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ORIGINAL PAPER

Hot-water pretreatment of cattails for extraction of cellulose

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Abstract To date in the US, production of renewable fuels, particularly ethanol, is primarily from food crops that are high in sugar and starch. The use of arable land for fuel rather than food production and the use of a food source for fuel rather than food have created issues in pricing and availability of traditional foods and feed. The use of cattails to produce biofuel will add value to land and also reduce emissions of greenhouse gases by replacing petroleum products. In order to investigate the feasibility of converting cattails into cellulosic ethanol, a hot-water pretreatment process was studied using a Dionex accelerated solvent extractor (ASE) varying treatment temperature and time. The pretreatment at 190°C for more than 10 min could effectively dissolve the xylan fraction of cattails as soluble oligomers. Both the glucose yield and xylose yield obtained from the pretreated cattails increased with the escalation of the final pretreatment temperature, treatment time or enzyme loading. When cattails were pretreated at 190°C for 15 min, the highest glucose yield of 77.6% from the cellulose was achieved in 48 h using a cellulase loading of 60 FPU/g glucan. The yeast *Saccharomyces cerevisiae* (ATCC 24858) was able to ferment glucose released by cattail cellulose, resulting in approximately 88.7 ± 2.8% of the theoretical ethanol yield. The higher enzyme loading of 60 FPU/g glucan will significantly increase costs. It is recommended that further studies be carried out using cattails as a feedstock for bio-fuels, especially to optimize the economics of biological conversion processes for cattails with regard to reducing enzyme usage, energy input, glucose yield and xylose yield.

Keywords Biomass · Cattails · Hot-water pretreatment · Hydrolysis · Fermentation

Introduction

The US Department of Energy predicts that the use of foreign petroleum, which currently feeds 56% of our demand, will grow to 68% by 2025 [1]. 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 dependence on non-renewable foreign energy sources. One such measure is the conversion of under-utilized lignocellulosic biomass sources, such as corn stover, bagasse, pulp and paper waste, switchgrass and the like, into liquid fuels and chemicals that can partially replace petroleum and petrochemicals [2–7].

To date in the US, production of renewable fuels, particularly ethanol, is primarily from food crops that are high in sugar and starch. Even given the economic viability of corn ethanol, government subsidies are still required [8, 9]. While this has had some impact on our energy source portfolio, its application 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

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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 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 [2, 4, 5]. However, another viable feedstock could be aquatic plants obtained from constructed wetlands.

The wetland plants under consideration in this paper are the *Typha* species, commonly known as cattails. Cattails have been identified as a particularly suitable biomass crop for wetlands because of their superiority in productivity (40+ metric ton/ha standing crops), pest resistance, adaptability, and chemical composition [10]. Cattails are often among the first wetland plants to colonize areas of newly exposed wet mud. They typically grow 1–7 m tall, and have spongy, strap-like leaves and starchy, creeping rhizomes [11]. The leaves are alternate and mostly basal on 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 young plants, they contain about 6% protein and 50% total digestible nutrients, with lower levels as the plants mature. With regard to lignin-cellulosic material, Küçük et al. [12] 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.

Hot-water pretreatment is often called autohydrolysis, and the major advantages are less expense, lower corrosion to equipment, less xylose degradation, and thus fewer byproducts, including inhibitory compounds in the extracts [13]. Hot-water pretreatment has been shown to improve enzyme digestibility of various lignocellulosic biomass [2–5, 14, 15]. Hot-water pretreatment enhances enzyme digestibility of the biomass by penetrating the cell structure, hydrating cellulose, and removing hemicellulose. In our previous study, when a 1-l Parr reactor was used to treat cattails with hot water and dilute sulfuric acid, about 21.5 and 42.3% of the cellulose (raw cattails basis) was dissolved into soluble form. Then the pretreated cattails were hydrolyzed with cellulase at a loading of 70 FPU/g glucan, and the glucose yields of pretreated cattails were approximately 79 and 95%, respectively, of the available glucan [16]. The heating rate of the Parr reactor used for the pretreatment was slow (4°C/min). It has been reported that the heating rate, as governed by the mode of heat transfer, is an important factor affecting the hydrothermal treatment process [17]. For this study, in order to minimize the heat transfer-related artifacts and optimize the pretreatment processes, cattails underwent a hot-water pretreatment process in an accelerated solvent extractor, which provides a heating rate of 25°C/min. *Saccharomyces cerevisiae* (ATCC 24858) was then used to test the fermentability of glucose enzymatically degraded from cattail cellulose.

Materials and methods

Materials

The aerial portions of 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.

Biomass analytical procedures

Compositional analysis of biomass was carried out using the laboratory analytical procedures (LAPs) developed by the National Renewable Energy Laboratory [18]. 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. The composition of untreated cattails and pretreated cattails is listed in Table 1.

Pretreatment of the feedstock

A Dionex ASE 350 Accelerated Solvent Extractor (Dionex Corporation, Sunnyvale, CA), which provides a heating rate of 25°C/min, was used for hot-water pretreatment of biomass below 190°C. Approximately 2.0 g of ground biomass (composed of cattails) was placed into a tared 66 ml Dionium extraction cell containing a glass fiber filter. Then the appropriate number of 150 ml collection vials were weighed and placed onto the ASE system. The extractor passed 60 ml de-ionized water into the cell-containing biomass. Then the cell was heated to the desired temperature (130–190°C) at a heating rate of 25°C/min, and the desired temperature was maintained for 5–15 min. After pretreatment, 40 ml of de-ionized water was passed into the cell to rinse the biomass. The resulting extractive and the rinsing water (total about 100 ml) were collected in the collection vials. The extraction cell was cooled down to 25°C by sitting at room temperature for 30 min. The biomass residue after pretreatment and the collected liquid were pooled into a 250-ml Erlenmeyer flask, and then tested.

Table 1 Biomass composition of cattails before and after hot-water pretreatment (air-dried, % by weight)

Pretreatment conditions	Cattails	130°C 5 min	130°C 10 min	130°C 15 min	150°C 5 min	150°C 10 min	150°C 15 min	170°C 5 min	170°C 10 min	170°C 15 min	190°C 5 min	190°C 10 min	190°C 15 min
Cellulose	34.3	38.7	40.2	41.0	44.8	43.1	44.6	47.4	48.8	47.3	52.6	68.0	70.2
Xylan	11.6	14.5	15.0	14.1	13.3	11.8	11.6	10.1	11.3	10.4	10.7	0.0	0.0
Other sugars[a]	3.2	4.7	4.1	3.9	3.2	1.8	3.1	2.3	2.3	1.0	2.0	0.0	0.0
Klason lignin	26.4	27.0	27.3	28.2	29.2	30.2	29.1	30.7	30.4	33.1	24.2	24.5	24.1
Ash	4.0	3.5	3.3	3.6	3.3	3.1	3.4	3.2	3.3	2.9	3.2	3.6	4.0

Moisture-free basis

Biomass also contains acid-soluble lignin, extractives, acetyl acid groups, and uronic acid groups

[a] Other sugars represent galactan, arabina, and mannan

The yield percentage of each fraction from pretreatment is defined as:

Pretreated biomass (%) = (weight of pretreated biomass/weight of starting biomass) × 100.

Dissolved solids yield (%) = (1−weight of pretreated biomass/weight of starting biomass) × 100.

All experiments and analyses were performed in triplicate.

Chemical analysis

Liquid samples were filtered through 0.2 μm nylon membranes (Whatman 0.2 μm NYLw/GMF) 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% H_3PO_4 solution at a flow rate of 1 ml/min. The temperatures of the detector and column were maintained at 35 and 60°C, respectively. For each run, the external standards of glucose, arabinose, xylose, galactose, mannose, and ethanol were used.

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 glucan. Novozyme β-glucosidase (NS50010) at a loading of 4.5 CBU/g glucan and hemicellulase (NS22002) at a loading of 2.5 FBG/g glucan were also incorporated with the cellulase. Applying both cellulase and hemicellulase effectively enhances the digestibility of pretreated biomass [19, 20]. The pretreated biomass and the extractives 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 was 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 at room temperature by centrifugation at 2,600 RCF for 15 min. 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. 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 0.2 μm nylon membranes and analyzed by HPLC to determine the presence of ethanol and sugars.

Results and discussion

Component balance of hot-water pretreatments

Figure 1 shows how up to 46% of the cattails was dissolved during pretreatment over the temperature range of 130–190°C and time intervals of 5–15 min. The yield of extractable products obtained from the pretreatment process increased as the final temperature and severity factor increased. A high temperature pretreatment (190°C for 15 min with a severity factor of Log*R*0 3.8) resulted in the

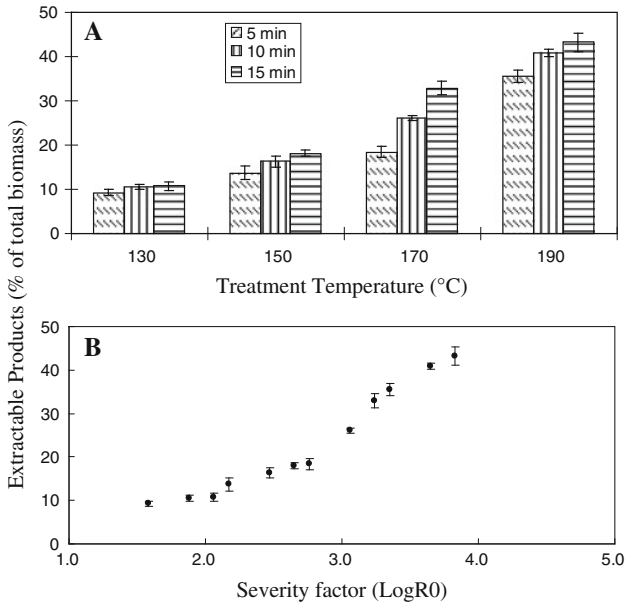

Fig. 1 Effect of treatment temperature and time on the yields of extractable products. **a** Extractable products yield vs. treatment temperature and time; **b** extractable products yield vs. severity factor (Log*R*0)

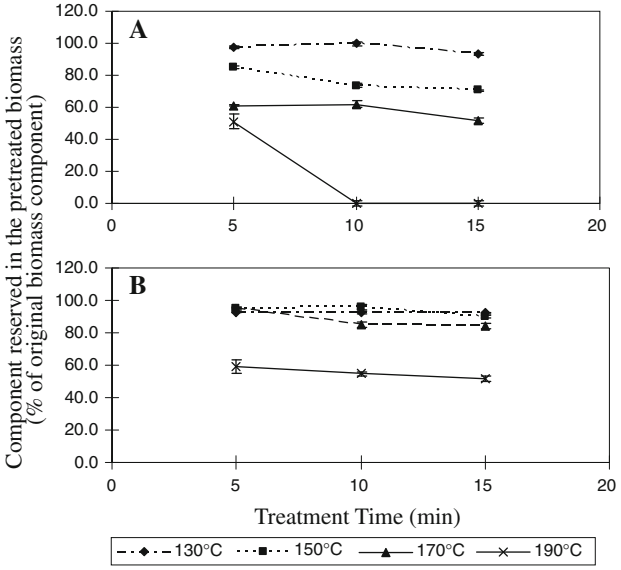

Fig. 2 Component balance of hot-water pretreatments. **a** Xylan; **b** Klason lignin

highest total extractables yield. 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 [2, 21]. The remaining soluble mass in the hydrolysate liquid was determined by difference. These results are shown in Fig. 2 and Table 1. Cattails without a pretreatment contained approximately 34% cellulose, 12% xylan, and 26% lignin. The cellulose portion of cattails remained almost intact during the hot-water pretreatment processes. When cattails were pretreated below 170°C, less than 50% of xylan was dissolved into the liquid solution. However, when cattails were pretreated at 190°C for more than 10 min, 100% of the xylan was removed. This finding is consistent with our previous study [16]. The xylan was solubilized as oligomers through pretreatment, and the monomeric form of sugars was not detectable in the extracted products. During the 130, 150, 170, and 190°C pretreatment processes, about 7–8%, 5–10%, 5–15%, and 41–48% of the Klason lignin also was dissolved, respectively, into soluble form (see Fig. 2b).

Hydrolysis of cellulose and xylan from cattails following hot-water pretreatments

The pretreatment processes were studied using the following treatment variables: reaction temperatures (130, 150, 170, and 190°C at 1,500 psi) and residence times (5, 10, and 15 min). The pretreated material was then hydrolyzed for 48 h with cellulase at 15 FPU/g glucan. The yields of fermentable sugars from the dissolution of the cattails are illustrated in Fig. 3. Both the glucose yield and xylose yield obtained from the pretreatment process increased with an increasing final temperature. When cattails were pretreated at 190°C for 15 min, nearly 59% of the cellulose was converted to fermentable glucose in 48 h. The highest xylose yield of 66.4% was reached under the same reaction conditions. The high temperature (190°C) pretreatment was more effective compared to the lower temperature pretreatments of 130, 150, and 170°C. The overall effectiveness of hot-water pretreatment was also a function of treatment temperature time. When cattails were pretreated at 190°C, the yield of glucose and xylose increased from 48.9 to 58.3% and 45.4 to 66.4%, respectively, as the treatment time increased from 5 to 15 min.

Effect of enzyme loadings on digestibility of pretreated cattails

Cattails pretreated at 190°C for 15 min were used for digestion to compare cellulase loading. An increase in release of sugars was observed as cellulase dosage was increased, as seen in Fig. 4. After 48 h of enzymatic hydrolysis, the glucose yields were 48.9, 58.3, and 77.6%, respectively, of the total cellulose following pretreatment with a cellulase loading of 7.5, 15, and 60 FPU/g glucan. The xylose yields were 57.7, 66.4, and 69.4%, respectively, of the total xylan. The highest glucose yield (77.6% of the cellulose) was obtained when applying a cellulase loading 60 FPU/g glucan with β-glucosidase at a loading of 4.5 CBU/g glucan and hemicellulase at a loading of 2.5 FBG/g glucan. An increase in glucose content of 19.3% of total cellulose was seen when

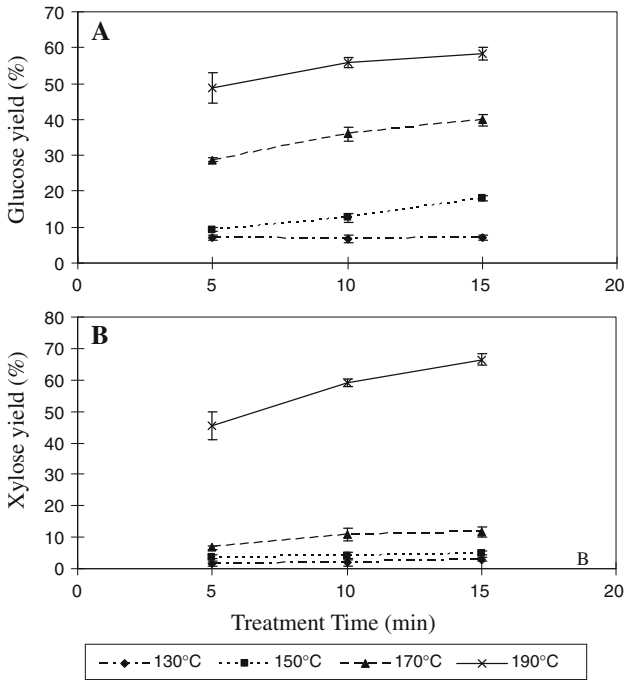

Fig. 3 Cellulose and xylan digestibility of cattails by hot-water pretreatments. Pretreated cattails, which were not washed, were hydrolyzed for 48 h with cellulase at a loading of 15 FPU/g glucan. **a** Glucose yield; **b** xylose yield

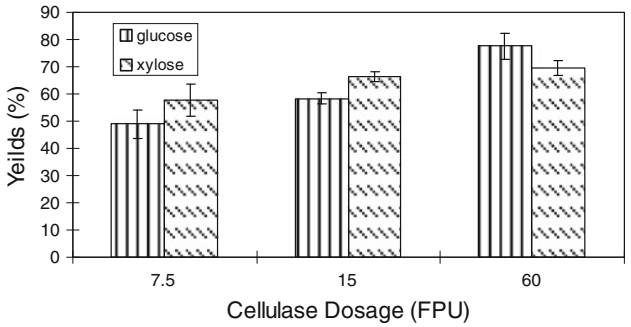

Fig. 4 Effect of cellulase dosage on the glucose and xylose yields of hot-water pretreated cattails. Cattails pretreated at 190°C for 15 min were used for digestion to compare cellulase loading

the cellulase loading was raised from 15 to 60 FPU/g glucan, while the glucose production increased by 9.4% of total cellulose when the cellulase loading was increased from 7.5 to 15 FPU/g glucan. The higher enzyme loading of 60 FPU/g glucan will significantly increase costs, and there is no economic justification for raising the cellulase level. One possible reason of the high enzyme dosage requirement is that the enzyme inhibitors were produced during the pretreatment process. Because the pretreated biomass was not washed to remove the inhibitors in this study, if further study shows that washing the pretreated biomass improves the sugar yields, this would suggest that inhibitors were formed. An alternative explanation may be that cattails are particularly recalcitrant. Liquid hot-water pretreatment may not be the most effective method for this biomass.

Fermentation of cellulose from base-pretreated cattails

Cattails were pretreated at 190°C for 15 min and then hydrolyzed for 2 days using the method described above. After a 2-day hydrolysis, the resulting hydrolysate was fermented at 30°C for 48 h by *Saccharomyces cerevisiae* (ATCC 24858). As shown in Fig. 5, the amount of glucose present in the hydrolysate was fermented within 6 h by *S. cerevisiae*. When diluted pretreated cattails (~1 g glucan/100 ml volume) were used, glucose to ethanol yields were approximately 88.7 ± 2.8% of the theoretical yield for this *S. cerevisiae* strain. This resulted in an ethanol concentration of approximately 0.34% w/v.

Comparison with the hot-water pretreatment of other feedstocks

In this study, when cattails were pretreated at 190°C for 15 min, the highest glucose yield of 77.6% of the cellulose was achieved in 48 h using a cellulase loading of 60 FPU/g glucan. The results are lower than that of corn stover, but similar to that of switchgrass and bagasse [2–4]. At optimal conditions, 90% of the cellulose from corn stover pretreated in hot water can be hydrolyzed to glucose. Both the switchgrass and bagasse pretreated in liquid hot water could result in approximately an 80% sugar yield. The glucose yield of cattails is higher than that of hybrid poplar, which is within a range of 54 and 67% [5]. The conversion of cattails to fermentable sugars and ethanol provides a cellulosic feedstock for production of fuel ethanol that may be grown on wetlands.

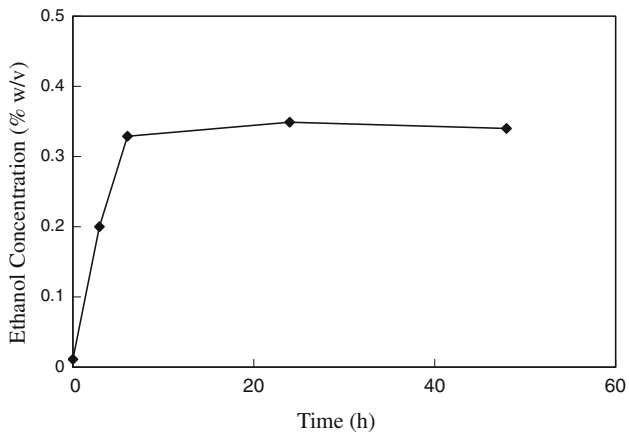

Fig. 5 Fermentation of cellulose from hot-water pretreated cattails. Cattails were pretreated at 190°C for 15 min and then enzymatically hydrolyzed. The resulting hydrolysate was then fermented at 30°C for 48 h using *S. cerevisiae* (ATCC 24858)

It is recommended that further studies be carried out using cattails as a feedstock for bio-fuels.

Conclusions

To investigate the feasibility of converting cattails into ethanol, a hot-water pretreatment process was studied using a Dionex accelerated solvent extractor, and varying treatment temperatures and times. Pretreating cattails at 190°C for more than 10 min could effectively dissolve the xylan fraction of cattails as soluble oligomers. Both the glucose yield and xylose yield obtained from the pretreatment process increased with increasing final temperatures, treatment times, or enzyme loading. When cattails were pretreated at 190°C for 15 min, the highest glucose yield of 77.6% of the cellulose was achieved in 48 h using a cellulase loading of 60 FPU/g glucan. The yeast *Saccharomyces cerevisiae* (ATCC 24858) was able to ferment the sugars released by cattail cellulose, resulting in an ethanol yield of approximately 88.7 ± 2.8% of the theoretical yield. It is recommended that further studies be carried out using cattails as a feedstock for bio-fuels, especially to optimize the economics of biological conversion processes for cattails with regard to reducing enzyme usage, energy input, glucose yield, and xylose yield.

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