

Dilute Acid Hydrolysis of Freshwater Fern (*Azolla* Sp.) For Production of Bioethanol

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Background & Objectives: For controlling the invasive growth and reduction of unfavorable effects of aggressor freshwater plant (*Azollasp.*) in Anzali wetland and using it as a new feedstock biomass, dilute acid hydrolysis of it was performed for production of bioethanol.

Methods: Samples were taken from different locations in Anzali wetland. After drying and milling, samples were digested by dilute acid and enzymatic methods. Dilute acid hydrolysis was done by autoclaving the ferns and also by a high pressure device (BatchSynth® Microwave synthesizer). Effect of different temperatures (in BatchSynth), times (in autoclave) and acid concentrations (in both) were compared. Cellulase treatment (Cellubrix®) was used for enzymatic hydrolysis of the residues. Reducing sugar, glucose and furfural contents were compared in hydrolysates.

Results: Highest amount of glucose (4.4% w/w) and reducing sugars (17.73%) were produced after autoclaving the samples for 60 and 20 min, respectively, by addition of 1.67% sulfuric acid. In Microwave Batchsynth system, maximum glucose (5.1% w/w) and reducing sugar (13.26% w/w) were produced at 200 and 180°C, respectively. Maximum amount of furfural (1.54 g/L), as an inhibitor of cell growth, was produced in microwave Batchsynth, while its content was insignificant after autoclaving the samples.

Conclusion: Statistical comparison showed significant ($P \leq 0.05$) difference in glucose production, which is important for fermentation. Total reducing sugars and glucose content were increased by optimizing acid concentration, time and temperature. By autoclaving, 1.67 % acid was best for total sugar and glucose production. By using Batchsynth microwave, increasing temperature led to increasing furfural, significantly ($P \leq 0.05$). Furfural strongly inhibits fermentation. Therefore, regarding energy and time consumption, inhibitor content and produced sugar, application of autoclave system for sugar production showed to be superior to high pressure microwave treatment of *Azolla*.

Keywords: Temperature; Acid Hydrolysis; *Azolla*; Biofuel