(54) Title: FRACTIONATION OF BIOMASS FOR CELLULOSIC ETHANOL AND CHEMICAL PRODUCTION

Figure 5
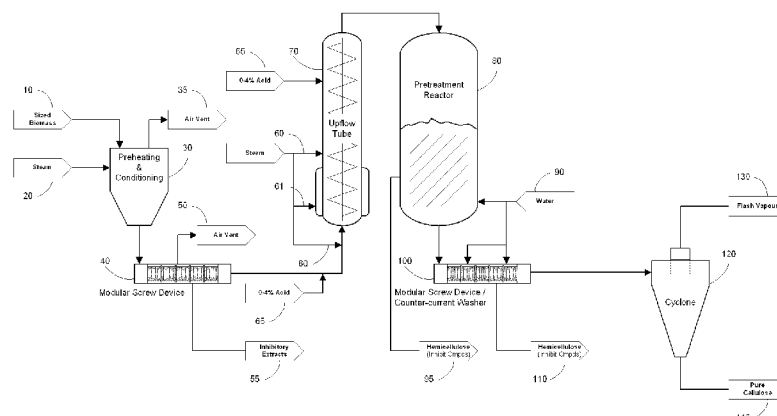
(57) Abstract: A process is defined for the continuous steam pretreatment and fractionation of corn cobs and low lignin lignocel-
lulosic biomass to produce a concentrated cellulose solid stream that is sensitive to enzymatic hydrolysis. Valuable chemicals are
recovered by fractionating the liquid and vapor stream composed of hydrolysis and degradation products of the hemicellulose.
Cellulosic derived glucose is produced for fermentation to biofuels. A hemicellulose concentrate is recovered that can be convert-
ed to value added products including ethanol.

Fractionation of Biomass for Cellulosic Ethanol and Chemical Production

FIELD OF THE INVENTION

5   The present invention generally relates to the production of ethanol from lignocellulosic biomass and in particular to a process for extracting cellulose and hemicellulose from corncobs, and a process for extracting cellulose and hemicellulose fractions from low lignin containing biomass.

BACKGROUND OF THE INVENTION

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Concerns over high oil prices, security of supply and global warming have raised the demand for renewable energy. Renewable energy is energy produced from plant derived biomass. Renewable energy applications such as fuel ethanol are seen as a valuable contribution to the reduction in fossil fuel consumption. Public policies have
15   supported the creation of a fuel ethanol industry largely based on the use of corn as a feedstock. The production of fuel ethanol helps to stabilize farm income and reduces farm subsidies. However, as demand increases for fuel ethanol, additional feedstocks such as lignocellulosic biomass are under consideration.

Fuel ethanol is created by the fermentation of starch derived sugars. The ethanol
20   is distilled and dehydrated to create a high-octane, water-free gasoline substitute. Fuel ethanol is blended with gasoline to produce a hybrid fuel, which has environmental advantages when compared to gasoline alone, and can be used in gasoline-powered vehicles manufactured since the 1980's. Most gasoline-powered vehicles can run on a blend consisting of gasoline and up to 10 percent ethanol, known as "E-10".

25   While corn is the major raw material for producing ethanol in North America, it is already apparent that large-scale use of ethanol for fuel will require new technologies that will allow the industry to expand its feedstock options to include cellulose.

Cellulosic ethanol is manufactured from lignocellulosic biomass. Lignocellulosic biomass may be grouped into four main categories: (1) wood residues (including sawmill
30   and paper mill discards), (2) municipal paper waste, (3) agricultural wastes (including corn stover, corn cobs and sugarcane bagasse), and (4) dedicated energy crops which

are mostly composed of fast growing tall, woody grasses such as switch grass and Miscanthus.

Lignocellulosic biomass is composed of three primary polymers that make up plant cell walls: Cellulose, hemicellulose and lignin. Cellulose is a polymer of D-glucose. Hemicellulose contains two different polymers i.e. xylan, a polymer of xylose and glucomannan, a polymer of glucose and mannose. Lignin is a polymer of guaiacylpropane- and syringylpropane units.

Cellulose fibers are locked into a rigid structure of hemicellulose and lignin. Lignin and hemicelluloses form chemically linked complexes that bind water soluble hemicelluloses into a three dimensional array, cemented together by lignin. Lignin covers the cellulose microfibrils and protects them from enzymatic and chemical degradation. These polymers provide plant cell walls with strength and resistance to degradation, which makes lignocellulosic biomass a challenge to use as a substrate for biofuel production. Variation in the content or organization of these polymers significantly affects the overall steps of cellulosic ethanol production.

Cellulose or β-1-4-glucan is a linear polysaccharide polymer of glucose made of cellobiose units. The cellulose chains are packed by hydrogen bonds into microfibrils. These fibrils are attached to each other by hemicelluloses, amorphous polymers of different sugars and are covered by lignin.

Hemicellulose is a physical barrier which surrounds the cellulose fibers and protects cellulose against degradation. There is evidence that hemicellulose, containing xylose polymers (xylan), limits the activity of cellulolytic enzymes, thereby lowering cellulose to glucose conversion rates. Thus for the production of fermentable sugars and ethanol, it is desirable to submit to the enzymatic hydrolysis a highly reactive cellulose low in xylan.

Lignin is a very complex molecule constructed of phenylpropane units linked in a three dimensional structure which is particularly difficult to biodegrade. Lignin is the most recalcitrant component of the plant cell wall. There are chemical bonds between lignin, hemicellulose and cellulose polymers. There is evidence that the higher the proportion of lignin, the higher the resistance to chemical and biological hydrolysis. Lignin and some soluble lignin derivatives inhibit enzymatic hydrolysis and fermentation processes. Thus, it is desirable to use a lignocellulosic feedstock which is low in lignin.

Published work on the various processes for the production of fermentable sugars from cellulosic biomass shows the existence of an inverse relationship between lignin content and the efficiency of enzymatic hydrolysis of sugar based polymers.

Lignocellulosic microfibrils are associated in the form of macrofibrils. This complicated structure and the presence of lignin provide plant cell walls with strength and resistance to degradation, which also makes these materials a challenge to use as substrates for the production of biofuel and bioproducts. Thus, pretreatment is necessary to produce highly reactive cellulose reacting well with catalysts such as enzymes.

Purified cellulose and lignin-free xylo-oligosaccharides are valuable for many purposes. Specifically, reactive cellulose extracted from biomass with low lignin content may be easily hydrolyzed to fermentable sugar monomers and then fermented to ethanol and other biofuels. Lignin-free xylo-oligosaccharides extracted from the hemicellulose fraction are valuable and may be easily used in the preparation of prebiotic substances for food and pharmaceutical applications.

The best method and conditions of pretreatment will vary and depend greatly on the type of lignocellulosic starting material used. Pretreatment configuration and operating conditions must be adjusted with respect to the content or organization of lignocellulosic polymers in the starting material, to attain optimal conversion of cellulose to fermentable sugars. The cellulose-to-lignin ratio is the main factor. Other parameters to consider are the content of hemicellulose, degree of acetylation of hemicellulose, cellulose-accessible surface area, degree of polymerization and crystallinity.

The lignin content of corncobs and certain hybrids of Miscanthus for example, is low i.e. 5% to 10%. Corncobs and some hybrids of Miscanthus are good candidates for the production of cellulosic ethanol due to their favorable ratios of cellulose: lignin:hemicellulose.   Corncobs and Miscanthus have ratios of 8:1:7 and 5:1:2, respectively.

An effective pretreatment should meet the following requirements: (a) production of reactive cellulosic fiber for enzymatic attack, (b) avoidance of cellulose and hemicelluloses destruction, and (c) avoidance of the formation of possible inhibitors for hydrolytic enzymes and fermenting microorganisms.

Several methods have been investigated for the pretreatment of lignocellulosic materials to produce reactive cellulose. These methods are classified into physical pretreatments, biological pretreatments and physicochemical pretreatments.

The prior art teaches that physical and biological pretreatments are not suitable for industrial applications. Physical methods such as milling, irradiation and extrusion are highly energy demanding and produce low grade cellulose. Also, the rates of known biological treatments are very low.

Pretreatments that combine both chemical and physical processes are referred to as physicochemical processes. These methods are among the most effective and include the most promising processes for industrial applications. Hemicellulose hydrolysis and lignin removal are often nearly complete. Increase in cellulose surface area, decrease in

5   cellulose degree of polymerization and crystallinity greatly increase overall cellulose reactivity. Treatment rates are usually rapid. These pretreatment methods usually employ hydrolytic techniques using acids (hemicellulose hydrolysis) and alkalis for lignin removal.

The steam explosion process is well documented. Batch and continuous processes have been tested at laboratory and pilot scale by several research groups and

10   companies. In steam explosion pretreatment, biomass is treated at high pressure, and high temperatures under acidic conditions i.e. 160 °C to 260 °C for 1 min to 20 min, at pH values < pH 4.0. The pressure of the pretreated biomass is suddenly reduced, which makes the materials undergo an explosive decompression leading to defibrization of the lignocellulosic fibers.

15   Steam explosion pretreatment is not very effective in dissolving lignin, but it does disrupt the lignin structure and increases the cellulose susceptibility to enzymatic hydrolysis. Steam explosion pretreatment generally results in extensive hemicellulose breakdown and, to a certain extent, to the degradation of xylose and glucose.

Steam explosion pretreatment has been successfully applied on a wide range of

20   lignocellulosic biomasses. Acetic acid, sulfuric acid or sulfur dioxide are the most commonly used catalysts.

In the autohydrolysis process, no added acid is required to reach pH values below 4.0. Acetic acid is released during the breakdown of acetylated hemicellulose resulting from the high pressure steam applied to the biomass during the cooking stage. The

25   degree of hemicellulose acetylation is variable among different sources of biomass. The hemicellulose content of corncobs is high. Much of the hemicellulose is acetylated, which means the breakdown and solubilization of the hemicellulose, which occurs during pretreatment, leads to the formation of acetic acid.

The pretreatment of biomass like Miscanthus which does not have a high degree

30   of acetylation requires the addition of acid prior to the steam heating process to achieve a degree of hemicellulose hydrolysis similar to the autohydrolysis pretreatment process for highly acetylated biomass. Dilute acid- or sulfur dioxide-catalyzed steam explosion pretreatments require the use of 0.1-4.0 % sulfuric acid or 0.5-4.0 % sulfur dioxide.

The presence of acetic acid reduces the need for acid catalysts, which is

35   beneficial to the pretreatment process. However, mineral acids, acetic acid and other

carboxylic acids are all powerful inhibitors of both the hydrolysis and the glucose fermentation process. Mineral and carboxylic acids may remain in the pretreated biomass and carry through to the hydrolysis and fermentation steps. A process is desired that includes a pretreatment step carried out at a pH values < pH 4.0 to maximize hemicellulose solubilization. However, after steam pretreatment, acid catalysts and pretreatment degradation products must be removed to enhance the digestibility of the cellulose in the enzymatic hydrolysis step and to enable a more rapid and complete conversion of glucose to ethanol in the fermentation step.

SUMMARY OF THE INVENTION

It is now an object of the present invention to provide a process which overcomes at least one of the above disadvantages.

The inventors have discovered that the catalytic activities of cellulolytic enzymes are specifically inhibited by soluble forms of hemicellulose i.e. soluble xylo-oligosaccharides and xylose. Thus products of hemicellulose decomposition released during biomass pretreatment which remain in the pretreated biomass, and carry through to the hydrolysis and fermentation steps, can negatively affect enzymatic conversion of cellulose to glucose.

As is apparent from the above discussion of known approaches, improving the overall ethanol yield and reducing enzyme usage or hydrolysis time are generally linked to increased operating costs. The increased costs may outweigh the value of the increased ethanol yield, rendering existing methods economically unacceptable.

The inventors have discovered that complete removal of the inhibitory compounds is neither required nor desirable for the achievement of the most economically viable pretreatment process. The inventors have identified a narrow range of extraction conditions for the removal of inhibitory compounds in which hemicelluloses and hemicellulose hydrolysis and degradation products and other inhibitors are still present, but reduced to a level where they have a much reduced inhibitory effect on the enzymes. In addition, the fractionation of the biomass still provides an economical amount of valuable hemicellulose. The extraction is achieved with a lower volume of diluent and level of dilution making the process much more cost effective. In effect, the extraction cost is significantly less than the value of the increased ethanol yield, lower enzyme dosages, and the reduced processing times achieved. When combined with the ideal pretreatment temperature, time and purging of impurities, an economical process to convert low lignin lignocellulosic biomasses to fermentable sugar is achieved.

In addition, the economics of ethanol production demand the maximization of the value in all the byproduct streams from the process. As an example, acetic acid may be recovered for sale as an industrial chemical. Also, xylo-oligosaccharides, (non digestible sugar oligomers made up of xylose units), have beneficial health properties; particularly their prebiotic activity. This makes them good candidates as high value added bioproducts. The xylo-oligosaccharides mixture derived from corncob autohydrolysis exhibits prebiotic potential similar to commercially available xylo-oligosaccharide products.

A novel process is described for the continuous steam explosion pretreatment of corncobs wherein no mineral acid is added and the amount of acetic acid released in the pretreatment step is controlled to maximize the efficiency of the steam exposing step.

A sufficient residence time is provided to ensure proper breakdown/hydrolysis of the hemicellulose and activation of the cellulose fraction.

The steam explosion pretreated corncob biomass is extracted under pressure prior to exiting the pretreatment reactor. Minimal water is used as an eluent to remove water soluble hemicellulose and cellulose degradation products such as, xylose, xylo-oligosaccharides, furans, fatty acids, sterols, ester, ethers and acetic acid.

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In addition, the economics of ethanol production from hemicellulose demand the maximization of the value in all the byproduct streams from the process. As an example, acetic acid may be recovered for sale as an industrial chemical. Extraction refers in general to a single or multiple step process of removing liquid portions from the fibers with or without addition or utilization of an eluent, (the diluting step) Typically the extraction is

enhanced by use of a mechanical compressing device such as a modular screw device. The eluent can be recycled to increase the economy of its use or used for example in the known process of counter current washing as an example. Liquefied components in the steam treated lignocellulosic biomass and the dissolved components are subsequently removed from the fibrous solids. Generally this removes most of the dissolved compounds, the wash water, primarily consisting of hemicellulose hydrolysis and degradation products that are inhibitory to downstream hydrolysis and fermentation steps.

The extracting system in general uses a device that employs a mechanical pressing or other means to separate solids from liquid or air from solids. This can be accomplished under pressure as described above and/or under atmospheric pressure accomplished with several different types of machines that vary and the detail of which is not essential to this invention.

The extract stream containing the xylo-oligosaccharide fraction is collected and concentrated to the desired dryness for further applications. A final refining step is required for producing xylo-oligosaccharides with a degree of purity suitable for pharmaceuticals, food and feed, and agricultural applications. Vacuum evaporation can be applied in order to increase the concentration and simultaneously remove volatile compounds such as acetic acid and flavors or their precursors. Solvent extraction, adsorption and ion-exchange precipitation have been proposed by those skilled in the art.

A balance must be maintained between the removal of the water soluble components (xylo-oligosaccharide fraction) and the need to minimize the amount of washing/eluent water added. It is the desire to minimize water use, as the xylo-oligosaccharide fraction must be concentrated for its eventual use, which requires equipment and energy, both of which must be minimized.

In the new process, pressurized activated cellulose is flashed into a cyclone by rapidly releasing the pressure to ensure an explosive decompression of the pretreated biomass into fibrous solids and vapors. This opens up the fibres to increase accessibility for the enzymes. Purified cellulose with a low level of residual hemicellulose can be sent to the hydrolysis and fermentation stages.

A novel process is also described for the continuous steam explosion pretreatment of low lignin biomass (< 12 %) wherein the recovery of both cellulose and hemicellulose is maximized by carefully choosing pH, temperature, and retention time of the exposing step.

The biomass is preferably chopped or ground and preheated with live steam at atmospheric pressure prior to the pretreatment step. Air is removed from the biomass by

pressing. Liquefied inhibiting extracts can be removed at this time. Acid is added, if required, to lower the pH to the desired value for catalyzing the breakdown/hydrolysis of the hemicellulose and activating the cellulose fraction during the cooking step. Pressed impregnated biomass is then cooked with steam at elevated temperatures and pressures

5   for a preselected amount of time.

A sufficient residence time is provided to ensure proper breakdown/hydrolysis of the hemicellulose and activation of the cellulose fraction. During pretreatment purging of condensate and venting of volatiles occurs continuously.

The pretreated biomass is extracted under pressure prior to exiting the

10   pretreatment reactor or after exiting or both. Minimal water is used as an eluent to remove water soluble or water emulsified hemicellulose and cellulose hydrolysis and degradation products such as, xylose, xylo-oligosaccharides, furans, fatty acids, sterols, ester, ethers and acetic acid.

Extraction refers in general to a single or multiple step process of removing liquid

15   portions from the fibers with or without addition or utilization of an eluent, (the diluting step). Typically the extraction is enhanced by use of a mechanical compressing device such as a modular screw device. The eluent can be recycled to increase the economy of its use or used for example in the known process of counter current washing. Soluble and suspended or emulsified components in the steam treated lignocellulosic biomass are

20   removed from the fibrous solids. The subsequent eluent wash water, containing hemicellulose products that are inhibitory to downstream hydrolysis and fermentation steps is sent to a recovery step.

The extracting system generally uses a device that employs mechanical pressing or other means to separate solids from liquid. This can be accomplished under pressure

25   as described above and/or under atmospheric pressure accomplished with several different types of machines, the details of which are not essential to this invention.

The extract stream containing the xylo-oligosaccharide fraction is collected and concentrated to the desired dryness for further applications. A final refining step is required for producing xylo-oligosaccharides with a degree of purity suitable for

30   pharmaceuticals, food and feed, and agricultural applications. Vacuum evaporation can be applied in order to increase the concentration and simultaneously remove volatile compounds such as acetic acid and flavors or their precursors. Solvent extraction, adsorption and ion-exchange precipitation have been proposed by those skilled in the art.

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35   components (xylo-oligosaccharide fraction) and the need to minimize the amount of

washing/eluent water added. It is desirable to minimize water use, as the xylo-oligosaccharide fraction must eventually be concentrated.

In the new process, pressurized activated cellulose is flashed into a cyclone by rapidly releasing the pressure to ensure an explosive decompression of the pretreated

5   biomass into fibrous solids and vapors. This opens up the fibres to increase accessibility for the enzymes. Purified cellulose with a low level of residual hemicellulose can be sent to the hydrolysis and fermentation stages.

BRIEF DESCRIPTION OF THE DRAWINGS

10   Other objects and advantages of the invention will become apparent upon reading the detailed description and upon referring to the drawings in which:

Figure 1 shows a process diagram of the continuous pretreatment unit proposed in the example.

Figure 2 shows the total percentage recovery of cellulose and hemicellulose

15   produced during the fractionation of corncobs.

Figure 3 illustrates the susceptibility of pretreated corncob cellulose to enzymatic hydrolysis i.e. cellulose to glucose conversion.

Figure 4 shows hydrolysis and fermentation results using pretreated corncobs produced at pilot scale (2.5 metric tonnes, 17 % consistency).

20   Figure 5 is a process diagram of the continuous steam explosion fractionation system used to produce activated cellulose and lignin free solutions of oligosaccharides. utilizing acid addition.

Figure 6 shows the impact on pretreated biomass pH of sulfuric acid added in conjunction with the acetic acid released from hemicellulose breakdown during steam

25   explosion pretreatment.

Figure 7 shows the total percentage recovery of cellulose and hemicellulose produced during high pressure fractionation of corncobs.

Figure 8 shows the total percentage recovery of cellulose and hemicellulose produced during low pressure fractionation of corncobs.

30   Figure 9 shows hydrolysis and fermentation results using pretreated corncobs produced at pilot scale and low pressure.

Figure 10 shows the total percentage recovery of cellulose and hemicellulose in solid and liquid fractions produced over the fractionation of Miscanthus.

Figure 11 illustrates the susceptibility of pretreated cellulose from Miscanthus (Example 3) to enzymatic hydrolysis (cellulose to glucose conversion) and fermentability of hydrolyzed cellulose (glucose to ethanol conversion).

Figure 12 shows cellulose conversion times at various levels of digestion versus

5   severity index for Miscanthus Biomass with 1.6% sulfuric acid to a pH of 3.8.

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DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

Before explaining the present invention in detail, it is to be understood that the invention is not limited to the preferred embodiments contained herein. The invention is capable of other embodiments and of being practiced or carried out in a variety of ways. It

15   is to be understood that the phraseology and terminology employed herein are for the purpose of description and not of limitation.

The abbreviations used in the figures have the following meaning:

°C, temperature in degree Celsius

ms, millisecond

20   DM, Dry matter

SI, Severity Index

t $_{90\%}$,Time to reach 90 % of the maximum theoretical cellulose to glucose conversion.

Pretreatment of Lignocellulosic Biomass

25   This invention is a new process for fractionating lignocellulosic biomass from corncobs into two main components, specifically a cellulose-rich corncob fibre and a xylo-oligosaccharides-rich solution. The cellulose-rich component is valuable for many purposes. Specifically it may be more easily hydrolyzed to glucose which in turn may be more easily fermented to ethanol or other biofuels than in previous processes.

30   A preferred aspect of the invention is a continuous process for the pretreatment of corncobs that generates highly reactive cellulose prehydrolysate with a reduced content of compounds which have an inhibiting effect on cellulose hydrolysis and glucose fermentation.

Another preferred aspect of the invention is a process for the pretreatment of

35   corncobs, for generating a lignin free solution of xylo-oligosaccharides with a ratio of xylo-

oligosaccharide to acetic acid and volatile compounds from hemicellulose degradation of greater than 4.

The preferred process of the invention includes the steps of exposing ground, preheated corncob biomass to steam at 170 °C to 220 °C at 100 to 322 psig for 2 to 300 minutes without the use of mineral acid catalysts. The pretreatment preferably includes the continuous purging of volatile and liquid compounds. The exposing step preferably steam treats the biomass to a temperature and hold time for a Severity Index of 3.8 to 4.1, the Severity Index being calculated according to the equation:

Severity Index = Log x Exp {(Temperature °C − 100)/14.75) x Retention Time (min).

The exposing step most preferably has a severity index of 4.0.

As shown in figure 6 the exposing of corn cobs to a severity index of 4.0 leads to a final pH of 3.5 to 4.0.

The process also includes extraction of the steam treated fibres with/or without eluent addition under pressure to remove water soluble hemicelluloses, acids and hemicellulose and cellulose degradation products. As an option these inhibitors may be extracted after pretreatment or both during and after. The extraction of the soluble biomass from the fiber preferably results in 4% to 10% xylose based sugars consisting of monomers and oligosaccharides remaining in the prehydrolysis fibers.

The extracted fibers, also referred to as prehydrolysate, are separated from the gaseous reaction products in a cyclone separator, collected at the bottom of the separator, then shredded and diluted to the desired consistency and subsequently transported to the enzymatic hydrolysis step.

The prehydrolysate is diluted with water to 10-30% consistency and then reacted with cellulase enzymes to produce glucose. The glucose rich solution is readily utilized in the subsequent fermentation step where an organism converts the glucose into ethanol.

EXAMPLE

In the following example, reference numbers refer to features of the pretreatment system and process streams, as shown in Figure 1.

Continuous steam explosion pretreatment of corncobs is carried out in a steam explosion pretreatment system.

Corncobs 10 are received, stored, cleaned, ground (0.5 to 1 cm^3 particle size) and fed through a V shaped hopper and screw auger (not shown). The corncob moisture is adjusted to 50 % DM.

Prepared corncobs are preheated with live steam 20 at atmospheric pressure, in a holding bin or preheating and conditioning container 30 to a temperature of about 95 °C for about 10-60 minutes. Air and steam are vented through an air vent 35 from the preheating and conditioning container 30.

5   Preheated corncobs are compressed in a first modular screw device 40 to remove air 50 through an air vent and inhibitory extracts 5. The corncobs are then fed into a pressurized upflow tube 70.

Pressurized saturated steam at a temperature of 205 C is injected upstream of and/or into the upflow tube 70 by direct injection 60 and/or indirect injection of steam 61 in

10   a jacketed section of the up flow tube until the desired cooking pressure is reached.

Corncobs are moved through the upflow tube with the aid of a screw conveyor/mixer (3 min) and are discharged into a pretreatment reactor 80.

Corncobs are continuously discharged from the pretreatment reactor 80 to a second pressurized modular screw device 100 after a residence time of 5 min at 205 °C

15   in the pretreatment reactor 80.

During the residence time, condensate and cooking liquids collected at the bottom of the pretreatment reactor are purged through a purge discharge control valve 95.

Pretreated corncobs are washed with water eluent under pretreatment pressure. Hot water 90 is added to dilute the pretreated corncobs as the fiber is discharged from the

20   pretreatment reactor. Further hot water is also added along the pressing device 100 to reach a ratio of about 6:1 wash water eluent to corncobs and achieve a greater extraction of hemicellulose. The extracted hemicellulose solution 110 is collected and concentrated to the desired dryness for further applications.

The pressurized washed corncobs are flashed into a cyclone 120. The solids, i.e.

25   purified cellulose, collected at the bottom of the cyclone separator are subjected to further processing i.e. shredded and then diluted with fresh water to the desired consistency for hydrolysis and fermentation.

The gaseous components are collected, condensed 130 and fed to a condensate tank. Any gaseous emissions from the steam gun, the cyclone separator and other parts

30   of the setup are collected and treated in an environmental control unit (not shown). Cleaned gases are exhausted to atmosphere from the environmental control unit.

In order to simulate this new process, steam explosion pretreatment of corncobs was followed by batch washing at pilot scale with a 97% recovery of cellulose (Figure 2).

Extracted cellulose from the pilot scale pretreatment was highly susceptible to

35   enzymatic hydrolysis. 80 % of the maximum theoretical cellulose to glucose conversion

was achieved in 60 h. 90 % conversion of the 17 % consistency slurry was reached in 95 h, using only 0.23 % load of commercial cellulases product (Figure 3).

In Figure 3, the dashed line represents the trend of eleven enzymatic hydrolysis experiments carried out at three different scales (i.e. 1 kg, 300 kg and 2500 kg). These

5   enzymatic hydrolysis experiments were carried out at 17 % consistency, 50 °C and pH 5.0. The pH adjustment chemical used was aqueous ammonia (30 %). Commercially available lignocellulolytic enzyme was used at a load of 0.23% weight/weight on incoming cob feedstock.

Samples of the continuously pretreated corncobs were hydrolyzed and fermented

10   in a 2.5 metric tonne batch hydrolysis and fermentation trial (Figure 4). The results were in accordance with the lower scale pilot and the laboratory scale results (Figure 3). A concentration of 100 g/L glucose was reached at $t_{90\%}$ i.e. 100 hours hydrolysis of 17 % consistency slurry, using only 0.23 % load of commercial cellulase product.

The fermentability of the hydrolyzed cellulose was high. A concentration of 4.9 %

15   alcohol was reached in 20 hours (Figure 4).

In Figure 4, hydrolysis was carried out at 50 °C, pH 5.0 and 0.5 % enzyme load. Fermentation was carried out at 33 °C, pH 5.3, using an industrial-grade C6-fermenting yeast. Hydrolysis and fermentation pH adjustment was carried out using aqueous ammonia (30 %). Grey circles indicate glucose concentration. Black squares indicate

20   ethanol concentration.

The production of soluble xylo-oligosaccharides was equivalent to 12 % of the weight of raw corncobs processed at pilot scale. 63 % of the original content of corncobs hemicellulose was converted to volatile degradation products (Figure 2). 66 % of these volatiles were flashed off during the step of explosive decompression.

25   81 % of the hemicellulose remaining in the corncobs prehydrolysate after autohydrolysis was collected through the prehydrolysate water washing step. The resulting lignin free solution contained dissolved solids of which 87 % were sugars, including 63 % of xylo-oligosaccharides (w/w) on a dry matter basis.

30   Pretreatment of Low Lignin Hemicellulosic Biomass

This invention is a new process for fractionating low lignin cellulosic biomass from different sources into two main components, specifically cellulose and xylo-oligosaccharides. The purified cellulose component is valuable for many purposes. Specifically it may then be more easily hydrolyzed to glucose which in turn may be more

35   easily fermented to ethanol or other biofuels than in previous processes.

- 13 -

A preferred aspect of the invention is a process for the pretreatment of feedstocks with low lignin content for generating highly reactive cellulose prehydrolysate with a reduced content of compounds which have an inhibiting effect on cellulose hydrolysis and glucose fermentation such as hemicellulose hydrolysis and degradation products.

Another preferred aspect of the invention is a process for the pretreatment of low lignin biomass, for generating a lignin free solution of xylo-oligosaccharides with a ratio of xylo-oligosaccharide to acetic acid and volatile compounds from hemicellulose degradation of greater than 4.

In Figure 6, grey diamonds and the dashed line show that enough acetic acid was released from agricultural residues i.e. corncob hemicellulose to ensure a pH value of 3.8 during the cooking step, hence, there was no need for the addition of mineral catalysts. The black squares and the plain line show that 1.6 % by weight DM sulfuric acid had to be added to an energy crop, i.e. Miscanthus, biomass to ensure a pH value of 3.8 during steam explosion pretreatment. As can be seen in Figure 6, 0% sulfuric acid addition with corn cob biomass and 1.6% sulfuric acid addition to Miscanthus biomass are both between a pH level of 3.5-4.0.

Process Description

The preferred process of the invention (Figure 5) includes the following steps:

Step 1 Feed Preparation

Low lignin biomass 10 is received, stored, cleaned and ground (0.5 to 1 cm^3 particle size) through a V shaped hopper and screw auger (not shown).

Biomass moisture is adjusted to a desired range of 30-60 % at this stage.

Step 2 Preheating

Prepared biomass is preheated with live steam 20 at atmospheric pressure, in a holding bin 30 to a temperature of about 95 °C for 10 to 60 minutes.

Air and steam are vented 35 from the bin.

Step 3 Heating and Catalyst(s) addition

Preheated biomass is compressed in a modular screw device 40 to remove air 50 liquefied inhibitory extracts and excess water and then fed into a pressurized up flow tube 70.

The tube is sized to provide a 3 to 15 minutes holdup time. The dry matter content of the biomass varies from 30 % to 60 % prior to the addition of steam and catalyst.

The biomass is further heated upstream of and in the up flow tube 70 to a pretreatment temperature of 170 $^{\circ}$C to 220 $^{\circ}$C by direct steam injection 60 or by indirect steam 61 in a jacketed section of the up flow tube.

Mineral acids or acid gases 65 are blended with the biomass in an amount up to 4% to obtain the desired value of pretreatment pH that ranges from pH 1.0 to pH 5.0; preferably pH 3.0 to pH 4.0; to catalyze the removal of hemicellulose and to activate the cellulose. The pH is measured after the desired exposure is reached.

The acid addition 65 is made through a set of one or more nozzles after the screw device and/or in along the length of the up flow tube.

The treated biomass moves through the up flow tube with the aid of a screw conveyor/mixer and is discharged into the pretreatment reactor 80.

Step 4 Pretreatment

The preheated and acidified biomass is discharged from the up flow tube 70 into the pretreatment reactor 80. The pretreatment reactor is sized to allow a residence time of 5 to 70 minutes. The low lignin biomass is preferably held at the target temperature to achieve a Severity Index of 3.5 to 4.0, the Severity Index being calculated according to the equation:

Severity Index = Log x Exp {(Temperature $^{\circ}$C – 100)/14.75) x Retention Time (min).

Most preferably the severity index is 3.6.

Step 5 Washing/pressing

Pressurized biomass, still at the pressure of the pretreatment reactor is diluted with wash eluent water 90 as it exits the pretreatment reactor and the water with solubilized and suspended compounds is pressed from the biomass as it passes through the modular screw device 100, still under pressure. Further hot eluent water may also be added along the pressing device to achieve a greater extraction of soluble hemicellulose hydrolysis and degradation products and acid catalyst(s). The temperature of the wash water may vary.

In this washing stage the majority of the hemicellulose fraction 110 is removed. The hemicellulose removal efficiency may vary from 50% to 90% or greater. The water washing system in general uses a device that employs pressing or other means to

separate solids form liquids. This can be accomplished with several different types of machines, which are well known to a person of skill in the art and need not be described in detail. A balance must be maintained between the removal of the water soluble components (Xylo-oligosaccharide fraction) and the need to minimize the amount of washing/eluent water added. It is the desire to minimize water use, as the xylo-oligosaccharide fraction must be concentrated for its eventual use. It is preferred to achieve a final xylose content of the prehydrolysate of 4% to 10%, the xylose is present as xylooligosaccharides and xylose.

A final refining step is required for producing pure xylo-oligosaccharides with a degree of purity suitable for fermentation, biofuels, pharmaceuticals, food and feed and agricultural applications. Vacuum evaporation (not shown) can be applied in order to increase the concentration and simultaneously remove volatile compounds and acid catalyst(s) such as furans, acetic acid, sulfuric acid or sulfur dioxide.

Solvent extraction, adsorption and ion-exchange precipitation have been proposed by those skilled in the art. Acid catalyst(s) and wash water are recycled and reused in the pretreatment process.

Step 6 Recovery of purified cellulose

Pressurized washed cellulose with a low level of hemicellulose is flashed into a cyclone 120. The solids fraction i.e. purified cellulose, collected at the bottom of the cyclone separator can be sent to the hydrolysis and fermentation stages.

Gaseous components are collected, condensed 130 and fed to the condensate station (not shown). Any gaseous emissions from the vessels, the cyclone separator and other parts of the setup are collected and treated in an environmental control unit (not shown). Cleaned gases are exhausted to atmosphere from the unit.

EXAMPLE 1 – High pressure pretreatment of corncobs

Steam explosion pretreatment of corncobs was carried out in a steam explosion pretreatment system pressurized with saturated steam at a temperature of 205 °C. No

acid was added to the corncobs during the heating step. The overall retention time of corncob pretreatment is 8 min e.g. 3 min in an up flow tube, 5 min in a pretreatment reactor at pH 3.8. Corncob acidification resulted from the release of acetic acid from hemicellulose breakdown.

5   Pretreated corncobs were water washed.

Cellulose extraction from corncobs was carried out at pilot scale with a percentage recovery of 92 % (Figure 7).

59 % of the incoming hemicellulose was recovered after high pressure pretreatment of corncobs. 52 % of incoming hemicellulose was collected into the xylo-

10   oligosaccharides solution (Figure 7). The resulting lignin free solution contained 89 % sugars, including 66 % of xylo-oligosaccharides (w/w) on a dry matter basis.

EXAMPLE 2 – Low pressure pretreatment of corncobs

Steam explosion pretreatment of corncobs was carried out in a steam explosion

15   pretreatment system pressurized with saturated steam at a temperature of 170 °C. No acid was added to the corncobs during the heating step. The overall retention time of corncobs pretreatment was 85 min e.g. 15 min in an up flow tube, 70 min in a pretreatment reactor at pH 3.8. Corncob acidification resulted from the release of acetic acid from hemicellulose breakdown.

20   Pretreated corncobs were water washed.

Cellulose extraction from corncobs was carried out at pilot scale with a percentage recovery of 92 % (Figure 8).

51 % of incoming hemicellulose was recovered after low pressure pretreatment of corncobs. 43 % of incoming hemicellulose was collected in the xylo-oligosaccharides

25   solution (Figure 8). The resulting lignin free solution contained 88 % sugars, including 65 % of xylo-oligosaccharides (w/w) on a dry matter basis.

After explosive decompression, the solid fraction from high or low pressure pretreatment i.e. purified cellulose was collected at the bottom of cyclone separator, shredded and then diluted with fresh water up to 17 % consistency.

30   Extracted cellulose from high and low pressure continuous pilot scale pretreatment of corncobs was highly susceptible to enzymatic hydrolysis. Digestibility of cellulose pretreated at high and low pressure was similar (Figure 3). 80 % of the maximum theoretical cellulose to glucose conversion was achieved in 60 h. 90 % conversion of the 17 % consistency slurry was reached in 95 h, using only 0.23 % load of

35   commercial cellulases product (Figure 3).

In Figure 3, the dashed line represents the trend of six duplicate enzymatic hydrolysis experiments carried out at three different scales (i.e. 1 kg, 300 kg and 2500 kg) with cellulose extracted at high or low pressure. These enzymatic hydrolysis experiments were carried out at 17 % consistency, 50 °C and pH 5.0. The pH adjustment chemical

5   used was aqueous ammonia (30 %). Commercially available lignocellulolytic enzyme was used at a load of 0.23 % weight/weight on incoming cob feedstock.

At pilot scale (2.5 metric tonne fed batch hydrolysis and fermentation trial, Figure 9) a concentration of 100 g/L glucose representing 91% conversion of the cellulose was reached after 100 hours hydrolysis of a 17 % consistency slurry from low

10   pressure pretreatment.

In Figure 9, hydrolysis was carried out at 50 °C, pH 5.0 and 0.5 % enzyme load. Fermentation was carried out at 33 °C, pH 5.3, using an industrial grade C6-fermenting yeast. Hydrolysis and fermentation pH adjustment was carried out using aqueous ammonia (30 %). Grey circles indicate glucose concentration. Black squares indicate

15   ethanol concentration.

Fermentability of the hydrolyzed cellulose was evaluated by adding enough C6-industrial grade commercial yeast to reach a concentration of 10^8 yeast cells per gram hydrolysate at 33°C, pH 5.3 when 90 % of the maximum theoretical cellulose to glucose conversion was reached. pH adjustment was carried out with aqueous ammonia (30 %)

20   prior to yeast addition.

Fermentability of the hydrolyzed cellulose was high. A concentration of 4.9 % alcohol was reached in 20 hours (Figure 10).

EXAMPLE 3 – Low pressure pretreatment of Miscanthus

25   Steam explosion pretreatment of Miscanthus was carried out in a system pressurized with saturated steam at a temperature of 170 °C. Miscanthus fibers were impregnated with sulfuric acid in the amount of 1.6 % by weight DM during the heating step. The overall retention time of the Miscanthus pretreatment was 30 min at pH 3.8.

Pretreated Miscanthus was water washed.

30   Cellulose extraction from Miscanthus was carried out at pilot scale with a percentage recovery in the solid fraction of 95 % (Figure 10).

45 % of the incoming hemicellulose was recovered after pretreatment of Miscanthus. 40 % of the incoming hemicellulose was collected in the xylo-oligosaccharides solution (Figure 10). The resulting lignin free solution contained 85 %

35   sugars, including 62 % of xylo-oligosaccharides (w/w) on a dry matter basis.

Extracted cellulose from pilot scale pretreatment of Miscanthus was highly susceptible to enzymatic hydrolysis. 80 % of the maximum theoretical cellulose to glucose conversion was achieved in 73 h. 90 % conversion of the 17 % consistency slurry was reached in 105 h, using a 1 % load of commercial cellulase (Figure 11).

5   In Figure 11, hydrolysis was carried out at 50 °C, pH 5.0, using commercially available lignocellulolytic enzyme product at a load of 1.0 % weight/weight on incoming cob feedstock. Fermentation was carried out at 33 °C, pH 5.3 using an industrial-grade C6-fermnenting yeast.

A concentration of 88 g/L glucose representing 90% conversion of cellulose was
10   reached after 100 hours of hydrolysis of a 17 % consistency slurry.

Fermentability of the hydrolyzed cellulose was evaluated by adding enough C6-industrial grade commercial yeast to reach a concentration of 10^8 yeast cells per gram hydrolysate at 33°C, pH 5.3. The time needed to reach 90 % of the maximum theoretical cellulose to glucose conversion was determined. pH adjustment was carried out with
15   aqueous ammonia (30 %) prior to yeast addition.

The fermentability of the hydrolyzed cellulose was high. A concentration of 4.1 % alcohol was reached in 30 hours (Figure 9).

Figure 12 shows cellulose conversion times at various levels of digestion versus Severity Index. In particular, Figure 12 shows the amount of time for enzyme digestion of
20   70%, 80%, and 90% of the cellulose for Miscanthus biomass which was steam heated with 1.6% sulphuric acid to achieve a pH of 3.8, which is the same pH as the prehydrolysate of corn cob biomass with 0% sulfuric acid, as shown in Figure 6. In the example shown in Figure 12, Miscanthus was steam heated at various Severity Indices and ,as shown in Figure 12, the ideal Severity Index is about 3.6 which results in the
25   enzymatic conversion of cellulose in the least amount of time.

The inventors have discovered that the ideal Severity Index used for miscanthus was not as expected. Pretreatment of corn cobs releases sufficient acetic acid for autohydrolysis at a Severity Index of 4.0 with a pH of about 3.8 and provides the best enzymatic hydrolysis time. If the same process conditions of temperature, pressure, pH,
30   and time is duplicated for Miscanthus, simply by trying to reach the same pH with acid, this leads to significantly lower cellulose digestibility. This result was unexpected and is well illustrated in Figure 12 which clearly indicates that the ideal Severity Index for the lowest enzymatic cellulose digestion of miscanthus at about pH 3.8 (1.6% sulphuric acid) is 3.6. Thus, as was first thought processing Miscanthus, a low acetyl low lignin biomass,
35   at the same pH as corn cobs in itself is not sufficient as the pretreatment must also be

adjusted to more gentle conditions. It is expected that, for other feedstocks that require less acid than Miscanthus but still require additional acid to achieve the same pH, the optimal Severity index would range between 3.6 to 4.0 as these other feedstocks get closer to corn cobs which work best at a Severity Index of 4.0.

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CLAIMS:

1.   A continuous process for fractionation of lignocellulosic biomass having a lignin content of less than 12%, comprising the steps of:

5   a) exposing the lignocellulosic biomass to steam in a reaction vessel at a preselected temperature and a preselected reaction pressure, for a preselected exposure time, and at a selected pH value for removing a hemicellulose fraction of the lignocellulosic biomass and activating a cellulose fraction of the lignocellulosic biomass to obtain a prehydrolyzed lignocellulosic biomass;

10 b) purging liquid condensate and vapor generated during the exposure step to remove and collect a first liquid stream with water soluble compounds and a first vapor stream with volatile chemicals;

c) extracting liquid containing hemicellulose hydrolysis and degradation components from the prehydrolysed lignocellulosic biomass as a hemicellulose degradation stream;

15 d) rapidly releasing the reaction pressure after the extracting step to afford explosive decompression of the prehydrolyzed lignocellulosic biomass into fibrous solids, vapor and condensate; and

e) collecting the vapor and condensate from the explosive decompression for separation and recovery of byproducts.

20

2.   The process of claim 1, wherein an acid catalyst(s) is added during the exposing step.

3.   The process of claim 2, wherein the lignocellulosic biomass is selected from the

25 group consisting of miscanthus, switchgrass, corn cob, prairie grass, sorghum straw, corn stover, and wheat straw.

4.   The process of claim 2, wherein the lignocellulosic biomass is miscanthus.

30 5.   The process of claim 2, wherein the steam pretreatement is carried out at a temperature of 170 °C to 205 °C.

6.   The process of claim 2, wherein the steam pretreatment is carried out for less than 90 min at a pH value of 3.0 to 4.0.

7.   The process of claim 2, wherein the steam pretreatment is carried out for less than 90 minutes at a pH value of pH 3.5-3.9.

8.   The process of claim 1, wherein the pH value is adjusted using pH adjustment chemical(s) or acid catalysts.

9.   The process of claim 8, wherein the acid catalyst is all or in part acetic acid released from the breakdown of the hemicellulose fraction of the lignocellulosic biomass.

10.   The process of claim 8, wherein pH adjustment chemical(s) or acid catalyst(s) include mineral acids or acid gases blended with the biomass in an amount of up to 4%.

11.   The process of claim 2, wherein a severity index of 3.5 to 4.0 is maintained during the exposing step, the severity index being calculated according to the equation: Severity Index = Log x Exp {(Temperature °C – 100)/14.75) x Retention Time (min).

12.   The process of claim 11, wherein the severity index is maintained at 3.6.

13.   The process of claim 11, wherein the exposing step is carried out at the reaction temperature of 170 °C, the reaction pressure of 100 psig, and for the time interval of 25-85 minutes.

14.   The process of claim 11, wherein the exposing step is carried out at the reaction temperature of 170 °C, the reaction pressure of 100 psig, and for the time interval of 34.5 minutes.

15.   The process of claim 11, wherein the Severity Index is about 3.6 and the pH after the retention time is 3.0 to 4.0.

16.   The process of claim 1, wherein a Severity Index of 3.8 to 4.1 is maintained during the exposing step, the severity index being calculated according to the equation: Severity Index = Log x Exp {(Temperature °C – 100)/14.75) x Retention Time (min).

17.   The process of claim 16, wherein the Severity Index is maintained at 4.0.

18.   The process of claim 17, wherein the pH after the retention time is 3.0 to 4.0.

19.   The process of claim 17, wherein the exposing step is carried out at the reaction temperature of 205 °C, the reaction pressure of 235 psig, and for the time interval of 8 minutes.

20.   The process of claim 17, wherein the exposing step is carried out at the preselected reaction temperature of 170 °C, the preselected reaction pressure of 100 psig, and for the preselected exposure time of 85 minutes.

21.   The process of claim 17, wherein the exposing step is controlled to achieve a pH of 3.0 to 4.0 at the end of the preselected exposure time.

22.   The process of claim 17, wherein the lignocellulosic biomass has an acetyl content selected to achieve a pH of 3.5 to 4.0 at the end of preselected exposure time.

23.   The process of claim 21, wherein the lignocellulosic biomass is corn cob.

24.   The process of claim 1, where the lignocellulosic biomass is selected from the group consisting of corn cobs, sugar cane bagasse, switchgrass, prairie grass, sorghum bagasse, corn stover, and wheat straw.

25.   The process of claim 1, wherein the process is carried out in a pretreatment exposing system and volatile compounds are removed continuously by venting the pretreatment exposing system.

26.   The process of claim 1, wherein the process is carried out in a pretreatment exposing system and the purging of the liquid condensate takes place continuously at purging points in the pretreatment exposing system.

27.   The process of claim 1, wherein solubilized degradation byproducts of hemicellulose created in the exposing step are extracted and removed from the pretreated lignocellulosic biomass under pressure prior to explosive decompression.

28.   The process of claim 26, wherein an eluent is added to the pretreated lignocellulosic biomass prior to the step of extracting and removing the hemicellulose under pressure.

29.   The process of claim 26, wherein the process is carried out in a pretreatment unit having a pretreatment reactor with an outlet connected to a solid-liquid separation device, and wherein wash water is added at a bottom of the pretreatment reactor and/or along the solid-liquid separation device to achieve a greater extraction of a soluble hemicellulose fraction of the lignocellulosic biomass.

30.   The process of claim 1, wherein solubilized byproducts of hemicellulose degradation created in the exposing step are extracted and removed from the solid portion both before and after explosive decompression, with or without the addition of an eluent.

31.   The process of claim 1, wherein solubilized byproducts of hemicellulose degradation created in the exposing step are extracted and removed from the pretreated lignocellulosic biomass with or without the addition of an eluent to produce a solid cellulose rich fiber containing 4% to 10% xylose content as xylose and xylose oligosaccharides.

32.   The process of claim 1, wherein solubilized byproducts of hemicellulose degradation created in the pretreatment exposing step are extracted and removed from the pretreated lignocellulosic biomass with or without the addition of an eluent to produce a solid cellulose rich fiber containing 6% +/- 1% xylose content as xylose and xylose oligosaccharides.

33.   The process of claim 1, wherein the removal of hydrolyzed and degraded hemicellulose and cellulose degradation products in the liquid phase is enhanced by using a mechanical compression device selected from the group consisting of a MSD, a drainer screw, a filter press, a belt press, and a filter to separate liquid from solid fibers to achieve a target 6% +/- 1% xylose content as xylose and xylose oligosaccharides.

34.   The process of claim 1, wherein pretreated fibrous solids are extracted with water as eluent, and the eluent water with hemicellulose hydrolysis and degradation

components is subsequently separated for producing a hemicellulose or lignin free xylose and xylo-oligosaccharides solution including other chemicals which inhibit enzymatic hydrolysis.

35.   The process of claim 1, wherein extracted fibrous solids are separated from the liquid by mechanical processing selected from the group consisting of compressing, filtering, centrifuging, and combinations thereof.

36.   The process of claim 1, wherein the lignocellulosic biomass is counter current washed with water eluent to enhance the hemicellulose hydrolysis and degradation extraction.

37.   The process of claim 1, wherein the water as eluent is derived from recycle streams of the process, or recycled eluent water.

38.   The process of claim 1, wherein hemicellulose hydrolysis and degradation product fractions in the hemicellulose degradation stream are collected for value added purposes.

39.   The process of claim 1, wherein the acetic acid in the vapor purge stream is recycled back to the exposing step to enhance the hemicellulose hydrolysis and degradation.

40.   The process of claim 1, wherein acetic acid in the first vapor stream is collected for value added purposes.

41.   The process of claim 1, wherein acetic acid in the first vapor stream and the hemicellulose degradation stream is collected for value added purposes.

42.   The process of claim 1, wherein furfural is produced in the exposing step, the furfural is condensed from the vapor purge stream and other streams and is collected for value added purpose.

43.   The process of claim 1, wherein the lignocellulosic biomass is pre-steamed prior to the exposing step with steam for 10 to 60 min at a temperature of up to 99 Celsius to

remove air and adjust a moisture content of the lignocellulosic biomass to between 30 and 60%.

1/12
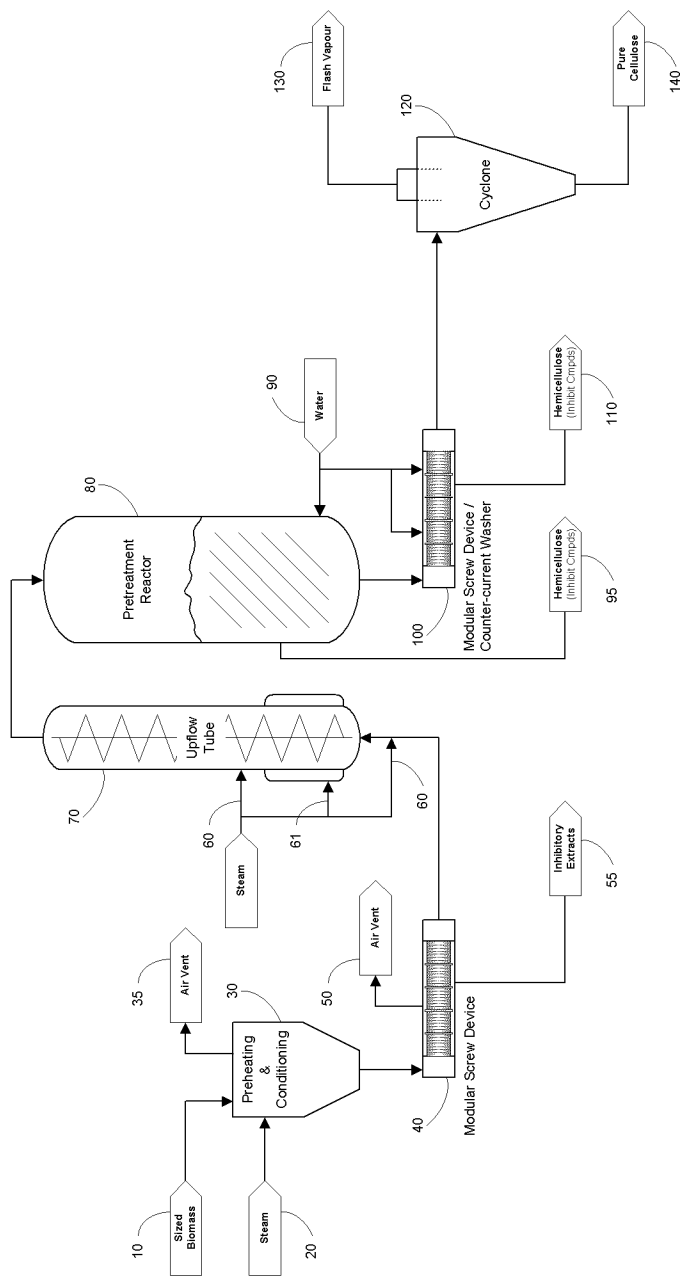
Figure 1

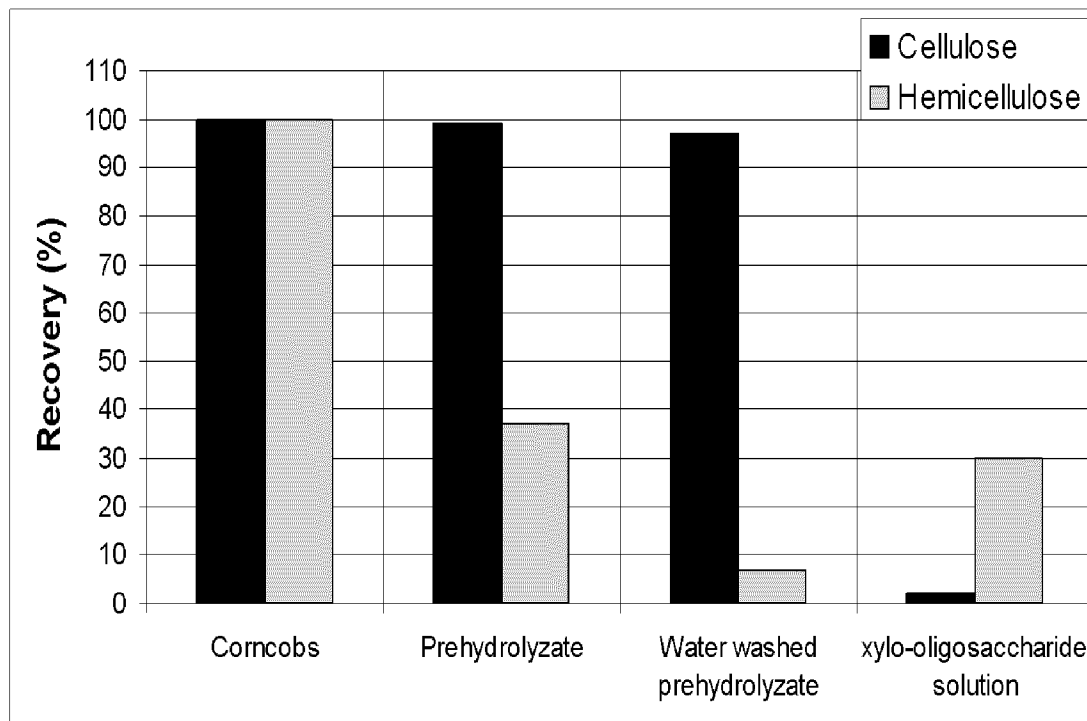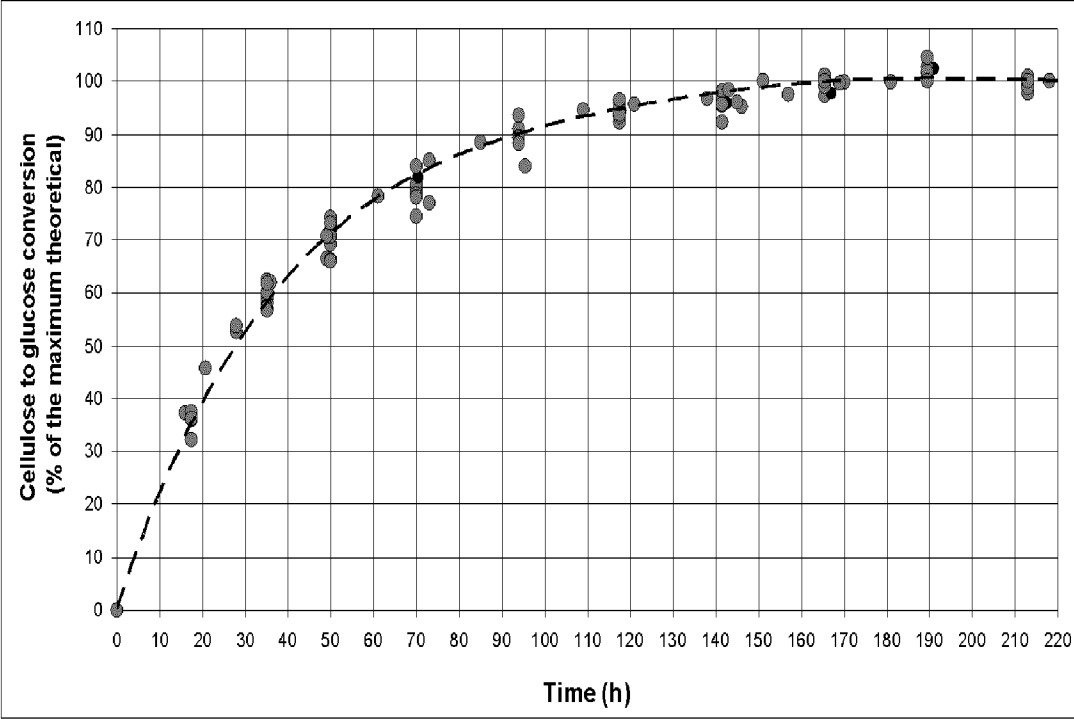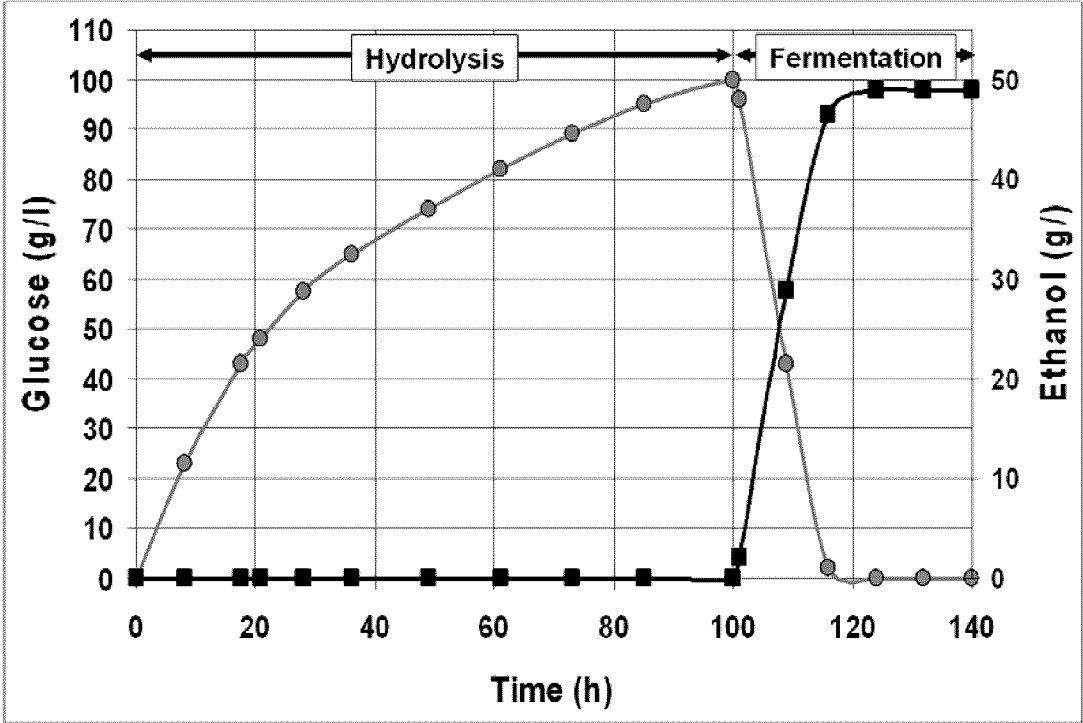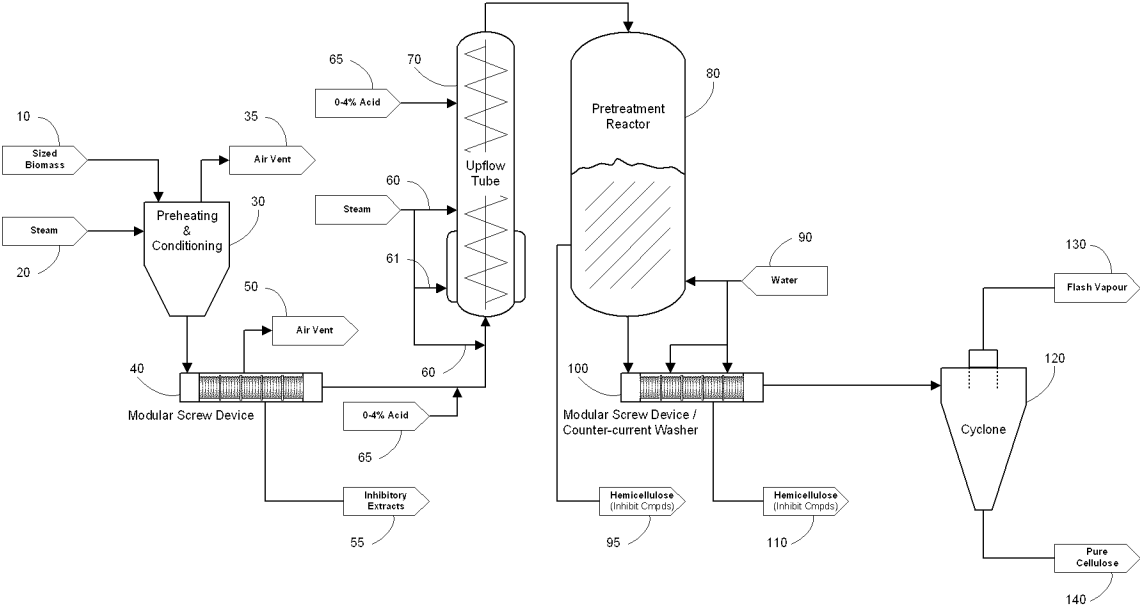**Figure 2**

Figure 3

Figure 4

Figure 5
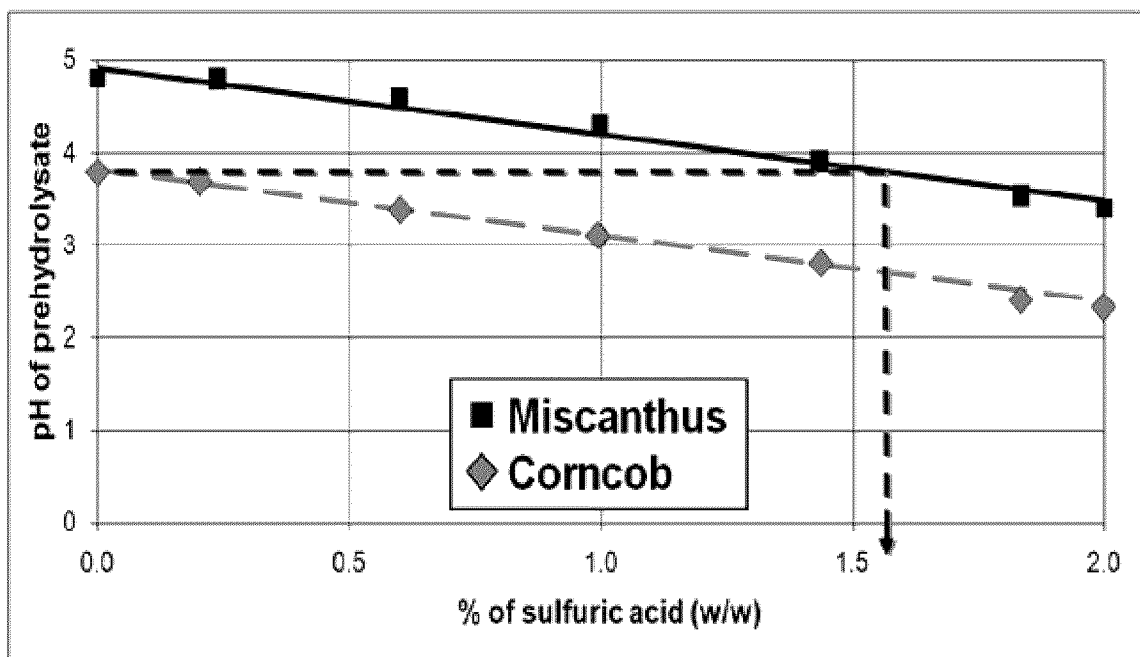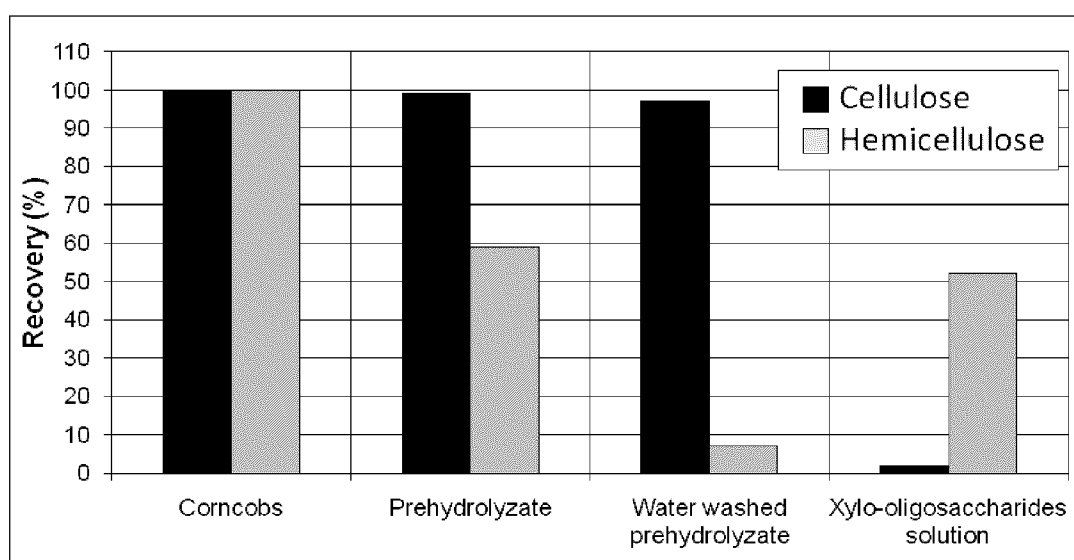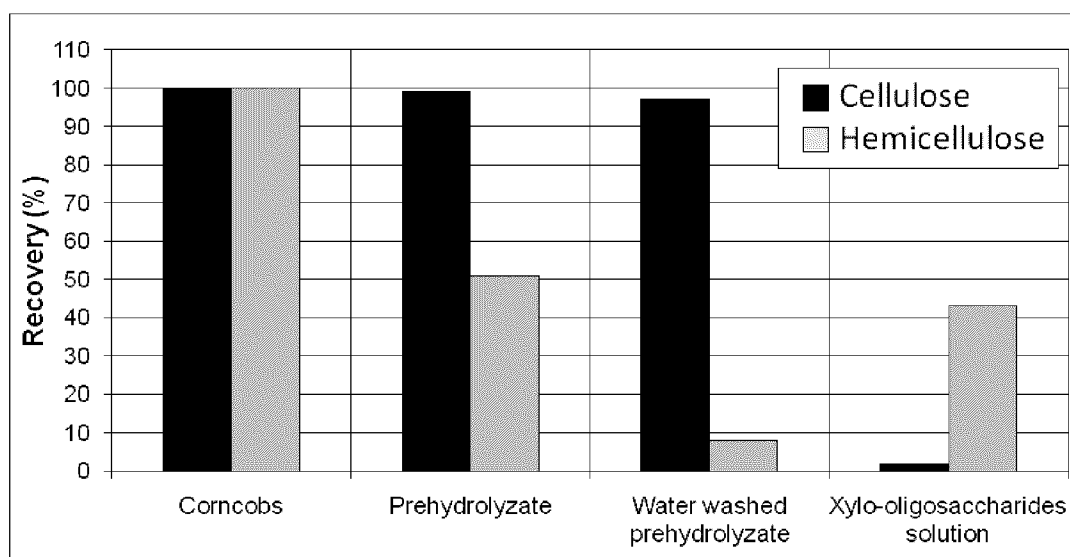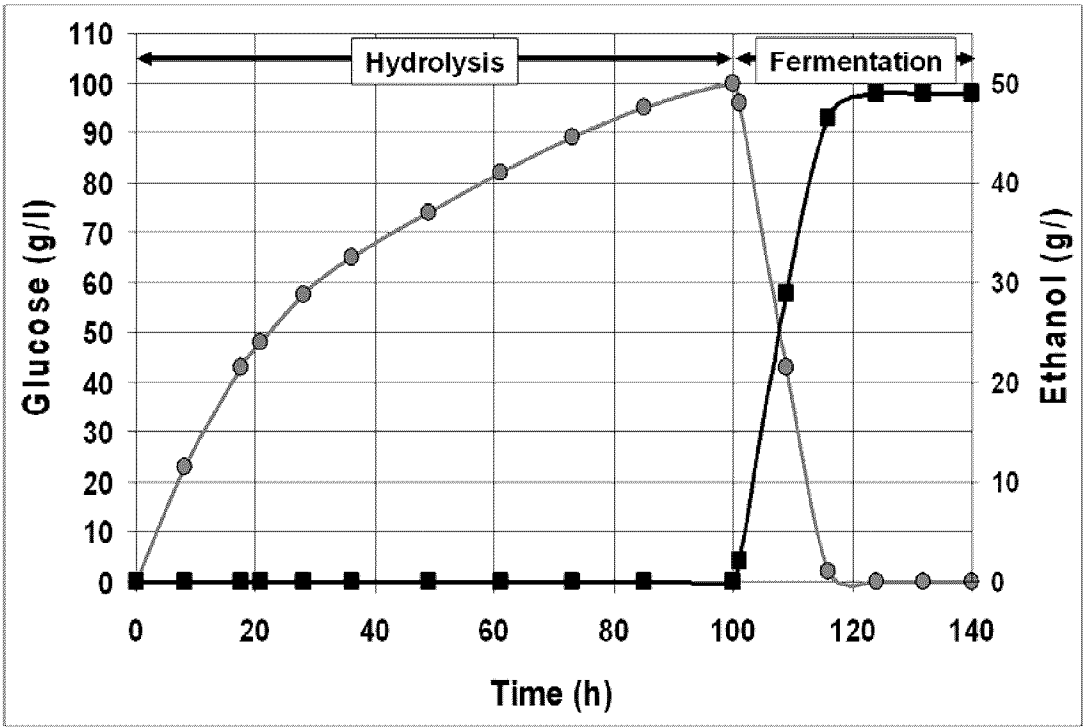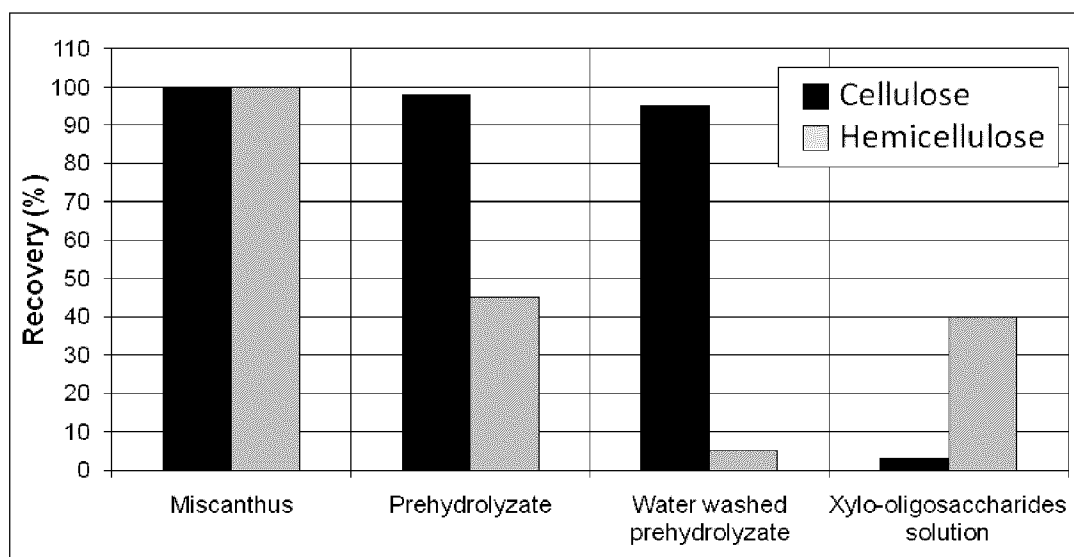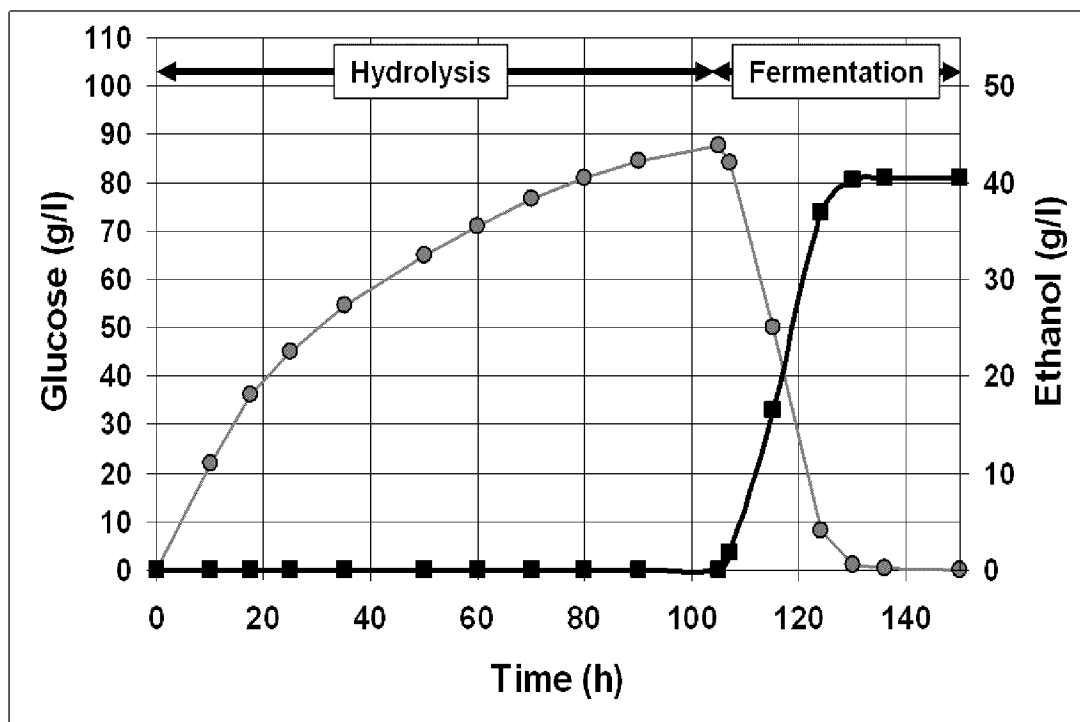
Figure 6

Figure 7

Figure 8

Figure 9

Figure 10

Figure 11

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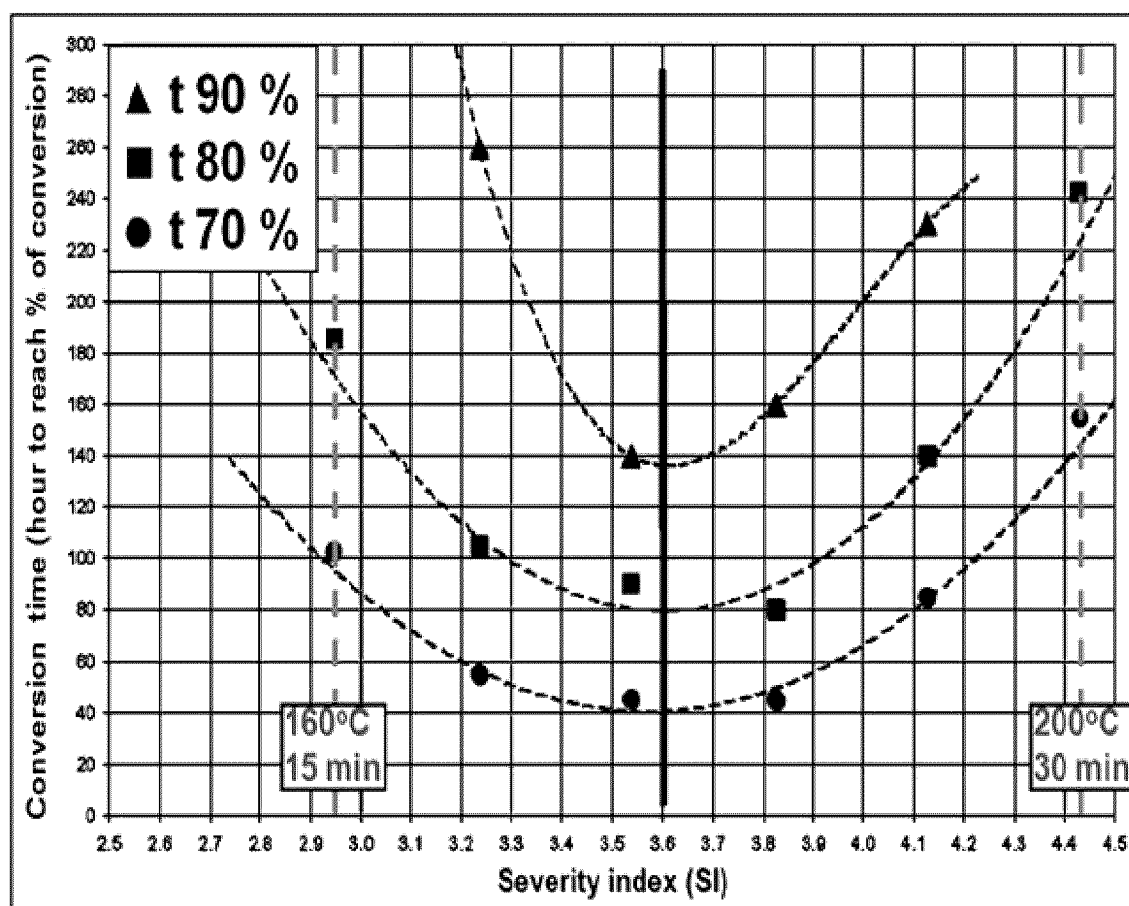
FIGURE 12

A.   CLASSIFICATION OF SUBJECT MATTER
       IPC: *C08H 8/00* (2010.01) , *B01D 36/00* (2006.01)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
       IPC: *C08H 8/00* (2010.01) , *B01D 36/00* (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)
Canadian Patent Database, Epoque (epodoc and cl txten databases), WEST, Scopus

Keywords: lignin, lignocellulosic, cellulose, hemicellulose, steam, explosion, explosive, decompression, biomass, purge, vent, venting, wash, washing, extract, extraction, separate, separation, xylose, hydrolyze

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CA 2 685 177 (South et al.) 13 November 2008 (13-11-2008) entire document	1-43
A,P	CA 2 678 976 (Benson et al.) 17 March 2010 (17-03-2010) entire document	1-43
A	CA 2 615 844 (Holm et al.) 25 January 2007 (25-01-2007) entire document	1-43
A	US 4,461,648 (Foody) 24-July-1984 (24-07-1984) entire document	1-43

[ ] Further documents are listed in the continuation of Box C.	[X] See patent family annex.

*	Special categories of cited documents :	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier application or patent but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 June 2010 (17-06-2010)	8 July 2010 (08-07-2010)

Name and mailing address of the ISA/CA	Authorized officer
Canadian Intellectual Property Office Place du Portage I, C114 - 1st Floor, Box PCT 50 Victoria Street Gatineau, Quebec K1A 0C9 Facsimile No.: 001-819-953-2476	Pierre Tessier (819) 934-0073

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