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Ultrasonic-Assisted Alkaline Pretreatment for Enhanced Bioethanol Production from Water Hyacinth (Eichhornia crassipes) Biomass

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Abstract: Water hyacinth (Eichhornia crassipes) is an abundant lignocellulosic biomass with strong potential as a feedstock for bioethanol production, but its relatively high lignin content limits the accessibility of cellulose and inhibits subsequent hydrolysis. This study evaluates ultrasonic-assisted alkaline pretreatment using NaOH concentrations of 1 M and 2 M and treatment times of 10, 15, 20, 25, and 30 minutes at 60°C and 60 W. The pretreated biomass was analyzed for lignin, cellulose, and hemicellulose using the Chesson method, followed by hydrolysis with 3% H2SO4, neutralization using NaOH, fermentation with 5% Saccharomyces cerevisiae for 120 hours, distillation, and characterization. The 2 M NaOH–25 min condition gave the lowest observed lignin fraction (5.4%), hemicellulose fraction (10.8%), and the highest observed cellulose fraction (75.1%). The corresponding selected distillate showed an alcohol-refractometer reading of 14%, density of 0.980 g/mL, pH 6.5, and distillate volume of 42 mL. GC-MS identified ethanol at a retention time of 1.289 min with a reported peak area of 73.02%; this peak area is interpreted qualitatively and is not treated as an ethanol concentration. The 14% value is therefore reported as an alcohol-refractometer reading rather than a fully validated ethanol concentration. Because independent experimental replicates and controls isolating the ultrasonic contribution were not included, the 2 M–25 min condition is described as the best observed condition rather than a statistically optimized condition. Further validation with replicate experiments and quantitative analytical calibration is required.

Keywords: Bioethanol; Water hyacinth; Ultrasonic-assisted pretreatment; Alkaline pretreatment; Delignification

1. Introduction

The increasing consumption of fossil fuels has strengthened the need to develop renewable energy sources that can reduce dependence on conventional fuels. Biomass is one of the renewable resources available for biofuel development.

Biofuels offer advantages related to renewable feedstock availability and the potential for lower environmental impacts. Bioethanol is a liquid biofuel commonly produced through hydrolysis and fermentation of carbohydrate-containing biomass [1].

Bioethanol feedstock can be derived from starchy, sugary,

or lignocellulosic biomass [1]. Water hyacinth (Eichhornia crassipes) is a rapidly growing aquatic plant that can be utilized as a lignocellulosic feedstock [2]. The main obstacle to utilizing lignocellulosic biomass is the structural association of lignin with carbohydrates, which can limit access to cellulose; therefore, pretreatment is commonly applied before hydrolysis.

Previous studies have investigated bioethanol production from water hyacinth and reported that the resulting alcohol content depends on hydrolysis and fermentation conditions. Wahyuni et al. [3] reported changes in bioethanol production with fermentation of water hyacinth, while Wulandari et al. [2] investigated the effects of H2SO4 concentration and fermentation time on bioethanol production from water hyacinth. These findings support the need to evaluate pretreatment conditions before hydrolysis and fermentation.

Previous studies have investigated alkaline and ultrasound-assisted pretreatment of lignocellulosic biomass [4]–[6]. However, limited information is available regarding the combined effects of ultrasonic-assisted alkaline pretreatment duration and NaOH concentration on the measured lignocellulosic composition of water hyacinth under the experimental conditions used in this study.

2. Materials and Methods

2.1. Tools and Materials

The tools used in this study included a 3-L ultrasonic cleaner (60 W ultrasonic power), reflux apparatus, condenser, round-bottom flask, hot plate, hydrolysis apparatus, distillation apparatus, thermometer, Brix refractometer, 0–80% alcohol refractometer, furnace, blender, fermentor, and Erlenmeyer flask. The operating ultrasonic frequency, effective delivered power in the sample, power density, and detailed temperature-control procedure were not documented in the available experimental record.

The materials used in this study included water hyacinth (Eichhornia crassipes), distilled water, H2SO4, NaOH, and yeast (Saccharomyces cerevisiae).

2.2. Research Flow Diagram

The overall research flow is presented in Figure 1.

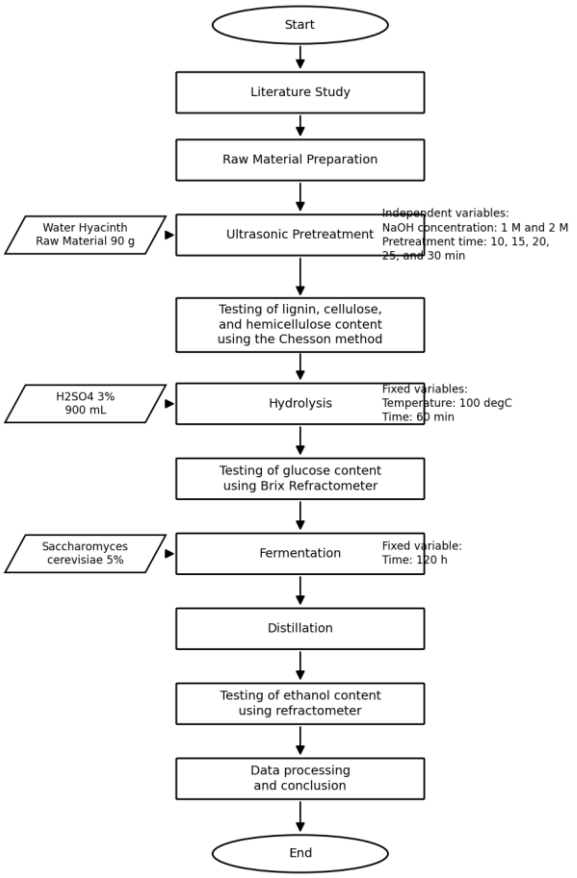

Figure 1. Research flow diagram.

2.3. Research Procedure

Water hyacinth was cleaned, dried, and ground into powder. A total of 90 g of water hyacinth powder was pretreated using NaOH solution at concentrations of 1 M and 2 M with a solid-to-solution ratio of 1:10 (w/v), using a 3-L ultrasonic cleaner at 60°C and 60 W power, with time variations of 10, 15, 20, 25, and 30 minutes. The pretreated samples were then washed to neutral pH, filtered, dried at 70°C for 2 hours, and analyzed for lignin, cellulose, and hemicellulose content using the Chesson method. The samples were subsequently hydrolyzed using 3% H2SO4 solution at 100°C for 60 minutes. After hydrolysis, the hydrolysate was neutralized using NaOH before fermentation. The exact amount of NaOH and the final target pH for this neutralization were not specified in the available experimental record. The soluble-solids reading of the hydrolysate was measured using a Brix refractometer. The hydrolysate was fermented with 5% Saccharomyces cerevisiae, corresponding to 4.5 g relative to the initial 90 g biomass, for 120 hours at 30–35°C under anaerobic conditions. The fermentation liquid was then distilled at 78°C to obtain distillate, which was characterized using a 0–80% alcohol refractometer, density measurement, pH measurement, and distillate-volume measurement. The alcohol refractometer reading is reported as an instrument reading rather than a fully validated ethanol concentration.

3. Results and Discussion

3.1. Effect of NaOH Concentration and Pretreatment Time on Lignin Content

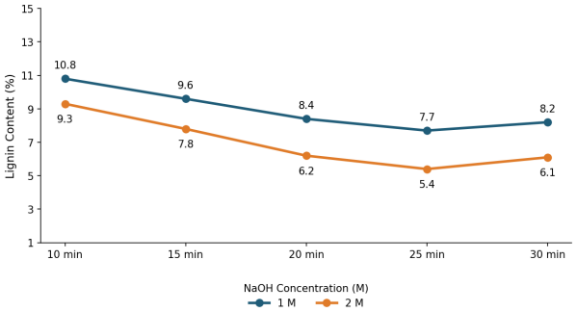

Figure 2. Effect of time and NaOH concentration on lignin content.

Based on Figure 2, the measured lignin fraction decreased with increasing pretreatment time and NaOH concentration. At 2 M, the lowest observed lignin fraction of 5.4% was obtained at 25 minutes. This result is consistent with alkaline pretreatment promoting disruption of lignin–carbohydrate associations and lignin solubilization [4], while ultrasound can enhance contact between the alkaline solution and biomass [4], [5]. At 30 minutes, the measured lignin fraction slightly increased. Because the experiment did not include independent replicates or controls isolating ultrasound from alkaline treatment, this change is interpreted cautiously as a trend in the measured composition rather than proof of an ultrasound-specific degradation mechanism. Thus, 2 M for 25 minutes is identified as the best observed condition for the lowest measured lignin fraction, not as a statistically optimized condition.

3.2. Effect of NaOH Concentration and Pretreatment Time on Hemicellulose Content

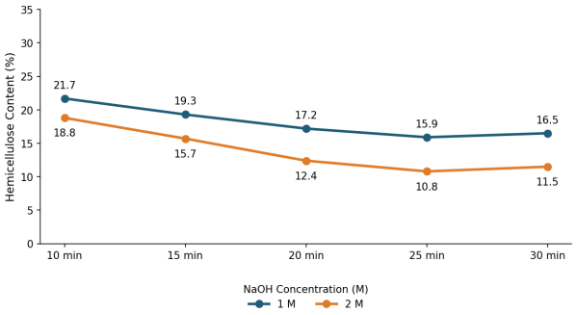

Figure 3. Effect of time and NaOH concentration on hemicellulose content.

Based on Figure 3, the measured hemicellulose fraction generally decreased with increasing pretreatment severity. The lowest observed value was 10.8% at 2 M NaOH for 25 minutes. This trend is consistent with alkaline pretreatment and ultrasound-assisted disruption of lignocellulosic structure [4]–[6]. At 30 minutes, a slight increase was observed at both NaOH concentrations. Because the study did not include independent replicates, the observed variation is discussed as an experimental trend rather than statistically significant evidence.

3.3. Effect of NaOH Concentration and Pretreatment Time on Cellulose Content

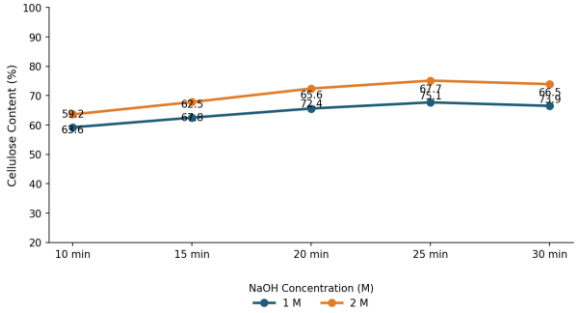

Figure 4. Effect of time and NaOH concentration on cellulose content.

3.*4. Effect of NaOH Concentration and Pretreatment Time on Alcohol-Refractometer Reading*

Based on Figure 4, the measured cellulose fraction increased with increasing pretreatment severity and reached the highest observed value of 75.1% at 2 M NaOH for 25 minutes. This increase is interpreted as a relative compositional change after removal or reduction of other fractions, rather than evidence that the absolute mass of cellulose increased. Alkaline pretreatment can alter the lignocellulosic matrix and increase the relative cellulose fraction [4], [7]. At 30 minutes, the measured cellulose fraction slightly decreased at both NaOH concentrations. Without independent mass-balance measurements or replicate experiments, the decrease cannot be assigned conclusively to cellulose degradation. The result is therefore treated as an observed trend under the tested conditions.

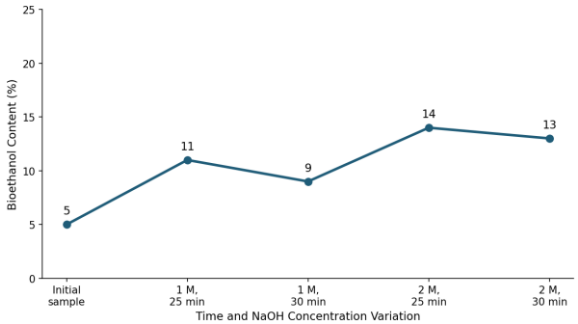

Figure 5. Alcohol-refractometer readings for the reported downstream distillate samples.

Based on Figure 5 and the available downstream data, the highest observed alcohol-refractometer reading was 14% for the 2 M NaOH–25 min condition. The reported distillate volume for this selected sample was 42 mL, with density 0.980

g/mL and pH 6.5. These downstream data were not obtained for all ten pretreatment combinations; the thesis reports characterization for five samples. Therefore, this result is not presented as a complete factorial time-by-NaOH ethanol response. The 14% value is an alcohol-refractometer reading and is not treated as fully validated ethanol concentration. The trend is discussed only as the highest observed reading among the reported downstream samples.

3.5. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The GC-MS analysis showed eleven detected chromatogram peaks. A peak at a retention time of 1.289 minutes was identified as ethanol (C2H5OH), with a reported peak area of 73.02%. This supports the qualitative identification of ethanol in the distillate. However, peak area represents detector response and cannot be converted directly to ethanol concentration without appropriate calibration. Therefore, the 14% value is reported separately as the alcohol-refractometer reading, while GC-MS is treated as qualitative confirmation of ethanol presence. Complete GC-MS operating conditions and the original chromatogram were not documented in the available manuscript record and should be supplied if available for final submission.

Several additional peaks were detected, indicating that compounds other than ethanol were present in the analyzed distillate. The result therefore indicates that the distillate was not characterized as pure ethanol, and further purification and quantitative validation would be required for stronger purity claims.

3.6. Analysis of Distillate Characteristics and Fuel Relevance

The selected distillate showed an alcohol-refractometer reading of 14%, while GC-MS qualitatively identified ethanol at a retention time of 1.289 minutes with a reported peak area of 73.02%. These measurements are complementary but are not numerically equivalent. The alcohol-refractometer value should not be interpreted as a validated ethanol purity without calibration and temperature-controlled validation.

The observed 14% alcohol-refractometer reading indicates that the distillate was not demonstrated to be high-purity ethanol. The presence of additional GC-MS peaks also indicates that other compounds were detected. Improvements in hydrolysis, fermentation control, distillation, and/or dehydration could be investigated in future work, supported by replicate experiments and quantitative analytical methods.

4. Conclusion

Ultrasonic-assisted alkaline pretreatment changed the measured lignocellulosic composition of water hyacinth under the tested conditions. The best observed condition was 2 M NaOH for 25 minutes, giving a measured lignin fraction of 5.4%, hemicellulose fraction of 10.8%, and cellulose fraction of 75.1%. Among the five downstream samples reported in the thesis, the corresponding selected distillate gave the highest observed alcohol-refractometer reading of 14%, with density 0.980 g/mL, pH 6.5, and distillate volume 42 mL. GC-MS qualitatively identified ethanol, but the reported 73.02% peak area is not interpreted as ethanol concentration. Because the experiment did not include independent replicates, ultrasound-only/alkaline-only controls, or quantitative calibration of the alcohol refractometer, the 2 M–25 min condition is not claimed as a statistically optimized or fuel-grade condition. Further work should include replicate experiments, appropriate controls, quantitative ethanol calibration, and complete GC-MS operating conditions.

4. Conclusion

Ultrasonic pretreatment using NaOH solution affects the chemical composition of water hyacinth biomass and the resulting bioethanol content. The optimum conditions were obtained at a NaOH concentration of 2 M for 25 minutes, with a lignin content of 5.4%, hemicellulose content of 10.8%, cellulose content of 75.1%, and the highest bioethanol content of 14%. These results indicate that the combination of ultrasonic pretreatment and NaOH can improve the effectiveness of delignification, so that the biomass-to-bioethanol conversion process proceeds better. To increase the bioethanol content, further optimization of the pretreatment conditions is required, along with control of the fermentation and purification processes, so that the efficiency of biomass conversion and the purity of the resulting bioethanol can be improved. These findings highlight the potential application of ultrasonic-assisted alkaline pretreatment for improving renewable bioethanol production from lignocellulosic biomass.

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