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Abstract:

The patent refers to the field of 'polysaccharides; derivatives thereof'. A method for treating biomass was developed that uses an apparatus which moves a biomass and dilute aqueous ammonia mixture through reaction chambers without compaction. The apparatus moves the biomass using a non-compressing piston. The resulting treated biomass is saccharified to produce fermentable sugars.

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Description:

[0001] Government rights statement

[0002] The invention support by the United States Government, Department of energy contract number of the signing 04-03-CA-70224 and DE-FC36-03GO13146 constraint. The Government of the United States have an inevitable right of this invention.

[0003] Invention field

[0004] Provides a method for the treatment of a biomass, the method includes a kind of the designated equipment. The method using the apparatus in the biomass non-compaction state moves into and through the reactor, the temperature and pressure in the appropriate implementation with dilute ammonia water impregnated biomass and the processing method for the reaction of the same.

[0005] Background Art

[0006] Fibrous and wooden cellulosic raw materials and agricultural residues such as garbage, wood, forestry refuse, the sludge from the papermaking industry, and municipal and industrial solid waste, provides tremendous potential renewable raw materials, used for production of valuable products, such as fuel and other chemical products. By the carbohydrate polymer (including cellulose, hemicellulose, dextran and lignin) of a fiber of the fiber and the garbage and for the feeding of various chemical, mechanical and enzymatic methods for processing in order to release the main to hexose and pentaglucose, they then can carry out the fermentation in order to produce useful products.

[0007] First of all, the biomass material to perform processing in order to prepare SACCHARIDASE more easy to use fibrous and wood fibrous material of the carbohydrate polymer, the process is commonly referred to as pre-treatment. Then the pre-treatment of biomass under the conditions in the presence of glucoamylase of further hydrolysis in the hydrolysis in the product to release oligose and/or monosaccharide. For from the pretreatment of the biomass in the fermentation of sugar production SACCHARIDASE usually includes one or more a glycosidase such as hydrolysis of cellulose glycocidase, hydrolyze hemicellulose glycocidase, and starch hydrolysis glycocidase, and peptidase, lipase, lignin enzyme and/or ferulic acid esterase. The saccharifying enzyme used for biomass processing and method refer to Lynd, L.R. People (Microbiol.Mol.Biol.Rev. (2002) 66 : 506-577).

[0008] Expectations for cost-effective large-scale processing biomass system and/or method. Processing the biomass needs to be a high concentration of heavy material in order to produce the needed high concentration can be fermented sugar, the fermentable sugar used in the fermentation into the product. Therefore, including the biomass part high dry weight move through the reactor, while at the same time maintaining through the handling of chemicals used for preparing capacity and optimizing the capacity of the saccharification of biomass, in addition to making use of a minimum number of chemicals and energy input to implement material mobile, this is a method of treating biomass one of the challenges. Also wish to include the low cost of capital equipment method. Does not need mixing or reactor of the reactor of the method can provide a lower cost of capital equipment and a lower energy input.

[0009] Has been described that does not require mixing or reactor rotating system and for moving biomass through the reactor the specific equipment. US4186658 discloses used for conveying equipment of the particulate matter, said particulate matter such as wood chips, straw, bagasse, and other fibrous material, said substance that this apparatus will be pressed into a solid \"block\" shape. Screw conveyor for the material to pre-load, and by reciprocating piston further compaction. Compacted mass is very compact so that it can effectively prevent blow back in the system. Then the block to the expected can be used for material processing in the apparatus. Biomass material dense block will not be able to make the pre-treatment reactant to achieve the best penetration.

[0010] Similarly, US4136207 discloses used for preparing and having improve ruminant oxybenzoates method of the cellulose material, the material begins to the compaction states from the machinery. Then it does not exist under the condition of the chemical reagent through the high-pressure steam treatment, and further compacting to form a biomass solid block, the escape through the inlet to prevent steam. Small part of the material and then is discharged, the pressure is reduced rapidly. The biomass is pressed into block would not be used in the pretreatment of the chemical reagent to achieve the best penetration.

[0011] US6176176 disclosed for processing equipment of cellulose material, the apparatus used in the extrusion machine cylinder rotatable screw. In the pressurized liquid ammonia is put in the cylinder, and the fibrous material mixed in the drum, and then the cylinder head is by a durable, liquid ammonia into a gas, so that the wood fiber material of the chemical explosive expansion. In large-scale commercial use in the method of the extrusion machine is very expensive, therefore cannot provide economical method.

[0012] In shared and common NA11/402757 pending US disclosed in in for treatment of biomass fermentation of sugar production method, the method using low intensity biomass pre-treating high concentration of ammonia water.

[0013] Therefore need to be used for the treatment of biomass system and/or method, the trimming respawn substance moving the reactor by low-cost, at the same time with maximum penetrability chemical reactant to prepare the biomass for saccharification.

[0014] Invention overview

[0015] The present invention provides for a method of treatment of biomass before saccharification, through the method of the present invention pre-processing biomass, comprising subsequently and through saccharifications the pre-treatment of biomass can be prepared by the hydrolysis of the sugar product. In one aspect, a method for the treatment of a biomass, the method comprises:

[0016] A) providing biomass;

[0017] B) use the feeding machine non-compaction (a) in the biomass into the apparatus, the apparatus including:

[0018] I) cylindrical drum, the cylindrical drum has a is equipped with the piston of the end of the 1st and 2nd is equipped with the tail end of the discharging valve;

[0019] Ii) optionally a branch pipe, said branch pipe in a branch pipe is connected with the end of the tail end of the 1st close to the cylindrical cylinder on the cylindrical cylinder, and in the said branch pipe of the branch pipe is not connected with the end of the valve can be sealed;

[0020] Iii) cylindrical cylinder or at least of the branch pipe 2 a the port can be sealed;

[0021] Iv) optionally a valve, said valve in the cylindrical cylinder of the cylinder is divided into separate chamber and the 1st 2nd chamber, the 1st chamber having is equipped with the piston of a cylinder the end of the 1st, and the 2nd chamber having is equipped with a discharge valve of a cylinder the end of the 2nd; and

[0022] V) is connected to the cylinder on the end of the 2nd discharge valve of a flash drum;

[0023] Wherein the biomass into the cylindrical cylinder or optionally into the is connected to the cylindrical of the cylinder in the tube states ;

[0024] C) closing the cylindrical cylinder and branch line (if any);

[0025] D) optionally via a cylindrical in the cylinder apply a vacuum to the at least one port;

[0026] E) a cylindrical drum or middle of the branch pipe of the at least one port by adding containing an aqueous ammonia solution, with respect to the cylinder is added in an amount of the dry weight of the biomass in less than about 12 weight percent, so as to generate biomass and ammonia-water mixture, in addition the relative to the dry weight of the biomass, said biomass with the weight of the ammonia-water mixture is at least about 15 weight percent of high solid concentration, and through the cylindrical cylinder or a branch pipe (if any) to the 2nd port of the steam, in order to make the temperature in the cylinder is between about to reach the 85 [...] and about 180 the between [...].

[0027] F) closing the cylindrical cylinder and branch line (if any) to provide the ports in the non-permeable chamber;

[0028] G) in said impermeable chamber said biomass with an ammonia-water mixture is maintained at the suitable temperature for a period of time under, the time is between about 30 seconds and about 4 hours;

[0029] H) optionally the displacement of the piston through the said biomass with an ammonia-water mixture in the cylinder to the cylindrical moving 2nd chamber in (if they exist), wherein the compacted material can not be stated, and maintain between about 2 minutes and 4 hours for a period of time between the; and

[0030] I) through the piston will move said biomass with an ammonia-water mixture through (g) or (h) of the impermeable cylindrical cylinder and through the discharge valve into the flash tank;

[0031] Wherein the production of the biomass treated.

[0032] In another aspect, a method for the treatment of a biomass, the method comprises:

[0033] A) providing a mixture of biomass and containing an aqueous ammonia solution, wherein, relative to the dry weight of the biomass of the gross weight of biomass and ammonia-water mixture is at least about 15 weight percent, and the amount of ammonia

relative to the dry weight of the biomass, less than about 12 weight percent;

[0034] B) use the feeding machine non-compaction (a) the biomass with an ammonia-water mixture into the apparatus, the apparatus including:

[0035] I) cylindrical cylinder, the cylinder is equipped with the piston of the end of the 1st and 2nd is equipped with the tail end of the discharging valve;

[0036] Ii) optionally a branch pipe, said branch pipe in a branch pipe is connected with the end of the tail end of the 1st close to the cylindrical cylinder on the cylindrical cylinder, and in the said branch pipe of the branch pipe is not connected with the end of the valve can be sealed;

[0037] Iii) cylindrical cylinder or at least of the branch pipe 2 a the port can be sealed;

[0038] Iv) valve, the valve in the cylindrical cylinder of the cylinder is divided into separate 2nd 1st chamber and the chamber, the chamber having 1st the 1st with the tail end of the piston in the cylinder, and the 2nd chamber having is equipped with a discharge valve of a cylinder the end of the 2nd; and

[0039] V) is connected to the cylinder on the end of the 2nd discharge valve of a flash drum;

[0040] Wherein the cylindrical drum of the biomass into the 1st chamber or optionally into the is connected to the cylindrical of the cylinder in the tube states ;

[0041] C) closing said 1st in the cylinder chamber and the branch pipe (if any);

[0042] D) optionally at least one port through the vacuum is applied;

[0043] E) or the branch pipe through the 1st chamber in at least one of the 1st port (if existing) adding steam in order to make the indoor temperature reach the is between about 85 the and about [...] the 180 between [...] ;

[0044] F) closing the 1st chamber and the branch pipe (if existing) to provide the ports in the 1st chamber can not be penetration;

[0045] G) in said impermeable 1st chamber in said biomass with an ammonia-water mixture will be kept in the appropriate temperature for a period of time, the time is between about 30 seconds and about 4 hours;

[0046] H) optionally, the displacement of the piston through said biomass with an ammonia-water mixture through the opening of the valve can be moved through the infiltration of the 1st chamber into the cylindrical in the cylinder in the 2nd chamber, wherein the compacted material can not be stated,

[0047] I) optionally, the opening or closing of the valve in order to form the 2nd-impermeable chamber and keep the biomass and ammonia-water mixture is between about 2 minutes and about 4 hours for a period of time between the; and

[0048] J) in step (g) or the step (i) through the displacement of the piston after said biomass with an ammonia-water mixture moving through the discharge valve into the flash tank;

[0049] The compacted material can not be stated and so as to produce the treated biomass.

[0050] Additional aspects of the present invention has been in accordance with the method of the present invention processing biomass, comprising through saccharifications biomass and of a hydrolysis product of fermentable sugar, the biomass has been through the method of this invention for the processing.

[0051] Biomass means any fibrous and/or wooden cellulosic material, can include biological energy crops, agricultural residue, municipal solid refuse, industrial solid waste, yard refuse, wood and forestry refuse or their combination. In order to reduce the size, increasing the surface area of the exposed, and/or increasing biomass in present in the cellulose, hemicellulose and/or oligose availability, can be in the step (a) before the application of energy to the biomass.

[0052] Description of drawings

[0053] Figure 1 is a schematic diagram of the apparatus used in the present invention one of the embodiments.

[0054] Figure 2 is a schematic diagram of the apparatus used in the present invention the 2nd embodiment.

[0055] Figure 3 is a schematic diagram of a discharge valve of the gradual expansion of the venturi tube an embodiment, the valve is closed.

[0056] Figure 4 is a schematic diagram of Figure 3 the gradual expansion of the embodiments of of the venturi tube, said valve opening.

[0057] Figure 5 is a schematic diagram of V-type port valve of the gradual expansion of the venturi tube an embodiment.

[0058] Figure 6 is a schematic diagram of type swing check valve of the gradual expansion of the venturi tube an embodiment, the valve in the closed in A, B in the opening.

[0059] Invention details

[0060] The applicant in the reference literature all the integrity of the reference content in the introduction of the disclosure. Furthermore, when the quantity, concentration, or other value or parameter in order to range, preferred range or, preferably, the upper limit value and lower limit value when the tabular form, should be understood as the same by any the high range limit or preferred value and any lower limit or range of any of the preferred values of all the range on the form, and no matter the stated range are individually open. Where the given herein is a numerical value range, the range is intended to include its end point, is located in the and all within the scope of an integer and a fractional, unless otherwise noted. When defining a range, not intended to be limited in the scope of this invention specific values of the listed.

[0061] The present invention provides for processing biomass in order to make it pass saccharifications to the production method for the fermentation of sugar. In order to easily can be through the production of valuable products, such as fuel and other chemical

products. Through the pre-treatment, saccharification and fermentation step, can be the use of renewable biomass (including waste biomass) to production of valuable chemical products, the chemical product demand of the oil can be reduced.

[0062] Definition:

[0063] The use of benzene in many terms. The following definition is given.

[0064] \"Biomass\" means any cellulosic or wood cellulosic material, including a cellulosic material, and optionally further comprising hemicellulose, lignin, starch, oligosaccharide and/or monosaccharide material. Biomass may also include additional components, such as proteins and/or lipid. As stated in this invention, the biomass can be derived from the single source, or derived from biomass may include a mixture of more than one kind of sources; such as biomass may include corn cobs and corn stalk or of fibrous mixture, or a mixture of grass and leaves. Biomass including, but not limited to, biological energy crops, agricultural residue, municipal solid refuse, industrial solid refuse, the sludge from the papermaking industry, garden refuse, wood and forestry refuse. Biomass examples include, but are not limited to, corn, corn cobs, crop residues such as corn shell, corn straw, corn fiber, grass, wheat, wheat straw, hay, stalk, switchgrass, waste paper, bagasse, sorghum, soybean shell or stalk , obtained from cereal, tree, branch, root, leaf, wood chips, sawdust, shrubs and underbrush, vegetables, fruit, flower and the ruminant animal manure component of the article. In one embodiment, the biomass used in the present invention includes a carbohydrate value relatively high biomass, their relative-intensive, and/or is relatively easy to collect, transport, storage and/or processing. In the present invention in one embodiment, the available biomass includes corn cobs, corn straw, corn fiber and bagasse.

[0065] The term \"fermentable sugar\" or \"sugar\" means into a target fermentation can be readily of the monosaccharide oligose and chemical products.

[0066] The term \"wood fiber\" means a material containing lignin and cellulose. Wooden cellulosic material can also include the hemicellulose.

[0067] The term \"fibrous\" means a material containing cellulose.

[0068] The term \"glycosylated\" from the polysaccharide in production can be fermented sugar.

[0069] Biomass removed \"dry weight\" means all or substantially all the weight of the biomass after the water content. In accordance with the normally dry weight of the test materials (ASTM) standards association E1756-01 (Standard TestMethod for Determination Biomass Solids Total of in) or paper pulp and paper-making industry and technology association, the standard Inc. (TAPPI) T-412om -02 (Moisturein Pulp, and Paper Paperboard) is measured.

[0070] The \"containing an aqueous ammonia solution\" means in an aqueous medium using ammonia (NH_3), comprising ammonium ion compound (NH_4^+) such as ammonium hydroxide or ammonium sulphate, when the degradation to release ammonia compound such as urea, and combinations of them.

[0071] The term \"treatment\" means the process of the reactant material, wherein the material changes the physical and/or chemical nature.

[0072] The term \"reactant\" be used in the processing method to change the target material under the condition of the physical and/or chemical nature of the composition.

[0073] For saccharification can be \" the enzyme gathers the living body \" in order to produce the biomass mixture of fermentable sugar combination of enzymes. Usually saccharifications the enzyme gathers the living body can include one or more glycocidase, the glycosidase can be selected from hydrolysis of cellulose glycocidase, hydrolyze hemicellulose glycocidase and starch hydrolysis xylosides enzymatics. Saccharifications the enzyme gathers lives of the other pulley body including peptidase, lipase, lignin enzyme and the ferulic acid esterase.

[0074] The terminology of the about the biomass \"processing\" or \"pre-processing\" associated with the following method. With the reactant to processing biomass in order to form the treated biomass product, which can also be referred to as processing in order to form the pre-treatment of biomass in order to form or pre-pre-processing of the biomass. The use of \"pre\" is used to distinguish the saccharifications biomass prior to biomass processing of.

[0075] Method of treating biomass

[0076] In shared and co-pending US Patent application # 11/402757 NA disclosed in in for treatment of biomass fermentation of sugar production method, the method including the use of low-strength ammonia water to pre-treating high concentration of biomass. The applicant has already developed with low intensity, ammonia and high of the substance concentration conditions of the new method for the effective treatment of biomass. The applicants have discovered, the method of the present invention surprisingly very successful, this is because to avoid compaction of biomass at any stage, and therefore in the biomass compaction in the system with processing the reactant to improve the penetrability of the raw material. In the in the system of the compacting biomass, biomass compaction state can be eliminated, improving the reaction of the reactant with the processing thereof, but this requires high energy input and thus improves the cost of the system. In the method of the present invention, does not need to remove compacted state steps or method.

[0077] In order to reduce the cost of processing the raw material of a large scale, have developed the method of the present invention, wherein the adding of the biomass non-compaction state in the fixed equipment, and will be in the same state non-compaction moving through the apparatus. non-compaction state by keeping the biomass, biomass material not be crushed natural small hole and the channel. In the method of the present invention comprises processing the reactant for use in ammonia and steam. These reactant non-compaction natural biomass capable of passing through the small hole and the channel, the fiber of the raw material and the wood fiber material provides rapid full effect. The processing method for processing high-efficient production of biomass, said biomass after effective saccharifications in order to produce the fermentable sugar, and therefore the units of each enzyme dosage to the reaction time of the biomass carbohydrate to solution high conversion rate.

[0078] Reference to Figure 1 and Figure 2 can be preferably a schematic diagram of geographical xie Ben method of treating biomass of the invention, the piston/cylinder apparatus for displaying a schematic diagram of the two embodiments, and the following in the treatment process of this invention in the description of using the device. For graphic clarity, these Figures are simplified, wherein some components omitted as shown in Figure 3 and Figure 4 the flange in. Figure 1 is the apparatus in the test-scale reactor. It comprises a

horizontal cylindrical shape room (10), with an open states the cylinder room the end of the 1st order to be used for adding biomass (11), after adding biomass by inserting the movable plug (12) to seal it, the movable plug used for a certain type of piston. Cylindrical chamber having 1st can seal port (13) in order to be used for adding an aqueous ammonia solution, 2nd port can be sealed (14) in order to be used for the cylindrical steam to the biomass shape room , the port can be sealed and 3rd (15) in order to be used for applying a vacuum. Steam is injected to increase the temperature of the biomass and ammonia-water mixture in order to be used for the treatment reaction. Heat insulation jacket (16) covering the cylindrical shape room.

[0079] In biomass, applied vacuum, and containing ammonia water solution and steam after joining, sealing port (13, 14, and 15) and is kept at the required temperature. After a certain period of time, by moving the valve shaft (19) of the cylinder the end of the 2nd cylindrical opening (18) of the previous in closed discharge valve (17). Valve shaft extends through the adjacent the flash drum (21) separating the downward in the inside of the bent tube (20) in the hole, the distal flash drum and passes through the packing gland (22) until the actuator (23). Through the cylindrical plug of the cylinder at the end of the 1st 1st end of the mobile, the biomass and ammonia mixture pushes through the discharge valve (17). Biomass through the discharge valve and through the bent pipe (20) into the flash drum (21) in. The upper cover of the opening at the bottom of the flash drum (24) allow to lead to pre-processing of the biomass. The port at the top of the flash drum (25) allow steam to escape, and through the pipe (26) is connected to the condenser (27) upper.

[0080] The invention embodiment of fig. 1 shown in its in the present invention apparatus and method for use in the treatment of the embodiment of described further below. The reactor type piston of the piston equipped with a horizontal orientation 5.1 cm × 68.6 cm stainless steel cylinder. Four O-ring for sealing the piston to the cylinder, the piston and the back of the discharge period the piston is pressurized with nitrogen (the most sastrowardoyo 5600kPa). 68.6 cm cylinder equipped with eight multi-purpose port, along the top surface and a bottom surface 4 a, they allow for vacuum, ammonia injection, steam injection, and is inserted into the method to measure the temperature of the thermocouple. Reactor-cylinder is equipped with a steam jacket is used for uniform heating of the cylinder. The reactor-cylinder directly connected to the vertically-oriented 15.2 cm × 61 cm stainless steel of the flash drum. The end of through the tapered nozzle and base shear valve arrangement of the cylinder is

separated from the flash evaporation groove. The diameter of the cutting die head end valve is 3.5 cm. Tapered nozzle and is adjustable on the base of the back pressure of the, most testing use -138kPa (measuring pressure) to the back pressure, make it enter 10.2 cm diameter in the air of the cylinder, the cylinder and the end of the conical nozzle is connected to the shear valve. Shearing of the valve the end of the tapered spout can be retracted a maximum of 1.6 cm, allowing the discharge of the flash tank. Shear valve outlet in the end of the pipe to guide the treatment of solid downwardly into the bottom of the flash drum, wherein the solid can be through hits the slot easily remove the dome at the bottom of the bolt. The upper part of the flash drum to a steam chest flange is processed into the flash drum with the axis of the slot is at right angle to the special outlet, this causes the release of the steam along the corner path in the discharge device, helps to prevent the generation and water droplets left over from the biomass particles into the exhaust gas in the condenser. Along the flash drum three strip-like electric heater (as the 60 [...]) and insulation, so that the heat treatment of solid shem steams in the heating container, better method for the analog commercial scale.

[0081] In another embodiment, small type piston reactor constructed as described above, besides 45.7 cm outside the cylinder, no steam jacket, the three strip-like electric heater, is covered with a silicone-filled glass fiber jacket of 2.5 cm thick as a heat-insulation glass fiber mat, and three multi-purpose port. Other part includes the large cylinder-piston reactor used in a flash drum, shear valve, and the bent tube.

[0082] Figure 2 the device is shown in the reactor design of a commercial scale. It includes in the end of the 1st (33) fitted with a piston (34) and the end of the 2nd (41) fitted with a discharge valve (40) of the horizontal cylindrical cylinder. The heat insulation treatment of the cylinder and has a non-permeable wall. The branch pipes (31) is connected to the 1st end close to, and the valve (35) is positioned in the feed valve is not connected to the end of the branch pipe. The hopper (30) is connected to the branch pipe of the tail end of the valve. Biomass is added through the hopper. non-compaction can be used to control the flow-guiding device from the hopper (30) the biomass is added to the branch pipe (31) in. Branch line has 1st sealing port (36) and 2nd seal port (37), they are used for when the ammonia water and steam moving when to the cylindrical cylinder, to the biomass in the branch pipe. 2nd valve (38) is divided into cylindrical 1st shape room the cylinder (32) and the 2nd cylinder shape room (39). Biomass and ammonia-water mixture through the branch pipe into the 1st

chamber, wherein by adding the steam reach the required temperature and pressure. The piston is moved through the non-permeable cylinder to promote biomass and ammonia-water mixture from chamber through opening of the 1st 2nd valve (38) into the 2nd chamber, and the 2nd chamber (39) through the contents of the opened discharge valve (40) is transferred to the flash drum (42) in. 2nd chamber is previously moved to the chamber under the conditions of use and the required time of dealing with the reaction of the biomass and the ammonia-water mixture. Then turn off 2nd valve (38) and is pulled back to the piston (34) in order to 1st cylindrical shape room (32) to a loaded substance and repeat the cycle process. In flash drum (42) in, biomass move through the downward bent pipe (43). The upper cover of the opening at the bottom of the flash drum (44) allow to lead to pre-processing of the biomass. The port at the top of the flash drum (45) allows the ammonia steam from escaping, and through the pipe (46) is connected to the condenser (47) the upper.

[0083] Can use the carbon steel or stainless steel to manufacture the device. Cylindrical cylinder can be as shown in Figure 1 and 2 is horizontal the, or it may be a vertical type. As shown in Figure 2 with vertical of the cylinder of the branch pipe and the hopper will be adapted to allow the biomass into the cylinder chamber, such as at less than 90 degrees of angle. Those of skill in the art will be able to easily dispose the device with the vertical cylinder. For example, the vertical-type cylinder can be positioned in the flash drum and is connected with the above, the connection is not a downward bend, because flow through the discharge valve will have already the downwards. Determining flash tank is vertical or horizontal and technical personnel in this field within the range of the capacity. In the method of the present invention the vertical groove is more suitable for ammonia treatment, it is conducive to removing and trapping ammonia released by the flash tank.

[0084] Figure 1 and 2 two embodiments in the similar function, adding biomass and make the mobile through the reactor under non-compaction state. The plan with a chamber 1 of the embodiment is a one-time processing of the biomass sample batch system. Figure 2 with the embodiments of the two separately through the valve chamber, allows the semi-continuous or batch operation, the biomass is loaded at the same time by a plurality of times. In the 2nd embodiment, full load after 2nd chamber, wherein each of the continuous biomass loading into the 2nd chamber associated with each piston displacement cycle through the discharge orifice to discharge the corresponding volume. 2nd chamber for a certain period of time in the number of times the cycle of piston displacement, and hence the 2nd chamber size, with

each of the biomass sample related to the required dwell time. The dwell time in the following text with reference to the method of the invention processing temperature and time for further discussion.

[0085] The method of the present invention is particularly suitable for processing with respect to the treatment reaction of the biomass, ammonia and steam mixture of the weight of the higher biomass dry weight of the biomass. High expectations the treatment of heavy concentration of biomass in order to offer the saccharified hydrolysate concentration of sugar production of the biomass. The provision of such biomass the characteristics of the method of the invention it is not extruded, thus allowing the concentration of effective processing of high dry weight of the biomass. The method of this invention is used for the initial dry weight of the biomass, biomass and ammonia water of the total weight of the mixture of at least about 15%. More typical, biomass dry weight of at least about 20% and can be at least about 30%, 45%, 50%, or higher. The percentage dry weight of the biomass can be changed, and different types of biomass from the best percentage can be different. For example, when using the corn cobs, expect at least about 24% of the dry weight percentage, in order to offer saccharifications pretreatment of sufficient concentration to fermentation of biomass to produce the fermentable sugars to ethanol. More suitable is at least about 30% of the biomass of corn. The technology in the field of decision to high sugar hydrolysis product for the production method of the invention in a particular type of biomass in the preferred dry weight percentage.

[0086] The sources can be directly obtained from biomass, or the application of energy in order to reduce substantially the size of the, increase the surface area of the exposed, and/or increasing biomass in present in the cellulose, hemicellulose and/or the availability of the oligosaccharide. For this purpose the energy device, including those not be crushed biomass or the compacted apparatus, so as not to undermine the biomass ultrastruct. For example, can be cut, chopped, or truncating garrulous biomass. When the in order to not be crushed biomass way shear Ultramicro structure can also be used when the jaw crusher. In the method of the present invention pre-processing before the toothed disc type purifier can also be used to reduce the size of the biomass.

[0087] In the processing method of the invention used in the biomass feeding machine non-compaction mobile to the cylindrical cylinder. In the most simple case, the feeder means non-

compaction biomass packed into the cylindrical opening of the cylinder in the 1st end. If the there are two chambers in the cylinder, in the 1st chamber can be loaded. The method described in the embodiment herein, the use of as shown in Figure 1 of the reactor. In Figure 2 of the reactor is shown in hopper feeder non-compaction. Hopper can be a self-unloading, and/or can be equipped with the do not provide pressure strength of the flow-guiding device. May, for example, use a plurality of types of live bottom box guide wheel, followed by flow metering conveyor such as various types of drag chain, chain bucket elevator, or spiral rotary conveyor (such as the Acrison Device). In 1st into the cylindrical chamber the amount of biomass is limited, in order to allow to allow biomass expansion space, the expansion may be added to the ammonia and steam.

[0088] Vacuum can be added to comprising the cylindrical cylinder of the biomass. If the there are two chambers in the cylinder, the vacuum can be applied to the 1st chamber containing biomass. Usually if a vacuum is applied, the pressure-reduced to less than approximately 20kPa. In the branch pipe through the cylindrical cylinder or the one or more ports to an aqueous ammonia solution containing, the added in an amount of ammonia relative to the chamber on a dry weight of the biomass, less than about 12 weight percent. Good distribution suitable for using more than one port for ammonia solution is substantially evenly distributed to contact with the biomass. If the there are two chambers in the cylinder, the ammonia solution is added containing the biomass in the 1st chamber. More appropriate the amount of addition of ammonia relative to the chamber can also be dry weight of the biomass is between about 4% and about 6% between. Can be preheated ammonia solution, this will contribute to increased temperature of the biomass. In an alternative embodiment, cylindrical 1st shape room the ammonia is mixed with the biomass before. Biomass and ammonia water can be mixed in the container into the cylindrical chamber 1st. For example, ammonia and water pump inlet can be through a built-in heater, then enter in paddle type stirrer containing biomass. With ammonia-water mixture is then loaded into the biomass 1st in the cylindrical chamber, wherein the steam is injected after closure room. As the other option, can be premixed biomass, ammonia, and the steam and into the cylindrical chamber 1st. Below under the temperature and pressure of the, many ammonia will evaporate into a steam, vapor permeation in the pretreated biomass. Furthermore, since the collection can be flash tank of the circulation wet-ammonia steam injection in order to form a part of the total adding ammonia.

[0089] In the method of the present invention, containing an aqueous ammonia solution can also optionally include at least one additional alkali, such as sodium hydroxide, sodium carbonate, potassium hydroxide, and potassium carbonate. Can be added to the at least one additional alkali, to the biomass dry weight added in an amount of up to 10 weight percent. For example can be by using additional alkali to biomass in and of the acid, so as to provide metal ion in order to be used for glucoamylase or the fermentation medium.

[0090] Because in the method of the present invention raw material is not compacted, it will not block the steam channel, biomass compaction in use in the system of the steam channel will be blocked. So joining steam chamber closed before the steam is injected. Sealing in addition to one or more of the steam to the port of the other port. The cylinder as the piston or at the end of the 1st of the piston and close the valve plug in position. The valve can be any opening or closing valve, such as poppet valve or the rotary knife gate valve.

[0091] In the cylinder through one or more of the valve or the steam to the branch pipe, needs to be added can improve the steam quantity of biomass and ammonia-water mixture temperature to the required temperature point. If the there are two chambers in the cylinder, the steam to the chamber containing the biomass in 1st. More suitable is the use of a more than one port, and inter-port have a certain distance, in order to steam distribution to the biomass in contact with it. Adding steam in order to make the biomass and ammonia temperature of the mixture is increased to between 85 the and about [...] the 180 between [...]. If required, can be through 2nd cylindrical shape room (if existing) adding additional the ports in the steam in order to keep the required temperature. The apparatus may include a heating jacket, steam jacket, heater, or heat insulation jacket to facilitate to improve and/or maintain a temperature. Heating jacket or steam jacket is especially suitable for small-scale reactor, and heat-insulating jacket is suitable for large-scale reactor. Heating can be in different stages, including the preheated before the treatment or pretreatment of the cylinder.

[0092] Below 85 the temperature of [...] , ammonia water of low intensity for the required time of processing will be very long. The processing time required to reduce the increase of temperature. For example, in 85 the under [...] may be required for the processing time is between about two hours and about four hours, in 180 the processing may be carried out under [...] only a few minutes. In Figure 2 the reactor in batch feeding cycle function of the

need to have sufficient time to carry out a plurality of times of feeding. The thus selected for a time limited time and temperature combination, the combined time enough to function the embodiment of the reactor, and the moderate temperature can provide economical method. In the moderate temperature lower, low cost can be used of the low-pressure steam. The condition is more appropriate is between about 120 the [...] and about 160 the temperature processing of [...] is between about 60 minutes and about 5 min, time is reduced along with the increase of the temperature. In particular, the appropriate conditions is between about 140 the [...] and about 150 the temperature processing of [...] is between about 30 minutes and about 10 min, time is reduced along with the increase of the temperature. To the type of pre-processing of biomass can also influence processing in the method of the present invention the best time and temperature, those of skill in the art can easily evaluate it.

[0093] The biomass in the reactor room temperature to maintain a required dwell time is the time. When using only the 1st chamber of the reactor, the residence time in the 1st chamber. When using has the 2nd 1st chamber and the reactor chamber, the time of the 1st chamber is sufficient to only in the 2nd chamber moves to the mixture of the biomass and reactant combined before, the dwell time in the 2nd chamber. In this case, the time of the 1st chamber can be about 30 seconds, the time of 2nd chamber can be between about 2 minutes and 4 hours.

[0094] In the method of the present invention using steam to reach the temperature of the biomass in the reactor room pressure is between about 60kPa and about 750kPa between. More typical, pressure is between about 300kPA and 600kPA between. With respect to the other known pre-treatment method such as US 5037663 the AFEX method, wherein use 1150kPa to 4250kPa pressure, or US 4461648 the steam jet method, wherein the about 1800kPa to about 5600kPa pressure (such as this article chart 1 shown), these pressure to be relatively low pressure. Under the pressure of the more moderate operating the method of the present invention provides low cost system, which can use low pressure steam.

[0095] In the method of the present invention, the biomass non-compaction state the mobile through the 1st chamber and the 2nd chamber (if they exist). This can use the piston and non-permeable cylindrical shape room to complete. For the purposes of the present disclosure, may include any of the piston can be used as products of the piston, such as the plug is pushed into the chamber, and any type of standard piston. Can use any applied pressure

sufficient to move the biomass of the method, the chart 1 in the type of reactor of illustration the plug is pushed into the chamber. The method is in particular suitable for after the end of the plug inserted in the static closed provides the room , such as a cylinder head of the bolt, between the closed and the plug is then introduced into the nitrogen in order to accumulate pressure and moves the plug. The spigot can be through other device to move, such as the use of is connected to the hydraulic, pneumatic, or electric actuator with a push rod.

[0096] The cylinder of the apparatus is not permeable (close all ports and valve), wherein there is no sealing of the wall penetration, so the liquid from flowing out of the cylinder. Liquid retained in the piston under the condition of without compacting biomass moving them. In the processing method of this invention the liquid is limited, the chamber wall can be lubricated, the flow of non-compaction in response to the pressure of the piston. In fact, piston pressure can be temporarily slightly extruded biomass, like sponge, the biomass extrusion to the aperture and the degree of collapse of the channel. After piston pressure is removed, the biomass can be absorption liquid does not enter the pressed in the small hole and the channel. In order to help the biomass flow, such as the lubricating fluid can be room the soap leads in vegetable oil. The wall of the chamber can be made to improve the fluidity of the rifle, which is not continuous portion such as angled groove by friction can be reduced, thus reducing yield stress and improve the biomass flow. The biomass non-compaction maintains generation of the handling of the hole filled with a liquid, this will promote the subsequent saccharifications.

[0097] In the method of the present invention, the temperature processing required after the time required for, biomass and ammonia-water mixture will move through the cylindrical cylinder at the end of the discharge valve into the flash tank. Biomass in the required temperature during the reaction with ammonia water and closing the discharge valve, and then opens the discharge valve the biomass through. As shown in Figure 2, the piston in the 1st chamber after produced the pressure in, in order to replace the content of chamber 1st 2nd all contents of the room, in the reactor the two chambers and the discharge valve opening between the 1st and 2nd chamber at the same time the opening of valve.

[0098] Can be used for the discharge valve of the V-shaped port rotary valve, swing check valve, and lifting the discharge valve to instantiate. As shown in Figure 1, in the small-scale reactor is particularly useful in driving the piston is of the lift valve type discharge valve,

wherein the valve base is Hardside upstream side of the discharge hole, and the valve base relatively soft the downstream side sealing piece leans against the hard surface of the valve rod, when the valve rod is retracted to open the valve, valve base other than continued to increase flow area.

[0099] The most suitable lifting valve-typed discharge valve will be connected to a progressive expansion of the venturi tube a. Progressive expansion of the venturi tube a lift valve of the embodiment in Figure 3 shown in, the venturi tube as shown in Figure 1 the discharge valve which is applicable to small-sized reactor. This valve with the conical nozzle and shear valve arrangement of the base. In order to avoid clogging, the design as shown in Figure 3 (closed position) and Figure 4 (the open position) shown in the progressive expansion of the venturi tube in order to accelerate the solid through the venturi tube of the fixed outer cone (50) of the venturi tube and a movable inner taper (51) to expand smoothly between the gap, wherein the movable inner cone is arranged on a valve shaft (52) on the end of the. The outer cone of the venturi tube is inserted in at the reactor room (54 ; equivalent to Figure 1 of the in 10) outlet of the flange (53) and flash drum inlet flange (55) is generally between the annular venturi tube. Conical in the venturi tube (51) is in the reactor outlet valve shaft (52) at the end of the noses. Conical in the venturi tube and the valve shaft is located in the discharge elbow (56 ; equivalent to Figure 1 the in 20) internal, located in flash drum discharge elbow pipe (57 ; equivalent to Figure 1 the in 21) internal. The valve shaft is connected to the actuator (58) to control the mobile. The actuator can is able to move the valve shaft of the front and rear flat any device, such as electric, pneumatic or hydraulic motor, pneumatic valve actuator, or hydraulic piston. When the valve shaft in its farthest left position, the internal cone of the base against the outer edge of the outer cone to the inner edge of the seal the reactor during the processing period discharge end. When the discharge time of the reactor, to move to the right to provide flash vaporization Wen Qiu valve shaft in the opening of the pipe to a required size.

[0100] The design provides regional the flash evaporation of a certain length, in the direction of flow in the region expansion smoothly. In this design, progressive opening of the biomass solid along the axis of the annular conical nozzle accelerating, this avoids the sudden blockage of the expansion in the radial direction.

[0101] The progressive expansion of the venturi tube another embodiment in Figure 5 is

shown in, the venturi tube is suitable for use as a discharge valve, especially in as shown in Figure 2 used in a large-scale reactor. This is of a V-shaped port embodiment, wherein the flash expansion machine of the venturi tube in the valve body. flash vaporization Wen Qiu pipe in the fixed body (70) from the reaction chamber in (72) the narrow portions of the outlet end (71) and expanded to the flash drum (74) expanding part of the entry (73). The center of rotation of the cock (75) is the opening of the angle (76), which when in the position of the opening of the narrow portion with the reactor chamber (71) are aligned, and expanded to flash drum (73). The rotation centre (75) screw-on half-in order to prevent the alignment of the near the valve of the faucet.

[0102] Progressive expansion of the venturi tube of another embodiment in fig. 6 is shown in, the venturi tube is suitable for use as a discharge valve, especially in as shown in Figure 2 used in a large-scale reactor. This is the embodiment of the swing type check valve, it has a conical nozzle (80), is suitable for in the reactor room (72) and flash drum (74) between the entrance of a narrow joint (81) (Figure 6A). The conical nozzle is for connection to the shaft (83) of the arm (82) is, the shaft passes through the packing gland until the rotary valve actuator. The rotation direction of the shaft as shown by an arrow of dotted line in, the mobile arm clockwise to open the joint, form a progressive expansion of the venturi tube (Figure 6B). For the progressive expansion of the venturi tube of the swing check valve of another embodiment, the diameter of the cone-shaped nozzle can be a plurality of feet, the mobile a distance counter-clockwise in order to open only a few inches of the valve (less than 8 cm).

[0103] Biomass and ammonia mixture moving through the discharge valve into the flash tank, the flash tank will be able to maintain the vacuum. A flash tank in the ammonia released from the treatment of biomass and cooling biomass, to prepare for saccharification. Can use any typical flash drum, its has tangential or volute inlet, to provide the most suitable for the separation of the function of the pipe. Particularly suitable to flash several times are sequentially exerted on the different pressures, with the release of ammonia from the pretreated biomass. For example, the flash evaporation pressure 1st time close to atmospheric pressure, most of the free ammonia is usually removed and the material is cooled to about the 100 [...]. 2nd-time flash pressure is less than approximately 20kPa, remove the remaining free ammonia and the material is cooled to about the 50 [...] , the temperature is saccharifications the required temperature.

[0104] From the flash tank through the relief valve in the mixture of the biomass and ammonia of ammonia release from the flash tank steam can recovery, and can be recycled. From the low-pressure flash steam can be used without intermediate cooling standard steam re-compression equipment (such as a turbine or steam jet pump) recycling. Thus ammonia steam may not be condensed directly cycle is used for processing, or may be condensed prior to reuse. In the latter case, as shown in Figure 1 the steam is added to the collected in the condenser.

[0105] Reducing treatment of biomass will reduce the ammonia in acid consumption and to reduce the pH, the acid used for adjusting the pH of the saccharification enzyme activity to achieve the satisfactory level. This is it is desirable, because large can lead to the addition of an acid to form a salt, its concentration to glucoamylase or to inhibit microbial growth. On the other hand, residual biomass can be used as a nitrogen source, ammonia, in order to maintain the growth of the microorganism in the period of fermentation. Therefore, the remaining ammonia can reduce or eliminate nitrogen source for the fermentation during the need for supplementing culture medium. Removing at least a portion of the ammonia usually, this reduces the pH but leave some nitrogen, these the nitrogen uses in for subsequent fermentation to provide the kind of nutrient material.

[0106] When the pre-treatment of biomass in accumulated at the bottom of the flash drum, can be used for the agitator type, can be connected to the stirrer states the propellor type the bottom of the flash drum. hits the slot usually through the bottom of the flash drum a cover member is removed from the bottom of the pre-processing of the biomass. For continuous extraction of mechanical device overlaying the pre-processing of the biomass, is especially suitable. In order to in the apparatus of the present invention processing multi-batch of biomass, a batch of biomass and ammonia can be in-cylinder indoor, however another number of in the flash tank. In the two chamber device, the material can be several group at the same time in the two chambers is and flash evaporation. Furthermore, can be removed in a flash tank before the collection of multi-batch pre-processing of the biomass.

[0107] After treatment, the mixture of the product usually includes ammonia, biomass and partly degradation number of fermentable sugar. Can be removed from the flash tank comprises a soluble portion and a water insoluble part and all of the pre-treatment of biomass using them in saccharifications in the reaction. As the other option, before

saccharification can be the biomass mixture from the pre-processed row go some liquid in the saccharification reaction in order to maintain a high level of the biomass dry weight. After processing of the liquid there may be excessive, especially when the need for a large amount of steam and maintain the biomass processing is all the more so when the temperature of.

[0108] In another alternative embodiment, the biomass solid can be in the method of the present invention by processing the re-circulation.

[0109] Saccharification

[0110] The method of the present invention for processing biomass in the presence of glucoamylase (may be called saccharifications the enzyme gathers the living body) further to carry out hydrolysis under the condition of, in the product in order to release the hydrolysis of the oligosaccharide and/or monosaccharide. The saccharifying enzyme used for biomass processing and method refer to Lynd, L.R. People (Microbiol.Mol.Biol.Rev. (2002) 66 : 506-577).

[0111] Before in saccharification, can be treated by the pre-processing of the biomass in order to change the pH, composition or temperature make saccharifications the enzyme gathers lives body an active enzyme. Can be through adding solid or liquid form to change the pH of the acid. As the other option, can be utilized in the recovery of carbon dioxide from the fermentation (CO_2) to reduce pH. For example, if there is sufficient liquid, can be collected from the fermentation tank CO_2 and into the flash tank at the top of the pre-treated product of biomass through the pre-treatment space or bubble, monitored at the same time until the pH reaches the required pH. The mentioned temperature reaches below the temperature of the suitable for saccharification enzyme activity. In the saccharification process can be added to the enzymatic activity of any cofactors.

[0112] Saccharifications the enzyme gathers the living body include one or more enzyme, the enzyme is mainly selected from (but) \"glucosidase\" such, the enzyme hydrolysis Biose, monosaccharide, or polysaccharide ether linkage, exists in the generalized \"hydrolase\" (EC 3.) of the EC classified enzyme in 3.2.1. x (Nomenclature Enzyme 1992, Academic Press, San Diego, CA Supplement with 1 (1993), Supplement 2 (1994), Supplement 3 (1995, Supplement 4 (1997) andSupplement5 [in Eur.J.Biochem. (1994) 223 : 1-5, Eur.J.Biochem.

(1995) 232 : 1-6, Eur.J.Biochem. (1996) 237 : 1-5, Eur.J.Biochem. (1997) 250 : 1-6, and Eur.J.Biochem. (1999) 264 : 610-650 in]). In the method of the present invention can be a glucosidase can be used according to their classification biomass component of hydrolysis. The method of the present invention can be used a glucosidase including hydrolysis of cellulose glycocidase (for example, cellulase, endoglucanase, outer glucanase, cellobiohydrolase, β-glucosidase enzyme), hydrolyze hemicellulose glycocidase (for example, xylanases, inner xylanses, outside wood enzyme, β-wood gathers the glycosidase , arabinoses wood/enzyme, mannanase, galactase, pectase, glucuronic acid enzyme), and starch hydrolysis glycocidase (for example, amylase, α-amylase, β-amylase, glucoamylase, α-glucosidase, isoamylase). Furthermore, other active agents can be added to the saccharifications the enzyme gathers lives body, such as a peptidase (EC 3.4.x .y), lipase (EC 3.1.1. x and 3.1.4. x), (EC 1.11.1 .x) lignin enzyme , and isoferulate sour esterase (EC 3.1.1 . 73), from biomass in order to help release the polysaccharide in the other component. This field is well known, a microorganism producing polysaccharide hydrolase often show some activity, such as a cellulose degradation, the active is composed of a plurality of enzyme or a group of enzyme catalysis with different substrate specificities. Therefore, \"cellulase\" from microorganisms belonging to a, can help to all enzymes cellulose degradation activity. Purification of the enzyme depends on access programme, commercial or non-commercial enzyme preparation such as cellulase can include a variety of enzyme. Therefore, the method of the invention can include the enzyme gathers the living bodyenzyme activity medicinal preparation saccharification \"cellulase\", for example, however it is recognized that this activity can be more than one type of enzyme catalysis. SACCHARIDASE can be commercial acquisition, Spezyme such as is obtained from Cellulase CP (GenencorInternational, Rochester, NY) and Multifect Xylanses (Genencor). Furthermore, through the biological method to prepare SACCHARIDASE can, including the use of recombinant microorganisms method.

[0113] Those of skill in the art will know how to determine the use of an effective amount of enzyme the enzyme gathers lives body, how to adjust the conditions and in order to obtain the best enzyme activity. Those of skill in the art will also know how to optimize the the enzyme gathers lives body in the necessary activity of the enzyme, obtained under the conditions in the selection of a given pre-processing the best saccharifications effect of the product.

[0114] Preferably, saccharifications the reaction pH SACCHARIDASE the best temperature and under or close to the optimum pH and temperature conditions. In the method of this invention, saccharifications the enzyme gathers body using the best temperature in about 15 the [...] to about 100 the within the range of [...]. In another embodiment, at about the optimum temperature the 20 [...] to about 80 the within the range of [...]. The optimum pH can be between about 2 to about 11 within the range of. Another embodiment, in the method of this invention, saccharifications the enzyme gathers body to be the best at about pH 4 to about 10 in the range of.

[0115] Saccharifications can be about several minutes to about 120 hours, preferably from about several minutes to about 48 hours. The reaction time will depend on the organella enzyme density and, a substrate already in use and environmental conditions such as temperature and pH. Those of skill in the art to easily decide specific substrate and saccharifications the enzyme gathers the temperature of the organism used, the most optimal pH conditions and time.

[0116] Saccharifications can be in batches or in a continuous process. Saccharifications can also be one-step or multiple steps. For example, glycosylated the required different enzyme can exhibit different optimum pH or temperature. The available enzyme under a certain temperature and pH first processing, subsequent use different enzyme under different temperature and/or pH of the 2nd or 3rd time (or more times) processing. Furthermore, the continuous different enzyme steps for the treatment can be carried out in the same pH and/or temperature, or at different pH and temperature, used in, for example, a relatively high stable under pH and temperature and higher activity of the hemicellulase treatment, the pH and temperature at a relatively low activity, cellulase treatment.

[0117] Saccharifications after sugar of biomass can be by measuring the solubility of the release of monosaccharides and oligosaccharides for monitoring. Measuring monosaccharides and oligosaccharides method is known in the field. For example, the concentration of the reducing sugar can be used 1, 3-dinitro salicylic acid (DNS) detection analysis method (Miller, G.L. , Anal.Chem. (1959) 31 : 426-428) to be measured. As the other option, such as the general method described in part, by using a suitable HPLC column to measuring sugar.

[0118] Fermentation

[0119] Suitable microorganism can use biomass release fermentable sugar to produce the target chemical products. After the sugar, but before the fermentation, can be through, for example, evaporation can be concentrated saccharifications mixture in order to improve the concentration of the sugar. Optionally, the liquid in the saccharification product can be from a batch or continuous method for the separation of solid in. Optionally, liquid or all the saccharification product can be sterilized before fermentation. Fermentation period depends on the use of microorganisms and saccharifications use during pH, pH can be adjusted to a level suitable for fermentation. Furthermore, required by the growth of the microorganism can be used for the additional nutrient material to supplement saccharifications mixture. Supplementary agent can include, for example, yeast extract, specific amino acid, phosphate, nitrogen source, salt, and trace elements. Can also include through specific biological catalyst required to produce the component of the specific products, such as for retaining the plasmid to antibiotic or enzyme catalytic reaction the necessary cofactors. Can also include additional sugar in order to improve the total sugar concentration. Saccharification mixture can be used as the component of the fermentation broth, for example, preparation is between about 100% and about 10% of the final culture medium between.

[0120] Depending on the use condition of the fermenting microorganisms, can also adjust the temperature and/or the top of the gas space. Fermentation may be aerobic or anaerobic. The saccharification took place after fermentation can be, or can be through the synchronous saccharification and fermentation process the (SSF) with the glycosylated deuterostomia. SSF can causes the saccharification production of the sugar content is kept a low level, thus reducing potential SACCHARIDASE product inhibition, reduce the usability of the sugar contaminating microorganisms, pre-treatment of biomass and improve to monosaccharides and/or oligose conversion.

[0121] Can be prepared by fermentation of the target chemical products including, for example, acid, alcohol, alkane, alkene, aromatic, aldehyde, ketone, biological polymer, protein, peptide, amino acid, vitamin, antibiotic, and medicine. includes mellowly , but not limited to, methanol, ethanol, propanol, isopropanol, butanol, ethylene glycol, propylene glycol, butanediol, glycerol, erythritol, xylitol, and sorbitol. Acid include acetic acid, lactic acid, propionic acid, 3-hydroxy-propionic acid, butyric acid, gluconic acid, itaconic acid, citric

acid, succinic acid and levulic acid. Amino acid includes glutamic acid, aspartic acid, methionine, lysine, glycine, arginine, threonine, phenylalanine, tyrosine. Additional target chemical products including methane, ethylene, acetone and industrial enzyme.

[0122] Can be through the one or more kind of appropriate biological catalyst in a one-step or multi-step barmy the chemical product into a target fermentation of sugars. Biological catalyst can be selected from bacteria, filamentous fungi and yeast microorganism. Biological catalyst can be a wild-type microorganism or recombinant microorganism, and include Escherichia, fermentation unit cell bacterium, a yeast, composition rayon , the Pichia pastoris, Streptomyces, bacillus, Lactobacillus, and Clostridium. In another embodiment, biological catalyst can be selected from recombinant Escherichia coli, Pseudomonas motion fermentation, thermophillus fat bacillus, beer yeast, thermophillus clostridiales, high-temperature hydrogenogen, and trunk pichia.

[0123] Have already been described many kinds of used for fermentation to produce the target chemical biological catalyst, and can be found, the production by mutation, or by recombinant methods to engineer other biological catalyst. Any use of the method of the present invention the saccharification processing biomass production of expandable yeast sugar biological catalyst can be used for preparing the known can be the goal of production by fermentation chemical products.

[0124] Production, including ethanol and butanol, the bio-fuel is particularly of concern at the catalyst. For example, by producing solvent clostridiales the carbohydrate fermentation into acetone, butanol, and ethanol (fermentation ABE) is known as (Jones and Woods (1986) Microbiol.Rev. 50 : 484-524). US 5192673 are described for the use of acetone butanol clostridiales mutant strain producing high-content butanol, acetone and ethanol fermentation process. US 6358717 are described for the use of a Clostridium beijerinckii mutant strain producing high-content butanol, acetone and ethanol method. A total of and co-pending Patent application WO2007/041269 and WO 2007/050671 respectively discloses genetic engineering production of microbial host 1-butanol and isobutanol. A total of and co-pending US Patent application # 11/741892 and # 11/741916 discloses in genetic engineering microorganisms to produce in the host 2-butanol. By a microorganism host according to the disclosed method can be from the use of the method of the present invention in producing a hydrolyzed product of fermentation production of isobutyl alcohol, 1-butanol or 2-butanol.

[0125] Already the use of genetically modified Escherichia coli strains as a biological catalyst for alcohol production (Underwood and others, (2002) Appl.Environ.Microbiol. 68 : 6263-6272). In US 2003/0162271 A1 describes has improved ethanol production strain aeromonad motion fermentation genetic modification. In shared and co-pending U.S. Patent application for 60/847813 and 60/847856 are respectively described in for ethanol production fermentation aeromonad the movement of the further engineered strain and its role in ethanol production. Fermentation aeromonad through the movement according to the disclosed method can be from the use of the method of the present invention in producing a hydrolyzed product of fermentation to produce ethanol.

[0126] The recombinant strain Escherichia coli (Zhou, and others, (2003) Appl.Environ.Microbiol. 69 : 399-407), bacillus natural strain (US20050250192), and Rhizopus oryzae (Tay and Yang (2002) Biotechnol.Bioeng. 80:1-12) lactic acid production in the fermentation process. Already using Escherichia coli recombinant strain as biological catalyst in fermentation production of 1, 3 propanediol (US 6013494, US 6514733) and adipic acid (Niu and others, (2002) Biotechnol.Prog. 18 : 201-211). Use of recombinant clostridiales (Cheryan, and others, (1997) Adv.Appl.Microbiol. 43:1-33) and new identification of yeast strain (Freer (2002) World J.Microbiol.Biotechnol. 18 : 271-275) preparing acetic acid by fermentation. In US 6159738 discloses by recombinant Escherichia coli producing succinic acid and other bacteria, such as the person Lin ((2005) Metab.Eng. 7 : 116-127) through the disclosed in producing succinic acid mutant recombinant Escherichia coli. Torulopsis the yeast mutant strain (Li and others, (2001) Appl.Microbiol.Technol. 55 : 680-685) and Escherichia coli mutant strain (Yokota, and others, (1994) Biosci.Biotech.Biochem. 58 : 2164-2167) preparation of the pyruvic acid. Already using Escherichia coli recombinant strain as biological catalyst used for producing the P-hydroxy cinnamic acid (US20030170834) and quinic acid (US20060003429).

[0127] In the fermentation process has been the use of propionic acid c acid bacillus mutant strain producing propionic acid (Suwannakham and Yang (2005) Biotechnol.Bioeng. 91 : 325-337), and has already been using cream butyric acid clostridiales preparation butyric acid (Wu and Yang (2003) Biotechnol.Bioeng. 82:93-102). Already by fermentation from Clostridium strain 17crl (Janssen (2004) Arch.Microbiol. 182 : 482-486) preparation of threonine in Dahurica and propanol. Pullulan bud has been to use short stem mildew optically (Anantassiadis and others, (2005) Biotechnol.Bioeng. 91 : 494-501) through aspergilliosis

aspegillus mutant strain (Singh, and others, (2001) Indian J.Exp.Biol. 39 : 1136-43) preparation gluconate. By oxidation of glucose acid bacillus mutant strain preparation of 5-keto-D-gluconate (Elfari and others, (2005) Appl Microbiol.Biotech. 66 : 668-674), through the soil preparation of itaconic acid mutant strain (Reddy and Singh (2002) Bioresour.Technol. 85:69-71), through aspergilliosis aspegillus mutant strain producing the citric acid (Ikram-Ul-Haq and others, (2005) Bioresour.Technol. 96 : 645-648), and through high in candidia FTI 20037 production of xylitol (Mus satto and Roberto (2003) J.Appl.Microbiol. 95 : 331-337). By recombinant Rhodococcus erythropolis and raises richly rolls Stone Bacteriol (Gorenflo and others, (2001) Biomacromolecules 2:45-57) production including 4-hydroxy-pentanoic acid ethyl ester and significant amount 3-hydroxy butyric acid 3-hydroxy-valeric acid biological polyester. By recombinant Escherichia coli (Ui and others, (2004) Lett.Appl.Microbiol. 39 : 533-537) preparation L-2, 3-butanediol.

[0128] Can be through the use of corynebacteria, bacillus brevis, and Serratia auxotrophic strain and of amino acid analogues-resistant strain fermentation production of the amino acid. For example, published Japanese Patent 56008596 described using histidine analogue resistance strain to produce histidine, EP 136359 describes the use of recombinant strain producing serine. Published Japanese Patent 47004505 and 51019037 has described the use of the tryptophan analogs-resistant strain to produce tryptophan. Published Japanese Patent 47038995, 51006237, 54032070 feungreek described using analogue resistance strain to produce isoleucine. Published Japanese Patent 56010035 described that uses phenyl alanine analogue resistance strain to produce phenyl alanine. The need has been described using phenyl alanine growth of tyrosine-resistant strain (Agr.Chem.Soc.Japan 50 (1) R79-R87 (1976), or recombinant strain (EP263515, EP332234) production of tyrosine, and the use of L-arginine analogue resistance strain to produce arginine (Agr.Biol.Chem. (1972) 36 : 1675-1684, Japanese Patent published 54037235 and 57150381). The strain of the Escherichia coli ATCC31882, 31883, and 31884 in producing phenyl-alanine by fermentation. US 6962805 described in the recombinant coryneform bacterium producing glutamic acid. The Okamoto and Ikeda (2000) J.BiosciBioeng 89:87-79 through the described in mutant strain of Escherichia coli producing threonine. Through cardiocrinum corynebacteria mutant strain producing methionine (Kumar, and others, (2005) Bioresour.Technol. 96 : 287-294).

[0129] By biological catalyst also has already prepared the useful peptide, enzyme, and other protein (for example, refer to US6861237, US6777207, US6228630).

[0130] In the embodiments 5 in examples of pre-processing and saccharifications biomass into a fermentable sugar, the glucocorticoid is then fermented into a target chemical product used for producing ethanol in corn cobs from the pre-processed, as the using motion fermentation aeromonad into ethanol fermentation of sugars, biological catalyst. The method of the present invention also can be used for the production from biomass 1, 3-propylene glycol. If a total of and co-pending US Patent application # 11/403087 embodiment of 10 the stated, the use of the method of the present invention processing biomass can be saccharifications; after in saccharification, the use of Escherichia coli to produce 1, 3-propylene glycol.

[0131] By biological catalyst preparation in the fermentation process of the target chemical products can use a plurality of the field and the known method. Can be by centrifugation, filtration, micro-filtration, and nanofiltration separation from other fermentation component product. Can be through the ion exchange, solvent extraction, or electrodialytic to extract the product. Product separation can be used to help of flocculating agent. A specific example is can be used known fermentation field ABE from the fermentation culture medium the method of separation of biological production in 1-butanol (refer to for example Durre, Appl.Microbiol.Biotechnol. 49 : 639-648 (1998), Groot and others, Process.Biochem. 27:61-75 (1992), and reference herein). For example, by centrifugation, filtration, decanting from the fermentation culture medium for removing solid. Furthermore, can use such as distillation, azeotropic distillation, liquid-liquid extraction, absorption, stripping, film evaporation, or the evaporation method is separated from the fermentation culture medium in 1-butanol. The organic solvent through the reaction mixture, distillation, extraction and column chromatography, can be purified from the fermentation culture medium 1, 3-propanediol (United States Patent 5,356,812). In this is particularly good method for a kind of organic solvent is cyclohexane (United States Patent 5,008,473). Amino acid can be through such as ion exchange resin adsorption and/or crystallization method of collecting from the fermentation medium.

[0132] Embodiment

[0133] General method and material

[0134] Using the following abbreviations:

[0135] \"HPLC\" is high performance liquid chromatography, \"C\" is degrees Celsius, \"kPa\" is thousands pascals, is m \"m\", \"mm\" is millimeter, is kW \"kW\", \"μm\" is micron, \"μL\" is microliter, \"ml\" is ml, is L \"L\", \"min\" is minute, is millimoles \"mM\", \"cm\" is cm, \"g\" is grams, \"kg\" is kg, \"wt\" is the weight, is hours \"hr\", \"temp\" or \"T\" is temperature, \"theoret\" is a theoretical, is \"pretreat\" pre-treatment, \"DWB\" is the dry weight of the biomass, \"ASME\" is the American Society of mechanical engineers (AmericanSociety of Engineers Mechanical), \"s.s.\" is the stainless steel, \"in\" or \"\" \" is-inch.

[0136] Sulfuric acid, ammonium hydroxide, acetic acid, acetamide, yeast extract, glucose, xylose, sorbitiol, $MgSO_4 \cdot 7H_2O$, Sigma-Aldrich phosphoric acid and citric acid from (St.Louis, MO) commercially-available.

[0137] In the described embodiment the processing referred to as pre-treatment.

[0138] Small cylindrical piston reactor

[0139] Small cylindrical piston reactor (piston/barrel type reactor) is composed of the piston equipped with a horizontal orientation 5.1 cm × 45.7 cm stainless steel cylinder. Four O-ring for sealing the piston to the cylinder, the piston and the back of the discharge period the piston is pressurized with nitrogen. 45.7 cm cylinder is equipped with the three multi-purpose port, permits use of the vacuum, ammonia injection, steam injection, and is inserted into the method to measure the temperature of the thermocouple. When the steam is injected in order to avoid the excessive steam condensation, three strip heater for external heating cylinder, and using a covered with a full glass fiber jacket of silicone of 2.5 cm thick glass fiber mat insulation.

[0140] The reactor-cylinder directly connected to the vertically-oriented 15.2 cm × 61 cm stainless steel of the flash drum. The end of through the tapered nozzle and base shear valve arrangement of the cylinder is separated from the flash evaporation groove. The diameter of the die for cutting the end of the is valve 3.5 cm. On the tapered nozzle and a back pressure regulating to about 138kPa the back pressure of the (measuring pressure), make it enter 10.2 cm diameter in the air of the cylinder, the cylinder and the end of the conical nozzle is connected to the shear valve. Shearing of the valve the end of the tapered spout can be

retracted a maximum of 1.6 cm, allowing the discharge of the flash tank. Shear valve outlet at the end of the elbow guide pretreatment solid downwardly into the bottom of the flash tank, wherein the solid can be through hits the slot easily remove the dome at the bottom of the bolt. The upper part of the flash drum to a steam chest flange is processed into the flash drum with the axis of the slot is at right angle to the special outlet, this causes the release of steam along the corner path into a discharge device, helps to prevent the generation and water droplets left over from the biomass particles into the exhaust gas in the condenser.

[0141] Large-scale type piston to reactor

[0142] The piston reactor (with ASME code) 2nd processed into a cylinder of the same diameter (5.1 cm), but longer length (68.6 cm) in order to keep the additional volume of the biomass. Four O-ring for sealing the piston to the cylinder, the piston and the back of the discharge period the piston is pressurized with nitrogen. 68.6 cm cylinder equipped with eight multi-purpose port, along the top surface and a bottom surface 4 a, they allow for vacuum, ammonia injection, steam injection, and is inserted into the method to measure the temperature of the thermocouple. Reactor-cylinder is equipped with a steam jacket is used for uniform heating of the cylinder. The reactor-cylinder directly connected to the vertically-oriented 15.2 cm × 61 cm stainless steel of the flash drum. The end of through the tapered nozzle and base shear valve arrangement of the cylinder is separated from the flash evaporation groove. The diameter of the die for cutting the end of the is valve 3.5 cm. Tapered nozzle and is adjustable on the base of the back pressure of the, most testing use -138kPa (measuring pressure) to the back pressure, make it enter 10.2 cm diameter in the air of the cylinder, the cylinder and the end of the conical nozzle is connected to the shear valve. Shearing of the valve the end of the tapered spout can be retracted a maximum of 1.6 cm, allowing the discharge of the flash tank. Shear valve outlet at the end of the elbow guide pretreatment solid downwardly into the bottom of the flash tank, wherein the solid can be through hits the slot easily remove the dome at the bottom of the bolt. The upper part of the flash drum to a steam chest flange is processed into the flash drum with the axis of the slot is at right angle to the special outlet, this causes the release of the steam along the corner path in the discharge device, helps to prevent the generation and water droplets left over from the biomass particles into the exhaust gas in the condenser. Along the flash drum three strip-like electric heater (as the 60 [...]) and insulation, thermal pre-treatment of the solid shem steams in the heating container, better method for the analog commercial scale.

[0143] Steam jet reactor batch digestive system

[0144] 4 liter steam jet reactor (Autoclave Engineers, Erie, Pa) of the steam with the jacket of the reactor is, the length is 102 mm schedule 80 of the Hastelloy Tube, with two spherical valve closed. The additional electric heater is placed on the reactor all exposed, not on the surface of the with the jacket, and the temperature control to the preset temperature. Can also be directly into the steam in order to make the biomass rapidly reach the pre-treatment temperature. Regulating and controlling steam pressure in order to keep the necessary pre-treatment temperature. Necking at the bottom of the reactor to 51 mm. All pre-treatment material through the die head can be replaced at the bottom of the reactor discharge, and is collected on a nylon (Hotfill) 0.21m ^{3} bag, the nylon sleeve in a thick wall, with the jacket in the low temperature flash evaporation.

[0145] Pre-treatment and enzyme hydrolysis reactor (PEHReactor)

[0146] 9-L PEHReactor (originating from NREL, Golden, CO; refer to co-pending U.S. Patent application for 11/402464) has about 15 cm × 51 cm stainless steel reaction vessel, and 3.2LPEHReactor with 15 cm × 18 cm stainless steel reaction vessel. Each container has passes through the reaction vessel used for introducing into the longitudinal center of the spray gun by processing the reactant. To use the rotary joint to one end of the spray gun is connected to the container on the port of the covering member, the container is used for the container is connected with an additional port. Four guide plate equal to the length of the container wall, is connected to the wall of the vertical. When the container is rotated, flow guiding plate and a 3.2 cm × 3.2 cm ceramic grinding medium cylinder (E.R.Advanced Ceramics, East Palestine, OH), floating in the container together with the freedom of the raw material and the reactant to apply the mechanical mixing, in the reactant unassimilable to the biomass. The use of seven of the small roller in the reactor, in the large-scale reactor using twenty-two drum. The provides a rotary mechanism is PEHReactor Cell-ProductionRoller Apparatus Bellco (Bellco Technology, Vineland, NJ) on, the reactor has a roller apparatus, provides heating of the temperature control chamber. The external source through the cover is connected to the connecting port of the spray gun to apply vacuum and pressure of the reaction container.

[0147] Batch saccharifications reactor

[0148] Batch saccharifications reactor is a 15-L fermentation tank (B.Braun BiotechInternational, Allentown, Pa), through BioStat ED data control unit to control the same, and related control module, the module comprises a circulating pump, acid pump and inlet, solenoid-valve, used for temperature control of the heat exchanger, steam supply, process water, gas supply control valve and a filter, and a back pressure control valve and exhaust gas filter. The fermentation tank is equipped with two 11.4 cm diameter of the three leaf efficient Ligntnin A-310 impeller. From the bottom of the reactor of the impeller at the bottom of the 7.6 cm (it cannot more near the bottom, because it is near a shaft bottom there is a large seal system for the bottom-transmission shaft penetration), from the bottom of the reactor the upper impeller 22.9 cm. The fermentation tank container has 19.0 cm diameter and 55.9 cm the maximum height of. Install the four removable flow guide plate, each flow guide plate has a 1.6 cm width and 48.3 cm length, to the top and from the bottom of the container -7.6 cm. The APV cam pump (M1/028/06-type), 1-1/2-inch (3.81 cm) of the flexible hose and teflonlike flow observation indicator a pump circulation loop is connected to the fermentation tank system on the top and the bottom of the port. Pump circulation loop with CF8M valve body, 316s. s. Spheroid, and a PTFE 1-1/2 inch (3.81 cm) Valmicro and SVF full port ball valve is separated from the fermentation vessel. Furthermore, V-shaped port shear valve (Triac control) is located downstream of the mechanical, the pump port at the top of the separation of the fermentation tank before the ball valve. In a recirculation period, the valve is gradually close to a maximum of 60° in order to provide greater recirculation preoonditioning solid shear.

[0149] Analysis method

[0150] Cellulose quantitative

[0151] The field of use of the well-known methods, such as ASTM E1758-01 \"Standard method for thedetermination of carbohydrates by HPLC\" determination in each of the starting biomass the amount of cellulose in the sample.

[0152] Measuring sugar, acetamide, lactic acid and acetic acid content

[0153] By HPLC (Agilent Model 1100, Agilent Technologies, PaloAlto, CA) using Bio-Rad HPX-87P and Bio-Rad HPX-87H column (Bio-RadLaboratories, Hercules, CA) and the

protection of the appropriate measuring column in saccharification liquid or fermentation broth of soluble sugar (glucose, fiber two ponds , xylose, galactose, arabinose, and mannose), acetic acid, and ethanol. If required, measurement samples and pH with sulfuric acid to the 5-6. Then the sample through the 0.2 the injection m directly enters the HPLC vial in the filter. HPLC operation conditions are as follows:

[0154] HPX-87P (used for carbohydrate):

[0155] Injection volume: the 10-50 L, limit depends on the concentration and detector

[0156] Mobile phase: HPLC-grade water, the 0.2 m, filter and degassing

[0157] Flow rate: 0.6 ml/min

[0158] Column temperature is: the 80-85 [...] , protection the column column is warm <the 60 [...]

[0159] Detector temperature: as far as possible, mainly the column column is warm of the proximity of the

[0160] Detector: refractive index

[0161] Running time: 35 minutes with data acquisition time of 15 minutes after the operation time (the later eluting compound possible adjustment)

[0162] Aminex HPX-87H Biorad (used for carbohydrate, acetic acid and ethanol)

[0163] Injection volume: the 5-10 L, limit depends on the concentration and detector

[0164] Mobile phase: 0.01N sulfuric acid, the 0.2 m, filter and degassing

[0165] Flow rate: 0.6 ml/min

[0166] Column temperature is: the 55 [...]

[0167] Detector temperature: of, as far as possible close to the column temperature

[0168] Detector: refractive index

[0169] Running time: 25-75 minutes of data acquisition time

[0170] After the end of the operation, according to the standard curve determination each compound concentration in the sample.

[0171] Embodiment 1

[0172] In small type piston corn cob pre-treated in a reactor

[0173] with jaw the interval is approximately 0.95 cm jaw crusher (2.2kW motor) corn processing a complete, then the crusher (1.5kW motor, Inc Franklin Miller. , Livingston, NJ) processing, for subsequently equipped with a 1.9 cm Sweco the United States standard sieve screen of the screen, so that the integrity of corncob broken into more small block. In the small cylinder piston reactor (such as general the method states) loading 115g (based on dry weight) crushing the corncob, the corn cob is arranged by removing the end of the reactor of the piston. In order to close the end of the original position of the piston. Vacuum the reaction vessel in order to decompress to <10kPa (0.1bar), dilute ammonium hydroxide solution is injected into the reactor, so that the ammonia concentration is 4g or 6g each 100g biomass dry weight (as shown in table 1 illustrated), the concentration of biomass dry weight 50g each 100g total biomass ammonia-water mixture. After the injected ammonia solution, injecting the steam into the reactor in order to bring the temperature to the 145 [...]. The biomass at a temperature of 20 minutes, and is then opened to the piston will be discharged into the flash tank. In 20 minutes of monitoring the temperature of the pre-processing period, such as the necessary steam is injected. Through the flash drum to the bottom of the pre-harvest of corn cob. Remove the excessive free liquid, the remainder of the solid used for saccharification.

[0174] If general the method states , would be about 470g added to the biomass pretreatment of reactor PEHR 3.2-L for saccharification. PH4.8 through into the 1M citric acid buffer

solution by adding citric acid monohydrate is added, the content of the pH is adjusted to about 5.5. Once reach the desired pH, the 12.9 mg/g cellulose or 25.8 mg/g cellulose of Spezyme Cellulase CP (GenencorInternational, Rochester, NY) and 4.2 mg active protein/g cellulose or 8.4 mg active protein/g cellulose the enzyme gathers the living body the hemicellulase (Diversa; San Diego, CA) is added to the reactor, the β-glucosidase by states the enzyme to gather the living body , xylanses, β-wood poly glycocidase and arabinoses glucoside enzyme composition. Adding buffer solution, the enzyme and water the final mixture in the reactor by 23g dry biomass/100g preonditioning biomass and saccharifications the enzyme gathers of a mixture of many. Reactor in the culture case the 50 [...] , 19rpm rotary culturing the 72 hours. In the following table 1 the yield is given in percentage of theoretical yield to release form.

[0175] Table 1: reactor type piston in the pre-treatment of the saccharification yield of corn.

[0176]

[0177] Embodiment 2

[0178] Different time in the large-scale type piston pre-treatment in the reactor

[0179] The cylinder jacket, the steam added to the large-scale type piston cylinder of the reactor (such as general the method states) the preheating to -130 [...]. The flash evaporation strip heater for preheat to -60 the receiver [...]. Accurately construct 1 crushing the corn cob. The corn cob of these (175g, based on dry weight) into the large-scale type reactor, the reactor is removed in the tail end of the piston. In order to close the end of the original position of the piston. The reaction container and a flash receiver vacuum in order to decompress to <10kPa, dilute ammonium hydroxide solution is injected into the reactor, so that the ammonia concentration is 6g/100g biomass dry weight, the concentration of biomass dry weight 45g/100g total biomass ammonia-water mixture. After injecting ammonia water, injecting the steam into the reactor in order to bring the temperature to the 145 [...]. By monitoring the temperature and steam is injected as necessary, the temperature of the mixture is kept under 10 or 20 minutes, and then opens the piston will be discharged into the preheating flash evaporation groove thereof. The flash evaporation groove until the vacuum reaches -59 the flash receiver [...]. Three 10 minutes pre-processing and six 20 minutes pre-

processing, all the material in the preconditioning of the same time. When the from the flash receiver when the harvest product, pretreatment of the free liquid from separated solids, and they add back the used for saccharification. Subsequently, such as embodiment 1 the small PEHReactor saccharfied pre-processing a sample. All the saccharification uses 12.9 mg/g cellulose of Spezyme Cellulase and CP 4.2 mg active protein/g cellulose of consortia the hemicellulase (Diversa) in the 50 [...] , pH5.5 carried out under the conditions of 72 hours, the containing xylanase states the enzyme to gather the living body , , β-xylosidase, arabinoses glucoside enzyme , and β-glucosidase enzyme. In the following table 2 to yield given in percentage of theoretical yield release form.

[0180] Table 2:in the large-scale type piston of the pre-processing in the reactor the saccharification yield of corn.

[0181]

Pretreatment time (minutes)	grapes grape sugar monomer emission (% theoretical value)	Total glucose release amount (% theoretical value)	Xylose monomer emission (% theoretical value)	Total xylose release amount (% theoretical value)
10	68.2	79.5	32.1	77.0
20	68.0	83.2	39.1	84.3

[0182] Embodiment 3

[0183] In the large-scale type piston carry on the preconditioning in the reactor, and the steam injection comparing

[0184] Accurately construct 1, the corn cob particle size reduction. Accurately construct 2 the large-scale type piston carry on the preconditioning in the reactor. In order to carry on the preconditioning in the steam jet, into the first corn 9-L in PEHReactor. Rotating contact ice through the outer surface of the reactor cooling to 4 the [...]. Vacuum the reaction vessel and the diluted solution of ammonium hydroxide is injected into the reactor, the ammonium hydroxide solution in 4 the cold chamber [...] in through pre-cooling and the tube is immersed in ice-water bath, the ammonia concentration in the reactor 6g/100g biomass dry weight, the concentration of biomass dry weight of the total biomass and ammonia mixture 45g/100g.

exerts the ice the rotating reaction and the surface of the container 4 the lower rotary [...] 30 minutes, the ammonia and the corn cob of cooling to PEHReactor the 4 [...]. At this moment the content is transferred to the such as a general method in the steam jet reactor. In the steam jet reactor after the addition of ammonia to the mixture, by direct injection of steam to the temperature rise to the 145 [...]. Corncob and ammonia mixture is kept at a temperature of 20 minutes, the mixture is then discharged into the flash tank.

[0185] From the large-scale type piston reactor and steam jet reactor sample collecting pre-processing of corn, such as embodiment 1 carrying out the saccharifications. the saccharification uses 12.9 mg/g cellulose of Spezyme Cellulase CP (Genencor) and 4.2 mg of active protein/g cellulose of consortia the hemicellulase (Diversa), the β-glucosidase by states the enzyme to gather the living body , xylanses, β-xylosidase, a and arabinoses glucoside enzyme. Reactor in the culture case the 50 [...] , 19rpm culturing the 72 hours. In each of the pre-treatment material in the reactor the glucose yield such as in the following table 3 is shown.

[0186] Table 3:in the large-scale type piston reactor or steam jet reactor of the pre-treated in the yield of sugar corn.

[0187]

The pretreatment reactor	Conc DWB in the reactor	Pretreatment time (minutes)	Pre-treatment temperature (°C)	Glucose monomer emission (% theoretical value)	Total glucose release amount (% theoretical value)	Xylose monomer emission (% theoretical value)	Total xylose release amount (% theoretical value)
Piston reactor	50%	20	145	68.0	83.2	39.1	84.3
Steam injection	60%	40	150	65	77	48	82

[0188] Embodiment 4

[0189] In the reactor the large-scale type piston pre-treating corn cob and fiber blend

[0190] Accurately construct 1 crushing the corn cob. In the reactor the large-scale type piston separate pretreatment crushing corncob, Cargill corn pretreatment crushing and Bran and 80 (Cargill, Minnetonka, MN) blend. Bran the Cargill corn cob and 80 corn fiber combined, mixed sample so that the fiber accounts for the total dry biomass of approximately 33%. In any case, will 175g (based on dry weight) in the raw material added to the reactor. Basic accurately construct 2 carrying out the pre-treatment. However, in these experiment, after the addition of the ammonia solution, the injection steam to the temperature is raised to 145 the content before [...] maintain reactor 10 minutes. After the steam is injected, the necessary steam is injected through the temperature is kept at 145 the under [...] 10 minutes. After the pretreatment, the sample is discharged into the flash groove of the piston.

[0191] From the large-scale type piston in flash drum of the reactor and the corn cob of corn collecting pre-treatment of the fiber blend of the sample, and in accurately construct 1 in the small-sized PEHReactor saccharification. Adding biomass, the volume of the reactor until the full 20%. With 12.9 mg/g cellulose of Spezyme Cellulase CP (Genencor) and 15 mg/g cellulose of xylanase Multifect carry out saccharification (Genencor). The culture case PEHReactor in the 50 [...] , 19rpm culture 72 hours. Pre-treatment material the resulting glucose and xylose yield such as in the following table 4 is shown.

[0192] Table 4:in the reactor in the large-scale type piston corn cob pre-treatment of the sample with bran and saccharification yield.

[0193]

Raw materials	Conc DWB in the reactor	Glucose monomer emission (% theoretical value)	Total glucose release amount (% theoretical value)	Xylose monomer emission (% theoretical value)	Total xylose release amount (% theoretical value)
Corncob only	45%	40.2	67.2	29.4	83.9

[0194]

Raw materials	Conc DWB in the reactor	Glucose monomer emission (% theoretical value)	Total glucose release amount (% theoretical value)	Xylose monomer emission (% theoretical value)	Total xylose release amount (% theoretical value)
Corn bran + 80	45%	37.0	65.4	21.6	77.2

[0195] Embodiment 5

[0196] Large-scale type piston from the pre-treated in a reactor producing ethanol in corn

[0197] Accurately construct 2 the pre-processing of corncob 10 minutes. A total of 17 such pre-processing. To be obtained from 4 corn pretreatment time of pre-injection in order to be used for saccharifications, so as to provide for batch saccharifications initial hydrolysis product. To be obtained from the remaining 13 time pre-treatment of production and running corn cob injection in order to be used in the batch saccharification.

[0198] In order to begin batch saccharifications, such as the general method the batch saccharifications reactor firstly adding hydrolysis product in the reactor is filled with 1st to the bottom of the of the impeller. The hydrolysis product through the pre-treatment of saccharification 2.8-L rocking bottle preparation of corn. These rocking bottled enters 465g preonditioning solid, 1000 ml de-ionized water, and 28.4 mg Spezyme CP/g cellulose and 4.2 mg of active protein/g cellulose (Diversa) consortia the hemicellulase, the the enzyme gathers the living body including b-glucosidase, xylanses, b-xylosidase and arabinoses glucoside enzyme. Before the enzyme, using 8.5% H_3PO_4 will be adjusted to pH 5. The shake flask maintained at the 50 and [...] in the rotary shaker 150rpm oscillation 48 hours, at this moment the hydrolyzed product into the batch reactor.

[0199] After adding the initial hydrolysis product, the pre-treatment of biomass mixture with ammonia (-700g) 1st and the like of the sample is added to the reactor. Adding 8.5% H_3PO_4 set to maintain pH 5.5. The pH after re-adjusting to the preset value, adding 28.4 mg Spezyme CP/g cellulose and 4.2 mg of active protein/g cellulose (Diversa) consortia the hemicellulase, the the enzyme gathers the living body including b-glucosidase, xylanses, b-xylosidase and arabinoses glucoside enzyme. In t=4, 8, 12, 22, 26, 30 and 34 hours by adding

pre-processing of the biomass and ammonia the additional sample mixture, Spezyme CP the enzyme gathers the living body cellulase and hemicellulase. Adding enzyme approximately in general 1 hours after the circulation loop start, and run about 1 hours until the subsection 22 hours by adding solid. In the 26 hour and 30 hours after feeding, so in about 50 minutes of operation of the pump and opening 30 minutes. In 34 hours after feeding, so in about 3 hours of operation of the pump and opening 30 minutes. The repeats t=29, 33, 47 and 49 hours running 30 minutes. Total saccharifications time is 120 hours. At this moment the hydrolysis product contains -60 g/l glucose monomer, 25 g/l xylose monomer and 10 g/l acetic acid.

[0200] The hydrolysis product is used for motion fermentation monad strain ZW800 or ZW658 (ATCC# PTA-7858) fermentation. ZW658 is motion fermentation monad strain, which have been engineered for fermenting xylose to ethanol, in shared and-co-pending U.S. Patent application for 60/847813 the in the described. ZW658 sequence transposition events via the two operon ZW1 (ATCC# 31821) integrated into the genome, then through the screening medium comprising xylose to the choice of the construction, the is states the operon P_{gap} xy1AB and P_{gap} taltkt, they contain four encoding xylose isomerase, ketoxylose kinase, the xylose alcoholasetransfers the aldehyde alcoholase and transfers the alkone using gene. ZW800 is provided with coding for glucose-fructose oxidoreductase ZW658 inactivated gene of strain, it is also in shared and co-pending U.S. Patent application for 60/847813 described in.

[0201] Fermentation in the sterilization of the 1 liter fermentation tank (BIOSTAT B-DCU system, Sartorius Inc BBISystem. , Bethlehem, Pennsylvania, USA) to, initial working volume is 500 ml. In the inoculum is added to the fermentation tank, content is 10% (v/v), so that the sample of broth after OD_{600} -1. Hydrolysis product and the balance of water in a proportion of 80% or 40% (v/v). Adding additional glucose and xylose in order to make them in the broth in the final concentration of 92 g/l and 82 g/l. Broth also supplemented with 10 mm sorbitol, and 1 g/l $MgSO_4 \cdot 7H_2O$. In the 33 [...] , pH5.8, the stirring speed 150rpm carried out under the condition of 72 hours of fermentation. ZW800 strain in the analogy mechanism to the final ethanol 40% in the product of hydrolysis of 8 g/l, in 80% in the product of hydrolysis of 7 g/l. ZW658 in the analogy mechanism to the final ethanol 40% in the product of hydrolysis of 8 g/l, in 80% in the product of hydrolysis of 6.5 g/l.

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Claims:

Claim 1: 1. Method for the treatment of a biomass, the method comprising: A) providing biomass; B) the use of the feeding machine non-compaction (a) in the biomass into the apparatus, the apparatus including: I) cylindrical drum, the cylindrical drum has a is equipped with the piston of the end of the 1st and 2nd is equipped with the tail end of the discharging valve; Ii) optionally a branch pipe, said branch pipe in a branch pipe is connected to the end of the 1st to the tail end of the cylindrical drum on the cylindrical cylinder, and in the said branch pipe of the branch pipe is not connected with the end of the valve can be sealed; Iii) in the cylindrical cylinder or at least of the branch pipe 2 a the port can be sealed; Iv) optionally

a valve, the valve in the cylindrical cylinder of the cylinder is divided into separate 2nd 1st chamber and the chamber, the chamber having 1st the 1st with the tail end of the piston in the cylinder, and the 2nd chamber having is equipped with a discharge valve of a cylinder the end of the 2nd; and V) is connected to the discharge valve of the cylinder on the end of the 2nd a flash drum; Wherein the biomass into the cylindrical cylinder or optionally into the is connected to the cylindrical of the cylinder in the tube states ; C) closing the cylindrical cylinder and branch line (if any); D) optionally via the cylindrical in the cylinder apply a vacuum to the at least one port; E) the cylindrical cylinder or through the middle of the branch pipe to the at least one port containing an aqueous ammonia solution, relative to the quantity of the dry weight of the biomass in the cylinder is less than about 12 weight percent, so as to prepare the mixture of the biomass and ammonia, and in addition the relative to the dry weight of the biomass, said biomass with the weight of the ammonia-water mixture is at least about 15 weight percent of high solid concentration, and through the cylindrical cylinder or a branch pipe (if any) to the 2nd port of the steam, in order to make the cylinder temperature reaches between about 85 the and about [...] the 180 between [...] ; F) closing the cylindrical cylinder and branch pipe, and in (if any) of the port, in order to offer the impermeable chamber; G) in said impermeable chamber said biomass with an ammonia-water mixture will be kept in the appropriate temperature for a period of time, the time is between about 30 seconds and about 4 hours; H) optionally through the displacement of the piston said biomass with an ammonia-water mixture will be moved to the 2nd chamber in the cylinder (if they exist), wherein the compacted material can not be stated, and maintain between about 2 minutes and 4 hours for a period of time between the; and I) through the piston will move said biomass with an ammonia-water mixture through (g) or (h) of the impermeable cylindrical cylinder and through the discharge valve into the the groove states the flash vaporization ; Wherein the production of the treated biomass.

Claim 2: 2. Method for the treatment of a biomass, the method comprising: A) providing a mixture of biomass and containing ammonia, Wherein, with respect to the dry weight of the biomass, said biomass with an ammonia-water mixture for gross weight of at least about 15 weight percent, and the ammonia water relative to the amount of dry weight of the biomass is less than about 12 weight percent; B) the use of the feeding machine non-compaction (a)

the biomass with an ammonia-water mixture into the apparatus, the apparatus including: I) cylindrical drum, the cylindrical drum has a is equipped with the piston of the end of the 1st and 2nd is equipped with the tail end of the discharging valve; Ii) optionally a branch pipe, said branch pipe in a branch pipe is connected to the end of the 1st to the tail end of the cylindrical drum on the cylindrical cylinder, and in the said branch pipe of the branch pipe is not connected with the end of the valve can be sealed; Iii) in the cylindrical cylinder or at least of the branch pipe 2 a the port can be sealed; Iv) valve, the valve in the cylindrical cylinder of the cylinder is divided into separate 2nd 1st chamber and the chamber, the chamber having 1st the 1st with the tail end of the piston in the cylinder, and the 2nd chamber having is equipped with a discharge valve of a cylinder the end of the 2nd; and V) is connected to the discharge valve of the cylinder on the end of the 2nd a flash drum; Wherein the biomass into the cylindrical cylinder of the 1st chamber or optionally into the is connected to the cylindrical of the cylinder in the tube states ; C) closing the cylinder in the main pipe and the branch of the 1st chamber (if any); D) optionally at least one port through the vacuum is applied; E) the 1st chamber or through the branch pipe (if existing) in 1st port of at least one of adding steam, in order to make the indoor temperature reach the is between about 85 the and about [...] the 180 between [...] ; F) closing said 1st chamber and the branch pipe (if existing) to provide the ports in the 1st chamber can not be penetration; G) in said impermeable 1st chamber in said biomass with an ammonia-water mixture will be kept in the appropriate temperature for a period of time, the time is between about 30 seconds and about 4 hours; H) optionally, through the displacement of the piston said biomass with an ammonia-water mixture will move through the open valve from the cylindrical in the cylinder is not permeable 1st 2nd in the room into the chamber, wherein the compacted material can not be stated; I) optionally, closing the opening of the valve in order to form the 2nd-impermeable chamber and keeps the biomass and ammonia-water mixture is between about 2 minutes and about 4 hours for a period of time between the; and J) in step (g) or the step (i) through the displacement of the piston after said biomass with an ammonia-water mixture will be moved through the discharge valve into the the states the flash vaporization groove; wherein the stated material can not be compacted and so as to produce the treated biomass.

Claim 3: 3. Claim 1 of the method, in the step (a), (b), (c), (d), (e), (f), (g), and (h) in one or more

of the steps in step (i) repeating at least one time before.

Claim 4: 4. Claim 2 of the method, in the step (a), (b), (c), (d), (e), (f), (g), (h), and (i) one or a plurality of steps in the step (j) repeating at least one time before.

Claim 5: 5. Claim 1 or 2 of the method, which does not include the step of compaction state elimination.

Claim 6: 6. Claim 1 or 2 of the method, wherein the ammonia water with respect to the dry weight of the biomass is between about 4% and about 6% between.

Claim 7: 7. Claim 1 or 2 of the method, wherein the biomass to be prepared with respect to the dry weight of the biomass and of the weight of the ammonia-water mixture is at least about 20%.

Claim 8: 8. Claim 7 the method, wherein the biomass to be prepared with respect to the dry weight of the biomass and of the weight of the ammonia-water mixture is at least about 30%.

Claim 9: 9. Claim 8 the method, wherein the biomass to be prepared with respect to the dry weight of the biomass and of the weight of the ammonia-water mixture is at least about 50%.

Claim 10: 10. Claim 1 or 2 of the method, wherein the suitable temperature is between about 120 the [...] and about 160 the between [...].

Claim 11: 11. Claim 10 the method, wherein the suitable temperature is between about 140 the [...] and about 150 the between [...].

Claim 12: 12. Claim 1 or 2 of the method, wherein said (b) of which is equipped with a feeder non-compactionconduction current wheelnon-compaction the hopper.

Claim 13: 13. Claim 1 or 2 of the method, wherein the 1st cylindrical shape room with at least one valve is closed.

Claim 14: 14. Claim 13 the method, wherein the 1st 1st used in room valve is closed in order to close and the 2nd feeder states the non-compaction valve is closed in order to close (h) of the 2nd chamber.

Claim 15: 15. Claim 1 or 2 of the method, wherein said discharge valve is a progressive expansion of the venturi tube.

Claim 16: 16. Claim 1 or 2 of the method, wherein the biomass is selected from switchgrass, waste paper, the sludge from the papermaking industry, corn, corn cobs, corn shell, corn fiber, corn straw, grass, wheat, wheat straw, hay, barley, barley straw, stalk, bagasse, sorghum, soybean, obtained from cereal, tree, branch, root, leaf, wood chips, sawdust, shrubs and underbrush, vegetables, fruits, flowers and animal manure component of the article.

Claim 17: 17. Claim 16 the method, wherein the biomass is selected from the group consisting of corn cobs, corn straw, corn fiber, corn shell, bagasse, sawdust, switchgrass, wheat straw, hay, stalk, and grass.

Claim 18: 18. Claim 17 the method, wherein the biomass is selected from the group consisting of corn cobs, corn straw, corn fiber, sawdust, and bagasse.

Claim 19: 19. Claim 1 or 2 of the method, wherein the biomass is derived from a plurality of raw materials.

Claim 20: 20. By claim 1 or 2 of the production method by the processing of the biomass.

Claim 21: 21. Through saccharifications the treated biomass and the production of the hydrolysis product, wherein the processed biomass of claim 1 or 2 the method of preparation.

Independent Claims:

First Claim:

Claim 1: 3. Claim 1 of the method, in the step (a), (b), (c), (d), (e), (f), (g), and (h) in one or more of the steps in step (i) repeating at least one time before.

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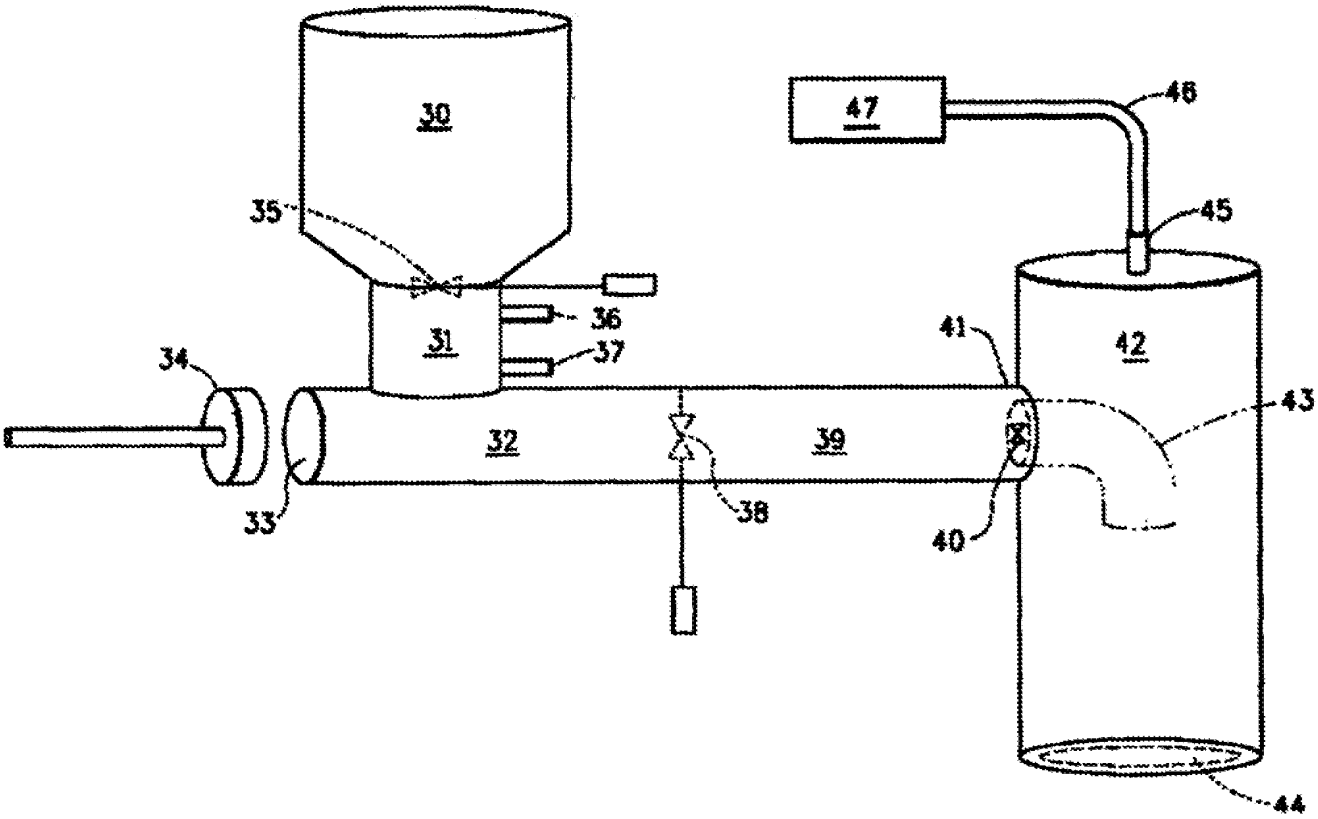

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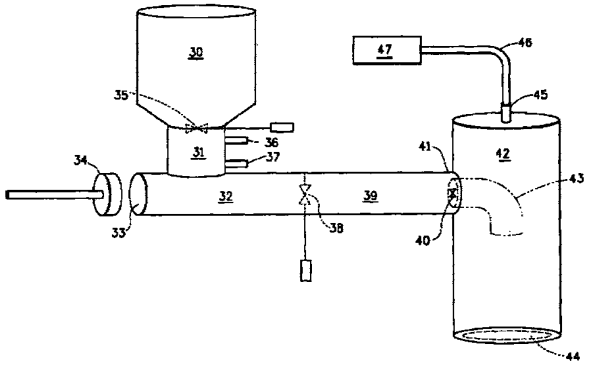
(54)发明名称

生物质处理方法

(57)摘要

本发明开发了用于处理生物质的方法，所述方法使用设备在非压实状态下将生物质与稀释氨水混合物移动通过反应室。所述设备使用非压实活塞来移动生物质。将所得的经处理的生物质进行糖化以生产可发酵糖。



CN 101835804 A

1. 用于处理生物质的方法，所述方法包括：

a) 提供生物质；

b) 使用非压实喂料机将所述 (a) 的生物质装入到设备中，所述设备包括：

i) 圆柱形圆筒，所述圆柱形圆筒具有配备有活塞的第一末端和配备有排放阀的第二末端；

ii) 任选的支管，所述支管在一个支管末端连接到靠近所述圆柱形圆筒第一末端的圆柱形圆筒上，并且所述支管在未连接的支管末端具有可密封的阀门；

iii) 所述圆柱形圆筒或支管中的至少 2 个可密封的端口；

iv) 任选的阀门，所述阀门在所述圆柱形圆筒中将圆筒分成单独的第一室和第二室，所述第一室具有配备有所述活塞的圆筒第一末端，并且所述第二室具有配备有排放阀的圆筒第二末端；以及

v) 连接至在所述圆筒第二末端的排放阀上的闪蒸槽；

其中将所述生物质装入到所述圆柱形圆筒中或任选地装入到连接至所述圆柱形圆筒的所述支管中；

c) 关闭所述圆柱形圆筒和支管（如果存在的话）；

d) 任选地经由所述圆柱形圆筒中的至少一个端口施加真空；

e) 通过所述圆柱形圆筒或者支管中的所述至少一个端口加入含氨水溶液，加入的量相对于所述圆筒中生物质的干重为小于约 12 重量百分比，从而制备生物质与氨水的混合物，并且此外其中所述生物质的干重相对于所述生物质与氨水混合物的重量为至少约 15 重量百分比的高固体浓度，并通过所述圆柱形圆筒或支管（如果存在的话）中的所述第二端口加入蒸汽，以使所述圆筒内温度达到介于约 85℃和约 180℃之间；

f) 关闭所述圆柱形圆筒和支管（如果存在的话）中的端口，以提供不可渗透的室；

g) 在所述不可渗透的室中将所述生物质与氨水混合物保持在合适温度下一段时间，所述时间介于约 30 秒和约 4 小时之间；

h) 任选地通过所述活塞的位移将所述生物质与氨水混合物移动到所述圆柱形圆筒中的第二室（如果存在的话），其中所述生物质不被压实，并保持介于约 2 分钟和 4 小时之间的一段时间；以及

i) 通过所述活塞将所述生物质与氨水混合物移动通过 (g) 或 (h) 的所述不可渗透的圆柱形圆筒并通过所述排放阀进入到所述闪蒸槽中；

其中生产出经处理的生物质。

2. 用于处理生物质的方法，所述方法包括：

a) 提供生物质和含氨水溶液的混合物，

其中所述生物质的干重相对于所述生物质与氨水混合物的总重为至少约 15 重量百分比，并且所述氨水的量相对于生物质的干重为小于约 12 重量百分比；

b) 使用非压实喂料机将所述 (a) 的生物质与氨水混合物装入到设备中，所述设备包括：

i) 圆柱形圆筒，所述圆柱形圆筒具有配备有活塞的第一末端和配备有排放阀的第二末端；

ii) 任选的支管，所述支管在一个支管末端连接到靠近所述圆柱形圆筒第一末端的圆

柱形圆筒上，并且所述支管在未连接的支管末端具有可密封的阀门；

iii) 所述圆柱形圆筒或支管中的至少 2 个可密封的端口；

iv) 阀门，所述阀门在所述圆柱形圆筒中将圆筒分成单独的第一室和第二室，所述第一室具有配备有所述活塞的圆筒第一末端，并且所述第二室具有配备有排放阀的圆筒第二末端；以及

v) 连接至在所述圆筒第二末端的排放阀上的闪蒸槽；

其中将所述生物质装入到所述圆柱形圆筒的所述第一室中或任选地装入到连接至所述圆柱形圆筒的所述支管中；

c) 关闭所述圆筒中的所述第一室和支管（如果存在的话）；

d) 任选地经由所述至少一个端口施加真空；

e) 通过在所述第一室或支管（如果存在的话）中的至少一个第一端口加入蒸汽，以使所述室内的温度达到介于约 85℃和约 180℃之间；

f) 关闭所述第一室和支管（如果存在的话）中的端口以提供不可渗透的第一室；

g) 在所述不可渗透的第一室中将所述生物质与氨水混合物保持在合适温度下一段时间，所述时间介于约 30 秒和约 4 小时之间；

h) 任选地，通过活塞的位移将所述生物质与氨水混合物移动通过开启的阀门从所述圆柱形圆筒中的不可渗透的第一室进入到第二室中，其中所述生物质不被压实；

i) 任选地，关闭所述开启的阀门以形成第二不可渗透的室并保持所述生物质与氨水混合物介于约 2 分钟和约 4 小时之间的一段时间；以及

j) 在步骤 (g) 或步骤 (i) 之后通过所述活塞的位移将所述生物质与氨水混合物移动通过所述排放阀进入到所述闪蒸槽中；其中所述生物质不被压实并且以此生产出经处理的生物质。

3. 权利要求 1 的方法，其中步骤 (a)、(b)、(c)、(d)、(e)、(f)、(g)、和 (h) 中的一个或多个步骤在步骤 (i) 之前重复至少一次。

4. 权利要求 2 的方法，其中步骤 (a)、(b)、(c)、(d)、(e)、(f)、(g)、(h)、和 (i) 中的一个或多个步骤在步骤 (j) 之前重复至少一次。

5. 权利要求 1 或 2 的方法，其中不包括消除压实状态的步骤。

6. 权利要求 1 或 2 的方法，其中所述氨水相对于生物质的干重为介于约 4％和约 6％之间。

7. 权利要求 1 或 2 的方法，其中所述生物质的干重相对于将要制备的生物质与氨水混合物的重量为至少约 20％。

8. 权利要求 7 的方法，其中所述生物质的干重相对于将要制备的生物质与氨水混合物的重量为至少约 30％。

9. 权利要求 8 的方法，其中所述生物质的干重相对于将要制备的生物质与氨水混合物的重量为至少约 50％。

10. 权利要求 1 或 2 的方法，其中所述合适的温度为介于约 120℃和约 160℃之间。

11. 权利要求 10 的方法，其中所述合适的温度为介于约 140℃和约 150℃之间。

12. 权利要求 1 或 2 的方法，其中所述 (b) 的非压实喂料机是配备有非压实导流轮的料斗。

13. 权利要求 1 或 2 的方法，其中所述第一圆柱形室用至少一个阀门关闭。

14. 权利要求 13 的方法，其中所述第一室用第一阀门关闭以关闭所述非压实喂料机并用第二阀门关闭以关闭 (h) 的所述第二室。

15. 权利要求 1 或 2 的方法，其中所述排放阀是渐进扩展文丘里管。

16. 权利要求 1 或 2 的方法，其中所述生物质选自柳枝稷、废纸、来自造纸业的淤渣、玉米粒、玉米芯、玉米壳、玉米纤维、玉米秸秆、草、小麦、小麦秸秆、干草、大麦、大麦秸秆、稻秆、蔗渣、高粱、大豆、获取自谷物、树、枝、根、叶、木屑、锯末、灌木及矮树丛、蔬菜、水果、花和动物粪肥的研磨物的组分。

17. 权利要求 16 的方法，其中生物质选自玉米芯、玉米秸秆、玉米纤维、玉米壳、蔗渣、锯末、柳枝稷、小麦秸秆、干草、稻秆、和草。

18. 权利要求 17 的方法，其中生物质选自玉米芯、玉米秸秆、玉米纤维、锯末、和蔗渣。

19. 权利要求 1 或 2 的方法，其中所述生物质来源于多种原料。

20. 通过权利要求 1 或 2 的方法生产的经处理的生物质。

21. 通过糖化经处理的生物质而生产的水解产物，其中所述经处理的生物质通过权利要求 1 或 2 的方法制备。

生物质处理方法

[0001] 政府权利声明

[0002] 本发明由美国政府支持，受与能源部签署的合同号 04-03-CA-70224 和 DE-FC36-03GO13146 的约束。美国政府拥有本发明的必然权利。

发明领域

[0003] 提供了一种处理生物质的方法，所述方法包括一种指定设备。该方法使用所述设备以非压实状态将生物质移动进入并通过反应器，其中实施在适度温度和压力下用稀氨水浸渍生物质并与其反应的处理方法。

背景技术

[0004] 纤维质的和木质纤维质原料以及垃圾例如农业残余物、木材、林业垃圾、来自造纸业的淤渣、和市政及工业固体垃圾，提供了潜力巨大的可再生原料，用于生产有价值的产品如燃料和其他化学制品。由碳水化合物聚合物（包括纤维素、半纤维素、葡聚糖和木质素）组成的纤维质的和木质纤维质的给料以及垃圾一般用多种化学、机械和酶方法进行处理以释放主要的己糖和戊糖，它们然后能进行发酵以产生有用产物。

[0005] 首先，将生物质原料进行处理以制备糖化酶更易利用的纤维质和木质纤维质材料的碳水化合物聚合物，该过程通常称为预处理。然后将预处理生物质在存在糖化酶的条件下进一步水解以在水解产物中释放低聚糖和 / 或单糖。用于从预处理生物质中生产可发酵糖的糖化酶通常包括一种或多种糖苷酶例如纤维素水解糖苷酶、半纤维素水解糖苷酶、和淀粉水解糖苷酶，以及肽酶、脂肪酶、木素酶和 / 或阿魏酸酯酶。用于生物质处理的糖化酶和方法参见 Lynd, L. R. 等人 (Microbiol. Mol. Biol. Rev. (2002) 66 :506-577)。

[0006] 期望有用于经济有效地大规模处理生物质的系统和 / 或方法。需要将生物质进行处理使之成为浓缩的高干重材料以经济地生产需要的高浓度可发酵糖，所述可发酵糖用于发酵成产品。因此，包括将生物质高干重部分移动通过反应器，同时保持处理化学品的透过能力和优化制备用于糖化的生物质的能力，此外还使用最少的化学品和能量输入来实施材料移动，这是生物质处理方法的一个挑战。还希望有包括低资本成本设备的方法。不需要搅拌或反应器旋转的反应器的方法可提供更低的资本成本设备和更低的能量输入。

[0007] 已经描述了不需要搅拌或反应器旋转的系统和用于移动生物质通过反应器的具体设备。US4186658 公开了用于传送颗粒物质的设备，所述颗粒物质如木屑、麦秸、蔗渣、和其他纤维材料，该设备将所述物质压实成一种固体“块”状。用螺杆传送器将所述材料进行预压，并用往复活塞进一步压实。压实的块状物非常致密使得它能够有效地防止系统中的倒吹。然后可将所述块给料到设备中用于材料加工。生物质材料致密块将不能使预处理反应物实现最佳的穿透性。

[0008] 同样的，US4136207 公开了用于制备具有提高反刍动物消化性的纤维素材料的方法，该方法从机械压实所述材料开始。然后它在不存在化学试剂的情况下经过高压蒸汽处理，并进一步压实以形成生物质固体块，它阻止蒸汽通过入口逸出。然后排出小部分材料，

使之迅速降低压力。将生物质压实成块状将不能使预处理中使用的化学试剂实现最佳的穿透性。

[0009] US6176176 公开了用于处理纤维素材料的设备，该设备使用安装在挤出机圆筒中的可旋转螺杆。将加压液氨装入圆筒中，并与木质纤维质材料在圆筒中混合，然后使其通过加热模头排出圆筒，液氨变成气体，使得含氨的木质纤维质材料发生爆炸性膨胀。在大规模商业方法中使用挤出机将是非常昂贵的，因此不能提供经济型方法。

[0010] 在共有的和共同未决的 US NA11/402757 中公开了用于处理生物质以生产可发酵糖的方法，该方法使用低强度氨水预处理高浓度生物质。

[0011] 因此需要用于处理生物质的系统和 / 或方法，其将高干重生物质移动通过低成本的反应器，同时使得化学反应物具有最大穿透性以将生物质预备用于糖化。

[0012] 发明概述

[0013] 本发明提供用于在糖化前处理生物质的方法、通过本发明的方法预处理的生物质、以及包含随后通过糖化该预处理生物质制备的可发酵糖的水解产物。在一个方面，用于处理生物质的方法，该方法包括 ：

[0014] a) 提供生物质 ；

[0015] b) 使用非压实喂料机将 (a) 的生物质装入设备中，所述设备包括 ：

[0016] i) 圆柱形圆筒，该圆柱形圆筒具有配备有活塞的第一末端和配备有排放阀的第二末端 ；

[0017] ii) 任选的支管，所述支管在一个支管末端连接到靠近圆柱形圆筒第一末端的圆柱形圆筒上，并且所述支管在未连接的支管末端具有可密封的阀门 ；

[0018] iii) 圆柱形圆筒或支管中的至少 2 个可密封的端口 ；

[0019] iv) 任选的阀门，所述阀门在圆柱形圆筒中将圆筒分成单独的第一室和第二室，所述第一室具有配备有活塞的圆筒第一末端，并且所述第二室具有配备有排放阀的圆筒第二末端 ；以及

[0020] v) 连接至在圆筒第二末端的排放阀上的闪蒸槽 ；

[0021] 其中将所述生物质装入到所述圆柱形圆筒中或任选地装入到连接至所述圆柱形圆筒的所述支管中 ；

[0022] c) 关闭所述圆柱形圆筒和支管（如果存在的话）；

[0023] d) 任选地经由圆柱形圆筒中的至少一个端口施加真空 ；

[0024] e) 通过圆柱形圆筒或者支管中的所述至少一个端口加入含氨水溶液，加入的量相对于圆筒中生物质的干重为小于约 12 重量百分比，从而生成生物质和氨水的混合物，此外其中所述生物质的干重相对于所述生物质与氨水混合物的重量为至少约 15 重量百分比的高固体浓度，并通过所述圆柱形圆筒或支管（如果存在的话）中的所述第二端口加入蒸汽，以使圆筒内温度达到介于约 85℃和约 180℃之间。

[0025] f) 关闭圆柱形圆筒和支管（如果存在的话）中的端口以提供不可渗透的室 ；

[0026] g) 在所述不可渗透的室中保持所述生物质与氨水混合物在合适温度下一段时间，所述时间介于约 30 秒和约 4 小时之间 ；

[0027] h) 任选地通过所述活塞的位移将所述生物质与氨水混合物移动到圆柱形圆筒中的第二室（如果存在的话）中，其中所述生物质不被压实，并保持介于约 2 分钟和 4 小时之

间的一段时间；以及

[0028] i) 通过所述活塞将所述生物质与氨水混合物移动通过 (g) 或 (h) 的所述不可渗透的圆柱形圆筒并通过排放阀进入到闪蒸槽中；

[0029] 其中生产出经处理过的生物质。

[0030] 在另一方面，用于处理生物质的方法，该方法包括：

[0031] a) 提供生物质和含氨水溶液的混合物，其中生物质的干重相对于生物质与氨水混合物的总重为至少约 15 重量百分比，并且氨水的量相对于生物质的干重为小于约 12 重量百分比；

[0032] b) 使用非压实喂料机将 (a) 的生物质与氨水混合物装入到设备中，所述设备包括：

[0033] i) 圆柱形圆筒，该圆筒具有配备有活塞的第一末端和配备有排放阀的第二末端；

[0034] ii) 任选的支管，所述支管在一个支管末端连接到靠近圆柱形圆筒第一末端的圆柱形圆筒上，并且所述支管在未连接的支管末端具有可密封的阀门；

[0035] iii) 圆柱形圆筒或支管中的至少 2 个可密封的端口；

[0036] iv) 阀门，所述阀门在圆柱形圆筒中将圆筒分成单独的第一室和第二室，所述第一室具有配备有所述活塞的圆筒第一末端，并且所述第二室具有配备有排放阀的圆筒第二末端；以及

[0037] v) 连接至在圆筒第二末端的排放阀上的闪蒸槽；

[0038] 其中将所述生物质装入到圆柱形圆筒的第一室中或任选地装入到连接至所述圆柱形圆筒的所述支管中；

[0039] c) 关闭圆筒中的所述第一室和支管（如果存在的话）；

[0040] d) 任选地经由所述至少一个端口施加真空；

[0041] e) 通过第一室或支管中的至少一个第一端口（如果存在的话）加入蒸汽以使所述室内的温度达到介于约 85℃和约 180℃之间；

[0042] f) 关闭第一室和支管（如果存在的话）中的端口以提供不可渗透的第一室；

[0043] g) 在所述不可渗透的第一室中将所述生物质与氨水混合物保持在合适温度下一段时间，所述时间介于约 30 秒和约 4 小时之间；

[0044] h) 任选地，通过活塞的位移将所述生物质与氨水混合物通过开启的阀门移动通过所述不可渗透的第一室进入到圆柱形圆筒中的第二室中，其中所述生物质不被压实，

[0045] i) 任选地，关闭开启的阀门以形成第二不可渗透的室并保持生物质与氨水混合物介于约 2 分钟和约 4 小时之间的一段时间；以及

[0046] j) 在步骤 (g) 或步骤 (i) 之后通过活塞的位移将所述生物质与氨水混合物移动通过排放阀进入到闪蒸槽中；

[0047] 其中所述生物质不被压实并且以此生产出经处理的生物质。

[0048] 本发明的附加方面涉及已经依照本发明的方法制备的处理生物质，以及包含通过糖化生物质制备的可发酵糖的水解产物，所述生物质已经通过本发明的方法进行了处理。

[0049] 生物质指任何纤维质和 / 或木质纤维质材料，可包括生物能作物、农业残余物、市政固体垃圾、工业固体垃圾、庭院垃圾、木材和林业垃圾或它们的组合。为了减小尺寸、增加暴露的表面积、和 / 或提高存在于生物质中的纤维素、半纤维素和 / 或低聚糖的可及性，可

在步骤 (a) 之前对生物质施加能量。

附图说明

[0050] 图 1 为用于本发明的设备的一个实施方案的示意图。

[0051] 图 2 为用于本发明的设备的第二实施方案的示意图。

[0052] 图 3 为用作排放阀的渐进扩展文丘里管的一个实施方案的示意图，所述阀门关闭。

[0053] 图 4 为图 3 的渐进扩展文丘里管的实施方案的示意图，所述阀门开启。

[0054] 图 5 为 V 型端口阀门渐进扩展文丘里管的一个实施方案的示意图。

[0055] 图 6 为旋启式止回阀渐进扩展文丘里管的一个实施方案的示意图，所述阀门在 A 中关闭，在 B 中开启。

[0056] 发明详述

[0057] 申请人特别将所有引用的参考文献的完整内容引入本公开内容中。此外，当数量、浓度或其他数值或参数以范围、优选范围或优选上限数值和优选下限数值的列表形式给出时，其应理解为具体地公开由任何范围上限或优选数值和任何范围下限或优选数值的任何一对所构成的所有范围，而不管所述范围是否被单独地公开。凡在本文中给出某一数值范围之处，该范围均旨在包含其端点，以及位于该范围内的所有整数和分数，除非另行指出。当定义一个范围时，不旨在将本发明的范围限定于所列举的具体数值。

[0058] 本发明提供用于处理生物质以使其经过糖化来生产可发酵糖的方法。所述糖可经过发酵以生产有价值的产品如燃料和其他化学制品。通过预处理、糖化和发酵步骤，可使用可再生的生物质（包括垃圾生物质）来生产有价值的化学制品，该化学制品可减少对油的需求。

[0059] 定义：

[0060] 在苯公开中使用了许多术语。给出了如下定义。

[0061] “生物质”指任何纤维质或木质纤维质材料，包括包含纤维素的材料，并且任选地还包含半纤维素、木质素、淀粉、低聚糖和 / 或单糖的材料。生物质也可包括附加组分如蛋白质和 / 或脂质。如本发明所述，生物质可来源于单一来源，或者生物质可包括来源于一种以上来源的混合物；例如生物质可包括玉米芯和玉米秸秆或纤维的混合物，或草和叶的混合物。生物质包括但不限于生物能作物、农业残余物、市政固体垃圾、工业固体垃圾、来自造纸业的淤渣、庭院垃圾、木材和林业垃圾。生物质的实例包括但不限于玉米粒、玉米芯、作物残余如玉米壳、玉米秸秆、玉米纤维、草、小麦、小麦秸秆、干草、稻秆、柳枝稷、废纸、蔗渣、高粱杆、大豆外壳或秆、获取自谷物、树、枝、根、叶、木屑、锯末、灌木及矮树丛、蔬菜、水果、花和反刍动物动物粪肥的研磨物的组分。在一个实施方案中，用于本发明的生物质包括具有相对较高碳水化合物值的生物质，它们相对密集，和 / 或相对易于收集、运输、贮存和 / 或处理。在本发明的一个实施方案中，可用的生物质包括玉米芯、玉米秸秆、玉米纤维和蔗渣。

[0062] 术语“可发酵糖”或“糖”指能易于被发酵成目标化学制品的低聚糖和单糖。

[0063] 术语“木质纤维质”指包含木质素和纤维素的材料。木质纤维质材料也可包含半纤维素。

[0064] 术语“纤维质”指包含纤维素的材料。

[0065] 术语“糖化”指从多糖中生产可发酵糖。

[0066] 生物质的“干重”是指移除全部或基本上全部水分后的生物质重量。干重通常依照美国材料与试验协会 (ASTM) 标准 E1756-01(Standard TestMethod for Determination of Total Solids in Biomass) 或纸浆与造纸工业技术协会，Inc.(TAPPI) 标准 T-412om-02(Moisturein Pulp，Paper and Paperboard) 进行测量。

[0067] “含氨水溶液”指在含水介质中使用氨气 (NH_3)、包含铵离子的化合物 (NH_4^+) 如氢氧化铵或硫酸铵、当降解时释放氨的化合物如尿素、以及它们的组合。

[0068] 术语“处理”指反应物作用于材料的过程，其中改变了所述材料的物理和 / 或化学性质。

[0069] 术语“反应物”指能够在处理方法使用的条件下改变目标材料的物理和 / 或化学性质的组合物。

[0070] 用于糖化的“酶聚生体”是能作用于生物质混合物以生产可发酵糖的酶的组合。通常糖化酶聚生体可包括一种或多种糖苷酶，所述糖苷酶可选自纤维素水解糖苷酶、半纤维素水解糖苷酶和淀粉水解糖苷酶。糖化酶聚生体中的其他酶可包括肽酶、脂肪酶、木素酶和阿魏酸酯酶。

[0071] 关于生物质的术语“处理”或“预处理”与以下方法相关。用反应物来处理生物质以形成经处理的生物质产品，也可将其称作处理以形成预处理生物质或预处理以形成预处理的生物质。使用“预”来区分在糖化生物质之前进行的生物质处理。

[0072] 生物质处理方法

[0073] 在共有的和共同未决的 US NA 专利申请 #11/402757 中公开了用于处理生物质以生产可发酵糖的方法，该方法包括使用低强度氨水来预处理高浓度生物质。申请人已经开发了使用低强度氨水和高生物质浓度条件来有效处理生物质的新方法。申请人发现，本发明的方法令人惊讶地很成功，这是因为避免在任何阶段压实生物质，并因此使得在包括生物质压实的系统中处理反应物具有对生物质改善的穿透性。在其中压实生物质的系统中，能够消除生物质的压实状态，改善其与处理反应物的反应，但这需要高能量输入并因此提高了所述系统的成本。在本发明的方法中，不需要消除压实状态的步骤或方法。

[0074] 为了降低大规模生物质处理的成本，已经开发了本发明的方法，其中在非压实状态下将生物质加入到固定设备中，并将其在非压实状态下移动通过该设备。通过保持生物质的非压实状态，不压碎生物质材料的天然小孔和通道。在本发明的方法中使用的处理反应物包括氨水和蒸汽。这些反应物能够穿过非压实的天然生物质的小孔和通道，对生物质的纤维质和木质纤维质材料提供迅速完全的效应。该处理方法高效生产处理生物质，所述生物质经过有效糖化以生产可发酵糖，因此它导致每酶剂量和单位反应时间内生物质碳水化合物到解聚糖的高转化率。

[0075] 参考图 1 和图 2 中的示意图可最好地理解本发明的生物质处理方法，所述示意图显示活塞 / 圆筒型设备的两个实施方案，以及以下在本发明处理方法中使用该设备的描述。为图示清楚起见，这些附图进行了简化，其中省略了一些元件如图 3 和图 4 中的凸缘。图 1 中的设备是测试规模的反应器。它包括水平的圆柱形室 (10)，所述圆筒室具有开放式第一末端以用于加入生物质 (11)，加入生物质后通过插入可移动塞 (12) 将其密封，将该可移动塞用作某种类型的活塞。圆柱形室具有第一可密封端口 (13) 以用于加入含氨水溶

液、第二可密封端口 (14) 以用于将蒸汽加入到圆柱形室的生物质中、以及第三可密封端口 (15) 以用于施加真空。注入蒸汽来提高生物质与氨水混合物的温度以用于处理反应。隔热夹套 (16) 覆盖所述圆柱形室。

[0076] 在装入生物质、施加真空、以及加入含氨水溶液和蒸汽之后，密封端口 (13、14、和 15) 并保持所需温度。在一段时间后，通过移动阀门轴 (19) 开启圆柱形圆筒第二末端 (18) 中的以前关闭的排放阀 (17)。阀门轴延伸穿过在邻近闪蒸槽 (21) 中的向下的内部分离弯管 (20) 中的孔，并穿过在闪蒸槽远侧的填料压盖 (22) 直至致动器 (23)。通过将圆柱形圆筒第一末端的塞子朝第一末端移动，把生物质与氨水混合物挤过排放阀 (17)。生物质通过排放阀并通过弯管 (20) 进入到闪蒸槽 (21) 中。覆盖在闪蒸槽底部开口的上盖 (24) 允许通向预处理生物质。在闪蒸槽顶部的端口 (25) 允许蒸汽逸出，并通过管子 (26) 连接到冷凝器 (27) 上。

[0077] 本文实例中的图 1 所示设备及其在本发明的处理方法中使用的实施方案在下文中进行进一步描述。筒式活塞反应器由配备有水平取向活塞的 5.1cm×68.6cm 的不锈钢圆筒组成。用四个 O 形环将活塞密封到筒上，并在排放期间在活塞背面用氮气为活塞加压（最多约 5600kPa）。68.6cm 的圆筒配备有八个多用途端口，沿着顶部表面和底部表面各 4 个，它们允许用真空、氨水注射、蒸汽注射、和插入热电偶的方法测量筒内温度。反应器圆筒配备有蒸汽夹套用于圆筒的均匀加热。将反应器圆筒直接连接到垂直取向的 15.2cm×61cm 的不锈钢闪蒸槽上。通过锥形喷嘴和底座末端剪切阀排列从闪蒸槽上分离圆筒。末端阀剪切模头的直径是 3.5cm。锥形喷嘴和底座上的背压是可调的，大多数测试使用～ 138kPa（测量压力）的背压进行，使其进入 10.2cm 直径的空气圆筒中，该圆筒与末端剪切阀的锥形嘴相连。末端剪切阀门的锥形嘴能缩回最多 1.6cm，允许排放闪蒸槽中的颗粒。末端剪切阀出口的弯管引导处理固体向下进入到闪蒸槽的底部，其中所述固体可通过打开槽底部的圆顶螺栓轻易移除。闪蒸槽的上部汽室凸缘加入一个具有被加工成与闪蒸槽轴线呈直角的狭槽的特殊出口，这引起释放的蒸汽沿着拐角路径进入到排出装置中，有助于防止产生的生物质颗粒遗留和水滴进入到排气冷凝器中。沿着闪蒸槽加上三个带状电加热器（设为 60℃）和隔热物，使得热处理的固体闪蒸到加热容器中，更好的模拟商业规模的方法。

[0078] 在另一个实施方案中，如上所述构建小型筒式活塞反应器，它除了具有 45.7cm 的圆筒之外，无蒸汽夹套、三个带状电加热器、覆盖有充满硅氧烷的玻璃纤维夹套的 2.5cm 厚作隔热用的玻璃纤维垫、以及三个多用途端口。其他部件包括用于大型圆筒活塞反应器的闪蒸槽、剪切阀门、和弯管。

[0079] 图 2 所示的设备是商业规模的反应器设计。它包括在第一末端 (33) 装配有活塞 (34) 并在第二末端 (41) 装配有排放阀 (40) 的水平圆柱形圆筒。圆筒进行了隔热处理并具有不可渗透的壁。将支管 (31) 连接到靠近第一末端处，并且将阀 (35) 即进料阀定位于支管的未连接末端。将料斗 (30) 连接到支管的阀门末端。通过料斗加入生物质。可以用非压实的流动引导装置来控制从料斗 (30) 将生物质加入到支管 (31) 中。支管具有第一密封端口 (36) 和第二密封端口 (37)，它们用于当氨水和蒸汽移动到圆柱形圆筒中时，将其加入到支管中的生物质中。第二阀门 (38) 将圆筒分成第一圆柱形室 (32) 和第二圆柱形室 (39)。生物质与氨水混合物通过支管进入第一室，其中通过加入蒸汽达到所需的温度和压力。将活塞移动通过不可渗透的圆筒来推动生物质和氨水混合物从第一室通过开启的第

二阀门 (38) 进入第二室，并将第二室 (39) 的内容物通过开启的排放阀 (40) 转移到闪蒸槽 (42) 中。第二室的内容物是以前移动到该室中并保持在使用条件下处理反应必需时间的生物质与氨水混合物。然后关闭第二阀门 (38) 并拉回活塞 (34) 以便第一圆柱形室 (32) 能够再装载物质并重复循环过程。在闪蒸槽 (42) 中，生物质移动通过向下的弯管 (43)。覆盖在闪蒸槽底部开口的上盖 (44) 允许通向预处理生物质。在闪蒸槽顶部的端口 (45) 允许氨蒸汽逸出，并通过管子 (46) 连接到冷凝器 (47) 上。

[0080] 可使用碳钢或不锈钢制造该设备。圆柱形圆筒可以如图 1 和 2 所示是水平式的，或者可以是竖式的。如图 2 所示的具有竖式圆筒的支管和料斗将进行改装以允许将生物质装入到圆筒室中，例如在小于 90 度的角度。本领域的技术人员将能够容易地配置该具有竖式圆筒的设备。例如，可将竖式圆筒定位在闪蒸槽上面并进行连接，该连接不通过向下的弯管，因为流经排放阀将已经向下定向了。确定闪蒸槽是竖式的还是水平式的也在本领域技术人员的能力范围内。在本发明的方法中竖式槽更适合氨处理，它有利于移除和捕集闪蒸槽中释放的氨气。

[0081] 图 1 和 2 中的两个实施方案功能相似，加入生物质并使其在非压实状态下移动通过反应器。具有一个室的图 1 的实施方案是一次加工一个生物质样本的分批系统。图 2 的实施方案具有通过阀门分开的两个室，允许半连续或分批补料运行，其中同时进行多次生物质装载。在该第二实施方案中，第二室满载荷之后，其中每个连续生物质装载进入第二室的每个活塞位移循环伴随通过排放孔来排放对应的体积。第二室中一定时间内活塞位移循环的次数，以及因此的第二室尺寸，与每个生物质样本所需的停留时间有关。停留时间在下文中与参照本发明方法处理的温度和时间进行进一步讨论。

[0082] 本发明的方法尤其适合处理相对于处理反应的生物质、氨水和蒸汽混合物重量的生物质干重较高的生物质。期望的是处理高干重浓度的生物质以提供糖化后将生产高糖浓度水解产物的生物质。提供上述生物质的本发明方法的特点是不挤压，因此允许有效处理高干重浓度的生物质。本发明的方法使用的生物质的初始干重为生物质与氨水混合物总重的至少约 15％。更典型的，生物质干重为至少约 20％并可以是为至少约 30％，45％，50％，或更高。生物质的干重百分比可以变化，并且不同类型生物质的最佳百分比可以不同。例如，当使用玉米芯时，期望至少约 24％的干重百分比，以提供糖化的预处理生物质来生产浓度足以发酵成乙醇的可发酵糖。更合适的是至少约 30％的玉米芯生物质。本领域的技术人员易于决定用于生产高糖水解产物的本发明方法中的特定类型生物质的优选干重百分比。

[0083] 可直接使用获取自来源的生物质，或施加能量到生物质上以减小尺寸、增加暴露的表面积、和 / 或提高存在于生物质中的纤维素、半纤维素和 / 或低聚糖的可用性。用于此目的的能量设备包括那些不压碎或压实生物质的设备，以便不破坏生物质的超微结构。例如，可切碎、剁碎、或削碎生物质。当以不压碎超微结构的方式剪切生物质时也可使用颚式破碎机。在以本发明的方法预处理之前也可使用齿状盘式精炼器来减小生物质尺寸。

[0084] 在本发明的处理方法中使用非压实喂料机将生物质移动到圆柱形圆筒中。在最简单的情况下，非压实喂料机指用手将生物质装入到圆柱形圆筒的开启的第一末端中。如果在圆筒中有两个室，可装载到第一室中。该方法描述于本文实施例中，使用如图 1 所示的反应器。在图 2 的反应器中示出的非压实喂料机是料斗。料斗可以是自卸式，和 / 或可配备有不提供压实力的流动引导装置。例如可使用多种类型的活底箱导流轮，随后是流量计量

输送器如多种类型的牵引链、链斗升降机、或螺旋旋转输送器（如Acrison®装置）。在第一圆柱形室中装入的生物质的量是有限的，以便留出允许生物质膨胀的空间，所述膨胀可能在加入氨水和蒸汽时发生。

[0085] 可将真空加入到包含生物质的圆柱形圆筒上。如果在圆筒中有两个室，可将真空施加于包含生物质的第一室。通常如果施加真空，则压力减少至小于约20kPa。通过圆柱形圆筒或者支管中的一个或多个端口将含氨水溶液加入，加入的量使得氨相对于室中生物质的干重为小于约12重量百分比。更适合使用分布好的一个以上的端口以便氨溶液基本上平均分配到生物质中与其接触。如果在圆筒中有两个室，将氨溶液加入到包含生物质的第一室中。更合适加入氨的量也可以是相对于室中生物质的干重介于约4％和约6％之间。可预热氨溶液，这将有助于提高生物质的温度。在一个可供选择的实施方案中，氨水溶液在装入第一圆柱形室之前与生物质混合。生物质和氨水可在容器中混合并装入到第一圆柱形室中。例如，可将氨水泵入并通过一个内嵌式加热器，然后进入包含生物质的桨式搅拌器中。然后将生物质与氨水混合物装入第一圆柱形室中，其中在关闭室后注入蒸汽。作为另外一种选择，可预混生物质、氨、和蒸汽并把它们加入到第一圆柱形室中。在下文所述的温度和压力下，许多氨水将蒸发成蒸汽，蒸汽渗透到进行预处理的生物质中。此外，可将收集自闪蒸槽的循环湿氨蒸汽注入以形成总加入氨的一部分。

[0086] 在本发明的方法中，含氨水溶液也任选地包含至少一种附加的碱，如氢氧化钠、碳酸钠、氢氧化钾、和碳酸钾。可加入所述至少一种附加碱，加入的量相对于生物质干重为最多10重量百分比。例如可利用附加碱来中和生物质中的酸，从而提供金属离子以用于糖化酶或发酵培养基。

[0087] 因为在本发明的方法中生物质未被压实，它不会阻塞蒸汽通道，而在使用压实生物质的系统中则会阻塞蒸汽通道。因此加入蒸汽的室在蒸汽注入前关闭。密封除一个或多个加入蒸汽的端口之外的其他端口。将圆筒第一末端的活塞或作为活塞使用的塞子就位并关闭阀门。使用的阀门可以是任何开启或关闭型阀门，如提升阀或旋转刀闸阀。

[0088] 通过圆柱形圆筒中的一个或多个阀门或者支管将蒸汽加入，需要加入的蒸汽量能提高生物质与氨水混合物温度至所需温度点。如果在圆筒中有两个室，则将蒸汽加入到包含生物质的第一室中。更合适的是使用一个以上的端口，并且端口间有一定间距，以便蒸汽分配到生物质中与其接触。加入蒸汽以使生物质与氨水混合物的温度提高至介于85℃和约180℃之间。如需要的话，可通过第二圆柱形室（如果存在的话）中的端口加入附加蒸汽以保持所需温度。所述设备可包括加热夹套、蒸汽夹套、带状加热器、或隔热夹套以利于提高和/或保持温度。加热夹套或蒸汽夹套尤其适合小型反应器，而隔热夹套适合大型反应器。加热可在不同阶段进行，包括在处理或预处理前预热圆筒。

[0089] 在低于85℃的温度下，用低强度氨水进行处理所需的时间将非常长。处理所需的时间随温度升高而减少。例如，在85℃下进行处理可能需要的时间介于约两小时和约四小时之间，而在180℃下进行处理可能仅需几分钟。在图2反应器中使用的分批给料循环功能需要足够的时间来进行多次给料。因此期望选择用于有时间限制的时间和温度组合，该组合的时间足够使用反应器的实施方案发挥功能，而适中的温度可提供经济型方法。在适中温度下，可使用成本更低的低压蒸汽。更合适的条件是在介于约120℃和约160℃的温度下处理介于约60分钟和约5分钟之间，时间随着温度升高而减少。尤其合适的条件是在介于

约 140℃和约 150℃的温度下处理介于约 30 分钟和约 10 分钟之间，时间随着温度升高而减少。要预处理的生物质的类型也能影响本发明的方法中处理的最佳时间和温度，本领域的技术人员能够容易地对其进行评估。

[0090] 反应器室中的生物质保持在所需温度的时间是停留时间。当使用只有第一室的反应器时，则停留时间发生在第一室中。当使用具有第一室和第二室的反应器时，则第一室中的时间仅仅足以在将混合物移动至第二室之前使生物质和反应物结合，停留时间发生在第二室中。在该情况下，第一室中的时间可以是约 30 秒，第二室中的时间可以介于约 2 分钟和 4 小时之间。

[0091] 在本发明的方法中使用蒸汽使生物质达到所述温度导致反应器室中压力介于约 60kPa 和约 750kPa 之间。更典型，压力介于约 300kPA 和 600kPA 之间。相对于其他已知的预处理方法如 US 5037663 所述的 AFEX 方法，其中使用 1150kPa 至 4250kPa 的压力，或者如 US 4461648 所述的使用蒸汽喷射的方法，其中使用约 1800kPa 至约 5600kPa 的压力（如本文图 1 所示），这些压力是相对低的压力。在更适中的压力下运行本发明的方法提供成本较低的系统，因为可使用压力更低的蒸汽。

[0092] 在本发明的方法中，在非压实状态下将生物质移动通过第一室和第二室（如果存在的话）。这可使用活塞和不可渗透的圆柱形室来完成。对于本公开内容而言，活塞可包括任何可用作活塞的制品，如推入室中的塞子，以及任何类型的标准活塞。可以使用任何施加足以移动生物质的压力的方法，将图 1 中例示的反应器类型的塞子推入室中。尤其合适的方法是在插入塞子后提供室末端的静态闭合，例如有螺栓的圆柱体头部，然后在闭合和塞子之间导入氮气以积聚压力并移动塞子。所述塞子可通过其他装置移动，例如使用连接到液压式、气动式、或电动式致动器上的推杆。

[0093] 所述设备的圆筒是不可渗透的（闭合所有端口和阀门），其中无未密封的壁渗透，因此液体不流出圆筒。液体保留使得活塞在不压实生物质的情况下移动它们。本发明的处理方法中的液体是有限的，它可润滑室壁，使得非压实的流动响应于活塞压力发生。事实上，活塞压力可临时轻微地挤压生物质，如同海绵一样，不把生物质挤压到小孔和通道塌陷的程度。移除活塞压力之后，生物质可再吸收液体进入不受挤压的小孔和通道中。为了帮助生物质流动，可将润滑液体如植物油皂导入室中。可通过在室内壁上制作膛线来提高流动性，其中加装不连续部分如成角的凹槽可减少摩擦，因此降低屈服应力并改善生物质流动性。非压实的生物质移动保持了处理生成的充满液体的孔，这会促进随后的糖化。

[0094] 在本发明的方法中，在所需温度处理所需时间后，将生物质与氨水混合物移动通过圆柱形圆筒末端的排放阀进入到闪蒸槽中。生物质在所需温度与氨水反应期间关闭排放阀，然后开启排放阀使生物质通过。如图 2 所示，在活塞在第一室中产生压力之后，为了用第一室内容物置换第二室的全部内容物，在两室反应器中排放阀开启与在第一和第二室之间的阀门开启同时进行。

[0095] 可使用的排放阀用 V 型端口回转阀、旋启式止回阀、和提升排放阀进行例示。如图 1 所示，在小型反应器中尤其有用的是活塞驱动的提升阀型排放阀，其中阀门底座的硬面上游侧是排放孔，而阀门底座的较软下游侧密封件靠着硬面的阀门压杆，当阀门压杆缩回开启阀门时，阀门底座以外的流动区域持续增加。

[0096] 最合适的提升阀型排放阀将连接一个渐进扩展文丘里管。渐进扩展文丘里管提升

阀的一个实施方案在图 3 中示出，该文丘里管排放阀适用于如图 1 所示的小型反应器。这个阀门加上圆锥形喷嘴和底座末端剪切阀排列。为了避免堵塞，设计如图 3（关闭位置）和图 4（打开位置）所示的渐进扩展文丘里管以加速固体通过文丘里管的固定外圆锥 (50) 和文丘里管的可移动内圆锥 (51) 之间的平稳扩大的间隙，其中将可移动内圆锥安装在阀门轴 (52) 的末端上。文丘里管外圆锥是夹在反应器室 (54 ；等同于图 1 中的 10) 出口的凸缘 (53) 和闪蒸槽入口凸缘 (55) 之间的一般呈环形的文丘里管。文丘里管内圆锥 (51) 是在反应器出口阀门轴 (52) 末端的鼻形物。文丘里管内圆锥和阀门轴位于排放弯管 (56 ；等同于图 1 中的 20) 内部，排放弯管位于闪蒸槽 (57 ；等同于图 1 中的 21) 内部。将阀门轴连接到致动器 (58) 上以控制移动。致动器可以是能够前后平移动阀门轴的任何装置，如电动式、气动式或液压式马达、气动式阀门致动器、或液压式活塞。当阀门轴在其最远的左侧位置时，内圆锥底座的外边缘靠着外圆锥内边缘以在处理期间密封反应器的排放末端。当排放反应器的时候，向右移动阀门轴以提供闪蒸文丘里管所需大小的开口。

[0097] 该设计提供一定长度的闪蒸区域，该区域在流动方向上平稳地扩展。在该设计中，生物质固体沿着渐进开口的环形锥形嘴的轴线加速，这避免了导致堵塞的突然径向扩展。

[0098] 渐进扩展文丘里管的另一个实施方案在图 5 中示出，该文丘里管适合作为排放阀使用，尤其是在如图 2 所示的大型反应器中使用。这是 V 型端口旋塞的实施方案，其中将闪蒸扩展文丘里管机器加工到阀体中。在闪蒸文丘里管固定主体 (70) 中有从反应室 (72) 出口端开始的狭窄部分 (71) 和扩展至闪蒸槽 (74) 入口的扩展部分 (73)。在旋塞的旋转中心 (75) 是成角度的开口 (76)，它当在开口位置时与反应器室狭窄部分 (71) 对齐，并扩展至闪蒸槽 (73)。将旋转中心 (75) 旋上一半以阻止靠近阀门的旋塞的对齐。

[0099] 渐进扩展文丘里管的另一个实施方案在图 6 中示出，该文丘里管适合作为排放阀使用，尤其是在如图 2 所示的大型反应器中使用。这是旋启式止回阀的实施方案，它有锥形嘴 (80)，适合在反应器室 (72) 和闪蒸槽 (74) 入口之间的狭窄接头 (81)（图 6A）。该锥形嘴位于连接到轴 (83) 的臂 (82) 上，所述轴穿过填料压盖直至回转阀致动器。该轴的旋转方向如虚线箭头所示，它逆时针旋转移动臂以开启接头，形成渐进扩展的文丘里管（图 6B）。在用于渐进扩展文丘里管的旋启式止回阀的另一个实施方案中，锥形嘴的直径可以是若干英尺，它逆时针移动一段距离以开启仅有几英寸的阀门（小于 8cm）。

[0100] 生物质和氨混合物移动通过排放阀进入闪蒸槽中，该闪蒸槽能够保持真空。在闪蒸槽中氨从处理生物质中释放出来并冷却生物质，为糖化作准备。可使用任何典型的闪蒸槽，其具有切向或涡形入口，为分离弯管提供最合适的功能。尤其合适的是以不同压力依次施加数次闪蒸，以从预处理生物质中释放氨。例如，第一次闪蒸的压力接近大气压，通常移除大多数游离氨并将材料冷却至约 100℃。第二次闪蒸压力小于约 20kPa，移除剩余的游离氨并将材料冷却至约 50℃，该温度是糖化所需的温度。

[0101] 在闪蒸槽中从通过排放阀的生物质和氨混合物中释放的氨蒸汽可从闪蒸槽中回收，并可循环利用。来自低压闪蒸的蒸汽可以使用无中间冷却的标准蒸汽再压缩设备（如涡轮或蒸汽喷射泵）循环利用。因此氨蒸汽可不冷凝直接循环用于处理，或者可以在再使用前进行冷凝。在后一种情况下，将收集的蒸汽加到如图 1 所示的冷凝器中。

[0102] 减少处理生物质中的氨将会降低 pH 并减少酸用量，所述酸用于调节 pH 使其达到令糖化酶活性使人满意的水平。这是令人期望的，因为大量加入酸可导致形成盐，其浓度对

糖化酶或对微生物生长造成抑制。另一方面，生物质中残留的氨可作为氮源使用，以维持发酵期间微生物的生长。因此剩余的氨可减少或消除在发酵期间用氮源补充培养基的需要。通常移除至少一部分氨，这降低了 pH 但留下一些氮，这些氮用于为随后的发酵提供此种营养物质。

[0103] 当预处理生物质在闪蒸槽底部积聚时，可以用桨式搅拌器搅拌，所述桨式搅拌器可连接到闪蒸槽底部。通常通过打开槽底部的覆盖件从闪蒸槽底部移除预处理生物质。用于连续提取预处理生物质的活底机械装置尤其适用。为了在本发明的设备中处理多批生物质，一批生物质和氨可以在圆筒室内，然而另一批在闪蒸槽内。在两室设备中，数批物料可以同时在两室内和闪蒸槽内。此外，可以在移除前在闪蒸槽内收集多批预处理生物质。

[0104] 在处理后，产品通常包括氨的混合物、部分降解的生物质和一些可发酵糖。可以从闪蒸槽中移除包括可溶性部分和不溶性部分的全部预处理生物质并在糖化反应中利用它们。作为另外一种选择，在糖化前可以从预处理生物质混合物中排走一些液体以便糖化反应中的生物质干重保持高水平。处理后可能存在过多的液体，尤其是当需要大量蒸汽提高并维持生物质处理的温度时更是如此。

[0105] 在另一个可供选择的实施方案中，生物质固体可在本发明的方法中通过处理再循环。

[0106] 糖化

[0107] 用本发明的方法处理的生物质在存在糖化酶（可称为糖化酶聚生体）的情况下进一步进行水解，以释放水解产物中的低聚糖和 / 或单糖。用于生物质处理的糖化酶和方法参见 Lynd, L.R. 等人 (Microbiol.Mol.Biol.Rev.(2002)66 :506-577)。

[0108] 在糖化之前，可处理经预处理的生物质以改变 pH、组合物或温度使得糖化酶聚生体中的酶将有活性。可通过加入固体或液体形式的酸来改变 pH。作为另外一种选择，可利用可从发酵中回收的二氧化碳 (CO_2) 来降低 pH。例如，如果存在足够液体，可从发酵罐中收集 CO_2 并将其通入闪蒸槽中的预处理产物顶部空间或起泡通过预处理生物质，同时监控 pH 直至达到所需的 pH。可使温度达到下文提到的适合糖化酶活性的温度。可加入糖化过程中使用的酶活性所需的任何辅因子。

[0109] 糖化酶聚生体包括一种或多种酶，所述酶主要选自（但非排他性地）“糖苷酶”类，所述酶水解二糖、单糖、和多糖的醚键，存在于广义“水解酶”(EC 3.) 的分类酶 EC 3.2.1.x 中 (Enzyme Nomenclature 1992, Academic Press, San Diego, CA with Supplement 1(1993), Supplement 2(1994), Supplement 3(1995, Supplement 4(1997) andSupplement5[分别在 Eur.J.Biochem.(1994)223 :1-5, Eur.J.Biochem.(1995)232 :1-6, Eur.J.Biochem.(1996)237 :1-5, Eur.J.Biochem.(1997)250 :1-6, 和 Eur.J.Biochem.(1999)264 :610-650 中])。本发明的方法中可用的糖苷酶可根据它们水解的生物质组分进行分类。本发明的方法可用的糖苷酶包括纤维素水解糖苷酶（例如，纤维素酶、内葡聚糖酶、外葡聚糖酶、纤维二糖水解酶、β - 葡萄糖苷酶）、半纤维素水解糖苷酶（例如，木聚糖酶、内木聚糖酶、外木聚糖酶、β - 木聚糖苷酶、阿拉伯糖基木聚糖酶、甘露聚糖酶、半乳糖酶、果胶酶、葡糖醛酸酶）、和淀粉水解糖苷酶（例如，淀粉酶、α - 淀粉酶、β - 淀粉酶、葡萄糖淀粉酶、α - 葡萄糖苷酶、异淀粉酶）。此外，可将其他活性剂加入到糖化酶聚生体中，如肽酶 (EC 3.4.x.y)、脂肪酶 (EC 3.1.1.x 和 3.1.4.x)、木素酶 (EC 1.11.1.x)、和阿魏酸酯

酶 (EC 3.1.1.73)，以帮助从生物质的其他组分中释放多糖。本领域熟知的是，生产多糖水解酶的微生物常常表现出某种活性，如纤维素降解，该活性由若干种酶或一组具有不同底物特异性的酶催化。因此，来自微生物的“纤维素酶”可包括一组酶，所有酶可有助于纤维素降解活性。取决于获取酶时利用的纯化方案，商业或非商业酶制剂如纤维素酶可包括多种酶。因此，本发明方法的糖化酶聚生体可包括酶活性剂例如“纤维素酶”，然而人们认识到该活性可被一种以上的酶催化。糖化酶可商业获取，如获取自 Spezyme®CP 的纤维素酶 (GenencorInternational，Rochester，NY) 和 Multifect®木聚糖酶 (Genencor)。此外，糖化酶可通过生物方法制备，包括使用重组微生物方法。

[0110] 本领域的技术人员将懂得如何测定在酶聚生体中使用的酶的有效量，以及如何调节条件以获得最佳酶活性。本领域的技术人员也将懂得如何优化在酶聚生体中的此类酶的所需活性，以在选择条件下获得给定预处理产物的最佳糖化效果。

[0111] 优选地，糖化反应在糖化酶的最佳温度和 pH 下或接近此最佳 pH 和温度的条件下进行。在本发明的方法中，糖化酶聚生体使用的最佳温度在约 15℃至约 100℃的范围内。在另一个实施方案中，最佳温度在约 20℃至约 80℃的范围内。最佳 pH 可在约 2 至约 11 的范围内。另一个实施方案中，在本发明的方法中，糖化酶聚生体使用的最佳 pH 在约 4 至约 10 的范围内。

[0112] 糖化可进行约若干分钟至约 120 小时，优选约若干分钟至约 48 小时。反应时间将取决于酶浓度和比活性、已经使用的底物和环境条件例如温度和 pH。本领域的技术人员能够容易地决定特定底物和糖化酶聚生体使用的温度、pH 和时间的最佳条件。

[0113] 糖化可分批进行或以连续方法进行。糖化也可一步进行或多步进行。例如，糖化所需的不同酶可表现出不同的最佳 pH 或温度。可用酶在某个温度和 pH 下进行首次处理，随后使用不同酶在不同温度和 / 或 pH 下进行第二次或第三次 (或更多次) 处理。此外，用不同酶在连续步骤中进行的处理可以在相同 pH 和 / 或温度下进行，或在不同 pH 和温度下进行，例如使用在较高 pH 和温度下稳定的和活性更高的半纤维素酶处理，随后用在较低 pH 和温度下有活性的纤维素酶处理。

[0114] 糖化之后的生物质的糖的溶解度可通过测量释放的单糖和低聚糖进行监控。测量单糖和低聚糖的方法是本领域熟知的。例如，还原糖的浓度可使用 1，3- 二硝基水杨酸 (DNS) 检测分析法 (Miller，G.L.，Anal.Chem.(1959)31 :426-428) 进行测定。作为另外一种选择，如本文在一般方法部分所述，可通过 HPLC 使用合适柱来测量糖。

[0115] 发酵

[0116] 合适的微生物可使用生物质释放的可发酵糖来生产目标化学制品。在糖化之后，但是在发酵之前，可通过例如蒸发浓缩糖化混合物以提高可发酵糖的浓度。任选地，在糖化产物中的液体可从分批或连续方法中的固体中分离。任选地，液体或全部糖化产物可在发酵前灭菌。取决于发酵期间使用的微生物和糖化期间使用的 pH，可将 pH 调节至适于发酵的水平。此外，可用微生物生长所需的附加营养物质来补充糖化混合物。补充剂可包括例如酵母提取物、特定氨基酸、磷酸盐、氮源、盐、和痕量元素。也可包括通过特定生物催化剂来生产特定产品所需的组分，如用于保留质粒的抗生素或酶催化反应所需的辅因子。也可包括附加的糖以提高总糖浓度。可使用糖化混合物作为发酵肉汤的组分，例如，制备介于约 100％和约 10％之间的最终培养基。

[0117] 取决于发酵微生物使用的条件，也可调节温度和 / 或顶部空间气体。发酵可以是有氧的或厌氧的。发酵可以在糖化之后发生，或可以通过同步糖化和发酵过程 (SSF) 使其与糖化同时发生。SSF 能够使糖化生产的糖含量保持低水平，因此减少潜在的糖化酶产物抑制，减少污染微生物的糖可用性，并改善预处理生物质向单糖和 / 或低聚糖的转化。

[0118] 可通过发酵制备的目标化学制品包括例如酸、醇、烷烃、烯烃、芳族、醛、酮、生物高聚物、蛋白质、肽、氨基酸、维生素、抗生素、和药物。醇包括但不限于甲醇、乙醇、丙醇、异丙醇、丁醇、乙二醇、丙二醇、丁二醇、甘油、赤藓醇、木糖醇、和山梨醇。酸包括乙酸、乳酸、丙酸、3- 羟基丙酸、丁酸、葡萄糖酸、衣康酸、柠檬酸、琥珀酸和乙酰丙酸。氨基酸包括谷氨酸、天冬氨酸、甲硫氨酸、赖氨酸、甘氨酸、精氨酸、苏氨酸、苯基丙氨酸和酪氨酸。附加的目标化学制品包括甲烷、乙烯、丙酮和工业酶。

[0119] 可通过一种或多种合适的生物催化剂在一步或多步发酵中把糖发酵成目标化学制品。生物催化剂可以是选自细菌、丝状真菌和酵母的微生物。生物催化剂可以是野生型微生物或重组微生物，并包括埃希氏菌属、发酵单胞菌属、糖酵母属、假丝酵母属、毕赤酵母属、链霉菌属、芽孢杆菌属、乳酸杆菌、和梭菌属。在另一个实施方案中，生物催化剂可以选自重组大肠杆菌、运动发酵单胞菌、嗜热脂肪芽胞杆菌、啤酒糖酵母、嗜热梭菌、高温产氢菌、和树干毕赤酵母。

[0120] 已经描述了多种用于发酵生产目标化学制品的生物催化剂，并可发现、通过突变生产、或通过重组方法来工程化其他生物催化剂。任何使用利用本发明的方法糖化处理生物质生产的可发酵糖的生物催化剂可以被用于制备已知可通过发酵生产的目标化学制品。

[0121] 生产包括乙醇和丁醇在内的生物燃料的生物催化剂是尤其受关注的。例如，通过产溶剂梭菌将碳水化合物发酵成丙酮、丁醇、和乙醇 (ABE 发酵) 是为人熟知的 (Jones 和 Woods(1986)Microbiol.Rev.50 :484-524)。US 5192673 描述了用于使用丙酮丁醇梭菌突变株生产高含量丁醇、丙酮和乙醇的发酵方法。US 6358717 描述了用于使用拜氏梭菌突变株生产高含量丁醇、丙酮和乙醇的方法。共有的和共同未决的专利申请 WO2007/041269 和 WO 2007/050671 分别公开了遗传工程的微生物宿主中生产 1- 丁醇和异丁醇。共有的和共同未决的美国专利申请 #11/741892 和 #11/741916 公开了在遗传工程的微生物宿主中来生产 2- 丁醇。通过微生物宿主按照公开的方法可从使用本发明的方法生产的水解产物中发酵生产异丁醇、1- 丁醇或 2- 丁醇。

[0122] 已经使用遗传修饰的大肠杆菌菌株作为乙醇生产的生物催化剂 (Underwood 等人，(2002)Appl.Environ.Microbiol.68 :6263-6272)。在 US 2003/0162271A1 中描述了已经改善了乙醇生产的遗传修饰运动发酵单胞菌菌株。在共有的和共同未决的美国专利申请 60/847813 和 60/847856 中分别描述了用于乙醇生产的进一步工程化的运动发酵单胞菌乙醇生产菌株及其作用。通过运动发酵单胞菌按照公开的方法可以从使用本发明的方法生产的水解产物中发酵生产乙醇。

[0123] 通过大肠杆菌重组菌株 (Zhou 等人，(2003)Appl.Environ.Microbiol.69 :399-407)、芽孢杆菌属天然菌株 (US20050250192)、和米根霉 (Tay 和 Yang(2002)Biotechnol.Bioeng.80 :1-12) 在发酵过程中生产乳酸。已经使用大肠杆菌重组菌株作为生物催化剂在发酵中生产 1,3 丙二醇 (US 6013494，US 6514733) 和己二酸 (Niu 等人，(2002)Biotechnol.Prog.18 :201-211)。使用重组梭菌 (Cheryan 等人，(1997)Adv.

Appl.Microbiol.43:1-33) 和新鉴定的酵母菌株 (Freer(2002)World J.Microbiol. Biotechnol.18:271-275) 通过发酵制备乙酸。在 US 6159738 中公开了通过重组大肠杆菌和其他细菌生产琥珀酸,在 Lin 等人 ((2005)Metab.Eng.7:116-127) 中公开了通过突变型重组大肠杆菌生产琥珀酸。已经通过光滑球拟酵母突变株 (Li 等人,(2001)Appl. Microbiol.Technol.55:680-685) 和大肠杆菌突变株 (Yokota 等人,(1994)Biosci. Biotech.Biochem.58:2164-2167) 制备了丙酮酸。已经使用大肠杆菌重组菌株作为生物催化剂用于生产对 - 羟基肉桂酸 (US20030170834) 和奎尼酸 (US20060003429)。

[0124] 已经在发酵过程中使用丙酸丙酸杆菌突变株生产丙酸 (Suwannakham 和 Yang(2005)Biotechnol.Bioeng.91:325-337),并已经使用酪丁酸梭菌制备丁酸 (Wu 和 Yang(2003)Biotechnol.Bioeng.82:93-102)。已经通过发酵从梭菌属菌株 17crl(Janssen(2004)Arch.Microbiol.182:482-486) 的苏氨酸中制备了丙酸盐和丙醇。已经使用酵母样普鲁兰出芽短梗霉 (Anantassiadis 等人,(2005)Biotechnol.Bioeng.91:494-501) 通过曲霉菌曲霉突变株 (Singh 等人,(2001)Indian J.Exp.Biol.39:1136-43) 制备葡萄糖酸。通过氧化葡萄糖酸杆菌突变株制备 5- 酮基 -D- 葡萄糖酸 (Elfari 等人,(2005)Appl Microbiol.Biotech.66:668-674),通过土曲霉突变株制备衣康酸 (Reddy 和 Singh(2002)Bioresour.Technol.85:69-71),通过曲霉菌曲霉突变株生产柠檬酸 (Ikram-Ul-Haq 等人,(2005)Bioresour.Technol.96:645-648),并通过高里假丝酵母 FTI 20037 生产木糖醇 (Mus satto 和 Roberto(2003)J.Appl.Microbiol.95:331-337)。通过重组红串红球菌和富养罗尔斯通氏菌 (Gorenflo 等人,(2001)Biomacromolecules 2:45-57) 生产包含 4- 羟基戊酸乙酯和显著量 3- 羟基丁酸 3- 羟基戊酸的生物聚酯。通过重组大肠杆菌 (Ui 等人,(2004)Lett.Appl.Microbiol.39:533-537) 制备 L-2,3- 丁二醇。

[0125] 可通过使用棒状杆菌、短杆菌、和沙雷氏菌的营养缺陷型菌株和氨基酸类似物抗性菌株发酵生产氨基酸。例如,日本专利公布 56008596 描述了使用组氨酸类似物抗性菌株生产组氨酸,EP 136359 描述了使用重组菌株生产组氨酸。日本专利公布 47004505 和 51019037 描述了使用色氨酸类似物抗性菌株生产色氨酸。日本专利公布 47038995、51006237、54032070 描述了使用异亮氨酸类似物抗性菌株生产异亮氨酸。日本专利公布 56010035 描述了使用苯基丙氨酸类似物抗性菌株生产苯基丙氨酸。已经描述了使用需要苯基丙氨酸生长的酪氨酸抗性菌株 (Agr.Chem.Soc.Japan 50(1)R79-R87(1976),或重组菌株 (EP263515,EP332234) 生产酪氨酸,以及使用 L- 精氨酸类似物抗性菌株生产精氨酸 (Agr. Biol.Chem.(1972)36:1675-1684,日本专利公布 54037235 和 57150381)。也通过在大肠杆菌菌株 ATCC31882、31883、和 31884 中通过发酵生产苯基丙氨酸。US 6962805 描述了在重组棒状杆菌中生产谷氨酸。在 Okamoto 和 Ikeda(2000)J.BiosciBioeng 89:87-79 中描述了通过人肠杆菌突变株生产苏氨酸。通过百合棒状杆菌突变株生产甲硫氨酸 (Kumar 等人,(2005)Bioresour.Technol.96:287-294)。

[0126] 通过生物催化剂也已经制备出有用的肽、酶、和其他蛋白质 (例如参见 US6861237、US6777207、US6228630)。

[0127] 在本文实施例 5 中举例说明预处理和糖化生物质成可发酵糖,随后将所述糖发酵成目标化学制品用于从预处理玉米芯中生产乙醇,使用运动发酵单胞菌作为将糖发酵成乙醇的生物催化剂。本发明的方法也能用于从生物质中生产 1,3- 丙二醇。如共有的和共同

未决的美国专利申请 #11/403087 的实施例 10 所述，使用本发明的方法处理的生物质可进行糖化；在糖化之后，使用大肠杆菌来生产 1,3- 丙二醇。

[0128] 通过生物催化剂在发酵过程中制备的目标化学制品可使用多种本领域已知的方法进行回收。可通过离心、过滤、微量过滤、和纳滤从其他发酵组分中分离产品。可通过离子交换、溶剂萃取、或电透析提取产品。可使用絮凝剂帮助产品分离。一个具体实例是可使用 ABE 发酵领域已知的方法从发酵培养基中分离生物生产的 1- 丁醇（参见例如 Durre, Appl. Microbiol. Biotechnol. 49 :639-648(1998), Groot 等 人, Process. Biochem. 27 : 61-75(1992)，以及本文参考）。例如，可通过离心、过滤、滗析等方法从发酵培养基中移除固体。然后，可使用诸如蒸馏、共沸蒸馏、液液萃取、吸附、汽提、膜蒸发、或全蒸发的方法从发酵培养基中分离 1- 丁醇。通过使反应混合物经过有机溶剂、蒸馏、和柱层析提取，可以从发酵培养基中纯化 1,3- 丙二醇（美国专利 5,356,812）。就此方法而言尤其好的一种有机溶剂是环己烷（美国专利 5,008,473）。氨基酸可以通过如离子交换树脂吸附和 / 或结晶的方法从发酵培养基中收集。

实施例

[0129] 一般方法和材料

[0130] 使用以下缩写：

[0131] “HPLC”是高效液相色谱，“C”是摄氏度，“kPa”是千帕斯卡，“m”是米，“mm”是毫米，“kW”是千瓦，“μm”是微米，“μL”是微升，“mL”是毫升，“L”是升，“min”是分钟，“mM”是毫摩尔，“cm”是厘米，“g”是克，“kg”是千克，“wt”是重量，“hr”是小时，“temp”或“T”是温度，“theoret”是理论，“pretreat”是预处理，“DWB”是生物质的干重，“ASME”是美国机械工程师学会 (AmericanSociety of Mechanical Engineers)，“s.s.”是不锈钢，“in”或“″”是英寸。

[0132] 硫酸、氢氧化铵、乙酸、乙酰胺、酵母提取物、葡萄糖、木糖、山梨醇、$MgSO_4 \cdot 7H_2O$、磷酸和柠檬酸从 Sigma-Aldrich(St. Louis, MO) 商购获得。

[0133] 在所述实施例中将处理称为预处理。

[0134] 小型筒式活塞反应器

[0135] 小型筒式活塞反应器（活塞 / 筒式反应器）由配备有水平取向活塞的 5.1cm×45.7cm 的不锈钢圆筒组成。用四个 O 形环将活塞密封到筒上，并在排放期间在活塞背面用氮气为活塞加压。45.7cm 的圆筒配备有三个多用途端口，允许用真空、氨水注射、蒸汽注射、和插入热电偶的方法测量筒内温度。当注入蒸汽时为了避免蒸汽冷凝过多，用三个带状加热器加热圆筒外部，并用一个覆盖有充满硅氧烷的玻璃纤维夹套的 2.5cm 厚的玻璃纤维垫隔热。

[0136] 将反应器圆筒直接连接到垂直取向的 15.2cm×61cm 的不锈钢闪蒸槽上。通过锥形喷嘴和底座末端剪切阀排列从闪蒸槽上分离圆筒。末端阀剪切模具的直径是 3.5cm。将锥形喷嘴和底座上的背压调节至约 138kPa（测量压力）的背压，使其进入 10.2cm 直径的空气圆筒中，该圆筒与末端剪切阀的锥形嘴相连。末端剪切阀门的锥形嘴能缩回最多 1.6cm，允许排放闪蒸槽中的颗粒。在末端剪切阀出口的弯管引导预处理固体向下进入闪蒸槽的底部，其中所述固体可通过打开槽底部的圆顶螺栓轻易移除。闪蒸槽的上部汽室凸缘加入一

个具有被加工成与闪蒸槽轴线呈直角的狭槽的特殊出口，这引起释放的蒸汽沿着拐角路径进入排出装置中，有助于防止产生的生物质颗粒遗留和水滴进入到排气冷凝器中。

[0137] 大型筒式活塞反应器

[0138] 将活塞反应器（印有代码 ASME）的第二圆筒加工成直径相同（5.1cm），但长度更长（68.6cm）以保持附加体积的生物质。用四个 O 形环将活塞密封到筒上，并在排放期间在活塞背面用氮气为活塞加压。68.6cm 的圆筒配备有八个多用途端口，沿着顶部表面和底部表面各 4 个，它们允许用真空、氨水注射、蒸汽注射、和插入热电偶的方法测量筒内温度。反应器圆筒配备有蒸汽夹套用于圆筒的均匀加热。将反应器圆筒直接连接到垂直取向的 15.2cm×61cm 的不锈钢闪蒸槽上。通过锥形喷嘴和底座末端剪切阀排列从闪蒸槽上分离圆筒。末端阀剪切模具的直径是 3.5cm。锥形喷嘴和底座上的背压是可调的，大多数测试使用～ 138kPa（测量压力）的背压进行，使其进入 10.2cm 直径的空气圆筒中，该圆筒与末端剪切阀的锥形嘴相连。末端剪切阀门的锥形嘴能缩回最多 1.6cm，允许排放闪蒸槽中的颗粒。在末端剪切阀出口的弯管引导预处理固体向下进入闪蒸槽的底部，其中所述固体可通过打开槽底部的圆顶螺栓轻易移除。闪蒸槽的上部汽室凸缘加入一个具有被加工成与闪蒸槽轴线呈直角的狭槽的特殊出口，这引起释放的蒸汽沿着拐角路径进入到排出装置中，有助于防止产生的生物质颗粒遗留和水滴进入到排气冷凝器中。沿着闪蒸槽加上三个带状电加热器（设为60℃）和隔热物，使得热预处理的固体闪蒸到加热容器中，更好的模拟商业规模的方法。

[0139] 蒸汽喷射反应器分批消化系统

[0140] 4 升蒸汽喷射反应器（Autoclave Engineers，Erie，PA）是带夹套的蒸汽反应器，由长度为 102mm schedule 80 的 Hastelloy®管组成，用两个球形阀闭合。将附加电加热器置于反应器所有暴露的、不带夹套的表面上，并将温度控制至预设温度。也可直接注入蒸汽以使生物质迅速达到预处理温度。调节并控制汽压以保持所需的预处理温度。将反应器底部颈缩至 51mm。所有预处理材料通过反应器底部的可置换模头放出，并收集在一个尼龙（Hotfill®）0.21m^3 袋中，该尼龙袋套在一个厚壁、带夹套的低温闪蒸槽中。

[0141] 预处理和酶水解反应器（PEHReactor）

[0142] 9-L PEHReactor（产自 NREL，Golden，CO；参见共同未决的美国专利申请 11/402464）具有大约 15cm×51cm 的不锈钢反应容器，而 3.2LPEHReactor 具有 15cm×18cm 不锈钢反应容器。每个容器具有穿过反应容器的纵向中心用于导入处理反应物的喷枪。使用旋转接头将喷枪连接到容器一端覆盖件的端口上，该容器具有用于容器连接的附加端口。四个导流板与容器壁等长，垂直连接到壁上。当容器旋转时，导流板和 3.2cm×3.2cm 的陶瓷研磨介质滚筒（E.R.Advanced Ceramics，East Palestine，OH）、在容器中的自由漂浮物一起对生物质和反应物施加机械混合，促使反应物同化到生物质中。在小型反应器中使用七个滚筒，在大型反应器中使用二十二个滚筒。将 PEHReactor 置于提供旋转机构的 Bellco Cell-ProductionRoller Apparatus（Bellco Technology，Vineland，NJ）上，该反应器具有滚轴设备，位于提供加热的温度控制舱中。可通过将外部来源连接到上盖中的喷枪连接端口上来对反应容器施加真空和压力。

[0143] 分批补料糖化反应器

[0144] 分批补料糖化反应器是一个 15-L 发酵罐（B.Braun BiotechInternational，

Allentown, PA)，通过 BioStat ED 数据控制单位对其进行控制，并且关联控制模块，所述模块包含循环泵、酸泵和碱泵、螺线管阀门、用于温度控制的换热器、蒸汽供应、处理水、供气控制阀门和过滤、和背压控制阀门以及排气过滤器。该发酵罐配备有两个 11.4cm 直径的三叶高效 Lignt nin A-310 叶轮。底部叶轮距反应器底部 7.6cm（它不能更靠近底部，因为靠近轴底部存在一个大的密封件系统用于底部 - 传动轴渗透），上部叶轮距反应器底部 22.9cm。该发酵罐容器具有 19.0cm 的直径和 55.9cm 的最大高度。安装四个可移除的导流板，每个导流板具有 1.6cm 的宽度和 48.3cm 的长度，并且从容器底部至顶部～ 7.6cm。将由 APV 凸轮泵 (M1/028/06 型）、1-1/2- 英寸 (3.81cm) 的挠性软管和特氟隆流动观测指示器组成的泵循环回路接到发酵罐系统的顶部和底部端口上。泵循环回路用具有 CF8M 阀体、316s. s. 球体、和 PTFE 底座的 1-1/2 英寸 (3.81cm) Valmicro 和 SVF 全端口球形阀从发酵容器上分离。此外，V 形端口剪切阀 (Triac 控制）位于凸轮泵下游，在将泵从发酵罐顶部端口分离的球形阀之前。在再循环期间，该阀门逐渐接近最多 60° 以提供更大的再循环预处理固体剪切。

[0145] 分析方法

[0146] 纤维素定量

[0147] 使用本领域熟知的方法如 ASTM E1758-01 “Standard method for thedetermination of carbohydrates by HPLC”测定在每个起始生物质样本中的纤维素的量。

[0148] 测量糖、乙酰胺、乳酸和乙酸含量

[0149] 通过 HPLC(Agilent Model 1100, Agilent Technologies, PaloAlto, CA) 使用 Bio-Rad HPX-87P 和 Bio-Rad HPX-87H 柱 (Bio-RadLaboratories,Hercules,CA) 以及合适的保护柱来测量糖化液体或发酵肉汤中的可溶性糖（葡萄糖、纤维二塘、木糖、半乳糖、阿拉伯糖、和甘露糖）、乙酸、和乙醇。如需要的话，测量样本 pH 并用硫酸将其调节至 5-6。然后使样本通过 0.2μm 的注射过滤器直接进入 HPLC 小瓶中。HPLC 运行条件如下：

[0150] HPX-87P（用于碳水化合物）：

[0151] 注射体积：10-50μL，取决于浓度和检测器限制

[0152] 流动相：HPLC 级水，0.2μm，过滤并脱气

[0153] 流速：0.6mL/ 分钟

[0154] 柱温：80-85℃，保护柱柱温＜ 60℃

[0155] 检测器温度：尽可能的接近主要柱柱温

[0156] 检测器：折射指数

[0157] 运行时间：35 分钟数据采集时间加上 15 分钟运行后时间（对后来的洗脱化合物进行可能的调节）

[0158] Biorad Aminex HPX-87H（用于碳水化合物、乙酸和乙醇）

[0159] 注射体积：5-10μL，取决于浓度和检测器限制

[0160] 流动相：0.01N 硫酸，0.2μm，过滤并脱气

[0161] 流速：0.6mL/ 分钟

[0162] 柱温：55℃

[0163] 检测器温度：尽可能的接近柱温

[0164] 检测器：折射指数

[0165] 运行时间：25-75 分钟的数据采集时间

[0166] 运行结束后，根据标准曲线测定每个化合物在样本中的浓度。

[0167] 实施例 1

[0168] 在小型筒式活塞反应器中预处理玉米芯

[0169] 用颚间距为大约 0.95cm 的颚式粉碎机（2.2kW 马达）处理完整玉米芯，然后用破碎机（1.5kW 马达，Franklin Miller Inc.，Livingston，NJ）处理，随后用配备有 1.9cm 美国标准筛网的 Sweco 筛网进行筛分，使完整玉米芯破碎成更小的块。在小型筒式活塞反应器（如一般方法所述）中装入 115g（基于干重）破碎的玉米芯，用手将玉米芯置于移除活塞的反应器末端。把活塞放回原处以堵上末端。将反应容器抽真空以减压至＜ 10kPa（0.1bar），将稀释氢氧化铵溶液注入到反应器中，使氨浓度为 4g 或 6g 每 100g 生物质干重（如表 1 所示），生物质干重浓度为 50g 每 100g 总生物质氨水混合物。在注入氨溶液后，将蒸汽注入到反应器中以将温度升至 145℃。生物质在该温度下保持 20 分钟，然后开启活塞将其排入闪蒸槽中。在 20 分钟预处理期间监控温度，如需要的话注入蒸汽。通过闪蒸槽底部来收获预处理的玉米芯。移除过多的游离液体，剩余下来的固体用于糖化。

[0170] 如一般方法所述，将约 470g 的预处理生物质加入到 3.2-L PEHR 反应器中用于糖化。通过注入 pH4.8 的 1M 柠檬酸缓冲液加上加入柠檬酸一水合物，将内容物的 pH 调节至大约 5.5。一旦达到所需的 pH，将 12.9mg/g 纤维素或 25.8mg/g 纤维素的 Spezyme®CP 纤维素酶（GenencorInternational，Rochester，NY）和 4.2mg 活性蛋白 /g 纤维素或 8.4mg 活性蛋白 /g 纤维素的半纤维素酶酶聚生体（Diversa；San Diego，CA）加入到反应器中，所述酶聚生体由 β－葡萄糖苷酶、木聚糖酶、β－木聚糖苷酶和阿拉伯糖苷酶组成。加入缓冲液、酶和水使得反应器中的最终混合物由 23g 干生物质 /100g 预处理生物质与糖化酶聚生体的混合物组成。反应器在培养箱中 50℃、19rpm 旋转下培养 72 小时。下表 1 中给出的收率是以理论收率百分比形式给出的释放量。

[0171] 表 1：在小型筒式活塞反应器中预处理的玉米芯糖化后的收率。

[0172]

氨 (g/100g DWB)	Spezyme® CP （mg/g 纤维素）	酶聚生体 (Diversa) （mg/g 纤维素）	葡萄糖单体释放量 (% 理论值)	总葡萄糖释放量 (% 理论值)	木糖单体释放量 (% 理论值)	总木糖释放量 (% 理论值)
4	25.8	8.4	78	90	50	80
6	12.9	4.2	65	77	48	72

[0173] 实施例 2

[0174] 不同时间在大型筒式活塞反应器中的预处理

[0175] 将蒸汽加到圆筒夹套中将大型筒式活塞反应器的圆筒（如一般方法所述）预热至～ 130℃。用带状加热器将闪蒸接收器预热至～ 60℃。如实施例 1 所述制备破碎玉米芯。用手将这些的玉米芯（175g，基于干重）装入到大型筒式反应器中，置于移除活塞的反应器末端。把活塞放回原处以堵上末端。将反应容器和闪蒸接收器抽真空以减压至＜ 10kPa，

将稀释氢氧化铵溶液注入到反应器中，使氨浓度为 6g/100g 生物质干重，生物质干重浓度为 45g/100g 总生物质氨水混合物。注入氨水之后，将蒸汽注入到反应器中以将温度升至 145℃。通过监控温度以及如需要的话注入蒸汽，使混合物该温度下保持 10 或 20 分钟，然后开启活塞将其排入预热闪蒸槽中。将闪蒸槽抽真空直至闪蒸接收器达到～ 59℃。进行三个 10 分钟预处理和六个 20 分钟预处理，最后将所有材料进行相同时间的预处理。当从闪蒸接收器收获产品时，将游离液体从预处理固体中分离出，并且不把它们再加回去用于糖化。随后如实施例 1 所述在小型 PEHReactor 中糖化预处理玉米芯的样本。所有糖化用 12.9mg/g 纤维素的 Spezyme®CP 纤维素酶和 4.2mg 活性蛋白 /g 纤维素的半纤维素酶聚生体 (Diversa) 在 50℃，pH5.5 条件下进行 72 小时，所述酶聚生体包含木聚糖酶、、β－木糖苷酶、阿拉伯糖苷酶、和 β－葡萄糖苷酶。下表 2 中给出的收率是以理论收率百分比形式给出的释放量。

[0176] 表 2：在大型筒式活塞反应器中预处理的玉米芯糖化后的收率。

[0177]

预处理时间（分钟）	葡萄糖单体释放量（%理论值）	总葡萄糖释放量（%理论值）	木糖单体释放量（%理论值）	总木糖释放量（%理论值）
10	68.2	79.5	32.1	77.0
20	68.0	83.2	39.1	84.3

[0178] 实施例 3

[0179] 在大型筒式活塞反应器中进行预处理，与蒸汽喷射进行比较

[0180] 如实施例 1 所述制备粒度减小的玉米芯。如实施例 2 所述在大型筒式活塞反应器中进行预处理。为了在蒸汽喷射中进行预处理，首先把玉米芯装入到 9-L PEHReactor 中。通过外表面旋转接触冰将反应器冷却至 4℃。将反应容器抽真空并将稀释氢氧化铵溶液注入到反应器中，所述氢氧化铵溶液在 4℃冷室中预冷并通过浸入冰水浴中的管子，反应器中氨浓度为 6g/100g 生物质干重，生物质干重浓度为 45g/100g 总生物质与氨水混合物。在旋转反应容器表面施加冰并在 4℃下旋转 30 分钟，将装入氨和玉米芯的 PEHReactor 冷却至 4℃。此时将内容物转移到如一般方法所述的蒸汽喷射反应器中。在蒸汽喷射反应器中加入氨与玉米芯混合物之后，通过直接注入蒸汽使温度上升至 145℃。玉米芯与氨混合物在该温度下保持 20 分钟，然后将混合物排入闪蒸槽中。

[0181] 从大型筒式活塞反应器和蒸汽喷射反应器中采集预处理玉米芯样本，如实施例 1 所述进行糖化。糖化用 12.9mg/g 纤维素的 Spezyme®CP 纤维素酶 (Genencor) 和 4.2mg 活性蛋白 /g 纤维素的半纤维素酶聚生体 (Diversa) 进行，所述酶聚生体由 β－葡萄糖苷酶、木聚糖酶、β－木糖苷酶、和阿拉伯糖苷酶组成。反应器在培养箱中 50℃、19rpm 下培养 72 小时。在每个反应器中的预处理材料所得的葡萄糖收率如下表 3 所示。

[0182] 表 3：在大型筒式活塞反应器或蒸汽喷射反应器中预处理的玉米芯的糖化收率。

[0183]

预处理反应器	反应器中 DWB conc	预处理时间（分钟）	预处理温度（℃）	葡萄糖单体释放量（%理论值）	总葡萄糖释放量（%理论值）	木糖单体释放量（%理论值）	总木糖释放量（%理论值）
活塞反应器	50%	20	145	68.0	83.2	39.1	84.3
蒸汽喷射	60%	40	150	65	77	48	82

[0184] 实施例 4

[0185] 在大型筒式活塞反应器中预处理玉米芯和纤维共混物

[0186] 如实施例 1 所述制备破碎玉米芯。在大型筒式活塞反应器中单独预处理破碎玉米芯，以及预处理破碎玉米芯和 Cargill Bran 80(Cargill，Minnetonka，MN) 的共混物。将破碎玉米芯和 Cargill Bran 80 玉米纤维合并，使得所述纤维占混合样本总干生物质的大约 33%。在任何情况下，将 175g（基于干重）的原料加入到反应器中。基本如实施例 2 所述进行预处理。然而，在这些实验中，在加入氨溶液后，在注入蒸汽以将温度升至 145℃之前保持反应器内容物 10 分钟。在注入蒸汽后，通过在需要时注入蒸汽使温度保持在 145℃下 10 分钟。在预处理后，开启活塞将样本排入闪蒸槽中。

[0187] 从大型筒式活塞反应器的闪蒸槽中采集预处理玉米芯和玉米芯与纤维共混物的样本，并在如实施例 1 所述的小型 PEHReactor 中进行糖化。将生物质加入，直至充满反应器体积的 20%。用 12.9mg/g 纤维素的 Spezyme®CP 纤维素酶 (Genencor) 和 15mg/g 纤维素的 Multifect 木聚糖酶 (Genencor) 进行糖化。PEHReactor 在培养箱中 50℃、19rpm 培养 72 小时。预处理材料的所得葡萄糖和木糖收率如下表 4 所示。

[0188] 表 4：在大型筒式活塞反应器中预处理的玉米芯以及玉米芯与麸皮样本的糖化收率。

[0189]

原料	反应器中 DWB conc	葡萄糖单体释放量（%理论值）	总葡萄糖释放量（%理论值）	木糖单体释放量（%理论值）	总木糖释放量（%理论值）
仅玉米芯	45%	40.2	67.2	29.4	83.9

原料	反应器中 DWB conc	葡萄糖单体释放量（%理论值）	总葡萄糖释放量（%理论值）	木糖单体释放量（%理论值）	总木糖释放量（%理论值）
玉米芯 + 麸皮 80	46%	37.0	65.4	21.6	77.2

[0190] 实施例 5

[0191] 从在大型筒式活塞反应器中预处理的玉米芯中生产乙醇

[0192] 如实施例 2 所述预处理玉米芯 10 分钟。进行总共 17 次此类预处理。将得自 4 次预处理的预处理玉米芯注入以用于糖化，从而提供用于分批补料糖化的初始水解产物。将得自其余 13 次生产运行的预处理玉米芯注入以用于分批补料糖化。

[0193] 为了开始分批补料糖化，在如一般方法所述的分批补料糖化反应器中首先加入水解产物以装满反应器第一叶轮的底部。该水解产物通过在 2.8-L 摇瓶中糖化预处理玉米芯进行制备。这些摇瓶装入 465g 预处理固体，1000mL 去离子水，以及 28.4mg Spezyme®CP/g 纤维素和 4.2mg 活性蛋白 /g 纤维素的半纤维素酶聚生体 (Diversa)，该酶聚生体包括 b- 葡萄糖苷酶、木聚糖酶、b- 木糖苷酶和阿拉伯糖苷酶。在加入酶之前，用 8.5% H_3PO_4 将 pH 调节至 5。将摇瓶保持在 50℃下并在旋转振荡器中以 150rpm 振荡 48 小时，此时将水解产物装入分批补料反应器中。

[0194] 加入初始水解产物之后，将预处理生物质与氨混合物 (～ 700g) 的第一等分试样加入到反应器中。加入 8.5% H_3PO_4 将 pH 保持设定值 5.5。将 pH 再调节至设定值之后，加入 28.4mg Spezyme®CP/g 纤维素和 4.2mg 活性蛋白 /g 纤维素的半纤维素酶聚生体 (Diversa)，该酶聚生体包括 b- 葡萄糖苷酶、木聚糖酶、b- 木糖苷酶和阿拉伯糖苷酶。在 t ＝ 4、8、12、22、26、30 和 34 小时加入预处理的生物质与氨混合物的附加等分试样、Spezyme® CP 纤维素酶和半纤维素酶酶聚生体。一般在加入酶约 1 小时后开启泵循环回路，并运行约 1 小时直至在第 22 小时加入固体。在 26 小时和 30 小时加料后，在加入酶后约 50 分钟开启泵并运行 30 分钟。在 34 小时加料后，在加入酶后约 3 小时开启泵并运行 30 分钟。泵也在 t ＝ 29、33、47 和 49 小时运行 30 分钟。总糖化时间为 120 小时。此时水解产物包含～ 60g/L 葡萄糖单体、25g/L 木糖单体和 10g/L 乙酸。

[0195] 该水解产物用于运动发酵单胞菌菌株 ZW800 或 ZW658(ATCC#PTA-7858) 发酵。ZW658 是运动发酵单胞菌菌株，它已经被工程化用于将木糖发酵成乙醇，在共有的和 - 共同未决的美国专利申请 60/847813 中对此进行了描述。ZW658 通过经由序列转座事件将两个操纵子整合到 ZW1(ATCC#31821) 基因组中，然后通过包含木糖的选择培养基筛选进行构建，所述操纵子是 P_{gap}xylAB 和 P_{gap}taltkt，它们包含四个编码木糖异构酶、木酮糖激酶、转醛醇酶和转酮醇酶的木糖利用基因。ZW800 是具有编码葡萄糖果糖氧化还原酶灭活基因的 ZW658 菌株，它也在共有的和共同未决的美国专利申请 60/847813 中进行了描述。

[0196] 发酵在灭菌的 1 升发酵罐 (BIOSTAT®B-DCU 系统，Sartorius BBISystem Inc.，Bethlehem，Pennsylvania，USA) 中进行，初始工作体积为 500mL。将种菌加入到发酵罐中，

含量为 10％ (v/v)，使得加样后肉汤的 OD_{600} ～ 1。水解产物与水的平衡比例为 80％或 40％ (v/v)。加入附加葡萄糖和木糖以使它们在肉汤中的终浓度各为 92g/L 和 82g/L。肉汤也补充有 10mM 山梨醇和 1g/L $MgSO_4 \cdot 7H_2O$。在 33℃、pH5.8、搅拌速度 150rpm 的条件下进行 72 小时的发酵。ZW800 菌株的最终乙醇滴定度为在 40％的水解产物中 8g/L，在 80％的水解产物中 7g/L。ZW658 的最终乙醇滴定度为在 40％的水解产物中 8g/L，在 80％的水解产物中 6.5g/L。

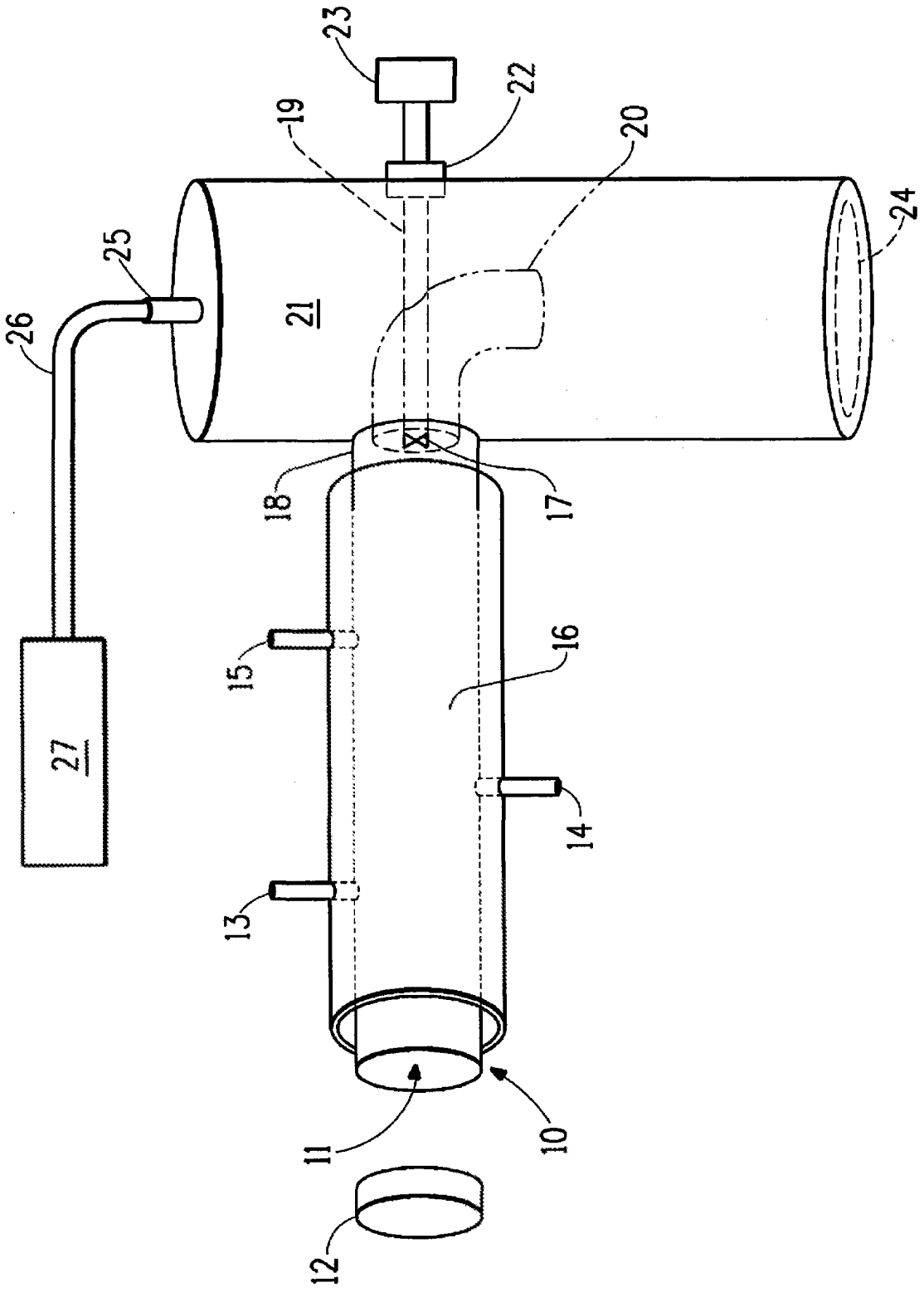


图 1

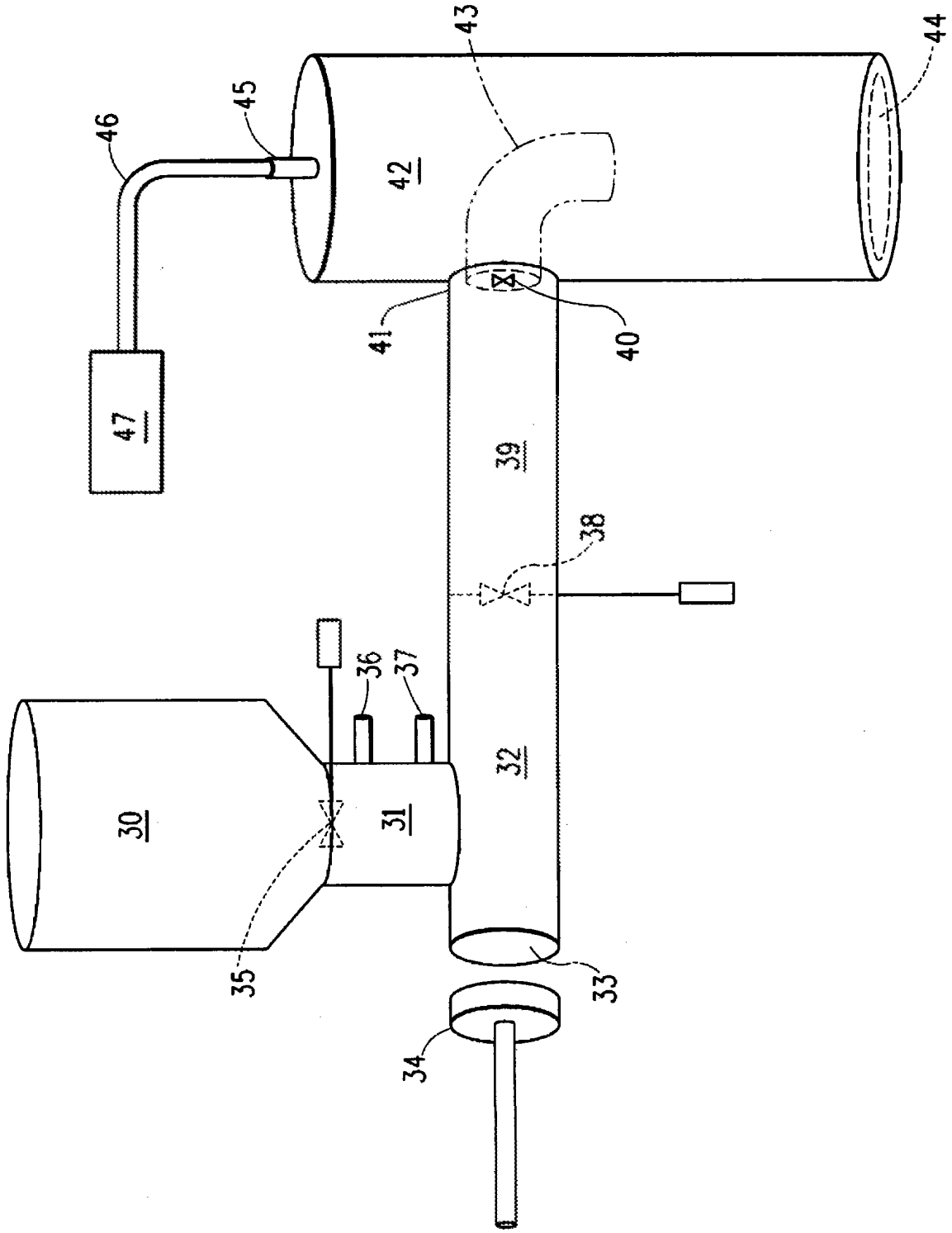


图 2

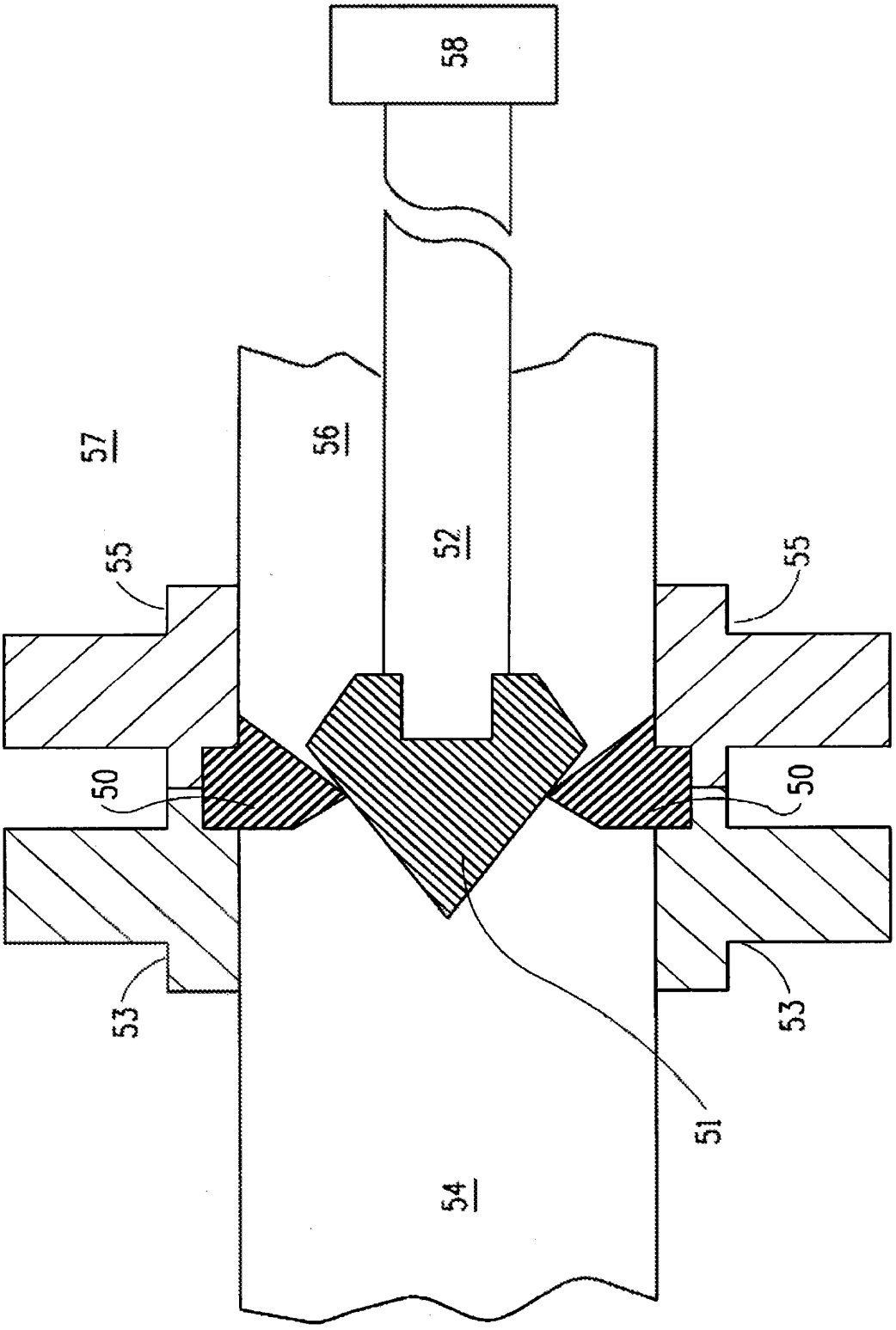


图 3

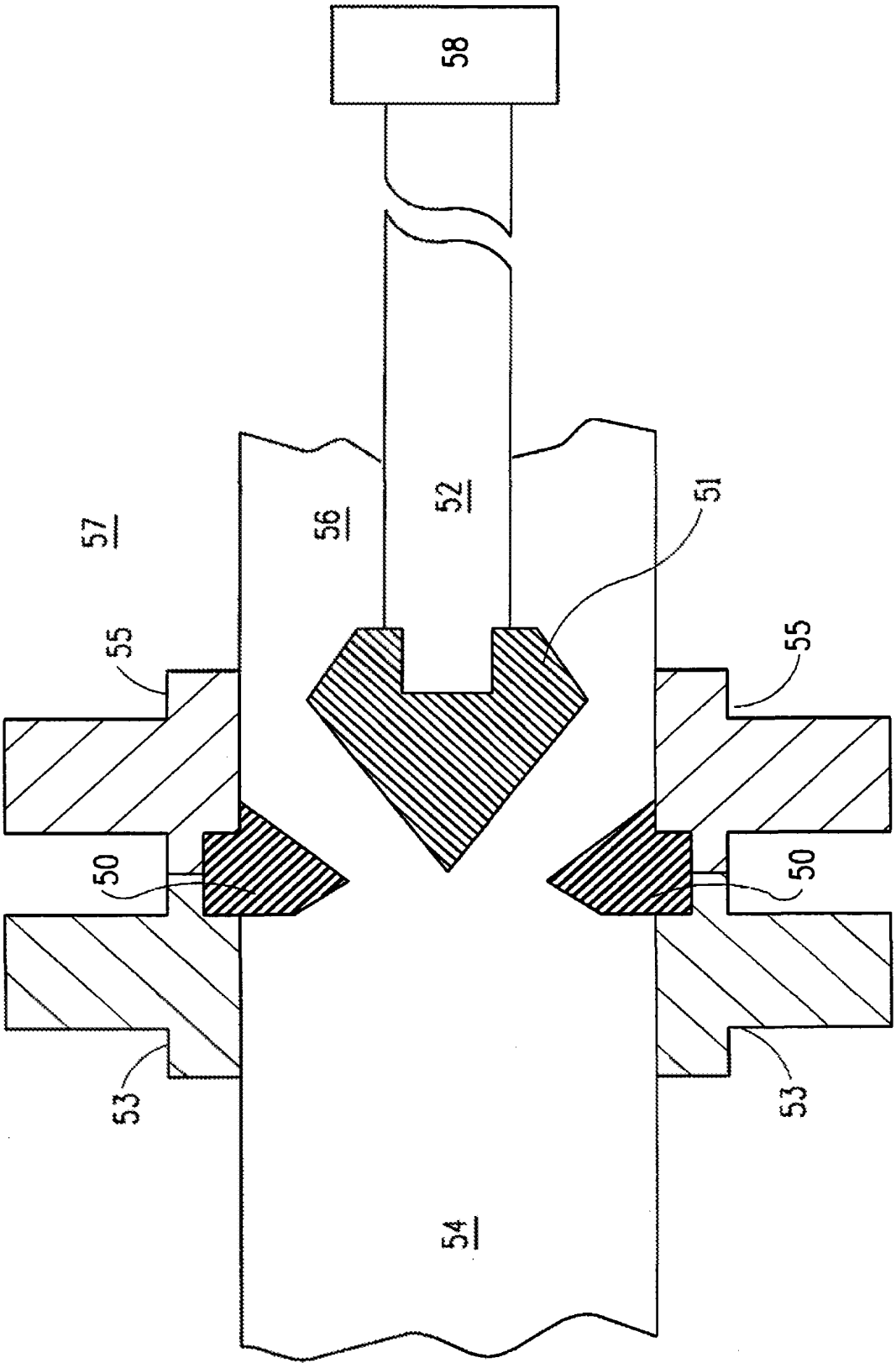


图 4

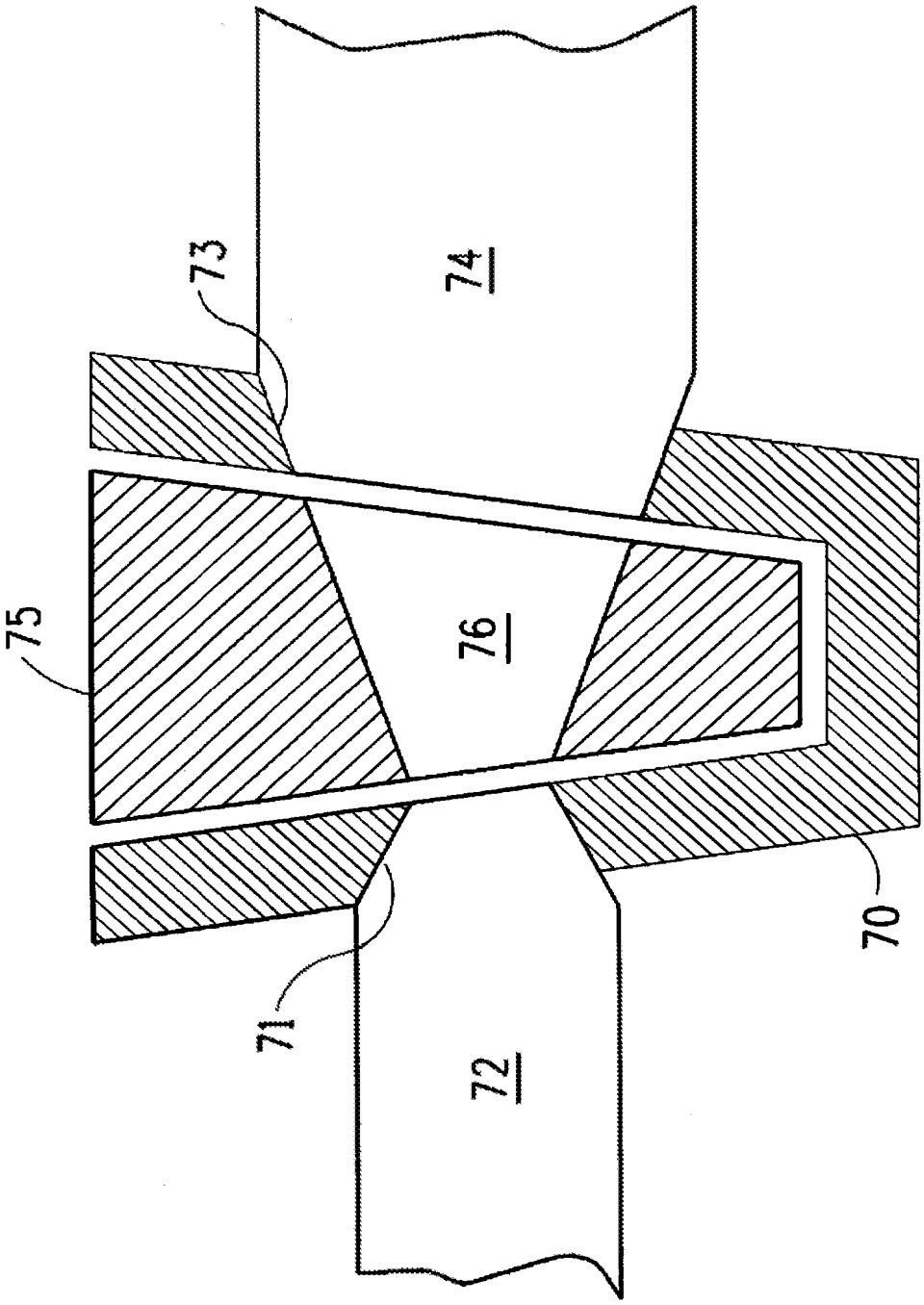


图 5

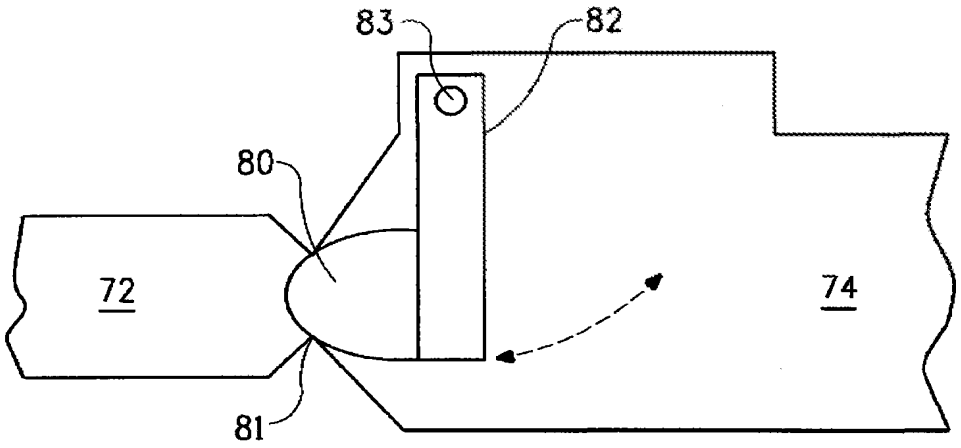


图 6A

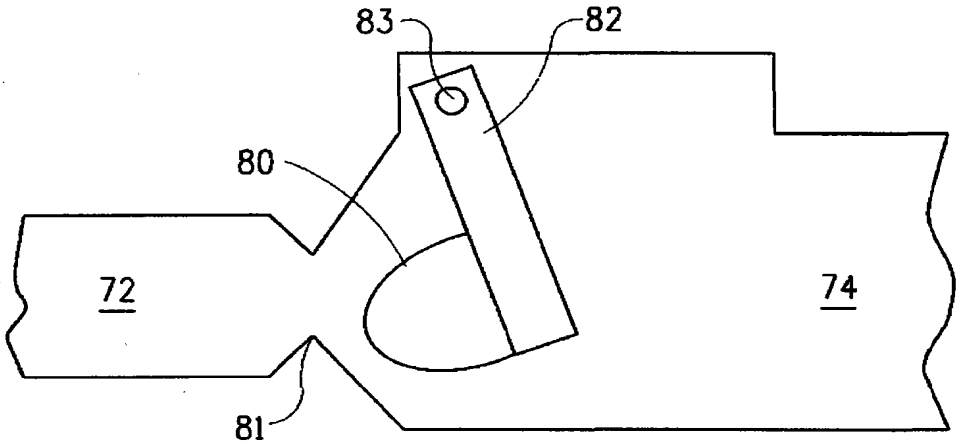


图 6B