

Alkali Pretreatment and Enzymatic Hydrolysis of Cattails from Constructed Wetlands

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Abstract: Problem statement: To date, production of liquid fuel, particularly ethanol, has only been economically feasible from food crops that are high in sugar and starch. However, the use of arable land for fuel rather than for food production and the use of a food source for fuel rather than as food have created issues in prices and availability of traditional foods and feed. The use of cattails to produce biofuel will add value to land and will also reduce emissions of greenhouse gases by replacing petroleum products. **Approach:** In order to investigate the feasibility of converting cattails into cellulosic ethanol, an alkali (NaOH) pretreatment process was studied using cattails from constructed wetlands on a North Carolina A and T Farm based on NaOH concentration and enzyme loading. **Results:** The alkali pretreatment method was able to effectively increase enzymatic digestibility of cattail cellulose; nearly 78% of the cellulose from raw cattails was converted to fermentable glucose in 48 h using a cellulase loading of 60 FPU g⁻¹ glucan. About 25.5, 37.4, 38.4, 42.4 and 55.9% of the lignin was removed with pretreatment in 0.5, 1, 2, 3 and 4% NaOH, respectively. The yeast *Saccharomyces cerevisiae* (ATCC 24858) was able to ferment the sugars released by cattail cellulose. **Conclusion:** The overall effectiveness of alkali pretreatment was a function of NaOH concentration and enzyme loading. NaOH concentrations in the range of 1-2% are recommended for the pretreatment of cattails. For cattails pretreated with 4% NaOH, no significant change in digestibility occurred when enzyme loading was increased beyond 15 FPU g⁻¹ glucan. It is recommended that further studies be carried out using cattails as a feedstock for biofuels, especially to optimize the economics of pretreatment processes for cattails in terms of energy input, enzyme loading, glucose yield and xylose yield.

Key words: Biomass, cattails, hydrolysis, pretreatment, alkali

INTRODUCTION

The US Department of Energy predicts that the use of foreign petroleum, which currently feeds 56% of our needs, will grow to 68% by 2025 (US Energy Information Administration, 2009). The economic consequences of this are self-evident, but recent geopolitical events and growing environmental concerns related to the global build-up of greenhouse gases have also become energy-related issues. Consequently, a serious interest in alternative energy sources is now being fostered for reducing this dependence on non-renewable foreign energy sources. One technology for doing so is the conversion of under-utilized lignocellulosic biomass sources, such as corn stover, bagasse, switchgrass, pulp and paper

waste and the like, into liquid fuels and chemicals that can partially replace petroleum and petrochemicals (Zhang *et al.*, 2008; 2009).

To date, in the US, production of liquid fuel, particularly ethanol, has only been economically feasible from food crops that are high in sugar and starch. While this has had some impact on our energy source portfolio, it has not been without several serious limitations. The use of arable land for fuel rather than for food production and the use of a food source for fuel rather than as food have created issues in prices and availability of traditional foods and feed. A more sustainable solution would be to use cellulosic feedstock, which often can be obtained as waste from food crops or from non-food plants grown on marginal land. To this end, the Federal government has been

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calling for research into ethanol production from a number of cellulosic sources. The most widely investigated of these sources thus far have been corn stover or crops grown specifically as energy crops, such as switchgrass and poplars (Mosiera *et al.*, 2005). However, another viable feedstock could be aquatic plants obtained from constructed wetlands.

The wetland plants under consideration in the present study are *Typha* species, commonly known as cattails. Cattails have been identified as a particularly suitable biomass crop for wetlands because of their superior productivity (40+ metric ton ha⁻¹ standing crops), pest resistance, adaptability and chemical composition (Pratt *et al.*, 1988). Cattails are often among the first wetland plants to colonize areas of newly exposed wet mud and typically grow to 1-7 m tall, with spongy, strap-like leaves and starchy, creeping stems (rhizomes) (Apfelbaum, 1985). The leaves are alternate and mostly basal to a simple, jointless stem that eventually bears the flowers. The rhizomes, which contain mostly starch, spread horizontally beneath the surface of muddy ground to start new upright growth. The spread of cattails is an important part of the conversion process of open water bodies to vegetated marshland and eventually to dry land. Cattails are sometimes eaten by cattle and have some nutritive value, as they contain about 6% protein and 50% total digestible nutrients as young plants, with lower levels as the plants mature. In terms of lignin-cellulosic material, Kucuk *et al.* (2005) reported that cattails contain 47.6% cellulose and 21.9% lignin. Based on this composition, it is possible that, after appropriate fractionations, cattails could be a good source of fuel ethanol.

The degradation of cellulose to fermentable sugars is inhibited by the co-occurrence of hemicellulose and lignin surrounding the cellulose fibers. Treatment with alkali can remove lignin, thus promoting hydrolysis and improving the glucose recovery from cellulose. The most commonly used alkali base is NaOH (Li *et al.*, 2004). Alkali pretreatment processes have the advantages of utilizing lower temperatures and pressures compared to other lignin removal technologies. For the present study, cattails from constructed wetlands on the North Carolina A&T Farm were processed with an alkali pretreatment process. *Saccharomyces cerevisiae* (ATCC 24858) was then used to test the fermentability of the sugars enzymatically degraded from cattail cellulose.

MATERIALS AND METHODS

The cattails, *Typha latifolia*, were chopped with pruning shears, dried at 70°C for 5 days and ground in a Wiley mill to 1 mm mesh size.

Table 1: Biomass composition^{a,b} of cattails before and after NaOH pretreatment

Concentration of NaOH (%)	0	0.5	1	2	3	4
Cellulose	32.3	37.2	42.5	40.0	42.3	45.0
Xylan	18.9	29.0	25.3	25.0	22.0	24.6
Other sugars ^c	5.7	3.4	3.0	3.1	3.0	3.2
Klason lignin	20.7	19.8	18.1	17.2	16.4	13.9
Ash	4.7	4.9	5.2	6.4	6.1	3.5

^a: Moisture free basis; ^b: Biomass also contains acid-soluble lignin, extractives, acetyl acid groups and uronic acid groups; ^c: Other sugars represents galactan, arabinan and mannan

Biomass analytical procedures: Compositional analysis of biomass was carried out using the Laboratory Analytical Procedures (LAPs) developed by the National Renewable Energy Laboratory. The moisture content of the biomass was determined by the method of LAP #001. The ash content of the biomass was determined by the method of LAP #005. Structural analyses of the samples were carried out according to the methods of LAP #002, LAP #003, LAP #017 and LAP #019. The composition of untreated cattails and pretreated cattails is listed in Table 1.

Pretreatment of the feedstock: About 50 g of dried, ground cattail was stirred into 0.5 L of NaOH (1-4%) and left at room temperature for 24 h. The mixture was then centrifuged at 2600 RCF for 20 min, the supernatant was decanted and the pellet was rinsed with water six times and twice with 0.05 M citric acid buffer (pH 4.8). Samples were centrifuged and supernatants decanted between rinses.

Chemical analysis: Liquid samples were filtered through 35 µm nylon membranes and analyzed by High Performance Liquid Chromatography (HPLC) (Waters, Milford, MA) with a KC-811 ion-exclusion column and a Waters 410 refractive index detector to determine the presence of glucose, arabinose, xylose, galactose, mannose and ethanol. The mobile phase was 0.1% H₃PO₄ solution at a flow rate of 1 mL min⁻¹. The temperatures of the detector and column were maintained at 35 and 60°C, respectively.

Digestibility test: Pretreated biomass samples were used in wet form for enzymatic digestibility tests. A control was prepared with an identical amount of cattail material that had not been pretreated. The total amount of glucose released after 48 h of hydrolysis was measured to calculate the enzymatic digestibility. The conditions of the enzymatic digestibility tests were 50 °C and pH 4.8 (0.05 M sodium citrate buffer). Screw-capped 250 mL Erlenmeyer flasks were used as reaction vessels and were agitated at 150 rpm in a constant temperature incubator shaker. Pretreated

cattails were hydrolyzed using a cellulase loading (Novozyme NS50013) of 7.5, 15, or 60 FPU g⁻¹ glucan. Novozyme β-glucosidase (NS50010) at a loading of 4.5 CBU g⁻¹ glucan and hemicellulase (NS22002) at a loading of 2.5 FBG g⁻¹ glucan were also incorporated with the cellulase. Biomass samples were loaded into the reactor to give an initial glucan concentration in the reactor of 1% (w/v) (i.e., 1 g-glucan/100 mL liquid).

Fermentation: *Saccharomyces cerevisiae* (ATCC 24858) was the yeast organism used to ferment the enzymatically released sugars. For ethanol production, 8 mL of seed culture were used to inoculate 40 mL YP medium in a 250 mL Erlenmeyer flask. The cultures were incubated in a shaker at 30°C and 200 rpm and grown aerobically overnight. The yeast was harvested by centrifugation at 2600 RCF for 15 min, at room temperature. The supernatant was discarded and the cells were transferred to 250 mL screw-capped Erlenmeyer flasks containing 100 mL of hydrolysate. The initial cell mass concentration prior to fermentation in each experiment was 8-9 g dry weight L⁻¹. The flasks were then tightly capped to allow fermentation to occur under largely anaerobic conditions. The cultures were placed in a shaker and incubated at 30°C. Fermentation samples were filtered through 35 μm nylon membranes and analyzed by HPLC to determine the presence of ethanol and sugars.

RESULTS

Component balance of NaOH pretreatments: Cattails were pretreated using NaOH (1-4% w/v) at room temperature for 24 h. Dry matter recoveries and compositional analyses of solids and liquids after the pretreatment step were used to develop a component balance for the pretreatment processes. The remaining soluble mass in the hydrolysate liquid was determined by difference. These results are shown in Fig. 1 and Table 1. Cattails without a pretreatment contained approximately 32% cellulose, 19% xylan and 21% lignin. During the 0.5, 1, 2, 3 and 4% NaOH pretreatment processes, about 25.5, 37.4, 38.4, 42.4 and 55.9% of the lignin was dissolved into soluble form, respectively. The cellulose portion of cattails was left almost intact during the pretreatment process. However, if the concentration of NaOH was higher than 3%, xylan was partially dissolved into the liquid solution.

Hydrolysis of cellulose from cattails following NaOH pretreatments: Cattails were pretreated using a 4% NaOH solution for 24 h and the pretreated material was then hydrolyzed for 48 h with cellulase at 60 FPU g⁻¹ glucan. The yields of fermentable sugars from the

dissolution of the cattails are illustrated in Fig. 2. Nearly 70% of the cellulose was converted to fermentable glucose in 6 h by 60 FPU g⁻¹ glucan.

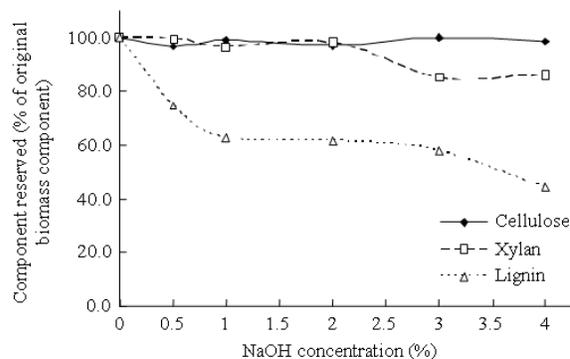


Fig. 1: Component balance of NaOH pretreatments cattails were pretreated with 0.5-4% NaOH

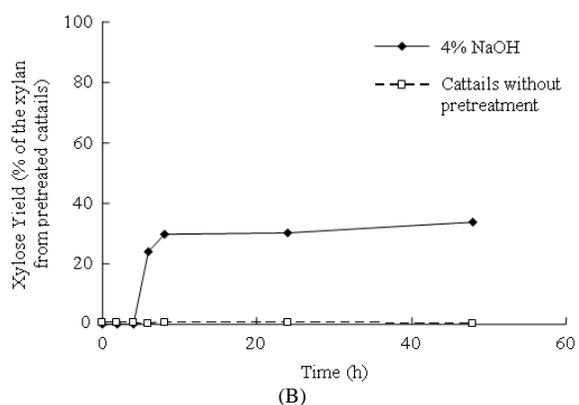
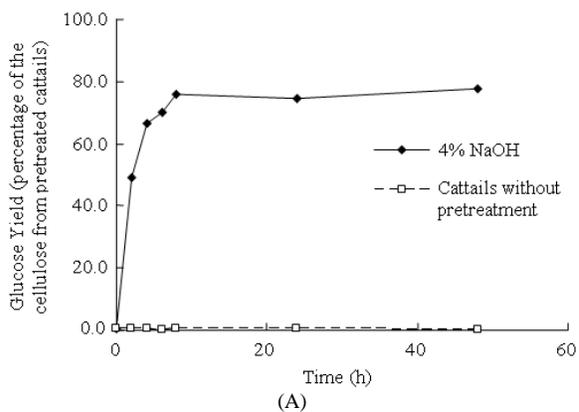


Fig. 2: Cellulose and xylan digestibility of cattails by NaOH pretreatments. Cattails were pretreated with 4% NaOH and the pretreated material was then hydrolyzed for 48 h with cellulase at 60 FPU g⁻¹ glucan. (A) Glucose yield; (B) Xylose yield

Table 2: Effect of NaOH concentration on the glucose and xylose yields of pretreated cattails

Sugar yield (%)	0.5% NaOH	1% NaOH	2% NaOH	3% NaOH	4% NaOH
Glucose	23.0±1.1	57.1±2.0	70.1±2.4	75.4±1.5	77.5±3.8
Xylose	11.5±0.4	28.6±1.9	30.4±0.5	34.8±2.0	33.6±1.9

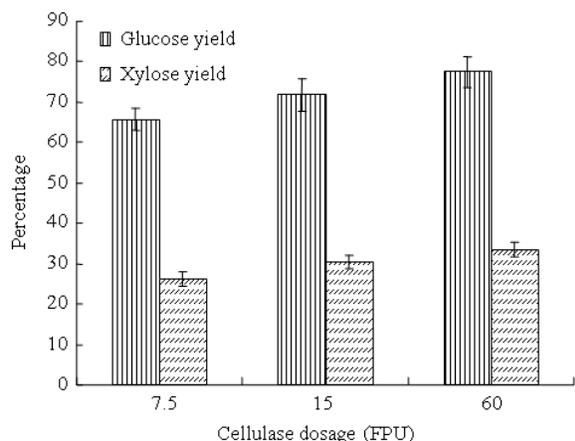


Fig. 3: Effect of cellulase dosage on the glucose and xylose yields of cattails pretreated with 4% NaOH. Cattails pretreated with 4% NaOH at room temperature for 24 h were used for digestion to compare cellulase loading. Error bars represent 95% confidence levels

However, no detectable xylose appeared within the first 4 h, but began to gradually appear, reaching a level corresponding to 30% of the total xylan by 8 h. At the end of 48 h of hydrolysis, the yields of glucose and xylose were approximately 78 and 34% of the cellulose and xylan from pretreated cattails, respectively.

Comparison of alkali pretreatments: The pretreated cattails were hydrolyzed for 48 h with cellulase at 60 FPU g⁻¹ glucan. The yields of glucose and xylose following enzymatic treatments of alkali-pretreated cattails are shown in Table 2. An increase in release of sugars was observed as NaOH concentration was increased. After 48 h of enzymatic hydrolysis, the glucose yields were 23.0, 57.1, 70.1, 75.4 and 77.5% of the total cellulose following pretreatment with 0.5, 1, 2, 3 and 4% (w/v) NaOH, respectively.

Effect of enzyme loadings on digestibility of pretreated cattails: Cattails pretreated with 4% NaOH at room temperature for 24 h were used for digestion to compare cellulase loading. The maximum glucose yield was achieved with a cellulase loading of 60 FPU g⁻¹ glucan. The difference in results between 60 and

15 FPU g⁻¹ of glucan were not statistically significant at a 95% confidence level (Fig. 3). In both cases, approximately 75% of the cellulose was converted to glucose and approximately 30% of the xylan was converted to monomeric xylose. When the cellulase loading was reduced to 7.5 FPU g⁻¹ of glucan, the glucose yield dropped to approximately 65.7%. However, the xylose yield only dropped to approximately 26%.

Fermentation of cellulose from base-pretreated cattails: Cattails were pretreated with 4% NaOH for 24 h and then hydrolyzed for six days using the method described above. After a six-day hydrolysis, the resulting hydrolysate was then fermented at 30°C for 48 h by *Saccharomyces cerevisiae* (ATCC 24858). Since only diluted pretreated cattails (~1 g glucan/100 mL volume) were used, glucose to ethanol yields were approximately 95% of the theoretical yield for this *S. cerevisiae* stain, resulting in a ethanol yield of approximately 0.36% w/v.

DISCUSSION

Using alkaline chemicals to remove lignin has long been known to improve cellulose digestibility. When applying a 0.5-4% NaOH solution, approximately 25.5-56% of the lignin was removed. And the alkali pretreatment increased the degree of enzymatic hydrolysis of cattails, while untreated cattails remained almost indigestible. The highest glucose yield (77.5% of the cellulose) was obtained when the cattails were pretreated with a 4% NaOH solution. An increase in glucose yield of only 3% was seen when the NaOH concentration was raised from 3-4%, while the glucose yield increased by 7.6% when the NaOH concentration was increased from 2-3%, by 22.8% when the NaOH concentration was increased from 1-2% and by 2.5 times when the NaOH concentration was increased from 0.5-1%. The increment in glucose yield decreased with the increase of NaOH concentration. Since sodium hydroxide and other bases are expensive and the recovery process is complex, lower ranges of 1-2% are recommended for the pretreatment of cattails.

The results indicate that cattails pretreated with 4% NaOH are digestible with similar results at enzyme loadings above 15 FPU g⁻¹ glucan. However, because higher enzyme loading will significantly increase cost, there is no economic justification for raising the cellulase level. An optimum cellulase loading needs to be selected according to the pretreatments used.

CONCLUSION

To investigate the feasibility of converting cattails into ethanol, alkali (NaOH) pretreatment was studied in cattails from constructed wetlands on the North Carolina A&T Farm. The alkali pretreatment method was able to effectively increase the enzymatic digestibility of cattail cellulose; nearly 78% of the cellulose was converted to fermentable glucose in 48 h using a cellulase loading of 60 FPU g⁻¹ glucan. About 25.5, 37.4, 38.4, 42.4 and 55.9% of the lignin could be removed by pretreatment with 0.5, 1, 2, 3 and 4% NaOH, respectively. The effectiveness of alkali pretreatment was a function of NaOH concentration and enzyme loading. NaOH concentrations in the range of 1-2% are recommended for the pretreatment of cattails. For cattails pretreated with 4% NaOH, no significant change in digestibility occurred when enzyme loading was increased beyond 15 FPU g⁻¹ glucan. It is recommended that further studies be carried out using cattails as a feedstock for biofuels, especially to optimize the economics of pretreatment processes for cattails in terms of energy input, enzyme loading, glucose yield and xylose yield.

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