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DESCRIPTION

BACKGROUND

[0001] In the production of pretreatment end-products from biomass, it is useful to carry out the pretreatment hydrolysis reactions as quickly and efficiently as possible. A major problem with hydrolysis of carbohydrate polymers and separation from lignin can be the potential to produce various byproducts that inhibit the later enzymatic attack on the cellulose or the fermentation of the resulting sugars. The risk of inhibitor formation and loss of desirable carbohydrates can increase as the pretreatment period lengthens. Furthermore, the energy requirements of a longer pretreatment time can increase the cost of the process.

[0002] Increased inhibitors can also raise costs due to prolonged enzymatic hydrolysis and fermentation times, and can affect yields of fermentation end-products. However, decreased pretreatment hydrolysis periods can mean incomplete separation of carbohydrates from lignins and proteinaceous materials. Further, the resulting incomplete hydrolysis of crystalline cellulose can reduce the carbohydrate available for enzymatic hydrolysis and further fermentation into desirable end-products. Such decreased yields of carbohydrate and subsequent products of fermentation can make the whole process of biomass utilization more expensive. Methods of processing biomass are disclosed in WO 2010/056940.

[0003] Methods and systems for pretreatment have typically involved treating the biomass under pressure and heat, with or without acid or alkali over long periods that can be an hour or more. Thus, a more rapid procedure that can process biomass efficiently is needed to reduce the costs of not only the pretreatment process, but also that of enzymatic hydrolysis and subsequent fermentation.

SUMMARY

[0004] The present invention provides a system for industrial scale pretreatment of at least one dry ton of biomass per day, the system comprising:

a barrel defining an inner chamber and comprising an inlet port near of first end of the barrel and an end flange plate at a second end of the barrel;

one or more rotatable screws configured to move the biomass through the inner chamber of the barrel and containing one or more sections configured to form one or more plugs from the biomass to separate the inner chamber of the barrel into two or more zones, including a feeder zone and a reaction zone; and

a pressure actuated discharge valve connected to the end flange plate and configured to open and close in response to pressure within the barrel, wherein the pressure actuated discharge valve is connected to an actuator that is operably coupled to a back pressure control unit operably coupled to one or more pressure gauges, thereby allowing for continuous production of a pretreated biomass composition comprising a liquid fraction comprising monosaccharides and solid particles comprising cellulose.

[0005] The present invention further provides an industrial scale method for pretreatment of at least one ton of biomass per day, the method comprising: (a) feeding the biomass at a rate of at least one dry metric ton (MT) of biomass per day into the system of the present invention, and (b) treating the biomass at an elevated temperature and pressure within the reaction zone for less than about 20 seconds to produce a pretreated biomass composition comprising a liquid fraction comprising monosaccharides and solid particles comprising cellulose.

[0006] In a first aspect, disclosed herein are industrial scale methods for pretreating at least one dry ton of biomass per day, the methods comprising: (a) feeding the biomass at a rate of at least one dry metric ton (MT) of biomass per day into an extrusion system comprising a barrel defining an inner chamber comprising a feeder zone and a reaction zone; and (b) treating the biomass at an elevated temperature and pressure within the reaction zone for less than about 20 seconds to produce a pretreated biomass composition comprising a liquid fraction comprising monosaccharides and solid particles comprising cellulose.

[0007] In some embodiments, the extrusion system further comprises one or more rotatable screws configured to move the biomass through the extrusion system from the feeder zone and through the reaction zone. In some embodiments, the one or more rotatable screws comprise one or more sections that are configured to form one or more plugs from the biomass to separate the inner chamber into two or more zones, including the feeder zone and the reaction zone. Some embodiments comprise one, two, or three rotatable screws. Some embodiments comprise two rotatable screws.

[0008] In some embodiments, the rate of biomass feeding is at least about 2 dry MT/day, 3 dry MT/day, 4 dry MT/day, 5 dry MT/day, 7.5 dry MT/day, 10 dry MT/day, 15 dry MT/day, 20 dry MT/day, 25 dry MT/day, 50 dry MT/day, 75 dry MT/day, dry 100 MT/day, 150 dry MT/day, or 200 dry MT/day.

[0009] Some embodiments further comprise adding a liquid to the biomass prior to the reaction zone. In some embodiments, the liquid is water. In some embodiments, the liquid is added in the feeder zone through one or more sealable ports on the barrel. In some embodiments, the liquid is added to increase the moisture content of the biomass to from about: 10- 90%, 15-85%, 20-80%, 30-70%, or about 40-60% w/v.

[0010] In some embodiments, the biomass is treated for less than 19, 18, 17, 16, 15, 14, 13, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 seconds in the reaction zone. In some embodiments, the biomass is treated for about 5 to 15 seconds in the reaction zone.

[0011] In some embodiments, the elevated temperature is about: 50-500 °C, 75-400 °C, 100-350 °C, 150-300°C, or 200-250°C. In some embodiments, the elevated temperature is about 150-300°C.

[0012] In some embodiments, the elevated pressure is about: 50-1000 PSI, 100-750 PSI, 200-600 PSI, 300-500 PSI or 350-450 PSI. In some embodiments, the elevated pressure is about 300-500 PSI.

[0013] Some embodiments further comprise injecting steam into the biomass to increase temperature and pressure. In some embodiments, the steam is injected in the reaction zone. In some embodiments, the steam is injected through one or more sealable ports in the barrel.

[0014] Some embodiments further comprise adding a chemical agent to the biomass in the reaction zone. In some embodiments, the chemical agent comprises an acid, a base, or a combination thereof. In some embodiments, the chemical agent comprises the acid that is sulfuric acid, peroxyacetic acid, lactic acid, formic acid, acetic acid, citric acid, phosphoric acid, hydrochloric acid, sulfurous acid, chloroacetic acid, dichloroacetic acid, trichloroacetic acid, trifluoroacetic acid, oxalic acid, benzoic acid, or a combination thereof. In some embodiments, the chemical agent comprises the acid that is sulfuric acid. In some embodiments, the chemical agent is added to a level of about: 0.1-20% w/v, 1-15% w/v, 1.5-10% w/v, 1-10% w/v, 1-5% w/v, or 2-4% w/v. In some embodiments, the chemical agent is added to a level of about 2-4% w/v.

[0015] In some embodiments, the liquid fraction comprises C5 monosaccharides in at least a 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% yield compared to the theoretical maximum based on the biomass. In some embodiments, the liquid fraction comprises C5 monosaccharides in at least a 70% yield compared to the theoretical maximum based on the biomass.

[0016] In some embodiments, the liquid fraction comprises C6 monosaccharides less than a 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, or 5% yield compared to the theoretical maximum based on the biomass. In some embodiments, the liquid fraction comprises C6 monosaccharides less than a 35% yield compared to the theoretical maximum based on the biomass.

[0017] In some embodiments, the solid particles have a size range of about: 1-500 μm, 1-250 μm, 1-200 μm, or 1-150 μm. In some embodiments, the solid particles have an average size of about 15-25 μm.

[0018] In some embodiments, the method produces low levels of one or more inhibitor compounds. In some embodiments, the one or more inhibitor compounds comprise formic acid, acetic acid, hydroxymethyl furfural (HMF), furfural, or a combination thereof. In some embodiments, less than 30, 25, 20, 15, 10, or 5 kg of formic acid is produced per MT of dry biomass. In some embodiments, less than 100, 80, 60, 50, 40, 30, 25, 20, 15, 10, or 5 kg of acetic acid is produced per MT of dry biomass. In some embodiments, less than 20, 15, 10, 7.5, 5, 4, 3, 2, or 1 kg of hydroxymethyl furfural (HMF) is produced per MT of dry biomass. In some embodiments, less than 20, 15, 10, 7.5, 5, 4, 3, 2, or 1 kg of furfural is produced per MT of dry biomass.

[0019] In some embodiments, the biomass comprises algae, corn, grass, straw, grain hulls, wood, bark, sawdust, paper, poplars, willows, switchgrass, alfalfa, prairie bluestem, sugar palms, nypa palm, cassava, milo, sorghum, sweet potatoes, molasses, tubers, roots, stems, sago, cassaya, tapioca, rice peas, beans, potatoes, beets, fruits, pits, sorghum, sugar cane, rice, wheat, whole grains, rye, barley, bamboo, seeds, oats, or a combination thereof, or a derivative or byproduct thereof. In some embodiments, the derivative or byproduct thereof comprises corn stover, corn cobs, corn mash, corn fiber, silage, bagasse, distiller's grains, distiller's dried solubles, distiller's dried grains, condensed distiller's solubles, distiller's wet grains, distiller's dried grains with solubles, fiber, fruit peels, rice straw, rice hulls, wheat straw, barley straw, seed hulls, oat hulls, food waste, municipal sewage waste, or a combination thereof. In some embodiments, the biomass comprises a woody biomass.

[0020] Some embodiments further comprise hydrolyzing the solid particles comprising cellulose with one or more enzymes to produce monosaccharides.

[0021] Also provided herein are pretreated biomass compositions produced by any of the methods

disclosed herein.

[0022] Also provided are sugar streams comprising C6 monosaccharides produced the enzymatic hydrolysis of the solid particles produced by any of the methods disclosed herein.

[0023] In a second aspect, disclosed herein are systems for industrial scale pretreatment of at least one dry ton of biomass per day, the systems comprising: (a) a barrel defining an inner chamber and comprising an inlet port near of first end of the barrel and an end flange plate at a second end of the barrel; (b) one or more rotatable screws configured to move the biomass through the inner chamber of the barrel and containing one or more sections configured to form one or more plugs from the biomass to separate the inner chamber of the barrel into two or more zones, including a feeder zone and a reaction zone; and (c) a pressure actuated discharge valve connected to the end flange plate and configured to open and close in response to pressure within the barrel, thereby allowing for continuous production of a pretreated biomass composition comprising a liquid fraction comprising monosaccharides and solid particles comprising cellulose.

[0024] Some embodiments comprise one, two, or three rotatable screws. Some embodiments comprise two rotatable screws.

[0025] Some embodiments further comprise a motor configured to rotate the one or more rotatable screws. In some embodiments, the motor is configured to rotate the one or more rotatable screws at about: 100, 250, 400, 500, 750, 1000, 1100, 1250, 1500, or 2000 RPMs.

[0026] In some embodiments, the system is capable of processing biomass at a rate at least about 2 dry MT/day, 3 dry MT/day, 4 dry MT/day, 5 dry MT/day, 7.5 dry MT/day, 10 dry MT/day, 15 dry MT/day, 20 dry MT/day, 25 dry MT/day, 50 dry MT/day, 75 dry MT/day, 100 dry MT/day, 150 dry MT/day, or 200 dry MT/day.

[0027] In some embodiments, the system further comprises a hopper connected to the inlet port for feeding the biomass into the feeder zone. In some embodiments, the hopper further comprises a feeder configured to move the biomass from the hopper through the inlet port. In some embodiments, the feeder is a delivery auger configured to distribute the biomass evenly into the feeder zone. In some embodiments, the hopper comprises one or more sealable ports configured to add a liquid to biomass in the hopper.

[0028] In some embodiments, the barrel further comprises one or more sealable ports configured to add liquid to biomass in the feeder zone.

[0029] In some embodiments, the rotatable screws are capable of conveying biomass through the reaction zone in less than about: 20, 19, 18, 17, 16, 15, 14, 13, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 seconds. In some embodiments, the rotatable screws are capable of conveying biomass through the reaction zone in about 5 to 15 seconds.

[0030] In some embodiments, the barrel further comprises one or more sealable ports configured to add steam to the reaction zone. In some embodiments, the barrel further comprises a heat jacket.

[0031] In some embodiments, the system is configured to maintain an elevated temperature in the reaction zone. In some embodiments, the elevated temperature is provided by steam, a heat jacket, or

a combination thereof. In some embodiments, the elevated temperature is about: 50-500 °C, 75-400 °C, 100-350 °C, 150-300°C, or 200-250°C.

[0032] In some embodiments, the system is configured to maintain an elevated pressure in the reaction zone. In some embodiments, the elevated pressure is maintained by addition of steam, liquid, biomass, or a combination thereof. In some embodiments, the elevated pressure is about: 50-1000 PSI, 100-750 PSI, 200-600 PSI, 300-500 PSI or 350-450 PSI.

[0033] In some embodiments, the barrel further comprises one or more sealable ports configured to add one or more chemical agents to the reaction zone. In some embodiments, the chemical agent comprises an acid, a base, or a combination thereof.

[0034] In some embodiments, the pressure actuated discharge valve comprises a poppet valve, a ball valve, a check valve, or a rotating knife-gate valve. In some embodiments, the pressure actuated discharge valve comprises a poppet valve.

[0035] In some embodiments, the pressure actuated discharge valve is connected to an actuator. In some embodiments, the actuator is a pneumatic actuator, a hydraulic actuator, an electro-mechanical actuator, or a combination thereof.

[0036] In some embodiments, the actuator is operably coupled to a back pressure control unit. In some embodiments, the back pressure control unit is operably coupled to one or more pressure gauges. In some embodiments, the one or more pressure gauges monitor pressure in the barrel via one or more sealable ports in the barrel. In some embodiments, at least one of the one or more pressure gauges is configured to monitor pressure within the reaction zone.

[0037] In some embodiments, the barrel further comprises one or more ports comprising a temperature gauge, a pressure gauge, or a combination thereof.

[0038] In some embodiments, the system further comprises a flash tank. In some embodiments, the flash tank collects the pretreated biomass composition as it exits the pressure actuated discharge valve.

[0039] In some embodiments, the system is configured to produce the liquid fraction comprising C5 monosaccharides in at least a 70% yield compared to the theoretical maximum based on the biomass.

[0040] In some embodiments, the system is configured to produce the liquid fraction comprising C6 monosaccharides in less than a 35% yield compared to the theoretical maximum based on the biomass.

[0041] In some embodiments, the system is configured to produce the solid particles in a size range of about: 1-500 µm, 1-250 µm, 1-200 µm, or 1-150 µm.

[0042] In some embodiments, the system is configured to produce the solid particles in an average size of about 15-25 µm.

[0043] In some embodiments, the biomass comprises a woody biomass.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

FIG. 1 is a schematic diagram of one embodiment of an apparatus for use in the present invention.

FIG. 2 is a horizontal fragmentary sectional view of an embodiment of an apparatus for use in the invention illustrating the barrel, screws, and end valve attached to a twin screw extruder of the invention.

FIG. 3 is a cross-sectional view similar to that of **FIG. 2** that depicts the extruder with the twin screws.

FIG. 4 is a cross-sectional view of the end plate of the extruder that abuts the end of the discharge valve.

FIG. 5 is a schematic cross-sectional view of the end plate of the extruder and the opening of the valve plate that abuts the end of the extruder.

FIG. 6 is a cross-sectional view of the end of the discharge valve plate that abuts the end of the extruder showing the seal facing.

FIG. 7 is a horizontal fragmentary sectional view of the transition from the discharge of the twin screws to the outlet orifice.

FIG. 8 is a horizontal sectional view of the housing for a valve assembly.

FIG. 9 is a schematic drawing of **FIG. 9** showing how the valve assembly fits into the housing.

FIG. 10 is a horizontal sectional view of the valve assembly in the housing.

FIG. 11 is a schematic drawing of one embodiment of a gradual expansion venturi used as a discharge valve with the valve mostly closed.

FIG. 12 is a schematic drawing of the gradual expansion venturi embodiment of **FIG. 11,** with the valve mostly open.

FIG. 13 is a graph showing the particle size distribution of Cherry sawdust biomass following pretreatment according to an embodiment of the methods disclosed herein.

DETAILED DESCRIPTION

[0045] As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a purified monomer" includes mixtures of two or more purified monomers. The term "comprising" as

used herein is synonymous with "including," "containing," or "characterized by," and is inclusive or open-ended and does not exclude additional, unrecited elements or method steps.

[0046] All numbers expressing quantities of ingredients, reaction conditions, and so forth used in the specification are to be understood as being modified in all instances by the term "about." Accordingly, unless indicated to the contrary, the numerical parameters set forth herein are approximations that can vary depending upon the desired properties sought to be obtained. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of any claims in any application claiming priority to the present application, each numerical parameter should be construed in light of the number of significant digits and ordinary rounding approaches.

[0047] Wherever the phrase "for example," "such as," "including" and the like are used herein, the phrase "and without limitation" is understood to follow unless explicitly stated otherwise. Therefore, "for example ethanol production" means "for example and without limitation ethanol production."

[0048] In this specification and in the claims that follow, reference will be made to a number of terms which shall be defined to have the following meanings. Unless characterized otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

Definitions

[0049] Unless characterized otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art.

[0050] "Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not. For example, the phrase "the medium can optionally contain glucose" means that the medium may or may not contain glucose as an ingredient and that the description includes both media containing glucose and media not containing glucose.

[0051] "About" means a referenced numeric indication plus or minus 10% of that referenced numeric indication. For example, the term about 4 would include a range of 3.6 to 4.4.

[0052] Fermentation is an anaerobic chemical process by which molecules such as glucose and xylose are broken down to release energy and fermentation end products are synthesized. Industrial fermentation processes begin with suitable microorganisms, such as yeasts and bacteria, and specified conditions, such as careful adjustment of nutrient concentration. The products are of many types: alcohols, glycerol, and carbon dioxide from yeast fermentation of various sugars; butyl alcohol, acetone, lactic acid, monosodium glutamate, and acetic acid from various bacteria; and citric acid, gluconic acid, and small amounts of antibiotics, vitamin B12, and riboflavin (vitamin B2) from mold fermentation. Ethyl alcohol and/or butanol are produced via the fermentation of starch or sugar and are important sources of liquid biofuel.

[0053] "Fermentive end-product" and "fermentation end-product" are used interchangeably herein to include biofuels, chemicals, compounds suitable as liquid fuels, gaseous fuels, triacylglycerols (TAGs),

reagents, chemical feedstocks, chemical additives, processing aids, food additives, bioplastics and precursors to bioplastics, and other products. Examples of fermentive end-products include but are not limited to 1,4 diacids (succinic, fumaric and malic), 2,5 furan dicarboxylic acid, 3 hydroxy propionic acid, aspartic acid, glucaric acid, glutamic acid, itaconic acid, levulinic acid, 3-hydroxybutyrolactone, glycerol, sorbitol, xylitol/arabinitol, butanediol, butanol, methane, methanol, ethane, ethene, ethanol, n-propane, 1-propene, 1-propanol, propanal, acetone, propionate, n-butane, 1-butene, 1-butanol, butanal, butanoate, isobutanal, isobutanol, 2-methylbutanal, 2-methylbutanol, 3-methylbutanal, 3-methylbutanol, 2-butene, 2-butanol, 2-butanone, 2,3-butanediol, 3-hydroxy-2-butanone, 2,3-butanedione, ethylbenzene, ethenylbenzene, 2-phenylethanol, phenylacetaldehyde, 1-phenylbutane, 4-phenyl-1-butene, 4-phenyl-2-butene, 1-phenyl-2-butene, 1-phenyl-2-butanol, 4-phenyl-2-butanol, 1-phenyl-2-butanone, 4-phenyl-2-butanone, 1-phenyl-2,3-butandiol, 1-phenyl-3-hydroxy-2-butanone, 4-phenyl-3-hydroxy-2-butanone, 1-phenyl-2,3-butanedione, n-pentane, ethylphenol, ethenylphenol, 2-(4-hydroxyphenyl)ethanol, 4-hydroxyphenylacetaldehyde, 1-(4-hydroxyphenyl) butane, 4-(4-hydroxyphenyl)-1-butene, 4-(4-hydroxyphenyl)-2-butene, 1-(4-hydroxyphenyl)-1-butene, 1-(4-hydroxyphenyl)-2-butanol, 4-(4-hydroxyphenyl)-2-butanol, 1-(4-hydroxyphenyl)-2-butanone, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-2,3-butandiol, 1-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 4-(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-2,3-butanonedione, indolylethane, indolylethene, 2-(indole-3-) ethanol, n-pentane, 1-pentene, 1-pentanol, pentanal, pentanoate, 2-pentene, 2-pentanol, 3-pentanol, 2-pentanone, 3-pentanone, 4-methylpentanal, 4-methylpentanol, 2,3-pentanediol, 2-hydroxy-3-pentanone, 3-hydroxy-2-pentanone, 2,3-pentanedione, 2-methylpentane, 4-methyl-1-pentene, 4-methyl-2-pentene, 4-methyl-3-pentene, 4-methyl-2-pentanol, 2-methyl-3-pentanol, 4-methyl-2-pentanone, 2-methyl-3-pentanone, 4-methyl-2,3-pentanediol, 4-methyl-2-hydroxy-3-pentanone, 4-methyl-3-hydroxy-2-pentanone, 4-methyl-2,3-pentanedione, 1-phenylpentane, 1-phenyl-1-pentene, 1-phenyl-2-pentene, 1-phenyl-3-pentene, 1-phenyl-2-pentanol, 1-phenyl-3-pentanol, 1-phenyl-2-pentanone, 1-phenyl-3-pentanone, 1-phenyl-2,3-pentanediol, 1-phenyl-2-hydroxy-3-pentanone, 1-phenyl-3-hydroxy-2-pentanone, 1-phenyl-2,3-pentanedione, 4-methyl-1-phenylpentane, 4-methyl-1-phenyl-1-pentene, 4-methyl-1-phenyl-2-pentene, 4-methyl-1-phenyl-3-pentene, 4-methyl-1-phenyl-3-pentanol, 4-methyl-1-phenyl-2-pentanol, 4-methyl-1-phenyl-3-pentanone, 4-methyl-1-phenyl-2-pentanone, 4-methyl-1-phenyl-2,3-pentanediol, 4-methyl-1-phenyl-2,3-pentanedione, 4-methyl-1-phenyl-3 -hydroxy-2-pentanone, 4-methyl-1-phenyl-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl) pentane, 1-(4-hydroxyphenyl)-1-pentene, 1-(4-hydroxyphenyl)-2-pentene, 1-(4-hydroxyphenyl)-3-pentene, 1-(4-hydroxyphenyl)-2-pentanol, 1-(4-hydroxyphenyl)-3-pentanol, 1-(4-hydroxyphenyl)-2-pentanone, 1-(4-hydroxyphenyl)-3-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanediol, 1-(4-hydroxyphenyl)-2-hydroxy-3-pentanone, 1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl) pentane, 4-methyl-1-(4-hydroxyphenyl)-2-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentene, 4-methyl-1-(4-hydroxyphenyl)-1-pentene, 4-methyl-1-(4-hydroxyphenyl)-3-pentanol, 4-methyl-1-(4-hydroxyphenyl)-2-pentanol, 4-methyl-1-(4-hydroxyphenyl)-3-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-pentanedione, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-pentanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3 -pentanone, 1-indole-3-pentane, 1-(indole-3)-1-pentene, 1-(indole-3)-2-pentene, 1-(indole-3)-3-pentene, 1-(indole-3)-2-pentanol, 1-(indole-3)-3-pentanol, 1-(indole-3)-2-pentanone, 1-(indole-3)-3-pentanone, 1-(indole-3)-2,3-pentanediol, 1-(indole-3)-2-hydroxy-3-pentanone, 1-(indole-3)-3-hydroxy-2-pentanone, 1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3-)pentane, 4-methyl-1-(indole-3)-2-pentene, 4-methyl-1-(indole-3)-3-pentene, 4-methyl-1-(indole-3)-1-pentene, 4-methyl-2-(indole-3)-3-pentanol, 4-methyl-1-(indole-3)-2-pentanol, 4-methyl-1-(indole-3)-3-pentanone, 4-methyl-1-(indole-3)-2-pentanone, 4-methyl-1-(indole-3)-2,3-pentanediol, 4-methyl-1-(indole-3)-2,3-pentanedione, 4-methyl-1-(indole-3)-3-hydroxy-2-pentanone, 4-methyl-1-(indole-3)-2-hydroxy-3-pentanone, n-hexane,

1-hexene, 1-hexanol, hexanal, hexanoate, 2-hexene, 3-hexene, 2-hexanol, 3-hexanol, 2-hexanone, 3-hexanone, 2,3-hexanediol, 2,3-hexanedione, 3,4-hexanediol, 3,4-hexanedione, 2-hydroxy-3-hexanone, 3-hydroxy-2-hexanone, 3-hydroxy-4-hexanone, 4-hydroxy-3-hexanone, 2-methylhexane, 3-methylhexane, 2-methyl-2-hexene, 2-methyl-3-hexene, 5-methyl-1-hexene, 5-methyl-2-hexene, 4-methyl-1-hexene, 4-methyl-2-hexene, 3-methyl-3-hexene, 3-methyl-2-hexene, 3-methyl-1-hexene, 2-methyl-3-hexanol, 5-methyl-2-hexanol, 5-methyl-3-hexanol, 2-methyl-3-hexanone, 5-methyl-2-hexanone, 5-methyl-3-hexanone, 2-methyl-3,4-hexanediol, 2-methyl-3,4-hexanedione, 5-methyl-2,3-hexanediol, 5-methyl-2,3-hexanedione, 4-methyl-2,3-hexanediol, 4-methyl-2,3-hexanedione, 2-methyl-3-hydroxy-4-hexanone, 2-methyl-4-hydroxy-3-hexanone, 5-methyl-2-hydroxy-3-hexanone, 5-methyl-3-hydroxy-2-hexanone, 4-methyl-2-hydroxy-3-hexanone, 4-methyl-3-hydroxy-2-hexanone, 2,5-dimethylhexane, 2,5-dimethyl-2-hexene, 2,5-dimethyl-3-hexene, 2,5-dimethyl-3-hexanol, 2,5-dimethyl-3-hexanone, 2,5-dimethyl-3,4-hexanediol, 2,5-dimethyl-3,4-hexanedione, 2,5-dimethyl-3-hydroxy-4-hexanone, 5-methyl-1-phenylhexane, 4-methyl-1-phenylhexane, 5-methyl-1-phenyl-1-hexene, 5-methyl-1-phenyl-2-hexene, 5-methyl-1-phenyl-3-hexene, 4-methyl-1-phenyl-1-hexene, 4-methyl-1-phenyl-2-hexene, 4-methyl-1-phenyl-3-hexene, 5-methyl-1-phenyl-2-hexanol, 5-methyl-1-phenyl-3-hexanol, 4-methyl-1-phenyl-2-hexanol, 4-methyl-1-phenyl-3-hexanol, 5-methyl-1-phenyl-2-hexanone, 5-methyl-1-phenyl-3-hexanone, 4-methyl-1-phenyl-2-hexanone, 4-methyl-1-phenyl-3-hexanone, 5-methyl-1-phenyl-2,3-hexanediol, 4-methyl-1-phenyl-2,3-hexanediol, 5-methyl-1-phenyl-3-hydroxy-2-hexanone, 5-methyl-1-phenyl-2-hydroxy-3-hexanone, 4-methyl-1-phenyl-3-hydroxy-2-hexanone, 4-methyl-1-phenyl-2-hydroxy-3-hexanone, 5-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-phenyl-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)hexane, 5-methyl-1-(4-hydroxyphenyl)-1-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexene, 5-methyl-1-(4-hydroxyphenyl)-3-hexene, 4-methyl-1-(4-hydroxyphenyl)-1-hexene, 4-methyl-1-(4-hydroxyphenyl)-2-hexene, 4-methyl-1-(4-hydroxyphenyl)-3-hexene, 5-methyl-1-(4-hydroxyphenyl)-2-hexanol, 5-methyl-1-(4-hydroxyphenyl)-3-hexanol, 4-methyl-1-(4-hydroxyphenyl)-2-hexanol, 4-methyl-1-(4-hydroxyphenyl)-3-hexanol, 5-methyl-1-(4-hydroxyphenyl)-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanediol, 5-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 4-methyl-1-(4-hydroxyphenyl)-3-hydroxy-2-hexanone, 4-methyl-1-(4-hydroxyphenyl)-2-hydroxy-3-hexanone, 5-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(4-hydroxyphenyl)-2,3-hexanedione, 4-methyl-1-(indole-3-)hexane, 5-methyl-1-(indole-3)-1-hexene, 5-methyl-1-(indole-3)-2-hexene, 5-methyl-1-(indole-3)-3-hexene, 4-methyl-1-(indole-3)-1-hexene, 4-methyl-1-(indole-3)-2-hexene, 4-methyl-1-(indole-3)-3-hexene, 5-methyl-1-(indole-3)-2-hexanol, 5-methyl-1-(indole-3)-3-hexanol, 4-methyl-1-(indole-3)-2-hexanol, 4-methyl-1-(indole-3)-3-hexanol, 5-methyl-1-(indole-3)-2-hexanone, 5-methyl-1-(indole-3)-3-hexanone, 4-methyl-1-(indole-3)-2-hexanone, 4-methyl-1-(indole-3)-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanediol, 4-methyl-1-(indole-3)-2,3-hexanediol, 5-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 5-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 4-methyl-1-(indole-3)-3-hydroxy-2-hexanone, 4-methyl-1-(indole-3)-2-hydroxy-3-hexanone, 5-methyl-1-(indole-3)-2,3-hexanedione, 4-methyl-1-(indole-3)-2,3-hexanedione, n-heptane, 1-heptene, 1-heptanol, heptanal, heptanoate, 2-heptene, 3-heptene, 2-heptanol, 3-heptanol, 4-heptanol, 2-heptanone, 3-heptanone, 4-heptanone, 2,3-heptanediol, 2,3-heptanedione, 3,4-heptanediol, 3,4-heptanedione, 2-hydroxy-3-heptanone, 3-hydroxy-2-heptanone, 3-hydroxy-4-heptanone, 4-hydroxy-3-heptanone, 2-methylheptane, 3-methylheptane, 6-methyl-2-heptene, 6-methyl-3-heptene, 2-methyl-3-heptene, 2-methyl-2-heptene, 5-methyl-2-heptene, 5-methyl-3-heptene, 3-methyl-3-heptene, 2-methyl-3-heptanol, 2-methyl-4-heptanol, 6-methyl-3-heptanol, 5-methyl-3-heptanol, 3-methyl-4-heptanol, 2-methyl-3-heptanone, 2-methyl-4-heptanone, 6-methyl-3-heptanone, 5-methyl-3-heptanone, 3-methyl-4-heptanone, 2-methyl-3,4-heptanediol, 2-methyl-3,4-heptanedione, 6-methyl-3,4-heptanediol, 6-methyl-

3,4-heptanedione, 5-methyl-3,4-heptanediol, 5-methyl-3,4-heptanedione, 2-methyl-3-hydroxy-4-heptanone, 2-methyl-4-hydroxy-3-heptanone, 6-methyl-3-hydroxy-4-heptanone, 6-methyl-4-hydroxy-3-heptanone, 5-methyl-3-hydroxy-4-heptanone, 5-methyl-4-hydroxy-3-heptanone, 2,6-dimethylheptane, 2,5-dimethylheptane, 2,6-dimethyl-2-heptene, 2,6-dimethyl-3-heptene, 2,5-dimethyl-2-heptene, 2,5-dimethyl-3-heptene, 3,6-dimethyl-3-heptene, 2,6-dimethyl-3-heptanol, 2,6-dimethyl-4-heptanol, 2,5-dimethyl-3-heptanol, 2,5-dimethyl-4-heptanol, 2,6-dimethyl-3,4-heptanediol, 2,6-dimethyl-3,4-heptanedione, 2,5-dimethyl-3,4-heptanediol, 2,5-dimethyl-3,4-heptanedione, 2,6-dimethyl-3-hydroxy-4-heptanone, 2,6-dimethyl-4-hydroxy-3-heptanone, 2,5-dimethyl-3-hydroxy-4-heptanone, 2,5-dimethyl-4-hydroxy-3-heptanone, n-octane, 1-octene, 2-octene, 1-octanol, octanal, octanoate, 3-octene, 4-octene, 4-octanol, 4-octanone, 4,5-octanediol, 4,5-octanedione, 4-hydroxy-5-octanone, 2-methyloctane, 2-methyl-3-octene, 2-methyl-4-octene, 7-methyl-3-octene, 3-methyl-3-octene, 3-methyl-4-octene, 6-methyl-3-octene, 2-methyl-4-octanol, 7-methyl-4-octanol, 3-methyl-4-octanol, 6-methyl-4-octanol, 2-methyl-4-octanone, 7-methyl-4-octanone, 3-methyl-4-octanone, 6-methyl-4-octanone, 2-methyl-4,5-octanediol, 2-methyl-4,5-octanedione, 3-methyl-4,5-octanediol, 3-methyl-4,5-octanedione, 2-methyl-4-hydroxy-5-octanone, 2-methyl-5-hydroxy-4-octanone, 3-methyl-4-hydroxy-5-octanone, 3-methyl-5-hydroxy-4-octanone, 2,7-dimethyloctane, 2,7-dimethyl-3-octene, 2,7-dimethyl-4-octene, 2,7-dimethyl-4-octanol, 2,7-dimethyl-4-octanone, 2,7-dimethyl-4,5-octanediol, 2,7-dimethyl-4,5-octanedione, 2,7-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyloctane, 2,6-dimethyl-3-octene, 2,6-dimethyl-4-octene, 3,7-dimethyl-3-octene, 2,6-dimethyl-4-octanol, 3,7-dimethyl-4-octanol, 2,6-dimethyl-4-octanone, 3,7-dimethyl-4-octanone, 2,6-dimethyl-4,5-octanediol, 2,6-dimethyl-4,5-octanedione, 2,6-dimethyl-4-hydroxy-5-octanone, 2,6-dimethyl-5-hydroxy-4-octanone, 3,6-dimethyloctane, 3,6-dimethyl-3-octene, 3,6-dimethyl-4-octene, 3,6-dimethyl-4-octanol, 3,6-dimethyl-4-octanone, 3,6-dimethyl-4,5-octanediol, 3,6-dimethyl-4,5-octanedione, 3,6-dimethyl-4-hydroxy-5-octanone, n-nonane, 1-nonene, 1-nonanol, nonanal, nonanoate, 2-methylnonane, 2-methyl-4-nonene, 2-methyl-5-nonene, 8-methyl-4-nonene, 2-methyl-5-nonanol, 8-methyl-4-nonanol, 2-methyl-5-nonanone, 8-methyl-4-nonanone, 8-methyl-4,5-nonanediol, 8-methyl-4,5-nonanedione, 8-methyl-4-hydroxy-5-nonanone, 8-methyl-5-hydroxy-4-nonanone, 2,8-dimethylnonane, 2,8-dimethyl-3-nonene, 2,8-dimethyl-4-nonene, 2,8-dimethyl-5-nonene, 2,8-dimethyl-4-nonanol, 2,8-dimethyl-5-nonanol, 2,8-dimethyl-4-nonanone, 2,8-dimethyl-5-nonanone, 2,8-dimethyl-4,5-nonanediol, 2,8-dimethyl-4,5-nonanedione, 2,8-dimethyl-4-hydroxy-5-nonanone, 2,8-dimethyl-5-hydroxy-4-nonanone, 2,7-dimethylnonane, 3,8-dimethyl-3-nonene, 3,8-dimethyl-4-nonene, 3,8-dimethyl-5-nonene, 3,8-dimethyl-4-nonanol, 3,8-dimethyl-5-nonanol, 3,8-dimethyl-4-nonanone, 3,8-dimethyl-5-nonanone, 3,8-dimethyl-4,5-nonanediol, 3,8-dimethyl-4,5-nonanedione, 3,8-dimethyl-4-hydroxy-5-nonanone, 3,8-dimethyl-5-hydroxy-4-nonanone, n-decane, 1-decene, 1-decanol, decanoate, 2,9-dimethyldecane, 2,9-dimethyl-3-decene, 2,9-dimethyl-4-decene, 2,9-dimethyl-5-decanol, 2,9-dimethyl-5-decanone, 2,9-dimethyl-5,6-decanediol, 2,9-dimethyl-6-hydroxy-5-decanone, 2,9-dimethyl-5,6-decanedionen-undecane, 1-undecene, 1-undecanol, undecanal. undecanoate, n-dodecane, 1-dodecene, 1-dodecanol, dodecanal, dodecanoate, n-dodecane, 1-decadecene, n-tridecane, 1-tridecene, 1-tridecanol, tridecanal, tridecanoate, n-tetradecane, 1-tetradecene, 1-tetradecanol, tetradecanal, tetradecanoate, n-pentadecane, 1-pentadecene, 1-pentadecanol, pentadecanal, pentadecanoate, n-hexadecane, 1-hexadecene, 1-hexadecanol, hexadecanal, hexadecanoate, n-heptadecane, 1-heptadecene, 1-heptadecanol, heptadecanal, heptadecanoate, n-octadecane, 1-octadecene, 1-octadecanol, octadecanal, octadecanoate, n-nonadecane, 1-nonadecene, 1-nonadecanol, nonadecanal, nonadecanoate, eicosane, 1-eicosene, 1-eicosanol, eicosanal, eicosanoate, 3-hydroxy propanal, 1,3-propanediol, 4-hydroxybutanal, 1,4-butanediol, 3-hydroxy-2-butanone, 2,3-butandiol, 1,5-pentane diol, homocitrate, homoisocitorate, b-hydroxy adipate, glutarate, glutarsemialdehyde, glutaraldehyde, 2-hydroxy-1-cyclopentanone, 1,2-cyclopentanediol, cyclopentanone, cyclopentanol, (S)-2-acetolactate, (R)-2,3-Dihydroxy-isovalerate, 2-oxoisovalerate, isobutyryl-CoA, isobutyrate, isobutyraldehyde, 5-amino

pentaldehyde, 1,10-diaminodecane, 1,10-diamino-5-decene, 1,10-diamino-5-hydroxydecane, 1,10-diamino-5-decanone, 1,10-diamino-5,6-decanediol, 1,10-diamino-6-hydroxy-5-decanone, phenylacetoaldehyde, 1,4-diphenylbutane, 1,4-diphenyl-1-butene, 1,4-diphenyl-2-butene, 1,4-diphenyl-2-butanol, 1,4-diphenyl-2-butanone, 1,4-diphenyl-2,3-butanediol, 1,4-diphenyl-3-hydroxy-2-butanone, 1-(4-hydeoxyphenyl)-4-phenylbutane, 1-(4-hydeoxyphenyl)-4-phenyl-1-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butene, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanol, 1-(4-hydeoxyphenyl)-4-phenyl-2-butanone, 1-(4-hydeoxyphenyl)-4-phenyl-2,3-butanediol, 1-(4-hydeoxyphenyl)-4-phenyl-3 -hydroxy-2-butanone, 1-(indole-3)-4-phenylbutane, 1-(indole-3)-4-phenyl-1-butene, 1-(indole-3)-4-phenyl-2-butene, 1-(indole-3)-4-phenyl-2-butanol, 1-(indole-3)-4-phenyl-2-butanone, 1-(indole-3)-4-phenyl-2,3-butanediol, 1-(indole-3)-4-phenyl-3-hydroxy-2-butanone, 4-hydroxyphenylacetoaldehyde, 1,4-di(4-hydroxyphenyl)butane, 1,4-di(4-hydroxyphenyl)-1-butene, 1,4-di(4-hydroxyphenyl)-2-butene, 1,4-di(4-hydroxyphenyl)-2-butanol, 1,4-di(4-hydroxyphenyl)-2-butanone, 1,4-di(4-hydroxyphenyl)-2,3-butanediol, 1,4-di(4-hydroxyphenyl)-3-hydroxy-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3 -)butane, 1-(4-hydroxyphenyl)-4-(indole-3)-1-butene, 1-di(4-hydroxyphenyl)-4-(indole-3)-2-butene, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanol, 1-(4-hydroxyphenyl)-4-(indole-3)-2-butanone, 1-(4-hydroxyphenyl)-4-(indole-3)-2,3-butanediol, 1-(4-hydroxyphenyl-4-(indole-3)-3-hydroxy-2-butanone, indole-3-acetoaldehyde, 1,4-di(indole-3-)butane, 1,4-di(indole-3)-1-butene, 1,4-di(indole-3)-2-butene, 1,4-di(indole-3)-2-butanol, 1,4-di(indole-3)-2-butanone, 1,4-di(indole-3)-2,3-butanediol, 1,4-di(indole-3)-3-hydroxy-2-butanone, succinate semialdehyde, hexane-1,8-dicarboxylic acid, 3-hexene-1,8-dicarboxylic acid, 3-hydroxy-hexane-1,8-dicarboxylic acid, 3-hexanone-1,8-dicarboxylic acid, 3,4-hexanediol-1,8-dicarboxylic acid, 4-hydroxy-3-hexanone-1,8-dicarboxylic acid, glycerol, fucoidan, iodine, chlorophyll, carotenoid, calcium, magnesium, iron, sodium, potassium, phosphate, lactic acid, acetic acid, formic acid, isoprenoids, and polyisoprenes, including rubber. Further, such products can include succinic acid, pyruvic acid, enzymes such as cellulases, polysaccharases, lipases, proteases, ligninases, and hemicellulases and may be present as a pure compound, a mixture, or an impure or diluted form.

[0054] Fermentation end-products can also include polyols or sugar alcohols; for example, methanol, glycol, glycerol, erythritol, threitol, arabitol, xylitol, ribitol, mannitol, sorbitol, dulcitol, fucitol, iditol, inositol, volemitol, isomalt, maltitol, lactitol, and/or polyglycitol.

[0055] The term "pH modifier" as used herein has its ordinary meaning as known to those skilled in the art and can include any material that will tend to increase, decrease or hold steady the pH of the broth or medium. A pH modifier can be an acid, a base, a buffer, or a material that reacts with other materials present to serve to raise, lower, or hold steady the pH. In one embodiment, more than one pH modifier can be used, such as more than one acid, more than one base, one or more acid with one or more bases, one or more acids with one or more buffers, one or more bases with one or more buffers, or one or more acids with one or more bases with one or more buffers. In one embodiment, a buffer can be produced in the broth or medium or separately and used as an ingredient by at least partially reacting in acid or base with a base or an acid, respectively. When more than one pH modifiers are utilized, they can be added at the same time or at different times. In one embodiment, one or more acids and one or more bases are combined, resulting in a buffer. In one embodiment, media components, such as a carbon source or a nitrogen source serve as a pH modifier; suitable media components include those with high or low pH or those with buffering capacity. Exemplary media components include acid- or base-hydrolyzed plant polysaccharides having residual acid or base, ammonia fiber explosion (AFEX) treated plant material with residual ammonia, lactic acid, corn steep solids or liquor.

[0056] The term "plant polysaccharide" as used herein has its ordinary meaning as known to those skilled in the art and can comprise one or more polymers of sugars and sugar derivatives as well as derivatives of sugar polymers and/or other polymeric materials that occur in plant matter. Exemplary plant polysaccharides include cellulose, starch, pectin, and hemicellulose. Others are chitin, sulfonated polysaccharides such as alginic acid, agarose, carrageenan, porphyran, furcelleran and funoran. Generally, the polysaccharide can have two or more sugar units or derivatives of sugar units. The sugar units and/or derivatives of sugar units can repeat in a regular pattern, or otherwise. The sugar units can be hexose units or pentose units, or combinations of these. The derivatives of sugar units can be sugar alcohols, sugar acids, amino sugars, etc. The polysaccharides can be linear, branched, cross-linked, or a mixture thereof. One type or class of polysaccharide can be cross-linked to another type or class of polysaccharide. The concentration of saccharides in a biomass containing plant polysaccharides such as cellulose, hemicellulose, starch, or pectin can be given in terms of monosaccharide equivalents. A monosaccharide equivalent concentration is the concentration of saccharides assuming complete hydrolysis of polysaccharides to monosaccharides.

[0057] The term "saccharification" as used herein has its ordinary meaning as known to those skilled in the art and can include conversion of plant polysaccharides to lower molecular weight species that can be utilized by the organism at hand. For some organisms, this would include conversion to monosaccharides, disaccharides, trisaccharides, and oligosaccharides of up to about seven monomer units, as well as similar sized chains of sugar derivatives and combinations of sugars and sugar derivatives.

[0058] The term "biomass" as used herein has its ordinary meaning as known to those skilled in the art and can include one or more biological materials that can be converted into a biofuel, chemical or other product. Biomass as used herein is synonymous with the term "feedstock" and includes corn syrup, molasses, silage, agricultural residues (corn stalks, grass, straw, grain hulls, fibers, bagasse, etc.), animal waste (manure from cattle, poultry, and hogs), Distillers Dried Solubles (DDS), Distillers Dried Grains (DDG), Condensed Distillers Solubles (CDS), Distillers Wet Grains (DWG), Distillers Dried Grains with Solubles (DDGS), woody materials (wood or bark, sawdust, timber slash, and mill scrap), municipal waste (waste paper, recycled toilet papers, yard clippings, etc.), and energy crops (poplars, willows, switchgrass, *Miscanthus* sp., alfalfa, prairie bluestem, algae, including macroalgae, etc.). One exemplary source of biomass is plant matter. Plant matter can be, for example, woody plant matter, including softwoods or hardwoods, non-woody plant matter, cellulosic material, lignocellulosic material, hemicellulosic material, carbohydrates, pectin, starch, inulin, fructans, glucans, corn, sugar cane, grasses such as rice, corn, barley, wheat, switchgrass, sorghum, high biomass sorghum, bamboo or the like, algae and material derived from these. Plants can be in their natural state or genetically modified, *e.g.,* to increase the cellulosic or hemicellulosic portion of the cell wall, or to produce additional exogenous or endogenous enzymes to increase the separation of cell wall components. Plant matter can also include plant cell culture or plant cell tissue culture. Plant matter can be further described by reference to the chemical species present, such as proteins, polysaccharides and oils. Polysaccharides include polymers of various monosaccharides and derivatives of monosaccharides including glucose, fructose, lactose, galacturonic acid, rhamnose, etc. Plant matter also includes agricultural waste byproducts or side streams such as pomace, corn steep liquor, corn steep solids, distillers grains, peels, pits, fermentation waste, straw, lumber, sewage, garbage and food leftovers. Peels can be citrus which include, but are not limited to, tangerine peel, grapefruit peel, orange peel, tangerine peel, lime peel and lemon peel. These materials can come from farms, forestry, industrial sources, households, etc. Another non-limiting example of biomass is animal matter, including, for example milk, meat, fat, animal processing waste, and animal waste. "Feedstock" is frequently used to

refer to biomass being used for a process, such as those described herein.

[0059] Biomass can be derived from agricultural crops, crop residues, trees, woodchips, sawdust, paper, cardboard, grasses, algae, municipal waste and other sources as described *supra.* In one embodiment, the biomass contains cellulosic, hemicellulosic, and/or lignocellulosic material. In one embodiment the biomass is woody (poplar, Eucalyptus, willow, pine, *etc.*). In another embodiment, the biomass is non-woody plant material, such as grasses, dicots, monocots, *etc.* Other biomasses include algal biomass, nonvascular plant biomass, and processed materials derived from plants; *e.g.,* hulls, distiller's grains, municipal sewage waste, and the like.

[0060] In one embodiment, a biomass composition comprising cellulose, hemicellulose, and/or lignocellulose comprises alfalfa, algae, bagasse, bamboo, corn stover, corn cobs, corn fiber, corn kernels, corn mash, corn steep liquor, corn steep solids, distiller's grains, distiller's dried solubles, distiller's dried grains, condensed distiller's solubles, distiller's wet grains, distiller's dried grains with solubles, eucalyptus, food waste, fruit peels, garden residue, grass, grain hulls, modified crop plants, municipal waste, oat hulls, paper, paper pulp, prairie bluestem, poplar, rice hulls, seed hulls, silage, sorghum, straw, sugarcane, switchgrass, wheat, wheat straw, wheat bran, de-starched wheat bran, willows, wood, plant cells, plant tissue cultures, tissue cultures, or a combination thereof.

[0061] The term "dry weight" of biomass is meant the weight of the biomass having all or essentially all water removed. Dry weight is typically measured according to American Society for Testing and Materials (ASTM) Standard E1 756-01 (Standard Test method for Determination of Total Solids in Biomass) or Technical Association of the Pulp and Paper Industry, Inc. (TAPPI) Standard T-412 om-02 (Moisture in Pulp, Paper and Paperboard).

[0062] The term "productivity" as used herein has its ordinary meaning as known to those skilled in the art and can include the mass of a material of interest produced in a given time in a given volume. Units can be, for example, grams per liter-hour, or some other combination of mass, volume, and time. In fermentation, productivity is frequently used to characterize how fast a product can be made within a given fermentation volume. The volume can be referenced to the total volume of the fermentation vessel, the working volume of the fermentation vessel, or the actual volume of broth being fermented. The context of the phrase will indicate the meaning intended to one of skill in the art. Productivity is different from "titer" in that productivity includes a time term, and titer is analogous to concentration. Titer and Productivity can generally be measured at any time during the fermentation, such as at the beginning, the end, or at some intermediate time, with titer relating the amount of a particular material present or produced at the point in time of interest and the productivity relating the amount of a particular material produced per liter in a given amount of time. The amount of time used in the productivity determination can be from the beginning of the fermentation or from some other time, and go to the end of the fermentation, such as when no additional material is produced or when harvest occurs, or some other time as indicated by the context of the use of the term. "Overall productivity" refers to the productivity determined by utilizing the final titer and the overall fermentation time.

[0063] The term "biocatalyst" as used herein has its ordinary meaning as known to those skilled in the art and can include one or more enzymes and/or microorganisms, including solutions, suspensions, and mixtures of enzymes and microorganisms. In some contexts this word will refer to the possible use of either enzymes or microorganisms to serve a particular function, in other contexts the word will refer to the combined use of the two, and in other contexts the word will refer to only one of the two. The context of the phrase will indicate the meaning intended to one of skill in the art. For example, a

biocatalyst can be a fermenting microorganism. The term biocatalyst includes fermenting microorganisms such as yeast, bacteria, or algae.

[0064] The terms "conversion efficiency" or "yield" as used herein have their ordinary meaning as known to those skilled in the art and can include the mass of product made from a mass of substrate. The term can be expressed as a percentage yield of the product from a starting mass of substrate. For the production of ethanol from glucose, the net reaction is generally accepted as:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2CO_2$$

and the theoretical maximum conversion efficiency, or yield, is 51% (wt.). Frequently, the conversion efficiency will be referenced to the theoretical maximum, for example, "80% of the theoretical maximum." In the case of conversion of glucose to ethanol, this statement would indicate a conversion efficiency of 41% (wt.). The context of the phrase will indicate the substrate and product intended to one of skill in the art.

[0065] "Pretreatment" or "pretreated" is used herein to refer to any mechanical, chemical, thermal, biochemical process or combination of these processes whether in a combined step or performed sequentially, that achieves disruption or expansion of the biomass so as to render the biomass more susceptible to attack by enzymes and/or microbes. In one embodiment, pretreatment includes removal or disruption of lignin so as to make the cellulose and hemicellulose polymers in the plant biomass more available to cellulolytic enzymes and/or microbes, for example, by treatment with acid or base. In one embodiment, pretreatment includes disruption or expansion of cellulosic and/or hemicellulosic material. Chemical pretreatment processes include, but are not limited to, bleaching, oxidation, reduction, acid treatment, base treatment, sulfite treatment, acid sulfite treatment, basic sulfite treatment, ammonia treatment, and hydrolysis. Thermal pretreatment processes include, but are not limited to, sterilization, ammonia fiber expansion or explosion ("AFEX"), steam explosion, holding at elevated temperatures, pressurized or unpressurized, in the presence or absence of water, and freezing. Biochemical processes include, but are not limited to, treatment with enzymes, including enzymes produced by genetically-modified plants, and treatment with microorganisms. Various enzymes that can be utilized include cellulase, amylase, β-glucosidase, xylanase, gluconase, and other polysaccharases; lysozyme; laccase, and other lignin-modifying enzymes; lipoxygenase, peroxidase, and other oxidative enzymes; proteases; and lipases. One or more of the mechanical, chemical, thermal, thermochemical, and biochemical processes can be combined or used separately. Such combined processes can also include those used in the production of paper, cellulose products, microcrystalline cellulose, and cellulosics and can include pulping, Kraft pulping, acidic sulfite processing. The feedstock can be a side stream or waste stream from a facility that utilizes one or more of these processes on a biomass material, such as cellulosic, hemicellulosic or lignocellulosic material. Examples include paper plants, cellulosics plants, distillation plants, cotton processing plants, and microcrystalline cellulose plants. The feedstock can also include cellulose-containing or cellulosic containing waste materials. The feedstock can also be biomass materials, such as wood, grasses, corn, starch, or saccharide, produced or harvested as an intended feedstock for production of ethanol or other products such as by biocatalysts.

[0066] Pretreatment of the biomass composition can be performed such that any solids are reduced in size. Reducing the size of solids in the biomass composition can be advantageous because smaller particles have larger surface area to volume ratios. Increasing the ratio of surface area to volume can be advantageous because it can, for example, increase the rate of particle wetting (*e.g.*, with water or

a chemical agent such as an acid or a base), increase the accessibility of enzymes to the polysaccharides in the biomass, enable the use of a smaller dose of enzymes during a hydrolysis of the biomass, enable the use of fewer or lower amounts of chemicals (*e.g.*, acids or bases) during a pretreatment and/or hydrolysis step, enable the use of weaker acids or bases in a pretreatment or hydrolysis step, enable the use of higher concentrations of solids in any further processing step *(e.g.,* during a hydrolysis step), and/or increase the yield of saccharides from the hydrolysis of the biomass.

[0067] Biomass compositions can be reduced in size to a mixture of particles having a uniform, or substantially uniform, size. Such mixtures can be referred to as homogeneous mixtures. Homogeneous mixtures of particles can have many advantages over mixtures of particles having heterogeneous sizes with respect to further pretreatment processes and/or during hydrolysis to produce saccharide streams. For example, heterogeneous mixtures of particles can experience uneven heating during thermal and thermochemical processing steps. Uneven heating can lead to overcooking (*e.g.*, charring/burning) of particles and/or undercooking of particles. Charring or burning of particles can reduce the yield of saccharide from the hydrolysis of the particles; this can be due to degradation or denaturation of saccharide polymers such as starch, hemicellulose, and/or cellulose. Undercooking of particles can lead to unhydrolyzed saccharide polymers (*e.g.*, starch, hemicellulose, cellulose) during enzymatic or chemical hydrolysis, which can also reduce the yield of saccharide. In contrast, uniform heating, wetting, chemical treatment (*e.g.*, acid or base treatment), and/or enzyme hydrolysis can be achieved with mixtures of particles having uniform sizes (*e.g.*, homogeneous mixtures).

[0068] "Sugar compounds", "sugar streams", "saccharide compounds", or "saccharide streams" is used herein to indicate mostly monosaccharide sugars, dissolved, crystallized, evaporated, or partially dissolved, including but not limited to hexoses and pentoses; sugar alcohols; sugar acids; sugar amines; compounds containing two or more of these linked together directly or indirectly through covalent or ionic bonds; and mixtures thereof. Included within this description are disaccharides; trisaccharides; oligosaccharides; polysaccharides; and sugar chains, branched and/or linear, of any length. A sugar stream can consist of primarily or substantially C6 sugars, C5 sugars, or mixtures of both C6 and C5 sugars in varying ratios of said sugars. C6 sugars have a six-carbon molecular backbone and C5 sugars have a five-carbon molecular backbone. The terms "sugar" and "saccharide" are used interchangeably herein.

[0069] A "liquid" or "aqueous" composition may contain solids and a "solids" composition may contain liquids. A liquid composition refers to a composition in which the material is primarily liquid, and a solids composition is one in which the material is primarily solid.

[0070] The term "kPa" refers to kilopascal, a unit of pressure. Standard atmospheric pressure, the pressure exerted by a 10 g mass resting on a 1 cm^2 area, is defined as 101.325 kPa. The term "psi" or "PSI" refers to pound-force per square inch. It is the pressure resulting from a force of one pound-force applied to an area of one square inch.

Description

[0071] The following description and examples illustrate some exemplary embodiments of the disclosure in detail. Those of skill in the art will recognize that there are numerous variations and modifications of this disclosure that are encompassed by its scope. Accordingly, the description of a

certain exemplary embodiment should not be deemed to limit the scope of the present disclosure.

[0072] Disclosed herein are methods for efficient, rapid treatment of biomass using high biomass concentration conditions. Unlike present methods, which can retain biomass materials in a chamber for a long period of time, it has been discovered that processing of these materials can avoid long retention times under thermal and chemical treatment, thereby avoiding the degradation of C5 sugars, proteins and lignins into undesirable products such as HMF and furfurals, while allowing the separation of carbohydrate materials, both monomeric and polymeric sugars, from other biomass components. The inhibitors usually formed during pretreatment are acetic acid (formed during the release of C5 sugars) and also formic acid, furfural and HMF. Formation of the latter three compounds is largely dependent on the temperature, pressure and biomass residence time during pretreatment.

[0073] Further, it has been discovered that the solubilization of crystalline cellulose is not impeded by the short exposure time. These methods can also allow biomass to be heated and pressurized uniformly for improved access of treatment reactants to the biomass. During this process, plugs can be produced that shear the biomass into smaller particles and further increase access of reactants to hydrolyze and release the C5 polymers while also releasing and solubilizing the C6 polymers. In one embodiment, the biomass is moved through a reaction zone wherein steam and pressure are applied, followed by the addition of acid, and finally release of the material to atmospheric pressure by a rapidly opening and closing an end valve. The whole process happens within seconds, resulting in a thermo-mechanical and chemically-hydrolyzed biomass with lower or reduced levels of inhibitors as compared to pretreatment methods known in the art.

[0074] In some cases, the pretreatment methods provided herein permit the release and depolymerization of sugars in a rapid time frame. The sugars can be released and depolymerized a within a very short period of time. The period of time can be less than 20 second. Generally, the time in the reaction zone can range from a second to less than 20 seconds. This provides continuously moving biomass through the tube resulting in a rapidly-pretreated biomass containing few, no, or substantially no inhibitors.

[0075] Described herein are improved, low cost, energy-efficient pretreatment devices and methods for the rapid processing of lignocellulose, cellulose, hemicellulose, and the like biomass materials prior to enzymatic hydrolysis, which includes a thermo-mechanical treatment with or without chemicals and a reaction extrusion controlled by a pressure-driven variable end valve. The methods disclosed herein can include the use of a device that comprises a cylindrical chamber divided into tubular zones, wherein biomass can be moved either continuously or in batches through the cylindrical chamber; reduced in size; and treated with pressure, heat, chemicals, or a combination thereof in the different tubular zones prior to being subjected to a rapid difference in temperature and pressure (*e.g.*, explosive decompression). The biomass can be moved by screw-type mechanism, such as a single, twin, or even triple screw as found in an extruder. Alternatively, the biomass can be moved by a mechanism such as a block or other mechanical pressure, differential hydrostatic pressure managed by air, oil, piston, vacuum, or gravity. These mechanisms can also have a function for pushing or driving forward or separating the biomass into chambers or zones for particular treatment or addition of materials.

[0076] In general, an extruder for use in this system includes an elongated barrel presenting a material inlet and a material outlet adjacent opposed ends thereof, with one or more elongated, axially rotatable screw(s) within the barrel which serves to advance the material from the inlet end to the outlet end

thereof. The screw is designed to smooth the flow of material while reducing it in size and various screw elements are arranged to increase or decrease the flow, or to form plugs of the biomass within the barrel. The screw(s) coupled with an end valve under pressure at the outlet, control the speed, pressure, and partly the temperature applied to the biomass as it moves through and out of the barrel.

[0077] It will be understood by those skilled in the art that various methods of processing biomass can be employed that are modified to include the herein described rapid treatment and hydrolysis to produce high levels of carbohydrates with low levels of inhibitors. For example the tube method of Andritz (US2014/0034260 A1) can be fitted with a separable inner chamber and the end valve process of this invention. The methods can be performed using a twin-screw extruder. Similarly, single or multiple screw extruders are adaptable to this method. Further, acid, alkali, or other chemicals can be used during pretreatment, or steam alone can be employed.

[0078] The systems and methods disclosed herein can be used for industrial scale pretreatment of biomass at a high rate of throughput. For example, it is estimated that biomass can move through and be processed in accordance with the following Table 1 by continuous operation of a twin screw extruder in accordance with some of the methods disclosed herein.

Table 1

Screw Diameter	Dry Matter Throughput
	Dry Tons/Day
30mm	3.3
52mm	17.0
92mm	94.4
124mm	231.1

[0079] In a general overview, the apparatus and its use in an extruder are described as follows. The barrel screw reactor can comprise a metal cylindrical barrel (which can be lined with specialty materials such as ceramic, glass, aluminum, hasteloy, titanium and the like) having a size that can range from, e.g., 30 mm to 220 mm diameter or larger equipped with one or more screws, oriented horizontally or vertically. The barrel can be divided into separate sections and can be equipped with multiple use ports along the top, side, and/or bottom surfaces. Such multiple use ports can be sealable ports. The multiple use ports can allow the injection of water, steam, acid or other chemicals. The multiple use ports can allow the insertion of thermocouples and/or pressure gauges for measurement of temperature and pressure inside the barrel. Additional ports can be added as required. The reactor barrel can be equipped with electric heating elements or a steam jacket for even heating of the barrel. Heating can alternatively or additionally be supplied by the injection of steam. The reactor barrel can be attached to a pipe that discharges into a flash tank or other container. The flash tank can be constructed using stainless steel. The barrel can be isolated from the flash tank by a pipe with a seat end having a pressure actuated discharge valve arrangement capable of continuously adjusting position depending upon the back pressure on the valve and the pressure within the system. The discharge valve arrangement can comprise a metal or ceramic sealing seat in between to allow for an explosive discharge of biomass. The pressure actuated valve arrangement can comprise a conical nozzle connected to a shaft (see in **FIGs. 1** and **2**). The diameter of the end valve can vary with the size of the machine, and typically ranges from 30 mm to 220 mm or larger. The conical nozzle can be connected to a shaft that is attached to an actuator in a backpressure generator. The actuator can

provide the pneumatic pressure that is regulated by the backpressure generator, which monitors the pressure. The pressure can be a high pressure such that no backflow occurs and there is a restricted flow of material out of the tube. The backpressure on the conical nozzle and seat can be adjustable. For example, operations can be performed using 50 psi to 600 psi (gauge pressure) of backpressure onto the shaft connected to the conical nozzle of the end shear valve. The cone of the end shearing valve can travel between a fully closed and a fully open position, and any intermediate position. A pipe at the outlet of the end shear valve can direct the treated solids down into the bottom of the flash tank, where the solids and vapor can be separated and easily removed.

[0080] FIG. 1 shows an embodiment of one type of a design of a reactor as provided herein. The reactor can be a commercial scale reactor. It comprises a horizontal cylindrical barrel **31** fitted with twin screws (not shown) and a discharge valve **17** attached at a special end flange **14** at the second end of the barrel. The barrel can be insulated and can have impermeable walls. A motor **24** for moving the screws can be attached near the first end. The motor can be, *e.g.,* an electrically-driven motor and gearbox combination, with or without pulleys and V-belts or any other mechanism to turn the screws. The motor can also be, for example and without being limiting, a synchronous torque motor. A hopper **20** can be attached to the inlet of the sealed end of the barrel **31.** Biomass can be added through the opening of the hopper **20.** The biomass can be any biomass as provided herein. There can be a feeder for non-compacting or compacting flow generation (not shown) such as a crammer to control biomass addition from the hopper **20** to the barrel **31.** The compacting and/or non-compacting feeder can be any compacting and/or non-compacting loader known in the art. For example, a non-compacting flow inducing feeder can be a non-compacting feeder or various types of live-bottom bin flow inducers followed by flow metering conveyors such as various types of drag chains, bucket elevators, or rotating helixes. In its simplest form a non-compacting feeder can refer to loading biomass by hand into an open first end of the cylindrical barrel. Compacting feeders can comprise mechanical compaction. Mechanical compaction can be achieved by provision of a mechanical compaction device such as a reciprocating plunger or screw feeder. The barrel **31** can have a first sealable port **29** for adding water to hydrate the biomass in the tube as it moves away from the hopper end. The screws can be designed with sections to form a high-shear plug **P1**, which can occur after the addition of water but prior to the addition of steam through the ports **32** and **33.** The high shear plug **P1** can break the biomass into smaller-sized particles (e.g., of about 10 to 200 microns). The plugs that are formed can separate the biomass into sections or zones that can be treated differently from one another. Movement of the screws through the impermeable barrel chamber **30** pushes the biomass and water mixture from the feeder zone into a first zone **91.** Ports **32**, **33** add pressurized steam (e.g., to about 300 psi to 600 psi) to the barrel after the first plug is formed, increasing the barrel pressure to a desired temperature and pressure (for example, 600 psi and a temperature of 253°C). A second plug **P2** can be formed prior to the addition of aqueous acid (or other chemical, *e.g.,* a base) and can separate the material into a second zone **92.** In this second zone, C6 polymers can be solubilized and/or C5 polymers can be hydrolyzed into monosaccharides. The thermo-mechanical conditions can be maintained in this zone. Following the acid (or alkali solution) addition through **34,** a third plug **P3** can be formed as the biomass moves through the cylindrical barrel chamber **30.** The acid (or alkali solution) can also be added after the third plug is formed. Further solubilization of sugars can occur in this third zone **93** and, if needed, more water can be added through another port (not shown) in the third zone **93.**

[0081] The end valve **17** at the far end of the third zone **93,** when seated, comprises a part of the reaction zone **R1**. The end valve can be under constant pressure. A back pressure regulator (*e.g.*, see **FIGs. 11** and **12)** can be added to the barrel cylinder **31** such that the back-pressure regulator

monitors the pressure at the end of the zone **93.** The monitoring can be continuous. The back pressure regulator acts to maintain a set pressure by opening and closing the end valve (e.g., on a continuous basis) **17** through a shaft **60** connected to an actuator (*e.g.*, see **FIGs. 12** and **13**). The actuator can be any actuator as provided herein. For example, the actuator can be a pneumatic actuator. The valve activity can occur rapidly to open the end valve **17** and release pressurized material into a pipe **62** that leads to the open flash tank **70,** thus blowing out the treated biomass and rapidly dropping the temperature and pressure from several hundred psi to atmospheric pressure. As the pressure drops, the back pressure regulator causes the actuator to close the end valve **17** via movement of the shaft **60.** The pressure differential can be varied depending on the amount needed to further solubilize the C6 sugars. When operating at ideal conditions, the end valve **17** may never be completely closed and never be completely open, the shaft **60** moving back and forth under the control of the actuator.

[0082] In some cases, any device as provided herein comprises an actuator. The actuator can be controlled by a regulator. The actuator on a device as provided herein can be any type of mechanical, electro-mechanical, linear, piezoelectric, ultrasonic, hydraulic, electrohydraulic, pneumatic, segmented spindle, moving coil, or moving iron controllable actuator or motor known in the art. In some cases, the actuator in a device as provided herein comprises a pneumatic actuator. The pneumatic actuator can be a piston actuator. In some cases, the actuator in a device as provided herein comprises a hydraulic actuator. Examples of mechanical actuators can include, screw type actuators (*e.g.,* leadscrew, screw jack, ball screw, or roller screw), wheel and axle *(e.g.,* hoist, winch, rack and pinion, chain drive, belt drive, rigid chain, or rigid belt), piston actuators, diaphragm actuators, or cam actuators. A regulator for controlling an actuator in any device as provided herein can be a pressure regulator. The pressure regulator can be a back-pressure regulator. The pressure regulator (*e.g.*, back-pressure regulator) can be a single stage regulator or double stage regulator. The pressure regulator can comprise a restricting element, a loading element, and a measuring element. The restricting element can be a valve such as a globe, butterfly or poppet valve. For precise control, a valve such as a linear globe can work well. Others types of valves can be a fast-opening globe, a ball, a butterfly, or an equal percentage globe valve.

[0083] The loading element can be a weight, a spring, or an actuator (*e.g.*, piston or diaphragm actuator) in combination with a spring. In some cases, the pressure regulator in any device provided herein is a pneumatic pressure regulator. The pneumatic pressure regulator works with a modified poppet valve. For example, an E/P pressure regulator, series ED05 (Bosch Rexroth AG) can be used. In some cases, the pressure regulator in any device provided herein is a hydraulic pressure regulator. The pressure regulator can also be in communication and/or comprise a flow meter for measuring flow rates within a device as provided herein. The flow meter can be a flow meter, rotometer or mass flow controller known in the art.

[0084] The three cylindrical chambers (zones) **91, 92,** and **93** within the barrel cylinder **31** and discharge pipe **62** through the valve to atmospheric pressure comprise the reaction zone **R1** where pretreatment of the biomass occurs. Pretreatment of the biomass inputted into the barrel cylinder **31** occurs within the barrel chamber **30** as long as the material is at elevated temperatures and pressures, so the reaction zone **R1** ends when the material is flashed to atmosphere. The thermochemical and mechanical pressure in this zone, as well as the residence time, can be varied in accordance with the type of biomass being pretreated. Those of skill in the art will recognize that biomass material with a high concentration of easily solubilized and hydrolyzed carbohydrate polymers could require less time and/or less pressure and temperature.

[0085] The residence time in the reaction zone can be very short as compared to other preteatment systems known in the art. The residence time in a reaction zone *(e.g.,* **FIG. 1, R1**) of a device as provided herein can be less than 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 35, 40, 45, 50, 55, or 60 seconds. The residence time in a reaction zone *(e.g.,* **FIG. 1, R1**) of a device as provided herein can be about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 35, 40, 45, 50, 55, or 60 seconds. The residence time in a reaction zone (*e.g.,* **FIG. 1, R1**) of a device as provided herein can be between about 1 second to about 2 , about 1 second to about 3 seconds, about 1 second to about 4 seconds, about 1 second to about 5 seconds, about 1 second to about 6 seconds, about 1 second to about 7 seconds, about 1 second to about 8 seconds, about 1 second to about 9 seconds, about 1 second to about 10 seconds, about 1 second to about 15 seconds, about 1 to about 20 seconds, about 2 second to about 4 seconds, about 2 second, to about 6 second, about 2 seconds to about 8 second, about 2 second, to about 10 seconds, about 2 seconds to about 15 second, about 2 seconds to about 20 seconds, about 5 seconds to about 10 seconds, about 5 seconds to about 10 seconds, about 5 seconds to about 15 seconds, about 5 seconds to about 20 seconds, about 10 second to about 12 second seconds, about 10 seconds to about 14 seconds, about 10 seconds, to about 16 seconds, about 10 seconds to about 18 seconds, about 10 seconds to about 20 seconds, about 15 seconds to about 20 seconds, about 20 seconds to about 30 seconds, about 30 seconds to about 45 seconds, or about 45 seconds to about 60 seconds. The pressure can vary from 0 to 800 psi, preferably from 300-700 psi. The temperature range is wide, from 100 to 260°C or more, preferably from 160-230°C. The temperature used often depends on the crystallinity of the cellulose fiber in the biomass; for example, softwood has a higher percent of crystalline cellulose and requires a temperature of 210-240°C. Acid may or may not be added to assist with the reaction and can range from 0 wt% of pure chemical per dry tonne of biomass to 8 wt% of pure chemical per dry tonne of biomass, preferably from 1 wt% to 5 wt%.

[0086] In another embodiment, biomass as provided herein can be pre-treated at an elevated temperature and/or pressure in a device as provided herein. In one embodiment, biomass is pre-treated at a temperature range of 20°C to 400°C. In another embodiment biomass is pretreated at a temperature of about 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 80°C, 90°C, 100°C, 120°C, 150°C, 200°C, 250°C, 300°C, 350°C, 400°C or higher. In another embodiment, elevated temperatures are provided by the use of steam, hot water, or hot gases. In one embodiment steam can be injected into a biomass containing vessel or barrel chamber. In another embodiment the steam, hot water, or hot gas can be injected into a vessel jacket such that it heats, but does not directly contact the biomass. In an additional embodiment heat can be externally applied using electric barrel heaters.

[0087] In another embodiment, biomass as provided herein can be pre-treated at an elevated temperature and/or pressure in a device as provided herein. In one embodiment, biomass is pre-treated at a pressure range of from 0 to 800 PSI. In some embodiments, heating the biomass pretreated in a device as provided herein is performed at a pressure higher than atmospheric. The pressure can be from about 25 PSI to about 800 PSI. The pressure can be from about 300 PSI and 500 PSI. The pressure can be about 400 PSI. For example, the pressure can be about 25-800, 25-700, 25-600, 25-500, 25-250 PSI, 25-225 PSI, 25-200 PSI, 25-175 PSI, 25-150 PSI, 25-125 PSI, 25-100 PSI, 25-75 PSI, 25-50 PSI, 50-225 PSI, 50-200 PSI, 50-175 PSI, 50-150 PSI, 50-125 PSI, 50-100 PSI, 50-75 PSI, 75-200 PSI, 75-175 PSI, 75-150 PSI, 75-125 PSI, 75-100 PSI, 100-175 PSI, 100-150 PSI, 100-125 PSI, 125-150 PSI, 25 PSI, 30 PSI, 35 PSI, 40 PSI, 45 PSI, 50 PSI, 55 PSI, 60 PSI, 65 PSI,

70 PSI, 75 PSI, 80 PSI, 85 PSI, 90 PSI, 95 PSI, 100 PSI, 105 PSI, 110 PSI, 115 PSI, 120 PSI, 125 PSI, 130 PSI, 135 PSI, 140 PSI, 145 PSI, 150 PSI, 155 PSI, 160 PSI, 165 PSI, 170 PSI, 175 PSI, 180 PSI, 185 PSI, 190 PSI, 195 PSI, 200 PSI, 205 PSI, 210 PSI, 215 PSI, 220 PSI, 225 PSI, 230 PSI, 235 PSI, 240 PSI, 245 PSI, 250 PSI, 300PSI, 350 PSI, 400 PSI, 450 PSI, 500 PSI, 550 PSI, 600 PS, 650 PSI, 700 PSI, 750 PSI, 800 PSI, 850 PSI, 900 PSI, 950 PSI, or 1000 PSI. In one embodiment, the pressure is from about 25 PSI to about 250 PSI. In another embodiment, the pressure is from about 75 PSI to about 200 PSI. In another embodiment, the pressure is from about 100 PSI to about 400 PSI.

[0088] In one embodiment, one or more acids can be combined, resulting in a buffer that can be used for conducting pretreatment of biomass as provided herein in a device as provided herein. In some instances, the pH can be lowered to neutral pH or acidic pH, for example a pH of 7.0, 6.5, 6.0, 5.5, 5.0, 4.5, 4.0, or lower. For example, the non-neutral aqueous medium used to pretreat biomass as provided herein in a device as provided herein can have a pH that is less than 7, 6.5, 6, 5.5, 5, 4.5, 4, 3.5, 3, 2.5, 2, 1.5, or 1. For example, the non-neutral aqueous medium can have a pH that is about 6.5, 6.4, 6.3, 6.2, 6.1, 6, 5.9, 5.8, 5.7, 5.6, 5.5, 5.4, 5.3, 5.2, 5.1, 5, 4.9, 4.8, 4.7, 4.6, 4.5, 4.4, 4.3, 4.2, 4.1, 4, 3.9, 3.8, 3.7, 3.6, 3.5, 3.4, 3.3, 3.2, 3.1, 3, 2.9, 2.8, 2.7, 2.6, 2.5, 2.4, 2.3, 2.2, 2.1, 2, 1.9, 1.8, 1.7, 1.6, 1.5, 1.4, 1.3, 1.2, 1.1, 1, 0.9, 0.8, 0.7, 0.6, 0.5, 0.4, 0.3, 0.2, 0.1, or lower. In some embodiments, the pH is lowered and/or maintained within a range of about pH 4.5 to about 7.1, or about 4.5 to about 6.9, or about pH 5.0 to about 6.3, or about pH 5.5 to about 6.3, or about pH 6.0 to about 6.5, or about pH 5.5 to about 6.9 or about pH 6.2 to about 6.7.

[0089] In some embodiments, pretreatment of a biomass as provided herein in a device as provided herein comprises hydration of the biomass composition in a non-neutral aqueous medium having a pH that is greater than 7. For example, the non-neutral aqueous medium can have a pH that is greater than 7, 7.5, 8, 8.5, 9, 9.5, 10, 10.5, 11, 11.5, 12, 12.5, 13, 13.5 or higher. For example, the non-neutral aqueous medium can have a pH that is about 7.5, 7.6, 7.7, 7.8, 7.9, 8, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10, 10.1, 10.2, 10.3, 10.4, 10.5, 10.6, 10.7, 10.8, 10.9, 11, 11.1, 11.2, 11.3, 11.4, 11.5, 11.6, 11.7, 11.8, 11.9, 12, 12.1, 12.2, 12.3, 12.4, 12.5, 12.6, 12.7, 12.8, 12.9, 13, 13.1, 13.2, 13.3, 13.4, 13.5, 13.6, 13.7, 13.8, 13.9, or higher. The non-neutral aqueous medium having a pH greater than 7 can comprise one or more bases such as sodium hydroxide, calcium hydroxide, potassium hydroxide, ammonia, ammonia hydroxide, hydrogen peroxide or a combination thereof. The one or more bases can be at any suitable concentration, such as any of the concentrations disclosed herein.

[0090] In some embodiments, pretreatment of a biomass composition comprises hydration of the biomass composition in a non-neutral aqueous medium comprises from about 0.1% to about 50% w/w or v/w by dry biomass weight of one or more acids or one or more bases. For example, the non-neutral aqueous medium can comprise about 25-50%, 10-50%, 10-25%, 5-50%, 5-25%, 5-10%, 4-50%, 4-25%, 4-10%, 4-5%, 3-50%, 3-25%, 3-10%, 3-5%, 3-4%, 2-50%, 2-25%, 2-10%, 2-5%, 2-4%, 2-3%, 1-50%, 1-25%, 1-10%, 1-5%, 1-4%, 1-3%, 1-2%, 0.5-50%, 0.5-25%, 0.5-10%, 0.5-5%, 0.5-4%, 0.5-3%, 0.5-2%, 0.5-1%, 0.5-%, 0.1-50%, 0.1-25%, 0.1-10%, 0.1-5%, 0.1-4%, 0.1-3%, 0.1-2%, 0.1-1%, 0.1-0.5%, 50%, 45%, 40%, 35%, 30%, 25%, 24%, 23%, 22%, 21%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9.5%, 9%, 8.5%, 8%, 7.5%, 7%, 6.5%, 6%, 5.5%, 5%, 4.9%, 4.8%, 4.7%, 4.6%, 4.5%, 4.4%, 4.3%, 4.2%, 4.1%, 4%, 3.9%, 3.8%, 3.7%, 3.6%, 3.5%, 3.4%, 3.3%, 3.2%, 3.1%, 3%, 2.9%, 2.8%, 2.7%, 2.6%, 2.5%, 2.4%, 2.3%, 2.2%, 2.1%, 2%, 1.9%, 1.8%, 1.7%, 1.6%, 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, or 0.1% of the one or more acids or the one or more bases. The one or more acids can be sulfuric acid, peroxyacetic acid, lactic acid, formic acid, acetic acid, citric acid, phosphoric acid, hydrochloric acid, sulfurous acid, chloroacetic acid,

dichloroacetic acid, trichloroacetic acid, trifluoroacetic acid, oxalic acid, benzoic acid, or a combination thereof. The one or more bases can be sodium hydroxide, calcium hydroxide, potassium hydroxide, ammonia, ammonia hydroxide, hydrogen peroxide or a combination thereof. In some embodiments, the non-neutral aqueous medium comprises the one or more acids or the one or more bases at from about 1% to about 5% v/w by dry biomass weight. In some embodiments, the non-neutral aqueous medium comprises sulfuric acid at from about 1% to about 5% v/w by dry biomass weight. In some embodiments, the non-neutral aqueous medium comprises sulfuric acid at about 1.8% v/w by dry biomass weight. In some embodiments, the non-neutral aqueous medium comprises sulfuric acid at about 1% v/w by dry biomass weight.

[0091] In the flash tank **70,** the biomass can move through a downward directed pipe. The pretreated biomass can be accessible from the flash tank **70.** In some cases, a device for pretreating biomass as provided herein can be designed to move the biomass to a separation step or to an enzyme hydrolysis tank. Vapors can be discharged through the open top of the flash tank **70** or, in the alternative, the flash tank **70** can be closed and vapors discharged through a pipe to another area or chamber. Alternatively, the pipe can be connected through a tubing to a condenser.

[0092] The apparatus (*e.g.*, barrel cylinder) can be constructed using carbon steel, stainless steel or any other material that is impervious to acid and alkali and that can withstand the pressures generated. It is also possible to have a chemically-inert coating on the inside of the chamber (*e.g.*, barrel cylinder inner chamber) that does not react with acid or alkali or any chemical that is used in the methods provided herein. The cylindrical barrel may be horizontal or vertical with modifications for loading biomass or providing the proper discharge pressure. One skilled in the art could readily configure the apparatus with a vertical barrel for proper operation.

[0093] In some cases, a device for pretreating biomass as provided herein comprises a twin-screw extruder. An example of a co-rotating, twin-screw extruder used for the methods provided herein is shown in **FIG. 2.** The twin-screw extruder in **FIG. 2** comprises barrel **31,** a horizontal cylindrical chamber **30,** which includes two screw-type feeder mechanisms **51** and **52.** The barrel comprises an open first end **11** for adding biomass. The biomass can be any biomass as provided herein. The overall extrusion apparatus **10** includes a primary feed hopper **20** to contain the biomass being added. Inside the hopper **20** is a delivery auger **13** to evenly distribute the material into the open first end **11.** There is a port **15** into which water can be added as the biomass enters the extruder chamber **30.** The cylindrical chamber **30** has another port **29** for water, sealable ports **32, 33** for the addition of pressurized steam and sealable ports **34, 35** for the addition of chemical reactant (*e.g.*, acid). A third port **36** can be added if additional steam is needed downstream of ports **34** and **35.** As in **FIG. 1,** the barrel chamber **30** is divided into 3 zones (not shown), produced by screw configurations, akin to **91, 92,** and **93** in **FIG. 1.** Pressurized steam is injected to raise the temperature and pressure of the biomass, and chemical for the chemical reaction, if necessary. Insulation **12** can be provided outside or as part of the barrel **31** that can encompass the barrel chamber **30** and maintain the desired temperature inside the chamber. Temperature gauges **83, 87** and pressure gauges **81, 85** are used to monitor temperature and pressure inside the chamber, respectively.

[0094] As biomass is loaded through the hopper **20** into the chamber **30,** thermo-mechanical pressure builds through the addition of steam and the configuration of plugs (due to the shape and movement of various sections of the screw mechanisms). The twin screw mechanism **51**, **52** moves the biomass through the tube to the opening **22** between the end plate **14** of the barrel chamber **30** and the discharge valve **89.**

[0095] As in **FIG. 1,** the device of **FIG. 2** produces three plugs **P1**, **P2, P3** during the process of moving biomass through the tube from one end to the other (see **FIG. 1**). While not shown in **FIG. 2, P1** is formed after the addition of water through port **29** prior to the addition of steam through the ports **32** and **33.** Ports **32**, **33** add pressurized steam at about 300 psi to 600 psi to the barrel after the first plug is formed increasing the barrel pressure to a desired temperature and pressure; for example, 600 psi and a temperature of 253°C. **P2** is formed prior to the addition of aqueous acid through ports **34** and **35,** while **P3** is usually formed after the addition of aqueous acid. Also like **FIG. 1, in FIG. 2** the zone within the barrel chamber **30** between **P1** and **P2** is zone **91,** while zone **92** is between **P2** and **P3** and zone **93** is between P3 and the area of discharge. The zone **91** between the first two plugs, zone **92** between plug **P2** and plug **P3,** the zone between plug **P3** and the area of discharge through the special end flange **14** collectively constitute the reaction zone **R1** (as shown in **FIG.1).** In some cases, chemicals used to assist in the pretreatment of the biomass (*e.g.*, acid or alkali or another chemical) are added between the second plug **P2** and third plug **P3** formed in the reaction zone **R1**.

[0096] In some cases, pretreatment of biomass using a device as depicted in **FIG. 2** entails sealing the ports in the device of **FIG. 2** following movement of biomass through each section and addition of aqueous solution comprising acid and steam and subsequently maintaining a desired temperature. As shown in **FIG. 2,** the discharge valve **17** can be partially seated in a metal or ceramic seal **89** such that the discharge valve is mostly closed. In operation, **(FIG.** 2 and **FIG. 12)** as pressure in the barrel chamber **30** builds to a certain point, the discharge valve **17** is pushed open (away from the barrel chamber). The valve shaft **60** extends through a tube to an actuator **72** within or attached to a back pressure generator (control unit) **73.** The mixture of biomass, sugars, and aqueous chemical is pushed through the discharge valve opening **22** by the movement of the twin screws **50, 51** in the chamber **30** from the first end towards the second (outlet) end and from the pressure buildup. The biomass passes through the discharge valve opening **22** and through a pipe **62** where it is collected in a flash tank **70** through an opening **71** in the top of the flash tank which allows access to pretreated biomass and the exit of vapors. There can also be a separate opening to allow discharge of vapors (not shown).

[0097] A cross section of the discharge end of the twin screw extruder shown in **FIG. 2** is depicted in **FIG. 3.** As shown in **FIG. 3,** the barrel **31** houses the twin screws **51, 52** which are turned by the shafts **26**, **28**. In one embodiment, a replaceable solid barrel insert **38** of corrosion and wear-resistant specialty metal or other compound can surround the screws. The extent of the blade edges of the twin screws **51, 52** and the boundaries of the bored holes through the barrel insert **38** are represented by dotted lines. There is 1 mm or so between the tips of the screw elements and the barrel insert **38.** In some instances, the dotted lines can be the actual barrel openings (instead of barrel **31**) and can be comprised of specialty metal inserts added for wear resistance and bored out to match the diameter of the screws. In one embodiment, a heating apparatus (not shown) is fitted around a whole cylindrical barrel. **FIG. 4** shows a cross section of the discharge end special face flange **14** that is attached to the end of the extruder. The flange **14** is connected to the extruder and the twin screws convey material onward to the discharge valve. **FIG. 5** depicts the transition from the two-screw opening in the extruder discharge end flange **14** to the single outlet of the valve opening **22** when looking from the end of the valve opening from the narrow end that connects with valve **17.** The opening expands to encompass the two-screw opening of flange **14. FIG. 6** further depicts the discharge valve end housing **19** with a single cylindrical opening **22** that connects adjacent to the extruder flange **14.** Pretreated biomass is discharged through the opening **22** surrounded by the metal or ceramic seal **89** which is secured in position to interact with the discharge valve mechanism. The ceramic or metal seal can also extend into the valve opening **22** and provide a coating or insert between the valve opening **22** and the valve

housing **19.**

[0098] The valve at the discharge end ("end valve") of an extruder as provided herein can be one of many different designs. The end valve used in this process can be one of several types that can be precisely monitored and controlled by a back pressure generator. The end valve can be unidirectional or bidirectional. In some cases, an end valve in an extruder as provided herein is unidirectional while the flow through the extruder is unidirectional. In some cases, an end valve in an extruder as provided herein is bidirectional while the flow through the extruder is unidirectional. **FIG. 7** is a horizontal view of the valve opening **22** in its housing **19** where it narrows from the two-screw openings of the extruder to the valve discharge end.

[0099] FIG. 8 is a horizontal view of the valve housing assembly **65** with the discharge pipe **62,** including the housing **63** that holds the valve opening and housing **19** as shown in **FIG. 7. FIG. 9** depicts the housing of **FIG. 8,** showing how the valve assembly is integrated with the housing. **FIG. 10** is a horizontal sectional view with the valve **17** and valve shaft **60** seated in the valve opening housing **19** within the housings **65** and **63.**

[0100] FIGS. 1 and **2** are embodiments that show the movement of the biomass through reactors using plugs to chamber different reactions and to reduce particle size. These embodiments can be varied to accommodate different types and sizes of biomass for optimal processing and the recovery of monosaccharides or even oligomers. For example, the size of the second chamber can be reduced if the biomass contains a small percentage of hemicellulose compared to the cellulosic portion. Residence time in any chamber can be varied and those of skill in the art will understand that the types of screw elements in sections that produce the plugs, and cut and move the processing materials forward and their placement can have an infinite number of permutations. The combinations used will depend on the type of biomass and the size of the particles desired for optimum pretreatment. Thus residence time, temperature, time, and chemical treatment can be unlimited using this method.

[0101] Screw sections incorporated into this system can include, for example, conveying elements for moving materials through the extruder and kneading block elements for forming plugs. One suitable system can be comprised of a single flight screw element with mixing grooves in the screw profile. The element can have a left hand (reversed conveying) or right hand (forward conveying pitched screw profile). These elements can comprise less mixing grooves and reduced groove depth to reduce the product cross flow between the screw profile channels, thus assisting to maintain a uniform pressure and movement of the biomass and reduce backflow. The screw elements can be comprised of various materials, including, for example, Stellite, Hasteloy, Inconell, PM steel, Chromium steel, and nitride steel, and/or can be manufactured with various surface coatings to reduce wear and abrasion. Examples of such elements can be found at Extricom GmbH (www.extricom.de). Those of skill in the art will understand the types of elements and their arrangement are unlimited and can be organized in many different patterns for specific biomass materials.

[0102] The initial dry weight of biomass used in the methods of this invention is at least about 10% of the total weight of the biomass and aqueous acid mixture. More typically, the dry weight of biomass is at least about 20%, and can be at least about 30%, 45%, 50%, or more. Feedstock biomass will typically range between 30 wt% and 90 wt% solids, and the biomass exiting the pretreater will typically range between 25 wt% and 40 wt% solids. The percent dry weight of biomass may vary and the optimal percent may be different for different types of biomass. For example, biomass of at least about 40% is desired when using sawdust (sawdust will get diluted with a small amount of steam

condensate), to provide pretreated biomass that is saccharified to produce fermentable sugars concentrated sufficiently for cost-effective fermentation to ethanol. More suitable is sawdust biomass that is at least about 30%. The preferred percent dry weight of a particular type of biomass for use in the present methods for producing a high sugars hydrolysate can be readily determined by one skilled in the art.

[0103] The biomass can be loaded into a feeder apparatus such as the hopper diagrammed in **FIG. 1,** which, in turn, feeds it to the reactor. The loading can be facilitated by use of a flow conveyor such as a screw conveyor, crammer, drag chain, bucket elevator, conveyor belt, or the like. The feeding of the biomass into the reactor can be made more uniform by the addition of a conical screw or the like, that allows the biomass to enter the reactor at a uniform rate and density that is helpful to keep the feeder apparatus from clogging.

[0104] Using this method, an aqueous solution comprising acid or base can comprise any concentration that is necessary to hydrolyze the carbohydrate polymers. Thus, for example, acid at a concentration of 0.01% to over 7 or 8%, or concentrations of 1%, 2%, 3%, 4%, 5%, 6% or anything in between can be used. In pretreatment devices as provided herein, ports, excluding the one or more through which steam is being added, can be sealed. Valves for use in the devices as provided herein can be any type of valve known in the art that can be opened or closed. The valves can be ball valves, poppet valves, check valves, or rotating knife-gate valves, or combinations thereof.

[0105] Steam can be added through one or more ports in the cylindrical barrel at the beginning of the reaction zone, after the first plug is formed, in an amount that is needed to raise the temperature of the biomass and aqueous acid mixture to the desired point. More than one port can be used, with ports being spaced so that steam contact is distributed over the biomass or to raise the temperature and pressure more quickly. Pressurized steam can be added to raise the temperature of the biomass and aqueous acid mixture to between about 80° C. and about 300° C, preferably between 160° C and 230° C. The temperature of the biomass and aqueous acid can be about 20°C, 25°C, 30°C, 35°C, 40°C, 45°C, 50°C, 55°C, 60°C, 65°C, 80°C, 90°C, 100°C, 120°C, 150°C, 200°C, 250°C, 300°C, 350°C, or 400°C. The temperature of the biomass and aqueous acid can be from about 20°C to about 400°C, about 50°C to about 350°C, about 80°C to about 300°C, about 100°C to about 250°C, or about 160°C to about 210°C. Additional steam can be added through a port between the second and third plug formation of cylindrical chamber, if needed, to maintain the desired temperature and pressure. The apparatus can include a heating jacket, steam jacket, band heaters, barrel heaters, or insulation jacket to contribute to raising and/or maintaining the temperature and pressure. Heating or steam jackets are particularly suited to small scale reactors while insulation jackets are suited to large scale reactors. Heating can occur at different stages, including preheating the barrel prior to treating or pretreating. The type of biomass being pretreated also can affect the optimum time and temperature for treatment in the present method, as can readily be assessed by one skilled in the art.

[0106] Bringing the biomass to the described temperatures using pressurized steam in these methods results in pressures within the reactor chamber that are between about 300 psi and about 1000 psi. More typically, pressure is between about 300 psi to 800 psi. The pressurized steam is added through the ports at about 300 to 600 psi. The pressures within the reactor chamber can be 25-250 PSI, 25-225 PSI, 25-200 PSI, 25-175 PSI, 25-150 PSI, 25-125 PSI, 25-100 PSI, 25-75 PSI, 25-50 PSI, 50-225 PSI, 50-200 PSI, 50-175 PSI, 50-150 PSI, 50-125 PSI, 50-100 PSI, 50-75 PSI, 75-200 PSI, 75-175 PSI, 75-150 PSI, 75-125 PSI, 75-100 PSI, 100-175 PSI, 100-150 PSI, 100-125 PSI, 125-150 PSI, 25 PSI, 30 PSI, 35 PSI, 40 PSI, 45 PSI, 50 PSI, 55 PSI, 60 PSI, 65 PSI, 70 PSI, 75 PSI, 80 PSI, 85 PSI, 90 PSI, 95

PSI, 100 PSI, 105 PSI, 110 PSI, 115 PSI, 120 PSI, 125 PSI, 130 PSI, 135 PSI, 140 PSI, 145 PSI, 150 PSI, 155 PSI, 160 PSI, 165 PSI, 170 PSI, 175 PSI, 180 PSI, 185 PSI, 190 PSI, 195 PSI, 200 PSI, 205 PSI, 210 PSI, 215 PSI, 220 PSI, 225 PSI, 230 PSI, 235 PSI, 240 PSI, 245 PSI, 250 PSI, 300PSI, 350 PSI, 400 PSI, 450 PSI, 500 PSI, 550 PSI, 600 PS, 650 PSI, 700 PSI, 750 PSI, 800 PSI, 850 PSI, 900 PSI, 950 PSI, or 1000 PSI. However, under certain circumstances a lower pressure could be desirable. For example, it takes little or no pressure to release C5 polymers from a C5-rich and/or lignin-free biomass.

[0107] In the embodiments of this invention (*e.g.*, **FIGs. 1** and **2**), following pre-treatment of a biomass as provided herein for the desired time at the proper pressure and temperature, the biomass and aqueous chemical or other mixture is moved through a discharge valve **17** at the end of the cylindrical barrel **30** into a flash tank **70.The** discharge valve **17** can be closed during biomass reaction with aqueous acid or other chemical at the desired temperature, then opened for passage of the biomass. In a twin screw chamber reactor, as exemplified in **FIG. 2,** the discharge valve **17** opens under pressure of the extruder with the opening of the valve between the end of the extruder and the valve chamber, after the steam and biomass has built up pressure in the reaction chamber, and discharges pretreated biomass to relieve the pressure to the point that the pressure delivered to the end valve through the shaft is greater. The use of the external valve is a great advantage over attempting to maintain homogeneous pressure in the barrel when using plug formation to maintain various zones. The zones can be maintained more easily when a release of pressurized material is controlled by a separately-responsive pressurized valve.

[0108] For example **FIG. 12,** illustrates an open valve **17** in a device as depicted in **FIG. 2,** which occurs when the pressure of the steam and biomass in the barrel chamber **30** (in **FIG. 2**) in the cylindrical barrel **31** is greater than the pressure delivered to the end valve **17.** As the pressure in the chamber is reduced, the shaft pressure on the end valve **17** pushes the valve towards closure against the end of the extruder, thus reducing the release of biomass into the flash tank **70** in **FIG. 2** and allowing pressure to build in the extruder again. In contrast, **FIG. 11** illustrates the position of the valve **17** when it is seated in the metal or ceramic seal **89** and the shaft pressure is greater than the pressure in the extruder. In combination, **FIGs. 11** and **12** depict a constant movement of the end valve forward and back as biomass is treated in the cylindrical barrel **31.** In some cases, the end valve **17** is never completely closed and never completely open. Thus, the end valve remains substantially open throughout operation while a consistent pressure is maintained in the extruder while biomass is treated and released.

[0109] The application described herein can be continuous, and the key to the outlet valve is the constant monitoring of the system pressure. There is a feedback loop that continuously adjusts the valve opening in order to maintain a specific system pressure. See **Figs. 11** and **12.** This can allows for a continuous flash process and the extruder can be continuously adjusted to maintain a desired pressure in the continuous process. Overall, this can be a more complex mechanism than normal pretreatment discharge systems, and can offers very tight, precise control of a continuous process.

[0110] Examples of discharge valves that can be used include poppet discharge valves, knife gate valves, seat valves, butterfly valves, rotary V-port valves, and the like. Particularly useful in a smaller scale reactor, can be a piston-operated linear globe or a poppet-type discharge valve, where a hard-faced upstream side of the valve seat is a ceramic discharge orifice, and a softer downstream side of the valve seat seals against a hard-faced valve plunger, with the flow area increasing continually beyond the valve seat when the valve plunger is retracted to open.

[0111] Most suitably, the poppet-type discharge valve would incorporate a gradual expansion venturi. One embodiment of a gradual expansion venturi poppet valve that is suitable for a biomass pretreatment reactor is diagrammed in **FIG. 10.** This valve incorporates a conical nozzle and a metal or ceramic seat end valve arrangement. To avoid plugging, the gradual expansion venturi as exemplified in **FIG. 11** (closed position) and **FIG. 12** (open position) can be designed to accelerate solids through a steadily enlarging gap between the stationary cylinder **89** of the venturi and the moveable inside cone **17** of the venturi that is mounted on the end of a valve shaft **60.** The discharge cylinder that the cone fits into can be generally seated into the discharge valve housing **19** at the reactor chamber exit. The venturi inside cone **89** can be the nose on the end of the valve shaft **60.** The valve shaft **60** can be attached to an actuator **72** for control of movement. The actuator **72** may be any device known in the art that can be able to move the valve shaft back and forth in a horizontal motion, such as an electric, pneumatic or hydraulic motor, pneumatic valve actuator, or hydraulic piston or any other type actuator known in the art and/or provided herein. The actuator, in turn, can be within or attached to an electronic pressure regulator **73** that receives a pressure signal from the reaction chamber. For example, an E/P pressure regulator, series ED05 (Bosch Rexroth AG) can be used. When the valve shaft is in its farthest leftward position, the outer edge of the inside cone seats against the inner edge of the metal or ceramic outside cylinder to seal the discharge end of the reactor. During pretreatment, the valve shaft can be moved to the right to provide the size of opening that is desired for the flash venturi. This design can provide a flash zone of some length which expands smoothly in the direction of flow. In this design, biomass solids can be accelerated down the axis of the gradually-opening annular cone until the pressure in the chamber can be released to the point where an electronic signal from the reaction chamber results in pressure delivered to the end valve that causes the end valve to move towards the chamber, closing the gap between the flash venturi and the metal or ceramic seat.

[0112] The treated biomass can be flashed through the discharge valve moving into a pipe that leads into the flash tank. Vapors can then be released and the biomass can be cooled in preparation for pH adjustment, solids separation and/or enzymatic hydrolysis. Any typical flash tank may be used. The flash tank can be The flashing can result in a drop in pressure from the pressure maintained inside the reaction zone *(e.g.,* the reaction zone depicted in **FIGs. 1** and **2**) to a pressure near atmospheric and can typically cool the biomass material to about 100° C. The temperature can then be reduced to about 50° C which can be desired for enzymatic saccharification. The biomass can be removed from the flash tank and saccharified in batches. Generally, at this point, the C5 polymers have been hydrolyzed into oligomers or monosaccharides, depending on the amount of acid or alkali used and the temperature and pressure maintained during the treatment in the reaction zones of the barrel chamber (see **FIGs. 1** and **2**), as well as hydrolysis of a certain fraction or percentage of amorphous regions and C6 polymers, leaving fragmented C6 crystalline lattices opened for saccharification (*e.g.*, enzymatic saccharification).

[0113] In some cases, the pH of a feedstock pretreated by the methods provided herein in a device as provided herein is adjusted prior to further treatment (*e.g.*, enzymatic saccharification). Alteration of the pH of a pretreated feedstock can be accomplished by washing the feedstock *(e.g.,* with water) one or more times to remove an alkaline or acidic substance, or other substance used or produced during pretreatment. Washing can comprise exposing the pretreated feedstock to an equal volume of water 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more times. In another embodiment, a pH modifier can be added. For example, an acid, a buffer, or a material that reacts with other materials present can be added to modulate the pH of the feedstock. In one embodiment, more than one pH modifier can be used, such as one or more bases, one or more bases with one or more

buffers, one or more acids, one or more acids with one or more buffers, or one or more buffers. When more than one pH modifiers are utilized, they can be added at the same time or at different times. Other non-limiting exemplary methods for neutralizing feedstocks treated with alkaline substances have been described, for example in U.S. Patent Nos. 4,048,341; 4,182,780; and 5,693,296.

[0114] In some cases, a system can be designed to hydrolyze and remove the C5 polymers in a first reaction chamber or zone prior to subjecting them to a strong acid treatment and/or high temperatures or pressures in a second reaction chamber zone. Dilute acid and hot water treatment methods can be used to solubilize all or a portion of the hemicellulose. Methods employing alkaline reagents can be used to remove all, most, or a portion of the lignin during the pretreatment step. The remaining C6 polymers and lignin residues can be treated at high acid concentrations and high temperatures and pressures without the formation of C5 byproducts, such as furfurals and acetic acid. This would result in a mixture of C6 polymers essentially without C5 sugars and inhibitors from hydrolysis of C5 polymers. A pure C6 stream of this type is desirable to produce particular end-products such as bioplastics and to supplement starch fermentation to ethanol and other biofuels.

[0115] In some cases, a biomass or feedstock as provided herein is subject to pretreatment using a device as provided herein such that the sugars (saccharides) produced from the pretreatment are separated and recovered for an end product as provided herein. The sugars separated and recovered can be used without a hydrolysis step. The sugars separated and recovered can be monosaccharides or saccharide oligomers or a combination thereof. The sugars (*e.g.*, monosaccharides and/or oligomers) can be C5 and/or C6 saccharides or a combination thereof. In some cases, the biomass subjected to pretreatment for the production of saccharide oligomers in corn fiber. The saccharide oligomers produced from the corn fiber can be C5 oligomers. In one embodiment, pretreatment of biomass or feedstock as provided herein produces a pretreated feedstock concentration of soluble oligomers in the pretreated feedstock that is 1%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99%. Examples of soluble oligomers include, but are not limited to, cellobiose and xylobiose. In one embodiment, the parameters of the pretreatment produce a concentration of soluble oligomers in the pretreated feedstock that is 30% to 90%. In one embodiment, the parameters of the pretreatment are such that the concentration of soluble oligomers in the pretreated feedstock is 45% to 80%.

Enzymatic hydrolysis

[0116] In one embodiment, the enzyme treatment is used to hydrolyze various higher saccharides (higher molecular weight) present in biomass to lower saccharides (lower molecular weight), such as in preparation for fermentation by biocatalysts such as yeasts to produce ethanol, hydrogen, or other chemicals such as organic acids including succinic acid, formic acid, acetic acid, and lactic acid. These enzymes and/or the hydrolysate can be used in fermentations to produce various products including fuels, and other chemicals.

[0117] In one example, the process for converting biomass material into ethanol includes pretreating the biomass material (*e.g.*, "feedstock"), hydrolyzing the pretreated biomass to convert polysaccharides to oligosaccharides, further hydrolyzing the oligosaccharides to monosaccharides, and converting the monosaccharides to biofuels and chemical products. Enzymes such as cellulases, polysaccharases, lipases, proteases, ligninases, and hemicellulases, help produce the

monosaccharides can be used in the biosynthesis of fermentation end-products. Biomass material that can be utilized includes woody plant matter, non-woody plant matter, cellulosic material, lignocellulosic material, hemicellulosic material, carbohydrates, pectin, starch, inulin, fructans, glucans, corn, algae, sugarcane, other grasses, switchgrass, bagasse, wheat straw, barley straw, rice straw, corncobs, bamboo, citrus peels, sorghum, high biomass sorghum, seed hulls, and material derived from these. The final product can then be separated and/or purified, as indicated by the properties for the desired final product. In some instances, compounds related to sugars such as sugar alcohols or sugar acids can be utilized as well.

[0118] Chemicals used in the methods of the present invention are readily available and can be purchased from a commercial supplier, such as Sigma-Aldrich. Additionally, commercial enzyme cocktails (*e.g.* Accellerase™ 1000, CelluSeb-TL, CelluSeb-TS, Cellic™, CTec, STARGEN™, Maxalig™, Spezyme.R™, Distillase.R™, G-Zyme.R™, Fermenzyme.R™, Fermgen™, GC 212, or Optimash™) or any other commercial enzyme cocktail can be purchased from vendors such as Specialty Enzymes & Biochemicals Co., Genencor, or Novozymes. Alternatively, enzyme cocktails can be prepared by growing one or more organisms such as for example a fungi *(e.g.* a *Trichoderma,* a *Saccharomyces,* a *Pichia,* a White Rot Fungus etc.), a bacteria (*e.g.* a *Clostridium,* or a coliform bacterium, a *Zymomonas* bacterium, *Sacharophagus degradans* etc.) in a suitable medium and harvesting enzymes produced therefrom. In some embodiments, the harvesting can include one or more steps of purification of enzymes.

[0119] In one embodiment, treatment of biomass following pretreatment of the biomass using methods and devices provided herein comprises enzyme hydrolysis. In one embodiment a biomass following pretreatment as provided herein is treated with an enzyme or a mixture of enzymes, *e.g.*, endonucleases, exonucleases, cellobiohydrolases, cellulase, beta-glucosidases, glycoside hydrolases, glycosyltransferases, lyases, esterases and proteins containing carbohydrate-binding modules. In one embodiment, the enzyme or mixture of enzymes is one or more individual enzymes with distinct activities. In another embodiment, the enzyme or mixture of enzymes can be enzyme domains with a particular catalytic activity. For example, an enzyme with multiple activities can have multiple enzyme domains, including for example glycoside hydrolases, glycosyltransferases, lyases and/or esterases catalytic domains.

[0120] In one embodiment, enzymes that degrade polysaccharides are used for the hydrolysis of biomass and can include enzymes that degrade cellulose, namely, cellulases. Examples of some cellulases include endocellulases and exo-cellulases that hydrolyze beta-1,4-glucosidic bonds.

[0121] In one embodiment, enzymes that degrade polysaccharides are used for the hydrolysis of biomass and can include enzymes that have the ability to degrade hemicellulose, namely, hemicellulases. Hemicellulose can be a major component of plant biomass and can contain a mixture of pentoses and hexoses, for example, D-xylopyranose, L-arabinofuranose, D-mannopyranose, Dglucopyranose, D-galactopyranose, D-glucopyranosyluronic acid and other sugars. In one embodiment, enzymes that degrade polysaccharides are used for the hydrolysis of biomass and can include enzymes that have the ability to degrade pectin, namely, pectinases. In plant cell walls, the cross-linked cellulose network can be embedded in a matrix of pectins that can be covalently cross-linked to xyloglucans and certain structural proteins. Pectin can comprise homogalacturonan (HG) or rhamnogalacturonan (RH).

[0122] In one embodiment, hydrolysis of biomass includes enzymes that can hydrolyze starch. Enzymes that hydrolyze starch include alpha-amylase, glucoamylase, beta-amylase, exo-alpha-1,4-glucanase, and pullulanase.

[0123] In one embodiment, hydrolysis of biomass comprises hydrolases that can include enzymes that hydrolyze chitin, namely, chitinase. In another embodiment, hydrolases can include enzymes that hydrolyze lichen, namely, lichenase.

[0124] In one embodiment, more than one of these steps can occur at any given time. For example, hydrolysis of the pretreated feedstock and hydrolysis of the oligosaccharides can occur simultaneously, and one or more of these can occur simultaneously to the conversion of monosaccharides to a fuel or chemical.

[0125] In another embodiment, an enzyme can directly convert the polysaccharide to monosaccharides. In some instances, an enzyme can hydrolyze the polysaccharide to oligosaccharides and the enzyme or another enzyme can hydrolyze the oligosaccharides to monosaccharides.

[0126] In another embodiment, the enzymes can be added to the fermentation or they can be produced by microorganisms present in the fermentation. In one embodiment, the microorganism present in the fermentation produces some enzymes. In another embodiment, enzymes are produced separately and added to the fermentation.

[0127] For the overall conversion of pretreated biomass to final product to occur at high rates, it is generally necessary for each of the necessary enzymes for each conversion step to be present with sufficiently high activity. If one of these enzymes is missing or is present in insufficient quantities, the production rate of an end product can be reduced. The production rate can also be reduced if the microorganisms responsible for the conversion of monosaccharides to product only slowly take up monosaccharides and/or have only limited capability for translocation of the monosaccharides and intermediates produced during the conversion to end product. Additions of fractions obtained from pretreatment and/or pretreatment and hydrolysis can increase initial or overall growth rates. In another embodiment, oligomers are taken up slowly by a biocatalyst, necessitating an almost complete conversion of polysaccharides and oligomers to monomeric sugars.

[0128] In another embodiment, the enzymes of the method are produced by a biocatalyst, including a range of hydrolytic enzymes suitable for the biomass materials used in the fermentation methods. In one embodiment, a biocatalyst is grown under conditions appropriate to induce and/or promote production of the enzymes needed for the saccharification of the polysaccharide present. The production of these enzymes can occur in a separate vessel, such as a seed fermentation vessel or other fermentation vessel, or in the production fermentation vessel where ethanol production occurs. When the enzymes are produced in a separate vessel, they can, for example, be transferred to the production fermentation vessel along with the cells, or as a relatively cell free solution liquid containing the intercellular medium with the enzymes. When the enzymes are produced in a separate vessel, they can also be dried and/or purified prior to adding them to the hydrolysis or the production fermentation vessel. The conditions appropriate for production of the enzymes are frequently managed by growing the cells in a medium that includes the biomass that the cells will be expected to hydrolyze in subsequent fermentation steps. Additional medium components, such as salt supplements, growth factors, and cofactors including, but not limited to phytate, amino acids, and peptides can also assist in

the production of the enzymes utilized by the microorganism in the production of the desired products.

Fermentation

[0129] The present disclosure also provides a fermentative mixture comprising: a cellulosic feedstock pre-treated with an alkaline or acid substance and at a temperature of from about 160°C to about 210°C; subsequently hydrolyzed with an enzyme mixture, and a microorganism capable of fermenting a five-carbon sugar and/or a six-carbon sugar. In one embodiment, the five-carbon sugar is xylose, arabinose, or a combination thereof. In one embodiment, the six-carbon sugar is glucose, galactose, mannose, or a combination thereof. In one embodiment, the alkaline substance is NaOH. In some embodiments, NaOH is added at a concentration of about 0.5% to about 2% by weight of the feedstock. In one embodiment, the acid is equal to or less than 2% HCl or H_2SO_4. In one embodiment, the microorganism is a *Rhodococcus* strain, a *Clostridium* strain, a *Trichoderma* strain, a *Saccharomyces* strain, a *Zymomonas* strain, or another microorganism suitable for fermentation of biomass. In another embodiment, the fermentation process comprises fermentation of the biomass using a microorganism that is *Clostridium phytofermentans, Clostridium algidixylanolyticum, Clostridium xylanolyticum, Clostridium cellulovorans, Clostridium cellulolyticum, Clostridium thermocellum, Clostridium josui, Clostridium papyrosolvens, Clostridium cellobioparum, Clostridium hungatei, Clostridium cellulosi, Clostridium stercorarium, Clostridium termitidis, Clostridium thermocopriae, Clostridium celerecrescens, Clostridium polysaccharolyticum, Clostridium populeti, Clostridium lentocellum, Clostridium chartatabidum, Clostridium aldrichii, Clostridium herbivorans, Acetivibrio cellulolyticus, Bacteroides cellulosolvens, Caldicellulosiruptor saccharolyticum, Rhodococcus opacus, Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes, Eubacterium cellulosolvens, Butyrivibrio fibrisolvens, Anaerocellum thermophilum, Halocella cellulolytica, Thermoanaerobacterium thermosaccharolyticum, Sacharophagus degradans, or Thermoanaerobacterium saccharolyticum.* In still another embodiment, the microorganism is genetically modified to enhance activity of one or more hydrolytic enzymes, such as a genetically-modified *Saccharomyces cerevisiae.*

[0130] In one embodiment a wild type or a genetically-improved microorganism can be used for chemical production by fermentation. Methods to produce a genetically-improved strain can include genetic modification, protoplast fusion, mutagenesis, and adaptive processes, such as directed evolution. For example, yeasts can be genetically-modified to ferment C5 sugars. Other useful yeasts are species of *Candida, Cryptococcus, Debaryomyces, Deddera, Hanseniaspora, Kluyveromyces, Pichia, Schizosaccharomyces,* and *Zygosaccharomyces. Rhodococcus* strains, such as *Rhodococcus opacus* variants are a source of triacylglycerols and other storage lipids. (See, *e.g.,* Waltermann, et al., Microbiology 146:1143-1149 (2000)). Other useful organisms for fermentation include, but are not limited to, yeasts, especially *Saccaromyces* strains and bacteria such as *Clostridium phytofermentans, Thermoanaerobacter ethanolicus, Clostridium thermocellum, Clostridium beijerinickii, Clostridium acetobutylicum, Clostridium tyrobutyricum, Clostridium thermobutyricum, Thermoanaerobacterium saccharolyticum, Thermoanaerobacter thermohydrosulfuricus, Clostridium acetobutylicum, Moorella ssp., Carboxydocella ssp., Zymomonas mobilis,* recombinant *E. Coli, Klebsiella oxytoca, Rhodococcus opacus* and *Clostridium beijerickii .*

[0131] An advantage of yeasts are their ability to grow under conditions that include elevated ethanol concentration, high sugar concentration, low sugar concentration, and/or operate under anaerobic

conditions. These characteristics, in various combinations, can be used to achieve operation with long or short fermentation cycles and can be used in combination with batch fermentations, fed batch fermentations, self-seeding/partial harvest fermentations, and recycle of cells from the final fermentation as inoculum.

[0132] In one embodiment, fed-batch fermentation is performed on the pre-treated and subsequently treated biomass to produce a fermentation end-product, such as alcohol, ethanol, organic acid, succinic acid, TAG, or hydrogen. In one embodiment, the fermentation process comprises simultaneous hydrolysis and fermentation (SSF) of the biomass using one or more microorganisms such as a *Rhodococcus* strain, a *Clostridium* strain, a *Trichoderma* strain, a *Saccharomyces* strain, a *Zymomonas* strain, or another microorganism suitable for fermentation of biomass. In another embodiment, the fermentation process comprises simultaneous hydrolysis and fermentation of the biomass using a microorganism that is *Clostridium algidixylanolyticum, Clostridium xylanolyticum, Clostridium cellulovorans, Clostridium cellulolyticum, Clostridium thermocellum, Clostridium josui, Clostridium papyrosolvens, Clostridium cellobioparum, Clostridium hungatei, Clostridium cellulosi, Clostridium stercorarium, Clostridium termitidis, Clostridium thermocopriae, Clostridium celerecrescens, Clostridium polysaccharolyticum, Clostridium populeti, Clostridium lentocellum, Clostridium chartatabidum, Clostridium aldrichii, Clostridium herbivorans, Clostridium phytofermentans, Acetivibrio cellulolyticus, Bacteroides cellulosolvens, Caldicellulosiruptor saccharolyticum, Ruminococcus albus, Ruminococcus flavefaciens, Fibrobacter succinogenes, Eubacterium cellulosolvens, Butyrivibrio fibrisolvens, Anaerocellum thermophilum, Halocella cellulolytica, Thermoanaerobacterium thermosaccharolyticum, Sacharophagus degradans,* or *Thermoanaerobacterium saccharolyticum.*

[0133] In one embodiment, the fermentation process can include separate hydrolysis and fermentation (SHF) of a biomass with one or more enzymes, such as a xylanases, endo-1,4-beta-xylanases, xylosidases, beta-D-xylosidases, cellulases, hemicellulases, carbohydrases, glucanases, endoglucanases, endo-1,4-beta-glucanases, exoglucanases, glucosidases, beta-D-glucosidases, amylases, cellobiohydrolases, exocellobiohydrolases, phytases, proteases, peroxidase, pectate lyases, galacturonases, or laccases. In one embodiment one or more enzymes used to treat a biomass is thermostable. In another embodiment a biomass is treated with one or more enzymes, such as those provided herein, prior to fermentation. In another embodiment a biomass is treated with one or more enzymes, such as those provided herein, during fermentation. In another embodiment a biomass is treated with one or more enzymes, such as those provided herein, prior to fermentation and during fermentation. In another embodiment an enzyme used for hydrolysis of a biomass is the same as those added during fermentation. In another embodiment an enzyme used for hydrolysis of biomass is different from those added during fermentation.

[0134] In some embodiments, fermentation can be performed in an apparatus such as bioreactor, a fermentation vessel, a stirred tank reactor, or a fluidized bed reactor. In one embodiment the treated biomass can be supplemented with suitable chemicals to facilitate robust growth of the one or more fermenting organisms. In one embodiment a useful supplement includes but is not limited to, a source of nitrogen and/or amino acids such as yeast extract, cysteine, or ammonium salts *(e.g.* nitrate, sulfate, phosphate etc.); a source of simple carbohydrates such as corn steep liquor, and malt syrup; a source of vitamins such as yeast extract; buffering agents such as salts (including but not limited to citrate salts, phosphate salts, or carbonate salts); or mineral nutrients such as salts of magnesium, calcium, or iron. In some embodiments redox modifiers are added to the fermentation mixture including but not limited to cysteine or mercaptoethanol.

[0135] In one embodiment the titer and/or productivity of fermentation end-product production by a microorganism is improved by culturing the microorganism in a medium comprising one or more compounds comprising hexose and/or pentose sugars. In one embodiment, a process comprises conversion of a starting material (such as a biomass) to a biofuel, such as one or more alcohols. In one embodiment, methods of the invention comprise contacting substrate comprising both hexose *(e.g.* glucose, cellobiose) and pentose *(e.g.* xylose, arabinose) saccharides with a microorganism that can hydrolyze C5 and C6 saccharides to produce ethanol. In another embodiment, methods of the invention comprise contacting substrate comprising both hexose (*e.g.* glucose, cellobiose) and pentose *(e.g.* xylose, arabinose) saccharides with *R. opacus* to produce TAG.

[0136] In some embodiments of the present invention, batch fermentation with a microorganism of a mixture of hexose and pentose saccharides using the methods of the present invention provides uptake rates of about 0.1-8 g/L/h or more of hexose and about 0.1-8 g/L/h or more of pentose (xylose, arabinose, etc.). In some embodiments of the present invention, batch fermentation with a microorganism of a mixture of hexose and pentose saccharides using the methods of the present invention provides uptake rates of about 0.1, 0.2, 0.4, 0.5, 0.6 0.7, 0.8, 1, 2, 3, 4, 5, or 6 g/L/h or more of hexose and about 0.1, 0.2, 0.4, 0.5, 0.6 0.7, 0.8, 1, 2, 3, 4, 5, or 6 g/L/h or more of pentose.

[0137] In one embodiment, a method for production of ethanol or another alcohol produces about 10 g/l to 120 gain 40 hours or less. In another embodiment a method for production of ethanol produces about 10 g/l, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, 31 g/L, 32 g/L, 33 g/L, 34 g/L, 35 g/L, 36 g/L, 37 g/L, 38 g/L, 39 g/L, 40 g/L, 41 g/L, 42 g/L, 43 g/L, 44 g/L, 45 g/L, 46 g/L, 47 g/L, 48 g/L, 49 g/L, 50 g/L, 51 g/L, 52 g/L, 53 g/L, 54 g/L, 55 g/L, 56 g/L, 57 g/L, 58 g/L, 59 g/L, 60 g/L, 61 g/L, 62 g/L, 63 g/L, 64 g/L, 65 g/L, 66 g/L, 67 g/L, 68 g/L, 69 g/L, 70 g/L, 71 g/L, 72 g/L, 73 g/L, 74 g/L, 75 g/L, 76 g/L, 77 g/L, 78 g/L, 79 g/L, 80 g/L, 81 g/L, 82 g/L, 83 g/L, 84 g/L, 85 g/L, 86 g/L, 87 g/L, 88 g/L, 89 g/L, 90 g/L, 91 g/L, 92 g/L, 93 g/L, 94 g/L, 95 g/L, 96 g/L, 97 g/L, 98 g/L, 99 g/L, 100 g/L, 110 g/l, 120 g/l, or more alcohol in 40 hours by the fermentation of biomass. In another embodiment, alcohol is produced by a method comprising simultaneous fermentation of hexose and pentose saccharides. In another embodiment, alcohol is produced by a microorganism comprising simultaneous fermentation of hexose and pentose saccharides.

[0138] In another embodiment, the level of a medium component is maintained at a desired level by adding additional medium component as the component is consumed or taken up by the organism. Examples of medium components included, but are not limited to, carbon substrate, nitrogen substrate, vitamins, minerals, growth factors, cofactors, and biocatalysts. The medium component can be added continuously or at regular or irregular intervals. In one embodiment, additional medium component is added prior to the complete depletion of the medium component in the medium. In one embodiment, complete depletion can effectively be used, for example to initiate different metabolic pathways, to simplify downstream operations, or for other reasons as well. In one embodiment, the medium component level is allowed to vary by about 10% around a midpoint, in one embodiment, it is allowed to vary by about 30% around a midpoint, and in one embodiment, it is allowed to vary by 60% or more around a midpoint. In one embodiment, the medium component level is maintained by allowing the medium component to be depleted to an appropriate level, followed by increasing the medium component level to another appropriate level. In one embodiment, a medium component, such as vitamin, is added at two different time points during fermentation process. For example, one-half of a total amount of vitamin is added at the beginning of fermentation and the other half is added at

midpoint of fermentation.

[0139] In another embodiment, the nitrogen level is maintained at a desired level by adding additional nitrogen-containing material as nitrogen is consumed or taken up by the organism. The nitrogen-containing material can be added continuously or at regular or irregular intervals. Useful nitrogen levels include levels of about 5 to about 10 g/L. In one embodiment levels of about 1 to about 12 g/L can also be usefully employed. In another embodiment levels, such as about 0.5, 0.1 g/L or even lower, and higher levels, such as about 20, 30 g/L or even higher are used. In another embodiment a useful nitrogen level is about 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 23, 24, 25, 26, 27, 28, 29 or 30 g/L. Nitrogen can be supplied as a simple nitrogen-containing material, such as an ammonium compounds (*e.g.* ammonium sulfate, ammonium hydroxide, ammonia, ammonium nitrate, or any other compound or mixture containing an ammonium moiety), nitrate or nitrite compounds (*e.g.* potassium, sodium, ammonium, calcium, or other compound or mixture containing a nitrate or nitrite moiety), or as a more complex nitrogen-containing material, such as amino acids, proteins, hydrolyzed protein, hydrolyzed yeast, yeast extract, dried brewer's yeast, yeast hydrolysates, distillers' grains, soy protein, hydrolyzed soy protein, fermentation products, and processed or corn steep powder or unprocessed protein-rich vegetable or animal matter, including those derived from bean, seeds, soy, legumes, nuts, milk, pig, cattle, mammal, fish, as well as other parts of plants and other types of animals. Nitrogen-containing materials useful in various embodiments also include materials that contain a nitrogen-containing material, including, but not limited to mixtures of a simple or more complex nitrogen-containing material mixed with a carbon source, another nitrogen-containing material, or other nutrients or non-nutrients, and AFEX treated plant matter.

[0140] In another embodiment, the carbon level is maintained at a desired level by adding sugar compounds or material containing sugar compounds ("Sugar-Containing Material") as sugar is consumed or taken up by the organism. The sugar-containing material can be added continuously or at regular or irregular intervals. In one embodiment, additional sugar-containing material is added prior to the complete depletion of the sugar compounds available in the medium. In one embodiment, complete depletion can effectively be used, for example to initiate different metabolic pathways, to simplify downstream operations, or for other reasons as well. In one embodiment, the carbon level (as measured by the grams of sugar present in the sugar-containing material per liter of broth) is allowed to vary by about 10% around a midpoint, in one embodiment, it is allowed to vary by about 30% around a midpoint, and in one embodiment, it is allowed to vary by 60% or more around a midpoint. In one embodiment, the carbon level is maintained by allowing the carbon to be depleted to an appropriate level, followed by increasing the carbon level to another appropriate level. In some embodiments, the carbon level can be maintained at a level of about 5 to about 120 g/L. However, levels of about 30 to about 100 g/L can also be usefully employed as well as levels of about 60 to about 80 g/L. In one embodiment, the carbon level is maintained at greater than 25 g/L for a portion of the culturing. In another embodiment, the carbon level is maintained at about 5 g/L, 6 g/L, 7 g/L, 8 g/L, 9 g/L, 10 g/L, 11 g/L, 12 g/L, 13 g/L, 14 g/L, 15 g/L, 16 g/L, 17 g/L, 18 g/L, 19 g/L, 20 g/L, 21 g/L, 22 g/L, 23 g/L, 24 g/L, 25 g/L, 26 g/L, 27 g/L, 28 g/L, 29 g/L, 30 g/L, 31 g/L, 32 g/L, 33 g/L, 34 g/L, 35 g/L, 36 g/L, 37 g/L, 38 g/L, 39 g/L, 40 g/L, 41 g/L, 42 g/L, 43 g/L, 44 g/L, 45 g/L, 46 g/L, 47 g/L, 48 g/L, 49 g/L, 50 g/L, 51 g/L, 52 g/L, 53 g/L, 54 g/L, 55 g/L, 56 g/L, 57 g/L, 58 g/L, 59 g/L, 60 g/L, 61 g/L, 62 g/L, 63 g/L, 64 g/L, 65 g/L, 66 g/L, 67 g/L, 68 g/L, 69 g/L, 70 g/L, 71 g/L, 72 g/L, 73 g/L, 74 g/L, 75 g/L, 76 g/L, 77 g/L, 78 g/L, 79 g/L, 80 g/L, 81 g/L, 82 g/L, 83 g/L, 84 g/L, 85 g/L, 86 g/L, 87 g/L, 88 g/L, 89 g/L, 90 g/L, 91 g/L, 92 g/L, 93 g/L, 94 g/L, 95 g/L, 96 g/L, 97 g/L, 98 g/L, 99 g/L, 100 g/L, 101 g/L, 102 g/L, 103 g/L, 104 g/L, 105 g/L, 106 g/L, 107 g/L, 108 g/L, 109 g/L, 110 g/L, 111 g/L, 112 g/L, 113 g/L, 114 g/L, 115 g/L, 116

g/L, 117 g/L, 118 g/L, 119 g/L, 120 g/L, 121 g/L, 122 g/L, 123 g/L, 124 g/L, 125 g/L, 126 g/L, 127 g/L, 128 g/L, 129 g/L, 130 g/L, 131 g/L, 132 g/L, 133 g/L, 134 g/L, 135 g/L, 136 g/L, 137 g/L, 138 g/L, 139 g/L, 140 g/L, 141 g/L, 142 g/L, 143 g/L, 144 g/L, 145 g/L, 146 g/L, 147 g/L, 148 g/L, 149 g/L, or 150 g/L.

[0141] The carbon substrate, like the nitrogen substrate, is necessary for cell production and enzyme production, but unlike the nitrogen substrate, it serves as the raw material for production of end products. Frequently, more carbon substrate can lead to greater production of end products. In another embodiment, it can be advantageous to operate with the carbon level and nitrogen level related to each other for at least a portion of the fermentation time. In one embodiment, the ratio of carbon to nitrogen is maintained within a range of about 30:1 to about 10:1. In another embodiment, the ratio of carbon nitrogen is maintained from about 20:1 to about 10:1 or more preferably from about 15:1 to about 10:1. In another embodiment the ratio of carbon nitrogen is about 30:1, 29:1, 28:1, 27:1, 26:1, 25:1, 24:1, 23:1, 22:1, 21:1, 20:1, 19:1, 18:1, 17:1, 16:1, 15:1, 14:1, 13:1, 12:1, 11:1, 10:1, 9:1, 8:1, 7:1, 6:1, 5:1, 4:1, 3:1, 2:1, or 1:1.

[0142] Maintaining the ratio of carbon and nitrogen ratio within particular ranges can result in benefits to the operation such as the rate of metabolism of carbon substrate, which depends on the amount of carbon substrate and the amount and activity of enzymes present, being balanced to the rate of end product production. Balancing the carbon to nitrogen ratio can, for example, facilitate the sustained production of these enzymes such as to replace those which have lost activity.

[0143] In another embodiment, the amount and/or timing of carbon, nitrogen, or other medium component addition can be related to measurements taken during the fermentation. For example, the amount of monosaccharides present, the amount of insoluble polysaccharide present, the polysaccharase activity, the amount of product present, the amount of cellular material (for example, packed cell volume, dry cell weight, etc.) and/or the amount of nitrogen (for example, nitrate, nitrite, ammonia, urea, proteins, amino acids, etc.) present can be measured. The concentration of the particular species, the total amount of the species present in the fermentor, the number of hours the fermentation has been running, and the volume of the fermentor can be considered. In various embodiments, these measurements can be compared to each other and/or they can be compared to previous measurements of the same parameter previously taken from the same fermentation or another fermentation. Adjustments to the amount of a medium component can be accomplished such as by changing the flow rate of a stream containing that component or by changing the frequency of the additions for that component. For example, the amount of saccharide can be increased when the cell production increases faster than the end product production. In another embodiment the amount of nitrogen can be increased when the enzyme activity level decreases.

[0144] In another embodiment, a fed batch operation can be employed, wherein medium components and/or fresh cells are added during the fermentation without removal of a portion of the broth for harvest prior to the end of the fermentation. In one embodiment a fed-batch process is based on feeding a growth limiting nutrient medium to a culture of microorganisms. In one embodiment the feed medium is highly concentrated to avoid dilution of the bioreactor. In another embodiment the controlled addition of the nutrient directly affects the growth rate of the culture and avoids overflow metabolism such as the formation of side metabolites. In one embodiment the growth limiting nutrient is a nitrogen source or a saccharide source.

[0145] In various embodiments, particular medium components can have beneficial effects on the

performance of the fermentation, such as increasing the titer of desired products, or increasing the rate that the desired products are produced. Specific compounds can be supplied as a specific, pure ingredient, such as a particular amino acid, or it can be supplied as a component of a more complex ingredient, such as using a microbial, plant or animal product as a medium ingredient to provide a particular amino acid, promoter, cofactor, or other beneficial compound. In some cases, the particular compound supplied in the medium ingredient can be combined with other compounds by the organism resulting in a fermentation-beneficial compound. One example of this situation would be where a medium ingredient provides a specific amino acid which the organism uses to make an enzyme beneficial to the fermentation. Other examples can include medium components that are used to generate growth or product promoters, etc. In such cases, it can be possible to obtain a fermentation-beneficial result by supplementing the enzyme, promoter, growth factor, etc. or by adding the precursor. In some situations, the specific mechanism whereby the medium component benefits the fermentation is not known, only that a beneficial result is achieved.

[0146] In one embodiment, a fermentation to produce a fuel is performed by culturing a strain of *R. opacus* biocatalyst in a medium having a supplement of lignin component and a concentration of one or more carbon sources. The resulting production of end product such as TAG can be up to 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, and in some cases up to 10-fold and higher in volumetric productivity than a process using only the addition of a relatively pure saccharide source, and can achieve a carbon conversion efficiency approaching the theoretical maximum. The theoretical maximum can vary with the substrate and product. For example, the generally accepted maximum efficiency for conversion of glucose to ethanol is 0.51 g ethanol/g glucose. In one embodiment a biocatalyst can produce about 40-100% of a theoretical maximum yield of ethanol. In another embodiment, a biocatalyst can produce up to about 40%, 50%, 60%, 70%, 80%, 90%, 95% and even 100% of the theoretical maximum yield of ethanol. In one embodiment a biocatalyst can produce up to about 1 %, 2 %, 3 %, 4 %, 5 %, 6 %, 7 %, 8 %, 9 %, 10 %, 11 %, 12 %, 13 %, 14 %, 15 %, 16 %, 17 %, 18 %, 19 %, 20 %, 21 %, 22 %, 23 %, 24 %, 25 %, 26 %, 27 %, 28 %, 29 %, 30 %, 31 %, 32 %, 33 %, 34 %, 35 %, 36 %, 37 %, 38 %, 39 %, 40 %, 41 %, 42 %, 43 %, 44 %, 45 %, 46 %, 47 %, 48 %, 49 %, 50 %, 51 %, 52 %, 53 %, 54 %, 55 %, 56 %, 57 %, 58 %, 59 %, 60 %, 61 %, 62 %, 63 %, 64 %, 65 %, 66 %, 67 %, 68 %, 69 %, 70 %, 71 %, 72 %, 73 %, 74 %, 75 %, 76 %, 77 %, 78 %, 79 %, 80 %, 81 %, 82 %, 83 %, 84 %, 85 %, 86 %, 87 %, 88 %, 89 %, 90 %, 91 %, 92 %, 93 %, 94 %, 95 %, 96 %, 97 %, 98 %, 99 %, 99.99 %, or100 % of a theoretical maximum yield of a fuel. It can be possible to obtain a fermentation-beneficial result by supplementing the medium with a pretreatment or hydrolysis component. In some situations, the specific mechanism whereby the medium component benefits the fermentation is not known, only that a beneficial result is achieved.

[0147] Various embodiments offer benefits relating to improving the titer and/or productivity of fermentation end-product production by a biocatalyst by culturing the organism in a medium comprising one or more compounds comprising particular fatty acid moieties and/or culturing the organism under conditions of controlled pH.

[0148] In one embodiment, the pH of the medium is controlled at less than about pH 7.2 for at least a portion of the fermentation. In one embodiment, the pH is controlled within a range of about pH 3.0 to about 7.1 or about pH 4.5 to about 7.1, or about pH 5.0 to about 6.3, or about pH 5.5 to about 6.3, or about pH 6.0 to about 6.5, or about pH 5.5 to about 6.9 or about pH 6.2 to about 6.7. The pH can be controlled by the addition of a pH modifier. In one embodiment, a pH modifier is an acid, a base, a buffer, or a material that reacts with other materials present to serve to raise of lower the pH. In one embodiment, more than one pH modifier can be used, such as more than one acid, more than one

base, one or more acid with one or more bases, one or more acids with one or more buffers, one or more bases with one or more buffers, or one or more acids with one or more bases with one or more buffers. When more than one pH modifiers are utilized, they can be added at the same time or at different times. In one embodiment, one or more acids and one or more bases can be combined, resulting in a buffer. In one embodiment, media components, such as a carbon source or a nitrogen source can also serve as a pH modifier; suitable media components include those with high or low pH or those with buffering capacity. Exemplary media components include acid- or base-hydrolyzed plant polysaccharides having with residual acid or base, AFEX treated plant material with residual ammonia, lactic acid, corn steep solids or liquor.

[0149] In one embodiment, a constant pH can be utilized throughout the fermentation. In one embodiment, the timing and/or amount of pH reduction can be related to the growth conditions of the cells, such as in relation to the cell count, the end product produced, the end product present, or the rate of end product production. In one embodiment, the pH reduction can be made in relation to physical or chemical properties of the fermentation, such as viscosity, medium composition, gas production, off gas composition, etc.

Recovery of Fermentive End Products

[0150] In another aspect, methods are provided for the recovery of the fermentive end products, such as an alcohol (*e.g.* ethanol, propanol, methanol, butanol, etc.) another biofuel or chemical product. In one embodiment, broth will be harvested at some point during of the fermentation, and fermentive end product or products will be recovered. The broth with end product to be recovered will include both end product and impurities. The impurities include materials such as water, cell bodies, cellular debris, excess carbon substrate, excess nitrogen substrate, other remaining nutrients, other metabolites, and other medium components or digested medium components. During the course of processing the broth, the broth can be heated and/or reacted with various reagents, resulting in additional impurities in the broth.

[0151] In one embodiment, the processing steps to recover end product frequently includes several separation steps, including, for example, distillation of a high concentration alcohol material from a less pure alcohol-containing material. In one embodiment, the high concentration ethanol material can be further concentrated to achieve very high concentration alcohol, such as 98% or 99% or 99.5% (wt.) or even higher. Other separation steps, such as filtration, centrifugation, extraction, adsorption, etc. can also be a part of some recovery processes for alcohol as a product or biofuel, or other biofuels or chemical products.

[0152] In one embodiment a process can be scaled to produce commercially useful biofuels. In another embodiment biocatalyst is used to produce an alcohol, *e.g.*, ethanol, butanol, propanol, methanol, or a fuel such as hydrocarbons hydrogen, TAG, and hydroxy compounds. In another embodiment biocatalyst is used to produce a carbonyl compound such as an aldehyde or ketone *(e.g.* acetone, formaldehyde, 1-propanal, etc.), an organic acid, a derivative of an organic acid such as an ester (*e.g.* wax ester, glyceride, etc.), 1, 2-propanediol, 1, 3-propanediol, lactic acid, formic acid, acetic acid, succinic acid, pyruvic acid, or an enzyme such as a cellulase, polysaccharase, lipases, protease, ligninase, and hemicellulase.

[0153] TAG biosynthesis is widely distributed in nature and the occurrence of TAG as reserve compounds is widespread among plants, animals, yeast and fungi. In contrast, however, TAGs have not been regarded as common storage compounds in bacteria. Biosynthesis and accumulation of TAGs have been described only for a few bacteria belonging to the actinomycetes group, such as genera of *Streptomyces, Nocardia, Rhodococcus, Mycobacterium, Dietzia* and *Gordonia,* and, to a minor extent, also in a few other bacteria, such as *Acinetobacter baylyi* and *Alcanivorax borkumensis.* Since the mid-1990's, TAG production in hydrocarbon-degrading strains of those genera has been frequently reported. TAGs are stored in spherical lipid bodies as intracellular inclusions, with the amounts depending on the respective species, cultural conditions and growth phase. Commonly, the important factor for the production of TAGs is the amount of nitrogen that is supplied to the culture medium. The excess carbon, which is available to the culture after nitrogen exhaustion, continues to be assimilated by the cells and, by virtue of oleaginous bacteria possessing the requisite enzymes, is converted directly into lipid. The compositions and structures of bacterial TAG molecules vary considerably depending on the bacterium and on the cultural conditions, especially the carbon sources. See, Brigham CJ, et al. (2011) J Microbial Biochem Technol S3:002.

[0154] In one embodiment, useful biochemicals can be produced from non-food plant biomass, with a steam or hot-water extraction technique that is carried out by contacting a charge of non-food plant pretreated biomass material such as corn stover or sorghum with water and/or acid (with or without additional process enhancing compounds or materials), in a pressurized vessel at an elevated temperature up to about 160 -220° C. and at a pH below about 7.0, to yield an aqueous (extract solution) mixture of useful sugars including long-chain saccharides (sugars), acetic acid, and lignin, while leaving the structural (cellulose and lignin) portion of the lignocellulosic material largely intact. In combination, these potential inhibitory chemicals especially sugar degradation products are low, and the plant derived nutrients that are naturally occurring lignocellulosic-based components are also recovered that are beneficial to a C5 and C6 fermenting organism. Toward this objective, the aqueous extract is concentrated (by centrifugation, filtration, solvent extraction, flocculation, evaporation), by producing a concentrated sugar stream, apart from the other hemicellulose (C5 rich) and cellulosic derived sugars (C6 rich) that are channeled into a fermentable stream.

[0155] In another embodiment, following enzyme/acid hydrolysis, additional chemical compounds that are released are recovered with the sugar stream resulting in a short-chain sugar solution containing xylose, mannose, arabinose, rhamnose, galactose, and glucose (5 and 6-carbon sugars). The sugar stream, now significantly rich in C5 and C6 substances can be converted by microbial fermentation or chemical catalysis into such products as triacylglycerol or TAG and further refined to produce stream rich in JP8 or jet fuels . If C5 sugar percentage correction has not been performed, it can be performed before fermentation to satisfy desired combination of C5 and C6 sugars for the fermentation organism and corresponding end product.

EXAMPLES

[0156] The following examples serve to illustrate certain embodiments and aspects and are not to be construed as limiting the scope thereof.

Example 1. Pretreatment of Biomass using a Twin Screw Extruder

[0157] A twin screw extruder as diagrammed in **FIG. 2** was used to perform four continuous runs of 224, 695, 1100, and 977 hours each. The extruder was run with indirect heating through the reactor walls until the end of the experiment. A flow rate of up to 136 kg/hr was reached through the extruder with direct steam injection to supply process heat. The materials selected were acid resistant. The feed was metered through a weight belt feeder and fell into a crammer feeder supplying the barrel of the extruder. Two screws intermeshed and provided rapid heat and mass transfer when steam and acid were injected through steam and acid ports connected to the cylindrical barrel of the extruder as shown in **FIG. 2.** The steam and acid supplying ports were sealed by reverse-flow sections in the screws. A hydraulically operated pressure control valve was seated in a ceramic seal and pressure was controlled to maintain as constant a pressure as possible in the reaction section of the extruder.

[0158] The solids were exposed to high temperature and pressure and low pH for a maximum of about 10 seconds in the reaction zone as shown in the extruder depicted in **FIG. 2** before being exploded into the flash tank. Residence time in the reaction zone was controlled by the rotational time of the screws. The surge chamber above the screws in the pump feeder acted as a flash vessel, where hot water is vaporized, cooling the product and removing some of the low-boiling inhibitors, such as furfural. Under these conditions, extrusion did not appear to produce enough furfural or HMF to inhibit yeast growth or fermentation, as long as overcooking was avoided with short reaction periods. HMF and furfural, reversion inhibitors, were formed in small amounts during this pretreatment (e.g., a total of 0.3 to 0.5 wt. % of the dry pretreated product).

Example 2. Particle Size Following Pretreatment with a Twin Screw Extruder

[0159] This run was conducted to evaluate the particle size reduction that takes place during biomass pretreatment in a modified twin screw extruder. Cherry sawdust, with an average particle size of about 3 mm x 3 mm x 1 mm and an average moisture content of 31% was used as the raw biomass feedstock. The cherry biomass was fed into a ZSK-30 twin screw extruder, manufactured by Coperion, essentially as described in Example 1. The processing parameters used for the experiment are presented in Table 2.

Table 2 - Particle Size Distribution Experimental Parameters

	Mass Throughput	Pressure	Temp.	Acid Addition	Water Addition	Residence Time
Feedstock	Dry g/min	psig	°C	g/min	g/min	seconds
Cherry Sawdust	398.4	400	231	7.6	1134	10

[0160] The cherry sawdust was processed on a continuous basis. The final moisture content of the processed cherry sawdust was about 76.8%. Once steady state was achieved a sample of the pretreated material was collected for particle size analysis. The sample was analyzed through a Mie Scattering theory using a Horiba LA-920, capable of measuring particle diameters from 0.02 µm to 2000 µm. The results indicated a significant particle size reduction occurring throughout the pretreatment process. The average particle size was reduced from 3 mm in the raw material to 20.75 µm in the pretreated effluent. A summary of the particle size distribution is presented in **FIG. 13.**

Example 3

Analysis of biomass slurry after pretreatment

[0161] Table 3 is a summary of various types of biomass pretreated at an optimized set of conditions, with the analysis of the resulting sugar and organic acid composition of the liquid fraction coming off of pretreatment as slurry of biomass. Water or steam was added to adjust to the desired solids content for pretreatment.

[0162] Wet distillers grain at 68.10% moisture was fed into a twin screw extruder crammer. Conditions in the extruder were set at a steam valve pressure of 39.0 kg/hr, 232°C, and an end valve pressure of 400 psi, with 4.0% H_2SO_4. Biomass exited the extruder as 21.8% total solids slurry. For analysis post pretreatment, the moisture content of the biomass slurry was determined using a moisture analyzer-balance. A sample of the homogenous slurry was centrifuged for 5 minutes at 6000rpm, and the supernatant used for standard HPLC sample preparation. All sugar and organic acid analysis was performed on a Shimadzu HPLC system with a RID-10A detector and a BIORAD Aminex HPX-87H column (300x7.8mm). The mobile phase was 0.01N H_2SO_4 and the HPLC had an 8 point calibration from 0.1g/L to 50.0g/L.

[0163] Cherry sawdust was resifted at 34.4% moisture. Conditions in the extruder system were set at a steam valve pressure of 27.2 kg/hr, 232°C, and an end valve pressure of 400psi, with 2.0% H_2SO_4. Biomass exited the system as 25.9% total solids homogeneous slurry. Biomass slurry was cooled and then analyzed as described above.

[0164] Red Maple measuring 22.50% moisture was fed to the extruder system at a steam valve pressure of 30.4 kg/hr, 232°C, and an end valve pressure of 400psi, with 2.0% H_2SO_4. Biomass exited the extruder system as 29.1% total solids homogeneous slurry. The biomass slurry was cooled and then analyzed as described above.

[0165] Yellow Poplar at 37.58% moisture was fed to the extruder system at a steam valve pressure of 30.4 kg/hr, 232°C, and an end valve pressure of 400psi, with 2.0% H_2SO_4. Biomass exited the extruder system as 26.8% total solids homogeneous slurry. The biomass slurry was cooled and then analyzed as described above.

[0166] The analyses of the liquid fraction of pretreated slurries produced a hemicellulose-rich extraction from the lignocellulose biomass, and a low concentration of organic acids and inhibitors.

Table 3. Pretreatment conditions and composition of liquid fraction of pretreated material; kg/MT indicates kilograms per dry metric ton of biomass.

ID	Raw Feedstock	Temp. (°C)	End Valve Pressure (PSI)	Acid Dosing (%)	Steam Valve (lbs/hr)	Solids (%)	Glucose (kg/MT)
WDG150123-S11	Wet Distillers Grains	232	400	4.0	95	21.8	70.4
HW150730-	Cherry	232	400	2.0	67	25.9	70.3

ID	Raw Feedstock	Temp. (°C)	End Valve Pressure (PSI)	Acid Dosing (%)	Steam Valve (lbs/hr)	Solids (%)	Glucose (kg/MT)
R10B6							
HW150728-R10B3	Red Maple	232	400	2.0	67	29.1	101.2
HW150618-R9B1	Yellow Poplar	232	400	2.0	67	26.8	131.1

ID	Raw Feedstock	Xylose (kg/MT)	Arabinose (kg/MT)	Formic Acid (kg/MT)	Acetic Acid (kg/MT)	HMF (kg/MT)	Furfural (kg/MT)
WDG150123-S11	Wet Distillers Grains	127.4	77.9	27.6*	12.1	0.0	0.6
HW150730-R10B6	Cherry	212.1	14.2	10.4	29.4	0.6	1.8
HW150728-R10B3	Red Maple	204.4	15.3	11.7	37.2	1.9	3.0
HW150618-R9B1	Yellow Poplar	247.6	0.0	8.3	56.1	2.8	5.8

*Indicates amount of glycerol (kg/MT) in liquid fraction of processed Wet Distillers Grains.

[0167] Table 4 provides a summary of the sugar compositional analysis of the various feedstocks outlined in Table 3 and Table 5.

Compositional Analysis

[0168] Wet distillers grain: corn grain sugar composition was analyzed using a bone dried sample of feedstock. The NREL LAP for "Determination of Structural Carbohydrates and Lignin in Biomass" (NREL/TP-510-42618: A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, and D. Crocker) was followed to quantify the total theoretical available sugars within the raw feedstock by using a 72% sulfuric acid hydrolysis for 1 hour at 35°C followed by a 4% sulfuric acid hydrolysis for 1 hour at 249°C in an autoclave. Sugar analysis was performed on a Shimadzu HPLC system with a RID-10A detector and a BIORAD Aminex HPX-87H column (300x7.8mm). The mobile phase was 0.01N H_2SO_4 and the HPLC had an 8 point calibration from 0.1g/L to 50.0g/L.

[0169] Cherry, red maple, and yellow poplar were analyzed for sugar composition post pretreatment. The compositional analysis of theoretical monomeric sugars was determined via enzymatic hydrolysis with an overdosed amount of cellulase enzyme cocktail to hydrolyze all possible sugars present in the cellulose and hemicellulose fractions. Sugar analysis was performed on a Shimadzu HPLC system with a RID-10A detector and a BIORAD Aminex HPX-87H column (300x7.8mm). The mobile phase was 0.01N H_2SO_4 and the HPLC had an 8 point calibration from 0.1g/L to 50.0g/L.

Table 4. Compositional analysis of raw feedstocks

Feedstock	Average Cellulose (kg/MT)	Average Hemicellulose (kg/MT)	Average Lignin (kg/MT)	Average Glucose (kg/MT)	Average Xylose (kg/MT)	Average Arabinose (kg/MT)	Total Avail. Sugar (kg/MT)
Wet Distillers Grains	154.1	228.1	209.2	169.6	171.9	79.0	420.5
Cherry	411.5	216.4	372.1	452.7	238.0	16.0	706.7
Red Maple	450.1	206.4	343.5	495.1	227.0	14.2	736.3
Yellow Poplar	446.8	226.1	327.1	491.5	248.7	0.0	740.2

[0170] Table 5 summarizes sugar conversions from pretreatment and subsequent enzymatic hydrolysis as described *infra.* Conversion efficiencies were calculated as a percentage of the compositional analysis of monomeric sugars, taken as a theoretical maximum sugar yield.

Enzymatic Hydrolysis

[0171] The moisture content of the pretreated biomass slurry was determined using a moisture analyzer-balance. Water was added to produce a slurry of 15% total solids and the slurry was mixed to homogeneity. The pH of the slurry was adjusted to 5.2 using 10M NaOH and then 100g aliquots of slurry were transferred into individual 250mL shake flasks. Commercially available cellulase enzyme was added at 8kg of protein per metric ton of dry solids into each 250mL shake flask. Hydrolysis was carried out in a Kuhner incubator shaker (Climo-Shaker ISF4-X) at 50°C and 200rpm for the duration of 72 hours.

[0172] Samples were taken at multiple time points throughout hydrolysis and after 72 hours, conversion efficiencies were calculated. The hydrolysate samples were collected for analysis and centrifuged. The supernatant was analyzed for monomeric sugars, and organic acids and inhibitors via HPLC as described *supra.*

Table 5. Sugar recovery from pretreatment and enzymatic hydrolysis, calculated as a percentage of the compositional analysis of monomeric sugars.

Feedstock	Glucose Conversion (%) Pretreatment	Glucose Conversion (%) Enzyme Hydrolysis	Xylose Conversion (%) Pretreatment	Xylose Conversion (%) Enzyme Hydrolysis
Wet Distillers Grains	41.5	97.0	74.1	87.1
Cherry	15.5	90.1	89.1	98.8
Red Maple	20.5	90.6	90.1	99.6
Yellow Poplar	30.6	85.8	96.3	97.0

Feedstock	Arabinose Conversion (%) Pretreatment	Arabinose Conversion (%) Enzyme Hydrolysis	Total Sugar Conversion (%) Pretreatment	Total Sugar Conversion (%) Enzyme Hydrolysis
Wet Distillers Grains	98.7	99.0	65.7	94.4
Cherry	88.8	98.1	42.0	93.2
Red Maple	100.0	100.0	43.6	92.9
Yellow Poplar			51.2	91.4

Example 4.

[0173] In addition to the treatments described *supra,* numerous other types of feedstocks were processed through the herein defined apparatus. For example, waste paper from a waste management facility was ground in a Fitzmill to pass through a 2.36 mm screen and blended to 1.25wt% in water prior to being processed. Other feedstocks included corn fiber at 25% solids, bagasse (16 runs), softwoods (33 runs), rice straw (12 runs), wheat straw (8 runs), mixed straws, barley straw (8 runs) and oat hulls (10 runs).

REFERENCES CITED IN THE DESCRIPTION

Cited references

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- WO2010056940A [0002]
- US20140034260A1 [0077]
- US4046341A [0113]
- US4182780A [0113]
- US5693296A [0113]

Non-patent literature cited in the description

- **WALTERMANN et al.**Microbiology, 2000, vol. 146, 1143-1149 [0130]
- **BRIGHAM CJ et al.**J Microbial Biochem Technol, 2011, vol. S3, 002- [0153]

Patentkrav

1. System til forbehandling af mindst ét tørton biomasse pr. dag i industriel skala, hvilket system omfatter:

en tromle (31), der definerer et indre kammer (30) og omfatter en indløbsåbning (11) nær ved en første ende af tromlen og en endeflangeplade (14) i en anden ende af tromlen;

én eller flere roterbare snekker (51, 52), der er konfigureret til at bevæge biomassen gennem tromlens indre kammer og indeholder ét eller flere afsnit, som er konfigureret til at danne én eller flere propper (P1, P2, P3) ud fra biomassen med henblik på at separere tromlens indre kammer i to eller flere zoner (91, 92, 93), herunder en tilførselszone og en reaktionszone (R1); og

en trykaktiveret udledningsventil (17), der er forbundet med endeflangepladen (14) og konfigureret til at åbne og lukke som reaktion på tryk inden i tromlen, hvor den trykaktiverede udledningsventil (17) er forbundet med en aktuator (72), som operabelt er koblet til en modtryksstyreenhed (73), som operabelt er koblet til én eller flere trykmålere (81, 85), hvilket derved muliggør kontinuerlig fremstilling af en forbehandlet biomassesammensætning, der omfatter en flydende fraktion, som omfatter monosaccharider, og faste partikler, der omfatter cellulose.

2. System ifølge krav 1, der omfatter én, to eller tre roterbare snekker.

3. System ifølge krav 1 eller 2, der endvidere omfatter en motor (24), som er konfigureret til at rotere den ene eller flere roterbare snekker (51, 52), eventuelt hvor motoren er konfigureret til at rotere den ene eller flere roterbare snekker ved ca.: 100, 250, 400, 500, 750, 1000, 1100, 1250, 1500 eller 2000 omdrejninger pr. minut.

4. System ifølge et hvilket som helst af kravene 1-3, hvor systemet er i stand til at behandle biomasse ved en hastighed på mindst ca. 2 tørton/dag, 3 tørton/dag, 4 tørton/dag, 5 tørton/dag, 7,5 tørton/dag, 10 tørton/dag, 15 tørton/dag, 20 tørton/dag, 25 tørton/dag, 50 tørton/dag, 75 tørton/dag, 100 tørton/dag, 150 tørton/dag eller 200 tørton/dag.

5. System ifølge et hvilket som helst af kravene 1-4, hvor systemet yderligere omfatter en tragt (20), der er forbundet med indløbsåbningen (11), til tilførsel af biomassen ind i tilførselszonen; og eventuelt hvor tragten yderligere omfatter et tilførselselement, som er konfigureret til at bevæge biomassen fra tragten gennem indløbsåbningen, og eventuelt er tilførselselementet en tilførselssnegl, som er konfigureret til at fordele biomassen jævnt i tilførselszonen.

6. System ifølge et hvilket som helst af kravene 1-5, hvor tromlen yderligere omfatter:

én eller flere lukbare åbninger, som er konfigureret til at tilsætte damp (åbningerne 32, 33) til reaktionszonen, at tilsætte væske til biomassen (åbning 29) i tilførselszonen eller begge dele;

én eller flere lukbare åbninger (34, 35), der er konfigureret til at tilsætte ét eller flere kemiske midler til reaktionszonen, og eventuelt hvor det kemiske middel omfatter en syre, en base eller en kombination deraf, og eventuelt hvor det kemiske middel omfatter svovlsyre;

én eller flere åbninger, der omfatter en temperaturmåler (83, 87), en trykmåler (81, 85) eller en kombination deraf; eller

en hvilken som helst kombination deraf.

7. System ifølge et hvilket som helst af kravene 1-6, hvor systemet er konfigureret til at opretholde en forhøjet temperatur i reaktionszonen ved: 50-500 °C, 75-400 °C, 100-350 °C, 150-300 °C eller 200-250 °C, og eventuelt hvor den forhøjede temperatur tilvejebringes med damp, en varmekappe eller en kombination deraf.

8. System ifølge et hvilket som helst af kravene 1-7, hvor systemet er konfigureret til at opretholde et forhøjet tryk i reaktionszonen ved: 3,45-68,95 bar (50-1000 PSI), 6,89-51,71 bar (100-750 PSI), 13,79-41,37 bar (200-600 PSI), 20,68-34,47 bar (300-500 PSI) eller 24,13-31,03 bar (350-450 PSI), og eventuelt hvor det forhøjede tryk opretholdes ved tilsætning af damp, væske, biomasse eller en kombination deraf.

9. System ifølge et hvilket som helst af kravene 1-8, hvor den trykaktiverede udledningsventil (17) omfatter en tallerkenventil, en kugleventil, en kontraventil eller en roterende kniv-sluseventil.

10. System ifølge et hvilket som helst af kravene 1-9, hvor aktuatoren 2. (72) er en pneumatisk aktuator, en hydraulisk aktuator, en elektromekanisk aktuator eller en kombination deraf.

11. System ifølge et hvilket som helst af kravene 1-10, hvor den ene eller flere trykmålere (81, 85) er konfigureret til at monitorere tryk i tromlen via én eller flere lukbare åbninger i tromlen.

12. System ifølge krav 11, hvor mindst én af den ene eller flere trykmålere (81, 85) er konfigureret til at monitorere tryk inden i reaktionszonen.

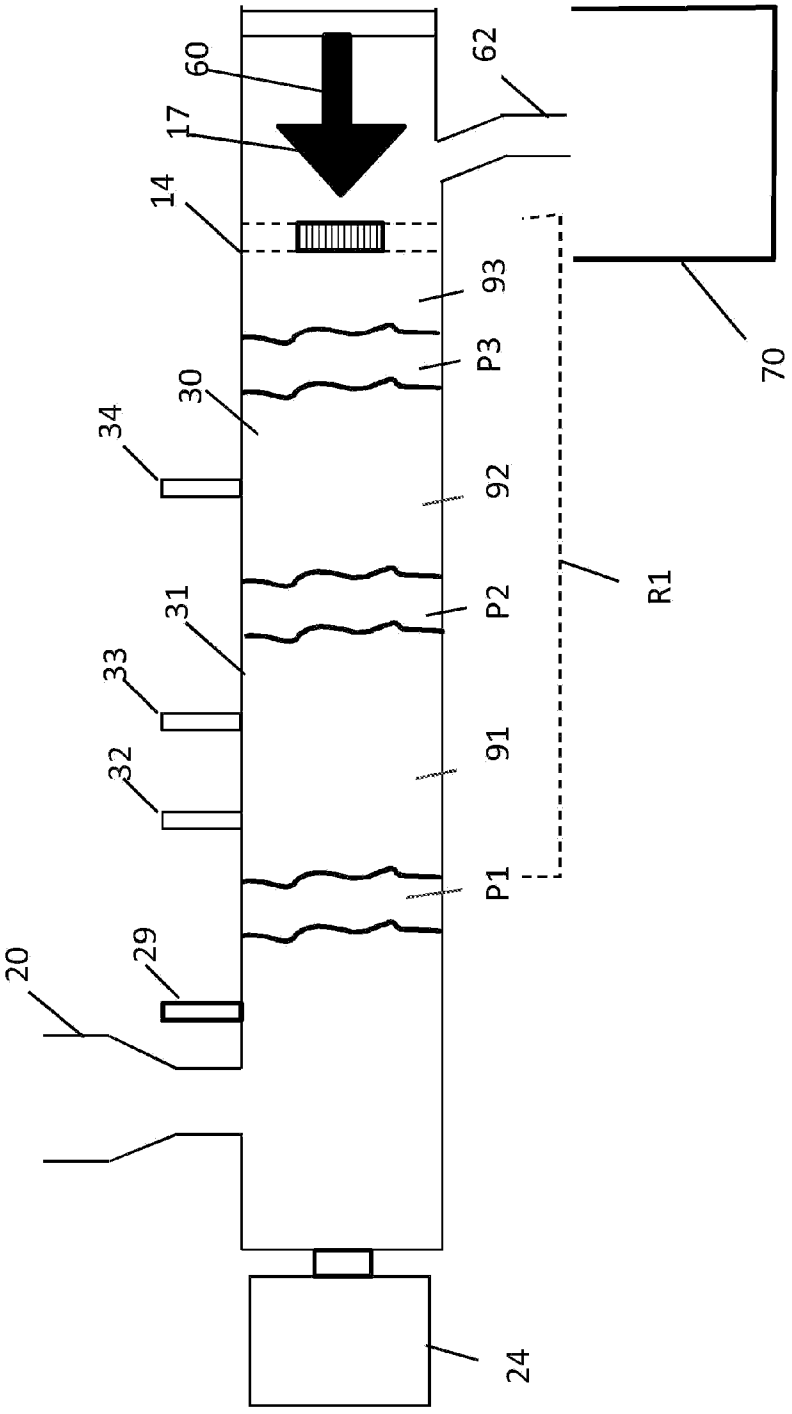
13. System ifølge et hvilket som helst af kravene 1-12, hvor systemet er konfigureret til fremstille de faste partikler i et størrelsesinterval på ca.: 1-500 µm, 1-250 µm, 1-200 µm eller 1-150 µm og eventuelt med en gennemsnitlig størrelse på ca. 15-25 µm.

14. System ifølge et hvilket som helst af kravene 9-13, hvor den trykaktiverede udledningsventil (17) er en udledningsventil af tallerkentypen, der inkorporerer en venturi med gradvis ekspansion.

15. Fremgangsmåde til forbehandling af mindst ét ton biomasse pr. dag i industriel skala, hvilken fremgangsmåde omfatter: (a) tilførsel af biomassen ved en hastighed på mindst ét tørton (t) biomasse pr. dag i systemet ifølge et hvilket som helst af kravene 1-14 og (b) behandling af biomassen ved en forhøjet temperatur og et forhøjet tryk inden i reaktionszonen i mindre end ca. 20 sekunder til fremstilling af en forbehandlet biomassesammensætning, der omfatter en flydende fraktion, som omfatter monosaccharider, og faste partikler, der omfatter cellulose.

DRAWINGS

FIG. 1



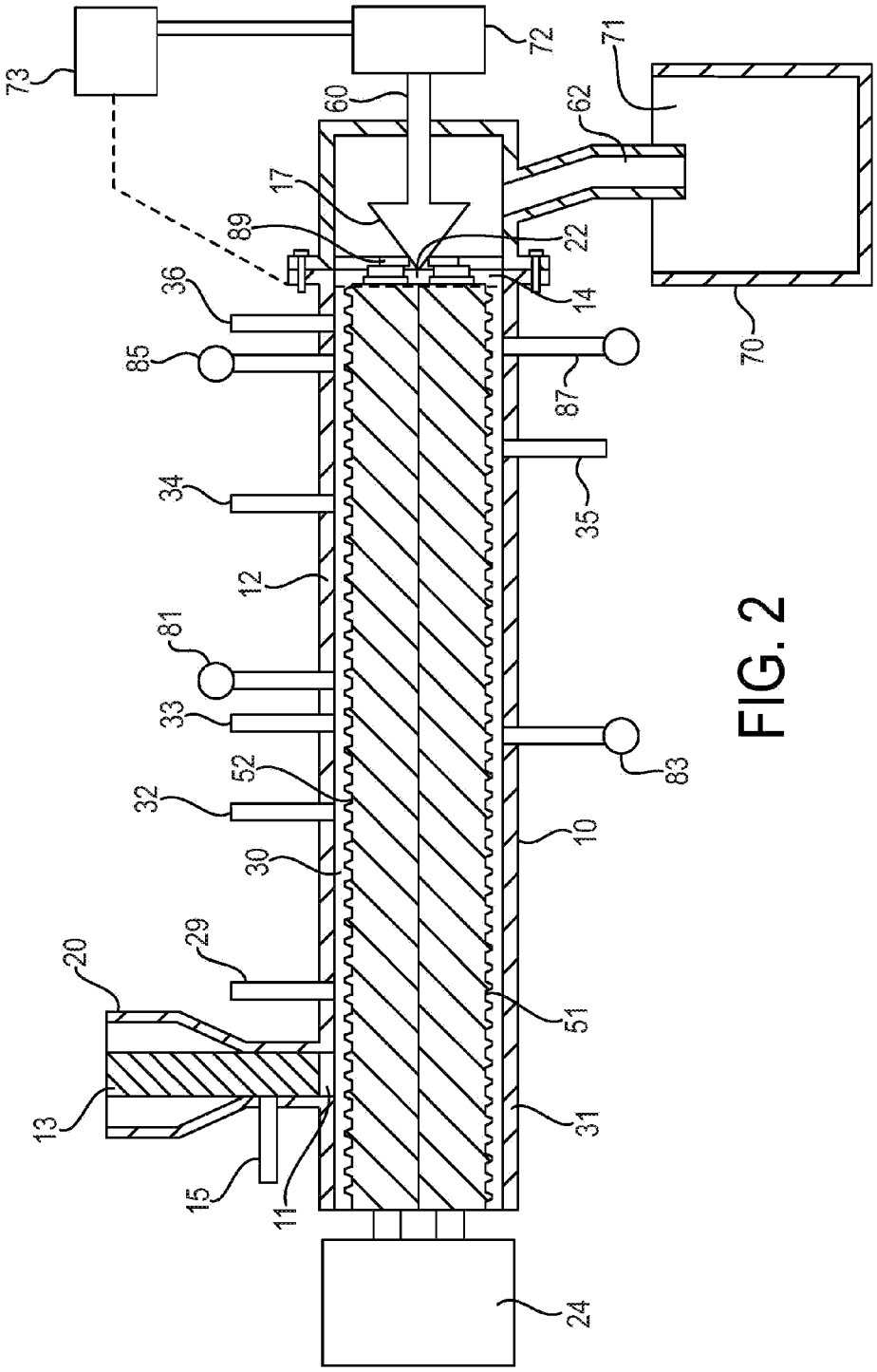
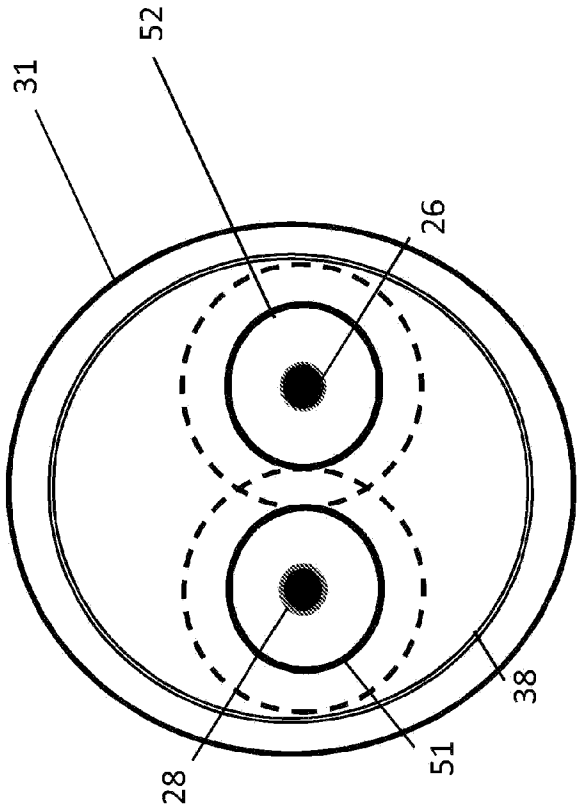

73
72
71
60
62
17
89
22
36
14
85
70
87
34
35
12
81
33
83
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32
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29
20
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31
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11
24

FIG. 2

FIG. 3



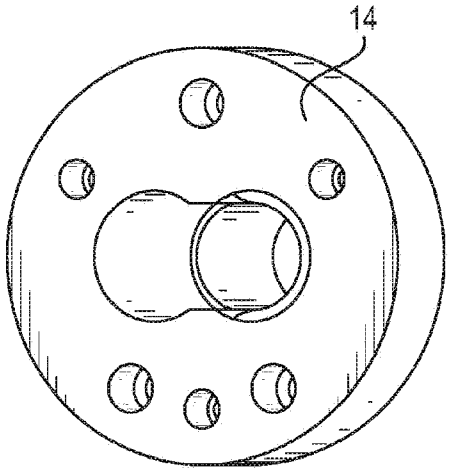


FIG. 4

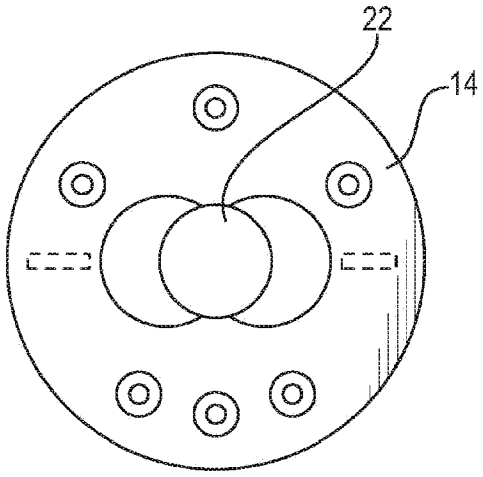


FIG. 5

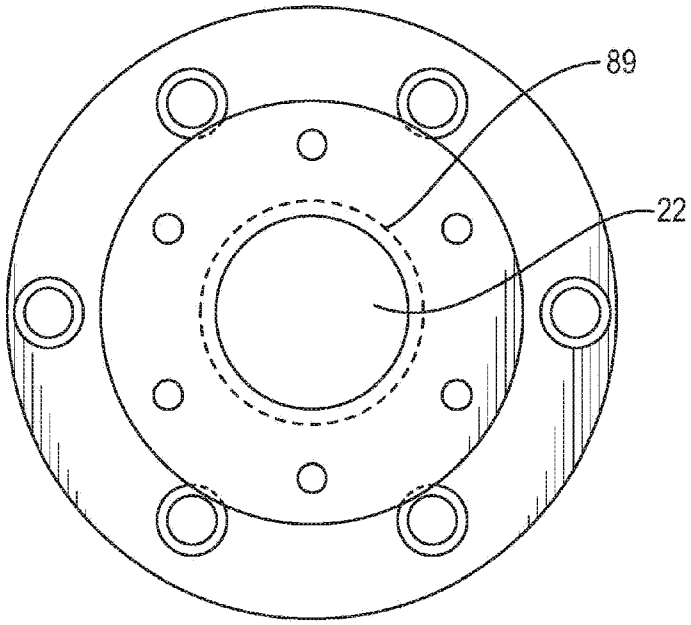


FIG. 6

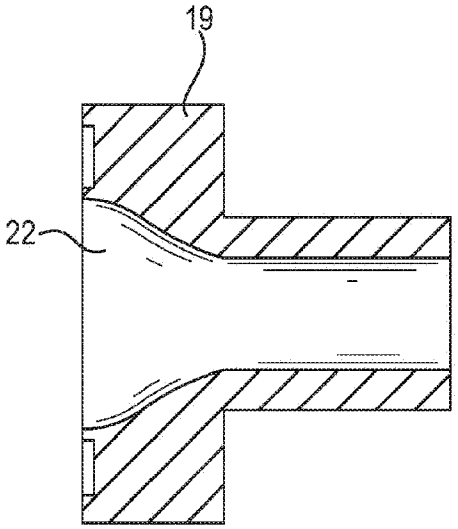


FIG. 7

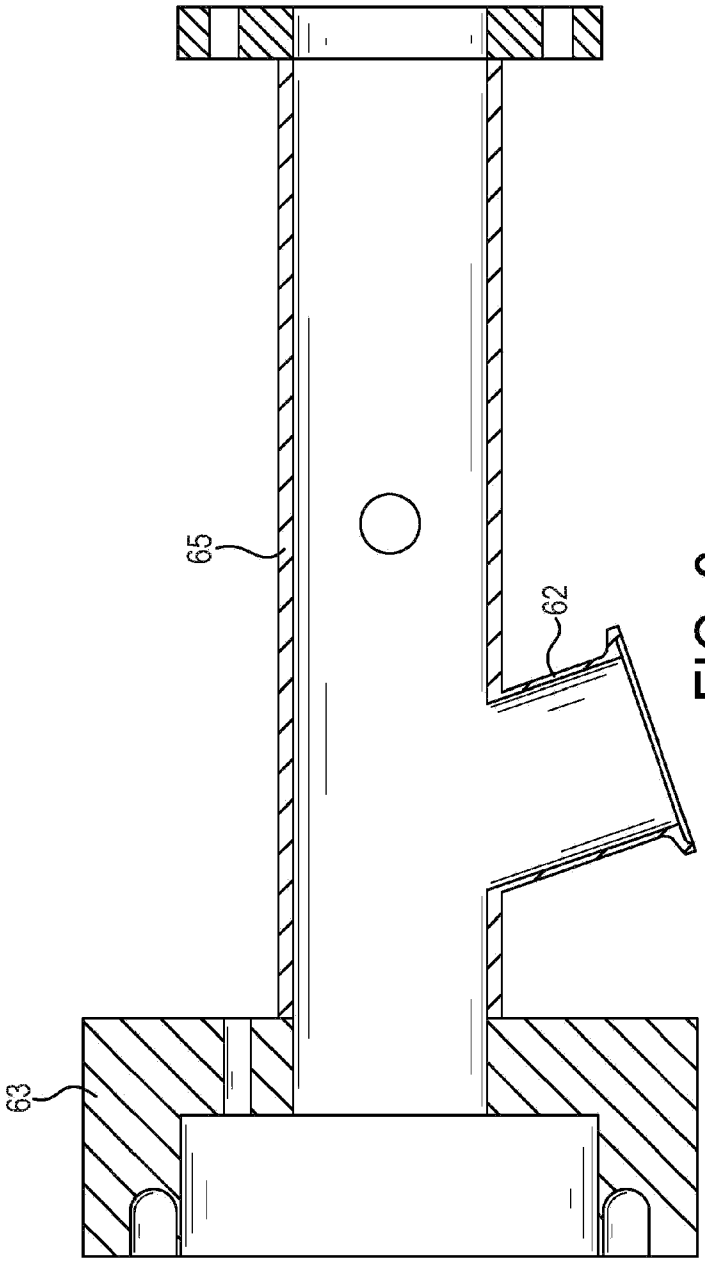


FIG. 8

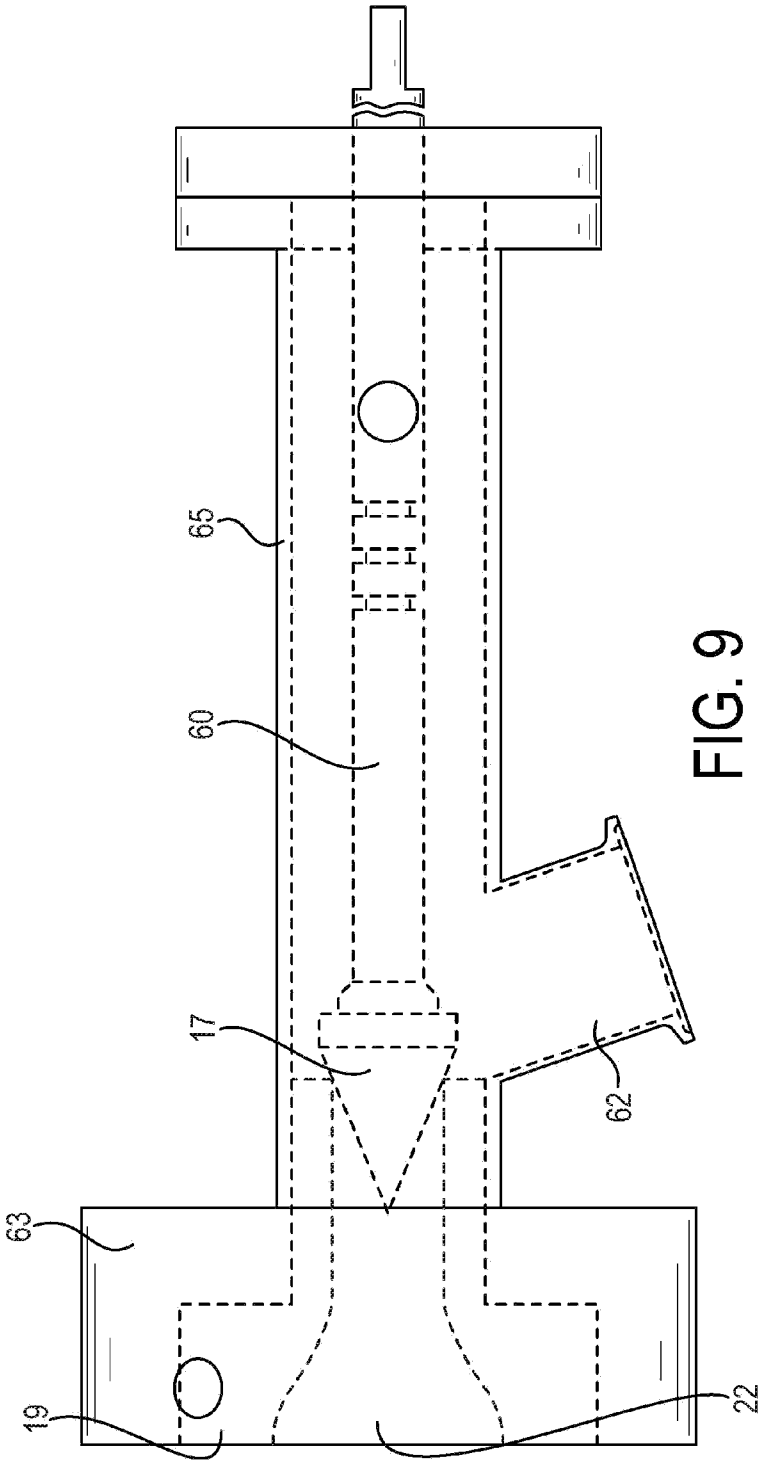


FIG. 9

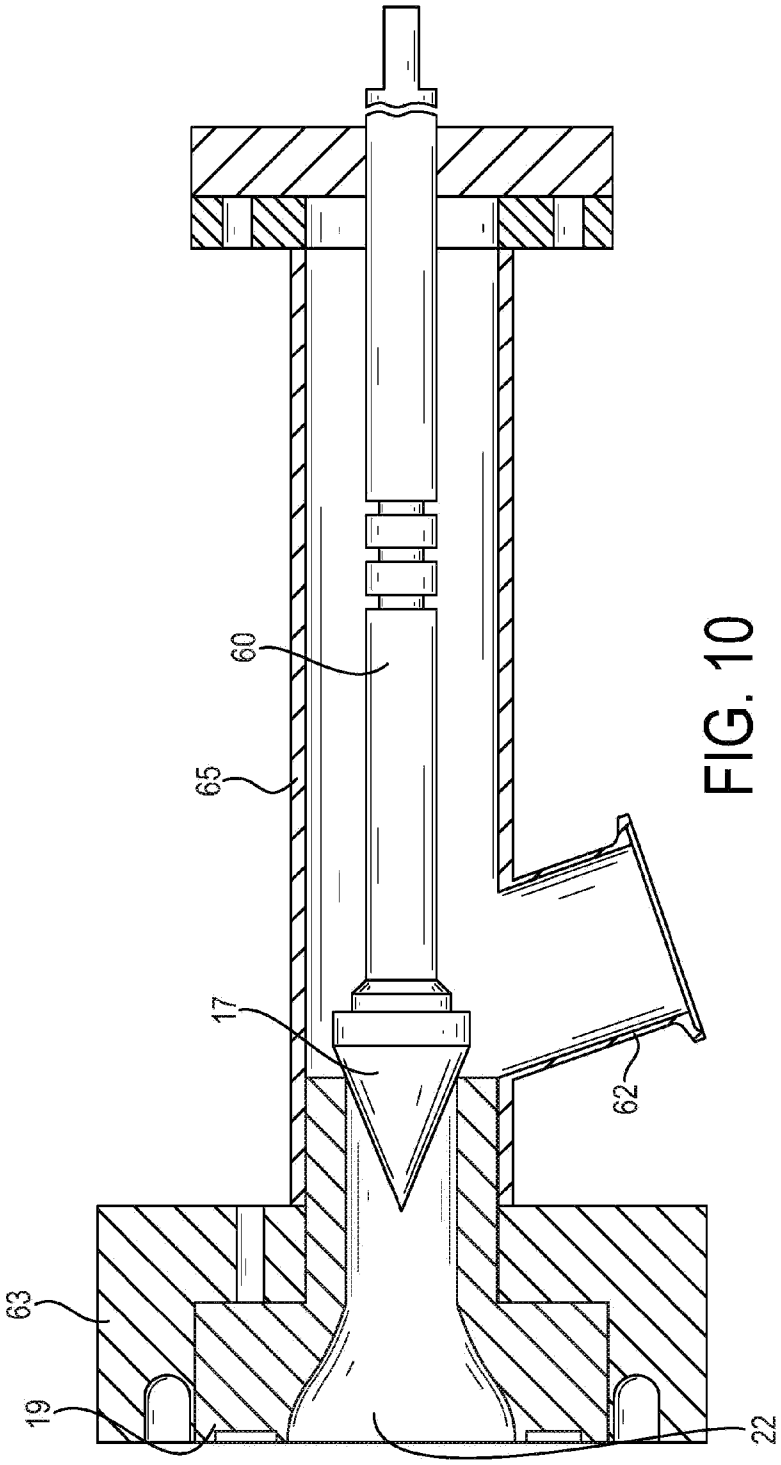


FIG. 10

FIG. 11

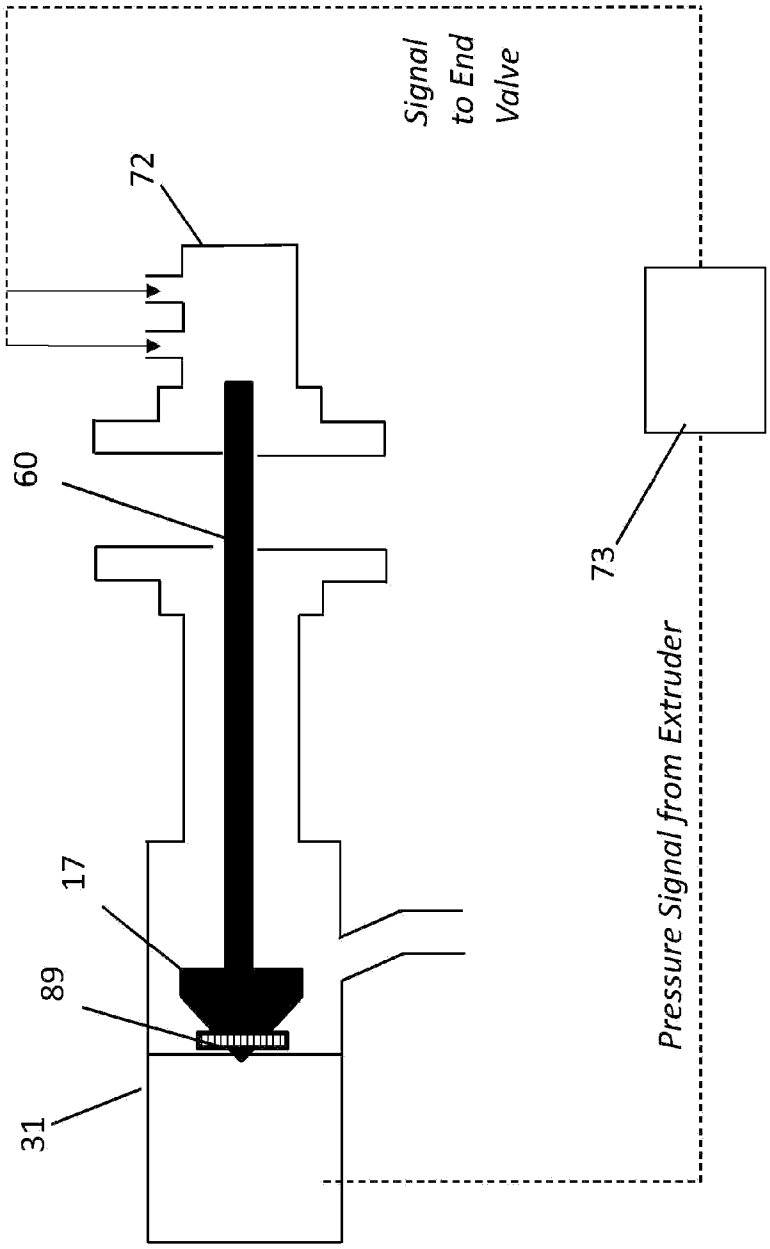

Signal to End Valve
72
60
17
89
31
73
Pressure Signal from Extruder

FIG. 12

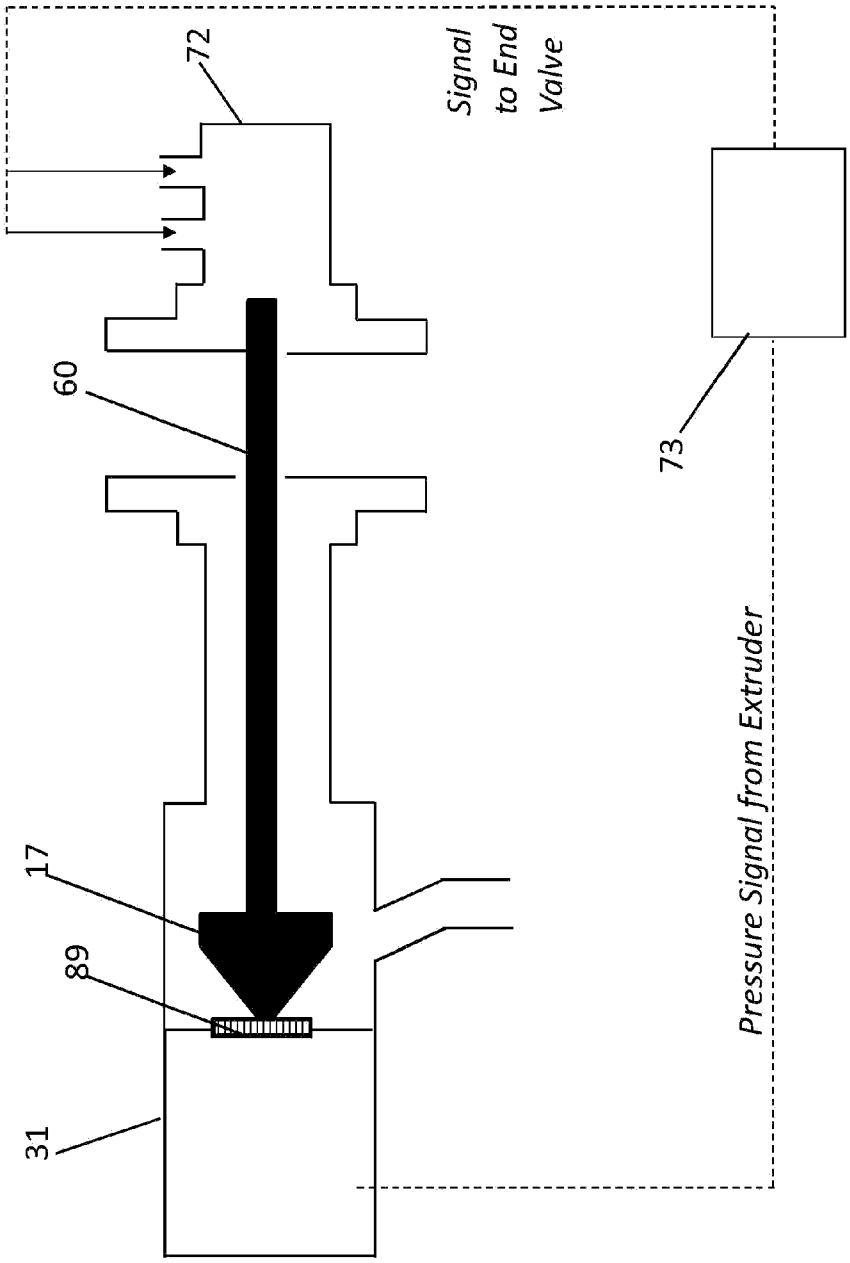
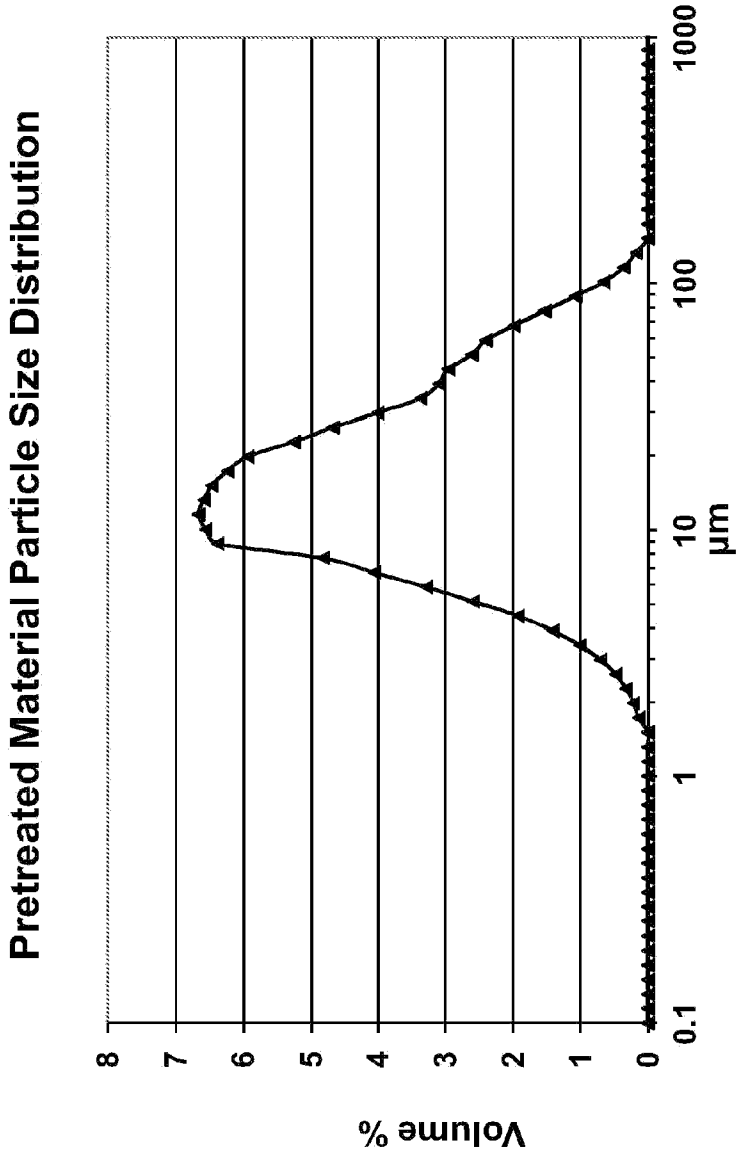

72
Signal to End Valve
60
73
17
89
31
Pressure Signal from Extruder

FIG. 13


Pretreated Material Particle Size Distribution
Volume %
0
1
2
3
4
5
6
7
8
0.1
1
10
100
1000
µm