# WATER HYACINTH – A POTENTIAL FEEDSTOCK FOR THE PRODUCTION OF BIOETHANOL AS TRANPORT FUEL

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**Introduction:** Fuel ethanol from lignocellulosic biomass is emerging as the single most important technology for production of renewable transport fuels. The cost of raw material is a significant factor affecting the biomass to ethanol process and the availability of cheap sustainable biomass resources is a major issue world wide. Water hyacinth is an aquatic weed listed as one of the most productive plants on earth. It grows faster than any other tested plants and can double in as little as 6 days. The biomass productivities of this plant is one of the highest in plant kingdom and an acre of water hyacinth can weigh more than 200 tons making it one of the most potent sources of biomass for fuel applications if exploited properly. The plant is a typical menace infesting the backwaters of Kerala state in southern part of India. Technology for ethanol production from water hyacinth biomass if developed, will serve the purpose of weed removal as well as generation of economy and such technologies are eagerly awaited. The major technical limitation in this direction is the saccharification of the biomass. The aim of the present study was to evaluate the effects of various process parameters for their effects on the pretreatment and saccharification of water hyacinth biomass using commercial cellulase preparation as well as onsite produced enzymes. The saccharified biomass was tested for its suitability in producing bio-ethanol.

**Methods:** Water hyacinth (WH) **w**as washed with tap water and leaves and stalks were dried in sunlight after removing the roots. Dried plant material was milled in a knife mill (particle size, < 1 mm) and stored in an air tight container at room temperature until used. Pretreatment was carried out in Erlenmeyer flasks by mixing biomass with different acids [mineral acids (HCl / H2SO4, 2% v/v) and organic acids (acetic acid / formic acid, 30% v/v)] and autoclaved (121°C, 15 lb) for 60 min. After pretreatment, samples were neutralized with 1N NaOH followed by washing with tap water and dried in air at 30C. Effectiveness of pretreatment was checked by sugar release from biomass after enzymatic hydrolysis. Most effective pretreatment agent was selected for further optimization of process parameters. Influence of various parameters (acid concentration, biomass loading, temperature and incubation time) on pretreatment was optimized by a stepwise experiment where specified parameter was changed by keeping all other parameters constant.

Enzymatic saccharification was carried out in stoppered flaks of acid pretreated WH (2 g) by adding commercial cellulase. Flasks were incubated at 50$^{o}$C in a shaking water bath set at 120 rpm. After incubation, samples were centrifuged to remove unhydrolyzed residue. Supernatant was used to estimate reducing sugar analysis by 2, 5 dinitrosalicylic acid method. Optimization of process parameters for enzymatic hydrolysis (incubation time, biomass loading, effect of surfactant, surfactant concentration and enzyme concentration) was carried out.

**Results:** Among different mineral and organic acids tested for pretreatment, H2SO4 gave maximum reducing sugar (0.327 g/g pretreated biomass) followed by HCl (0.277g/g). Compared to mineral acids, organic acids were not a good pretreatment reagent; reducing sugar yields were very less for acetic acid (0.097g/g) and formic acid (0.088g/g). Hence, H2SO4 was selected for further optimization of process parameters. Optimization of acid concentration and biomass loading for pretreatment (Table 1) showed that 4% (w/v) H2SO4 with 10% (w/w) biomass loading gave maximum reducing sugar yield (0.342 g/g).

Effect of incubation time on hydrolysis showed that maximum reducing sugar (0.487g/g) was produced after 24 h of incubation. Solid loading (12.5%) gave maximum reducing sugar (0.671 g/g); also, solid loading (10 - 17.5%) gave almost same amount of reducing sugar. Among surfactants used as additives to improve enzymatic hydrolysis of acid pretreated WH, Triton X-100 gave maximum reducing sugar (0.686g/g) followed by Tween-80 (0.647g/g) and PEG (0.482g/g); Triton X-100 (0.1%) gave maximum reducing sugar (0.689g/g).

**Conclusions:** WH could be potential low-cost biomass for the production of bioethanol.

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