mobilis* (Dien et al. 2003) to produce bioethanol. It is highly soluble in water and is hygroscopic, thus requiring an energy-intensive distillation process to separate it from the mixture during the extraction process.



a.2 Biobutanol

Biobutanol is produced by fermentation of starch or sugars by the action of *Clostridium acetobutylicum*. It is long chain alcohol and fairly nonpolar, thus non-hygroscopic compared to ethanol, and less energy is needed for its production.

a3 Biomethanol

Though an alcohol, biomethanol is not produced by microbial fermentation. Biomethanol is produced by the mechanical breakdown and gasification of biomass, which upon subsequent release is condensed into liquid form by catalysis under high pressure (Fig. 1b).

2. Biogas and Biohydrogen

Biogas is produced from decomposed organic substances by pyrolysis in the presence of anaerobic bacteria, releasing gaseous substances (generally a mixture of hydrogen, methane and carbon-dioxide). Recently, there has been speculation of the need to develop nitrogen-fixing bacteria capable of producing biogas.

5. Alternative Uses

Water hyacinth can be used in farming as an organic fertilizer and as a mulch crop. There has been an increase in demand for organic foods in the developed world. Besides, it helps to conserve soil moisture and nutrient reclamation. The plants can be turned into compost and used as a fertilizer. The plant tends to retain most of the nutrients (reviewed by Gunnarsson and Petersen, 2007) when dried thus decreasing our needs on chemical fertilizer. The time taken in composting is only 30 days (Polprasert et al., 1980) compared to other crop plants, which can take up to 2-3 months. They can be converted into ash (40%) [Reviewed by Gunnarson and Petersen, 2007] which is rich in micronutrients and thus can be used in soil enrichment. Thus, compost from water hyacinth tends to improve physical, chemical and biological properties of soil. They are relatively easy to transport and cost very little. They are also used in traditional medicine as their root and leaf extracts are known to cure certain diseases (swelling, burning, hemorrhage, and goiters). They are also used to treat certain inflammatory conditions of veterinary animals. They are also used as a vegetable crop (leaves, petiole and flowers) in some parts of the world (Phillipines, Java) and are known to be rich in carotene, protein and carbohydrates. In addition they can be used as a feed for livestock as a roughage component (Gunnarsson and Petersen, 2007). They can be used to grow mushroom, vegetable and floriculture plants can be grown on water hyacinth rafts. The green biomass can be used as an animal fodder and also used to make hay and silage (Lindsey and Hirt, 1999a). They are also used in furniture manufacture, which are in high demand and available through online stores (typical examples, <http://www.balifurnish.com> and <http://www.whup.20m.com>). They are also used to manufacture building board. They can be also be used in processing coffee beans and even as laundry detergents (Hassan et al., 2006). Further they can be used in paper production and also in grease- proof paper manufacture (De Groote et al., 2003). They are used as briquettes for cook stoves. Rural folks also use the plant to manufacture rope or coir. In some South Asian countries they are used to make floating vegetable gardens. Water hyacinths can be used in reclamation of water bodies containing high levels of lead and mercury. Al Ramalli et al., (2005) have demonstrated that even dried hyacinth roots are very effective in removing arsenate from the

contaminated water system. They can thus grow in highly polluted water bodies. They can be used to produce cellulase enzymes (Lindsey and Hirt, 1999b). Water hyacinths also provide food sources to aquatic fauna like snails, fish, crabs etc.

6. Spread and Efficient Control Strategies

Water hyacinth grows at an extremely rapid pace (almost producing 2 tonnes of biomass per acre and population doubles every 5 -15d, Craft et al., 2003). Since it adversely affects natural flora and fauna, considerable efforts have been given for its eradication. Besides, indirectly they tend to become breeding grounds for various disease causing insects and pests. The use of chemical agents, like 2,4-D and other herbicides including glyphosate and diquat is common; however, the use of chemicals is largely discouraged as its long term effect of human health, and the ecosystem is unknown. In the future, plants may become more tolerant to such chemicals and slowly these chemicals may become ineffective, thus requiring higher dosage, which may increase costs associated with application. Further, the treated area may become uninhabitable for other plant species. Physical eradication is largely used; this is not entirely effective, limited by a certain area which can be covered in a day, besides costs and human resources involved, which may become a bottleneck in various locations. However, when water hyacinth is used for biofuel production, this will be perhaps the best method to both control and harvest them. The use of biological agents like weevils (*Neochetina eichhorniae*, *Neochetina bruchi*), some native weevils, moths (*Sameodes albiguttalis*) and fungus [*Cercospora piaropi* and *Acremonium zonatum*] (van Thelen et al., 1994; Grodowitz, 1998) have yielded in encouraging results, and authors have reported complete control, but in areas where there are temperature fluctuations, insects need to be reintroduced every new season. Moreover wetland ecosystems are complex entities, therefore introduced insects may out-compete other native insect species, thereby decreasing the biodiversity.

7. Further Discussion and 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. Now is the time to look for new material sources of biofuels, which are naturally amenable to processing during extraction of biofuel, thus reducing costs drastically and substituting fossil fuels in all aspects. Water hyacinth has long been seen as an invasive species all over the globe and considerable amount of resources have been spent for their control. However, they have certain qualities which can be utilized to produce biofuels (both bioethanol to power vehicles and motors, biogas to generate electricity) as the plants are low in lignin content and have rapid growth rate. This could be of potential interest in south-east Asian countries where this species tends to occur so frequently. Besides they do not compete with food crops for land diversification. They tend to grow well in water bodies even with low nutrient regime and can be easily mechanically harvested. There are so many alternative uses of these plant species and time has come to look at the plant from a different viewpoint and utilize their potential as much as possible. This tends to generate ample employment particularly in developing world. And the growth rate is so tremendous that there will be no dearth of biomass in the long run. Further, they do not compete with arable crops in term of land resources and needs little to no care to grow them. There are certain concerns which must be addressed, controlling its rapid proliferation, and preventing marshes from becoming breeding grounds for various disease- spreading insect and pests, and its interference with day-to-

day activities of mankind, like blockage of irrigation channels, fishing, and recreating activities like boating.

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Floating vegetable garden from water hyacinth: A floating garden is built using water hyacinth as a base on which vegetables can be grown. It is cheap and sustainable. Firstly, a floating raft is built using water hyacinth to collect the water hyacinth. The rafts vary in size depending on the space available and size of the family. Typically the length will be around 8 m, the width from 1.5 to 2m, and the height will be less than one metre. Water hyacinth as a potential biofuel crop. *Electr. J. Environ.* Water hyacinth was used as substrate for bioethanol production in the present study. Combination of acid pretreatment and enzymatic hydrolysis was the most effective process for sugar production that resulted in the production of 402.93 mg reducing sugar at optimal condition. A regression model was built to optimize the fermentation factors according to response surface method in saccharification and fermentation (SSF) process. The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Acknowledgments.