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Description

TECHNICAL FIELD

[0001] This invention relates to a method for producing a combustible fuel.

BACKGROUND

[0002] Various carbohydrates, such as cellulosic and lignocellulosic materials, e:g., in fibrous form, are produced, processed, and used in large quantities in a number of applications. Often such materials are used once, and then discarded as waste, or are simply considered to be waste materials, e.g., sewage, bagasse, sawdust, and stover.

[0003] Various cellulosic and lignocellulosic materials, their uses, and applications have been described in U.S. Patent Nos. 7,307,108, 7,074,918, 6,448,307, 6,258,876, 6,207,729, 5,973,035 and 5,952,105; and in various patent applications, including "FIBROUS MATERIALS AND COMPOSITES," PCT/US2006/010648, filed on March 23, 2006, and "FIBROUS MATERIALS AND COMPOSITES," U.S. Patent Application Publication No. 2007/0045456.

[0004] Because cellulosic and lignocellulosic materials are so widely available, and waste cellulosic and lignocellulosic materials require disposal, it would be advantageous to put such materials to good use. The use of cellulosic and lignocellulosic materials to make biofuels such as ethanol is being considered, but as yet has not been implemented commercially on a large scale.

[0005] Van Zyl et al (Adv Biochem Engin/Biotechnol, 2007, 108:205-235) describes consolidated bioprocessing for bioethanol production using *Saccharomyces cerevisiae.*

[0006] US 4,769,082 discloses that the efficiency of enzymatic saccharification and fermentation of cellulose in a waste cellulose resource can be increased by a preliminary treatment with ionizing radiation.

[0007] Mohagheghi et al (Applied Biochemistry and Biotechnology, vol. 98-100, 1 April 2002, pp 885-898) describes the cofermentation of glucose, xylose and arabinose by genomic DNA-integrated xylose/arabinose fermenting strain of Zymomonas mobilis AX101.

[0008] Fujita et al (Applied and Environmental Microbiology, vol. 70, no. 2, 1 February 2004, pp 1207-1212) discloses the synergistic saccharification and direct fermentation to ethanol of amorphous cellulose by use of an engineered yeast strain codisplaying three types of cellulolytic enzyme.

SUMMARY

[0009] The subject matter of the invention is set out in the appended claims.

[0010] In some instances, functionalized biomass is more soluble and more readily utilized by microorganisms in comparison to biomass that has not been functionalized. In addition, many of the functionalized materials described herein are less prone to oxidation and can have enhanced long-term stability (e.g., oxidation in air under ambient conditions). Many of the products obtained, such as ethanol or n-butanol, can be utilized as a fuel for powering cars, trucks, tractors, ships or trains, e.g., as an internal combustion fuel or as a fuel cell feedstock. Many of the products obtained can also be utilized to power aircraft, such as planes, e.g., having jet engines or helicopters. In addition, the products described herein can be utilized for electrical power generation, e.g., in a conventional steam generating plant or in a fuel cell plant.

[0011] The invention derives from the realization that the addition of biomass, which is a functionalized cellulosic or lignocellulosic material, to a mixture including a low molecular weight sugar can facilitate conversion of the low molecular weight sugar to a combustible fuel such as ethanol. The inventors have found that including the biomass in a mixture with a low molecular weight sugar, a solvent or solvent system and a microorganism significantly improves the yield of a product obtained by conversion of the sugar, for example an alcohol such as ethanol, without conversion or depletion of the biomass itself. Including the biomass also can prevent incomplete, sluggish, or "stuck" product conversion, e.g., by fermentation.

[0012] The biomass is not in itself be converted to the product (such as ethanol), along with the low molecular weight sugar.

[0013] In some cases, the biomass may be the remnants of a cellulosic or lignocellulosic material that has been saccharified, e.g., lignin and/or other materials that are left over after cellulose has been converted to sugar.

[0014] Thus, in one aspect, the invention features a method that includes converting a low molecular weight sugar, or a material that includes a low molecular weight sugar, in a mixture comprising a biomass which has been irradiated prior to mixing, a microorganism, and a solvent or a solvent system, e.g., water or a mixture of water and an organic solvent, to a product, for example, other than sugar. Examples of solvents or solvent systems include water, hexane, hexadecane, glycerol, chloroform, toluene, ethyl acetate, petroleum ether, liquefied petroleum gas (LPG), ionic liquids and mixtures thereof. The solvent or solvent system can be in the form of a single phase or two or more phases. The

biomass can be, e.g., in fibrous form.

[0015] In some instances, having a biomass material treated by irradiation present during production of a product, such as ethanol, can enhance the production rate of the product. Without wishing to be bound by any particular theory, it is believed that having a solid present, such as a high surface area and/or high porosity solid, can increase reaction rates by increasing the effective concentration of solutes and providing a substrate on which reactions can occur.

[0016] For example, an irradiated biomass material, e.g., a paper fiber, can be added to a fermentation process, such as during a corn-ethanol fermentation or a sugarcane extract fermentation, to increase the rate of production by 10, 15, 20, 30, 40, 50, 75, 100 percent or more, e.g., 150 percent. The biomass material can have a high surface area, high porosity, and/or low bulk density. In some embodiments, the biomass is present in the mixture from about 0.5 percent to about 50 percent by weight, such as between about 1 percent and about 25 percent by weight, or between about 2 percent and about 12.5 percent by weight. In other embodiments, the biomass is present in amounts greater than about 0.5 percent by weight, such as greater than about 1, 2, 3, 4, 5, 6, 7, 8, 9, or even greater than about 10 percent by weight.

[0017] Because the biomass material is not itself consumed during the conversion process, the biomass material can be reused in multiple batch processes, or can be used continuously for the production of a relatively large volume of the product.

[0018] Some implementations include one or more of the following features.

[0019] The biomass may comprise a fibrous material. Converting can include allowing the microorganism to convert at least a portion of the low molecular weight sugar to ethanol. The converting comprises fermentation. The microorganism can comprise a yeast, e.g., selected from the group consisting of S. cerevisiae and P. stipitis, or a bacterium such as Zymomonas mobilis. Converting can exhibit a % performance of at least 140%, in some cases at least 170%.

[0020] The method includes irradiating the fibrous biomass prior to mixing, with ionizing radiation, at a total dosage of at least 5 Mrad. Irradiating can be performed using a particle beam. Irradiating can be conducted under conditions selected to reduce the molecular weight of the biomass.

[0021] The biomass can have a bulk density of less than about 0.5 g/cm^3. The biomass can have a BET surface area of greater than 0.25 m^2/g, and/or a length to diameter ratio of at least 5. The biomass can have a porosity greater than 50%, e.g., greater than 70%.

[0022] The method can further include physically preparing the biomass, e.g., by shearing, or by reducing the size of the biomass by stone grinding, mechanical ripping or tearing, pin grinding, or air attrition milling. The biomass can have internal fibers, and can have been sheared to an extent that its internal fibers are substantially exposed.

[0023] The biomass is or includes a cellulosic or lignocellulosic material. For example, the biomass can be selected from the group consisting of paper, paper products, paper waste, wood, particle board, sawdust, agricultural waste, sewage, silage, grasses, rice hulls, bagasse, cotton, jute, hemp, flax, bamboo, sisal, abaca, straw, corn cobs, corn stover, switchgrass, alfalfa, hay, rice hulls, coconut hair, cotton, seaweed, algae, and mixtures thereof.

[0024] Materials are disclosed herein that include a plurality of saccharide units arranged in a molecular chain, wherein from about 1 out of every 2 to about 1 out of every 250 saccharide units includes a carboxylic acid group, or an ester or salt thereof. In another aspect, materials include a plurality of such molecular chains. For example, about 1 out of every 8, 1 out of every 10, 1 out of every 50, or 1 out of every 100 saccharide units of each chain can include a carboxylic acid group, or an ester or salt thereof. In some embodiments, the saccharide units can include 5 or 6 carbon saccharide units. Each chain can have between about 10 and about 200 saccharide units, e.g., between about 10 and about 100 or between about 10 and about 50. For example, each chain can include hemicellulose or cellulose. In some embodiments, each chain also includes saccharide units that include nitroso, nitro, or nitrile groups.

[0025] In some embodiments, the average molecular weight of the materials relative to PEG standards can be from about 1,000 to about 1,000,000, such as between 1,500 and 200,000 or 2,000 and 10,000. For example, the average molecular weight of the materials relative to PEG standards can be less than about 10,000.

[0026] Examples of biomass feedstock include paper, paper products, paper waste, wood, particle board, sawdust, agricultural waste, sewage, silage, grasses, rice hulls, bagasse, cotton, jute, hemp, flax, bamboo, sisal, abaca, straw, corn cobs, corn stover, switchgrass, alfalfa, hay, rice hulls, coconut hair, cotton, synthetic celluloses, seaweed, algae, or mixtures of these. The biomass can be or can include a natural or a synthetic material.

[0027] Examples of fuels include one or more of hydrogen, alcohols, and hydrocarbons. For example, the alcohols can be ethanol, n-propanol, isopropanol, n-butanol, or mixtures of these.

[0028] Irradiation can be, e.g., performed utilizing an ionizing radiation, such as gamma rays, a beam of electrons, or ultraviolet C radiation having a wavelength of from about 100 nm to about 280 nm. Irradiation can be performed using multiple applications of radiation. The ionizing radiation can include electron beam radiation. For example, the radiation can be applied at a total dose of between about 10 Mrad and about 150 Mrad, such as at a dose rate of about 0.5 to about 10 Mrad/day, or 1 Mrad/s to about 10 Mrad/s. In some embodiments, irradiating includes applying two or more radiation sources, such as gamma rays and a beam of electrons.

[0029] In some embodiments, the biomass includes a first cellulose having a first number average molecular weight and the carbohydrate material comprises a second cellulose having a second number average molecular weight lower

than the first number average molecular weight. For example, the second number average molecular weight is lower than the first number average molecular weight by more than about one-fold. In some embodiments, the first cellulose has a first crystallinity, and the second cellulose has a second crystallinity lower than the first crystallinity. For example, the second crystallinity can be lower than the first crystallinity by more than about 10 percent.

[0030] In some embodiments, the first cellulose can have a first level of oxidation and the second cellulose has a second level of oxidation higher than the first level of oxidation.

[0031] The biomass material can further include a buffer, such as sodium bicarbonate or ammonium chloride, an electrolyte, such as potassium chloride or sodium chloride a growth factor, such as biotin and/or a base pair such as uracil, a surfactant, a mineral, or a chelating agent.

[0032] In some embodiments, the methods include pretreating with one or more other pretreatment methods in addition to irradiation. For example, the two or more different pretreatment methods can include radiation and sonication, radiation and oxidation, and radiation and pyrolization. Optionally, pretreating the biomass can include steam explosion.

[0033] To further aid in the reduction of the molecular weight of the biomass, an enzyme, e.g., a cellulolytic enzyme, or a chemical, e.g., sodium hypochlorite, an acid, a base or a swelling agent, can be utilized with any method described herein. The enzyme and/or chemical treatment can occur before, during or after irradiation or other pretreatment.

[0034] When a microorganism is utilized, it can be a natural microorganism or an engineered microorganism. For example, the microorganism can be a bacterium, a fungus, e.g., a yeast, a plant or a protist, e.g., an algae, a protozoa or a fungus-like protist, e.g., a slime mold. When the organisms are compatible, mixtures may be utilized.

[0035] Examples of products that may be produced using the methods disclosed herein include mono- and polyfunctional C1-C6 alkyl alcohols, mono- and poly-functional carboxylic acids, C1-C6 hydrocarbons, and combinations thereof. Specific examples of suitable alcohols include methanol, ethanol, propanol, isopropanol, butanol, ethylene glycol, propylene glycol, 1,4-butane diol, glycerin, and combinations thereof. Specific example of suitable carboxylic acids include formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, palmitic acid, stearic acid, oxalic acid, malonic acid, succinic acid, glutaric acid, oleic acid, linoleic acid, glycolic acid, lactic acid, γ-hydroxybutyric acid, and combinations thereof. Examples of suitable hydrocarbons include methane, ethane, propane, pentane, n-hexane, and combinations thereof. Many of these products may be used as fuels. Other products are described in U.S. Provisional Application Serial No. 61/139,453. Products or co-products produced can be products intended to be used as produced or the products produced can be intermediates for any other process described herein or any process described in any application described herein.

[0036] Examples of microorganisms that may be used to produce useful products include bacteria, yeasts, or combinations thereof. For example, the microorganism can be a bacterium, a fungus, e.g., a yeast, a plant or a protist, e.g., an algae, a protozoa or a fungus-like protist, e.g., a slime mold.

[0037] In any of the methods disclosed herein, radiation may be applied from a device that is in a vault.

[0038] The term "fibrous material," as used herein, is a material that includes numerous loose, discrete and separable fibers. For example, a fibrous material can be prepared from a bleached Kraft paper fiber source by shearing, e.g., with a rotary knife cutter.

[0039] The term "screen," as used herein, means a member capable of sieving material according to size. Examples of screens include a perforated plate, cylinder or the like, or a wire mesh or cloth fabric.

[0040] The term "pyrolysis," as used herein, means to break bonds in a material by the application of heat energy. Pyrolysis can occur while the subject material is under vacuum, or immersed in a gaseous material, such as an oxidizing gas, e.g., air or oxygen, or a reducing gas, such as hydrogen.

[0041] Oxygen content is measured by elemental analysis by pyrolyzing a sample in a furnace operating at 1300 °C or above.

[0042] The term "biomass" includes any non-fossilized, i.e., renewable, organic matter. The various types of biomass include plant biomass (defined below), microbial biomass, animal biomass (any animal by-product, animal waste, etc.) and municipal waste biomass (residential and light commercial refuse with recyclables such as metal and glass removed). The term biomass also includes virgin or post consumer cellulosic materials, such as rags and towels fabricated from cotton or a cotton blend.

[0043] The term "plant biomass" and "lignocellulosic biomass" refer to virtually any plant-derived organic matter (woody or non-woody). Plant biomass can include, but is not limited to, agricultural or food crops (e.g., sugarcane, sugar beets or corn kernels) or an extract therefrom (e.g., sugar from sugarcane and corn starch from corn), agricultural crops and agricultural crop wastes and residues such as corn stover, wheat straw, rice straw, sugar cane bagasse, cotton and the like. Plant biomass further includes, but is not limited to, trees, woody energy crops, wood wastes and residues such as softwood forest thinnings, barky wastes, sawdust, paper and pulp industry waste streams, wood fiber, and the like. Additionally, grass crops, such as switchgrass and the like have potential to be produced on a large-scale as another plant biomass source. For urban areas, the best potential plant biomass feedstock includes yard waste (e.g., grass clippings, leaves, tree clippings, and brush) and vegetable processing waste.

[0044] "Lignocellulosic feedstock," is any type of plant biomass such as, but not limited to, non-woody plant biomass,

cultivated crops, such as, but not limited to, grasses, for example, but not limited to, C4 grasses, such as switchgrass, cord grass, rye grass, miscanthus, reed canary grass, or a combination thereof, or sugar processing residues such as bagasse, or beet pulp, agricultural residues, for example, soybean stover, corn stover, rice straw, rice hulls, barley straw, corn cobs, wheat straw, canola straw, rice straw, oat straw, oat hulls, corn fiber, recycled wood pulp fiber, sawdust, hardwood, for example aspen wood and sawdust, softwood, or a combination thereof. Further, the lignocellulosic feedstock may include cellulosic waste material such as, but not limited to, newsprint, cardboard, sawdust, and the like.

[0045] Lignocellulosic feedstock may include one species of fiber or alternatively, lignocellulosic feedstock may include a mixture of fibers that originate from different lignocellulosic feedstocks. Furthermore, the lignocellulosic feedstock may comprise fresh lignocellulosic feedstock, partially dried lignocellulosic feedstock, fully dried lignocellulosic feedstock or a combination thereof.

[0046] For the purposes of this disclosure, carbohydrates are materials that are composed entirely of one or more saccharide units or that include one or more saccharide units. The saccharide units can be functionalized about the ring with one or more functional groups, such as carboxylic acid groups, amino groups, nitro groups, nitroso groups or nitrile groups and still be considered carbohydrates. Carbohydrates can be polymeric (e.g., equal to or greater than 10-mer, 100-mer, 1,000-mer, 10,000-mer, or 100,000-mer), oligomeric (e.g., equal to or greater than a 4-mer, 5-mer, 6-mer, 7-mer, 8-mer, 9-mer or 10-mer), trimeric, dimeric, or monomeric. When the carbohydrates are formed of more than a single repeat unit, each repeat unit can be the same or different.

[0047] Examples of polymeric carbohydrates include cellulose, xylan, pectin, and starch, while cellobiose and lactose are examples of dimeric carbohydrates. Examples of monomeric carbohydrates include glucose and xylose.

[0048] Carbohydrates can be part of a supramolecular structure, e.g., covalently bonded into the structure. Examples of such materials include lignocellulosic materials, such as those found in wood.

[0049] A starchy material is one that is or includes significant amounts of starch or a starch derivative, such as greater than about 5 percent by weight starch or starch derivative. For purposes of this disclosure, a starch is a material that is or includes an amylose, an amylopectin, or a physical and/or chemical mixture thereof, e.g., a 20:80 or 30:70 percent by weight mixture of amylose to amylopectin. For example, rice, corn, and mixtures thereof are starchy materials. Starch derivatives include, e.g., maltodextrin, acid-modified starch, base-modified starch, bleached starch, oxidized starch, acetylated starch, acetylated and oxidized starch, phosphate-modified starch, genetically-modified starch and starch that is resistant to digestion.

[0050] For purposes of this disclosure, a low molecular weight sugar is a carbohydrate or a derivative thereof that has a formula weight (excluding moisture) that is less than about 2,000, e.g., less than about 1,800, 1,600, less than about 1,000, less than about 500, less than about 350 or less than about 250. For example, the low molecular weight sugar can be a monosaccharide, e.g., glucose or xylose, a disaccharide, e.g., cellobiose or sucrose, or a trisaccharide.

[0051] A combustible fuel is a material capable of burning in the presence of oxygen. Examples of combustible fuels include ethanol, n-propanol, n-butanol, hydrogen and mixtures of any two or more of these.

[0052] Swelling agents as used herein are materials that cause a discernable swelling, e.g., a 2.5 percent increase in volume over an unswollen state of cellulosic and/or lignocellulosic materials, when applied to such materials as a solution, e.g., a water solution. Examples include alkaline substances, such as sodium hydroxide, potassium hydroxide, lithium hydroxide and ammonium hydroxides, acidifying agents, such as mineral acids (e.g., sulfuric acid, hydrochloric acid and phosphoric acid), salts, such as zinc chloride, calcium carbonate, sodium carbonate, benzyltrimethylammonium sulfate, and basic organic amines, such as ethylene diamine.

[0053] A "sheared material," as used herein, is a material that includes discrete fibers in which at least about 50% of the discrete fibers have a length/diameter (L/D) ratio of at least about 5, and that has an uncompressed bulk density of less than about 0.6 g/cm^3. A sheared material is thus different from a material that has been cut, chopped or ground.

[0054] Changing a molecular structure of a biomass feedstock, as used herein, means to change the chemical bonding arrangement, such as the type and quantity of functional groups or conformation of the structure. For example, the change in the molecular structure can include changing the supramolecular structure of the material, oxidation of the material, changing an average molecular weight, changing an average crystallinity, changing a surface area, changing a degree of polymerization, changing a porosity, changing a degree of branching, grafting on other materials, changing a crystalline domain size, or an changing an overall domain size.

[0055] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0056] Other features and advantages of the invention will be apparent from the following detailed description, and from the claim.

DESCRIPTION OF DRAWINGS

[0057]

FIG. 1 is a block diagram illustrating conversion of biomass into products and co-products.
FIG. 2 is block diagram illustrating conversion of a fiber source into a first and second fibrous material.
FIG. 3 is a cross-sectional view of a rotary knife cutter.
FIG. 4 is block diagram illustrating conversion of a fiber source into a first, second and third fibrous material.
FIG. 5 is block diagram illustrating densification of a material.
FIG. 6 is a perspective view of a pellet mill.
FIG. 7A is a densified fibrous material in pellet form.
FIG. 7B is a transverse cross-section of a hollow pellet in which a center of the hollow is in-line with a center of the pellet.
FIG. 7C is a transverse cross-section of a hollow pellet in which a center of the hollow is out of line with the center of the pellet.
FIG. 7D is a transverse cross-section of a tri-lobal pellet.
FIG. 8 is a block diagram illustrating a treatment sequence for processing feedstock.
FIG. 9 is a perspective, cut-away view of a gamma irradiator housed in a concrete vault.
FIG. 10 is an enlarged perspective view of region R of FIG. 9.
FIG. 11A is a block diagram illustrating an electron beam irradiation feedstock pretreatment sequence.
FIG. 11B is a schematic representation of biomass being ionized, and then oxidized or quenched.
FIG. 12 is a schematic view of a system for sonicating a process stream of cellulosic material in a liquid medium.
FIG. 13 is a schematic view of a sonicator having two transducers coupled to a single horn.
FIG. 14 is a block diagram illustrating a pyrolytic feedstock pretreatment system.
FIG. 15 is a cross-sectional side view of a pyrolysis chamber.
FIG. 16 is a cross-sectional side view of a pyrolysis chamber.
FIG. 17 is a cross-sectional side view of a pyrolyzer that includes a heated filament.
FIG. 18 is a schematic cross-sectional side view of a Curie-Point pyrolyzer.
FIG. 19 is a schematic cross-sectional side view of a furnace pyrolyzer.
FIG. 20 is a schematic cross-sectional top view of a laser pyrolysis apparatus.
FIG. 21 is a schematic cross-sectional top view of a tungsten filament flash pyrolyzer.
FIG. 22 is a block diagram illustrating an oxidative feedstock pretreatment system.
FIG. 23 is block diagram illustrating a general overview of the process of converting a fiber source into a product, e.g., ethanol.
FIG. 24 is a cross-sectional view of a steam explosion apparatus.
FIG. 25 is a schematic cross-sectional side view of a hybrid electron beam/sonication device.
FIG. 26 is a scanning electron micrograph of a fibrous material produced from polycoated paper at 25 X magnification. The fibrous material was produced on a rotary knife cutter utilizing a screen with 1/8 inch openings.
FIG. 27 is a scanning electron micrograph of a fibrous material produced from bleached Kraft board paper at 25 X magnification. The fibrous material was produced on a rotary knife cutter utilizing a screen with 1/8 inch openings.
FIG. 28 is a scanning electron micrograph of a fibrous material produced from bleached Kraft board paper at 25 X magnification. The fibrous material was twice sheared on a rotary knife cutter utilizing a screen with 1/16 inch openings during each shearing.
FIG. 29 is a scanning electron micrograph of a fibrous material produced from bleached Kraft board paper at 25 X magnification. The fibrous material was thrice sheared on a rotary knife cutter. During the first shearing, a 1/8 inch screen was used; during the second shearing, a 1/16 inch screen was used, and during the third shearing a 1/32 inch screen was used.
FIGS. 29A-29F are 3-D Raman spectra from the surface of fibers from samples P132, P132-10, P132-100, P-1e, P-30e, and P-100e, respectively.
FIG. 30 is a schematic side view of a sonication apparatus, while FIG. 31 is a cross-sectional view through the processing cell of FIG. 30.
FIG. 32 is a scanning electron micrograph at 1000 X magnification of a fibrous material produced from shearing switchgrass on a rotary knife cutter, and then passing the sheared material through a 1/32 inch screen.
FIGS. 33 and 34 are scanning electron micrographs of the fibrous material of FIG. 32 after irradiation with 10 Mrad and 100 Mrad gamma rays, respectively, at 1000 X magnification.
FIG. 35 is a scanning electron micrographs of the fibrous material of FIG. 32 after irradiation with 10 Mrad and sonication at 1000 X magnification.
FIG. 36 is a scanning electron micrographs of the fibrous material of FIG. 32 after irradiation with 100 Mrad and sonication at 1000 X magnification.

FIG. 37 is an infrared spectrum of Kraft board paper sheared on a rotary knife cutter.
FIG. 38 is an infrared spectrum of the Kraft paper of FIG. 37 after irradiation with 100 Mrad of gamma radiation.
FIGS. 38A-38I are ^{1}H-NMR spectra of samples P132, P132-10, P132-100, P-1e, P-5e, P-10e, P-30e, P-70e, and P-100e in Example 23. FIG. 38J is a comparison of the exchangeable proton at ~16ppm from FIGS. 38A-38I. FIG. 38K is a ^{13}C-NMR of sample P-100e. FIGS. 38L-38M are ^{13}C-NMR of sample P-100e with a delay time of 10 seconds. FIG. 38N is a ^{1}H-NMR at a concentration of 10% wt./wt. of sample P-100e.
FIG. 39 is a schematic view of a process for biomass conversion.
FIG. 40 is schematic view of another process for biomass conversion.

DETAILED DESCRIPTION

[0058] Low molecular weight sugars can be processed to produce fuels, e.g., fuels for internal combustion engines, jet engines or feedstocks for fuel cells. In addition, functionalized materials having desired types and amounts of functionality, such as carboxylic acid groups, enol groups, aldehyde groups, ketone groups, nitrile groups, nitro groups, or nitroso groups, can be prepared using the methods described herein. Such functionalized materials can be, e.g., more soluble, easier to utilize by various microorganisms or can be more stable over the long term, e.g., less prone to oxidation.

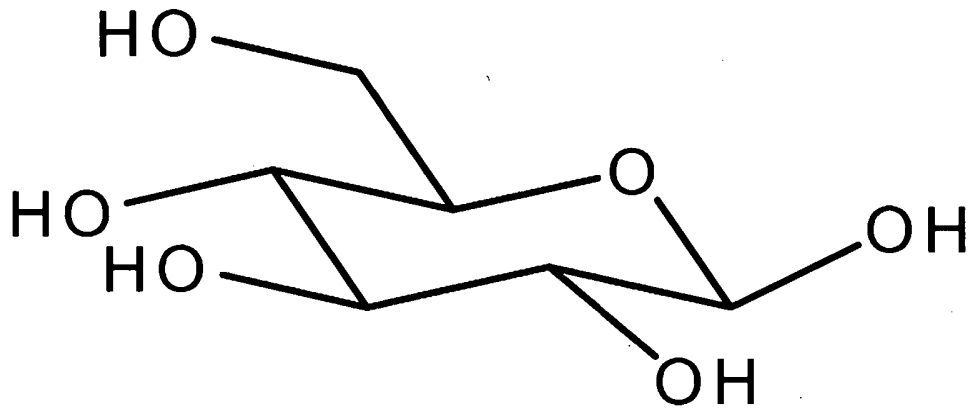
TYPES OF BIOMASS

[0059] The biomass material can be cellulosic or lignocellulosic materials, starchy materials.

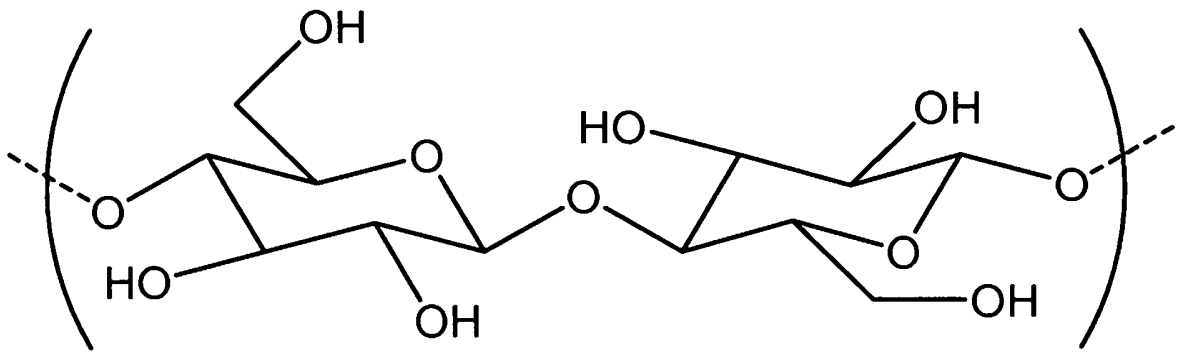
[0060] For example, such materials can include paper, paper products, wood, wood-related materials, particle board, grasses, rice hulls, bagasse, cotton, jute, hemp, flax, bamboo, sisal, abaca, straw, corn cobs, rice hulls, coconut hair, algae, seaweed, cotton, synthetic celluloses, or mixtures of any of these. Suitable materials include those listed in the Summary section, above.

[0061] Fiber sources include cellulosic fiber sources, including paper and paper products (e.g., polycoated paper and Kraft paper), and lignocellulosic fiber sources, including wood, and wood-related materials, e.g., particleboard. Other suitable fiber sources include natural fiber sources, e.g., grasses, rice hulls, bagasse, cotton, jute, hemp, flax, bamboo, sisal, abaca, straw, corn cobs, rice hulls, coconut hair; fiber sources high in α-cellulose content, e.g., cotton; and synthetic fiber sources, e.g., extruded yarn (oriented yarn or un-oriented yarn). Natural or synthetic fiber sources can be obtained from virgin scrap textile materials, e.g., remnants or they can be post consumer waste, e.g., rags. When paper products are used as fiber sources, they can be virgin materials, e.g., scrap virgin materials, or they can be post-consumer waste. Aside from virgin raw materials, post-consumer, industrial (e.g., offal), and processing waste (e.g., effluent from paper processing) can also be used as fiber sources. Also, the fiber source can be obtained or derived from human (e.g., sewage), animal or plant wastes. Additional fiber sources have been described in U.S. Patent Nos. 6,448,307, 6,258,876, 6,207,729, 5,973,035 and 5,952,105.

[0062] In some embodiments, the carbohydrate is or includes a material having one or more β-1,4-linkage and having a number average molecular weight between about 3,000 and 50,000. Such a carbohydrate is or includes cellulose (I), which is derived from (β-glucose 1) through condensation of $\beta(1\rightarrow4)$-glycosidic bonds. This linkage contrasts itself with that for $\alpha(1\rightarrow4)$-glycosidic bonds present in starch and other carbohydrates.



1

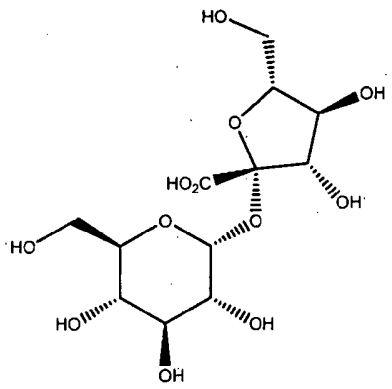


I

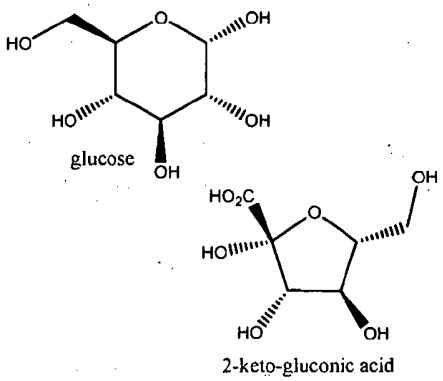
[0063] Biomass materials that include low molecular weight sugars can, e.g., include at least about 0.5 percent by weight of the low molecular sugar, e.g., at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 12.5, 25, 35, 50, 60, 70, 80, 90 or even at least about 95 percent by weight of the low molecular weight sugar. In some instances, the biomass is composed substantially of the low molecular weight sugar, e.g., greater than 95 percent by weight, such as 96, 97, 98, 99 or substantially 100 percent by weight of the low molecular weight sugar.

[0064] Biomass materials that include low molecular weight sugars can be agricultural products or food products, such as sugarcane and sugar beets, or an extract therefrom, e.g., juice from sugarcane or sugar beets. Biomass materials that include low molecular weight sugars can be substantially pure extracts, such as raw or crystallized table sugar (sucrose). Low molecular weight sugars include sugar derivatives. For example, the low molecular weight sugars can be oligomeric (e.g., equal to or greater than a 4-mer, 5-mer, 6-mer, 7-mer, 8-mer, 9-mer or 10-mer), trimeric, dimeric, or monomeric. When the carbohydrates are formed of more than a single repeat unit, each repeat unit can be the same or different.

[0065] Specific examples of low molecular weight sugars include cellobiose, lactose, sucrose, glucose and xylose, along with derivatives thereof. In some instances, sugar derivatives are more rapidly dissolved in solution or utilized by microbes to provide a useful material, such as ethanol or butanol. Several such sugars and sugar derivatives are shown below.

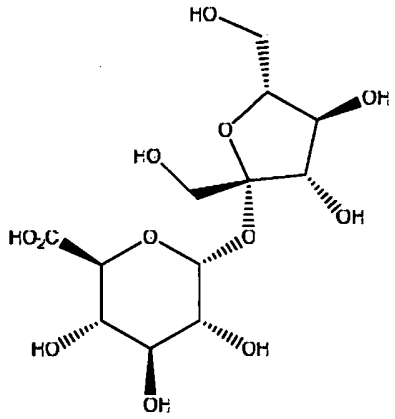


1'-MAS
(1'-monoacid of sucrose)

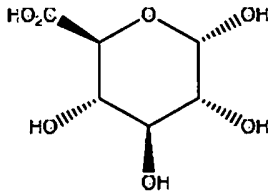


glucose

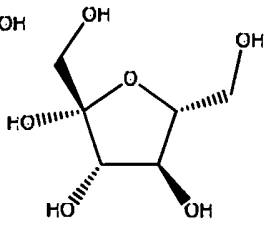
2-keto-gluconic acid



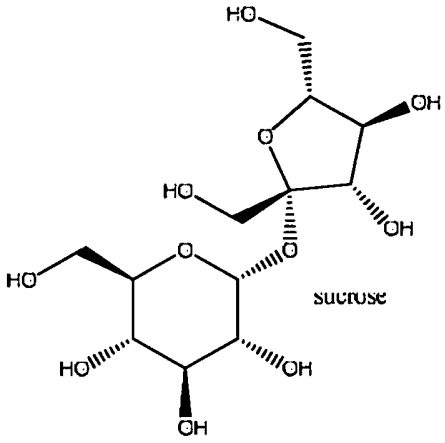
6-MAS
6-monoacid of sucrose



glucuronic acid



fructose



sucrose

[0066] Blends of any biomass materials described herein can be utilized for making any of the products described herein, such as ethanol. For example, blends of cellulosic materials and starchy materials can be utilized for making any product described herein.

SYSTEMS FOR TREATING BIOMASS

[0067] FIG. 1 shows a reference system 100 for converting biomass, particularly biomass with significant cellulosic and lignocellulosic components, into useful products and co-products. System 100 includes a feed preparation subsystem 110, a pretreatment subsystem 114, a primary process subsystem 118, and a post-processing subsystem 122. Feed preparation subsystem 110 receives biomass in its raw form, physically prepares the biomass for use as feedstock by downstream processes (e.g., reduces the size of and homogenizes the biomass), and stores the biomass both in its raw and feedstock forms. Biomass feedstock with significant cellulosic and/or lignocellulosic components can have a high average molecular weight and crystallinity that can make processing the feedstock into useful products (e.g., fermenting the feedstock to produce ethanol) difficult. For example, others have used acids, bases and enzymes to process cellulosic, lignocellulosic or starchy feedstocks. As described herein, in some embodiments, such treatments are unnecessary, or are necessary only in small or catalytic amounts.

[0068] Pretreatment subsystem 114 receives feedstock from the feed preparation subsystem 110 and prepares the feedstock for use in primary production processes by, for example, reducing the average molecular weight and crystallinity of the feedstock. Primary process subsystem 118 receives pretreated feedstock from pretreatment subsystem 114 and produces useful products (e.g., ethanol, other alcohols, pharmaceuticals, and/or food products). In some cases, the output of primary process subsystem 118 is directly useful but, in other cases, requires further processing provided by post-processing subsystem 122. Post-processing subsystem 122 provides further processing to product streams from primary process system 118 which require it (e.g., distillation and denaturation of ethanol) as well as treatment for waste

streams from the other subsystems. In some cases, the co-products of subsystems 114, 118, 122 can also be directly or indirectly useful as secondary products and/or in increasing the overall efficiency of system 100. For example, post-processing subsystem 122 can produce treated water to be recycled for use as process water in other subsystems and/or can produce burnable waste which can be used as fuel for boilers producing steam and/or electricity.

[0069] The optimum size for biomass conversion plants is affected by factors including economies of scale and the type and availability of biomass used as feedstock. Increasing plant size tends to increase economies of scale associated with plant processes. However, increasing plant size also tends to increase the costs (e.g., transportation costs) per unit of feedstock. Studies analyzing these factors suggest that the appropriate size for biomass conversion plants can range from 2000 to 10,000 dried tons of feedstock per day depending at least in part on the type of feedstock used. The type of feedstock can also impact plant storage requirements with plants designed primarily for processing feedstock whose availability varies seasonally (e.g., corn stover) requiring more on- or of-site feedstock storage than plants designed to process feedstock whose availability is relatively steady (e.g., waste paper).

PHYSICAL PREPARATION

[0070] In some cases, methods of processing begin with a physical preparation of the feedstock, e.g., size reduction of raw feedstock materials, such as by cutting, grinding, shearing or chopping. In some cases, loose feedstock (e.g., recycled paper, starchy materials, or switchgrass) is prepared by shearing or shredding. Screens and/or magnets can be used to remove oversized or undesirable objects such as, for example, rocks or nails from the feed stream.

[0071] Feed preparation systems can be configured to produce feed streams with specific characteristics such as, for example, specific maximum sizes, specific length-to-width, or specific surface areas ratios. As a part of feed preparation, the bulk density of feedstocks can be controlled (e.g., increased or decreased).

Size Reduction

[0072] In some embodiments, the material to be processed is in the form of a fibrous material that includes fibers provided by shearing a fiber source. For example, the shearing can be performed with a rotary knife cutter.

[0073] For example, and by reference to FIG. 2, a fiber source 210 is sheared, e.g., in a rotary knife cutter, to provide a first fibrous material 212. The first fibrous material 212 is passed through a first screen 214 having an average opening size of 1.59 mm or less (1/16 inch, 0.0625 inch) to provide a second fibrous material 216. If desired, fiber source can be cut prior to the shearing, e.g., with a shredder. For example, when a paper is used as the fiber source, the paper can be first cut into strips that are, e.g., 1/4- to 1/2-inch wide, using a shredder, e.g., a counter-rotating screw shredder, such as those manufactured by Munson (Utica, N.Y). As an alternative to shredding, the paper can be reduced in size by cutting to a desired size using a guillotine cutter. For example, the guillotine cutter can be used to cut the paper into sheets that are, e.g., 10 inches wide by 12 inches long.

[0074] In some embodiments, the shearing of fiber source and the passing of the resulting first fibrous material through first screen are performed concurrently. The shearing and the passing can also be performed in a batch-type process.

[0075] For example, a rotary knife cutter can be used to concurrently shear the fiber source and screen the first fibrous material. Referring to FIG. 3, a rotary knife cutter 220 includes a hopper 222 that can be loaded with a shredded fiber source 224 prepared by shredding fiber source. Shredded fiber source is sheared between stationary blades 230 and rotating blades 232 to provide a first fibrous material 240. First fibrous material 240 passes through screen 242, and the resulting second fibrous material 244 is captured in bin 250. To aid in the collection of the second fibrous material, the bin can have a pressure below nominal atmospheric pressure, e.g., at least 10 percent below nominal atmospheric pressure, e.g., at least 25 percent below nominal atmospheric pressure, at least 50 percent below nominal atmospheric pressure, or at least 75 percent below nominal atmospheric pressure. In some embodiments, a vacuum source 252 is utilized to maintain the bin below nominal atmospheric pressure.

[0076] Shearing can be advantageous for "opening up" and "stressing" the fibrous materials, making the cellulose of the materials more susceptible to chain scission and/or reduction of crystallinity. The open materials can also be more susceptible to oxidation when irradiated.

[0077] The fiber source can be sheared in a dry state, a hydrated state (e.g., having up to ten percent by weight absorbed water), or in a wet state, e.g., having between about 10 percent and about 75 percent by weight water. The fiber source can even be sheared while partially or fully submerged under a liquid, such as water, ethanol, isopropanol.

[0078] The fiber source can also be sheared in under a gas (such as a stream or atmosphere of gas other than air), e.g., oxygen or nitrogen, or steam.

[0079] Other methods of making the fibrous materials include, e.g., stone grinding, mechanical ripping or tearing, pin grinding or air attrition milling.

[0080] If desired, the fibrous materials can be separated, e.g., continuously or in batches, into fractions according to their length, width, density, material type, or some combination of these attributes.

[0081] For example, ferrous materials can be separated from any of the fibrous materials by passing a fibrous material that includes a ferrous material past a magnet, e.g., an electromagnet, and then passing the resulting fibrous material through a series of screens, each screen having different sized apertures.

[0082] The fibrous materials can also be separated, e.g., by using a high velocity gas, e.g., air. In such an approach, the fibrous materials are separated by drawing off different fractions, which can be characterized photonically, if desired. Such a separation apparatus is discussed in Lindsey et al, U.S. Patent No. 6,883,667.

[0083] The fibrous materials can irradiated immediately following their preparation, or they can may be dried, e.g., at approximately 105 °C for 4-18 hours, so that the moisture content is, e.g., less than about 0.5% before use.

[0084] If desired, lignin can be removed from any of the fibrous materials that include lignin. Also, to aid in the breakdown of the materials that include the cellulose, the material can be treated prior to irradiation with heat, a chemical (e.g., mineral acid, base or a strong oxidizer such as sodium hypochlorite) and/or an enzyme.

[0085] In some embodiments, the average opening size of the first screen is less than 0.79 mm (1/32 inch, 0.03125 inch), e.g., less than 0.51 mm (1/50 inch, 0.02000 inch), less than 0.40 mm (1/64 inch, 0.015625 inch), less than 0.23 mm (0.009 inch), less than 0.20 mm (1/128 inch, 0.0078125 inch), less than 0.18 mm (0.007 inch), less than 0.13 mm (0.005 inch), or even less than less than 0.10 mm (1/256 inch, 0.00390625 inch). The screen is prepared by interweaving monofilaments having an appropriate diameter to give the desired opening size. For example, the monofilaments can be made of a metal, e.g., stainless steel. As the opening sizes get smaller, structural demands on the monofilaments may become greater. For example, for opening sizes less than 0.40 mm, it can be advantageous to make the screens from monofilaments made from a material other than stainless steel, e.g., titanium, titanium alloys, amorphous metals, nickel, tungsten, rhodium, rhenium, ceramics, or glass. In some embodiments, the screen is made from a plate, e.g. a metal plate, having apertures, e.g., cut into the plate using a laser. In some embodiments, the open area of the mesh is less than 52%, e.g., less than 41%, less than 36%, less than 31%, less than 30%.

[0086] In some embodiments, the second fibrous is sheared and passed through the first screen, or a different sized screen. In some embodiments, the second fibrous material is passed through a second screen having an average opening size equal to or less than that of first screen.

[0087] Referring to FIG. 4, a third fibrous material 220 can be prepared from the second fibrous material 216 by shearing the second fibrous material 216 and passing the resulting material through a second screen 222 having an average opening size less than the first screen 214.

[0088] Generally, the fibers of the fibrous materials can have a relatively large average length-to-diameter ratio (e.g., greater than 20-to-1), even if they have been sheared more than once. In addition, the fibers of the fibrous materials described herein may have a relatively narrow length and/or length-to-diameter ratio distribution.

[0089] As used herein, average fiber widths (i.e., diameters) are those determined optically by randomly selecting approximately 5,000 fibers. Average fiber lengths are corrected length-weighted lengths. BET (Brunauer, Emmet and Teller) surface areas are multi-point surface areas, and porosities are those determined by mercury porosimetry.

[0090] The average length-to-diameter ratio of the second fibrous material 14 can be, e.g., greater than 8/1, e.g., greater than 10/1, greater than 15/1, greater than 20/1, greater than 25/1, or greater than 50/1. An average length of the second fibrous material 14 can be, e.g., between about 0.5 mm and 2.5 mm, e.g., between about 0.75 mm and 1.0 mm, and an average width (i.e., diameter) of the second fibrous material 14 can be, e.g., between about 5 μm and 50 μm, e.g., between about 10 μm and 30 μm.

[0091] In some embodiments, a standard deviation of the length of the second fibrous material 14 is less than 60 percent of an average length of the second fibrous material 14, e.g., less than 50 percent of the average length, less than 40 percent of the average length, less than 25 percent of the average length, less than 10 percent of the average length, less than 5 percent of the average length, or even less than 1 percent of the average length.

[0092] In some embodiments, a BET surface area of the second fibrous material is greater than 0.1 m^2/g, e.g., greater than 0.25 m^2/g, greater than 0.5 m^2/g, greater than 1.0 m^2/g, greater than 1.5 m^2/g, greater than 1.75 m^2/g, greater than 5.0 m^2/g, greater than 10 m^2/g, greater than 25 m^2/g, greater than 35 m^2/g, greater than 50m^2/g, greater than 60 m^2/g, greater than 75 m^2/g, greater than 100 m^2/g, greater than 150 m^2/g, greater than 200 m^2/g, or even greater than 250 m^2/g. A porosity of the second fibrous material 14 can be, e.g., greater than 20 percent, greater than 25 percent, greater than 35 percent, greater than 50 percent, greater than 60 percent, greater than 70 percent, e.g., greater than 80 percent, greater than 85 percent, greater than 90 percent, greater than 92 percent, greater than 94 percent, greater than 95 percent, greater than 97.5 percent, greater than 99 percent, or even greater than 99.5 percent.

[0093] In some embodiments, a ratio of the average length-to-diameter ratio of the first fibrous material to the average length-to-diameter ratio of the second fibrous material is, e.g., less than 1.5, e.g., less than 1.4, less than 1.25, less than 1.1, less than 1.075, less than 1.05, less than 1.025, or even substantially equal to 1.

[0094] In particular embodiments, the second fibrous material is sheared again and the resulting fibrous material passed through a second screen having an average opening size less than the first screen to provide a third fibrous material. In such instances, a ratio of the average length-to-diameter ratio of the second fibrous material to the average length-to-diameter ratio of the third fibrous material can be, e.g., less than 1.5, e.g., less than 1.4, less than 1.25, or

even less than 1.1.

[0095] In some embodiments, the third fibrous material is passed through a third screen to produce a fourth fibrous material. The fourth fibrous material can be, e.g., passed through a fourth screen to produce a fifth material. Similar screening processes can be repeated as many times as desired to produce the desired fibrous material having the desired properties.

Densification

[0096] Densified materials can be processed by any of the methods described herein, or any material described herein, e.g., any fibrous material described herein, can be processed by any one or more methods described herein, and then densified as described herein.

[0097] A material, e.g., a fibrous material, having a low bulk density can be densified to a product having a higher bulk density. For example, a material composition having a bulk density of 0.05 g/ cm^3 can be densified by sealing the fibrous material in a relatively gas impermeable structure, e.g., a bag made of polyethylene or a bag made of alternating layers of polyethylene and a nylon, and then evacuating the entrapped gas, e.g., air, from the structure. After evacuation of the air from the structure, the fibrous material can have, e.g., a bulk density of greater than 0.3 g/cm^3, e.g., 0.5 g/cm^3, 0.6 g/cm^3, 0.7 g/cm^3 or more, e.g., 0.85 g/ cm^3. After densification, the product can processed by any of the methods described herein, e.g., irradiated, e.g., with gamma radiation. This can be advantageous when it is desirable to transport the material to another location, e.g., a remote manufacturing plant, where the fibrous material composition can be added to a solution, e.g., to produce ethanol. After piercing the substantially gas impermeable structure, the densified fibrous material can revert to nearly its initial bulk density, e.g., greater than 60 percent of its initial bulk density, e.g., 70 percent, 80 percent, 85 percent or more, e.g., 95 percent of its initial bulk density. To reduce static electricity in the fibrous material, an anti-static agent can be added to the material.

[0098] In some embodiments, the structure, e.g., bag, is formed of a material that dissolves in a liquid, such as water. For example, the structure can be formed from a polyvinyl alcohol so that it dissolves when in contact with a water-based system. Such embodiments allow densified structures to be added directly to solutions that include a microorganism, without first releasing the contents of the structure, e.g., by cutting.

[0099] Referring to FIG. 5, a biomass material can be combined with any desired additives and a binder, and subsequently densified by application of pressure, e.g., by passing the material through a nip defined between counter-rotating pressure rolls or by passing the material through a pellet mill. During the application of pressure, heat can optionally be applied to aid in the densification of the fibrous material. The densified material can then be irradiated.

[0100] In some embodiments, the material prior to densification has a bulk density of less than 0.25 g/cm^3, e.g., 0.20 g/cm^3, 0.15 g/cm^3, 0.10 g/cm^3, 0.05 g/cm^3 or less, e.g., 0.025 g/cm^3. Bulk density is determined using ASTM D1895B. Briefly, the method involves filling a measuring cylinder of known volume with a sample and obtaining a weight of the sample. The bulk density is calculated by dividing the weight of the sample in grams by the known volume of the cylinder in cubic centimeters.

[0101] The preferred binders include binders that are soluble in water, swollen by water, or that has a glass transition temperature of less 25 °C, as determined by differential scanning calorimetry. By water-soluble binders, we mean binders having a solubility of at least about 0.05 weight percent in water. By water swellable binders, we mean binders that increase in volume by more than 0.5 percent upon exposure to water.

[0102] In some embodiments, the binders that are soluble or swollen by water include a functional group that is capable of forming a bond, e.g., a hydrogen bond, with the fibers of the fibrous material, e.g., cellulosic fibrous material. For example, the functional group can be a carboxylic acid group, a carboxylate group, a carbonyl group, e.g., of an aldehyde or a ketone, a sulfonic acid group, a sulfonate group, a phosphoric acid group, a phosphate group, an amide group, an amine group, a hydroxyl group, e.g., of an alcohol, and combinations of these groups, e.g., a carboxylic acid group and a hydroxyl group. Specific monomeric examples include glycerin, glyoxal, ascorbic acid, urea, glycine, pentaerythritol, a monosaccharide or a disaccharide, citric acid, and tartaric acid. Suitable saccharides include glucose, sucrose, lactose, ribose, fructose, mannose, arabinose and erythrose. Polymeric examples include polyglycols, polyethylene oxide, polycarboxylic acids, polyamides, polyamines and polysulfonic acids polysulfonates. Specific polymeric examples include polypropylene glycol (PPG), polyethylene glycol (PEG), polyethylene oxide, e.g., POLYOX®, copolymers of ethylene oxide and propylene oxide, polyacrylic acid (PAA), polyacrylamide, polypeptides, polyethylenimine, polyvinylpyridine, poly(sodium-4-styrenesulfonate) and poly(2-acrylamido-methyl-1-propanesulfonic acid).

[0103] In some embodiments, the binder includes a polymer that has a glass transition temperature less 25 °C. Examples of such polymers include thermoplastic elastomers (TPEs). Examples of TPEs include polyether block amides, such as those available under the tradename PEBAX®, polyester elastomers, such as those available under the tradename HYTREL®, and styrenic block copolymers, such as those available under the tradename KRATON®. Other suitable polymers having a glass transition temperature less 25 °C include ethylene vinyl acetate copolymer (EVA), polyolefins, e.g., polyethylene, polypropylene, ethylene-propylene copolymers, and copolymers of ethylene and alpha olefins, e.g.,

1-octene, such as those available under the tradename ENGAGE®. In some embodiments, e.g., when the material is a fiberized polycoated paper, the material is densified without the addition of a separate low glass transition temperature polymer.

[0104] In a particular embodiment, the binder is a lignin, e.g., a natural or synthetically modified lignin.

[0105] A suitable amount of binder added to the material, calculated on a dry weight basis, is, e.g., from about 0.01 percent to about 50 percent, e.g., 0.03 percent, 0.05 percent, 0.1 percent, 0.25 percent, 0.5 percent, 1.0 percent, 5 percent, 10 percent or more, e.g., 25 percent, based on a total weight of the densified material. The binder can be added to the material as a neat, pure liquid, as a liquid having the binder dissolved therein, as a dry powder of the binder, or as pellets of the binder.

[0106] The densified fibrous material can be made in a pellet mill. Referring to FIG. 6, a pellet mill 300 has a hopper 301 for holding undensified material 310 that includes a carbohydrate-containing material, such as cellulose. The hopper communicates with an auger 312 that is driven by variable speed motor 314 so that undensified material can be transported to a conditioner 320 that stirs the undensified material with paddles 322 that are rotated by conditioner motor 330. Other ingredients, e.g., any of the additives and/or fillers described herein, can be added at inlet 332. If desired, heat may be added while the fibrous material is in conditioner. After conditioned, the material passes from the conditioner through a dump chute 340, and to another auger 342. The dump chute, as controlled by actuator 344, allows for unobstructed passage of the material from conditioner to auger. Auger is rotated by motor 346, and controls the feeding of the fibrous material into die and roller assembly 350. Specifically, the material is introduced into a hollow, cylindrical die 352, which rotates about a horizontal axis and which has radially extending die holes 250. Die 352 is rotated about the axis by motor 360, which includes a horsepower gauge, indicating total power consumed by the motor. Densified material 370, e.g., in the form of pellets, drops from chute 372 and are captured and processed, such as by irradiation.

[0107] The material, after densification, can be conveniently in the form of pellets or chips having a variety of shapes. The pellets can then be irradiated. In some embodiments, the pellets or chips are cylindrical in shape, e.g., having a maximum transverse dimension of, e.g., 1 mm or more, e.g., 2 mm, 3 mm, 5 mm, 8 mm, 10 mm, 15 mm or more, e.g., 25 mm. Other convenient shapes include pellets or chips that are platelike in form, e.g., having a thickness of 1 mm or more, e.g., 2 mm, 3 mm, 5 mm, 8 mm, 10 mm or more, e.g., 25 mm; a width of, e.g., 5 mm or more, e.g., 10 mm, 15 mm, 25 mm, 30 mm or more, e.g., 50 mm; and a length of 5 mm or more, e.g., 10 mm, 15 mm, 25 mm, 30 mm or more, e.g., 50 mm.

[0108] Referring now FIG. 7A-7D, pellets can be made so that they have a hollow inside. As shown, the hollow can be generally in-line with the center of the pellet (FIG. 7B), or out of line with the center of the pellet (FIG. 7C). Making the pellet hollow inside can increase the rate of dissolution in a liquid after irradiation.

[0109] Referring now to FIG. 7D, the pellet can have, e.g., a transverse shape that is multi-lobal, e.g., tri-lobal as shown, or tetra-lobal, penta-lobal, hexa-lobal or deca-lobal. Making the pellets in such transverse shapes can also increase the rate of dissolution in a solution after irradiation.

[0110] Alternatively, the densified material can be in any other desired form, e.g., the densified material can be in the form of a mat, roll or bale.

Examples

[0111] In one example, half-gallon juice cartons made of un-printed white Kraft board having a bulk density of 20 lb/ft^3 can be used as a feedstock. Cartons can be folded flat and then fed into a shredder to produce a confetti-like material having a width of between 0.1 inch and 0.5 inch, a length of between 0.25 inch and 1 inch and a thickness equivalent to that of the starting material (about 0.075 inch). The confetti-like material can be fed to a rotary knife cutter, which shears the confetti-like pieces, tearing the pieces apart and releasing fibrous material.

[0112] In some cases, multiple shredder-shearer trains can be arranged in series with output. In one embodiment, two shredder-shearer trains can be arranged in series with output from the first shearer fed as input to the second shredder. In another embodiment, three shredder-shearer trains can be arranged in series with output from the first shearer fed as input to the second shredder and output from the second shearer fed as input to the third shredder. Multiple passes through shredder-shearer trains are anticipated to increase decrease particle size and increase overall surface area within the feedstream.

[0113] In another example, fibrous material produced from shredding and shearing juice cartons can be treated to increase its bulk density. In some cases, the fibrous material can be sprayed with water or a dilute stock solution of POLYOX™ WSR N10 (polyethylene oxide) prepared in water. The wetted fibrous material can then be processed through a pellet mill operating at room temperature. The pellet mill can increase the bulk density of the feedstream by more than an order of magnitude.

PRETREATMENT

Pretreatment Conditions

[0114] In some embodiments, the process does not include hydrolyzing the cellulosic and/or lignocellulosic material, such as with an acid or a base, e.g., a mineral acid, such as hydrochloric or sulfuric acid. If desired, some or none of the feedstock can include a hydrolyzed material. For example, in some embodiments, at least about seventy percent by weight of the feedstock is an unhydrolyzed material, e.g., at least at 95 percent by weight of the feedstock is an unhydrolyzed material. In some embodiments, substantially all of the feedstock is an unhydrolyzed material.

[0115] Any feedstock or any reactor or fermentor charged with a feedstock can include a buffer, such as sodium bicarbonate, ammonium chloride or Tris; an electrolyte, such as potassium chloride, sodium chloride, or calcium chloride; a growth factor, such as biotin and/or a base pair such as uracil or an equivalent thereof; a surfactant, such as Tween® or polyethylene glycol; a mineral, such as such as calcium, chromium, copper, iodine, iron, selenium, or zinc; or a chelating agent, such as ethylene diamine, ethylene diamine tetraacetic acid (EDTA) (or its salt form, e.g., sodium or potassium EDTA), or dimercaprol.

[0116] When radiation is utilized, it can be applied to any sample that is dry or wet, or even dispersed in a liquid, such as water. For example, irradiation can be performed on cellulosic and/or lignocellulosic material in which less than about 25 percent by weight of the cellulosic and/or lignocellulosic material has surfaces wetted with a liquid, such as water. In some embodiments, irradiating is performed on cellulosic and/or lignocellulosic material in which substantially none of the cellulosic and/or lignocellulosic material is wetted with a liquid, such as water.

[0117] In some embodiments, any processing described herein occurs after the cellulosic and/or lignocellulosic material remains dry as acquired or has been dried, e.g., using heat and/or reduced pressure. For example, in some embodiments, the cellulosic and/or lignocellulosic material has less than about five percent by weight retained water, measured at 25°C and at fifty percent relative humidity.

[0118] If desired, a swelling agent, as defined herein, can be utilized in any process described herein. In some embodiments, when a cellulosic and/or lignocellulosic material is processed using radiation, less than about 25 percent by weight of the cellulosic and/or lignocellulosic material is in a swollen state, the swollen state being characterized as having a volume of more than about 2.5 percent higher than an unswollen state, e.g., more than 5.0, 7.5, 10, or 15 percent higher than the unswollen state. In some embodiments, when radiation is utilized on a cellulosic and/or lignocellulosic material, substantially none of the cellulosic and/or lignocellulosic material is in a swollen state. In specific embodiments when radiation is utilized, the cellulosic and/or lignocellulosic material includes a swelling agent, and swollen cellulosic and/or lignocellulosic receives a dose of less than about 10 Mrad.

[0119] When radiation is utilized in any process, it can be applied while the cellulosic and/or lignocellulosic is exposed to air, oxygen-enriched air, or even oxygen itself, or blanketed by an inert gas such as nitrogen, argon, or helium. When maximum oxidation is desired, an oxidizing environment is utilized, such as air or oxygen.

[0120] When radiation is utilized, it may be applied to cellulosic and/or lignocellulosic material, under a pressure of greater than about 2.5 atmospheres, such as greater than 5, 10, 15, 20 or even greater than about 50 atmospheres.

Radiation Treatment

[0121] Irradiation can reduce the molecular weight and/or crystallinity of feedstock. In some embodiments, energy deposited in a material that releases an electron from its atomic orbital is used to irradiate the materials. The radiation may be provided by 1) heavy charged particles, such as alpha particles or protons, 2) electrons, produced, for example, in beta decay or electron beam accelerators, or 3) electromagnetic radiation, for example, gamma rays, x rays, or ultraviolet rays. In one approach, radiation produced by radioactive substances can be used to irradiate the feedstock. In some embodiments, any combination in any order or concurrently of (1) through (3) may be utilized. In another approach, electromagnetic radiation (e.g., produced using electron beam emitters) can be used to irradiate the feedstock. The doses applied depend on the desired effect and the particular feedstock. For example, high doses of radiation can break chemical bonds within feedstock components and low doses of radiation can increase chemical bonding (e.g., cross-linking) within feedstock components. In some instances when chain scission is desirable and/or polymer chain functionalization is desirable, particles heavier than electrons, such as protons, helium nuclei, argon ions, silicon ions, neon ions, carbon ions, phosphorus ions, oxygen ions or nitrogen ions can be utilized. When ring-opening chain scission is desired, positively charged particles can be utilized for their Lewis acid properties for enhanced ring-opening chain scission. For example, when oxygen-containing functional groups are desired, irradiation in the presence of oxygen or even irradiation with oxygen ions can be performed. For example, when nitrogen-containing functional groups are desirable, irradiation in the presence of nitrogen or even irradiation with nitrogen ions can be performed.

[0122] Referring to FIG. 8, in one reference method, a first material 2 that is or includes cellulose having a first number average molecular weight ($^{T}M_{N1}$) is irradiated, e.g., by treatment with ionizing radiation (e.g., in the form of gamma

radiation, X-ray radiation, 100 nm to 280 nm ultraviolet (UV) light, a beam of electrons or other charged particles) to provide a second material 3 that includes cellulose having a second number average molecular weight ($^{T}M_{N2}$) lower than the first number average molecular weight. The second material (or the first and second material) can be combined with a microorganism (e.g., a bacterium or a yeast) that can utilize the second and/or first material to produce a fuel that is or includes hydrogen, an alcohol (e.g., ethanol or butanol, such as n-, sec- or t-butanol), an organic acid, a hydrocarbon or mixtures of any of these.

[0123] Since the second material 3 has cellulose having a reduced molecular weight relative to the first material, and in some instances, a reduced crystallinity as well, the second material is generally more dispersible, swellable and/or soluble in a solution containing a microorganism. These properties make the second material 3 more susceptible to chemical, enzymatic and/or biological attack relative to the first material 2, which can greatly improve the production rate and/or production level of a desired product, e.g., ethanol. Radiation can also sterilize the materials.

[0124] In some embodiments, the second number average molecular weight (M_{N2}) is lower than the first number average molecular weight ($^{T}M_{N1}$) by more than about 10 percent, e.g., 15, 20, 25, 30, 35, 40, 50 percent, 60 percent, or even more than about 75 percent.

[0125] In some instances, the second material has cellulose that has as crystallinity ($^{T}C_{2}$) that is lower than the crystallinity ($^{T}C_{1}$) of the cellulose of the first material. For example, ($^{T}C_{2}$) can be lower than ($^{T}C_{1}$) by more than about 10 percent, e.g., 15, 20, 25, 30, 35, 40, or even more than about 50 percent.

[0126] In some embodiments, the starting crystallinity index (prior to irradiation) is from about 40 to about 87.5 percent, e.g., from about 50 to about 75 percent or from about 60 to about 70 percent, and the crystallinity index after irradiation is from about 10 to about 50 percent, e.g., from about 15 to about 45 percent or from about 20 to about 40 percent. However, in some embodiments, e.g., after extensive irradiation, it is possible to have a crystallinity index of lower than 5 percent. In some embodiments, the material after irradiation is substantially amorphous.

[0127] In some embodiments, the starting number average molecular weight (prior to irradiation) is from about 200,000 to about 3,200,000, e.g., from about 250,000 to about 1,000,000 or from about 250,000 to about 700,000, and the number average molecular weight after irradiation is from about 50,000 to about 200,000, e.g., from about 60,000 to about 150,000 or from about 70,000 to about 125,000. However, in some embodiments, e.g., after extensive irradiation, it is possible to have a number average molecular weight of less than about 10,000 or even less than about 5,000.

[0128] In some embodiments, the second material can have a level of oxidation ($^{T}O_{2}$) that is higher than the level of oxidation ($^{T}O_{1}$) of the first material. A higher level of oxidation of the material can aid in its dispersibility, swellability and/or solubility, further enhancing the materials susceptibility to chemical, enzymatic or biological attack. In some embodiments, to increase the level of the oxidation of the second material relative to the first material, the irradiation is performed under an oxidizing environment, e.g., under a blanket of air or oxygen, producing a second material that is more oxidized than the first material. For example, the second material can have more hydroxyl groups, aldehyde groups, ketone groups, ester groups or carboxylic acid groups, which can increase its hydrophilicity.

Ionizing Radiation

[0129] Each form of radiation ionizes the biomass via particular interactions, as determined by the energy of the radiation. Heavy charged particles primarily ionize matter via Coulomb scattering; furthermore, these interactions produce energetic electrons that may further ionize matter. Alpha particles are identical to the nucleus of a helium atom and are produced by the alpha decay of various radioactive nuclei, such as isotopes of bismuth, polonium, astatine, radon, francium, radium, several actinides, such as actinium, thorium, uranium, neptunium, curium, californium, americium, and plutonium.

[0130] When particles are utilized, they can be neutral (uncharged), positively charged or negatively charged. When charged, the charged particles can bear a single positive or negative charge, or multiple charges, e.g., one, two, three or even four or more charges. In instances in which chain scission is desired, positively charged particles may be desirable, in part, due to their acidic nature. When particles are utilized, the particles can have the mass of a resting electron, or greater, e.g., 500, 1000, 1500, or 2000 or more times the mass of a resting electron. For example, the particles can have a mass of from about 1 atomic unit to about 150 atomic units, e.g., from about 1 atomic unit to about 50 atomic units, or from about 1 to about 25, e.g., 1, 2, 3, 4, 5, 10, 12 or 15 amu. Accelerators used to accelerate the particles can be electrostatic DC, electrodynamic DC, RF linear, magnetic induction linear or continuous wave. For example, cyclotron type accelerators are available from IBA, Belgium, such as the Rhodotron® system, while DC type accelerators are available from RDI, now IBA Industrial, such as the Dynamitron®. Ions and ion accelerators are discussed in Introductory Nuclear Physics, Kenneth S. Krane, John Wiley & Sons, Inc. (1988), Krsto Prelec, FIZIKA B 6 (1997) 4, 177-206, Chu, William T., "Overview of Light-Ion Beam Therapy", Columbus-Ohio, ICRU-IAEA Meeting, 18-20 March 2006, Iwata, Y. et al., "Alternating-Phase-Focused IH-DTL for Heavy-Ion Medical Accelerators", Proceedings of EPAC 2006, Edinburgh, Scotland, and Leitner, C.M. et al., "Status of the Superconducting ECR Ion Source Venus", Proceedings of EPAC 2000, Vienna, Austria.

[0131] Electrons interact via Coulomb scattering and bremsstrahlung radiation produced by changes in the velocity of electrons. Electrons may be produced by radioactive nuclei that undergo beta decay, such as isotopes of iodine, cesium, technetium, and iridium. Alternatively, an electron gun can be used as an electron source via thermionic emission.

[0132] Electromagnetic radiation interacts via three processes: photoelectric absorption, Compton scattering, and pair production. The dominating interaction is determined by the energy of the incident radiation and the atomic number of the material. The summation of interactions contributing to the absorbed radiation in cellulosic material can be expressed by the mass absorption coefficient.

[0133] Electromagnetic radiation is subclassified as gamma rays, x rays, ultraviolet rays, infrared rays, microwaves, or radio waves, depending on its wavelength.

[0134] For example, gamma radiation can be employed to irradiate the materials. Referring to FIGS. 9 and 10 (an enlarged view of region R), a gamma irradiator 10 includes gamma radiation sources 408, e.g., ^{60}Co pellets, a working table 14 for holding the materials to be irradiated and storage 16, e.g., made of a plurality iron plates, all of which are housed in a concrete containment chamber (vault) 20 that includes a maze entranceway 22 beyond a lead-lined door 26. Storage 16 includes a plurality of channels 30, e.g., sixteen or more channels, allowing the gamma radiation sources to pass through storage on their way proximate the working table.

[0135] In operation, the sample to be irradiated is placed on a working table. The irradiator is configured to deliver the desired dose rate and monitoring equipment is connected to an experimental block 31. The operator then leaves the containment chamber, passing through the maze entranceway and through the lead-lined door. The operator mans a control panel 32, instructing a computer 33 to lift the radiation sources 12 into working position using cylinder 36 attached to a hydraulic pump 40.

[0136] Gamma radiation has the advantage of a significant penetration depth into a variety of material in the sample. Sources of gamma rays include radioactive nuclei, such as isotopes of cobalt, calcium, technicium, chromium, gallium, indium, iodine, iron, krypton, samarium, selenium, sodium, thalium, and xenon.

[0137] Sources of x rays include electron beam collision with metal targets, such as tungsten or molybdenum or alloys, or compact light sources, such as those produced commercially by Lyncean.

[0138] Sources for ultraviolet radiation include deuterium or cadmium lamps.

[0139] Sources for infrared radiation include sapphire, zinc, or selenide window ceramic lamps.

[0140] Sources for microwaves include klystrons, Slevin type RF sources, or atom beam sources that employ hydrogen, oxygen, or nitrogen gases.

[0141] Various other irradiating devices may be used in the methods disclosed herein, including field ionization sources, electrostatic ion separators, field ionization generators, thermionic emission sources, microwave discharge ion sources, recirculating or static accelerators, dynamic linear accelerators, van de Graaff accelerators, and folded tandem accelerators. Such devices are disclosed, for example, in U.S. Provisional Application Serial No. 61/073,665.

Electron Beam

[0142] In some embodiments, a beam of electrons is used as the radiation source. A beam of electrons has the advantages of high dose rates (e.g., 1, 5, or even 10 Mrad per second), high throughput, less containment, and less confinement equipment. Electrons can also be more efficient at causing chain scission. In addition, electrons having energies of 4-10 MeV can have a penetration depth of 5 to 30 mm or more, such as 40 mm.

[0143] Electron beams can be generated, e.g., by electrostatic generators, cascade generators, transformer generators, low energy accelerators with a scanning system, low energy accelerators with a linear cathode, linear accelerators, and pulsed accelerators. Electrons as an ionizing radiation source can be useful, e.g., for relatively thin piles of materials, e.g., less than 0.5 inch, e.g., less than 0.4 inch, 0.3 inch, 0.2 inch, or less than 0.1 inch. In some embodiments, the energy of each electron of the electron beam is from about 0.3 MeV to about 2.0 MeV (million electron volts), e.g., from about 0.5 MeV to about 1.5 MeV, or from about 0.7 MeV to about 1.25 MeV.

[0144] FIG. 11A shows a process flow diagram 3000 that includes various steps in an electron beam irradiation feedstock pretreatment sequence. In first step 3010, a supply of dry feedstock is received from a feed source. As discussed above, the dry feedstock from the feed source may be pre-processed prior to delivery to the electron beam irradiation devices. For example, if the feedstock is derived from plant sources, certain portions of the plant material may be removed prior to collection of the plant material and/or before the plant material is delivered by the feedstock transport device. Alternatively, or in addition, as expressed in optional step 3020, the biomass feedstock can be subjected to mechanical processing (e.g., to reduce the average length of fibers in the feedstock) prior to delivery to the electron beam irradiation devices.

[0145] In step 3030, the dry feedstock is transferred to a feedstock transport device (e.g., a conveyor belt) and is distributed over the cross-sectional width of the feedstock transport device approximately uniformly by volume. This can be accomplished, for example, manually or by inducing a localized vibration motion at some point in the feedstock transport device prior to the electron beam irradiation processing.

[0146] In some embodiments, a mixing system introduces a chemical agent 3045 into the feedstock in an optional process 3040 that produces a slurry. Combining water with the processed feedstock in mixing step 3040 creates an aqueous feedstock slurry that may be transported through, for example, piping rather than using, for example, a conveyor belt.

[0147] The next step 3050 is a loop that encompasses exposing the feedstock (in dry or slurry form) to electron beam radiation via one or more (say, N) electron beam irradiation devices. The feedstock slurry is moved through each of the N "showers" of electron beams at step 3052. The movement may either be at a continuous speed through and between the showers, or there may be a pause through each shower, followed by a sudden movement to the next shower. A small slice of the feedstock slurry is exposed to each shower for some predetermined exposure time at step 3053.

[0148] Electron beam irradiation devices may be procured commercially from Ion Beam Applications, Louvain-la-Neuve, Belgium or the Titan Corporation, San Diego, CA. Typical electron energies can be 1 MeV, 2 MeV, 4.5 MeV, 7.5 MeV, or 10 MeV. Typical electron beam irradiation device power can be 1 kW, 5 kW, 10 kW, 20 kW, 50 kW, 100 kW, 250 kW, or 500 kW. Effectiveness of depolymerization of the feedstock slurry depends on the electron energy used and the dose applied, while exposure time depends on the power and dose. Typical doses may take values of 1 kGy, 5 kGy, 10 kGy, 20 kGy, 50 kGy, 100 kGy, or 200 kGy.

[0149] Tradeoffs in considering electron beam irradiation device power specifications include cost to operate, capital costs, depreciation, and device footprint. Tradeoffs in considering exposure dose levels of electron beam irradiation would be energy costs and environment, safety, and health (ESH) concerns. Tradeoffs in considering electron energies include energy costs; here, a lower electron energy may be advantageous in encouraging depolymerization of certain feedstock slurry (see, for example, Bouchard, et al, Cellulose (2006) 13: 601-610). Typically, generators are housed in a vault, e.g., of lead or concrete or lead-lined concrete.

[0150] It may be advantageous to provide a double-pass of electron beam irradiation in order to provide a more effective depolymerization process. For example, the feedstock transport device could direct the feedstock (in dry or slurry form) underneath and in a reverse direction to its initial transport direction. Double-pass systems can allow thicker feedstock slurries to be processed and can provide a more uniform depolymerization through the thickness of the feedstock slurry.

[0151] The electron beam irradiation device can produce either a fixed beam or a scanning beam. A scanning beam may be advantageous with large scan sweep length and high scan speeds, as this would effectively replace a large, fixed beam width. Further, available sweep widths of 0.5 m, 1m, 2 m or more are available.

[0152] Once a portion of feedstock slurry has been transported through the N electron beam irradiation devices, it may be necessary in some embodiments, as in step 3060, to mechanically separate the liquid and solid components of the feedstock slurry. In these embodiments, a liquid portion of the feedstock slurry is filtered for residual solid particles and recycled back to the slurry preparation step 3040. A solid portion of the feedstock slurry is then advanced on to the next processing step 3070 via the feedstock transport device. In other embodiments, the feedstock is maintained in slurry form for further processing.

Electromagnetic Radiation

[0153] In embodiments in which the irradiating is performed with electromagnetic radiation, the electromagnetic radiation can have, e.g., energy per photon (in electron volts) of greater than 10^2 eV, e.g., greater than 10^3, 10^4, 10^5, 10^6, or even greater than 10^7 eV. In some embodiments, the electromagnetic radiation has energy per photon of between 10^4 and 10^7, e.g., between 10^5 and 10^6 eV. The electromagnetic radiation can have a frequency of, e.g., greater than 10^{16} hz, greater than 10^{17} hz, 10^{18}, 10^{19}, 10^{20}, or even greater than 10^{21} hz. In some embodiments, the electromagnetic radiation has a frequency of between 10^{18} and 10^{22} Hz, e.g., between 10^{19} to 10^{21} Hz.

Doses

[0154] The irradiating (with any radiation source or a combination of sources) is performed until the material receives a dose of at least 5.0 Mrad or at least 10.0 Mrad. In some embodiments, the irradiating is performed at a dose rate of between 5.0 and 1500.0 kilorads/hour, e.g., between 10.0 and 750.0 kilorads/hour or between 50.0 and 350.0 kilorads/hours.

[0155] In some embodiments, two or more radiation sources are used, such as two or more ionizing radiations. For example, samples can be treated, in any order, with a beam of electrons, followed by gamma radiation and UV light having wavelengths from about 100 nm to about 280 nm. In some embodiments, samples are treated with three ionizing radiation sources, such as a beam of electrons, gamma radiation, and energetic UV light.

[0156] Alternatively, in another example, a fibrous material that includes a cellulosic and/or lignocellulosic material is irradiated and, optionally, treated with acoustic energy, e.g., ultrasound.

[0157] In one example of the use of radiation as a pretreatment, half-gallon juice cartons made of un-printed polycoated white Kraft board having a bulk density of 20 lb/ft^3 are used as a feedstock. Cartons are folded flat and then fed into a

sequence of three shredder-shearer trains arranged in series with output from the first shearer fed as input to the second shredder, and output from the second shearer fed as input to the third shredder. The fibrous material produced by the shredder-shearer train can be sprayed with water and processed through a pellet mill operating at room temperature. The densified pellets can be placed in a glass ampoule, which is evacuated under high vacuum and then backfilled with argon gas. The ampoule is sealed under argon. Alternatively, in another example, the ampoule is sealed under an atmosphere of air. The pellets in the ampoule are irradiated with gamma radiation for about 3 hours at a dose rate of about 1 Mrad per hour to provide an irradiated material in which the cellulose has a lower molecular weight than the starting material.

Additives to Enhance Molecular Weight Breakdown During Irradiation

[0158] In some embodiments, prior to irradiation, various materials, e.g., solids or liquids, can be added to the biomass to enhance molecular weight reduction. In those instances in which a liquid is utilized, the liquid can be in contact with outer surfaces of the biomass and/or the liquid can be in interior portions of the biomass, e.g., infused into the biomass.

[0159] For example, the material can be a neutral weak base, such as alanine, ammonia, ammonia/water mixture, e.g., 25 percent by weight ammonia in water, water, methyl amine, dimethyl amine, trimethyl amine, pyridine, or a anionic base, such as a salt of acetic acid (e.g., sodium acetate), sodium carbonate, sodium bicarbonate or a salt of an ion of hydrogen sulfide (e.g., sodium hydrosulfide).

[0160] Alternatively, the material can be a neutral weak acid, such as formic acid, acetic acid, trichloroacetic acid, water, hydrogen sulfide or a cationic acid, such as an ammonium salt.

Quenching and Controlled Functionalization of Biomass

[0161] After treatment with one or more ionizing radiations, such as photonic radiation (e.g., X-rays or gamma-rays), e-beam radiation or particles heavier than electrons that are positively or negatively charged (e.g., protons or carbon ions), any of the carbohydrate-containing materials or mixtures described herein become ionized; that is, they include radicals at levels that are detectable with an electron spin resonance spectrometer. The current practical limit of detection of the radicals is about 1014 spin at room temperature. After ionization, any biomass material that has been ionized can be to reduce the level of radicals in the ionized biomass, e.g., such that the radicals are no longer detectable with the electron spin resonance spectrometer. For example, the radicals can be quenched by the application of a sufficient pressure to the biomass and/or by utilizing a fluid in contact with the ionized biomass, such as a gas or liquid, that reacts with (quenches) the radicals. The use of a gas or liquid to at least aid in the quenching of the radicals also allows the operator to control functionalization of the ionized biomass with a desired amount and kind of functional groups, such as carboxylic acid groups, enol groups, aldehyde groups, nitro groups, nitrile groups, amino groups, alkyl amino groups, alkyl groups, chloroalkyl groups or chlorofluoroalkyl groups. In some instances, such quenching can improve the stability of some of the ionized biomass materials. For example, quenching can improve the resistance of the biomass to oxidation. Functionalization by quenching can also improve the solubility of any biomass described herein, can improve its thermal stability, which can be important in the manufacture of composites and boards described herein, and can improve material utilization by various microorganisms. For example, the functional groups imparted to the biomass material by quenching can act as receptor sites for attachment by microorganisms, e.g., to enhance cellulose hydrolysis by various microorganisms.

[0162] FIG. 11B illustrates changing a molecular and/or a supramolecular structure of a biomass feedstock by pretreating the biomass feedstock with ionizing radiation, such as with electrons or ions of sufficient energy to ionize the biomass feedstock, to provide a first level of radicals. As shown in FIG. 11B, if the ionized biomass remains in the atmosphere, it will be oxidized, such as to an extent that carboxylic acid groups are generated by reacting with the atmospheric oxygen. In some instances with some materials, such oxidation is desired because it can aid in the further breakdown in molecular weight of the carbohydrate-containing biomass, and the oxidation groups, e.g., carboxylic acid groups can be helpful for solubility and microorganism utilization in some instances. However, since the radicals can "live" for some time after irradiation, e.g., longer than 1 day, 5 days, 30 days, 3 months, 6 months or even longer than 1 year, material properties can continue to change over time, which in some instances, can be undesirable. Detecting radicals in irradiated samples by electron spin resonance spectroscopy and radical lifetimes in such samples is discussed in Bartolotta et al., Physics in Medicine and Biology, 46 (2001), 461-471 and in Bartolotta et al., Radiation Protection Dosimetry, Vol. 84, Nos. 1-4, pp. 293-296 (1999). As shown in FIG. 11B, the ionized biomass can be quenched to functionalize and/or to stabilize the ionized biomass. At any point, e.g., when the material is "alive", "partially alive" or fully quenched, the pretreated biomass can be converted into a product, e.g., a fuel, a food, or a composite.

[0163] In some embodiments, the quenching includes an application of pressure to the biomass, such as by mechanically deforming the biomass, e.g., directly mechanically compressing the biomass in one, two, or three dimensions, or applying pressure to a fluid in which the biomass is immersed, e.g., isostatic pressing. In such instances, the deformation

of the material itself brings radicals, which are often trapped in crystalline domains, in sufficient proximity so that the radicals can recombine, or react with another group. In some instances, the pressure is applied together with the application of heat, such as a sufficient quantity of heat to elevate the temperature of the biomass to above a melting point or softening point of a component of the biomass, such as lignin, cellulose or hemicellulose. Heat can improve molecular mobility in the polymeric material, which can aid in the quenching of the radicals. When pressure is utilized to quench, the pressure can be greater than about 1000 psi, such as greater than about 1250 psi, 1450 psi, 3625 psi, 5075 psi, 7250 psi, 10000 psi or even greater than 15000 psi.

[0164] In some embodiments, quenching includes contacting the biomass with a fluid, such as a liquid or gas, e.g., a gas capable of reacting with the radicals, such as acetylene or a mixture of acetylene in nitrogen, ethylene, chlorinated ethylenes or chlorofluoroethylenes, propylene or mixtures of these gases. In other particular embodiments, quenching includes contacting the biomass with a liquid, e.g., a liquid soluble in, or at least capable of penetrating into the biomass and reacting with the radicals, such as a diene, such as 1,5-cyclooctadiene. In some specific embodiments, the quenching includes contacting the biomass with an antioxidant, such as Vitamin E. If desired, the biomass feedstock can include an antioxidant dispersed therein, and the quenching can come from contacting the antioxidant dispersed in the biomass feedstock with the radicals.

[0165] Other methods for quenching are possible. For example, any method for quenching radicals in polymeric materials described in Muratoglu et al., U.S. Patent Application Publication No. 2008/0067724 and Muratoglu et al., U.S. Patent No. 7,166,650, can be utilized for quenching any ionized biomass material described herein. Furthermore any quenching agent (described as a "sensitizing agent" in the above-noted Muratoglu disclosures) and/or any antioxidant described in either Muratoglu reference can be utilized to quench any ionized biomass material.

[0166] Functionalization can be enhanced by utilizing heavy charged ions, such as any of the heavier ions described herein. For example, if it is desired to enhance oxidation, charged oxygen ions can be utilized for the irradiation. If nitrogen functional groups are desired, nitrogen ions or ions that includes nitrogen can be utilized. Likewise, if sulfur or phosphorus groups are desired, sulfur or phosphorus ions can be used in the irradiation.

[0167] In some embodiments, after quenching any of the quenched materials described herein can be further treated with one or more of radiation, such as ionizing or non-ionizing radiation, sonication, pyrolysis, and oxidation for additional molecular and/or supramolecular structure change.

In particular embodiments, functionalized materials described herein are treated with an acid, base, nucleophile or Lewis acid for additional molecular and/or supramolecular structure change, such as additional molecular weight breakdown. Examples of acids include organic acids, such as acetic acid and mineral acids, such as hydrochloric, sulfuric and/or nitric acid. Examples of bases include strong mineral bases, such as a source of hydroxide ion, basic ions, such as fluoride ion, or weaker organic bases, such as amines. Even water and sodium bicarbonate, e.g., when dissolved in water, can effect molecular and/or supramolecular structure change, such as additional molecular weight breakdown.

Particle Beam Exposure in Fluids

[0168] In some cases, the cellulosic or lignocellulosic materials can be exposed to a particle beam in the presence of one or more additional fluids (e.g., gases and/or liquids). Exposure of a material to a particle beam in the presence of one or more additional fluids can increase the efficiency of the treatment.

[0169] In some embodiments, the material is exposed to a particle beam in the presence of a fluid such as air. Particles accelerated in any one or more of the types of accelerators disclosed herein (or another type of accelerator) are coupled out of the accelerator via an output port (e.g., a thin membrane such as a metal foil), pass through a volume of space occupied by the fluid, and are then incident on the material. In addition to directly treating the material, some of the particles generate additional chemical species by interacting with fluid particles (e.g., ions and/or radicals generated from various constituents of air, such as ozone and oxides of nitrogen). These generated chemical species can also interact with the material, and can act as initiators for a variety of different chemical bond-breaking reactions in the material. For example, any oxidant produced can oxidize the material, which can result in molecular weight reduction.

[0170] In certain embodiments, additional fluids can be selectively introduced into the path of a particle beam before the beam is incident on the material. As discussed above, reactions between the particles of the beam and the particles of the introduced fluids can generate additional chemical species, which react with the material and can assist in functionalizing the material, and/or otherwise selectively altering certain properties of the material. The one or more additional fluids can be directed into the path of the beam from a supply tube, for example. The direction and flow rate of the fluid(s) that is/are introduced can be selected according to a desired exposure rate and/or direction to control the efficiency of the overall treatment, including effects that result from both particle-based treatment and effects that are due to the interaction of dynamically generated species from the introduced fluid with the material. In addition to air, exemplary fluids that can be introduced into the ion beam include oxygen, nitrogen, one or more noble gases, one or more halogens, and hydrogen.

Irradiating Low Bulk Density Biomass Materials and Cooling Irradiated Biomass

[0171] During treatment of biomass materials with ionizing radiation, especially at high dose rates, such as at rates greater then 0.15 Mrad per second, e.g., 0.25 Mrad/s, 0.35 Mrad/s, 0.5 Mrad/s, 0.75 Mrad/s or even greater than 1 Mrad/sec, biomass materials can retain significant quantities of heat so that the temperature of the biomass materials becomes elevated. While higher temperatures can, in some embodiments, be advantageous, e.g., when a faster reaction rate is desired, it is advantageous to control the heating of the biomass to retain control over the chemical reactions initiated by the ionizing radiation, such as cross-linking, chain, scission and/or grafting, e.g., to maintain process control. Low bulk density materials, such as those having a bulk density of less than about 0.4 g/cm^3, e.g., less than about 0.35, 0.25 or less about 0.15 g/cm^3, especially when combined with materials that have thin cross-sections, such as fibers having small transverse dimensions, are generally easier to cool. In addition, photons and particles can generally penetrate further into and through materials having a relatively low bulk density, which can allow for the processing of larger volumes of materials at higher rates, and can allow for the use of photons and particles that having lower energies, e.g., 0.25 Mev, 0.5 MeV, 0.75 MeV or 1.0 MeV, which can reduce safety shielding requirements.

For example, in one method of changing a molecular and/or a supramolecular structure of a biomass feedstock, the biomass is pretreated at a first temperature with ionizing radiation, such as photons, electrons or ions (e.g., singularly or multiply charged cations or anions), for a sufficient time and/or a sufficient dose to elevate the biomass feedstock to a second temperature higher than the first temperature. The pretreated biomass is then cooled to a third temperature below the second temperature. Finally, if desired, the cooled biomass can be treated one or more times with radiation, e.g., with ionizing radiation. If desired, cooling can be applied to the biomass after and/or during each radiation treatment.

[0172] The biomass feedstock can be physically prepared as discussed above, e.g., by reducing one or more dimensions of individual pieces of the biomass feedstock so that the feedstock can be more efficiently processed, e.g., more easily cooled and/or more easily penetrated by an ionizing radiation.

[0173] In some implementations, the ionizing radiation is applied at a total dose of less than 25 Mrad or less than 10 Mrad, such as less than 5 Mrad or less than 2.5 Mrad, and at a rate of more than 0.25 Mrad per second, such as more than 0.5, 0.75 or greater than 1.0 Mrad/s, prior to cooling the biomass.

[0174] The pretreating of the biomass feedstock with ionizing radiation can be performed as the biomass feedstock is being pneumatically conveyed in a fluid, such as a in a gas, e.g., nitrogen or air. To aid in molecular weight breakdown and/or functionalization of the materials, the gas can be saturated with any swelling agent described herein and/or water vapor. For example, acidic water vapor can be utilized. To aid in molecular weight breakdown, the water can be acidified with an organic acid, such as formic, or acetic acid, or a mineral acid, such as sulfuric or hydrochloric acid.

[0175] The pretreating of the biomass feedstock with ionizing radiation can be performed as the biomass feedstock falls under the influence of gravity. This procedure can effectively reduce the bulk density of the biomass feedstock as it is being processed and can aid in the cooling of the biomass feedstock. For example, the biomass can be conveyed from a first belt at a first height above the ground and then can be captured by a second belt at a second level above the ground lower than the first level. For example, in some embodiments, the trailing edge of the first belt and the leading edge of the second belt define a gap. Advantageously, the ionizing radiation, such as a beam of electrons, protons, or other ions, can be applied at the gap to prevent damage to the biomass conveyance system.

[0176] Cooling of the biomass can include contacting the biomass with a fluid, such as a gas, at a temperature below the first or second temperature, such as gaseous nitrogen at or about 77 K. Even water, such as water at a temperature below nominal room temperature (e.g., 25 degrees Celsius) can be utilized.

[0177] Often advantageously, the biomass feedstock has internal fibers, and prior to irradiation with the ionizing radiation, the biomass feedstock has been sheared to an extent that its internal fibers are substantially exposed. This shearing can provide a low bulk density material having small cross-sectional dimensions, which can aid in the breakdown and/or functionalization of the biomass. For example, in some embodiments, the biomass is or includes discrete fibers and/or particles having a maximum dimension of not more than about 0.5 mm, such as not more than about 0.25 mm, not more than about 0.1 mm or not more than about 0.05 mm.

[0178] In some embodiments, the biomass feedstock to which the ionizing radiation is applied has a bulk density of less than about 0.35 g/cm^3, such as less than about 0.3, 0.25, 0.20, or less than about 0.15 g/cm^3 during the application of the ionizing radiation. In such embodiments, the biomass feedstock can be cooled, and then ionizing radiation can be applied to the cooled biomass. In some advantageous embodiments, the biomass feedstock is or includes discrete fibers and/or particles having a maximum dimension of not more than about 0.5 mm, such as not more than about 0.25 mm, not more than about 0.1 mm, not more than about 0.05 mm, or not more than about 0.025 mm.

Combined Irradiating, Pyrolyzing, Sonicating, and/or Oxidizing Devices

[0179] In some embodiments, it may be advantageous to combine two or more separate irradiation, sonication, pyrolyzation, and/or oxidation devices into a single hybrid machine. Using such a hybrid machine, multiple processes may

be performed in close juxtaposition or even simultaneously, with the benefit of increasing pretreatment throughput and potential cost savings.

[0180] For example, consider the electron beam irradiation and sonication processes. Each separate process is effective in lowering the mean molecular weight of cellulosic material by an order of magnitude or more, and by several orders of magnitude when performed serially.

[0181] Both irradiation and sonication processes can be applied using a hybrid electron beam/sonication device as is illustrated in FIG. 25. Hybrid electron beam/sonication device 2500 is pictured above a shallow pool (depth ~ 3-5 cm) of a slurry of cellulosic material 2550 dispersed in an aqueous, oxidant medium, such as hydrogen peroxide or carbamide peroxide. Hybrid device 2500 has an energy source 2510, which powers both electron beam emitter 2540 and sonication horns 2530.

[0182] Electron beam emitter 2540 generates electron beams, which pass though an electron beam aiming device 2545 to impact the slurry 2550 containing cellulosic material. The electron beam-aiming device can be a scanner that sweeps a beam over a range of up to about 6 feet in a direction approximately parallel to the surface of the slurry 2550.

[0183] On either side of the electron beam emitter 2540 are sonication horns 2530, which deliver ultrasonic wave energy to the slurry 2550. The sonication horns 2530 end in a detachable endpiece 2535 that is in contact with the slurry 2550.

[0184] The sonication horns 2530 are at risk of damage from long-term residual exposure to the electron beam radiation. Thus, the horns can be protected with a standard shield 2520, e.g., made of lead or a heavy-metal-containing alloy such as Lipowitz metal, which is impervious to electron beam radiation. Precautions must be taken, however, to ensure that the ultrasonic energy is not affected by the presence of the shield. The detachable endpieces 2535, which are constructed of the same material and attached to the horns 2530, are in contact with the cellulosic material 2550 during processing and are expected to be damaged. Accordingly, the detachable endpieces 2535 are constructed to be easily replaceable.

[0185] A further benefit of such a simultaneous electron beam and ultrasound process is that the two processes have complementary results. With electron beam irradiation alone, an insufficient dose may result in cross-linking of some of the polymers in the cellulosic material, which lowers the efficiency of the overall depolymerization process. Lower doses of electron beam irradiation and/or ultrasound radiation may also be used to achieve a similar degree of depolymerization as that achieved using electron beam irradiation and sonication separately. An electron beam device can also be combined with one or more of high-frequency, rotor-stator devices, which can be used as an alternative to ultrasonic sonication devices.

[0186] Further combinations of devices are also possible. For example, an ionizing radiation device that produces gamma radiation emitted from, e.g., ^{60}Co pellets, can be combined with an electron beam source and/or an ultrasonic wave source. Shielding requirements may be more stringent in this case.

[0187] The radiation devices for pretreating biomass discussed above can also be combined with one or more devices that perform one or more pyrolysis processing sequences. Such a combination may again have the advantage of higher throughput. Nevertheless, caution must be observed, as there may be conflicting requirements between some radiation processes and pyrolysis. For example, ultrasonic radiation devices may require the feedstock be immersed in a liquid oxidizing medium. On the other hand, as discussed previously, it may be advantageous for a sample of feedstock undergoing pyrolysis to be of a particular moisture content. In this case, the new systems automatically measure and monitor for a particular moisture content and regulate the same Further, some or all of the above devices, especially the pyrolysis device, can be combined with an oxidation device as discussed previously.

PRIMARY PROCESSES

Fermentation

[0188] The microorganism can be a natural microorganism or an engineered microorganism. For example, the microorganism can be a bacterium, a fungus, e.g., a yeast, a plant or a protist, e.g., an algae, a protozoa or a fungus-like protist, e.g., a slime mold. When the organisms are compatible, mixtures of organisms can be utilized.

[0189] During the fermentation are fermented to, e.g., ethanol, by a fermenting microorganism such as yeast. Suitable fermenting microorganisms have the ability to convert carbohydrates, such as glucose, xylose, arabinose, mannose, galactose, oligosaccharides or polysaccharides into fermentation products. Fermenting microorganisms include strains of the genus *Sacchromyces spp.* e.g., *Sacchromyces cerevisiae* (baker's yeast), *Saccharomyces distaticus, Saccharomyces uvarum;* the genus *Kluyveromyces,* e.g., species *Kluyveromyces marxianus, Kluyveromyces fragilis;* the genus *Candida,* e.g., *Candida pseudotropicalis,* and *Candida brassicae, Pichia stipitis* (a relative of *Candida shehatae,* the genus *Clavispora,* e.g., species *Clavispora lusitaniae* and *Clavispora opuntiae* the genus *Pachysolen,* e.g., species *Pachysolen tannophilus,* the genus *Bretannomyces,* e.g., species *Bretannomyces clausenii* (Philippidis, G. P., 1996, Cellulose bioconversion technology, in Handbook on Bioethanol: Production and Utilization, Wyman, C.E., ed., Taylor & Francis, Washington, DC, 179-212).

[0190] Commercially available yeast include, for example, Red Star®/Lesaffre Ethanol Red (available from Red Star/Lesaffre, USA) FALI® (available from Fleischmann's Yeast, a division of Burns Philip Food Inc., USA), SUPERSTART® (available from Alltech), GERT STRAND® (available from Gert Strand AB, Sweden) and FERMOL® (available from DSM Specialties).

[0191] Bacteria that can ferment biomass to ethanol and other products include, e.g., *Zymomonas mobilis* and *Clostridium thermocellum* (Philippidis, 1996, supra).

[0192] Fermentation of biomass to ethanol and other products may be carried out using certain types of thermophilic or genetically engineered microorganisms, such Thermoanaerobacter species, including *T. mathranii,* and yeast species such as *Pichia species.* An example of a strain of *T. mathranii* is A3M4 described in Sonne-Hansen et al. (Applied Microbiology and Biotechnology 1993, 38, 537-541) or Ahring et al. (Arch. Microbiol. 1997, 168, 114-119).

[0193] Yeast and *Zymomonas* bacteria can be used for fermentation or conversion. The optimum pH for yeast is from about pH 4 to 5, while the optimum pH for *Zymomonas* is from about pH 5 to 6. Typical fermentation times are about 24 to 96 hours with temperatures in the range of 26 °C to 40 °C, however thermophilic microorganisms prefer higher temperatures.

[0194] Mobile fermentors can be utilized, as described in U.S. Provisional Patent Application Serial 60/832,735, now Published International Application No. WO 2008/011598.

POST-PROCESSING

Distillation

[0195] After fermentation, the resulting fluids can be distilled using, for example, a "beer column" to separate ethanol and other alcohols from the majority of water and residual solids. The vapor exiting the beer column can be, e.g., 35% by weight ethanol and can be fed to a rectification column. A mixture of nearly azeotropic (92.5%) ethanol and water from the rectification column can be purified to pure (99.5%) ethanol using vapor-phase molecular sieves. The beer column bottoms can be sent to the first effect of a three-effect evaporator. The rectification column reflux condenser can provide heat for this first effect. After the first effect, solids can be separated using a centrifuge and dried in a rotary dryer. A portion (25%) of the centrifuge effluent can be recycled to fermentation and the rest sent to the second and third evaporator effects. Most of the evaporator condensate can be returned to the process as fairly clean condensate with a small portion split off to waste water treatment to prevent build-up of low-boiling compounds.

PRODUCTS / CO-PRODUCTS

Alcohols

[0196] The alcohol produced can be a monohydroxy alcohol, e.g., ethanol, or a polyhydroxy alcohol, e.g., ethylene glycol or glycerin. Examples of alcohols that can be produced include methanol, ethanol, propanol, isopropanol, butanol, e.g., n-, sec- or t-butanol, ethylene glycol, propylene glycol, 1, 4-butane diol, glycerin or mixtures of these alcohols.

[0197] Each of the alcohols produced by the plant have commercial value as industrial feedstock. For example, ethanol can be used in the manufacture of varnishes and perfume. As another example, methanol can be used as a solvent used as a component in windshield wiper fluid. As still another example, butanol can be used in plasticizers, resins, lacquers, and brake fluids.

[0198] Bioethanol produced by the plant is valuable as an ingredient used in the food and beverage industry. For example, the ethanol produced by the plant can be purified to food grade alcohol and used as a primary ingredient in the alcoholic beverages.

[0199] Bioethanol produced by the plant also has commercial value as a transportation fuel. The use of ethanol as a transportation fuel can be implemented with relatively little capital investment from spark ignition engine manufacturers and owners (e.g., changes to injection timing, fuel-to-air ratio, and components of the fuel injection system). Many automotive manufacturers currently offer flex-fuel vehicles capable of operation on ethanol/gasoline blends up to 85% ethanol by volume (e.g., standard equipment on a Chevy Tahoe 4 x 4).

[0200] Bioethanol produced by this plant can be used as an engine fuel to improve environmental and economic conditions beyond the location of the plant. For example, ethanol produced by this plant and used as a fuel can reduce greenhouse gas emissions from manmade sources (e.g., transportation sources). As another example, ethanol produced by this plant and used as an engine fuel can also displace consumption of gasoline refined from oil.

[0201] Bioethanol has a greater octane number than conventional gasoline and, thus, can be used to improve the performance (e.g., allow for higher compression ratios) of spark ignition engines. For example, small amounts (e.g., 10% by volume) of ethanol can be blended with gasoline to act as an octane enhancer for fuels used in spark ignition engines. As another example, larger amounts (e.g., 85% by volume) of ethanol can be blended with gasoline to further

increase the fuel octane number and displace larger volumes of gasoline.

[0202] Bioethanol strategies are discussed, e.g., by DiPardo in Journal of Outlook for Biomass Ethanol Production and Demand (EIA Forecasts), 2002; Sheehan in Biotechnology Progress, 15:8179, 1999; Martin in Enzyme Microbes Technology, 31:274, 2002; Greer in BioCycle, 61-65, April 2005; Lynd in Microbiology and Molecular Biology Reviews, 66:3, 506-577, 2002; Ljungdahl et al. in U.S. Patent No. 4,292,406; and Bellamy in U.S. Patent No. 4,094,742.

Organic acids

[0203] The organic acids produced can include monocarboxylic acids or polycarboxylic acids. Examples of organic acids include formic acid, acetic acid, propionic acid, butyric acid, valeric acid, caproic, palmitic acid, stearic acid, oxalic acid, malonic acid, succinic acid; glutaric acid, oleic acid, linoleic acid, glycolic acid, lactic acid, y-hydroxybutyric acid or mixtures of these acids.

EXAMPLES

[0204] The following Examples are intended to illustrate, and do not limit the teachings of this disclosure.

Reference Example 1 - Preparation Of Fibrous Material From Polycoated Paper

[0205] A 1500 pound skid of virgin, half-gallon juice cartons made of un-printed polycoated white Kraft board having a bulk density of 20 lb/ft^3 was obtained from International Paper. Each carton was folded flat, and then fed into a 3 hp Flinch Baugh shredder at a rate of approximately 15 to 20 pounds per hour. The shredder was equipped with two 12 inch rotary blades, two fixed blades and a 0.30 inch discharge screen. The gap between the rotary and fixed blades was adjusted to 0.10 inch. The output from the shredder resembled confetti having a width of between 0.1 inch and 0.5 inch, a length of between 0.25 inch and 1 inch and a thickness equivalent to that of the starting material (about 0.075 inch).

[0206] The confetti-like material was fed to a Munson rotary knife cutter, Model SC30. Model SC30 is equipped with four rotary blades, four fixed blades, and a discharge screen having 1/8 inch openings. The gap between the rotary and fixed blades was set to approximately 0.020 inch. The rotary knife cutter sheared the confetti-like pieces across the knife-edges, tearing the pieces apart and releasing a fibrous material at a rate of about one pound per hour. The fibrous material had a BET surface area of 0.9748 m^2/g +/-0.0167 m^2/g, a porosity of 89.0437 percent and a bulk density (@0.53 psia) of 0.1260 g/mL. An average length of the fibers was 1.141 mm and an average width of the fibers was 0.027 mm, giving an average L/D of 42:1. A scanning electron micrograph of the fibrous material is shown in FIG. 26 at 25 X magnification.

Reference Example 2 - Preparation Of Fibrous Material From Bleached Kraft Board

[0207] A 1500 pound skid of virgin bleached white Kraft board having a bulk density of 30 lb/ft^3 was obtained from International Paper. The material was folded flat, and then fed into a 3 hp Flinch Baugh shredder at a rate of approximately 15 to 20 pounds per hour. The shredder was equipped with two 12 inch rotary blades, two fixed blades and a 0.30 inch discharge screen. The gap between the rotary and fixed blades was adjusted to 0.10 inch. The output from the shredder resembled confetti having a width of between 0.1 inch and 0.5 inch, a length of between 0.25 inch and 1 inch and a thickness equivalent to that of the starting material (about 0.075 inch). The confetti-like material was fed to a Munson rotary knife cutter, Model SC30. The discharge screen had 1/8 inch openings. The gap between the rotary and fixed blades was set to approximately 0.020 inch. The rotary knife cutter sheared the confetti-like pieces, releasing a fibrous material at a rate of about one pound per hour. The fibrous material had a BET surface area of 1.1316 m^2/g +/- 0.0103 m^2/g, a porosity of 88.3285 percent and a bulk density (@0.53 psia) of 0.1497 g/mL. An average length of the fibers was 1.063 mm and an average width of the fibers was 0.0245 mm, giving an average L/D of 43:1. A scanning electron micrograph of the fibrous material is shown in FIG. 27 at 25 X magnification.

Reference Example 3 - Preparation Of Twice Sheared Fibrous Material From Bleached Kraft Board

[0208] A 1500 pound skid of virgin bleached white Kraft board having a bulk density of 30 lb/ft^3 was obtained from International Paper. The material was folded flat, and then fed into a 3 hp Flinch Baugh shredder at a rate of approximately 15 to 20 pounds per hour. The shredder was equipped with two 12 inch rotary blades, two fixed blades and a 0.30 inch discharge screen. The gap between the rotary and fixed blades was adjusted to 0.10 inch. The output from the shredder resembled confetti (as above). The confetti-like material was fed to a Munson rotary knife cutter, Model SC30. The discharge screen had 1/16 inch openings. The gap between the rotary and fixed blades was set to approximately 0.020 inch. The rotary knife cutter the confetti-like pieces, releasing a fibrous material at a rate of about one pound per hour.

The material resulting from the first shearing was fed back into the same setup described above and sheared again. The resulting fibrous material had a BET surface area of 1.4408 m^2/g +/- 0.0156 m^2/g, a porosity of 90.8998 percent and a bulk density (@0.53 psia) of 0.1298 g/mL. An average length of the fibers was 0.891 mm and an average width of the fibers was 0.026 mm, giving an average L/D of 34:1. A scanning electron micrograph of the fibrous material is shown in FIG. 28 at 25 X magnification.

Reference Example 4 - Preparation Of Thrice Sheared Fibrous Material From Bleached Kraft Board

[0209] A 1500 pound skid of virgin bleached white Kraft board having a bulk density of 30 lb/ft^3 was obtained from International Paper. The material was folded flat, and then fed into a 3 hp Flinch Baugh shredder at a rate of approximately 15 to 20 pounds per hour. The shredder was equipped with two 12 inch rotary blades, two fixed blades and a 0.30 inch discharge screen. The gap between the rotary and fixed blades was adjusted to 0.10 inch. The output from the shredder resembled confetti (as above). The confetti-like material was fed to a Munson rotary knife cutter, Model SC30. The discharge screen had 1/8 inch openings. The gap between the rotary and fixed blades was set to approximately 0.020 inch. The rotary knife cutter sheared the confetti-like pieces across the knife-edges. The material resulting from the first shearing was fed back into the same setup and the screen was replaced with a 1/16 inch screen. This material was sheared. The material resulting from the second shearing was fed back into the same setup and the screen was replaced with a 1/32 inch screen. This material was sheared. The resulting fibrous material had a BET surface area of 1.6897 m^2/g +/- 0.0155 m^2/g, a porosity of 87.7163 percent and a bulk density (@0.53 psia) of 0.1448 g/mL. An average length of the fibers was 0.824 mm and an average width of the fibers was 0.0262 mm, giving an average L/D of 32:1. A scanning electron micrograph of the fibrous material is shown in FIG. 29 at 25 X magnification.

Reference Example 5 - Preparation Of Densified Fibrous Material From Bleached Kraft Board Without Added Binder

[0210] Fibrous material was prepared according to Example 2. Approximately 1 lb of water was sprayed onto each 10 lb of fibrous material. The fibrous material was densified using a California Pellet Mill 1100 operating at 75 °C. Pellets were obtained having a bulk density ranging from about 7 $lb/ft^3$to about 15 lb/ft^3.

Reference Example 6 - Preparation Of Densified Fibrous Material From Bleached Kraft Board With Binder

[0211] Fibrous material was prepared according to Example 2.

[0212] A 2 weight percent stock solution of POLYOX™ WSR N10 (polyethylene oxide) was prepared in water.

[0213] Approximately 1 lb of the stock solution was sprayed onto each 10 lb of fibrous material. The fibrous material was densified using a California Pellet Mill 1100 operating at 75 °C. Pellets were obtained having a bulk density ranging from about 15 lb/ft^3 to about 40 lb/ft^3.

Reference Example 7 - Reducing the Molecular Weight of Cellulose in Fibrous Kraft Paper by Gamma Radiation with Minimum Oxidation

[0214] Fibrous material is prepared according to Example 4. The fibrous Kraft paper is densified according to Example 5.

[0215] The densified pellets are placed in a glass ampoule having a maximum capacity of 250 mL. The glass ampoule is evacuated under high vacuum (10^{-5} torr) for 30 minutes, and then back-filled with argon gas. The ampoule is sealed under argon. The pellets in the ampoule are irradiated with gamma radiation for about 3 hours at a dose rate of about 1 Mrad per hour to provide an irradiated material in which the cellulose has a lower molecular weight than the fibrous Kraft starting material.

Reference Example 8 - Educing the Molecular Weight of Cellulose in Fibrous Kraft Paper by Gamma Radiation with Maximum Oxidation

[0216] Fibrous material is prepared according to Example 4. The fibrous Kraft paper is densified according to Example 5.

[0217] The densified pellets are placed in a glass ampoule having a maximum capacity of 250 mL. The glass ampoule is sealed under an atmosphere of air. The pellets in the ampoule are irradiated with gamma radiation for about 3 hours at a dose rate of about 1 Mrad per hour to provide an irradiated material in which the cellulose has a lower molecular weight than the fibrous Kraft starting material.

Reference Example 9- Electron Beam Processing

[0218] Samples were treated with electron beam using a vaulted Rhodotron® TT200 continuous wave accelerator delivering 5 MeV electrons at 80 kW of output power. Table 1 describes the parameters used. Table 2 reports the nominal dose used for the Sample ID (in Mrad) and the corresponding dose delivered to the sample (in kgy).

Table 1. Rhodotron® TT 200 Parameters

Beam	
Beam Produced:	Accelerated electrons
Beam energy:	Nominal (fixed): 10 MeV (+0 keV-250 keV
Energy dispersion at 10 Mev:	Full width half maximum (FWHM) 300 keV
Beam power at 10 MeV:	Guaranteed Operating Range 1 to 80 kW
Power Consumption	
Stand-by condition (vacuum and cooling ON):	<15 kW
At 50 kW beam power:	<210 kW
At 80 kW beam power:	<260 kW
RF System	
Frequency:	107.5 ± 1 MHz
Tetrode type:	Thomson TH781
Scanning Horn	
Nominal Scanning Length (measured at 25-35 cm from window):	120 cm
Scanning Range:	From 30% to 100% of Nominal Scanning Length
Nominal Scanning Frequency (at max. scanning length):	100 Hz ± 5%
Scanning Uniformity (across 90% of Nominal Scanning Length)	± 5%

Table 2. Dosages Delivered to Samples

Total Dosage (Mrad) (Number Associated with Sample ID	**Delivered Dose (kgy)[1]**
1	9.9
3	29.0
5	50.4
7	69.2
10	100.0
15	150.3
20	198.3
30	330.9
50	529.0
70	695.9
100	993.6
[1]For example, 9.9kgy was delivered in 11 seconds at a beam current of 5mA and a line speed of 12.9 feet/minute. Cool time between treatments was around 2 minutes.	

Reference Example 10 - Methods of Determining Molecular Weight of Cellulosic and Lignocellulosic Materials by Gel Permeation Chromatography

[0219] Cellulosic and lignocellulosic materials for analysis were treated according to Example 4. Sample materials presented in the following tables include Kraft paper (P), wheat straw (WS), alfalfa (A), cellulose (C), switchgrass (SG), grasses (G), and starch (ST), and sucrose (S). The number "132" of the Sample ID refers to the particle size of the material after shearing through a 1/32 inch screen. The number after the dash refers to the dosage of radiation (MRad) and "US" refers to ultrasonic treatment. For example, a sample ID "P132-10" refers to Kraft paper that has been sheared to a particle size of 132 mesh and has been irradiated with 10 Mrad.

[0220] For samples that were irradiated with e-beam, the number following the dash refers to the amount of energy delivered to the sample. For example, a sample ID "P-100e" refers to Kraft paper that has been delivered a dose of energy of about 100 MRad or about 1000 kgy (Table 2).

Table 3. Peak Average Molecular Weight of Irradiated Kraft Paper

Sample Source	Sample ID	Dosage[1] (Mrad)	Ultrasound[2]	Average MW ± Std Dev.
Kraft Paper	**P132**	0	No	32853 ± 10006
	P132-10	10	"	61398 ± 2468**
	P132-100	100	"	8444 ± 580
	P132-181	181	"	6668 ± 77
	P132-US	0	Yes	3095 ± 1013

**slow doses of radiation appear to increase the molecular weight of some materials

[1]Dosage Rate = 1MRad/hour

[2]Treatment for 30 minutes with 20kHz ultrasound using a 1000W horn under re-circulating conditions with the material dispersed in water.

Table 4. Peak Average Molecular Weight of Irradiated Kraft Paper with E-Beam

Sample Source	Sample ID	Dosage (Mrad)	Average MW ± Std Dev.
Kraft Paper	**P-1e**	1	63489 ± 595
	P-5e	5	56587 ± 536
	P-10e	10	53610 ± 327
	P-30e	30	38231 ± 124
	P-70e	70	12011 ± 158
	P-100e	100	9770 ± 2

Table 5. Peak Average Molecular Weight of Gamma Irradiated Materials

Sample ID	Peak #	Dosage[1] (Mrad)	Ultrasound[2]	Average MW ± Std Dev.
WS132	1	0	No	1407411 ± 175191
	2	"	"	39145 ± 3425
	3	"	"	2886 ± 177
WS132-10*	1	10	"	26040 ± 3240
WS132-100*	1	100	"	23620 ± 453
A132	1	0	"	1604886 ± 151701
	2	"	"	37525 ± 3751
	3	"	"	2853 ± 490
A132-10*	1	10	"	50853 ± 1665
	2	"	"	2461 ± 117
A132-100*	1	100	"	38291 ± 2235
	2	"	"	2487 ± 15
SG132	1	0	"	1557360 ± 83693

(continued)

Sample ID	Peak #	Dosage[1] (Mrad)	Ultrasound[2]	Average MW ± Std Dev.
	2	"	"	42594 ± 4414
	3	"	"	3268 ± 249
SG132-10*	1	10	"	60888 ± 9131
SG132-100*	1	100	"	22345 ± 3797
SG132-10-US	1	10	Yes	86086 ± 43518
	2	"	"	2247 ± 468
SG132-100-US	1	100	"	4696 ± 1465

*Peaks coalesce after treatment

**Low doses of radiation appear to increase the molecular weight of some materials

[1]Dosage Rate = 1MRad/hour

[2]Treatment for 30 minutes with 20kHz ultrasound using a 1000W horn under re-circulating conditions with the material dispersed in water.

Table 6. Peak Average Molecular Weight of Irradiated Material with E-Beam

Sample ID	Peak #	Dosage	Average MW ± STD DEV.
A-1e	1	**1**	1004783 ± 97518
	2		34499 ± 482
	3		2235 ± 1
A-5e	1	**5**	38245 ± 346
	2		2286 ± 35
A-10e	1	**10**	44326 ± 33
	2		2333 ± 18
A-30e	1	**30**	47366 ± 583
	2		2377 ± 7
A-50e	1	**50**	32761 ± 168
	2		2435 ± 6
G-1e	1	**1**	447362 ± 38817
	2		32165 ± 779
	3		3004 ± 25
G-5e	1	**5**	62167 ± 6418
	2		2444 ± 33
G-10e	1	**10**	72636 ± 4075
	2		3065 ± 34
G-30e	1	**30**	17159 ± 390
G-50e	1	**50**	18960 ± 142
ST	1	**0**	923336 ± 1883
	2		150265 ± 4033
ST-1e	1	**1**	846081 ± 5180
	2		131222 ± 1687
ST-5e	1	**5**	90664 ± 1370
ST-10e	1	**10**	98050 ± 255
ST-30e	1	**30**	41884 ± 223
ST-70e	1	**70**	9699 ± 31
ST-100e	1	**100**	8705 ± 38

[0221] Gel Permeation Chromatography (GPC) is used to determine the molecular weight distribution of polymers. During GPC analysis, a solution of the polymer sample is passed through a column packed with a porous gel trapping

small molecules. The sample is separated based on molecular size with larger molecules eluting sooner than smaller molecules. The retention time of each component is most often detected by refractive index (RI), evaporative light scattering (ELS), or ultraviolet (UV) and compared to a calibration curve. The resulting data is then used to calculate the molecular weight distribution for the sample.

[0222] A distribution of molecular weights rather than a unique molecular weight is used to characterize synthetic polymers. To characterize this distribution, statistical averages are utilized. The most common of these averages are the "number average molecular weight" (M_n) and the "weight average molecular weight" (M_w).

[0223] Man is similar to the standard arithmetic mean associated with a group of numbers. When applied to polymers, M_n refers to the average molecular weight of the molecules in the polymer. M_n is calculated affording the same amount of significance to each molecule regardless of its individual molecular weight. The average M_n is calculated by the following formula where N_i is the number of molecules with a molar mass equal to M_i.

$$\overline{M}_n = \frac{\sum_i N_i M_i}{\sum_i N_i}$$

[0224] M_w is another statistical descriptor of the molecular weight distribution that places a greater emphasis on larger molecules than smaller molecules in the distribution. The formula below shows the statistical calculation of the weight average molecular weight.

$$\overline{M}_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i}$$

[0225] The polydispersity index or PI is defined as the ratio of M_w/M_n. The larger the PI, the broader or more disperse the distribution. The lowest value that a PI can be is 1. This represents a monodisperse sample; that is, a polymer with all of the molecules in the distribution being the same molecular weight.

[0226] The peak molecular weight value (M_P) is another descriptor defined as the mode of the molecular weight distribution. It signifies the molecular weight that is most abundant in the distribution. This value also gives insight to the molecular weight distribution.

[0227] Most GPC measurements are made relative to a different polymer standard. The accuracy of the results depends on how closely the characteristics of the polymer being analyzed match those of the standard used. The expected error in reproducibility between different series of determinations, calibrated separately, is ca. 5-10% and is characteristic to the limited precision of GPC determinations. Therefore, GPC results are most useful when a comparison between the molecular weight distributions of different samples is made during the same series of determinations.

[0228] The lignocellulosic samples required sample preparation prior to GPC analysis. First, a saturated solution (8.4% by weight) of lithium chloride (LiCl) was prepared in dimethyl acetamide (DMAc). Approximately 100 mg of the sample was added to approximately 10 g of a freshly prepared saturated LiCl/DMAc solution, and the mixture was heated to approximately 150°C-170°C with stirring for 1 hour. The resulting solutions were generally light- to dark-yellow in color. The temperature of the solutions were decreased to approximately 100°C and heated for an additional 2 hours. The temperatures of the solutions were then decreased to approximately 50°C and the sample solution was heated for approximately 48 to 60 hours. Of note, samples irradiated at 100 MRad were more easily solubilized as compared to their untreated counterpart. Additionally, the sheared samples (denoted by the number 132) had slightly lower average molecular weights as compared with uncut samples.

[0229] The resulting sample solutions were diluted 1:1 using DMAc as solvent and were filtered through a 0.45 μm PTFE filter. The filtered sample solutions were then analyzed by GPC using the parameters described in Table 7. The peak average molecular weights (Mp) of the samples, as determined by Gel Permeation Chromatography (GPC), are summarized in Tables 3-6. Each sample was prepared in duplicate and each preparation of the sample was analyzed in duplicate (two injections) for a total of four injections per sample. The EasiCal® polystyrene standards PS1A and

PS1B were used to generate a calibration curve for the molecular weight scale from about 580 to 7,500,00 Daltons.

Table 7. GPC Analysis Conditions

Instrument:	Waters Alliance GPC 2000
Columns (3):	Plgel 10μ Mixed-B **S/N's:** 10M-MB-148-83; 10M-MB-148-84; 10M-MB-174-129
Mobile Phase (solvent):	0.5% LiCl in DMAc (1.0 mL/min.)
Column/Detector Temperature:	70 °C
Injector Temperature:	70 °C
Sample Loop Size:	323.5 μL

Reference Example 11. Time-Of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) Surface Analysis

[0230] Time-of-Flight Secondary Ion Mass Spectroscopy (ToF-SIMS) is a surface-sensitive spectroscopy that uses a pulsed ion beam (Cs or microfocused Ga) to remove molecules from the very outermost surface of the sample. The particles are removed from atomic monolayers on the surface (secondary ions). These particles are then accelerated into a "flight tube" and their mass is determined by measuring the exact time at which they reach the detector (i.e. time-of-flight). ToF-SIMS provides detailed elemental and molecular information about the surface, thin layers, interfaces of the sample, and gives a full three-dimensional analysis. The use is widespread, including semiconductors, polymers, paint, coatings, glass, paper, metals, ceramics, biomaterials, pharmaceuticals and organic tissue. Since ToF-SIMS is a survey technique, all the elements in the periodic table, including H, are detected. ToF-SIMS data is presented in Tables 8-11. Parameters used are reported in Table 12.

Table 8. Normalized Mean Intensities of Various Positive Ions of Interest (Normalized relative to total ion counts x 10000)

		P132		P132-10		P132-100	
m/z	**species**	**Mean**	**σ**	**Mean**	**σ**	**Mean**	**σ**
23	**Na**	257	28	276	54	193	36
27	**Al**	647	43	821	399	297	44
28	**Si**	76	45.9	197	89	81.7	10.7
15	**CH_3**	77.9	7.8	161	26	133	12
27	**C_2H_3**	448	28	720	65	718	82
39	**C_3H_3**	333	10	463	37	474	26
41	**C_3H_5**	703	19	820	127	900	63
43	**C_3H_7**	657	11	757	162	924	118
115	**C_9H_7**	73	13.4	40.3	4.5	42.5	15.7
128	**$C_{10}H_8$**	55.5	11.6	26.8	4.8	27.7	6.9
73	**$C_3H_9Si^*$**	181	77	65.1	18.4	81.7	7.5
147	**$C_5H_{15}OSi_2^*$**	72.2	33.1	24.9	10.9	38.5	4
207	**$C_5H_{15}O_3Si_3^*$**	17.2	7.8	6.26	3.05	7.49	1.77
647	**$C_{42}H_{64}PO_3$**	3.63	1.05	1.43	1.41	10.7	7.2

Table 9. Normalized Mean Intensities of Various Negative Ions of Interest (Normalized relative to total ion counts x 10000)

		P132		P132-10		P132-100	
m/z	**species**	**Mean**	**σ**	**Mean**	**σ**	**Mean**	**σ**
19	**F**	15.3	2.1	42.4	37.8	19.2	1.9
35	**Cl**	63.8	2.8	107	33	74.1	5.5
13	**CH**	1900	91	1970	26	1500	6
25	**C_2H**	247	127	220	99	540	7
26	**CN**	18.1	2.1	48.6	30.8	43.9	1.4

(continued)

		P132		P132-10		P132-100	
m/z	species	Mean	σ	Mean	σ	Mean	σ
42	CNO	1.16	0.71	0.743	0.711	10.8	0.9
46	NO_2	1.87	0.38	1.66	1.65	12.8	1.8

Table 10. Normalized Mean Intensities of Various Positive Ions of Interest (Normalized relative to total ion counts x 10000)

		P-1e		P-5e		P-10e		P-30e		P-70e		P-100e	
m/z	species	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
23	Na	232	56	370	37	241	44	518	57	350	27	542	104
27	Al	549	194	677	86	752	371	761	158	516	159	622	166
28	Si	87.3	11.3	134	24	159	100	158	32	93.7	17.1	124	11
15	CH_3	114	23	92.9	3.9	128	18	110	16	147	16	141	5
27	C_2H_3	501	205	551	59	645	165	597	152	707	94	600	55
39	C_3H_3	375	80	288	8	379	82	321	57	435	61	417	32
41	C_3H_5	716	123	610	24	727	182	607	93	799	112	707	84
43	C_3H_7	717	121	628	52	653	172	660	89	861	113	743	73
115	C_9H_7	49.9	14.6	43.8	2.6	42.2	7.9	41.4	10.1	27.7	8	32.4	10.5
128	$C_{10}H_8$	38.8	13.1	39.2	1.9	35.2	11.8	31.9	7.8	21.2	6.1	24.2	6.8
73	$C_3H_9Si^*$	92.5	3.0	80.6	2.9	72.3	7.7	75.3	11.4	63	3.4	55.8	2.1
147	$C_5H_{15}OSi_2^*$	27.2	3.9	17.3	1.2	20.4	4.3	16.1	1.9	21.7	3.1	16.3	1.7
207	$C_5H_{15}O_3Si_3^*$	6.05	0.74	3.71	0.18	4.51	0.55	3.54	0.37	5.31	0.59	4.08	0.28
647	$C_{42}H_{64}PO_3$	1.61	1.65	1.09	1.30	0.325	0.307	nd	~	0.868	1.31	0.306	0.334

Table 11. Normalized Mean Intensities of Various Negative Ions of Interest (Normalized relative to total ion counts x 10000)

		P-1e		P-5e		P-10e		P-30e		P-70e		P-100e	
m/z	species	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ	Mean	σ
13	CH	1950	72	1700	65	1870	91	1880	35	2000	46	2120	102
25	C_2H	154	47	98.8	36.3	157	4	230	17	239	22	224	19
19	F	25.4	1	24.3	1.4	74.3	18.6	40.6	14.9	25.6	1.9	21.5	2
35	Cl	39.2	13.5	38.7	3.5	46.7	5.4	67.6	6.2	45.1	2.9	32.9	10.2
26	CN	71.9	18.9	6.23	2.61	28.1	10.1	34.2	29.2	57.3	28.9	112	60
42	CNO	0.572	0.183	0.313	0.077	0.62	0.199	1.29	0.2	1.37	0.55	1.38	0.28
46	NO_2	0.331	0.057	0.596	0.255	0.668	0.149	1.44	0.19	1.92	0.29	0.549	0.1

Table 12. ToF-SIMS Parameters

Instrument Conditions:	
Instrument:	PHI TRIFT II
Primary Ion Source:	^{69}Ga
Primary Ion Beam Potential:	12 kV + ions 18 kV - ions
Primary Ion Current (DC):	2 na for P#E samples 600 pA for P132 samples
Energy Filter/CD:	Out/Out

(continued)

Instrument Conditions:	
Masses Blanked:	None
Charge Compensation:	On

[0231] ToF-SIMS uses a focused, pulsed particle beam (typically Cs or Ga) to dislodge chemical species on a materials surface. Particles produced closer to the site of impact tend to be dissociated ions (positive or negative). Secondary particles generated farther from the impact site tend to be molecular compounds, typically fragments of much larger organic macromolecules. The particles are then accelerated into a flight path on their way towards a detector. Because it is possible to measure the "time-of-flight" of the particles from the time of impact to detector on a scale of nano-seconds, it is possible to produce a mass resolution as fine as 0.00X atomic mass units (i.e. one part in a thousand of the mass of a proton). Under typical operating conditions, the results of ToF-SIMS analysis include: a mass spectrum that surveys all atomic masses over a range of 0-10,000 amu, the rastered beam produces maps of any mass of interest on a sub-micron scale, and depth profiles are produced by removal of surface layers by sputtering under the ion beam. Negative ion analysis showed that the polymer had increasing amounts of CNO, CN, and NO_2 groups.

Reference Example 12. X-Ray Photoelectron Spectroscopy (XPS)/Electron Spectroscopy for Chemical Analysis (ESCA)

[0232] X-Ray Photoelectron Spectroscopy (XPS) (sometimes called "ESCA") measures the chemical composition of the top five nanometers of surface; XPS uses photoionization energy and energy-dispersive analysis of the emitted photoelectrons to study the composition and electronic state of the surface region of a sample. X-ray Photoelectron spectroscopy is based upon a single photon in/electron out. Soft x-rays stimulate the ejection of photoelectrons whose kinetic energy is measured by an electrostatic electron energy analyzer. Small changes to the energy are caused by chemically-shifted valence states of the atoms from which the electrons are ejected; thus, the measurement provides chemical information about the sample surface.

Table 13. Atomic Concentrations (in %)[a,b]

Sample ID	Atom			
	C	O	Al	Si
P132 (Area1)	57.3	39.8	1.5	1.5
P132 (Area2)	57.1	39.8	1.6	1.5
P132-10 (Area 1)	63.2	33.5	1.7	1.6
P132-10 (Area 2)	65.6	31.1	1.7	1.7
P132-100 (Area 1)	61.2	36.7	0.9	1.2
P132-100 (Area 2)	61	36.9	0.8	1.3

[a] Normalized to 100% of the elements detected. XPS does not detect H or He.

Table 14. Carbon Chemical State (in % C)

	C-C, C-H	C-O	C=O	O-C=O
Sample ID				
P132 (Area1)	22	49	21	7
P132 (Area2)	25	49	20	6
P132-10 (Area 1)	34	42	15	9
P132-10 (Area 2)	43	38	14	5
P132-100 (Area 1)	27	45	15	9

(continued)

Sample ID	C-C, C-H	C-O	C=O	O-C=O
P132-100 (Area 2)	25	44	23	9

Table 15. Atomic Concentrations (in %)[a,b]

Sample ID	Atom				
	C	O	Al	Si	Na
P-1e (Area 1)	59.8	37.9	1.4	0.9	~
P-1e (Area 2)	58.5	38.7	1.5	1.3	~
P-5e (Area 1)	58.1	39.7	1.4	0.8	~
P-5e (Area 2)	58.0	39.7	1.5	0.8	~
P-10e (Area 1)	61.6	36.7	1.1	0.7	~
P-10e (Area 2)	58.8	38.6	1.5	1.1	~
P-50e (Area 1)	59.9	37.9	1.4	0.8	<0.1
P-50e (Area 2)	59.4	38.3	1.4	0.9	<0.1
P-70e (Area 1)	61.3	36.9	1.2	0.6	<0.1
P-70e (Area 2)	61.2	36.8	1.4	0.7	<0.1
P-100e (Area 1)	61.1	37.0	1.2	0.7	<0.1
P-100e (Area 2)	60.5	37.2	1.4	0.9	<0.1

a Normalized to 100% of the elements detected. XPS does not detect H or He.
[b] A less than symbol "<" indicates accurate quantification cannot be made due to weak signal intensity.

Table 16. Carbon Chemical State Table (in %C)

Sample ID	C-C, C-H	C-O	C=O	O-C=O
P-1e (Area 1)	29	46	20	5
P-1e (Area 2)	27	49	19	5
P-5e (Area 1)	25	53	18	5
P-5e (Area 2)	28	52	17	4
P-10e (Area 1)	33	47	16	5
P-10e (Area 2)	28	51	16	5
P-50e (Area 1)	29	45	20	6
P-50e (Area 2)	28	50	16	5
P-70e (Area 1)	32	45	16	6
P-70e (Area 2)	35	43	16	6
P-100e (Area 1)	32	42	19	7
P-100e (Area 2)	30	47	16	7

Table 17. Analytical Parameters

Instrument:	PHI Quantum 2000
X-ray source:	Monochromated Alk_{α} 1486.6 eV
Acceptance Angle:	±23°
Take-off angle:	45°
Analysis area:	1400 x 300 μm
Charge Correction:	C1s 284.8 eV

[0233] XPS spectra are obtained by irradiating a material with a beam of aluminum or magnesium X-rays while simultaneously measuring the kinetic energy (KE) and number of electrons that escape from the top 1 to 10 nm of the material being analyzed (see analytical parameters, Table 17). The XPS technique is highly surface specific due to the short range of the photoelectrons that are excited from the solid. The energy of the photoelectrons leaving the sample is determined using a Concentric Hemispherical Analyzer (CHA) and this gives a spectrum with a series of photoelectron peaks. The binding energy of the peaks is characteristic of each element. The peak areas can be used (with appropriate sensitivity factors) to determine the composition of the materials surface. The shape of each peak and the binding energy can be slightly altered by the chemical state of the emitting atom. Hence XPS can provide chemical bonding information as well. XPS is not sensitive to hydrogen or helium, but can detect all other elements. XPS requires ultra-high vacuum (UHV) conditions and is commonly used for the surface analysis of polymers, coatings, catalysts, composites, fibers, ceramics, pharmaceutical/medical materials, and materials of biological origin. XPS data is reported in Tables 13-16.

Reference Example 13. Raman Analysis

[0234] Raman spectra were acquired from the surface of fibers from samples: P132, P132-100, P-1e, and P-100e. The measurements were performed using a "LabRam" J-Y Spectrometer. A HeNe laser (632.8 nm wavelength) and 600 gr/mm grating were used for the measurements. The measurements were performed confocally using backscattering geometry (180°) under an Olympus BX40 microscope. The samples had a Raman spectrum typical of cellulose.

Reference Example 14. Scanning Probe Microscopy (SPM) Surface Analysis Using an Atomic Force Microscope (AFM)

[0235] The purpose of this analysis was to obtain Atomic Force Microscope (AFM) images of the samples in Tables 18 and 19 to measure surface roughness.

[0236] Scanning probe microscopy (SPM) is a branch of microscopy that forms images of surfaces using a physical probe that scans the specimen. An image of the surface is obtained by mechanically moving the probe in a raster scan of the specimen, line by line, and recording the probe-surface interaction as a function of position. The atomic force microscope (AFM) or scanning force microscope (SFM) is a very high-resolution type of scanning probe microscope, with demonstrated resolution of fractions of a nanometer, more than 1000 times better than the optical diffraction limit. The probe (or the sample under a stationary probe) generally is moved by a piezoelectric tube. Such scanners are designed to be moved precisely in any of the three perpendicular axes (x,y,z). By following a raster pattern, the sensor data forms an image of the probe-surface interaction. Feedback from the sensor is used to maintain the probe at a constant force or distance from the object surface. For atomic force microscopy, the sensor is a position-sensitive photodetector that records the angle of reflection from a laser bean focused on the top of the cantilever.

Table 18. Roughness Results for Gamma-Irradiated Samples

Sample ID	RMS (Å)	R_a (Å)	R_{max} (Å)
P132	927.2	716.3	8347.6
P132-10	825.7	576.8	11500
P132-100	1008	813.5	7250.7

Table 19. Roughness Results for Samples Irradiated with E-Beam

Sample ID	RMS (Å)	R_a (Å)	R_{max} (Å)
P-1e	1441.2	1147.1	8955.4
P-5e	917.3	727.5	6753.4
P-10e	805.6	612.1	7906.5
P-30e	919.2	733.7	6900
P-70e	505.8	388.1	5974.2
P-100e	458.2	367.9	3196.9

[0237] AFM images were collected using a NanoScope III Dimension 5000 (Digital Instruments, Santa Barbara, California, USA). The instrument was calibrated against a NIST traceable standard with an accuracy better than 2%. NanoProbe silicon tips were used. Image processing procedures involving auto-flattening, plane fitting or convolution were employed.

[0238] One 5μm x 5μm area was imaged at a random location on top of a single fiber. Perspective (3-D) views of these surfaces are included with vertical exaggerations noted on the plots (FIGS. 29A-29F). The roughness analyses were performed and are expressed in: (1) Root-Mean-Square Roughness, RMS; (2) Mean Roughness, Ra; and (3) Maximum Height (Peak-to-Valley), Rmax. Results are summarized in Tables 18 and 19.

Reference Example 15- Determining Crystallinity of Irradiated Materials by X-Ray Diffraction

[0239] X-ray diffraction (XRD) is a method by which a crystalline sample is irradiated with monoenergetic x-rays. The interaction of the lattice structure of the sample with these x-rays is recorded and provides information about the crystalline structure being irradiated. The resulting characteristic "fingerprint" allows for the identification of the crystalline compounds present in the sample. Using a whole-pattern fitting analysis (the Rietvelt Refinement), it is possible to perform quantitative analyses on samples containing more than one crystalline compound.

Table 20. XRD Data Including Domain Size and % Crystallinity

Sample ID	Domain Size (Å)	% Crystallinity
P132	55	55
P132-10	46	58
P132-100	50	55
P132-181	48	52
P132-US	26	40
A132	28	42
A132-10	26	40
A132-100	28	35
WS132	30	36
WS132-10	27	37
WS132-100	30	41
SG132	29	40
SG132-10	28	38
SG132-100	28	37
SG132-10-US	25	42
SG132-100-US	21	34

[0240] Each sample was placed on a zero background holder and placed in a Phillips PW1800 diffractometer using Cu radiation. Scans were then run over the range of 5° to 50° with a step size of 0.05° and a counting time of 2 hours each.

[0241] Once the diffraction patterns were obtained, the phases were identified with the aid of the Powder Diffraction File published by the International Centre for Diffraction Data. In all samples the crystalline phase identified was cellulose - Ia, which has a triclinic structure.

[0242] The distinguishing feature among the 20 samples is the peak breadth, which is related to the crystallite domain size. The experimental peak breadth was used to compute the domain size and percent crystallinity, which are reported in Table 4

[0243] Percent crystallinity (X_c %) is measured as a ratio of the crystalline area to the total area under the x-ray diffraction peaks,

$$X_c\% = \frac{A_C}{\{A_a + A_C\}} \times 100\%$$

where,

A_c = Area of crystalline phase

A_a = Area of amorphous phase

X_c = Percent of crystallinity

[0244] To determine the percent crystallinity for each sample it was necessary to first extract the amount of the amorphous phase. This is done by estimating the area of each diffraction pattern that can be attributed to the crystalline phase (represented by the sharper peaks) and the non-crystalline phase (represented by the broad humps beneath the pattern and centered at 22° and 38°).

[0245] A systematic process was used to minimize error in these calculations due to broad crystalline peaks as well as high background intensity. First, a linear background was applied and then removed. Second, two Gaussian peaks centered at 22° and 38° with widths of 10-12° each were fitted to the humps beneath the crystalline peaks. Third, the area beneath the two broad Gaussian peaks and the rest of the pattern were determined. Finally, percent crystallinity was calculated by dividing the area beneath the crystalline peak by the total intensity (after background subtraction). Domain size and % crystallinity of the samples as determined by X-ray diffraction (XRD) are presented in Table 20.

Reference Example 16 - Porosimetry Analysis of Irradiated Materials

[0246] Mercury pore size and pore volume analysis (Table 21) is based on forcing mercury (a non-wetting liquid) into a porous structure under tightly controlled pressures. Since mercury does not wet most substances and will not spontaneously penetrate pores by capillary action, it must be forced into the voids of the sample by applying external pressure. The pressure required to fill the voids is inversely proportional to the size of the pores. Only a small amount of force or pressure is required to fill large voids, whereas much greater pressure is required to fill voids of very small pores.

Table 21. Pore Size and Volume Distribution by Mercury Porosimetry

Sample ID	Total Intrusion Volume (mL/g)	Total Pore Area (m^2/g)	Median Pore Diameter (Volume) (μm)	Median Pore Diameter (Area) (μm)	Average Pore Diameter (4V/A) (μm)	Bulk Density @ 0.50 psia (g/mL)	Apparent (skeletal) Density (g/mL)	Porosity (%)
P132	6.0594	1.228	36.2250	13.7278	19.7415	0.1448	1.1785	87.7163
P132-10	5.5436	1.211	46.3463	4.5646	18.3106	0.1614	1.5355	89.4875
P132-100	5.3985	0.998	34.5235	18.2005	21.6422	0.1612	1.2413	87.0151
P132-181	3.2866	0.868	25.3448	12.2410	15.1509	0.2497	1.3916	82.0577
P132-US	6.0005	14.787	98.3459	0.0055	1.6231	0.1404	0.8894	84.2199
A132	2.0037	11.759	64.6308	0.0113	0.6816	0.3683	1.4058	73.7990
A132-10	1.9514	10.326	53.2706	0.0105	0.7560	0.3768	1.4231	73.5241
A132-100	1.9394	10.205	43.8966	0.0118	0.7602	0.3760	1.3889	72.9264
SG132	2.5267	8.265	57.6958	0.0141	1.2229	0.3119	1.4708	78.7961
SG132-10	2.1414	8.643	26.4666	0.0103	0.9910	0.3457	1.3315	74.0340
SG132-100	2.5142	10.766	32.7118	0.0098	0.9342	0.3077	1.3590	77.3593
SG132-10-US	4.4043	1.722	71.5734	1.1016	10.2319	0.1930	1.2883	85.0169
SG132-100-US	4.9665	7.358	24.8462	0.0089	2.6998	0.1695	1.0731	84.2010
WS132	2.9920	5.447	76.3675	0.0516	2.1971	0.2773	1.6279	82.9664
WS132-10	3.1138	2.901	57.4727	0.3630	4.2940	0.2763	1.9808	86.0484
WS132-100	3.2077	3.114	52.3284	0.2876	4.1199	0.2599	1.5611	83.3538
A-1e	1.9535	3.698	25.3411	0.0810	2.1130	0.3896	1.6299	76.0992
A-5e	1.9697	6.503	29.5954	0.0336	1.2117	0.3748	1.4317	73.8225
A-10e	2.0897	12.030	45.5493	0.0101	0.6948	0.3587	1.4321	74.9545
A-50e	2.1141	7.291	37.0760	0.0304	1.1599	0.3577	1.4677	75.6264
G-1e	2.4382	7.582	58.5521	0.0201	1.2863	0.3144	1.3472	76.6610
G-5e	2.4268	6.436	44.4848	0.0225	1.5082	0.3172	1.3782	76.9831
G-10e	2.6708	6.865	62.8605	0.0404	1.5562	0.2960	1.4140	79.0638
G-50e	2.8197	6.798	56.5048	0.0315	1.6591	0.2794	1.3179	78.7959
P-1e	7.7692	1.052	49.8844	22.9315	29.5348	0.1188	1.5443	92.3065
P-5e	7.1261	1.212	46.6400	12.3252	23.5166	0.1268	1.3160	90.3644
P-10e	6.6096	1.113	41.4252	17.4375	23.7513	0.1374	1.4906	90.7850
P-50e	6.5911	1.156	40.7837	15.9823	22.7974	0.1362	1.3302	89.7616
P-100e	5.3507	1.195	35.3622	10.7400	17.9063	0.1648	1.3948	88.1840
S	0.4362	0.030	102.8411	42.5047	57.8208	0.9334	1.5745	40.7160

(continued)

Sample ID	Total Intrusion Volume (mL/g)	Total Pore Area (m^2/g)	Median Pore Diameter (Volume) (μm)	Median Pore Diameter (Area) (μm)	Average Pore Diameter (4V/A) (μm)	Bulk Density @ 0.50 psia (g/mL)	Apparent (skeletal) Density (g/mL)	Porosity (%)
S-1e	0.3900	0.632	90.6808	0.0041	2.4680	0.9772	1.5790	38.1140
S-5e	0.3914	0.337	97.1991	0.0070	4.6406	0.9858	1.6052	38:5847
S-10e	0.4179	0.349	113.4360	0.0042	4.7873	0.9469	1.5669	39.5678
S-30e	0.4616	5.329	102.0559	0.0042	0.3464	0.9065	1.5585	41.8388
S-50e	0.5217	7.162	137.2124	0.0051	0.2914	0.8521	1.5342	44.4582
S-100e	0.8817	15.217	76.4577	0.0053	0.2318	0.6478	1.5105	57.1131
St	0.6593	17.631	4.2402	0.0053	0.1496	0.7.757	1.5877	51.1438
St-1e	0.6720	18.078	4.3360	0.0052	0.1487	0.7651	1.5750	51.4206
St-5e	0.6334	19.495	4.2848	0.0051	0.1300	0.7794	1.5395	49.3706
St-10e	0.6208	16.980	4.3362	0.0056	0.1462	0.7952	1.5703	49.3630
St-30e	0.6892	18.066	4.4152	0.0050	0.1526	0.7475	1.5417	51.5165
St-50e	0.6662	18.338	4.3759	0.0054	0.1453	0.7637	1.5548	50.8778
St-100e	0.6471	23.154	5.4032	0.0048	0.1118	0.7229	1.3582	46.7761

[0247] The AutoPore 9520 can attain a maximum pressure of 414 MPa or 60,000 psia. There are four low pressure stations for sample preparation and collection of macropore data from 0.2 psia to 50 psia. There are two high pressure chambers, which collect data from 25 psia to 60,000 psia. The sample is placed in a bowl-like apparatus called a penetrometer, which is bonded to a glass capillary stem with a metal coating. As mercury invades the voids in and around the sample, it moves down the capillary stem. The loss of mercury from the capillary stem results in a change in the electrical capacitance. The change in capacitance during the experiment is converted to volume of mercury by knowing the stem volume of the penetrometer in use. A variety of penetrometers with different bowl (sample) sizes and capillaries are available to accommodate most sample sizes and configurations. Table 22 below defines some of the key parameters calculated for each sample.

Table 22. Definition of Parameters

Parameter	Description
Total Intrusion Volume:	The total volume of mercury intruded during an experiment. This can include interstitial filling between small particles, porosity of sample, and compression volume of sample.
Total Pore Area:	The total intrusion volume converted to an area assuming cylindrical shaped pores.
Median Pore Diameter (volume):	The size at the 50th percentile on the cumulative volume graph.
Median Pore Diameter (area):	The size at the 50th percentile on the cumulative area graph.
Average Pore Diameter:	The total pore volume divided by the total pore area (4V/A).
Bulk Density:	The mass of the sample divided by the bulk volume. Bulk volume is determined at the filling pressure, typically 0.5 psia.
Apparent Density:	The mass of sample divided by the volume of sample measured at highest pressure, typically 60,000 psia.
Porosity:	(Bulk Density/ Apparent Density) x 100%

Reference Example 17 - Particle Size Analysis of Irradiated Materials

[0248] The technique of particle sizing by static light scattering is based on Mie theory (which also encompasses Fraunhofer theory). Mie theory predicts the intensity vs. angle relationship as a function of the size for spherical scattering particles provided that other system variables are known and held constant. These variables are the wavelength of incident light and the relative refractive index of the sample material. Application of Mie theory provides the detailed particle size information. Table 23 summarizes particle size using median diameter, mean diameter, and modal diameter as parameters.

Table 23. Particle Size by Laser Light Scattering (Dry Sample Dispersion)

Sample ID	Median Diameter (μm)	Mean Diameter (μm)	Modal Diameter (μm)
A132	380.695	418.778	442.258
A132-10	321.742	366.231	410.156
A132-100	301.786	348.633	444.169
SG132	369.400	411.790	455.508
SG132-10	278.793	325.497	426.717
SG132-100	242.757	298.686	390.097
WS132	407.335	445.618	467.978
WS132-10	194.237	226.604	297.941
WS132-100	201.975	236.037	307.304

[0249] Particle size was determined by Laser Light Scattering (Dry Sample Dispersion) using a Malvern Mastersizer 2000 using the following conditions:

Feed Rate:	35%
Disperser Pressure:	4 Bar
Optical Model:	(2.610, 1.000i), 1.000

[0250] An appropriate amount of sample was introduced onto a vibratory tray. The feed rate and air pressure were adjusted to ensure that the particles were properly dispersed. The key component is selecting an air pressure that will break up agglomerations, but does not compromise the sample integrity. The amount of sample needed varies depending on the size of the particles. In general, samples with fine particles require less material than samples with coarse particles.

Reference Example 18 - Surface Area Analysis of Irradiated Materials

[0251] Surface area of each sample was analyzed using a Micromeritics ASAP 2420 Accelerated Surface Area and Porosimetry System. The samples were prepared by first degassing for 16 hours at 40 °C. Next, free space (both warm and cold) with helium is calculated and then the sample tube is evacuated again to remove the helium. Data collection begins after this second evacuation and consists of defining target pressures, which control how much gas is dosed onto the sample. At each target pressure, the quantity of gas adsorbed and the actual pressure are determined and recorded. The pressure inside the sample tube is measured with a pressure transducer. Additional doses of gas will continue until the target pressure is achieved and allowed to equilibrate. The quantity of gas adsorbed is determined by summing multiple doses onto the sample. The pressure and quantity define a gas adsorption isotherm and are used to calculate a number of parameters, including BET surface area (Table 24).

Table 24. Summary of Surface Area by Gas Adsorption

Sample ID	Single point surface area (m^2/g)		BET Surface Area (m^2/g)
P132	@ P/Po= 0.250387771	1.5253	1.6897
P132-10	@ P/Po= 0.239496722	1.0212	1.2782
P132-100	@ P/Po= 0.240538100	1.0338	1.2622
P132-181	@ P/Po= 0.239166091	0.5102	0.6448
P132-US	@ P/Po= 0.217359072	1.0983	1.6793
A132	@ P/Po= 0.240040610	0.5400	0.7614
A132-10	@ P/Po= 0.211218936	0.5383	0.7212
A132-100	@ P/Po= 0.238791097	0.4258	0.5538
SG132	@ P/Po= 0.237989353	0.6359	0.8350
SG132-10	@ P/Po= 0.238576905	0.6794	0.8689
SG132-100	@ P/Po= 0.241960361	0.5518	0.7034
SG132-10-US	@ P/Po= 0.225692889	0.5693	0.7510
SG132-100-US	@ P/Po= 0.225935246	1.0983	1.4963
G-10-US			0.751
G100-US			1.496
G132-US			1.679
WS132	@ P/Po= 0.237823664	0.6582	0.8663
WS132-10	@ P/Po= 0.238612476	0.6191	0.7912
WS132-100	@ P/Po= 0.238398832	0.6255	0.8143
A-1e	@ P/Po=0.238098138	0.6518	0.8368
A-5e	@ P/Po=0.243184477	0.6263	0.7865
A-10e	@ P/Po=0.243163236	0.4899	0.6170
A-50e	@ P/Po=0.243225512	0.4489	0.5730
G-1e	@ P/Po=0.238496102	0.5489	0.7038
G-5e	@ P/Po=0.242792602	0.5621	0.7086
G-10e	@ P/Po=0.243066031	0.5021	0.6363
G-50e	@ P/Po=0.238291132	0.4913	0.6333
P-1e	@ P/Po=0.240842223	1.1413	1.4442
P-5e	@ P/Po=0.240789274	1.0187	1.3288
P-10e	@ P/Po=0.240116967	1.1015	1.3657
P-50e	@ P/Po=0.240072114	1.0089	1.2593
P-100e	@ P/Po=0.236541386	0.9116	1.1677
S	@ P/Po=0.225335038	0.0147	0.0279
S-1e	@ P/Po=0.217142291	0.0193	0.0372

(continued)

Sample ID	Single point surface area (m^2/g)		BET Surface Area (m^2/g)
S-5e	@ P/Po=0.133107838	0.0201	0.0485
S-10e	@ P/Po=0.244886517	0.0236	0.0317
S-30e	@ P/Po=0.237929400	0.0309	0.0428
S-50e	@ P/Po=0.245494494	0.0262	0.0365
S-100e	@ P/Po=0.224698551	0.0368	0.0506
St	@ P/Po=0.238324949	0.3126	0.4013
St-1e	@ P/Po=0.238432726	0.3254	0.4223
St-5e	@ P/Po=0.238363587	0.3106	0.4071
St-10e	@ P/Po=0.238341099	0.3205	0.4268
St-30e	@ P/Po=0.238629889	0.3118	0.4189
St-50e	@ P/Po=0.244630980	0.3119	0.3969
St-100e	@ P/Po=0.238421621	0.2932	0.3677

[0252] The BET model for isotherms is a widely used theory for calculating the specific surface area. The analysis involves determining the monolayer capacity of the sample surface by calculating the amount required to cover the entire surface with a single densely packed layer of krypton. The monolayer capacity is multiplied by the cross sectional area of a molecule of probe gas to determine the total surface area. Specific surface area is the surface area of the sample aliquot divided by the mass of the sample.

Reference Example 19 - Fiber Length Determination of Irradiated Materials

[0253] Fiber length distribution testing was performed in triplicate on the samples submitted using the Techpap MorFi LBO1 system. The average fiber length and width are reported in Table 25.

Table 25. Summary of Lignocellulosic Fiber Length and Width Data

Sample ID	Arithmetic Average (mm)	Average Length Weighted in Length (mm)	Statistically Corrected Average Length Weighted in Length (mm)	Width (micrometers) (μm)
P132-10	0.484	0.615	0.773	24.7
P132-100	0.369	0.423	0.496	23.8
P132-181	0.312	0.342	0.392	24.4
A132-10	0.382	0.423	0.650	43.2
A132-100	0.362	0.435	0.592	29.9
SG132-10	0.328	0.363	0.521	44.0
SG132-100	0.325	0.351	0.466	43.8
WS132-10	0.353	0.381	0.565	44.7
WS132-100	0.354	0.371	0.536	45.4

Reference Example 20 - Ultrasonic Treatment of Irradiated and Un-irradiated Switchgrass

[0254] Switchgrass was sheared according to Example 4. The switchgrass was treated by ultrasound alone or irradiation with 10 Mrad or 100 Mrad of gamma rays, and then sonicated. The resulting materials correspond to G132-BR (un-irradiated), G132-10-BR (10 Mrad and sonication) and G132-100-BR (100 Mrad and sonication), as presented in Table 1. Sonication was performed on each sample for 30 minutes using 20kHz ultrasound from a 1000W horn under re-circulating conditions. Each sample was dispersed in water at a concentration of about 0.10 g/mL.

[0255] FIGS. 30 and 31 show the apparatus used for sonication. Apparatus 500 includes a converter 502 connected to booster 504 communicating with a horn 506 fabricated from titanium or an alloy of titanium. The horn, which has a seal 510 made from VITON® about its perimeter on its processing side, forms a liquid tight seal with a processing cell

508. The processing side of the horn is immersed in a liquid, such as water, that has dispersed therein the sample to be sonicated. Pressure in the cell is monitored with a pressure gauge 512. In operation, each sample is moved by pump 517 from tank 516 through the processing cell and is sonicated. After, sonication, the sample is captured in tank 520. The process can be reversed in that the contents of tank 520 can be sent through the processing cell and captured in tank 516. This process can be repeated a number of times until a desired level of processing is delivered to the sample.

Reference Example 21 - Scanning Electron Micrographs of Un-irradiated Switchgrass in Comparison to Irradiated and Irradiated and Sonicated Switch-grasps

[0256] Switchgrass samples for the scanning electron micrographs were applied to carbon tape and gold sputter coated (70 seconds). Images were taken with a JEOL 6500 field emission scanning electron microscope.

[0257] FIG. 32 is a scanning electron micrograph at 1000 X magnification of a fibrous material produced from shearing switchgrass on a rotary knife cutter, and then passing the sheared material through a 1/32 inch screen.

[0258] FIGS. 33 and 34 are scanning electron micrographs of the fibrous material of FIG. 32 after irradiation with 10 Mrad and 100 Mrad gamma rays, respectively, at 1000 X magnification.

[0259] FIG. 35 is a scanning electron micrographs of the fibrous material of FIG. 32 after irradiation with 10 Mrad and sonication at 1000 X magnification.

[0260] FIG. 36 is a scanning electron micrographs of the fibrous material of FIG. 32 after irradiation with 100 Mrad and sonication at 1000 X magnification.

Reference Example 22- Fourier Transform Infrared (FT-IR) Spectrum of Irradiated and Unirradiated Kraft Paper

[0261] FT-IR analysis was performed on a Nicolet/Impact 400. The results indicate that samples P132, P132-10, P132-100, P-1e, P-5e, P-10e, P-30e, P-70e, and P-100e are consistent with a cellulose-based material.

[0262] FIG. 37 is an infrared spectrum of Kraft board paper sheared according to Example 4, while FIG. 38 is an infrared spectrum of the Kraft paper of FIG. 37 after irradiation with 100 Mrad of gamma radiation. The irradiated sample shows an additional peak in region A (centered about 1730 cm^{-1}) that is not found in the unirradiated material. Of note, an increase in the amount of a carbonyl absorption at ~1650 cm^{-1} was detected when going from P132 to P132-10 to P132-100. Similar results were observed for the samples P-1e, P-5e, P-10e, P-30e, P-70e, and P-100e.

Reference Example 23- Proton and Carbon-13 Nuclear Magnetic Resonance (^{1}H-NMR and ^{13}C-NMR) Spectra of Irradiated and Unirradiated Kraft Paper

Sample Preparation

[0263] The samples P132, P132-10, P132-100, P-1e, P-5e, P-10e, P-30e, P-70e, and P-100e were prepared for analysis by dissolution with DMSO-d_6 with 2% tetrabutyl ammonium fluoride trihydrate. The samples that had undergone lower levels of irradiation were significantly less soluble than the samples with higher irradiation. Unirradiated samples formed a gel in this solvent mixture, but heating to 60 °C resolved the peaks in the NMR spectra. The samples having undergone higher levels of irradiation were soluble at a concentration of 10% wt/wt.

Analysis

[0264] ^{1}H-NMR spectra of the samples at 15 mg/mL showed a distinct very broad resonance peak centered at 16ppm (FIGS. 38A-38J). This peak is characteristic of an exchangeable -OH proton for an enol and was confirmed by a "d_2O shake." Model compounds (acetylacetone, glucuronic acid, and keto-gulonic acid) were analyzed and made a convincing case that this peak was indeed an exchangeable enol proton. This proposed enol peak was very sensitive to concentration effects, and we were unable to conclude whether this resonance was due to an enol or possibly a carboxylic acid.

[0265] The carboxylic acid proton resonances of the model compounds were similar to what was observed for the treated cellulose samples. These model compounds were shifted up field to ~5-6 ppm. Preparation of P-100e at higher concentrations (~10% wt/wt) led to the dramatic down field shifting to where the carboxylic acid resonances of the model compounds were found (~6 ppm) (FIG. 38N). These results lead to the conclusion that this resonance is unreliable for characterizing this functional group, however the data suggests that the number of exchangeable hydrogens increases with increasing irradiation of the sample. Also, no vinyl protons were detected.

[0266] The ^{13}C NMR spectra of the samples confirm the presence of a carbonyl of a carboxylic acid or a carboxylic acid derivative. This new peak (at 168 ppm) is not present in the untreated samples (FIG. 38K). A ^{13}C NMR spectrum with a long delay allowed the quantitation of the signal for P-100e (FIGS. 38L-38M). Comparison of the integration of the carbonyl resonance to the resonances at approximately 100 ppm (the C1 signals) suggests that the ratio of the

carbonyl carbon to C1 is 1:13.8 or roughly 1 carbonyl for every 14 glucose units. The chemical shift at 100 ppm correlates well with glucuronic acid.

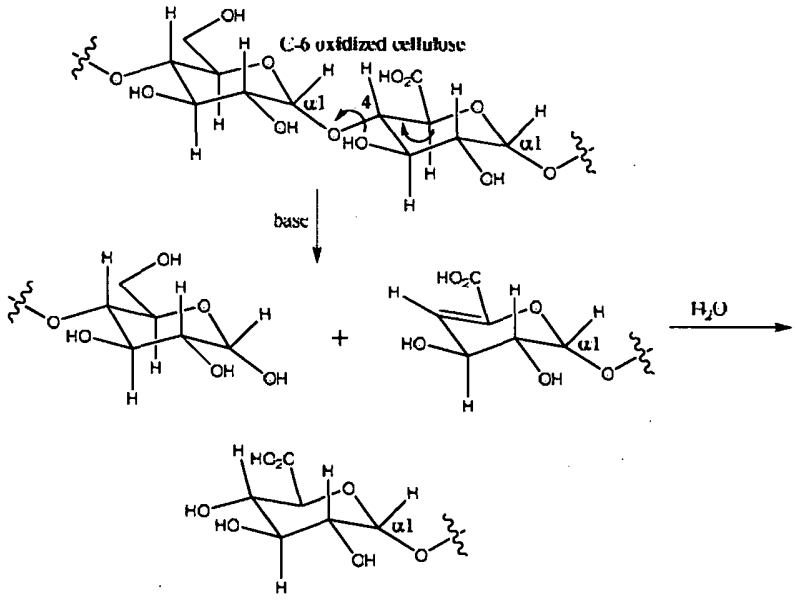
Titration

[0267] Samples P-100e and P132-100 (1g) were suspended in deionized water (25 mL). The indicator alizarin yellow was added to each sample with stirring. P-100e was more difficult to wet. Both samples were titrated with a solution of 0.2M NaOH. The end point was very subtle and was confirmed by using pH paper. The starting pH of the samples was ~ 4 for both samples. P132-100 required 0.4 milliequivalents of hydroxide, which gives a molecular weight for the carboxylic acid of 2500 amu. If 180 amu is used for a monomer, this suggests there is one carboxylic acid group for 13.9 monomer units. Likewise, P-100e required 3.2 milliequivalents of hydroxide, which calculates to be one carboxylic acid group for every 17.4 monomer units.

Conclusions

[0268] The C-6 carbon of cellulose appears to be oxidized to the carboxylic acid (a glucuronic acid derivative) in this oxidation is surprisingly specific. This oxidation is in agreement with the IR band that grows with irradiation at ~ 1740 cm^{-1}, which corresponds to an aliphatic carboxylic acid. The titration results are in agreement with the quantitative ^{13}C NMR. The increased solubility of the sample with the higher levels of irradiation correlates well with the increasing number of carboxylic acid protons. A proposed mechanism for the degradation of "C-6 oxidized cellulose" is provided below in Scheme 1.

Scheme 1



Reference Example 24 - Combination of Electron Beam and Sonication Pretreatment

[0269] Switchgrass is used as the feedstock and is sheared with a Munson rotary knife cutter into a fibrous material. The fibrous material is then evenly distributed onto an open tray composed of tin with an area of greater than about 500 in2. The fibrous material is distributed so that it has a depth of about 1 - 2 inches in the open tray. The fibrous material may be distributed in plastic bags at lower doses of irradiation (under 10 MRad), and left uncovered on the metal tray at higher doses of radiation.

[0270] Separate samples of the fibrous material are then exposed to successive doses of electron beam radiation to achieve a total dose of 1 Mrad, 2 Mrad, 3, Mrad, 5 Mrad, 10 Mrad, 50 Mrad, and 100 Mrad. Some samples are maintained under the same conditions as the remaining samples, but are not irradiated, to serve as controls. After cooling, the irradiated fibrous material is sent on for further processing through a sonication device.

[0271] The sonication device includes a converter connected to booster communicating with a horn fabricated from titanium or an alloy of titanium. The horn, which has a seal made from VITON® about its perimeter on its processing side, forms a liquid tight seal with a processing cell. The processing side of the horn is immersed in a liquid, such as

water, into which the irradiated fibrous material to be sonicated is immersed. Pressure in the cell is monitored with a pressure gauge. In operation, each sample is moved by pump through the processing cell and is sonicated.

[0272] To prepare the irradiated fibrous material for sonication, the irradiated fibrous material is removed from any container (e.g., plastic bags) and is dispersed in water at a concentration of about 0.10 g/mL. Sonication is performed on each sample for 30 minutes using 20 kHz ultrasound from a 1000 W horn under re-circulating conditions. After sonication, the irradiated fibrous material is captured in a tank. This process can be repeated a number of times until a desired level of processing is achieved based on monitoring the structural changes in the switchgrass. Again, some irradiated samples are kept under the same conditions as the remaining samples, but are not sonicated, to serve as controls. In addition, some samples that were not irradiated are sonicated, again to serve as controls. Thus, some controls are not processed, some are only irradiated, and some are only sonicated.

Example 25 - Microbial Testing of Pretreated Biomass

[0273] Specific lignocellulosic materials pretreated as described herein are analyzed for toxicity to common strains of yeast and bacteria used in the biofuels industry for the fermentation step in ethanol production. Additionally, sugar content and compatibility with cellulase enzymes are examined to determine the viability of the treatment process. Testing of the pretreated materials is carried out in two phases as follows.

Phase 1 :Toxicity and Sugar Content

[0274] Toxicity of the pretreated grasses and paper feedstocks is measured in yeast *Saccharomyces cerevisiae* (wine yeast) and *Pichia stipitis* (ATCC 66278) as well as.the bacteria *Zymomonas mobilis* (ATCC 31821) and *Clostridium thermocellum* (ATCC 31924). A growth study is performed with each of the organisms to determine the optimal time of incubation and sampling.

[0275] Each of the feedstocks is then incubated, in duplicate, with *S. cerevisiae, P. stipitis, Z. mobilis,* and *C. thermocellum* in a standard microbiological medium for each organism. YM broth is used for the two yeast strains, *S. cerevisiae* and *P. stipitis.* RM medium is used for *Z. mobilis* and CM4 medium for *C. thermocellum.* A positive control, with pure sugar added, but no feedstock, is used for comparison. During the incubation, a total of five samples is taken over a 12 hour period at time 0, 3, 6, 9, and 12 hours and analyzed for viability (plate counts for *Z. mobilis* and direct counts for *S. cerevisiae*) and ethanol concentration.

[0276] Sugar content of the feedstocks is measured using High Performance Liquid Chromatography (HPLC) equipped with either a Shodex™ sugar SP0810 or Biorad Aminex® HPX-87P column. Each of the feedstocks (approx. 5 g) is mixed with reverse osmosis (RO) water for 1 hour. The liquid portion of the mixture is removed and analyzed for glucose, galactose, xylose, mannose, arabinose, and cellobiose content. The analysis is performed according to National Bioenergy Center protocol *Determination of Structural Carbohydrates and Lignin in Biomass.*

Phase 2: Cellulase Compatibility

[0277] Feedstocks are tested, in duplicate, with commercially available Accellerase™ 1000, which contains a complex of enzymes that reduces lignocellulosic biomass into fermentable sugars, at the recommended temperature and concentration in an Erlenmeyer flask. The flasks are incubated with moderate shaking at around 200 rpm for 12 hours. During that time, samples are taken every three hours at time 0, 3, 6, 9, and 12 hours to determine the concentration of reducing sugars (Hope and Dean, Biotech J., 1974, 144:403) in the liquid portion of the flasks.

Example 26- Sugar Concentration Analysis using HPLC

[0278] 13 samples were analyzed for sugar concentration (HPLC) and toxicity against 3 microorganisms (*Pichia stipitis, Saccharomyces cerevisiae, and Zymomonas mobilis.* Table 26 lists the equipment used for these experiments. Table 27 and 28 provide a list of the sugars (including vendor and lot numbers) used to prepare the HPLC standard and the protocol used to prepare the HPLC standard, respectively.

Table 26. Equipment Utilized in Experiments

Equipment	Manufacturer, Name
pH meter	Orion
Shakers (2)	B. Braun Biotech, Certomat BS-1
HPLC	Waters, 2690 HPLC Module
Spectrophotometer	Unicam, UV300

(continued)

Equipment	Manufacturer, Name
YSI Biochem Analyzer	Interscience, YSI

Table 27. Sugars used in HPLC analysis

Sugar	Manufacturer	Ref #	Lot #
glucose		49140	1284892
xylose		95731	1304473 51707231
cellobiose	BioChemika	22150	130315714806191
arabinose		10840	1188979 24105272
mannose		63582	363063/1 22097
galactose.		48259	46032/1 33197

Table 28. Preparation of HPLC standards

Desired Concentration (mg/mL)	Volume of sugar solution	Volume of Nanopure Water (mL)	Total Volume (mL)
4	50 mL of 4 mg/mL	0	50
2	25 mL of 4 mg/mL	25	50
1	25 mL of 2 mg/mL	25	50
0.5	25 mL of 1 mg/mL	25	50
0.1	5 ml of 1 mg/mL	20	25
Verification Standard 1.5 mg/mL	18.75 mL of 4 mg/mL	31.25	50

Analysis

[0279] Each sample (1 gram) was mixed with reverse osmosis water at 200 rpm and 50 °C overnight. The pH of the sample was adjusted to between 5 and 6 and filtered through a 0.2 μm syringe filter. Samples were stored at -20 °C prior to analysis to maintain integrity of the samples. The observations made during the preparation of the samples are presented in Table 29.

Table 29. Observations During HPLC Sample Preparation

Sample	Amount used (g)	Water added (mL)	pH	Observations
P132	1	30	5.38	Fluffy, difficult to mix
P132-10	**1**	**25**	**6.77**	**Fluffy, difficult to mix**
P132-100	**1**	**20**	**3.19**	**pH is low, difficult to bring to pH 5.0, used 10 N NaOH**
P132-US	**0.3**	**5**	**6.14**	
A132	1	15	5.52	
A132-10	**1**	**15**	**4.9**	
A132-100	1	15	5.39	
SG132	1	15	5.59	
SG132-10	1	15	5.16	
SG132-100	**1**	**15**	**4.7**	
SG132-10-US	0.3	5	5.12	
SG132-100-US	**0.3**	**5**	**4.97**	

(continued)

Sample	Amount used (g)	Water added (mL)	pH	Observations
WS132	1	15	5.63	
WS132-10	1	15	5.43	
WS132-100	1	15	5.02	

*pH of these samples was adjusted to pH using 1N NaOH

[0280] Standards were prepared fresh from a 4 mg/mL stock solution of the 6 combined sugars, glucose, xylose, cellobiose, arabinose, mannose, and galactose. The stock solution was prepared by dissolving 0.400 grams of each sugar into 75 mL of nanopure water (0.3 micron filtered). Once dissolved, the stock solution was diluted to 100 mL using a volumetric flask and stored at -20 °C. Working standard solutions of 0.1, 0.5, 1, 2, and 4 mg/mL were prepared by serial dilution of the stock solution with nanopure water. In addition, a verification standard of 1.5 mg/mL was also prepared from the stock solution.

[0281] Sugar concentrations were analyzed according to the protocol *Determination of Structural Carbohydrates in Biomass* (NREL Biomass Program, 2006) and this protocol is incorporated herein by reference in its entirety. A SHODEX SUGAR SP0810 COLUMN with an Evaporative Light Scattering Detector was used. A verification standard (1.5 mg/mL of standard) was analyzed every 8 injections to ensure that the integrity of the column and detector were maintained during the experiment. The standard curve coefficient of variation (R^2 value) was at least 0.989 and the concentration of the verification standards were within 10% of the actual concentration. The HPLC conditions were as follows:

Table 30. HPLC Parameters

Injection volume:	20 μL
Mobile phase:	nanopure water*, 0.45 μm filtered and degassed
Flow rate:	0.5 mL/min
Column temperature:	85 °C
Detector temperature:	evaporator temperature 110 °C, nebulizer temperature 90 °C

*Initial tests noted that better separation was observed when using nanopure water than 15/85 acetonitrile:water in the mobile phase (manufacturer does not recommend using greater than 20% acetonitrile with this column).

Results

[0282] The results of the HPLC analysis are presented in Tables 31, 32, and 33.

Table 31. Sugar Concentration Expressed as mg/mL and mg/g of Extract

Sample ID	Xylose mW ~150 $C_5H_{10}O_5$ Mono		Arabinose mW ~150 $C_5H_{10}O_5$ Mono		Glucose mW~180 $C_6H_{12}O_6$ Mono		Galactose (see gluc) mg/mL:mg/g		Mannose (see gluc) mg/mL:mg/g		Cellobios mW~34 $C_{12}H_{22}O$ Disacc	
	mg /mL	mg/g	mg /mL	mg/g	mg /mL	mg/g	mg /mL	mg/g	mg /mL	mg/g	mg /mL	n
P												
P-132	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	(
P-132-10	0.00	0.00	0.00	0.00	0.34	8.60	0.00	0.00	0.00	0.00	00.33	8
P-132-100	0.35	7.04	0.00	0.00	0.34	6.14	0.00	0.00	0.00	0.00	0.36	7
P-132-BR	0.35	5.80	0.43	7.17	0.34	5.62	0.00	0.00	0.00	0.00	0.00	(
G												
G-132	0.39	5.88	0.38	5.73	0.84	12.66	0.34	5.04	0.92	13.76	0.00	(
G-132-10	0.50	7.50	0.41	6.18	1.07	16.04	0.35	5.19	0.98	14.66	0.00	(
G-132-100	0.00	0.00	0.37	5.54	0.41	6.14	0.00	0.00	0.55	8.28	0.45	6
G-132-10-US	0.34	5.73	0.39	6.45	0.33	5.43	0.00	0.00	0.00	0.00	0.00	(
G-132-100-US	0.00	0.00	0.37	6.22	0.35	5.90	0.33	5.43	0.40	6.70	0.39	6
A												
A-132	1.36	20.39	0.00	0.00	1.08	16.22	0.39	5.84	1.07	16.02	0.00	(
A-132-10	1.19	17.87	0.00	0.00	0.00	0.00	0.00	0.00	0.37	5.52	0.00	(
A-132-100	1.07	16.11	0.00	0.00	0.35	5.18	0.00	0.00	0.00	0.00	0.81	
WS												
WS-132	0.49	7.41	0.41	6.15	0.39	5.90	0.00	0.00	0.00	0.00	0.00	(
WS-132-10	0.57	8.49	0.40	5.99	0.73	10.95	0.34	5.07	0.50	7.55	0.00	(
WS-132-100	0.43	6.39	0.37	5.51	0.36	5.36	0.00	0.00	0.36	5.33	0.35	5

Table 32. Sugar Concentration Expressed at % of Paper

Sugar concentration (% of dry sample)	P132	P132-10	P132-100	P132-US
cellobiose	0.00	0.81	0.72	0.00
glucose	0.00	0.86	0.67	0.56
xylose	0.00	0.00	0.70	0.58
galactose	0.00	0.00	0.00	0.00
arabinose	0.00	0.00	0.00	0.72
mannose	0.00	0.00	0.00	0.00

Table 33. Sugar Concentration Expressed at % of Total Sample

Sugar concentration (% of dry sample)		A132	A132-10	A132-100	SG132	SG132-10	SG132-100	SG132-10-US	SG132-100-US	WS132	WS132-10	W132-100
cellobiose		0.00	0.00	1.22	0.00	0.00	0.67	0.00	0.65	0.00	0.00	0.53
glucose		1.62	0.00	0.52	1.27	1.60	0.61	0.54	0.59	0.59	1.10	0.54
xylose		2.04	1.79	1.61	0.59	0.75	0.00	0.57	0.00	0.74	0.85	0.64
galactose		0.58	0.00	0.00	0.50	0.52	0.00	0.00	0.54	0.00	0.51	0.00
arabinose		0.00	0.00	0.00	0.57	0.62	0.55	0.65	0.62	0.62	0.60	0.55
mannose		1.60	0.55	0.00	1.38	1.47	0.83	0.00	0.67	0.00	0.76	0.53

Example 27- Toxicity Study

[0283] Twelve samples were analyzed for toxicity against a panel of three ethanol-producing cultures. In this study, glucose was added to the samples in order to distinguish between starvation of the cultures and toxicity of the samples. A thirteenth sample was tested for toxicity against *Pichia stipitis.* A summary of the protocol used is listed in Table 32. A description of the chemicals and equipment used in the toxicity testing is reported in Tables 34-36.

Table 34. Conditions for Toxicity Testing

Variable	Organism		
	***Zymomonas mobilis* ATCC 31821**	***Saccharomyces cerevesiae* ATCC 24858**	***Pichia stipitis* NRRL Y-7124**
Test Repetition	Duplicate		
Inoculation Volume (mL)	1	0.1	1
Incubation Temperature	30 °C	25 °C	25 °C
Shaker Speed (rpm)	125	200	125
Erlenmeyer Flask Volume	250 mL	500 mL	250 mL
Media volume	100 mL	100 mL	100 mL
Total Incubation time (hours)	36	36	48
Ethanol Analysis (hours)	24, 30, 36	24, 30, 36	24, 36, 48
Cell Counts (hours)	24, 36	24 ,36	24, 48
pH	0 hours	0 hours	0 hours

Table 35. Reagents Used for Toxicity Testing

Media Component	Manufacturer	Reference #	Lot #
Urea	ScholAR Chemistry	9472706	AD-7284-43
Yeast Nitrogen Base	Becton Dickinson	291940	7128171
Peptone	Becton Dickinson	211677	4303198
Xylose	Fluka	95731	1304473 51707231
Glucose	Sigma	G-5400	107H0245
Yeast Extract (used for *S. cerevisiae*)	Becton Dickinson	288620	4026828
Yeast Extract (used for *P. stipitis* and *Z. mobilis*)	Becton Dickinson	212750	7165593
$MgSO_4$ $7H_2O$	Sigma	M5921	034K0066
$(NH_4)_2SO_4$	Sigma	A4418	117K5421
KH_2PO_4	Sigma	P5379	074K0160
YM Broth	Becton Dickinson	271120	6278265

Table 36. YSI Components Used in Shake Flask Study

Component	Catalog #	Lot #
YSI Ethanol Membrane	2786	07L100153
YSI Ethanol Standard (3.2 g/L)	2790	012711040
YSI Ethanol Buffer	2787	07M1000053, 07100215

[0284] Testing was performed using the three microorganisms as described below.

***Saccharomyces cerevisiae* ATCC 24858 (American Type Culture Collection)**

[0285] A slant of *S. cerevisiae* was prepared from a rehydrated lyophilized culture obtained from ATCC. A portion of the slant material was streaked onto an YM Broth + 20 g/L agar (pH 5.0) and incubated at 30 °C for 2 days. A 250 mL Erlenmeyer flask containing 50 mL of medium (20 g/L glucose, 3 g/L yeast extract, and 5.0 g/L peptone, pH 5.0) was inoculated with one colony from the YM plate and incubated for 24 hours at 25 °C and 200 rpm. After 23 hours of growth, a sample was taken and analyzed for optical density (600 nm in a UV spectrophotometer) and purity (Gram stain). Based on these results, one flask (called the Seed Flask) with an OD of 14.8 and clean Gram Stain was chosen to inoculate all of the test flasks.

[0286] The test vessels were 500 mL Erlenmeyer flasks containing 100 mL of the sterile medium described above. All flasks were autoclaved at 121 °C and 15 psi prior to the addition of the test materials. The test materials were not sterilized, as autoclaving will change the content of the samples. The test samples were added at the time of inoculation (rather than prior to) to reduce the possibility of contamination. In addition to the test samples, 1 mL (1% v/v) of seed flask material was added to each flask. The flasks were incubated as described above for 36 hours.

***Pichia stipitis* NRRL Y-7124 (ARS Culture Collection)**

[0287] A slant of *P. stipitis* was prepared from a rehydrated lyophilized culture obtained from ARS Culture Collection. A portion of the slant material was streaked onto an YM Broth + 20 g/L agar (pH 5.0) and incubated at 30 °C for 2 days. A 250 mL Erlenmeyer flask containing 100 mL of medium (40 g/L glucose, 1.7 g/L yeast nitrogen base, 2.27 g/L urea, 6.56 g/L peptone, 40 g/L xylose, pH 5.0) was inoculated with a small amount of plate material and incubated for 24 hours at 25 °C and 125 rpm. After 23 hours of growth, a sample was taken and analyzed for optical density (600 nm in a UV spectrophotometer) and purity (Gram stain). Based on these results, one flask (called the Seed Flask) at an optical density of 5.23 and with a clean Gram Stain was chosen to inoculate all of the test flasks.

[0288] The test vessels were 250 mL Erlenmeyer flasks containing 100 mL of the sterile medium described above. All flasks were autoclaved empty at 121 °C and 15 psi and filter sterilized (0.22 μm filter) media added to the flasks prior to the addition of the test materials. The test materials were not sterilized, as autoclaving will change the content of the samples and filter sterilization not appropriate for sterilization of solids. The test samples were added at the time of inoculation (rather than prior to) to reduce the possibility of contamination. In addition to the test samples, 1 mL (1% v/v) of seed flask material was added to each flask. The flasks were incubated as described above for 48 hours.

***Zymomonas mobilis* ATCC 31821 (American Type Culture)**

[0289] A slant of *Z. mobilis* was prepared from a rehydrated lyophilized culture obtained from ATTC. A portion of the slant material was streaked onto DYE plates (glucose 20 g/L, Yeast Extract 10 g/L, Agar 20 g/L, pH 5.4) and incubated at 30 °C and 5% CO_2 for 2 days. A 20 mL screw-cap test tube containing 15 mL of medium (25 g/L glucose, 10 g/L yeast extract, 1 g/L $MgSO_4$ $7H_2O$, 1 g/L $(NH_4)_2SO_4$, 2 g/L KH_2PO_4, pH 5.4) was inoculated with one colony and incubated for 24 hours at 30 °C with no shaking. After 23 hours of growth, a sample was taken and analyzed for optical density (600 nm in a UV spectrophotometer) and purity (gram stain). Based on these results, one tube (OD 1.96) was chosen to inoculate the second seed flask. The second seed flask was a 125 ml flask containing 70 mL of the media described above and was inoculated with 700 μL (1% v/v) and incubated for 24 hours at 30 °C with no shaking. After 23 hours of growth, a sample was taken and analyzed for optical density (600 nm in a UV spectrophotometer) and purity (gram stain). Based on these results, one flask (called the Seed Flask) with an OD of 3.72 was chosen to inoculate all of the test flasks.

[0290] The test vessels were 250 mL Erlenmeyer flasks containing 100 mL of the sterile medium described above with the exception of yeast extract at 5 g/L. All flasks were autoclaved empty at 121 °C and 15 psi and filter sterilized (0.22 μm filter) media added to the flasks prior to the addition of the test materials. The test materials were not sterilized, as autoclaving will change the content of the samples and filter sterilization not appropriate for sterilization of solids. The test samples were added at the time of inoculation to reduce the possibility of contamination. In addition to the test samples, 1 mL (1% v/v) of seed flask material was added to each flask. The flasks were incubated as described above for 36 hours

Analysis

[0291] Two samples were analyzed for cell concentration (using spread plating for *Z. mobilis* and direct counts (haemocytometer and microscope for *S. cerevisiae* and *P. stipitis*). Appropriately diluted samples of *Z. mobilis* were spread on Dextrose Yeast Extract (glucose 20 g/L, Yeast Extract 10 g/L, Agar 20 g/L, pH 5.4) plates, incubated at 30 °C and 5% CO2 for 2 days, and the number of colonies counted. Appropriately diluted samples of *S. cerevisiae* and *P. stipitis* were

mixed with 0.05% Trypan blue, loaded into a Neubauer haemocytometer. The cells were counted under 40 X magnification.

[0292] Three samples were analyzed for ethanol concentration using the YSI Biochem Analyzer based on the alcohol dehydrogenase assay (YSI, Interscience). Samples were centrifuged at 14,000 rpm for 20 minutes and the supernatant stored at -20 °C to preserve integrity. The samples were diluted to between 0-3.2 g/L ethanol prior to analysis. A standard of 3.2 g/L ethanol was analyzed approximately every 30 samples to ensure the integrity of the membrane was maintained during analysis. The optical density (600 nm) of the samples is not reported because the solid test samples interfered with absorbance measurement by increasing the turbidity of the samples and are inaccurate.

Results of Ethanol Analysis

[0293] Performance was used to compare each sample to the control for each microorganism (Tables 37-39). However, the % performance cannot be used to compare between strains. When comparing strains, the total concentration of ethanol should be used. When analyzing the data, a % performance of less than 80% may indicate toxicity when accompanied by low cell number. The equation used to determine % performance is:

$$\% \text{ Performance} = (\text{ethanol in the sample}/\text{ethanol in control}) \times 100$$

Table 37. Ethanol Concentration and % Performance Using *Saccharomyces cerevisiae*

	24 hours		30 hours		36 hours	
Sample #	Ethanol Concentration (g/L)	% Performance	Ethanol Concentration (g/L)	% Performance	Ethanol Concentration (g/L)	% Performance
P132	4.0	140	5.2	127	3.26	176
P132-10	4.2	147	5.1	125	3.86	209
P132-100	4.3	149	5.6	136	3.47	187
A132	5.5	191	6.5	160	5.24	283
A132-10	1.9	67	6.3	153	5.54	299
A132-100	4.4	154	5.6	137	4.04	218
G132	5.3	186	6.0	146	3.99	215
G132-10	5.2	180	6.4	156	4.63	250
G132-100	5.5	191	6.3	155	4.60	248
WS132	4.8	168	6.3	155	4.51	244
WS132-10	4.9	172	6.0	146	4.55	246
WS132-100	4.9	170	5.7	140	4.71	254
Control	2.9	100	4.1	100	1.85	100

Table 38. Ethanol Concentration and % Performance Using *Pichia stipitis*

	24 hours		36 hours		48 hours	
Sample #	**Ethanol Concentration (g/L)**	**% Performance**	**Ethanol Concentration (g/L)**	**% Performance**	**Ethanol Concentration (g/L)**	**% Performance**
P132	2.8	130	3.4	188	8.1	176
P132-10	7.3	344	11.9	655	15.8	342
P132-100	5.2	247	8.6	472	13.3	288
A132	12.2	575	14.7	812	14.9	324
A132-10	**15.1**	**710**	**18.7**	**1033**	**26.0**	**565**
A132-100	**10.9**	**514**	**16.7**	**923**	**22.2**	**483**
G132	8.0	375	12.9	713	13.3	288
G132-10	**10.1**	**476**	**16.0**	**884**	**22.3**	**485**
G132-100	**8.6**	**406**	**15.2**	**837**	**21.6**	**470**
WS132	9.8	460	14.9	820	17.9	389
WS132-10	7.8	370	16.1	890	19.3	418
WS132-100	9.1	429	15.0	829	15.1	328
Sample A*	**13.2**	**156**	**19.0**	**166**	**20.6**	**160**
Control	2.1	100	1.8	100	4.6	100

Samples in BOLD were the highest ethanol producers, over 20 g/L and similar to the concentrations in wood hydrolyzates (H.K. Sreenath and T.W. Jeffries Bioresource Technology 72 (2000) 253-260).

* Analyzed in later shake flask experiment.

Table 39. Ethanol Concentration and % Performance Using *Zymomonas mobilis*

Sample #	24 hours		30 hours		36 hours	
	Ethanol Concentration (g/L)	% Performance	Ethanol Concentration (g/L)	% Performance	Ethanol Concentration (g/L)	% Performance
P132	7.5	85	6.8	84	7.5	93
P132-10	7.5	85	4.8	59	6.8	84
P132-100	7.3	83	6.2	77	7.1	88
A132	9.6	109	8.3	103	9.1	112
A132-10	9.2	105	8.4	105	8.8	109
A132-100	8.2	93	7.6	94	7.6	93
WS132	7.9	89	7.1	88	7.7	94
WS132-10	8.2	93	6.8	85	7.3	90
WS132-100	8.7	98	6.9	86	8.3	102
G132	8.7	99	7.1	88	8.1	99
G132-10	7.8	88	7.0	88	7.3	90
G132-100	8.6	98	7.8	98	8.3	102
Control	8.8	100	8.0	100	8.1	100

Results from Cell Concentration Analysis

[0294] % Cells is used to compare each sample to the control for each organism (Tables 40-42). However, the % cells cannot be used to compare between strains. When comparing strains, the total concentration of cells should be used. When analyzing the data, a % performance of less than 70% may indicate toxicity when accompanied by low ethanol concentration. The equation used to determine % performance is:

$$\% \text{ cells} = (\text{number of cell in the sample/number of cells in control}) \times 100$$

Table 40. Results from Cell Concentration Analysis for *Saccharomyces cererisiae*

Sample #	24 hours		36 hours	
	Cell Concentration (x 10^8/mL)	% Cells	Cell Concentration (x 10^8/mL)	% Cells
P132	1.99	166	2.51	83
P132-10	2.51	209	1.91	63
P132-100	1.35	113	1.99	66
A132	3.80	316	2.59	85
A132-10	1.73	144	3.90	129
A132-100	3.98	331	2.51	83
G132	2.14	178	3.12	103
G132-10	2.33	194	2.59	85
G132-100	3.57	298	2.66	88
WS132	4.10	341	2.66	88
WS132-10	2.63	219	2.81	93
WS132-100	2.29	191	2.40	79
Control	1.20	100	3.03	100

Table 41. Results from Cell Concentration Analysis for *Pichia stipitis*

Sample #	24 hours		48 hours	
	Cell Concentration (x 10^8/mL)	% Cells	Cell Concentration (x 10^8/mL)	% Cells
P132	16.4	108	20.3	87
P132-10	11.5	76	9.5	41
P132-100	6.5	43	17.8	76
A132	7.1	47	10.2	44
A132-10	12.7	84	9.3	40
A132-100	11.8	78	18.3	78
G132	4.5	30	4.8	21
G132-10	22.8	151	9.8	42
G132-100	10.1	67	21.7	93
WS132	17.6	117	8.2	35
WS132-10	5.3	35	10.8	46
WS132-100	9.3	62	10.7	46

(continued)

Sample #	24 hours		48 hours	
	Cell Concentration (x 10^8/mL)	% Cells	Cell Concentration (x 10^8/mL)	% Cells
Control	15.1	100	23.4	100

Table 42. Results from Cell Concentration Analysis for *Zymomonas mobilis*

Sample #	24 hours		36 hours	
	Cell Concentration (x 10^8/mL)	% Cells	Cell Concentration (x 10^8/mL)	% Cells
P132	7.08	86	2.97	66
P132-10	21.80	264	4.37	98
P132-100	4.50	54	3.35	75
A132	6.95	84	1.99	44
A132-10	6.13	74	4.05	91
A132-100	9.60	116	4.20	94
G132	7.48	90	3.84	86
G132-10	14.75	178	2.89	65
G132-100	6.00	72	2.55	57
WS132	9.70	117	4.55	102
WS132-10	13.20	160	4.32	97
WS132-100	5.15	62	2.89	65
Control	8.27	100	4.47	100

Reference Example 28- Shake Flask Fermentation of Cellulose Samples Using *P. stipitis*

Summary

[0295] Thirteen samples were tested for ethanol production in *P. stipitis* culture without sugar added. They were tested in the presence and absence of cellulase (Accellerase 1000® enzyme complex, Genencor). Equipment,and reagents used for the experiment are listed below in Tables 43-45.

Table 43. Equipment and frequency of maintenance

Equipment	Manufacturer	Frequency of Maintenance
Shakers (2)	B. Braun Biotech, Certomat BS-1	Quarterly
Spectrophotometer	Unicam, UV300	Biannual
YSI Biochem Analyzer	Interscience, YSI	Monthly

Table 44. YSI Components used in shake flask study

Component	Catalog #	Lot #
YSI Ethanol Membrane	2786	07L100153
YSI Ethanol Standard (3.2 g/L)	2790	012711040
YSI Ethanol Buffer	2787	07M 1000053, 07100215

Table 45. Chemicals used for shake flask fermentation

Media Component	Manufacturer	Reference #	Lot #
Urea	ScholAR Chemistry	9472706	AD-7284-43
Yeast Nitrogen Base	Becton Dickinson	291940	7128171
Peptone	Becton Dickinson	211677	4303198
YM Broth	Becton Dickinson	271120	6278265
Accellerase® Enzyme complex	Genencor	Accellerase® 1000	1600794133
Xylose	BioChemika	95731	1304473 51707231
Glucose	Sigma	G-5400	107H0245

[0296] A slant of *P. stipitis* NRRL Y-7124 was prepared from a rehydrated lyophilized culture obtained from ARS Culture Collection. A portion of the slant material was streaked onto a Yeast Mold (YM) Broth + 20 g/L agar (pH 5.0) and incubated at 30 °C for 2 days. A 250 mL Erlenmeyer flask containing 100 mL of medium (40 g/L glucose, 1.7 g/L yeast nitrogen base, 2.27 g/L urea, 6.56 g/L peptone, 40 g/L xylose, pH 5.0) was inoculated with one colony and incubated for 24 hours at 25 °C and 100 rpm. After 23 hours of growth, a sample was taken and analyzed for optical density (600 nm in a UV spectrophotometer) and purity (Gram stain). Based on these results, one flask (called the Seed Flask) at an optical density of 6.79 and with a clean Gram stain was chosen to inoculate all of the test flasks.

[0297] The test vessels were 250 mL Erlenmeyer flasks containing 100 mL of medium (1.7 g/L yeast nitrogen base, 2.27 g/L urea, and 6.56 g/L peptone). No sugar (glucose or xylose) was added to the growth flask medium. All flasks were autoclaved empty at 121 °C and 15 psi and filter sterilized (0.22 μm filter) media added to the flasks prior to the addition of the test materials. The test materials were not sterilized, as autoclaving will change the content of the samples and filter sterilization is not appropriate for sterilization of solids. The test samples (listed in Table 46) were added at the time of inoculation (rather than prior to) to reduce the possibility of contamination. In addition to the test samples, 1 mL (1% v/v) of seed flask material was added to each flask. Flasks containing sample P132-100 required the addition of 0.4 mL 1 M NaOH to bring the pH to 5.0. The flasks were incubated at 30 °C and 150 rpm above for 96 hours.

[0298] One set of duplicate flasks per feedstock contained Accellerase® enzyme complex (1.25 mL per flask, highest recommended dosage is 0.25 mL per gram of biomass, Genencor) to attempt simultaneous saccharification and fermentation (SSF). The other set of duplicate flasks did not contain Accellerase® enzyme complex. A total of 52 flasks were analyzed.

[0299] Six control flasks were also analyzed. Positive control flasks contained SolkaFloc 200 NF Powdered Cellulose (lot # UA158072, International Fiber Corporation) at a concentration of 2.5 grams per 100 mL flask (25 grams per L) with and without addition of Accellerase® enzyme complex. In addition, a control containing sugars (glucose and xylose) only was used.

Table 46. The amount of each feedstock added to each flask

Xyleco Number	Amount added to Flask (g/100 mL)
P132	2.5
P132-10	2.5
P132-100	2.5
A132	5
A132-10	5
A132-100	5
G132	5
G132-10	5
G132-100	5
WS132	5
WS132-10	5
WS132-100	5
Sample A	5

Analysis

[0300] Samples were analyzed for ethanol concentration (Tables 47, 48, and 49) using the YSI Biochem Analyzer based on the alcohol dehydrogenase assay (YSI, Interscience). Samples were centrifuged at 14,000 rpm for 20 minutes and the supernatant stored at -20 °C. The samples were diluted to between 0-3.2 g/L ethanol prior to analysis. A standard of 2.0 g/L ethanol was analyzed approximately every 30 samples to ensure the integrity of the membrane was maintained during analysis.

Results

[0301]

Table 47. Results of Control Flasks

Control	Ethanol Concentration (g/L)			
	24 hours	36 hours	48 hours	96 hours
Containing Glucose, no cellulose, no enzyme	13.20	19.00	20.60	21.60
Containing Crystalline Cellulose (Solka Floc), no sugar, no enzyme	0.00	0.00	0.00	0.00
Containing Crystalline Cellulose (Solka Floc) at 25 g/L, no sugar, Accellerase® added	6.56	7.88	9.80	8.65

Table 48. Results of Shake Flasks without Accellerase® 1000 Enzyme Complex

Sample Number	Ethanol Concentration (g/L)			
	24 hours	36 hours	48 hours	96 hours
P132	0.09	0.00	0.00	0.12
P132-10	0.02	0.01	0.02	0.17
P132-100	0.09	0.01	0.00	0.02
A132	1.74	1.94	2.59	3.70
A132-10	1.82	2.36	2.30	2.96
A132-100	0.30	0.73	1.31	2.38
G132	0.40	0.09	0.24	0.42
G132-10	0.69	0.42	0.22	0.24
G132-100	0.19	0.05	0.05	0.21
WS132	0.47	0.50	0.68	0.65
WS132-10	0.47	0.49	0.34	0.92
WS132-100	0.14	0.07	0.08	0.22
Sample A	1.88	1.89	2.30	3.28

Table 49. Results of Shake Flasks with Accellerase® 1000 Enzyme Complex

Sample Number	Ethanol Concentration (g/L)			
	24 hours	36 hours	48 hours	96 hours
P132	7.04	8.72	9.30	5.80
P132-10	4.22	4.48	4.49	1.24
P132-100	3.18	4.28	4.70	3.35

(continued)

Sample Number	Ethanol Concentration (g/L)			
	24 hours	36 hours	48 hours	96 hours
A132	2.79	2.91	2.03	4.30
A132-10	3.31	1.62	2.11	2.71
A132-100	2.06	1.92	1.02	1.47
G132	0.87	0.40	0.32	0.44
G132-10	1.38	1.04	0.63	0.07
G132-100	2.21	2.56	2.34	0.12
WS132	1.59	1.47	1.07	0.99
WS132-10	1.92	1.18	0.73	0.23
WS132-100	2.90	3.69	3.39	0.27
Sample A	2.21	2.35	3.39	2.98

Reference Example 29- Cellulase Assay

Summary

[0302] Thirteen samples were tested for cellulase susceptibility using an industry cellulase (Accellerase® 1000, Genencor) under optimum conditions of temperature and pH.

Protocol

[0303] The protocol is a modification of the NREL "Laboratory Analytical Procedure LAP-009 *Enzymatic Saccharification of Lignocellulosic Biomass".* A sample of material was added to 10 mL 0.1 M sodium citrate buffer (pH 4.8) and 40 mg/mL tetracycline (to prevent growth of bacteria) in a 50 mL tube in duplicate. The amount of sample added to each tube is listed in Table 50. Some samples were difficult to mix (P132, P132-10, P132-100), so were added at a lower concentration. A positive control of 0.2 grams SolkaFloc 200 NF Powdered Cellulose (lot # UA158072, International Fiber Corporation) and a negative control (no sample) were also included. Enough reverse osmosis (RO) water to bring the volume to a total of 20 mL was added to the tubes. Both the sodium citrate buffer and water were heated to 50 °C prior to use.

[0304] Accellerase® 1000 enzyme was added to each tube at a dosage of 0.25 mL per gram of biomass (highest dosage recommended by Genencor). The tubes were incubated at 45° angle at 150 rpm and 50 degrees C (recommended by Genencor) for 72 hours. Samples were taken at 0, 3, 6, 9, 12, 18, 24, 48, and 72 hours (Table 52 and 53), centrifuged at 14,000 rpm for 20 minutes and the supernatant frozen at -20 °C. The glucose concentration in the samples was analyzed using the YSI Biochem Analyzer (Interscience) using the conditions described in Table 51. A glucose standard solution of 2.5 g/L was prepared by dissolving 2.500 grams glucose (Sigma Cat# G7528-5KG, Lot#:107H0245) in distilled water. Once dissolved, the total volume was brought to 1 L with distilled water in a volumetric flask. The standard was prepared fresh weekly and stored at 4 °C.

Table 50. Amount of Each Sample Added

Xyleco Number	Amount added to Tube (g/20 mL)
P132	0.5
P132-10	0.5
P132-100	0.5
A132	0.75
A132-10	0.75
A132-100	0.75

(continued)

Xyleco Number	Amount added to Tube (g/20 mL)
G132	0.75
G132-10	0.75
G132-100	0.75
WS132	0.75
WS132-10	0.75
WS132-100	0.75
Sample A	0.75
SolkaFloc 200NF (Control)	0.2
Negative Control	0

Table 51. YSI Components Used in Shake Flask Study

Component	Catalog #	Lot #
YSI Glucose Membrane	2365	07D100124
YSI Glucose Buffer	2357	014614A

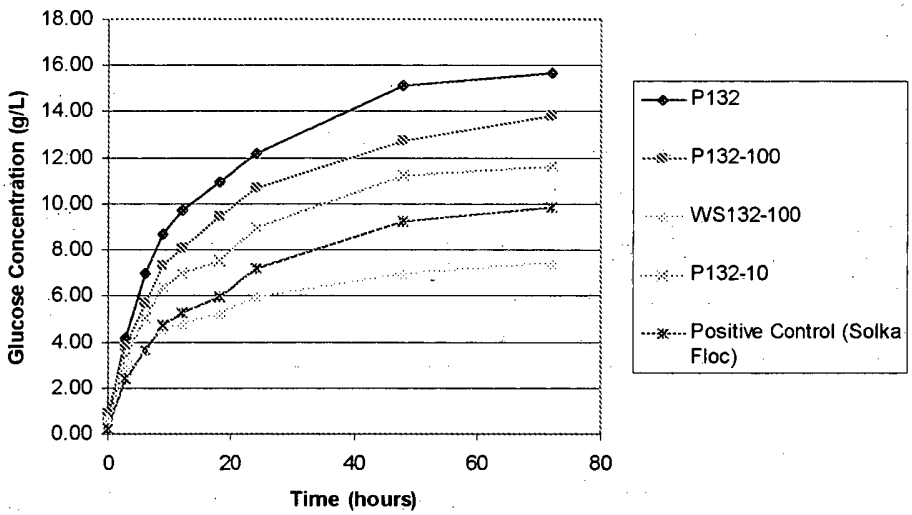
Results

[0305]

Table 52. Cellulase Assay Results

Sample Number	Glucose Concentration (mg/mL) at Incubation Time (hours)								
	0	3	6	9	12	18	24	48	72
P132	0.59	4.19	7.00	8.72	9.70	10.95	12.19	15.10	15.65
P132-10	0.36	3.37	5.08	6.39	6.98	7.51	8.99	11.25	11.65
P132-100	0.91	3.86	5.67	7.31	8.08	9.47	10.70	12.70	13.80
A132	0.39	1.51	1.92	2.40	2.64	3.04	3.30	3.90	4.06
A132-10	0.42	1.80	2.27	2.63	2.86	3.16	3.43	4.02	4.14
A132-100	0.46	2.09	2.72	3.16	3.43	3.78	4.09	4.84	5.26
G132	0.40	1.16	1.35	1.52	1.60	1.67	1.85	2.10	2.21
G132-10	0.34	1.34	1.64	1.95	2.03	2.09	2.36	2.77	3.02
G132-100	0.61	1.84	2.32	2.89	3.14	3.52	3.97	4.81	5.44
WS132	0.35	1.48	1.81	2.14	2.26	2.50	2.70	3.18	3.26
WS132-10	0.44	1.77	2.22	2.60	2.76	2.61	3.15	3.62	3.82
WS132-100	0.70	2.76	3.63	4.59	4.78	5.29	5.96	6.99	7.43
Sample A	0.42	1.09	1.34	1.55	1.69	1.66	2.17	2.96	3.71
Negative Control (no sample)	0.03	0.03	0.01	0.01	0.02	0.01	0.02	0.02	0.02
Positive Control (SolkaFloc)	0.17	2.38	3.65	4.71	5.25	5.98	7.19	9.26	9.86

Chart 1. Glucose Concentration (Top 4 producers)



[0306] The amount of cellulose digested in the tube was calculated as follows:

g/mL glucose x 20 mL (volume of sample) x 0.9 (to correct for the water molecule added upon hydrolysis of cellulose)

[0307] The percent of the total sample released as glucose (in Table 53 below) was calculated as follows:

g of cellulose digested/g of sample added (see Table 5 for details) * 100

Table 53. Cellulase Assay Results

Sample Number	Percent of the Total Sample Released as Glucose (%) at Incubation Time (h)								
	0	3	6	9	12	18	24	48	72
P132	2.02	14.98	25.16	31.36	34.85	39.38	43.81	54.29	56.27
P132-10	1.19	12.02	18.25	22.97	25.06	27.00	32.29	40.43	41.87
P132-100	3.17	13.79	20.38	26.28	29.02	34.06	38.45	45.65	49.61
A132	0.86	3.55	4.58	5.74	6.29	7.27	7.87	9.31	9.70
A132-10	0.94	4.25	5.42	6.29	6.82	7.56	8.18	9.60	9.89
A132-100	1.03	4.94	6.50	7.56	8.18	9.05	9.77	11.57	12.58
G132	0.89	2.71	3.22	3.62	3.79	3.98	4.39	4.99	5.26
G132-10	0.74	3.14	3.91	4.66	4.82	4.99	5.62	6.60	7.20
G132-100	1.39	4.34	5.54	6.91	7.49	8.42	9.48	11.50	13.01
WS132	0.77	3.48	4.32	5.11	5.38	5.98	6.43	7.58	7.78
WS132-10	0.98	4.18	5.30	6.22	6.58	6.24	7.51	8.64	9.12
WS132-100	1.61	6.55	8.69	10.99	11.42	12.67	14.26	16.73	17.78
Sample A	0.94	2.54	3.19	3.70	4.01	3.96	5.16	7.06	8.86
Positive Control (SolkaFloc)	1.29	21.15	32.72	42.30	47.07	53.73	64.53	83.16	88.56

Example 30- Shake Flask Fermentation Using *Pichia stipitis*

Summary

[0308] Shake flask fermentation using *Pichia stipitis* was performed using four cellulosic materials having the highest % performance from Table 36.

Protocol

[0309] Experiments were run under the parameters outlined in Tables 54-56.

Table 54. Equipment and Frequency of Maintenance

Equipment	Manufacturer, Name	Frequency of Maintenance
Shakers (2)	B. Braun Biotech, Certomat BS-1	Quarterly
Spectrophotometer	Unicam, UV300	Biannual
YSI Biochem Analyzer	Interscience, YSI	Monthly

Table 55. YSI Components Used in Shake Flask Study

Component	Reference #	Lot #
YSI Ethanol Membrane	2786	07M100361
YSI Ethanol Standard (3.2 g/L)	2790	1271040
YSI Ethanol Buffer	2787	07J100215

Table 56. Chemicals Used for Shake Flask Fermentation

Media Component	Manufacturer	Reference #	Lot #
Urea	ScholAR Chemistry	9472706	AD-7284-43
Yeast Nitrogen Base	Becton Dickinson	291940	7128171
Peptone	Becton Dickinson	211677	4303198
YM Broth	Becton Dickinson	271120	6278265
Xylose	Alfa Aesar	A10643	10130919
Glucose	Fisher Scientific	BP350-1	030064

Seed Development

[0310] For all the following shake flask experiments the seed flasks were prepared using the following procedure.

[0311] A working cell bank of *P. stipitis* NRRL Y-7124 was prepared from a rehydrated lyophilized culture obtained from ARS Culture Collection. Cryovials containing *P. stipitis* culture in 15 % v/v glycerol were stored at -75 °C. A portion of the thawed working cell bank material was streaked onto a Yeast Mold (YM) Broth + 20 g/L agar (pH 5.0) and incubated at 30 °C for 2 days. The plates were held for 2 days at 4 °C before use. A 250 mL Erlenmeyer flask containing 100 mL of medium (40 g/L glucose, 1.7 g/L yeast nitrogen base, 2.27 g/L urea, 6.56 g/L peptone, 40 g/L xylose, pH 5.0) was inoculated with one colony and incubated for 24 hours at 25 °C and 100 rpm. After 23 hours of growth, a sample was taken and analyzed for optical density (600 nm in a UV spectrophotometer) and purity (Gram stain). Based on these results, one flask (called the Seed Flask) at an optical density of between 4 and 8 and with a clean Gram stain was used to inoculate all of the test flasks.

[0312] Three experiments were run using samples A132-10, A132-100, G132-10, and G132-100. Experiment #1 tested these four samples for ethanol concentration at varying concentrations of xylose and at constant concentrations of glucose. Experiment #2 tested these four samples for ethanol concentration at double the concentration of feedstock used in the experiments of Table 36. Finally, experiment #3 tested these four samples for ethanol concentration while varying both the xylose and the glucose concentrations, simultaneously.

Experiment #1-Varying the Xylose Concentration

[0313] Four cellulosic samples (A132-10, A132-100, G132-10, and G132-100) were tested at varying xylose concentrations as listed in Table 57 below.

Table 57. Media Composition of Experiment #1 Flasks

Treatment	Xylose Concentration (g/L)	Glucose Concentration (g/L)
100 % Xylose	40.0	40.0
50 % Xylose	20.0	40.0
25 % Xylose	10.0	40.0
10 % Xylose	4.0	40.0
0 % Xylose	0.0	40.0

[0314] The test vessels (a total of 40, 250 mL Erlenmeyer flasks) contained 100 mL of medium. Five different types of media were prepared with the amount of xylose and glucose outlined in Table 57. In addition, the media contained 1.7 g/L yeast nitrogen base (Becton Dickinson # 291940) 2.27 g/L urea (ScholAR Chemistry #9472706), and 6.56 g/L peptone (Becton Dickinson #211677). All flasks were autoclaved empty at 121 °C and 15 psi and filter sterilized (0.22 μm filter) media was added to the flasks prior to the addition of the test materials. Flasks were held at room temperature for 4 days and inspected for contamination (cloudiness) prior to use. The test materials were not sterilized, as autoclaving will change the content of the samples and filter sterilization not appropriate for sterilization of solids. The test samples (A132-10, A132-100, G132-10, and G132-100 at 5 g per 100 mL) were added at the time of inoculation (rather than prior to) to reduce the possibility of contamination. In addition to the test samples, 1 mL (1% v/v) of seed flask material was added to each flask. The flasks were incubated at 30 °C and 150 rpm for 72 hours.

[0315] Unfortunately, one flask (sample A132-100 with 100% Xylose) was broken during the testing. Therefore, all results past 24 hours of incubation are reported as a single flask. After 72 hours of incubation, 100% of the original amount of cellulosic material (5.0 g) was added to the 100% Xylose flasks (7 flasks in total, one flask containing sample A132-100 was broken) and incubated as above for an additional 48 hours.

Table 58. Addition of Feedstock to 100 % Xylose Flasks at Incubation Time 72 hours

Feedstock	Added at 72 hours (grams)
A132-10	5
A132-100	5
G132-10	5
G132-100	5

Analysis

[0316] Samples were taken from the 40 test flasks at incubation times of 0, 6, 12, 24, 36, 48, and 72 hours. In addition, samples were taken at 24 and 48 hours post-addition of the second feedstock amount in the 100% Xylose flasks (see Table 58).

[0317] A total of 292 samples were analyzed for ethanol concentration using a YSI Biochem Analyzer based on the alcohol dehydrogenase assay (YSI, Interscience). Samples were centrifuged at 14,000 rpm for 20 minutes and the supernatant stored at -20 °C. Of note, time 0 samples required filtration through a 0.45 μm syringe filter. The samples will be diluted to between 0-3.2 g/L ethanol prior to analysis. A standard of 2.0 g/L ethanol was analyzed approximately every 30 samples to ensure the integrity of the membrane was maintained.

[0318] A total of 47 samples were analyzed for cell count. Samples will be taken at 72 hours incubation and 48 hours post-addition of more cellulosic material. Appropriately diluted samples were mixed with 0.05% Trypan blue and loaded into a Neubauer haemocytometer. The cells were counted under 40 X magnification.

Experiment #2- Analysis of 2 X Feedstock Concentration

[0319] The test vessels (a total of 8, 250 mL Erlenmeyer flasks) contained 100 mL of medium. The media contained 40 g/L glucose, 40 g/L xylose, 1.7 g/L yeast nitrogen base (Becton Dickinson # 291940) 2.27 g/L urea (ScholAR Chemistry #9472706), and 6.56 g/L peptone (Becton Dickinson #211677). Flasks were prepared as in Experiment #1. The test samples (A132-10, A132-100, G132-10, and G132-100 at 10 g per 100 mL) were added at the time of inoculation (rather

than prior to) to reduce the possibility of contamination. In addition to the test samples, 1 mL (1% v/v) of seed flask material was added to each flask. The flasks were incubated at 30 °C and 150 rpm above for 72 hours.

Analysis

[0320] Samples were from the 8 test flasks at an incubation time of 0, 6, 12, 24, 36, 48, and 72 hours. Ethanol analys3s of the 56 samples were performed as per experiment #1 and are reported in Table 59. A cell count was performed on the 72 hour sample as per experiment #1 and is presented in Table 60.

Table 59. Ethanol Concentration in Flasks with Double Feedstock

Sample Time	Concentration (g/L)			
	A132-10	A132-100	G132-10	G132-100
0	1.38	0.26	0.12	0.11
6	1.75	0.21	0.20	0.10
12	2.16	0.73	0.69	0.31
24	19.05	15.35	16.55	12.60
36	21.75	17.55	18.00	15.30
48	26.35	23.95	24.65	20.65
72	26.95	27.35	28.90	27.40

Table 60. Cell Concentration at 72 hour Incubation Time in Flasks with Double Feedstock

Sample	Cell Concentration (x 10^8/mL)
A132-10	4.06
A132-100	5.37
G132-10	5.18
G132-100	4.47

Experiment #3-Varying Xylose and Glucose Concentrations

[0321] Four cellulosic samples (A132-10, A132-100, G132-10, and G132-100) were tested at varying xylose and glucose concentrations as listed in the table below (Table 60).

Table 61. Media Composition of Experiment #3 Flasks

Treatment	Xylose Concentration (g/L)	Glucose Concentration (g/L)
50 % Sugar	20.0	20.0
25 % Sugar	10.0	10.0
10 % Sugar	4.0	4.0
0 % Sugar	0.0	0

[0322] The test vessels (a total of 32, 250 mL Erlenmeyer flasks) contained 100 mL of medium. Four different types of media were prepared with the amount of xylose and glucose outlined in Table 61. In addition, the media contained 1.7 g/L yeast nitrogen base (Becton Dickinson # 291940) 2.27 g/L urea (ScholAR Chemistry #9472706), and 6.56 g/L peptone (Becton Dickinson #211677). The flasks were prepared as per Experiment #1. The test samples (A132-10, A132-100, G132-10, and G132-100) were added at the time of inoculation (rather than prior to) to reduce the possibility of contamination. In addition to the test samples, 1 mL (1% v/v) of seed flask material was added to each flask. The flasks were incubated at 30 °C and 150 rpm for 72 hours.

Analysis

[0323] Samples were taken from the 32 test flasks at an incubation time of 0, 6, 12, 24, 36, 48, and 72 hours (see

Tables 62-65). A total of 224 samples were analyzed for ethanol concentration using the YSI Biochem Analyzer based on the alcohol dehydrogenase assay (YSI, Interscience). Samples were centrifuged at 14,000 rpm for 20 minutes and the supernatant stored at -20 °C. Of note, some of the samples required centrifugation and then filtration through a 0.45 μm syringe filter. The samples were diluted to between 0-3.2 g/L ethanol prior to analysis. A standard of 2.0 g/L ethanol was analyzed approximately every 30 samples to ensure the integrity of the YSI membrane was maintained.

Table 62. Ethanol Results Sample A132-10

Sample Time	Ethanol Concentration (g/L)								
	0 % Xylose	10 % Xylose	25 % Xylose	50 % Xylose	100 % Xylose	0 % Sugars*	10 % Sugars*	25 % Sugars*	50 % Sugars*
0	0.43	0.42	0.42	0.41	0.39	0.53	0.57	0.56	0.56
6	1.16	1.16	1.15	1.16	1.12	0.93	0.91	0.83	0.88
12	1.72	1.86	1.71	1.79	1.90	1.21	2.13	2.47	2.32
24	15.55	15.90	17.05	17.05	16.95	1.02	4.88	9.77	13.35
36	17.10	17.40	20.25	21.35	20.25	1.29	4.27	9.99	17.55
48	16.40	17.05	19.70	23.00	26.80	1.47	3.03	8.33	16.60
72	15.15	15.55	19.25	21.85	28.00	1.14	1.52	5.08	14.20
24 hours post-addition	--	--	--	--	23.15	--	--	--	--
48 hours post-addition	--	--	--	--	21.55	--	--	--	--
* Analysis from experiment #3.									

Table 63. Ethanol Results Sample A132-100

Sample Time	Ethanol Concentration (g/L)								
	0 % Xylose	10 % Xylose	25 % Xylose	50 % Xylose	100 % Xylose	0 % Sugars*	10 % Sugars*	25 % Sugars*	50 % Sugars*
0	0.11	0.09	0.17	0.20	0.18	0.12	0.14	0.09	0.13
6	0.13	0.15	0.15	0.15	0.14	0.10	0.11	0.11	0.13
12	0.88	1.00	1.18	1.25	0.89	0.18	1.58	1.55	1.57
24	15.90	15.70	16.50	16.05	14.60**	0.18	3.33	7.99	11.15
36	16.00	17.90	16.90	19.45	17.80**	0.21	2.85	8.37	16.10
48	15.75	16.70	19.30	22.15	27.00**	0.54	1.47	7.54	15.60
72	14.85	15.35	18.55	21.30	28.50**	0.78	0.51	4.47	12.90
24 hours post-addition	--	--	--	--	24.80**	--	--	--	--
48 hours post-addition	--	--	--	--	23.60**	--	--	--	--

* Analysis from experiment #3.
** All results based on analysis of one flask.

Table 64. Ethanol Results Sample G132-10

Sample Time	Ethanol Concentration (g/L)								
	0 % Xylose	10 % Xylose	25 % Xylose	50 % Xylose	100 % Xylose	0 % Sugars*	10 % Sugars*	25 % Sugars*	50 % Sugars*
0	0.09	0.08	0.08	0.08	0.08	0.05	0.05	0.05	0.06
6	0.14	0.13	0.14	0.14	0.13	0.11	0.12	0.11	0.12
12	1.01	0.96	1.00	0.87	1.14	0.48	1.60	1.79	1.71
24	15.90	15.70	16.30	16.05	14.60	0.13	3.96	8.54	11.10
36	15.10	17.45	16.80	18.75	22.15	0.09	3.02	8.69	16.55
48	15.95	16.90	19.25	21.10	24.00	0.07	2.05	8.10	16.50
72	13.50	15.80	18.55	21.25	26.55	0.09	0.11	5.55	14.15
24 hours post-addition	--	--	--	--	24.95	--	--	--	--
48 hours post-addition	--	--	--	--	24.20	--	--	--	--
* Analysis from experiment #3.									

Table 65. Ethanol Results Sample G132-100

Sample Time	Ethanol Concentration (g/L)								
	0 % Xylose	10 % w/v Xylose	25 % w/v Xylose	50 % w/v Xylose	100 % w/v Xylose	0 % Sugars*	10 % Sugars*	25 % Sugars*	50 % Sugar*
0	0.04	0.04	0.04	0.04	0.05	0.05	0.05	0.05	0.06
6	0.07	0.07	0.08	0.08	0.07	0.04	0.05	0.05	0.06
12	0.60	0.56	0.67	0.58	0.71	0.13	1.37	1.48	1.44
24	13.05	14.45	14.90	13.95	12.05	0.03	3.67	7.62	10.55
36	15.10	17.10	18.25	18.20	19.25	0.01	3.09	8.73	16.10
48	14.40	17.00	19.35	22.55	24.45	0.01	1.91	7.76	15.85
72	14.70	15.40	18.45	22.10	27.55	0.03	0.01	5.08	14.30
24 hours post-addition	--	--	--	--	25.20	--	--	--	--
48 hours post-addition	--	--	--	--	24.60	--	--	--	--

* Analysis from experiment #3.

[0324] Samples were taken at 72 hours incubation for cell counts (see Tables 66-67). Appropriately diluted samples were mixed with 0.05% Trypan blue and loaded into a Neubauer haemocytometer. The cells were counted under 40 X magnification.

Result

[0325] One seed flask was used to inoculate all Experiment #1 and #2 test flasks. The optical density (600 nm) of the seed flask was measured to be 5.14 and the cell concentration was 4.65 x 10^8 cells/mL (Tables 65-66). Therefore, the initial concentration of cells in the test flasks was approximately 4.65 x 10^6 cells/mL.

[0326] A second seed flask was used to inoculate Experiment #3 flasks. The optical density (600 nm) of the seed flask was 5.78 and the cell concentration was 3.75 x 10^8 cells/mL. Therefore, the initial concentration of cells in the test flasks was approximately 3.75 x 10^6 cell s/mL.

Table 66. Cell Counts at Incubation Time of 72 hours

Sample	Cell Concentration (x 10^8/mL)								
	0 % Xylose	10 % Xylose	25 % Xylose	50 % Xylose	100 % Xylose	0 % Sugar	10 % Sugar	25 % Sugar	50 % Sugar
A132-10	0.37	0.63	3.72	4.92	4.05	0.26	0.22	0.26	1.54
A132-100	0.99	1.07	0.99	0.78	1.97	0.03*	0.33	0.44	1.81
G132-10	0.95	4.50	2.67	2.67	3.82	0.01*	0.17	0.49	1.92
G132-100	6.53	4.02	4.84	4.47	5.29	0.01*	0.33	0.89	2.22
* Samples were heavily contaminated after 72 hours of growth. This is expected because the *Pichia* did not grow well without sugar added, and contaminants (from the non-sterile samples) were able to out-grow the *Pichia.*									

Table 67. Cell Counts at Incubation Time of 48 hours Post-Addition (100 % Xylose and Glucose)

Sample	Cell Concentration (x 10^8/mL)
A132-10	10.17
A132-100	3.38
G132-10	3.94
G132-100	6.53

Example 31- Toxicity Testing of Lignocellulosic Samples against *P. stipitis* and *S. cerevisiae*

Summary

[0327] Thirty-seven samples were analyzed for toxicity against two ethanol-producing cultures, *Saccharomyces cerevisiae* and *Pichia stipitis.* In this study, glucose was added to the samples in order to distinguish between starvation of the cultures and toxicity of the samples.

Table 68. Conditions for Toxicity Testing

Variable	Organism	
	***Saccharomyces cerevisiae* ATCC 24858**	***Pichia stipitis* NRRL Y-7124**
Inoculation Volume (mL)	0.5-1 (target 6-7 x 10^5 cells/mL)	1 (target 3-4 x 10^6 cells/mL)
Test Repetition	Single Flasks	
Incubation Temperature (±1°C)	25 °C	25 °C
Shaker Speed (rpm)	200	125
Type of Container	500 mL Erlenmeyer Flask	250 mL Erlenmeyer Flask

(continued)

Variable	Organism	
	***Saccharomyces cerevisiae* ATCC 24858**	***Pichia stipitis* NRRL Y-7124**
Media volume	100 mL	100 mL
Total Incubation time (hours)	72	72
Ethanol Analysis (hours)	0, 6, 12, 24, 36, 48, 72	0, 6, 12, 24, 36, 48, 72
Cell Counts (hours)	24, 72	24, 72
pH	0 hours	0 hours

Protocol

[0328] A summary of the protocol used is listed in Table 68. A description of the chemicals used in toxicity testing is listed in Table 69. Two control flasks (no sample added) were performed for each microorganism for each week of testing. A total of 82 flasks were analyzed.

[0329] During the experiments, no ethanol or cells appeared in the *P. stipitis* flasks containing samples C, C-1e, C-5e, and C-10e in the first 24 hours of incubation. In order to confirm the results, the test was repeated. The second test confirmed some inhibition of *P. stipitis* growth when samples C, C1E, C5E, and C10E were added to the flasks.

Table 69. Chemicals and Materials Used for Toxicity Testing

Media Component	Manufacturer	Reference #	Lot #
Urea	ScholAR Chemistry	9472706	AD-7284-43
Yeast Nitrogen Base	Becton Dickinson	291940	7128171
Peptone	Becton Dickinson	211677	4303198
Xylose	Alfa Aesar	A10643	10130919
Glucose	Sigma	G-5400	107H0245
Yeast Extract	Becton Dickinson	288620	4026828
YM Broth	Becton Dickinson	271120	6278265

Table 70. YSI Components Used in Toxicity Study

Component	Catalogue #
YSI Ethanol Membrane	2786
YSI Ethanol Standard (3.2 g/L)	2790
YSI Ethanol Buffer	2787

Test Samples

[0330] Seven test samples (all with the C designation) were ground using a coffee grinder suitable for small samples. The samples were ground to a consistent particle size (between samples) with the naked eye. Sample number C-100e ground easily to a small particle size.

[0331] All samples were added to the flasks at a concentration of 50 grams per liter with the exception of the six P samples (25 grams per liter). These samples were white to off-white in color and visually fluffy and the flasks would not mix properly (not enough free liquid) at the 50 grams per liter concentration. Samples S dissolved easily and could in the future be added to the flasks at a higher concentration. Samples A and G could be added at 100 grams per liter in the future.

[0332] Testing was performed using the two microorganisms as described below.

***Saccharomyces cerevisiae* ATCC 24858 (American Type Culture Collection)**

[0333] A working cell bank of *S. cerevisiae* ATCC 24858 was prepared from a rehydrated lyophilized culture obtained from American Type Culture Collection. Cryovials containing *S. cerevisiae* culture in 15 % v/v glycerol are stored at -75 °C. A portion of the thawed working cell bank material will be streaked onto a Yeast Mold (YM) Broth + 20 g/L agar (pH 5.0) and incubated at 30 °C for 2 days. A 250 mL Erlenmeyer flask containing 50 mL of medium (20 g/L glucose, 3 g/L yeast extract, and 5.0 g/L peptone, pH 5.0) was inoculated with one colony from the YM plate and incubated for 24 hours at 25 °C and 200 rpm. After 23 hours of growth, a sample was taken and analyzed for optical density (600 nm in a UV spectrophotometer) and purity (Gram stain). Based on these results, one flask (called the Seed Flask) with an OD of 9-15 and pure Gram stain was to be used for inoculating the growth flasks. After 23 hours of growth, the seed flask had a low OD (5.14) and cell count (1.35 x 10^8 cells/mL). Of note, the colony taken from the seed plate was smaller than usual. Therefore, 0.5 mL of seed material (as opposed to the planned 0.1 mL) was added to each test vessel.

[0334] The test vessels were 500 mL Erlenmeyer flasks containing 100 mL of the sterile medium described above. All flasks were autoclaved at 121 °C and 15 psi prior to the addition of the test materials. The test materials were not sterilized, as autoclaving would change the content of the samples. The test samples were added at the time of inoculation (rather than prior to) to reduce the possibility of contamination. In addition to the test samples, 0.5 -1.0 mL (0.5-1.0% v/v) of seed flask material was added to each flask. The flasks were incubated as described above for 72 hours.

***Pichia stipitis* (ARS Culture Collection)**

[0335] A working cell bank of *P. stipitis* NRRL Y-7124 was prepared from a rehydrated lyophilized culture obtained from ARS Culture Collection. Cryovials containing *P. stipitis* culture in 15 % v/v glycerol are stored at -75 °C. A portion of the thawed working cell bank material was streaked onto a Yeast Mold (YM) Broth + 20 g/L agar (pH 5.0) and incubated at 30 °C for 2 days. The plates were held for up to 5 days at 4 °C before use. A 250 mL Erlenmeyer flask containing 100 mL of medium (40 g/L glucose, 1.7 g/L yeast nitrogen base, 2.27 g/L urea, 6.56 g/L peptone, 40 g/L xylose, pH 5.0) was inoculated with one colony and incubated for 24 hours at 25 °C and 125 rpm. After 23 hours of growth, a sample was taken and analyzed for optical density (600 nm in a UV spectrophotometer) and purity (Gram stain). Based on these results, one flask (called the Seed Flask) at an optical density of 5-9 and with a pure Gram Stain was used to inoculate all of the test flasks.

[0336] The test vessels were 250 mL Erlenmeyer flasks containing 100 mL of the sterile medium described above. All flasks were autoclaved empty at 121 °C and 15 psi and filter sterilized (0.22 μm filter) medium added to the flasks prior to the addition of the test materials. The test materials were not sterilized, as autoclaving would change the content of the samples and filter sterilization not appropriate for sterilization of solids. The test samples were added at the time of inoculation (rather than prior to) to reduce the possibility of contamination. In addition to the test samples, I mL (1% v/v) of seed flask material was added to each flask. The flasks were incubated as described above for 72 hours.

Analysis

[0337] Samples were taken from seed flasks just prior to inoculation and each test flask at 24 and 72 hours and analyzed for cell concentration using direct counts. Appropriately diluted samples of *S. cerevisiae and P. stipitis* were mixed with 0.05% Trypan blue, loaded into a Neubauer haemocytometer. The cells were counted under 40 X magnification.

[0338] Samples were taken from each flask at 0, 6, 12, 24, 36, 48 and 72 hours and analyzed for ethanol concentration using the YSI Biochem Analyzer based on the alcohol dehydrogenase assay (YSI, Interscience). Samples were centrifuged at 14,000 rpm for 20 minutes and the supernatant stored at -20 °C. The samples will be diluted to 0-3.2 g/L ethanol prior to analysis. A standard of 2.0 g/L ethanol was analyzed approximately every 30 samples to ensure the integrity of the membrane was maintained during analysis.

Calculations

[0339] The following calculations were used to compare the cell counts and ethanol concentration to the control flasks.

$$\%\ \text{performance} = (\text{concentration of ethanol in test flask/ethanol in control}) * 100\%$$

cells = (number of cells in test flask/number of cells in control flask)*100

Results

[0340] The *S. cerevisiae* seed flask had an optical density (600 nm) of 5.14 and a cell concentration of 1.35 x 10^8 cells/mL. One half mL of seed flask material was added to each of the test flasks. Therefore, the starting cell concentration in each flask was 6.75 x 10^5/mL. During the second week of testing, the *S. cerevisiae* seed flask had an optical density (600 nm) of 4.87 and a cell concentration of 3.15 x 10^7 cells/mL. One mL of seed flask material was added to each of the test flasks. Therefore, the starting cell concentration in each flask was 6.30 x 10^5/mL. The pH of the *S. cerevisiae* flasks at a sample time of 0 hours is presented in Table 71. The pH of the flask contents was within the optimal pH for *S. cerevisiae* growth (pH 4-6): No pH adjustment was required.

Table 71. pH of *S. cerevisiae* flasks at sample time 0 hours

Sample Number	pH	Sample Number	pH
P	5.04	C	5.46
P1E	4.99	C1E	5.54
P5E	5.04	C5E	5.50
P10E	4.98	C10E	5.33
P50E	4.67	C30E	5.12
P100E	4.43	C50E	4.90
G	5.45	C100E	4.66
G1E	5.47	ST	5.11
G5E	5.46	ST1E	5.06
G10E	5.39	ST5E	4.96
G50E	5.07	ST10E	4.94
A	5.72	ST30E	5.68
A1E	5.69	ST50E	4.48
A5E	5.62	ST100E	4.23
A10E	5.61	control A	5.02
A50E	5.74	control B	5.04
S*	5.10		
S1E	5.08		
S5E	5.07		
S10E	5.04		
S30E	4.84		
S50E	4.57		
S100E	4.33		
* "S" refers to sucrose * "C" refers to corn * "ST" refers to starch			

[0341] The ethanol concentration and performance in the *S. cerevisiae* flasks are presented in Table 72 and 73. The highest ethanol concentrations were produced by the S series.

Table 72. Ethanol Concentration in *S. cerevisiae* flasks

Sample Number	Ethanol Concentration (g/L) at the following times (hours)						
	0	6	12	24	36	48	72
P	0.02	0.04	0.38	5.87	7.86	5.41	1.04
P1E	0.03	0.03	0.28	5.10	8.03	5.46	0.58
P5E	0.03	0.04	0.57	8.84	6.38	3.40	0.04
P10E	0.06	0.05	0.65	6.63	7.66	5.57	1.40
P50E	0.04	0.03	0.26	2.80	5.85	8.59	5.68
P100E	0.04	0.02	0.12	3.64	8.26	7.51	3.03
G	0.04	0.04	0.57	10.20	8.24	6.66	2.84
G1E	0.04	0.05	0.46	10.20	9.24	6.94	2.84
G5E	0.11	0.11	0.44	10.00	8.7	6.36	0.88
G10E	0.05	0.04	0.40	9.97	8.41	5.79	0.11
G50E	0.05	0.05	0.48	9.72	8.33	6.13	2.38
A	0.29	0.38	0.48	8.43	8.76	7.09	4.66
A1E	0.34	0.44	0.79	9.66	8.9	7.18	2.64
A5E	0.55	0.45	0.99	9.44	8.96	7.56	3.80
A10E	0.55	0.55	0.93	9.58	8.33	6.28	1.40
A50E	0.22	0.08	0.38	9.38	8.01	5.99	0.98
S	0.03	0.03	0.39	5.73	7.06	10.10	15.90
S1E	0.05	0.06	0.31	7.24	9.52	12.10	14.90
S5E	0.02	0.05	0.34	5.87	7.68	11.90	19.00
S10E	0.03	0.04	0.35	5.88	7.72	11.50	19.30
S30E	0.03	0.05	0.09	5.94	7.97	11.20	20.40
S50E*	0.13	0.19	0.47	5.46	7.96	13.00	18.30
S100E	0.11	0.10	0.21	7.00	10.6	13.80	12.70
C	0.01	0.04	0.32	8.47	7.57	5.48	6.40
C1E	0.00	0.06	0.37	8.93	7.86	5.99	1.37
C5E	0.03	0.05	0.48	9.32	7.92	5.69	1.41
C10E	0.02	0.04	0.52	9.14	7.67	5.34	0.35
C30E	0.02	0.05	0.28	9.15	8.15	5.84	2.47
C50E	0.03	0.06	0.44	9.31	7.79	5.78	1.79
C100E	0.03	0.06	0.58	9.06	6.85	5.95	1.09
ST	0.02	0.05	0.99	8.54	6.69	5.09	0.42
ST1E	0.03	0.04	0.70	8.87	7.29	4.81	1.04
ST5E	0.02	0.04	0.52	8.61	7.16	4.97	0.85
ST10E	0.02	0.05	0.33	8.97	7.05	5.26	0.68
ST30E	0.03	0.04	0.71	8.47	6.96	4.89	0.21
ST50E	0.04	0.07	0.34	8.46	8.19	7.04	3.20

(continued)

Sample Number	Ethanol Concentration (g/L) at the following times (hours)						
	0	6	12	24	36	48	72
ST100E	0.03	0.10	0.30	9.30	8.62	7.29	4.23
control A	0.01	0.07	0.85	5.92	8.18	7.81	6.26
control B	0.01	0.04	0.27	4.86	6.43	8.01	6.75
control A*	0.04	0.21	1.36	5.19	7.31	7.55	5.16
control B*	0.03	0.20	1.18	5.16	5.96	7.62	5.32
* analyzed week 2 See Table 72 for Sample Number key							

Table 73. Performance in *S. cerevisiae* flasks

Sample Number	Performance (%) at the following times (hours)			
	24	36	48	72
P	108.9	107.6	68.4	16.0
P1E	94.6	109.9	69.0	8.9
P5E	164.0	87.3	43.0	0.6
P10E	123.0	104.9	70.4	21.5
P50E	51.9	80.1	108.6	87.3
P100E	67.5	113.1	94.9	46.5
G	189.2	112.8	84.2	43.6
G1E	189.2	126.5	87.7	43.6
G5E	185.5	119.1	80.4	13.5
G10E	185.0	115.1	73.2	1.7
G50E	180.3	114.0	77.5	36.6
A	156.4	119.9	89.6	71.6
A1E	179.2	121.8	90.8	40.6
A5E	175.1	122.7	95.6	58.4
A10E	177.7	114.0	79.4	21.5
A50E	174.0	109.7	75.7	15.1
S	106.3	96.6	127.7	244.2
S1E	134.3	130.3	153.0	228.9
S5E	108.9	105.1	150.4	291.9
S10E	109.1	105.7	145.4	296.5
S30E	110.2	109.1	141.6	313.4
S50E*	105.5	119.9	171.3	349.2
S100E	129.9	145.1	174.5	195.1
C	157.1	103.6	69.3	98.3
C1E	165.7	107.6	75.7	21.0
C5E	172.9	108.4	71.9	21.7

(continued)

Sample Number	Performance (%) at the following times (hours)			
	24	36	48	72
C10E	169.6	105.0	67.5	5.4
C30E	169.8	111.6	73.8	37.9
C50E	172.7	106.6	73.1	27.5
C100E	168.1	93.8	75.2	16.7
ST	158.4	91.6	64.3	6.5
ST1E	164.6	99.8	60.8	16.0
ST5E	159.7	98.0	62.8	13.1
ST10E	166.4	96.5	66.5	10.4
ST30E	157.1	95.3	61.8	3.2
ST50E	157.0	112.1	89.0	49.2
ST100E	172.5	118.0	92.2	65.0
control A	109.8	112.0	98.7	96.2
control B	90.2	88.0	101.3	103.7
control A*	100.3	110.1	99.5	98.5
control B*	99.7	89.8	100.4	101.5
* analyzed week 2				

[0342] The cell concentration and % cells in the *S. cerevisiae* flasks are presented in Table 74. High cell counts were observed in all flasks; however, not all of the cells appear to be making ethanol.

Table 74. *S cerevisiae* Cell Counts and % Cells

Sample Number	Cell Count (cells x 10^8/ mL)		% Cells (count/ count control) *100	
	24 hours	72 hours	24 hours	72 hours
P	0.62	0.96	97.7	139.0
P1E	0.35	1.18	54.1	170.9
P5E	1.13	1.93	177.3	279.5
P10E	0.59	1.42	91.8	205.6
P50E	0.32	1.40	49.4	202.8
P100E	0.45	1.94	70.6	281.0
G	0.74	3.48	116.5	504.0
G1E	0.68	3.65	107.1	528.6
G5E	0.62	3.87	96.5	560.5
G10E	0.70	2.73	109.5	395.4
G50E	0.46	2.10	71.8	304.1
A	0.55	3.53	86.0	511.2
A1E	0.83	3.45	130.7	499.6
A5E	0.67	3.53	104.8	511.2
A10E	0.53	1.95	83.6	282.4

(continued)

Sample Number	Cell Count (cells x 10^8/ mL)		% Cells (count/ count control) *100	
	24 hours	72 hours	24 hours	72 hours
A50E	0.66	1.62	103.5	234.6
S	0.44	1.11	69.5	160.8
S1E	0.44	1.10	68.2	159.3
S5E	0.23	0.99	36.5	143.4
S10E	0.39	0.73	61.2	105.4
S30E	0.31	0.71	48.3	102.1
S50E*	0.44	0.90	86.5	196.5
S100E	0.53	0.84	82.4	121.7
C	0.45	1.81	70.6	262.1
C1E	0.71	2.40	110.6	347.6
C5E	0.53	2.33	83.6	337.4
C10E	0.77	1.55	120.0	224.5
C30E	0.75	1.80	117.6	260.7
C50E	0.64	1.70	100.1	246.2
C100E	0.81	1.51	127.1	218.7
ST	0.75	1.75	117.6	253.4
ST1E	0.57	1.36	89.4	197.0
ST5E	0.58	1.49	90.7	215.8
ST10E	0.61	1.32	95.4	191.2
ST30E	0.59	0.60	91.8	86.9
ST50E	0.59	1.30	91.8	188.3
ST100E	0.41	1.24	63.5	179.6
control A	0.81	0.79	127.1	114.1
control B	0.47	0.59	72.9	85.9
control A*	0.66	0.42	131.2	91.7
control B*	0.35	0.50	69.0	108.1

[0343] The *P. stipitis* seed flask had an optical density (600 nm) of 5.01 and a cell concentration of 3.30 x 10^8 cells/mL. One mL of seed flask material was added to each of the test flasks. Therefore, the starting cell concentration in each flask was 3.30 x 10^6/mL. During the second week of testing, the *P. stipitis* seed flask had an optical density (600 nm) of 5.45 and a cell concentration of 3.83 x 10^8 cells/mL. One mL of seed flask material was added to each of the test flasks. Therefore, the starting cell concentration in each flask was 3.83 x 10^6/mL. The pH of the *P. stipitis* flasks at a sample time of 0 hours is presented in Table 75. The pH of the flask contents was within the optimal pH for *P. stipitis* growth (pH 4-7). No pH adjustment was required.

Table 75. pH of *P. stipitis* Flasks at Sample Time 0 Hours

Sample Number	pH	Sample Number	pH
P	4.91	C	5.36
P1E	4.87	C1E	5.30
P5E	4.90	C5E	5.29

(continued)

Sample Number	pH	Sample Number	pH
P10E	4.78	C10E	5.06
P50E	4.46	C30E	4.89
P100E	4.24	C50E	4.70
G	5.45	C100E	4.59
G1E	5.43	ST	4.93
G5E	5.48	ST1E	4.90
G10E	5.32	ST5E	4.81
G50E	4:99	ST10E	4.83
A	5.69	ST30E	4.91
A1E	5.66	ST50E	4.24
A5E	5.60	ST100E	4.07
A10E	5.58	control A	4.93
A50E	5.69	control B	4.91
S	5.00		
S1E	4.94		
S5E	4.86		
S10E	4.78		
S30E	4.51		
S50E	4.27		
S100E	4.08		

[0344] The ethanol concentration and performance in the *P. stipitis* flasks are presented in Table 76 and 77. The highest ethanol concentrations were the G and A series. Flasks C-30e, C-50e, and C-100e also contained high concentrations of ethanol. The cell concentration and % cells in the *P. stipitis* flasks are presented in Table 78. Low cell concentrations were observed in the flasks with the S designations. Low cell counts were also observed in flasks containing samples C, C1E, C5E, and C10E at the 24 hour sample time.

Table 76. Ethanol concentration in *P. stipitis* flasks

Sample Number	Ethanol Concentration (g/L) at the following times (hours)						
	0	6	12	24	36	48	72
P	0.01	0.05	0.26	4.98	8.57	14.10	17.00
P1E	0.02	0.03	0.04	4.24	9.03	12.40	17.30
P5E	0.02	0.03	0.42	6.72	12.40	15.60	18.60
P10E	0.02	0.02	0.01	1.38	8.69	13.00	17.00
P50E	0.01	0.02	0.02	0.03	3.77	10.50	16.90
P100E	0.02	0.03	0.02	3.75	10.50	15.60	18.80
G	0.02	0.08	0.20	10.80	17.70	19.40	25.40
G1E	0.04	0.12	0.50	12.20	19.60	23.80	28.60
G5E	0.07	0.14	0.73	12.50	19.10	24.50	27.50
G10E	0.04	0.19	0.42	10.20	19.10	22.90	28.20

(continued)

Sample Number	Ethanol Concentration (g/L) at the following times (hours)						
	0	6	12	24	36	48	72
G50E	0.05	0.22	0.25	8.73	18.40	22.20	28.00
A	0.13	0.28	0.82	16.10	19.40	19.30	18.60
A1E	0.22	0.59	1.08	16.10	22.40	27.60	27.70
A5E	0.32	0.43	0.43	10.60	22.10	27.10	28.10
A10E	0.33	0.61	1.15	14.90	22.00	27.10	27.90
A50E	0.30	0.10	0.47	13.40	20.20	24.80	27.10
S	0.01	0.01	0.26	3.68	7.50	10.20	13.30
S1E	0.02	0.02	0.22	4.98	9.22	11.60	14.20
S5E	0.02	0.02	0.19	4.25	8.50	11.70	14.70
S10E	0.03	0.02	0.17	2.98	8.87	11.90	14.70
S30E	0.08	0.05	0.03	2.96	8.73	12.60	16.50
S50E	0.08	0.05	0.04	2.24	6.13	7.95	12.50
S100E	0.11	0.10	0.08	3.36	7.82	10.50	13.90
C*	0.02	0.03	0.05	0.23	1.66	2.68	6.57
C1E*	0.03	0.03	0.03	0.07	0.95	1.85	10.20
C5E*	0.03	0.02	0.04	0.05	0.37	1.59	4.80
C10E*	0.03	0.04	0.04	0.05	3.91	15.20	28.30
C30E	0.01	0.03	0.60	12.30	21.20	26.00	27.20
C50E	0.02	0.02	0.45	12.30	19.50	23.80	29.20
C100E	0.05	0.04	0.38	11.40	18.70	22.90	27.70
ST	0.03	0.03	0.37	6.69	10.70	13.50	10.90
ST1E	0.01	0.00	0.48	5.24	9.37	12.50	15.70
ST5E	0.02	0.03	0.29	5.45	10.10	11.90	14.70
ST10E	0.02	0.02	0.42	5.60	9.44	12.20	14.90
ST30E	0.05	0.04	0.73	5.70	9.50	12.10	15.20
ST50E	0.02	0.05	0.19	5.16	9.47	12.70	15.20
ST100E*	0.07	0.15	0.11	4.98	10.70	15.40	18.80
control A	0.02	0.03	0.37	4.05	7.50	9.24	11.50
control B	0.02	0.02	0.30	4.22	7.44	9.44	11.50
control A*	0.02	0.05	0.69	4.86	8.69	11.10	16.40
control B*	0.02	0.05	0.74	5.96	10.80	13.00	14.00
* analyzed week 2							

Table 77. Performance in *P. stipitis* flasks

Sample Number	Performance (%) at the following times (hours)			
	24	36	48	72
P	120.3	114.7	151.0	147.8
P1E	102.4	120.9	132.8	150.4
P5E	162.3	166.0	167.0	161.7
P10E	33.3	116.3	139.2	147.8
P50E	0.7	50.5	112.4	147.0
P100E	90.6	140.6	167.0	163.5
G	260.9	236.9	207.7	220.9
G1E	294.7	262.4	254.8	248.7
G5E	301.9	255.7	262.3	239.1
G10E	246.4	255.7	245.2	245.2
G50E	210.9	246.3	237.7	243.5
A	388.9	259.7	206.6	161.7
A1E	388.9	299.9	295.5	240.9
A5E	256.0	295.9	290.1	244.3
A10E	359.9	294.5	290.1	242.6
A50E	323.7	270.4	265.5	235.7
S	88.9	100.4	109.2	115.7
S1E	120.3	123.4	124.2	123.5
S5E	102.7	113.8	125.3	127.8
S10E	72.0	118.7	127.4	127.8
S30E	71.5	116.9	134.9	143.5
S50E	54.1	82.1	85.1	108.7
S100E	81.2	104.7	112.4	120.9
C*	4.2	17.0	22.2	43.2
C1E*	1.4	9.7	15.4	67.1
C5E*	0.9	3.8	13.2	31.6
C10E*	0.9	40.1	126.1	246.1
C30E	297.1	283.8	278.4	236.5
C50E	297.1	261.0	254.8	253.9
C100E	275.4	250.3	245.2	240.9
ST	161.6	143.2	144.5	94.8
ST1E	126.6	125.4	133.8	136.5
ST5E	131.6	135.2	127.4	127.8
ST10E	135.3	126.4	130.6	129.6
ST30E	137.7	127.2	129.6	132.2
ST50E	124.6	126.8	136.0	132.2

(continued)

Sample Number	Performance (%) at the following times (hours)			
	24	36	48	72
ST100E*	120.3	109.7	127.8	123.7
control A	97.8	100.4	98.9	100.0
control B	101.9	99.6	101.1	100.0
control A*	89.8	89.1	92.1	107.9
control B*	110.2	110.8	107.9	92.1
*analyzed in week 2				

Table 78. *P. stipitis* Cell Counts and % Cells

Sample Number	Cell Count (cells x 10^8/ mL)		% Cells (count/ count control) *100	
	24 hours	72 hours	24 hours	72 hours
P	2.78	11.00	80.6	148.0
P1E	2.10	7.20	60.9	96.9
P5E	2.93	9.68	84.9	130.3
P10E	1.42	7.73	41.2	104.0
P50E	0.33	8.63	9.6	116.2
P100E	1.58	8.25	45.8	111.0
G	1.50	14.20	43.5	191.1
G1E	3.90	8.10	113.0	109.0
G5E	2.93	6.45	84.9	86.8
G10E	4.35	13.30	126.1	179.0
G50E	3.75	11.60	108.7	156.1
A	7.43	8.55	215.4	115.1
A1E	4.13	9.53	119.7	128.3
A5E	3.68	9.75	106.7	131.2
A10E	4.50	7.50	130.4	100.9
A50E	6.23	5.33	180.6	71.7
S	3.53	5.55	102.3	74.7
S1E	3.00	3.30	87.0	44.4
S5E	3.68	3.00	106.7	40.4
S10E	1.73	5.78	50.1	77.8
S30E	2.55	5.48	73.9	73.8
S50E	2.63	6.15	76.2	82.8
S100E	2.25	4.43	65.2	59.6
C*	0.00	0.26	0.00	7.2
C1E*	0.00	0.36	0.00	9.9
C5E*	0.00	0.08	0.00	2.1

(continued)

Sample Number	Cell Count (cells x 10^8/ mL)		% Cells (count/ count control) *100	
	24 hours	72 hours	24 hours	72 hours
C10E*	0.00	5.85	0.00	160.7
C30E	5.78	4.20	167.5	56.5
C50E	3.40	7.35	98.6	98.9
C100E	1.98	6.60	57.4	88.8
ST	2.55	7.65	73.9	103.0
ST1E	2.00	8.70	58.0	117.1
ST5E	1.85	6.75	53.6	90.8
ST10E	1.83	5.40	53.0	72.7
ST30E	2.78	6.15	80.6	82.8
ST50E	1.33	3.45	38.6	46.4
ST100E*	4.35	3.83	59.8	105.2
control A	3.60	7.13	104.3	96.0
control B	3.30	7.73	95.7	104.0
control A*	7.50	3.23	103.0	88.7
control B*	7.05	4.05	96.8	111.3
* analyzed week 2				

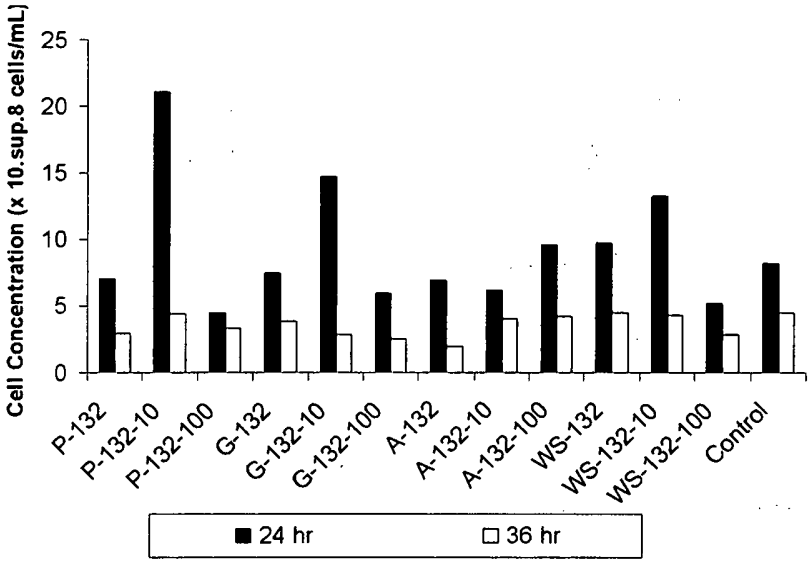
Cell Toxicity Results Summary

Zymomonas mobilis

[0345] As shown in Chart 1A, elevated cell numbers (e.g., greater than the control) were observed in samples containing P-132-10, G-132-10, and WS-132-10 at the 24 hour time point. Cell numbers in the presence of all other samples were comparable to the control. This observation indicates that the substrates were not toxic towards *Z. mobilis* for up to 24 hours after seeding.

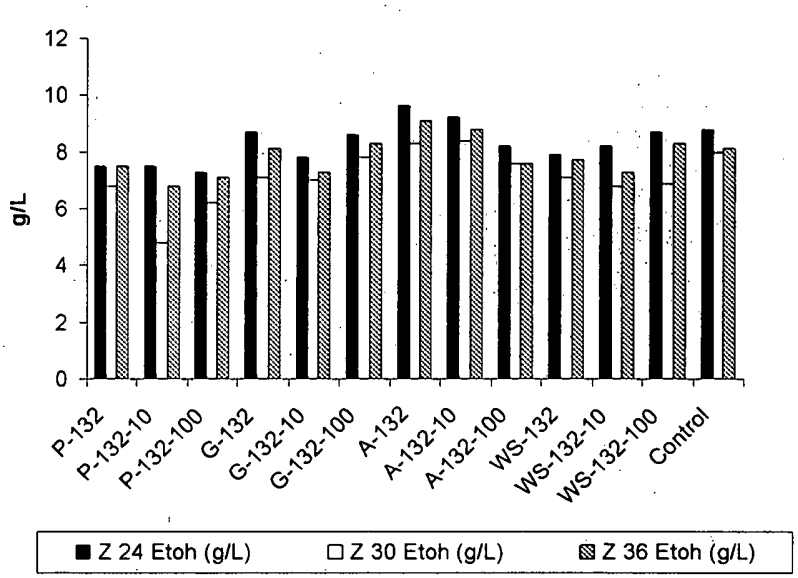
[0346] At the 36 hour time point, a decrease in cell numbers (e.g., due to a loss of cells or cell death) was observed for all samples, including the control. The greatest decrease in cell numbers was observed for those samples containing P-132-10, G-132-10. The likely cause of this effect is common to all samples, including the control. Thus, the cause of this effect is not the test substrates, as these vary in each sample, and are not present in the control. Possible explanations for this observation include inappropriate culture conditions (e.g., temperature, media compositions), or ethanol concentrations in the sample.

Chart 1A. Cell Concentrations for *Z. mobilis*



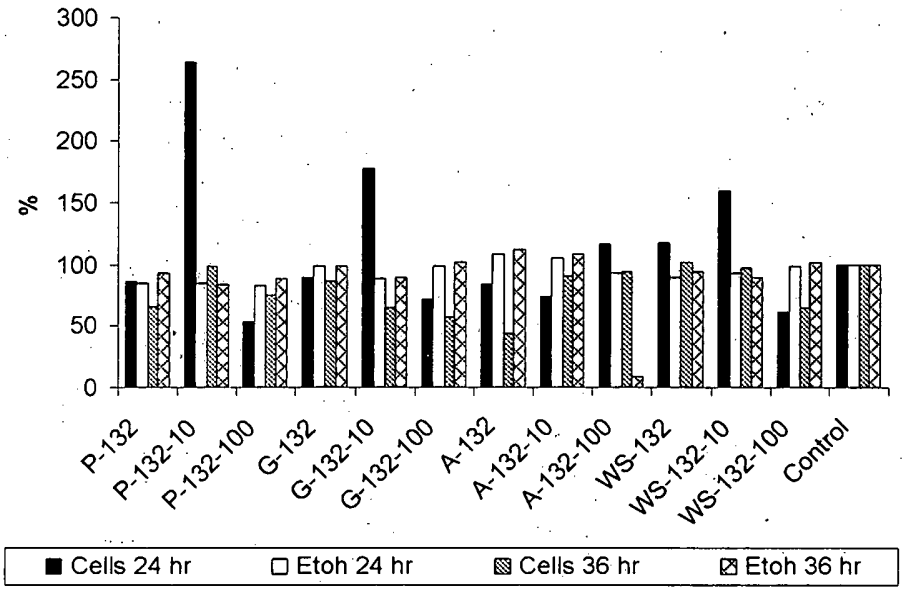
[0347] As shown in Chart 1B, all cells produced comparable amounts of ethanol (e.g., 5-10 g/L) at each time point, irrespective of the substrate. Consistent with the cell number data presented in Chart 1A, ethanol concentration in each sample peaked at the 24 hour time point. In contrast to the cell number data, ethanol concentration did not decrease at subsequent time points. This was expected as ethanol was not removed from the system. In addition, this data suggests that ethanol production in these samples may have resulted from fermentation of glucose in the culture media. None of the substrates tested appeared to increase ethanol production.

Chart 1B. Ethanol Concentrations for *Z. mobilis*



[0348] Together, Charts 1A and 1B suggest that ethanol concentrations above about 6 g/L may be toxic to *Z. mobilis.* This data is also presented as a percentage normalized against the control, as shown in Chart 1C.

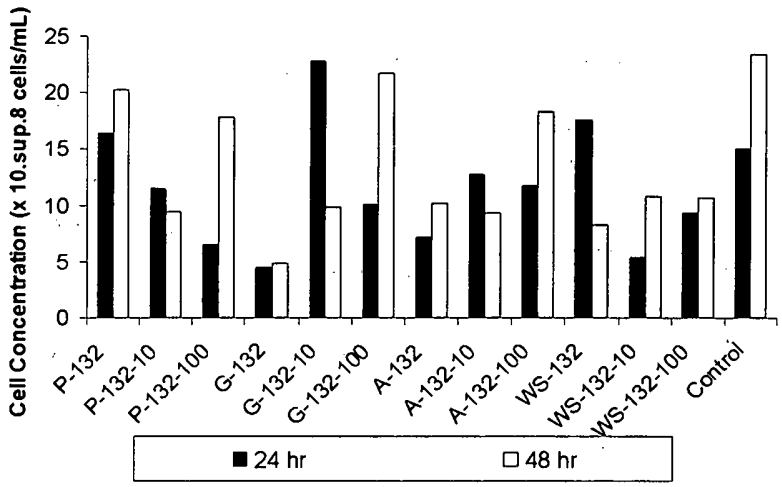
Chart 1C. % Growth and Ethanol Production for *Z. mobilis*



Pichia stipitis

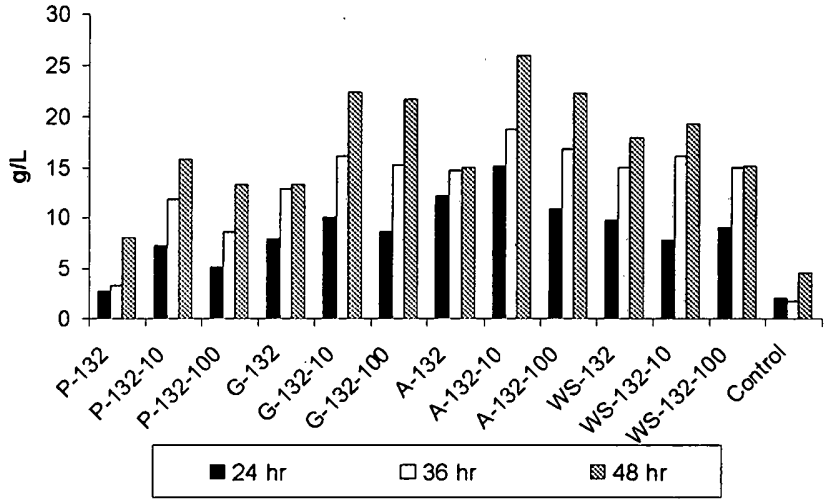
[0349] As shown in Chart 2A, cell numbers were comparable to the control. Furthermore, although slightly reduced cell numbers were present in samples containing G-132 and WS-132, reduced cell numbers were not observed for G-132-10, G-132-100, A-132-10, or A-132-100. Thus, it is unlikely that substrates G or A are toxic. Rather, the reduced cell numbers observed for G-132 and WS-132 are likely to have been caused by an experimental anomaly or by the presence of unprocessed substrate somehow impeding cell growth. Overall, this data suggests that glucose present in the control and experimental samples is likely to be sufficient to promote optimal *P. stipitis* growth, and that the presence of an additional substrate in the sample does not increase this growth rate. These results also suggest that none of the samples are toxic in *P. stipitis.*

Chart 2A. Cell concentrations for *P. stipitis*



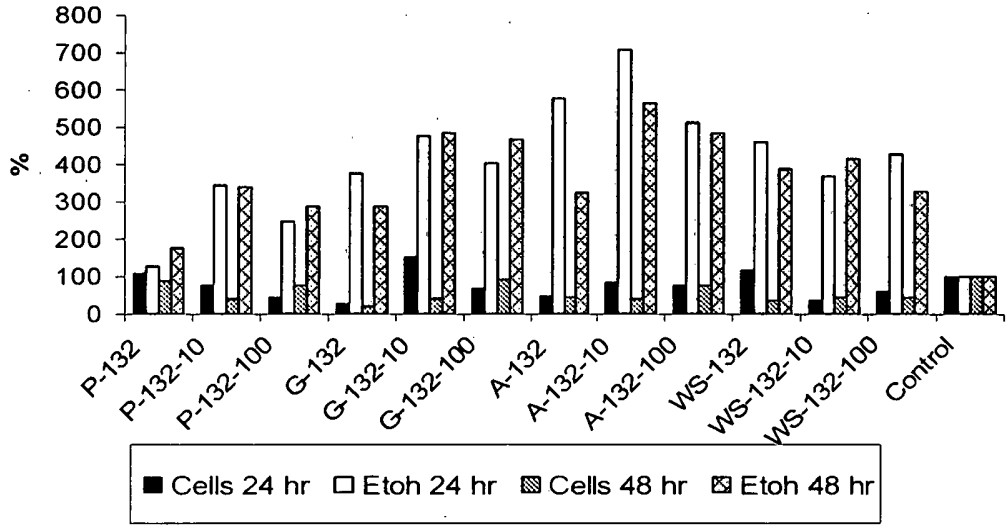
[0350] As shown in Chart 2B, despite the similar cell numbers reported in Chart 2B, greatly increased ethanol production was observed in all samples containing an experimental substrate. Ethanol concentrations increased over time for each of the three time points tested. The highest concentration of ethanol was observed for A-132-10 at the 48 hour time point (e.g., approximately 26.0 g/L). By comparing the substrate concentrations with the highest levels of ethanol production with the cell number data presented in Chart 2B, it can be seen that *P. stipitis* do not appear to be sensitive to increasing ethanol concentrations. Furthermore, ethanol production does not appear to be related to cell number, but rather appears to be related to the type of substrate present in the sample.

Chart 2B. Ethanol Concentrations for *P. stipitis*



[0351] Together, the results presented in Charts 2A and 2B suggest that the experimental substrates do not promote increased *P. stipitis* growth, however, they greatly increase the amount of ethanol produced by this cell type. This data is also presented as a percentage normalized against the control, as shown in Chart 2C.

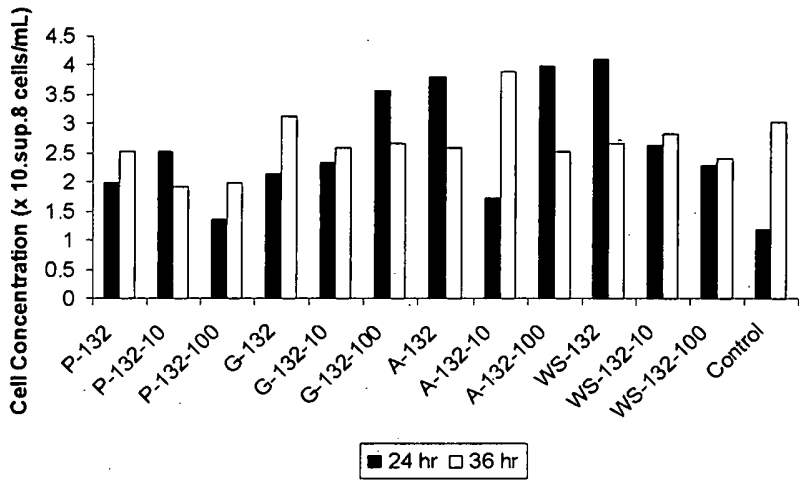
Chart 2C. % Growth and Ethanol Production for *P. stipitis*



Saccharomyces cerevisiae

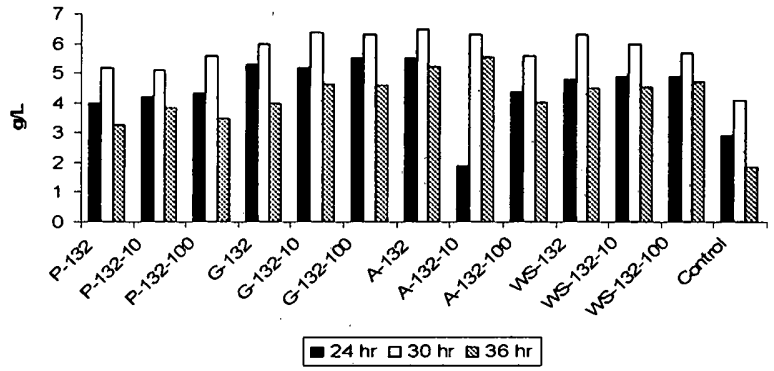
[0352] As shown in Chart 3A, G-132-100, A-132, A-132-10, A-132-100, and WS-132 promoted slightly elevated cell numbers compared to the control. No significant reductions in cell number were observed for any sample. These results suggest that none of the samples are toxic in *S. cerevisiae.*

Chart 3A. Cell Concentrations for *S. cerevisiae*



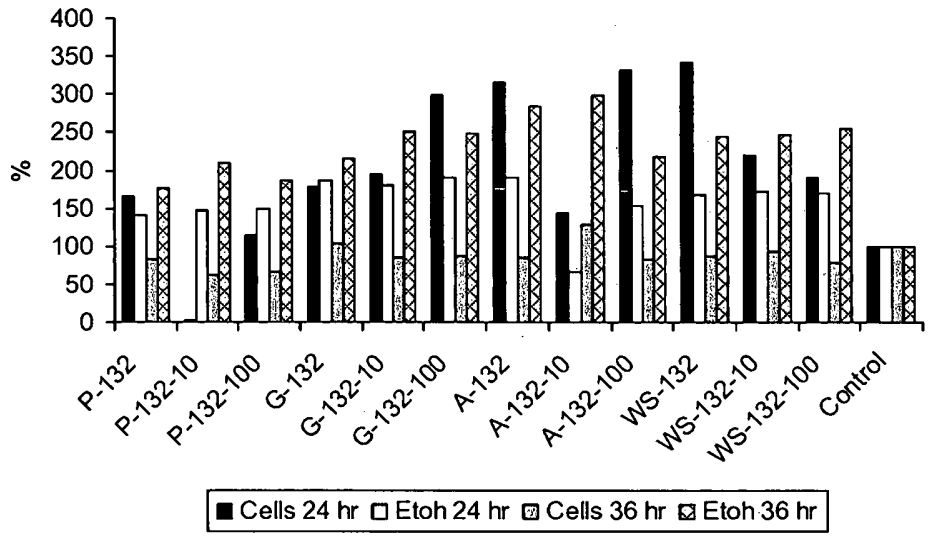
[0353] As shown in Chart 3B, increased ethanol production was observed in cells treated with each cell type compared to the control. Comparison of those samples containing the highest amount of ethanol with the cell number data presented in Chart 3A suggests that ethanol concentrations in excess of 5 g/L may have had an adverse effect on cell numbers. However, this observation is not the case for all samples.

Chart 3B. Ethanol Concentrations for *S. cerevisiae*



[0354] This data is also presented as a percentage normalized against the control, as shown in Chart 3C.

Chart 3C. % Growth and Ethanol Production for *S. cerevisiae*



[0355] In conclusion, none of the samples tested appeared to be toxic in *Z. mobilis, P. stipitis,* or *S. cerevisiae.* Furthermore, *P. stipitis* appeared to be the most efficient of the three cell types for producing ethanol from the experimental substrates tested.

Reference Example 32 - Alcohol Production Using Irradiation-Sonication Pretreatment

[0356] The optimum size for biomass conversion plants is affected by factors including economies of scale and the type and availability of biomass used as feedstock. Increasing plant size tends to increase economies of scale associated with plant processes. However, increasing plant size also tends to increase the costs (e.g., transportation costs) per unit of biomass feedstock. Studies analyzing these factors suggest that the appropriate size for biomass conversion plants can range from 2000 to 10,000 dried tons of biomass feedstock per day. The plant described below is sized to process 2000 tons of dry biomass feedstock per day.

[0357] FIG. 39 shows a process schematic of a biomass conversion system configured to process switchgrass. The feed preparation subsystem processes raw biomass feedstock to remove foreign objects and provide consistently sized particles for further processing. The pretreatment subsystem changes the molecular structure (e.g., reduces the average molecular weight and the crystallinity) of the biomass feedstock by irradiating the biomass feedstock, mixing the irradiated the biomass feedstock with water to form a slurry, and applying ultrasonic energy to the slurry. The irradiation and sonication convert the cellulosic and lignocellulosic components of the biomass feedstock into fermentable materials. The primary process subsystem ferments the glucose and other low weight sugars present after pretreatment to form alcohols.

Feed preparation

[0358] The selected design feed rate for the plant is 2,000 dry tons per day of switchgrass biomass. The design feed is chopped and/or sheared switchgrass.

[0359] Biomass feedstock, in the form of bales of switchgrass, is received by the plant on truck trailers. As the trucks are received, they are weighed and unloaded by forklifts. Some bales are sent to on-site storage while others are taken directly to the conveyors. From there, the bales are conveyed to an automatic unwrapping system that cuts away the plastic wrapping and/or net surrounding the bales. The biomass feedstock is then conveyed past a magnetic separator to remove tramp metal, after which it is introduced to shredder-shearer trains where the material is reduced in size. Finally, the biomass feedstock is conveyed to the pretreatment subsystem.

[0360] In some cases, the switchgrass bales are wrapped with plastic net to ensure they don't break apart when handled, and may also be wrapped in plastic film to protect the bale from weather. The bales are either square or round. The bales are received at the plant from off-site storage on large truck trailers.

[0361] Since switchgrass is only seasonally available, long-term storage is required to provide feed to the plant year-round. Long-term storage will likely consist of 400-500 acres of uncovered piled rows of bales at a location (or multiple

locations) reasonably close to the ethanol plant. On-site short-term storage is provided equivalent to 72 hours of production at an outside storage area. Bales and surrounding access ways as well as the transport conveyors will be on a concrete slab. A concrete slab is used because of the volume of traffic required to deliver the large amount of biomass feedstock required. A concrete slab will minimize the amount of standing water in the storage area, as well as reduce the biomass feedstock's exposure to dirt. The stored material provides a short-term supply for weekends, holidays, and when normal direct delivery of material into the process is interrupted.

[0362] The bales are off-loaded by forklifts and are placed directly onto bale transport conveyors or in the short-term storage area. Bales are also reclaimed from short-term storage by forklifts and loaded onto the bale transport conveyors.

[0363] Bales travel to one of two bale unwrapping stations. Unwrapped bales are broken up using a spreader bar and then discharged onto a conveyor, which passes a magnetic separator to remove metal prior to shredding. A tramp iron magnet is provided to catch stray magnetic metal and a scalping screen removes gross oversize and foreign material ahead of multiple shredder-shearer trains, which reduce the biomass feedstock to the proper size for pretreatment. The shredder-shearer trains include shredders and rotary knife cutters. The shredders reduce the size of the raw biomass feedstock and feed the resulting material to the rotary knife cutters. The rotary knife cutters concurrently shear the biomass feedstock and screen the resulting material.

[0364] Three storage silos are provided to limit overall system downtime due to required maintenance on and/or breakdowns of feed preparation subsystem equipment. Each silo can hold approximately 55,000 cubic feet of biomass feedstock (~3 hours of plant operation).

Pretreatment

[0365] A conveyor belt carries the biomass feedstock from the feed preparation subsystem 110 to the pretreatment subsystem 114. As shown in FIG. 40, in the pretreatment subsystem 114, the biomass feedstock is irradiated using electron beam emitters, mixed with water to form a slurry, and subjected to the application of ultrasonic energy. As discussed above, irradiation of the biomass feedstock changes the molecular structure (e.g., reduces the average molecular weight and the crystallinity) of the biomass feedstock. Mixing the irradiated biomass feedstock into a slurry and applying ultrasonic energy to the slurry further changes the molecular structure of the biomass feedstock. Application of the radiation and sonication in sequence may have synergistic effects in that the combination of techniques appears to achieve greater changes to the molecular structure (e.g., reduces the average molecular weight and the crystallinity) than either technique can efficiently achieve on its own. Without wishing to be bound by theory, in addition to reducing the polymerization of the biomass feedstock by breaking intramolecular bonds between segments of cellulosic and lignocellulosic components of the biomass feedstock, the irradiation may make the overall physical structure of the biomass feedstock more brittle. After the brittle biomass feedstock is mixed into a slurry, the application of ultrasonic energy further changes the molecular structure (e.g., reduces the average molecular weight and the crystallinity) and also can reduce the size of biomass feedstock particles.

Electron Beam Irradiation

[0366] The conveyor belt 491 carrying the biomass feedstock into the pretreatment subsystem distributes the biomass feedstock into multiple feed streams (e.g., 50 feed streams) each leading to separate electron beam emitters 492. In this embodiment, the biomass feedstock is irradiated while it is dry. Each feed stream is carried on a separate conveyor belt to an associated electron beam emitter. Each irradiation feed conveyor belt can be approximately one meter wide. Before reaching the electron beam emitter, a localized vibration is induced in each conveyor belt to evenly distribute the dry biomass feedstock over the cross-sectional width of the conveyor belt.

[0367] Electron beam emitter 492 (e.g., electron beam irradiation devices commercially available from Titan Corporation, San Diego, CA) are configured to apply a 100 kilo-Gray dose of electrons applied at a power of 300 kW. The electron beam emitters are scanning beam devices with a sweep width of 1 meter to correspond to the width of the conveyor belt. In some embodiments, electron beam emitters with large, fixed beam widths are used. Factors including belt/beam width, desired dose, biomass feedstock density, and power applied govern the number of electron beam emitters required for the plant to process 2,000 tons per day of dry feed.

Sonication

[0368] The irradiated biomass feedstock is mixed with water to form a slurry before ultrasonic energy is applied. There can be a separate sonication system associated with each electron beam feed stream or several electron beam streams can be aggregated as feed for a single sonication system.

[0369] In each sonication system, the irradiated biomass feedstock is fed into a reservoir 1214 through a first intake 1232 and water is fed into the reservoir 1214 through second intake 1234. Appropriate valves (manual or automated)

control the flow of biomass feedstock and the flow of water to produce a desired ratio of biomass feedstock to water (e.g., 10% cellulosic material, weight by volume). Each reservoir 1214 includes a mixer 1240 to agitate the contents of volume 1236 and disperse biomass feedstock throughout the water.

[0370] In each sonication system, the slurry is pumped (e.g., using a recessed impeller vortex pump 1218) from reservoir 1214 to and through a flow cell 1224 including an ultrasonic transducer 1226. In some embodiments, pump 1218 is configured to agitate the slurry 1216 such that the mixture of biomass feedstock and water is substantially uniform at inlet 1220 of the flow cell 1224. For example, the pump 1218 can agitate the slurry 1216 to create a turbulent flow that persists throughout the piping between the first pump and inlet 1220 of flow cell 1224.

[0371] Within the flow cell 1224, ultrasonic transducer 1226 transmits ultrasonic energy into slurry 1216 as the slurry flows through flow cell 1224. Ultrasonic transducer 1226 converts electrical energy into high frequency mechanical energy (e.g., ultrasonic energy), which is then delivered to the slurry through booster 48. Ultrasonic transducers are commercially available (e.g., from Hielscher USA, Inc. of Ringwood, New Jersey) that are capable of delivering a continuous power of 16 kilowatts.

[0372] The ultrasonic energy traveling through booster 1248 in reactor volume 1244 creates a series of compressions and rarefactions in process stream 1216 with an intensity sufficient to create cavitation in process stream 1216. Cavitation disaggregates components of the biomass feedstock including, for example, cellulosic and lignocellulosic material dispersed in process stream 1216 (e.g., slurry). Cavitation also produces free radicals in the water of process stream 1216 (e.g., slurry). These free radicals act to further break down the cellulosic material in process stream 1216. In general, about 250 MJ/m^3 of ultrasonic energy is applied to process stream 1216 containing fragments of poplar chips. Other levels of ultrasonic energy (between about 5 and about 4000 MJ/m^3, e.g., 10, 25, 50, 100, 250, 500, 750, 1000, 2000, or 3000) can be applied to other biomass feedstocks After exposure to ultrasonic energy in reactor volume 1244, process stream 1216 exits flow cell 24 through outlet 1222.

[0373] Flow cell 1224 also includes a heat exchanger 1246 in thermal communication with at least a portion of reactor volume 1244. Cooling fluid 1248 (e.g., water) flows into heat exchanger 1246 and absorbs heat generated when process stream 1216 (e.g., slurry) is sonicated in reactor volume 1244. In some embodiments, the flow of cooling fluid 1248 into heat exchanger 1246 is controlled to maintain an approximately constant temperature in reactor volume 1244. In addition or in the alternative, the temperature of cooling fluid 1248 flowing into heat exchanger 1246 is controlled to maintain an approximately constant temperature in reactor volume 1244.

[0374] The outlet 1242 of flow cell 1224 is arranged near the bottom of reservoir 1214 to induce a gravity feed of process stream 1216 (e.g., slurry) out of reservoir 1214 towards the inlet of a second pump 1230 which pumps process stream 1216 (e.g., slurry) towards the primary process subsystem.

[0375] Sonication systems can include a single flow path (as described above) or multiple parallel flow paths each with an associated individual sonication unit. Multiple sonication units can also be arranged to series to increase the amount of sonic energy applied to the slurry.

Primary Processes

[0376] A vacuum rotary drum type filter removes solids from the slurry before fermentation. Liquid from the filter is pumped cooled prior to entering the fermentors. Filtered solids are passed to the post-processing subsystem for further processing.

[0377] The fermentation tanks are large, low pressure, stainless steel vessels with conical bottoms and slow speed agitators. Multiple first stage fermentation tanks can be arranged in series. The temperature in the first stage fermentation tanks is controlled to 30 degrees centigrade using external heat exchangers. Yeast is added to the first stage fermentation tank at the head of each series of tanks and carries through to the other tanks in the series.

[0378] Second stage fermentation consists of two continuous fermentors in series. Both fermentors are continuously agitated with slow speed mechanical mixers. Temperature is controlled with chilled water in external exchangers with continuous recirculation. Recirculation pumps are of the progressive cavity type because of the high solids concentration.

[0379] Off gas from the fermentation tanks and fermentors is combined and washed in a counter-current water column before being vented to the atmosphere. The off gas is washed to recover ethanol rather than for air emissions control.

Post-Processing

Distillation

[0380] Distillation and molecular sieve adsorption are used to recover ethanol from the raw fermentation beer and produce 99.5% ethanol. Distillation is accomplished in two columns-the first, called the beer column, removes the dissolved CO2 and most of the water, and the second concentrates the ethanol to a near azeotropic composition.

[0381] All the water from the nearly azeotropic mixture is removed by vapor phase molecular sieve adsorption. Re-

generation of the adsorption columns requires that an ethanol water mixture be recycled to distillation for recovery.

[0382] Fermentation vents (containing mostly CO2, but also some ethanol) as well as the beer column vent are scrubbed in a water scrubber, recovering nearly all of the ethanol. The scrubber effluent is fed to the first distillation column along with the fermentation beer.

[0383] The bottoms from the first distillation contain all the unconverted insoluble and dissolved solids. The insoluble solids are dewatered by a pressure filter and sent to a combustor. The liquid from the pressure filter that is not recycled is concentrated in a multiple effect evaporator using waste heat from the distillation. The concentrated syrup from the evaporator is mixed with the solids being sent to the combustor, and the evaporated condensate is used as relatively clean recycle water to the process.

[0384] Because the amount of stillage water that can be recycled is limited, an evaporator is included in the process. The total amount of the water from the pressure filter that is directly recycled is set at 25%. Organic salts like ammonium acetate or lactate, steep liquor components not utilized by the organism, or inorganic compounds in the biomass end up in this stream. Recycling too much of this material can result in levels of ionic strength and osmotic pressures that can be detrimental to the fermenting organism's efficiency. For the water that is not recycled, the evaporator concentrates the dissolved solids into a syrup that can be sent to the combustor, minimizing the load to wastewater treatment.

Wastewater Treatment

[0385] The wastewater treatment section treats process water for reuse to reduce plant makeup water requirements. Wastewater is initially screened to remove large particles, which are collected in a hopper and sent to a landfill. Screening is followed by anaerobic digestion and aerobic digestion to digest organic matter in the stream. Anaerobic digestion produces a biogas stream that is rich in methane that is fed to the combustor. Aerobic digestion produces a relatively clean water stream for reuse in the process as well as a sludge that is primarily composed of cell mass. The sludge is also burned in the combustor. This screening /Anaerobic digestion / aerobic digestion scheme is standard within the current ethanol industry and facilities in the 1-5 million gallons per day range can be obtained as "off-the-shelf" units from vendors.

Combustor, Boiler, and Turbo-generator

[0386] The purpose of the combustor, boiler, and turbo-generator subsystem is to burn various by-product streams for steam and electricity generation. For example, some lignin, cellulose, and hemicellulose remains unconverted through the pretreatment and primary processes. The majority of wastewater from the process is concentrated to a syrup high in soluble solids. Anaerobic digestion of the remaining wastewater produces a biogas high in methane. Aerobic digestion produces a small amount of waste biomass (sludge). Burning these by-product streams to generate steam and electricity allows the plant to be self sufficient in energy, reduces solid waste disposal costs, and generates additional revenue through sales of excess electricity.

[0387] Three primary fuel streams (post-distillate solids, biogas, and evaporator syrup) are fed to a circulating fluidized bed combustor. The small amount of waste biomass (sludge) from wastewater treatment is also sent to the combustor. A fan moves air into the combustion chamber. Treated water enters the heat exchanger circuit in the combustor and is evaporated and superheated to 510°C (950°F) and 86 atm (1265 psia) steam. Flue gas from the combustor preheats the entering combustion air then enters a baghouse to remove particulates, which are landfilled. The gas is exhausted through a stack.

[0388] A multistage turbine and generator are used to generate electricity. Steam is extracted from the turbine at three different conditions for injection into the pretreatment reactor and heat exchange in distillation and evaporation. The remaining steam is condensed with cooling water and returned to the boiler feedwater system along with condensate from the various heat exchangers in the process. Treated well water is used as makeup to replace steam used in direct injection.

Claims

1. A method comprising:

converting a low molecular weight sugar, in a mixture comprising a biomass, a microorganism, and a solvent or solvent system, to a combustible fuel, further comprising irradiating the biomass prior to mixing with ionizing radiation at a total dosage of at least 5 Mrad, wherein the converting comprises fermentation wherein the biomass is not consumed during the fermentation, and wherein the biomass comprises a cellulosic or lignocellulosic material.

2. The method of claim 1 wherein converting comprises allowing the microorganism to convert at least a portion of the low molecular weight sugar to ethanol.

3. The method of claim 1 or 2 wherein the microorganism comprises a yeast, in particular a yeast selected from the group consisting of S. cerevisiae and P. stipitis, or wherein the microorganism comprises a bacterium, e.g., Zymomonas mobilis.

4. The method of claim 1 wherein irradiating is performed using a particle beam, such as a beam of electrons.

5. The method of any one of the above claims wherein the biomass has a bulk density of less than about 0.5 g/cm^3 prior to addition to the mixture, and, optionally, wherein the biomass is fibrous.

6. The method of any one of the above claims further comprising physically preparing the biomass, e.g., by shearing, or by reducing the size of the biomass by stone grinding, mechanical ripping or tearing, pin grinding, crushing, or air attrition milling.

7. The method of any one of the above claims wherein the biomass has a BET surface area of greater than 0.25 m^2/g.

8. The method of any one of the above claims wherein the biomass has a length to diameter ratio of at least 5.

9. The method of any one of the above claims, wherein the biomass is selected from the group consisting of paper, paper products, paper waste, wood, particle board, sawdust, agricultural waste, sewage, silage, grasses, rice hulls, bagasse, cotton, jute, hemp, flax, bamboo, sisal, abaca, straw, corn cobs, corn stover, switchgrass, alfalfa, hay, coconut hair, cotton, seaweed, algae, and mixtures thereof.

10. The method of any one of the above claims wherein the biomass has internal fibers, and wherein the biomass feedstock has been sheared to an extent that its internal fibers are substantially exposed.

11. The method of any one of the above claims wherein the biomass has a porosity greater than 50 percent, e.g., greater than 70 percent.

12. The method of any one of the above claims wherein the converting step exhibits a percent performance of at least 140 percent, e.g., at least 170 percent.

13. The method of any one of the above claims, wherein the fuel comprises an alcohol.

14. The method of Claim 13, wherein the alcohol comprises ethanol.

Patentansprüche

1. Verfahren, umfassend:

   Umwandeln eines Zuckers mit niedrigem Molekulargewicht in einem Gemisch, das eine Biomasse, einen Mikroorganismus und ein Lösungsmittel oder Lösungsmittelsystem umfasst, in einen Brennstoff, weiterhin umfassend das Bestrahlen der Biomasse mit ionisierender Strahlung mit einer Gesamtdosis von wenigstens 5 Mrad vor dem Mischen, wobei das Umwandeln eine Fermentation umfasst, wobei die Biomasse während der Fermentation nicht verbraucht wird, und wobei die Biomasse ein Cellulose- oder Lignocellulosematerial umfasst.

2. Verfahren nach Anspruch 1, wobei das Umwandeln umfasst, dem Mikroorganismus zu gestatten, wenigstens einen Teil des Zuckers mit niedrigem Molekulargewicht in Ethanol umzuwandeln.

3. Verfahren nach Anspruch 1 oder 2, wobei der Mikroorganismus eine Hefe, insbesondere eine Hefe, die aus der Gruppe bestehend aus S. cerevisiae und P. stipitis ausgewählt ist, umfasst oder wobei der Mikroorganismus ein Bakterium, z. B. Zymomonas mobilis, umfasst.

4. Verfahren nach Anspruch 1, wobei das Bestrahlen unter Verwendung eines Teilchenstrahls, wie z. B. eines Elektronenstrahls, durchgeführt wird.

5. Verfahren nach einem der obigen Ansprüche, wobei die Biomasse vor der Zugabe zu dem Gemisch eine Schüttdichte von weniger als 0,5 g/cm^3 aufweist und wobei die Biomasse optional faserig ist.

6. Verfahren nach einem der obigen Ansprüche, das weiterhin das physikalische Aufbereiten der Biomasse, z. B. durch Scheren oder durch Verringern der Größe der Biomasse durch Steinmahlen, mechanisches Zerreißen oder Reißen, Stiftmahlen, Brechen oder Reibungsmahlen unter Luftzufuhr, umfasst.

7. Verfahren nach einem der obigen Ansprüche, wobei die Biomasse eine BET-Oberfläche von über 0,25 m^2/g aufweist.

8. Verfahren nach einem der obigen Ansprüche, wobei die Biomasse ein Länge-Durchmesser-Verhältnis von wenigstens 5 aufweist.

9. Verfahren nach einem der obigen Ansprüche, wobei die Biomasse aus der Gruppe bestehend aus Papier, Papierprodukten, Papierabfall, Holz, Spanplatte, Sägemehl, Landwirtschaftsabfall, Abwasser, Silage, Gräsern, Reisschalen, Bagasse, Baumwolle, Jute, Hanf, Flachs, Bambus, Sisal, Abacä, Stroh, Maisspindeln, Maisstroh, Rutenhirse, Luzerne, Heu, Kokosfasern, Baumwolle, Seetang, Algen und Gemischen derselben ausgewählt ist.

10. Verfahren nach einem der obigen Ansprüche, wobei die Biomasse innere Fasern aufweist und wobei das Biomasseeinsatzgut in einem Ausmaß geschert wurde, dass seine inneren Fasern im Wesentlichen freiliegen.

11. Verfahren nach einem der obigen Ansprüche, wobei die Biomasse eine Porosität von über 50 Prozent, z. B. über 70 Prozent, aufweist.

12. Verfahren nach einem der obigen Ansprüche, wobei der Umwandlungsschritt eine prozentuale Leistung von wenigstens 140 Prozent, z. B. wenigstens 170 Prozent, aufweist.

13. Verfahren nach einem der obigen Ansprüche, wobei der Brennstoff einen Alkohol umfasst.

14. Verfahren nach Anspruch 13, wobei der Alkohol Ethanol umfasst.

Revendications

1. Procédé comprenant :

   la conversion d'un sucre à faible poids moléculaire, dans un mélange comprenant une biomasse, un micro-organisme, et un solvant ou système de solvants, en un carburant combustible, comprenant en outre l'irradiation de la biomasse avant le mélange avec un rayonnement ionisant à un dosage total d'au moins 5 Mrad, dans lequel la conversion comprend une fermentation sans consommation de la biomasse pendant la fermentation, et dans lequel la biomasse comprend un matériau cellulosique ou lignocellulosique.

2. Procédé selon la revendication 1, dans lequel la conversion comprend le fait de permettre au micro-organisme de convertir au moins une partie du sucre à faible poids moléculaire en éthanol.

3. Procédé selon la revendication 1 ou 2, dans lequel le micro-organisme comprend une levure, en particulier une levure choisie dans le groupe consistant en S. cerevisiae et P. stipitis, ou dans lequel le micro-organisme comprend une bactérie, par exemple Zymomonas mobilis.

4. Procédé selon la revendication 1, dans lequel l'irradiation est réalisée en utilisant un faisceau de particules, tel qu'un faisceau d'électrons.

5. Procédé selon l'une quelconque des revendications précédentes, dans lequel la biomasse a une masse volumique de moins d'environ 0,5 g/cm^3 avant l'addition au mélange, et, facultativement, dans lequel la biomasse est fibreuse.

6. Procédé selon l'une quelconque des revendications précédentes, comprenant en outre la préparation physique de la biomasse, par exemple par cisaillement, ou par réduction de la taille de la biomasse par broyage à la meule, déchirement ou déchiquetage mécanique, meulage au tourillon, concassage ou broyage par attrition.

7. Procédé selon l'une quelconque des revendications précédentes, dans lequel la biomasse a une surface spécifique BET de plus de 0,25 m^2/g.

8. Procédé selon l'une quelconque des revendications précédentes, dans lequel la biomasse a un rapport entre longueur et diamètre d'au moins 5.

9. Procédé selon l'une quelconque des revendications précédentes, dans lequel la biomasse est choisie dans le groupe consistant en le papier, les produits de papier, les déchets de papier, le bois, le panneau de particules, la sciure, les déchets agricoles, les eaux usées, le fourrage vert, les poacées, les balles de riz, la bagasse, le coton, le jute, le chanvre, le lin, le bambou, le sisal, l'abaca, la paille, les rafles de maïs, la paille de maïs, le panic érigé, la luzerne, le foin, les fibres de coco, le coton, le varech, les algues, et des mélanges de ceux-ci.

10. Procédé selon l'une quelconque des revendications précédentes, dans lequel la biomasse a des fibres internes, et dans lequel la charge d'alimentation de biomasse a été cisaillée dans une mesure telle que ses fibres internes sont sensiblement exposées.

11. Procédé selon l'une quelconque des revendications précédentes, dans lequel la biomasse a une porosité supérieure à 50 pour cent, par exemple supérieure à 70 pour dent.

12. Procédé selon l'une quelconque des revendications précédentes, dans lequel l'étape de conversion affiche une performance en pour cent d'au moins 140 pour cent, par exemple, d'au moins 170 pour cent.

13. Procédé selon l'une quelconque des revendications précédentes, dans lequel le carburant comprend un alcool.

14. Procédé selon la revendication 13, dans lequel l'alcool comprend de l'éthanol.

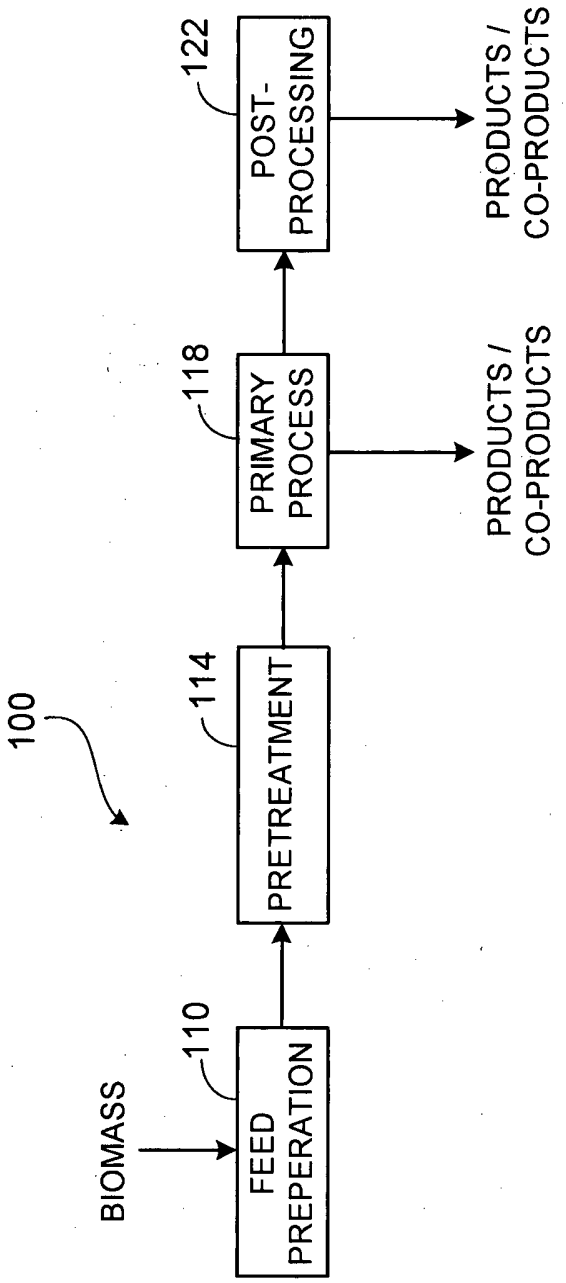

100
BIOMASS
110
FEED PREPERATION
114
PRETREATMENT
118
PRIMARY PROCESS
PRODUCTS / CO-PRODUCTS
122
POST-PROCESSING
PRODUCTS / CO-PRODUCTS

FIG. 1

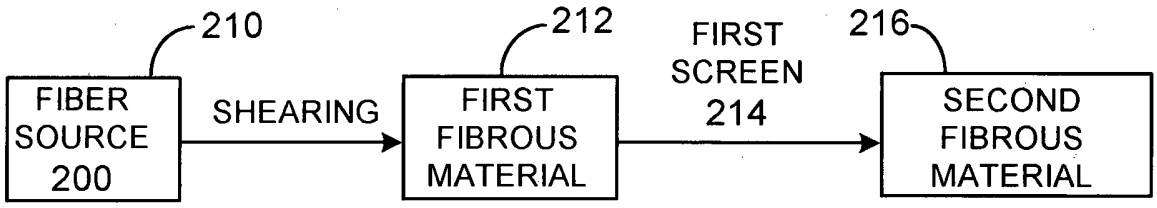

210
212
FIRST
SCREEN
214
216
FIBER
SOURCE
200
SHEARING
FIRST
FIBROUS
MATERIAL
SECOND
FIBROUS
MATERIAL

FIG. 2

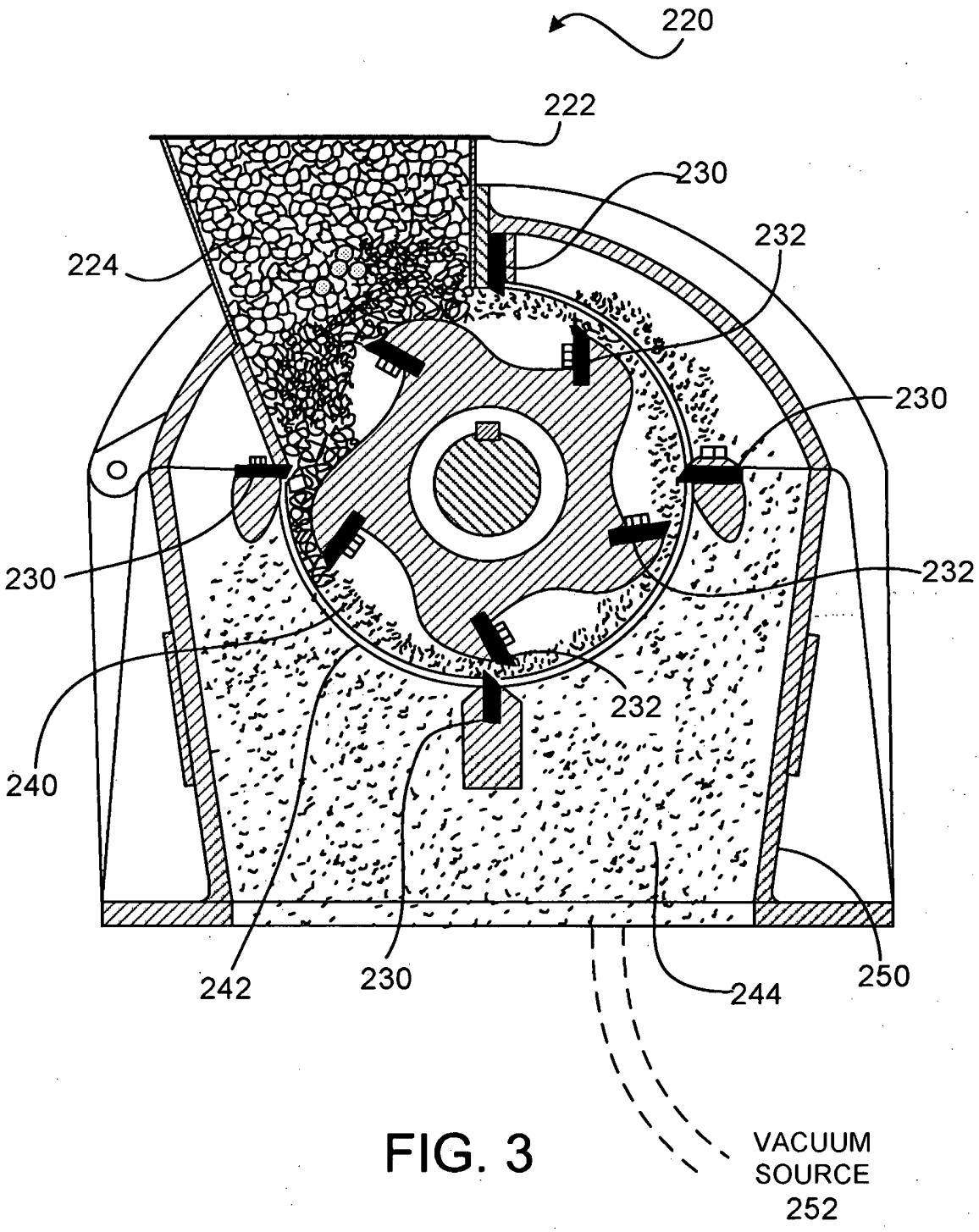

220
222
230
232
224
230
232
230
232
240
242
230
244
250
VACUUM
SOURCE
252

FIG. 3

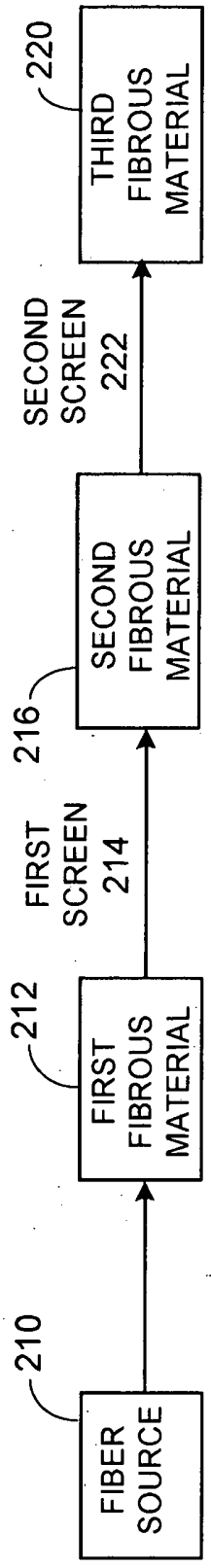

210
FIBER SOURCE
212
FIRST FIBROUS MATERIAL
FIRST SCREEN 214
216
SECOND FIBROUS MATERIAL
SECOND SCREEN 222
220
THIRD FIBROUS MATERIAL

FIG. 4

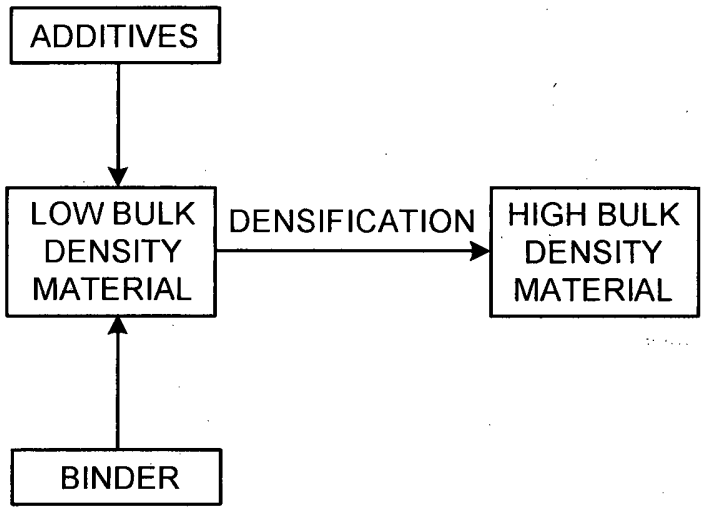

ADDITIVES
LOW BULK DENSITY MATERIAL
DENSIFICATION
HIGH BULK DENSITY MATERIAL
BINDER

FIG. 5

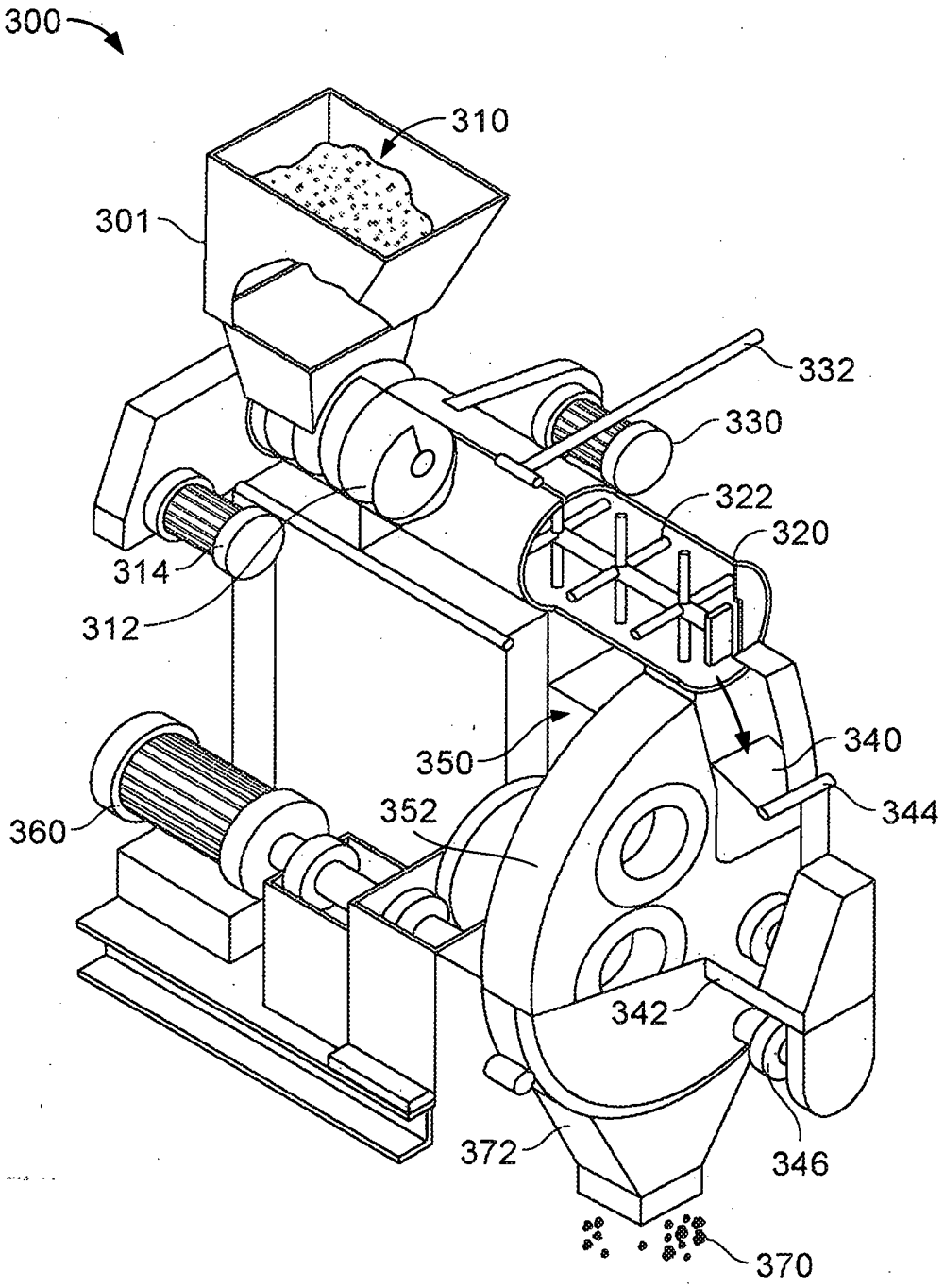

300
310
301
332
330
322
320
314
312
340
350
344
352
360
342
372
346
370

FIG. 6

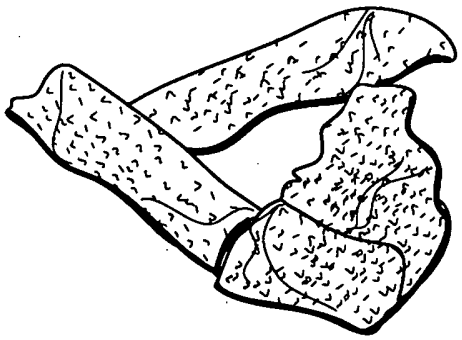


FIG. 7A

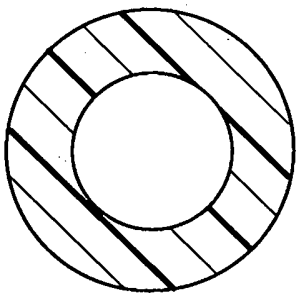


FIG. 7B

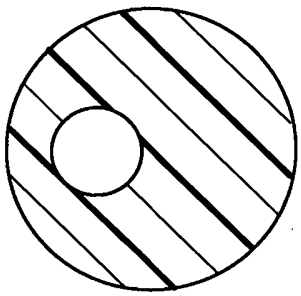


FIG. 7C

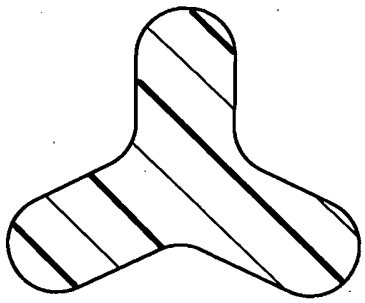


FIG. 7D

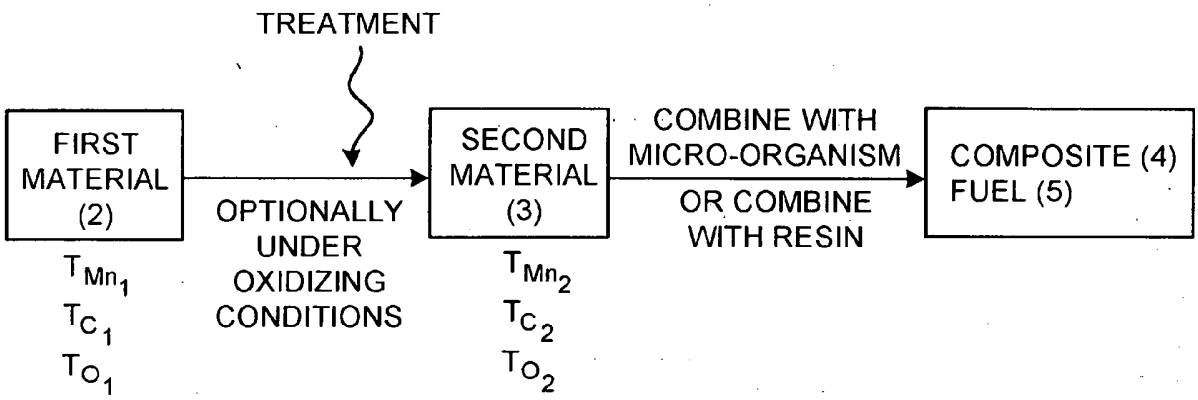

TREATMENT
FIRST MATERIAL (2)
T_{Mn_1}
T_{C_1}
T_{O_1}
OPTIONALLY UNDER OXIDIZING CONDITIONS
SECOND MATERIAL (3)
T_{Mn_2}
T_{C_2}
T_{O_2}
COMBINE WITH MICRO-ORGANISM
OR COMBINE WITH RESIN
COMPOSITE (4) FUEL (5)

FIG. 8

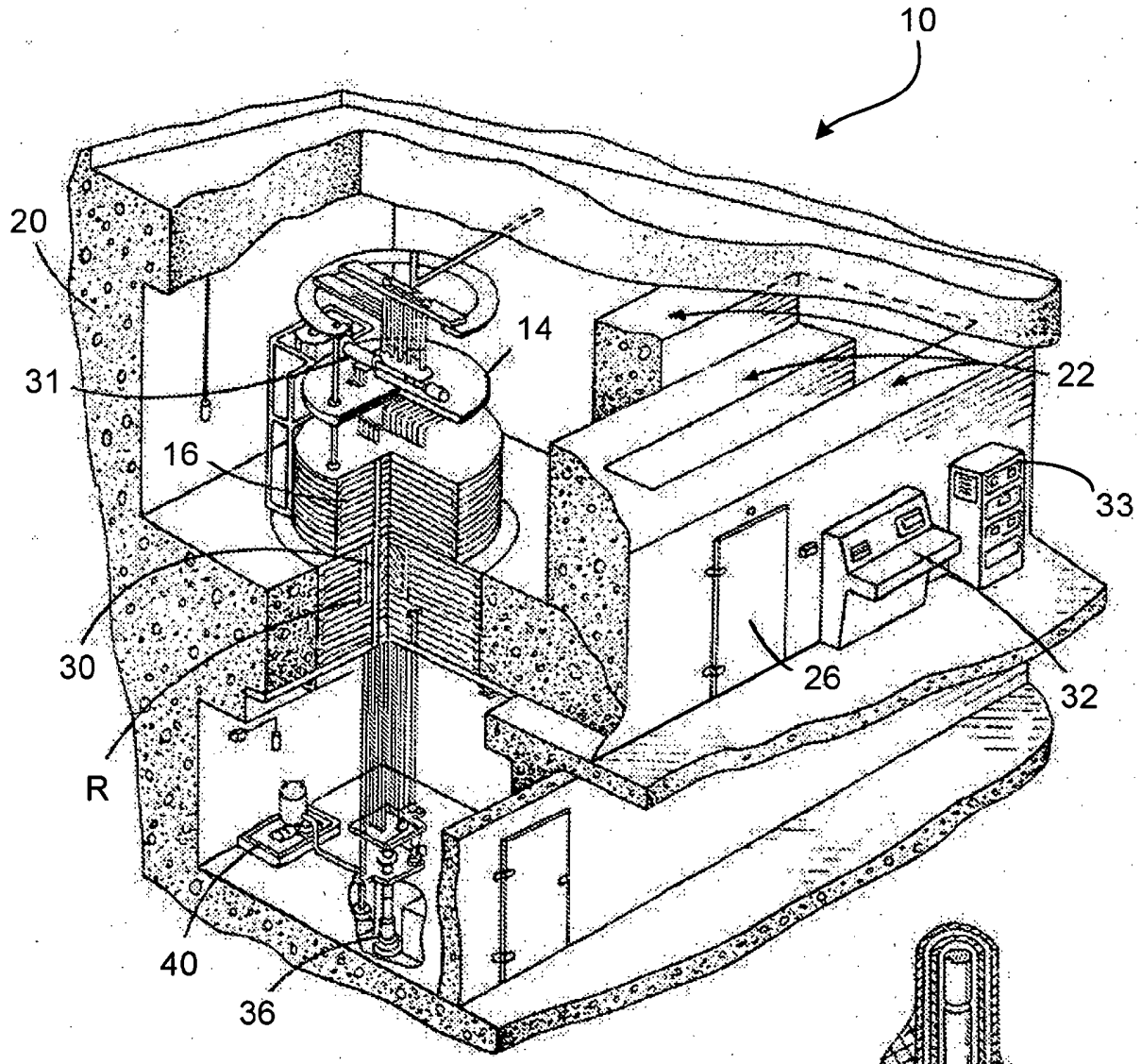


FIG. 9

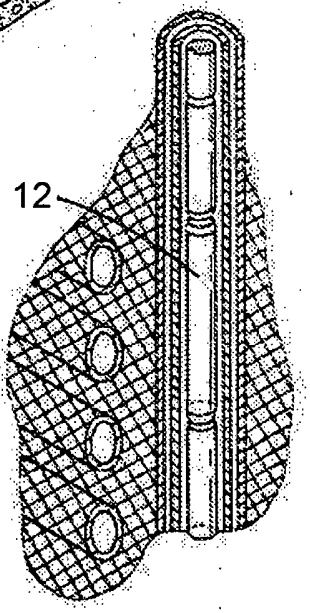


FIG. 10

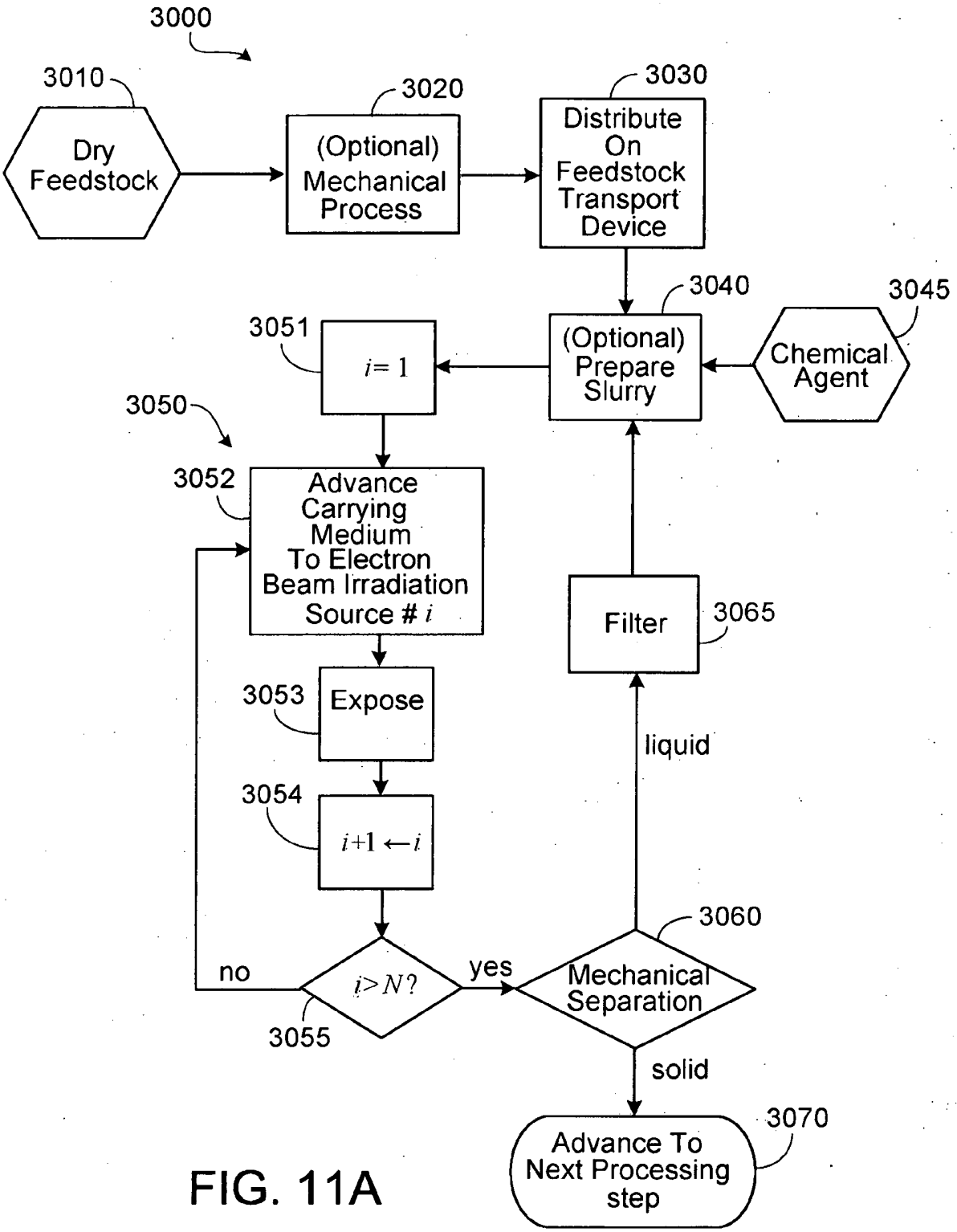

3000
3010
Dry Feedstock
3020
(Optional) Mechanical Process
3030
Distribute On Feedstock Transport Device
3040
(Optional) Prepare Slurry
3045
Chemical Agent
3051
i= 1
3050
3052
Advance Carrying Medium To Electron Beam Irradiation Source # i
3053
Expose
3054
i+1 ← i
i>N?
3055
no
yes
Mechanical Separation
3060
Filter
3065
liquid
solid
3070
Advance To Next Processing step

FIG. 11A

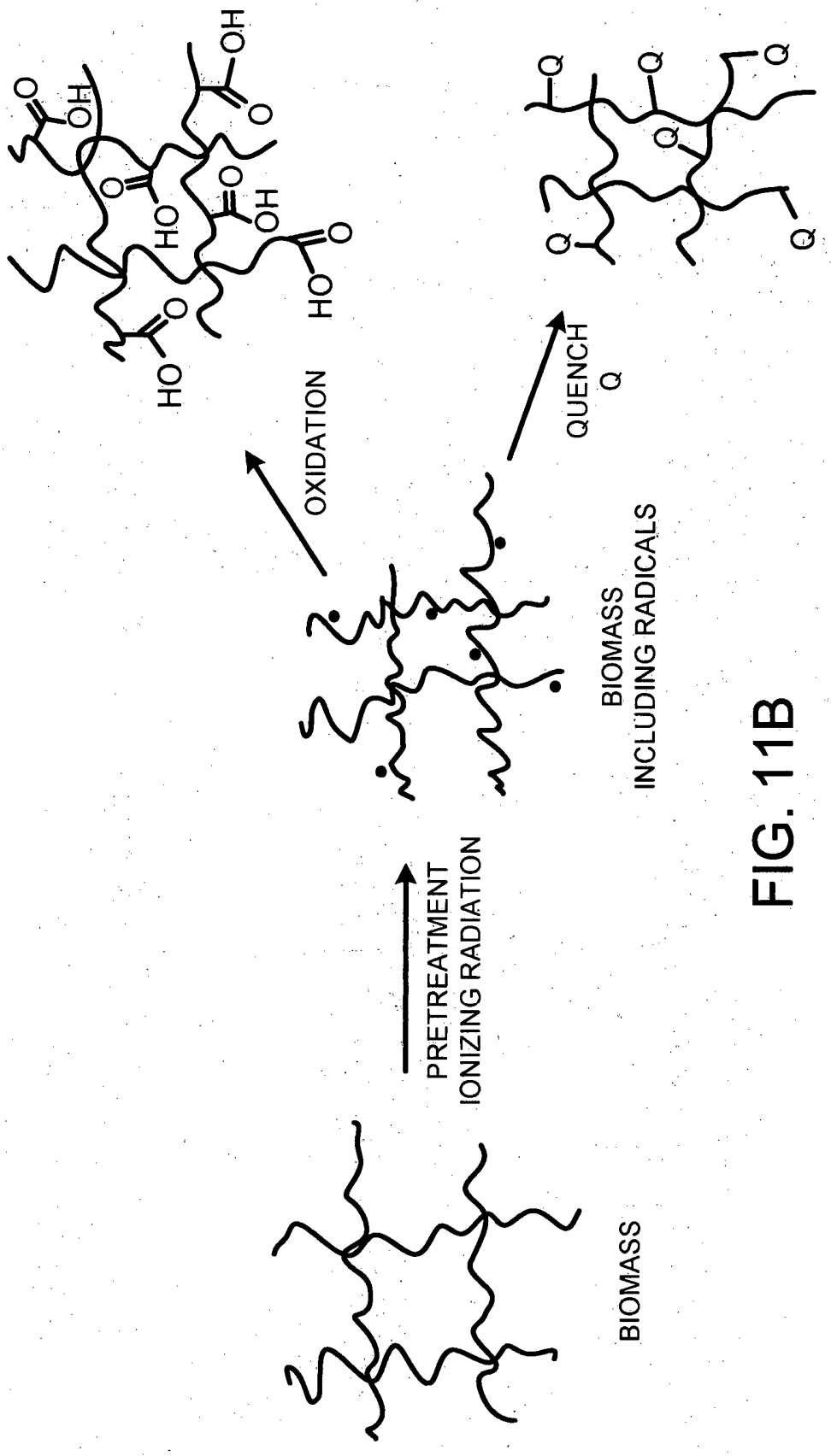

BIOMASS
PRETREATMENT
IONIZING RADIATION
BIOMASS
INCLUDING RADICALS
OXIDATION
QUENCH
Q

FIG. 11B

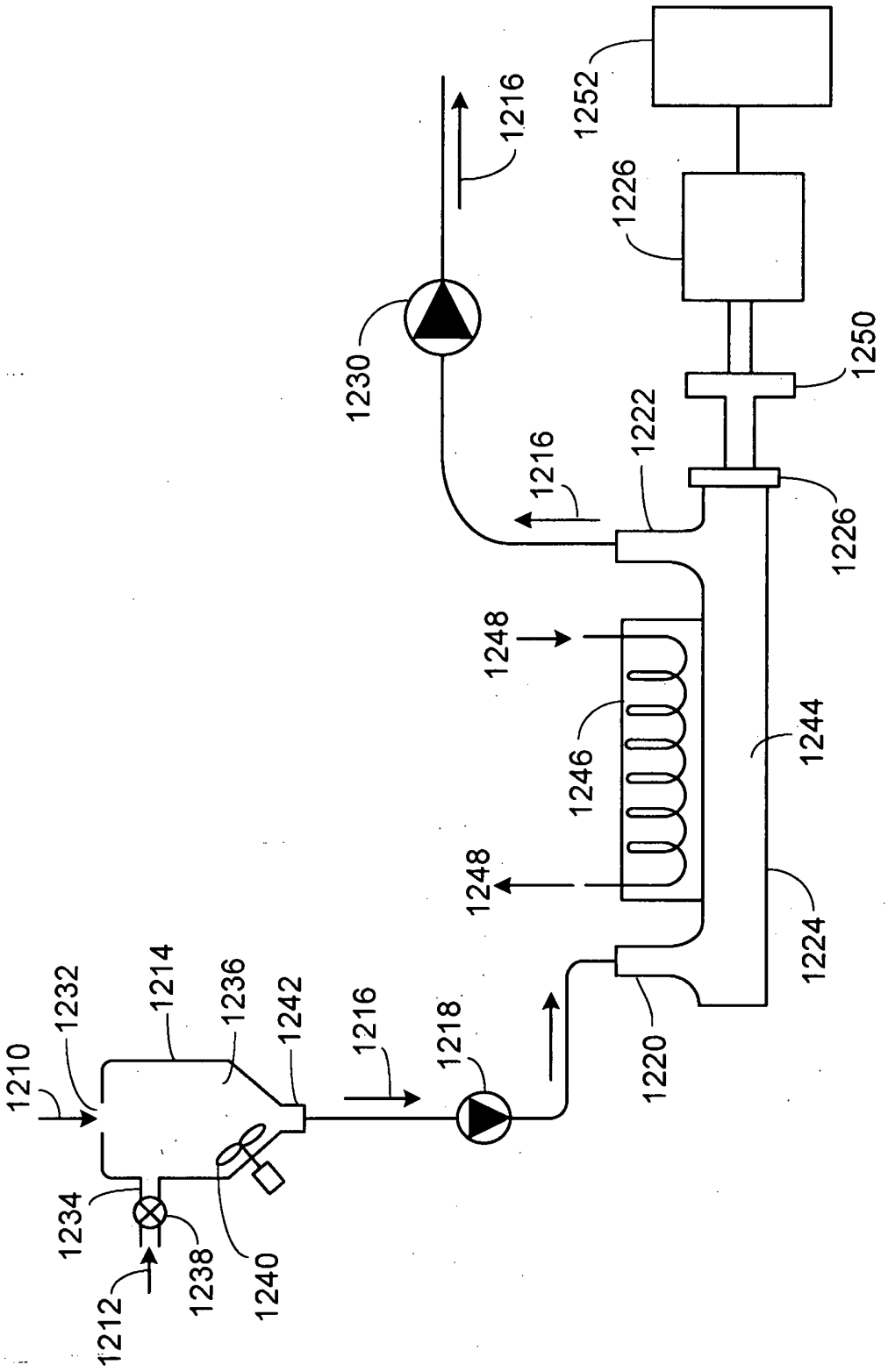

1252
1216
1226
1230
1250
1222
1216
1226
1248
1246
1244
1248
1224
1220
1218
1216
1242
1236
1214
1232
1210
1234
1238
1240
1212

FIG. 12

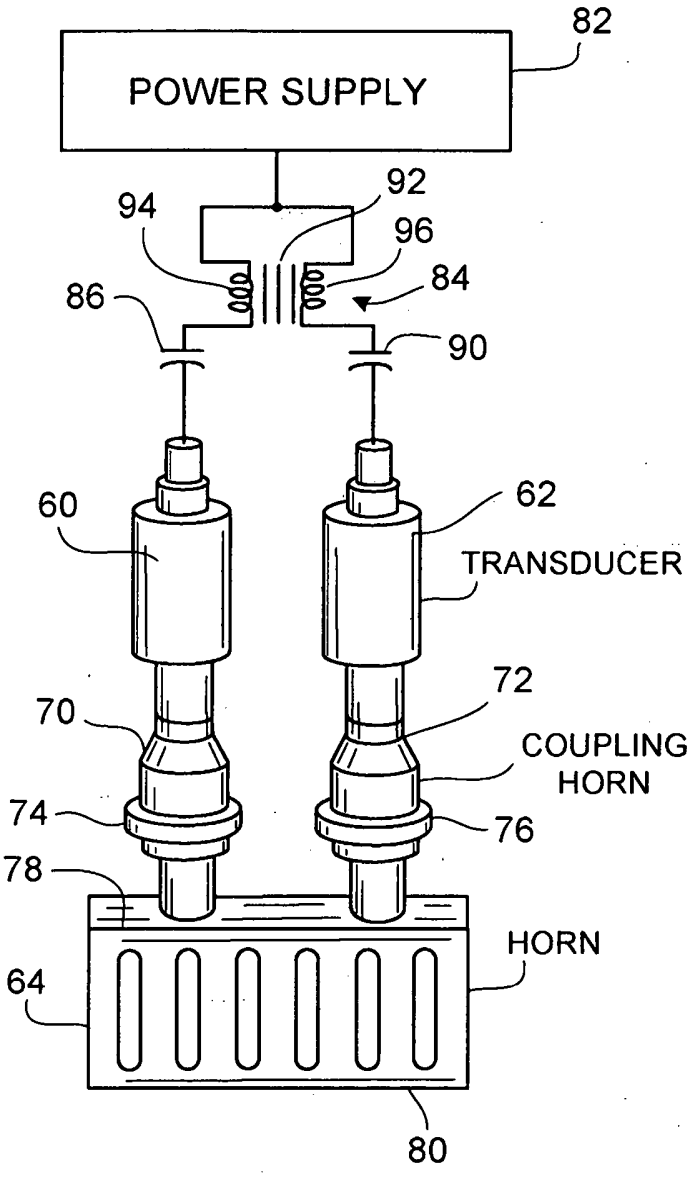

POWER SUPPLY
82
92
94
96
84
86
90
60
62
TRANSDUCER
72
70
COUPLING
HORN
74
76
78
HORN
64
80

FIG. 13

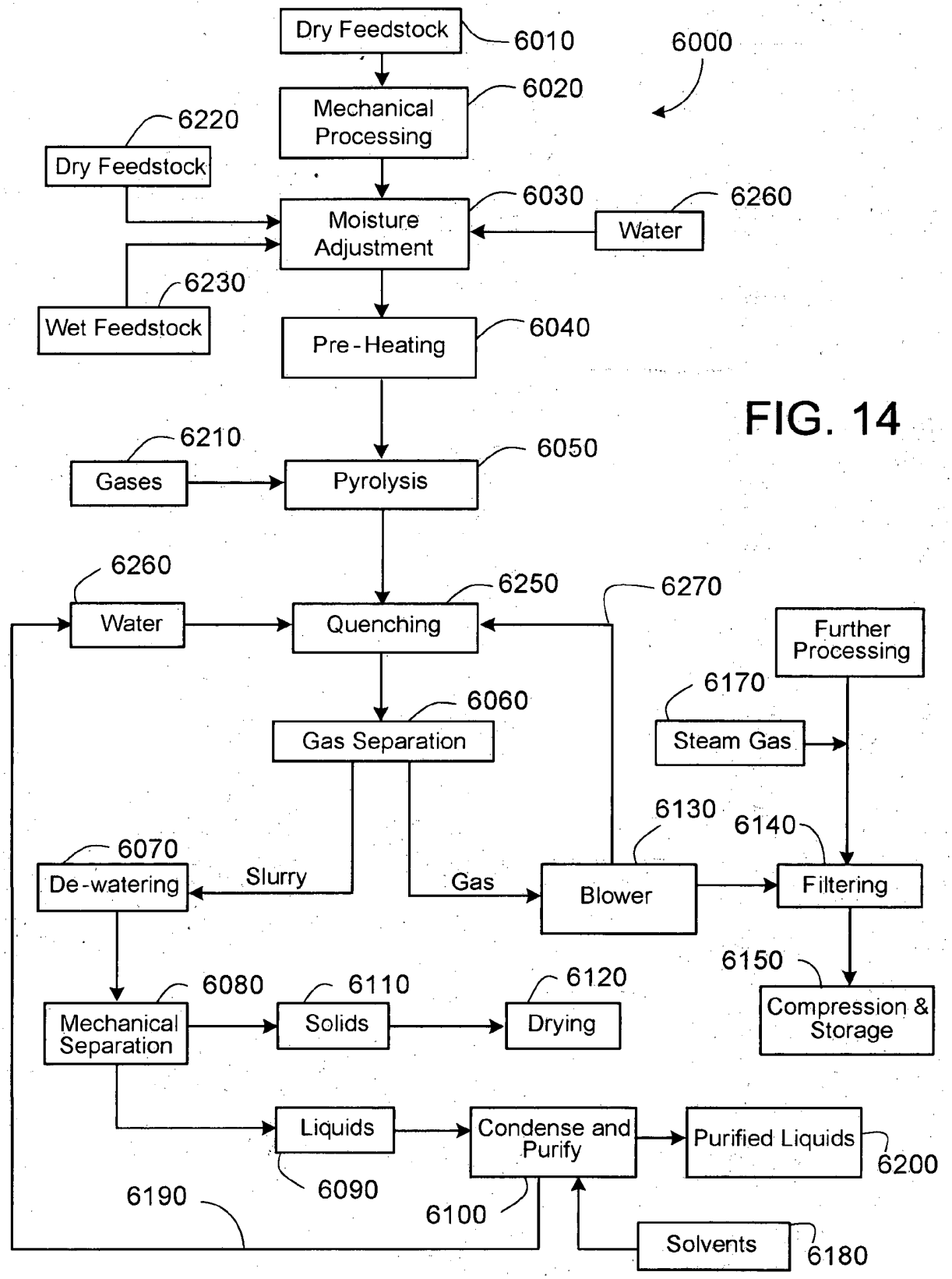

Dry Feedstock
6010
6000
Mechanical Processing
6020
6220
Dry Feedstock
Moisture Adjustment
6030
6260
Water
6230
Wet Feedstock
Pre-Heating
6040
6210
Gases
Pyrolysis
6050
6260
Water
Quenching
6250
6270
Further Processing
6170
Steam Gas
Gas Separation
6060
6130
6140
6070
De-watering
Slurry
Gas
Blower
Filtering
6150
Compression & Storage
6080
6110
6120
Mechanical Separation
Solids
Drying
Liquids
Condense and Purify
Purified Liquids
6200
6090
6190
6100
Solvents
6180

FIG. 14

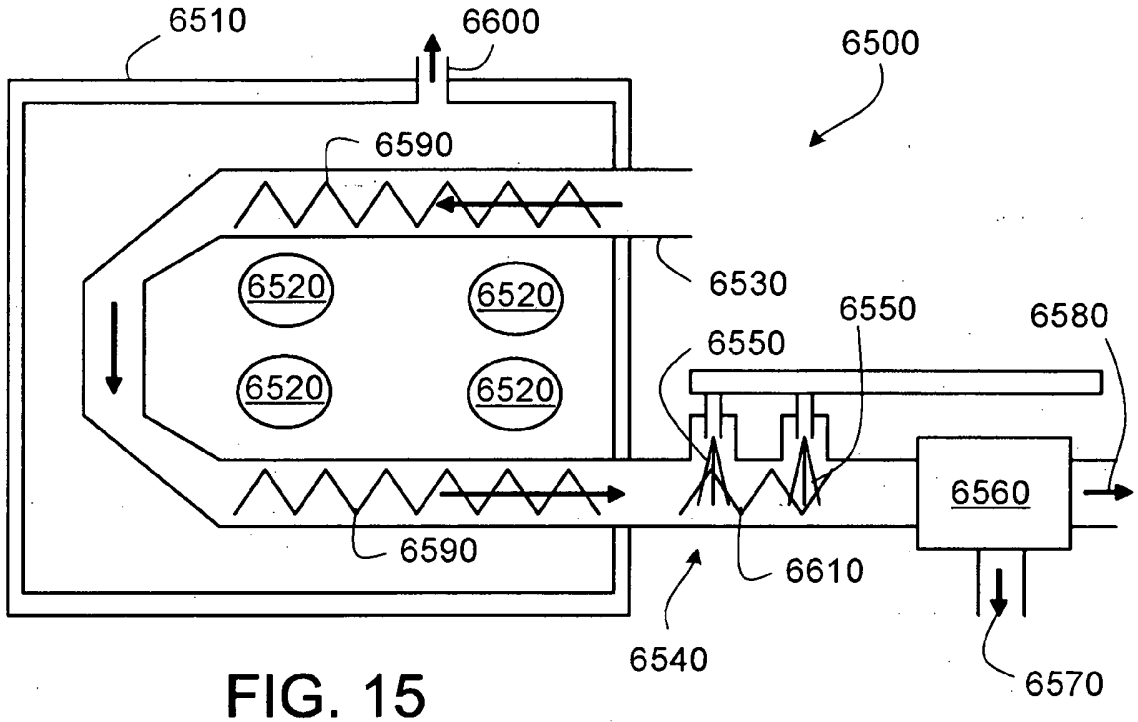

6510
6600
6500
6590
6520
6520
6520
6520
6530
6550
6550
6580
6560
6590
6610
6540
6570

FIG. 15

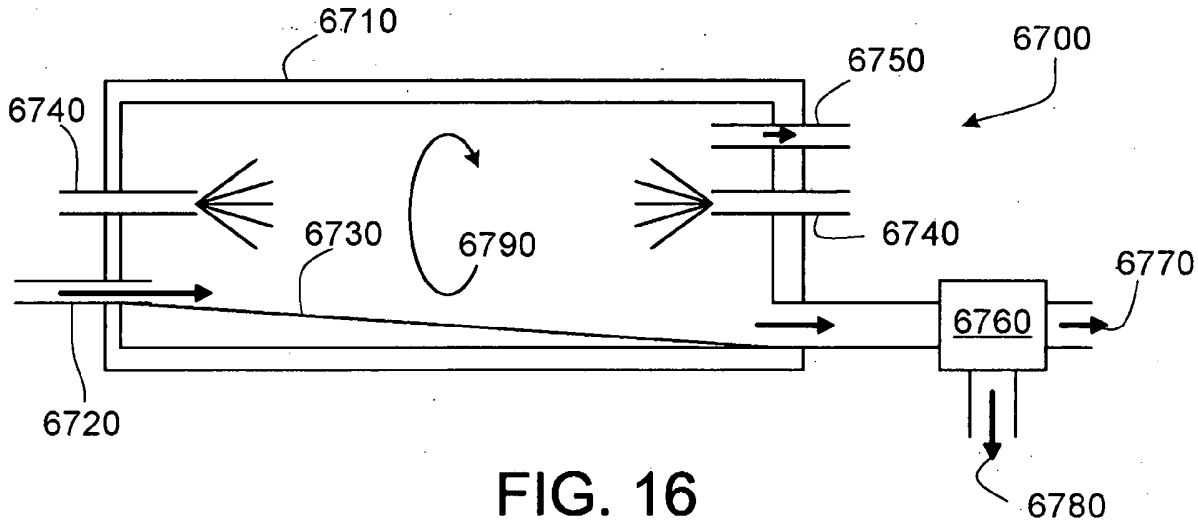

6710
6750
6700
6740
6730
6790
6740
6770
6760
6720
6780

FIG. 16

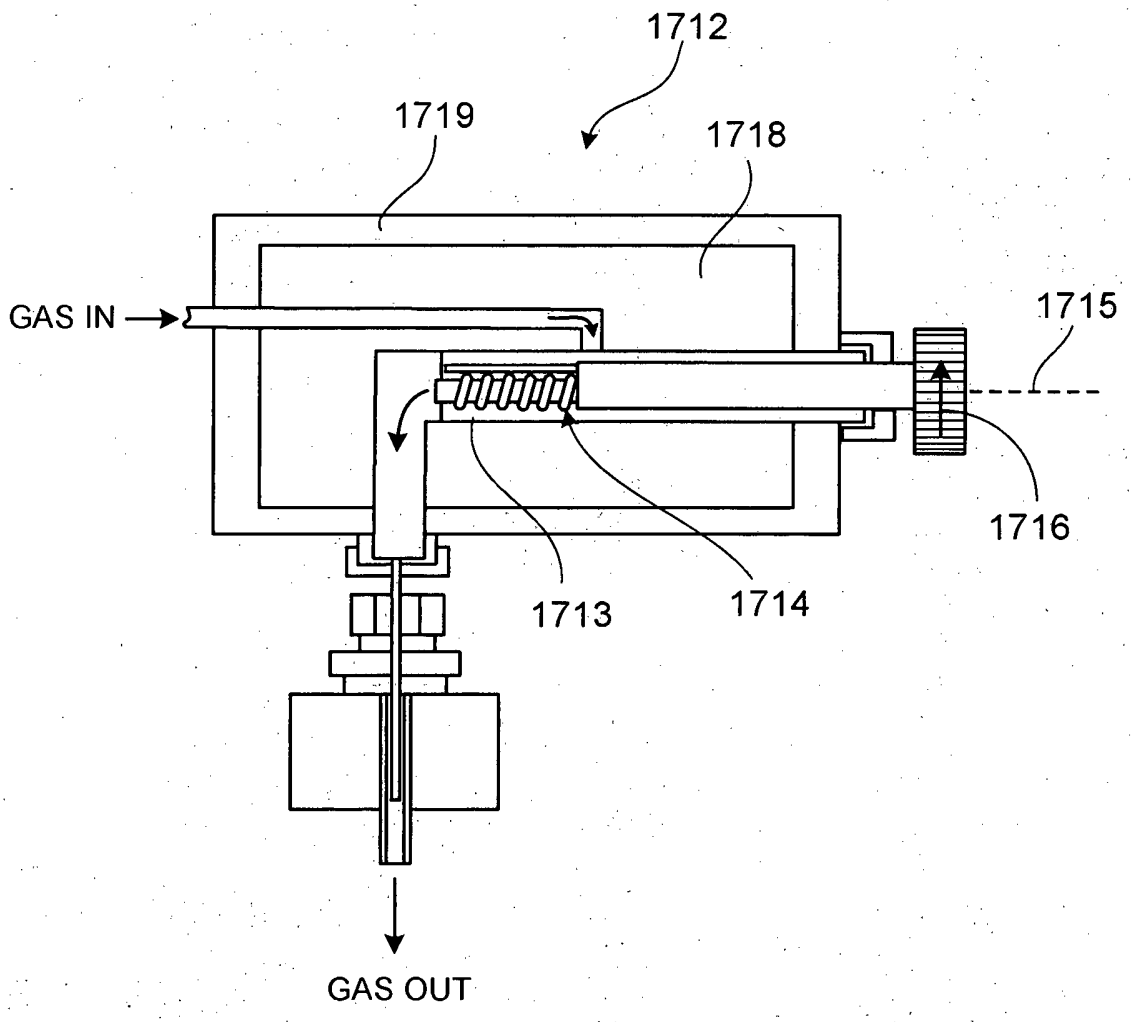


FIG. 17

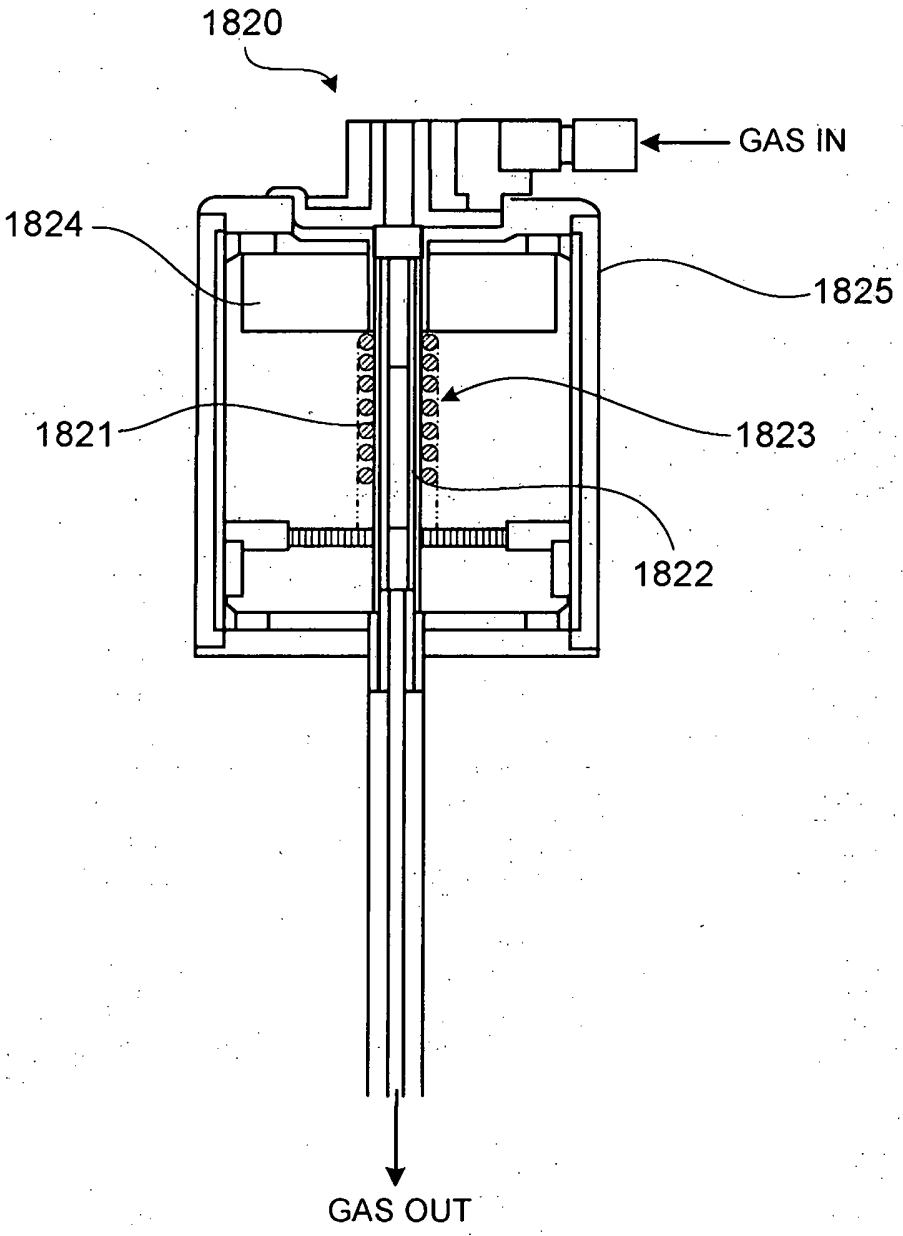


FIG. 18

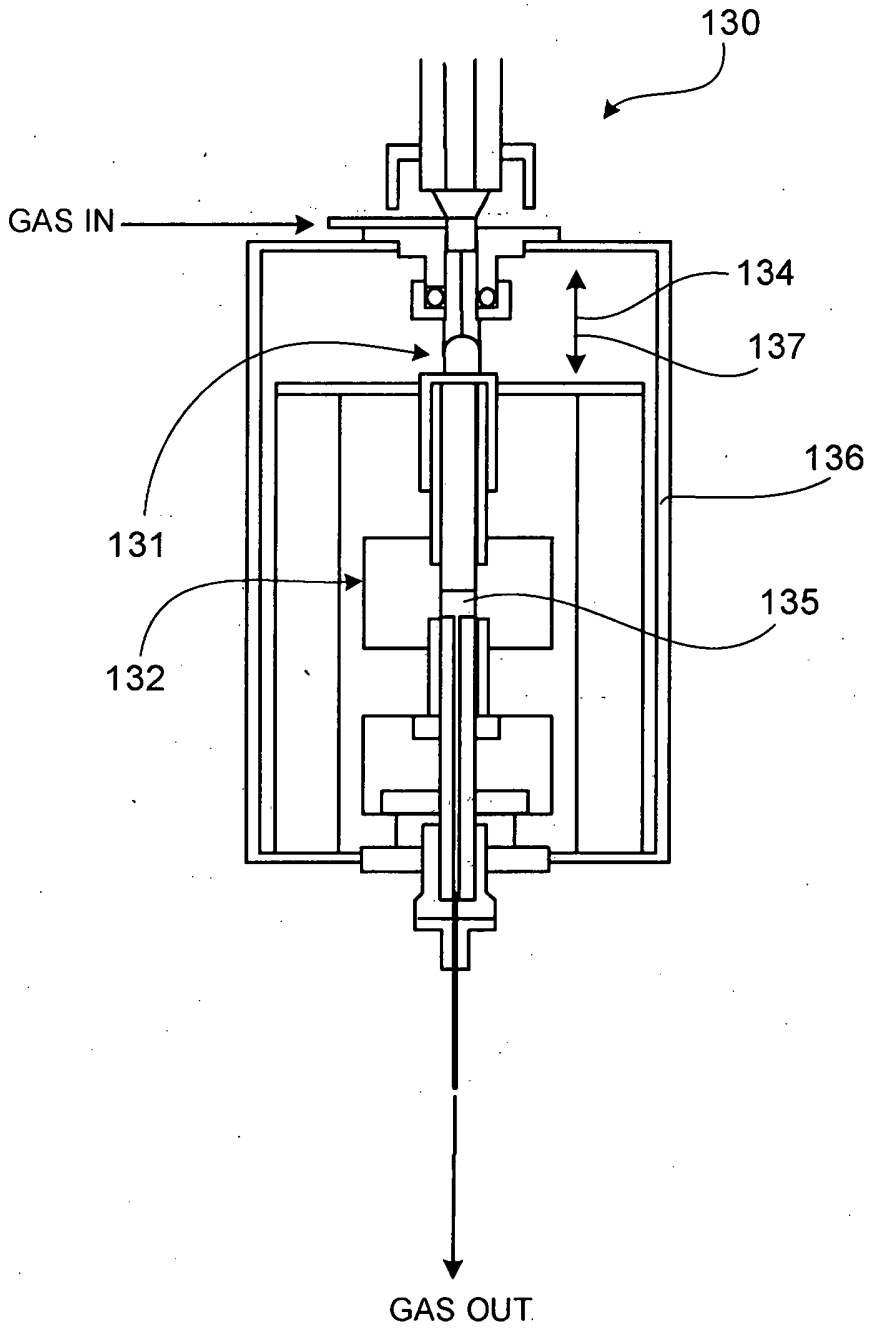


FIG. 19

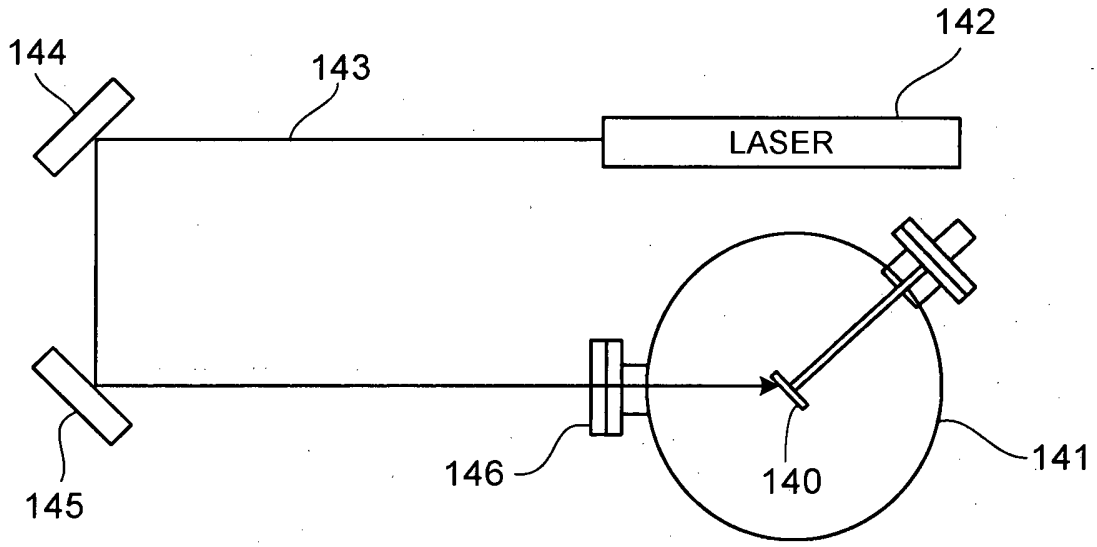


FIG. 20

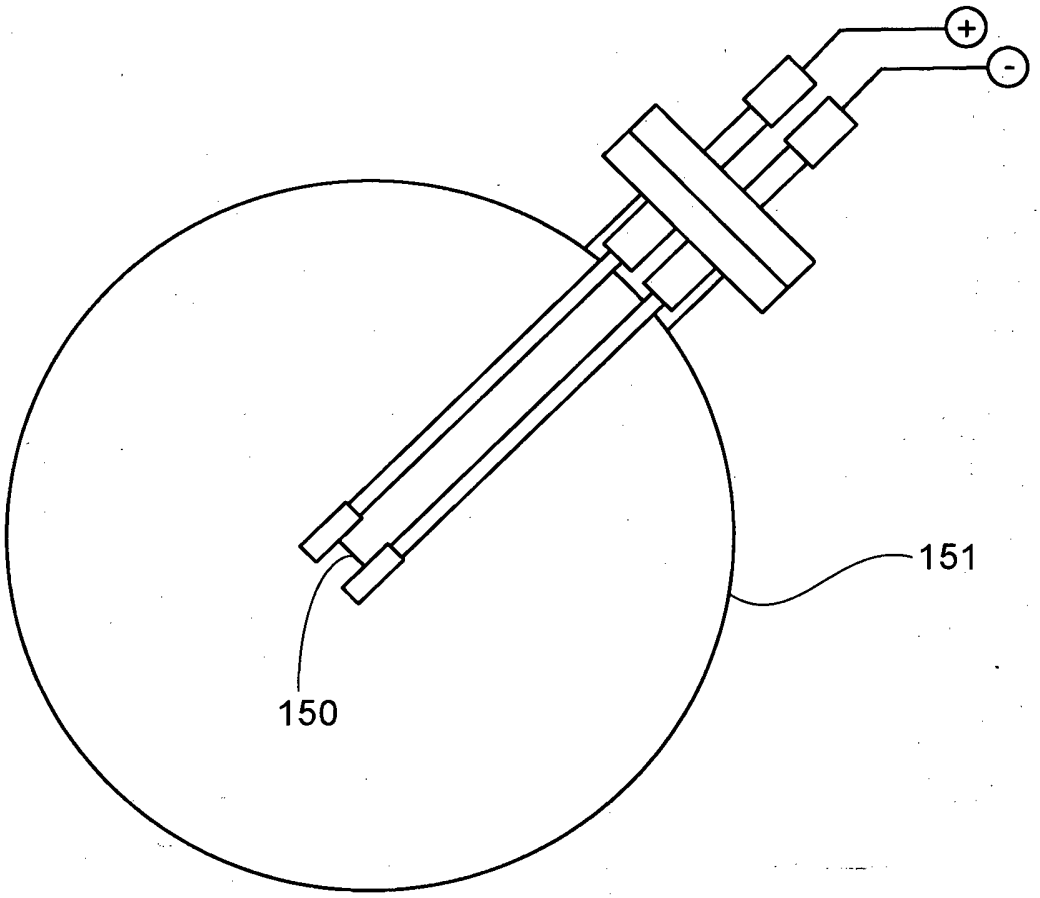


FIG. 21

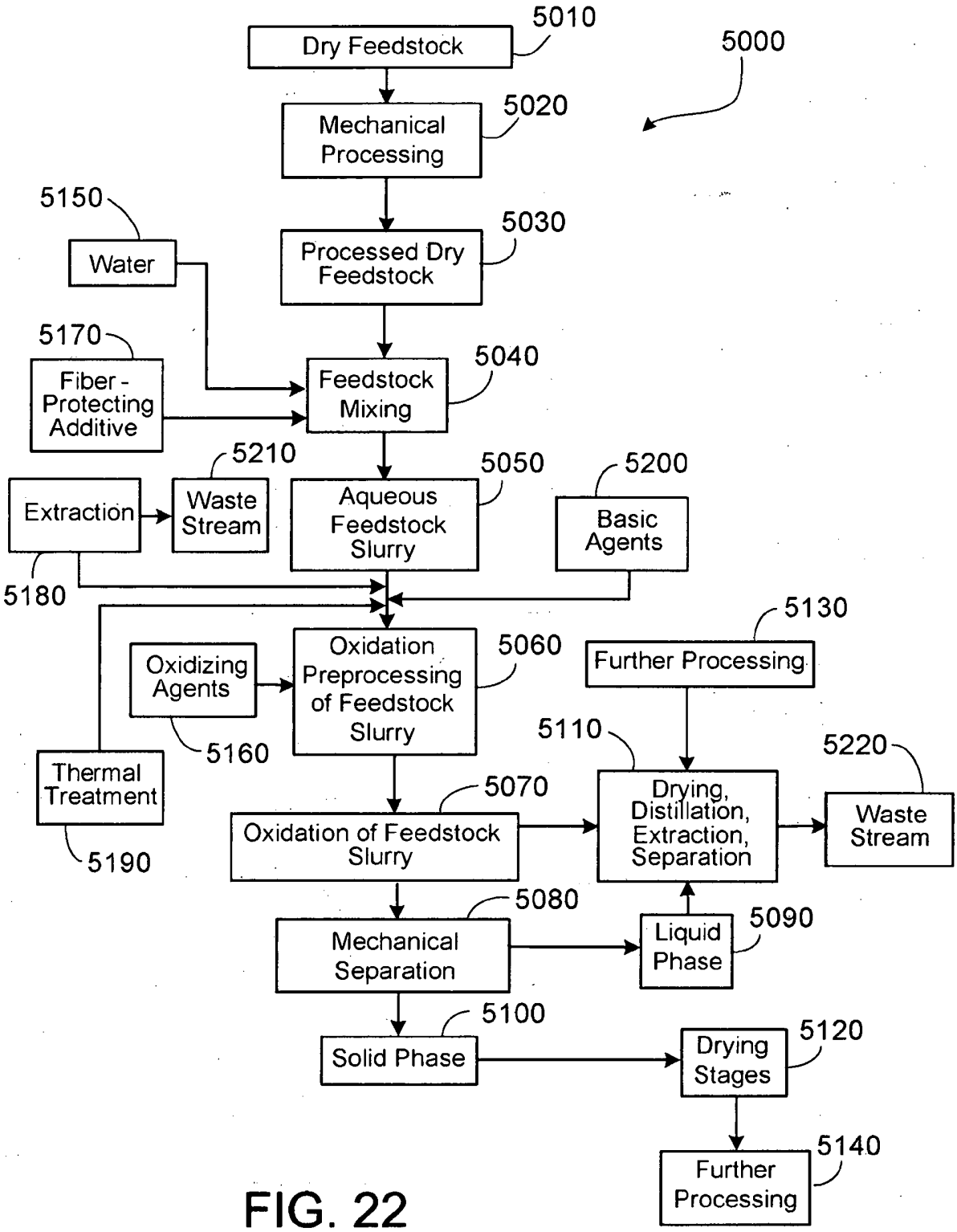

5010
Dry Feedstock
5000
Mechanical Processing
5020
5150
Water
Processed Dry Feedstock
5030
5170
Fiber - Protecting Additive
Feedstock Mixing
5040
5210
Extraction
Waste Stream
5050
Aqueous Feedstock Slurry
5200
Basic Agents
5180
5130
Oxidizing Agents
Oxidation Preprocessing of Feedstock Slurry
5060
Further Processing
5160
5110
5220
Thermal Treatment
5070
Drying, Distillation, Extraction, Separation
Waste Stream
Oxidation of Feedstock Slurry
5190
5080
5090
Mechanical Separation
Liquid Phase
5100
5120
Solid Phase
Drying Stages
5140
Further Processing

FIG. 22

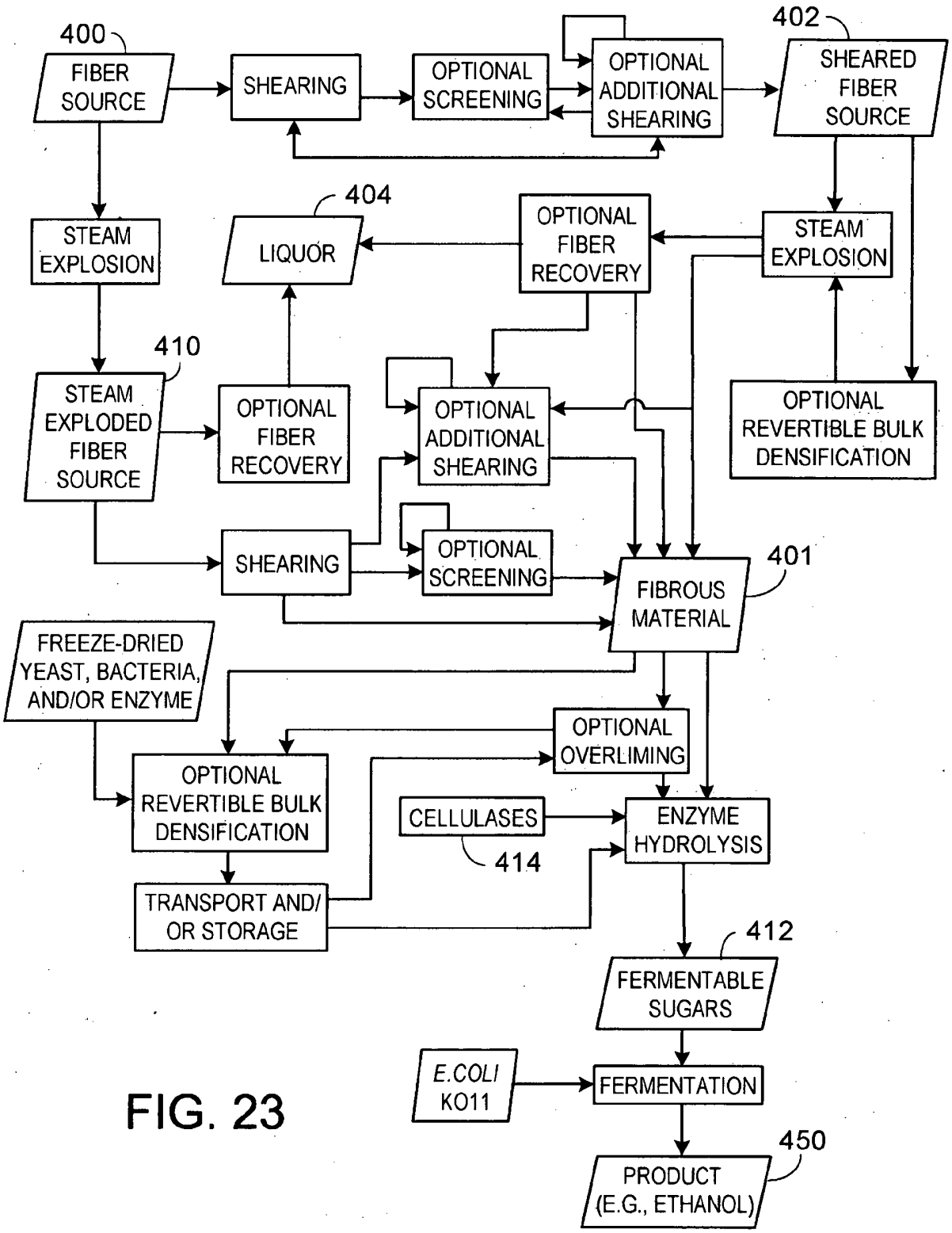

400
FIBER SOURCE
SHEARING
OPTIONAL SCREENING
OPTIONAL ADDITIONAL SHEARING
402
SHEARED FIBER SOURCE
STEAM EXPLOSION
404
LIQUOR
OPTIONAL FIBER RECOVERY
STEAM EXPLOSION
410
STEAM EXPLODED FIBER SOURCE
OPTIONAL FIBER RECOVERY
OPTIONAL ADDITIONAL SHEARING
OPTIONAL REVERTIBLE BULK DENSIFICATION
SHEARING
OPTIONAL SCREENING
401
FIBROUS MATERIAL
FREEZE-DRIED YEAST, BACTERIA, AND/OR ENZYME
OPTIONAL OVERLIMING
OPTIONAL REVERTIBLE BULK DENSIFICATION
CELLULASES
414
ENZYME HYDROLYSIS
TRANSPORT AND/ OR STORAGE
412
FERMENTABLE SUGARS
E.COLI KO11
FERMENTATION
450
PRODUCT (E.G., ETHANOL)

FIG. 23

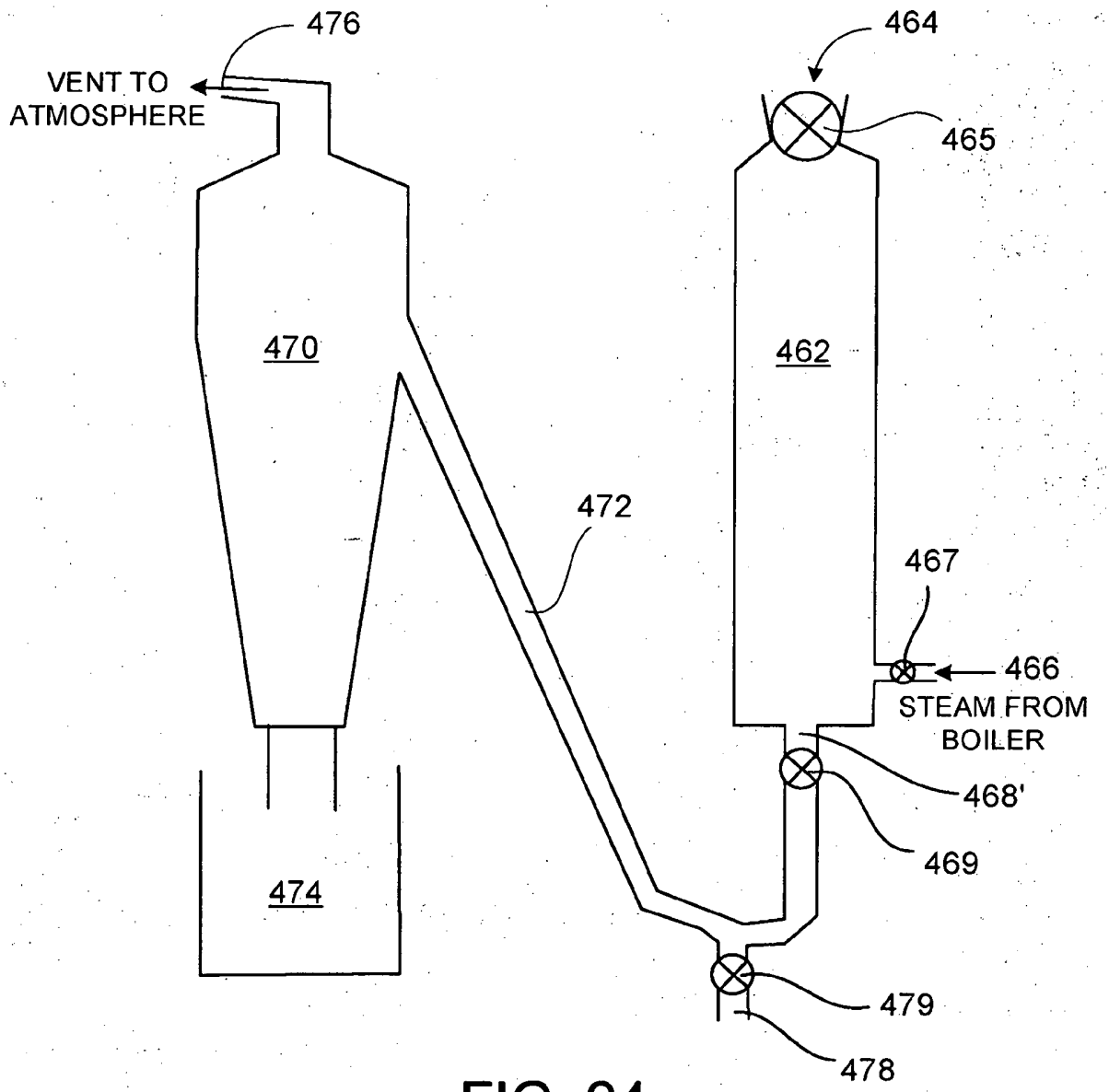

476
VENT TO
ATMOSPHERE
464
465
470
462
472
467
466
STEAM FROM
BOILER
468'
469
474
479
478

FIG. 24

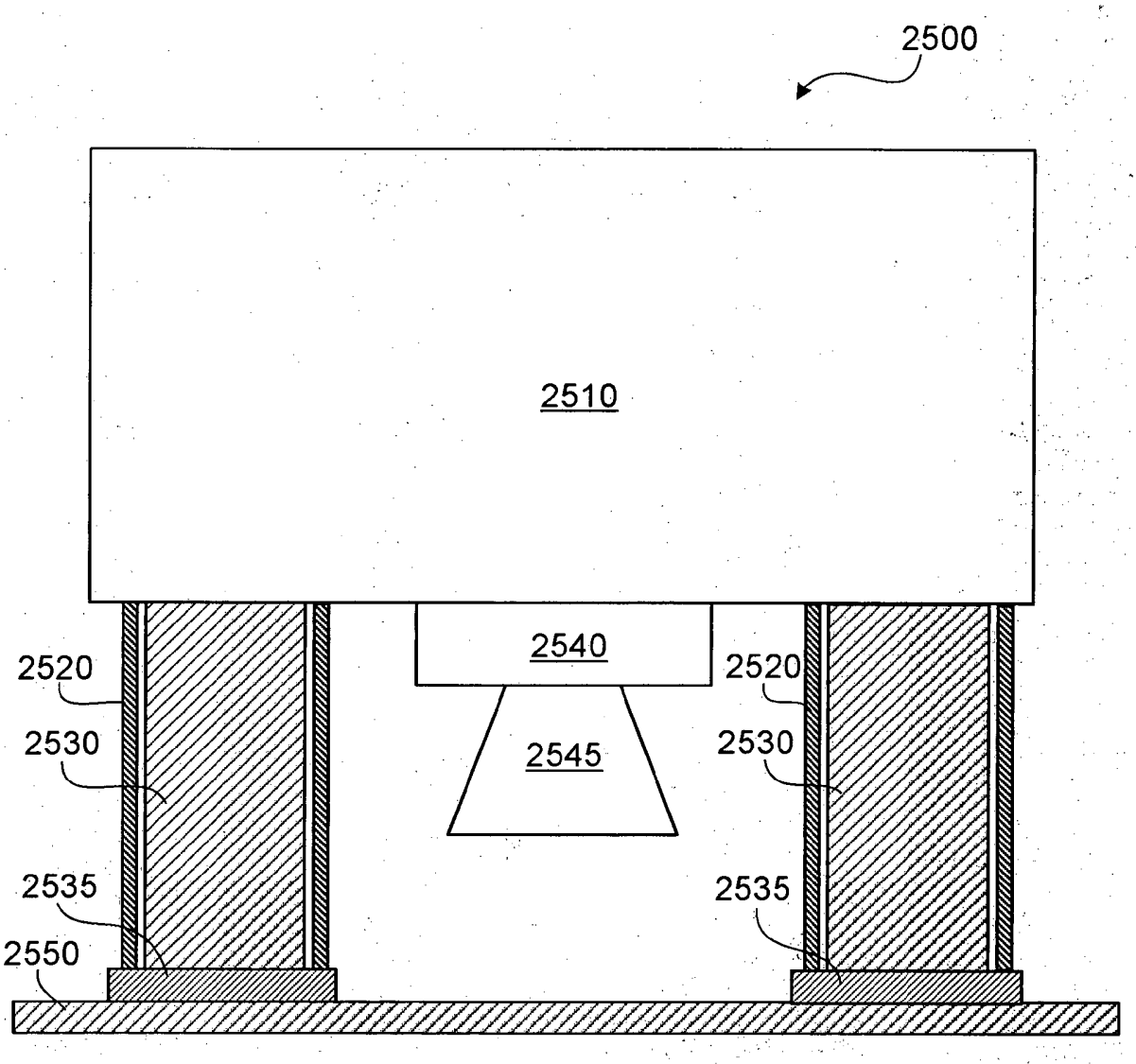


FIG. 25

FIG. 26

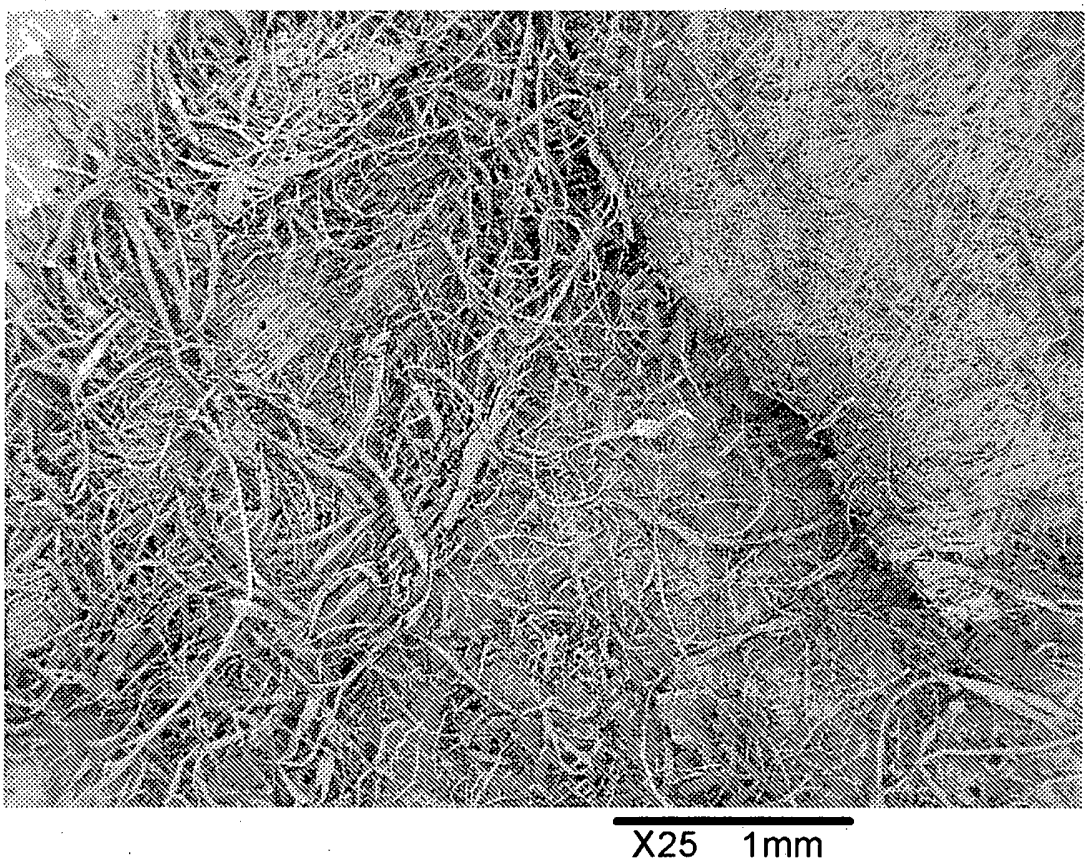


FIG. 27

FIG. 28

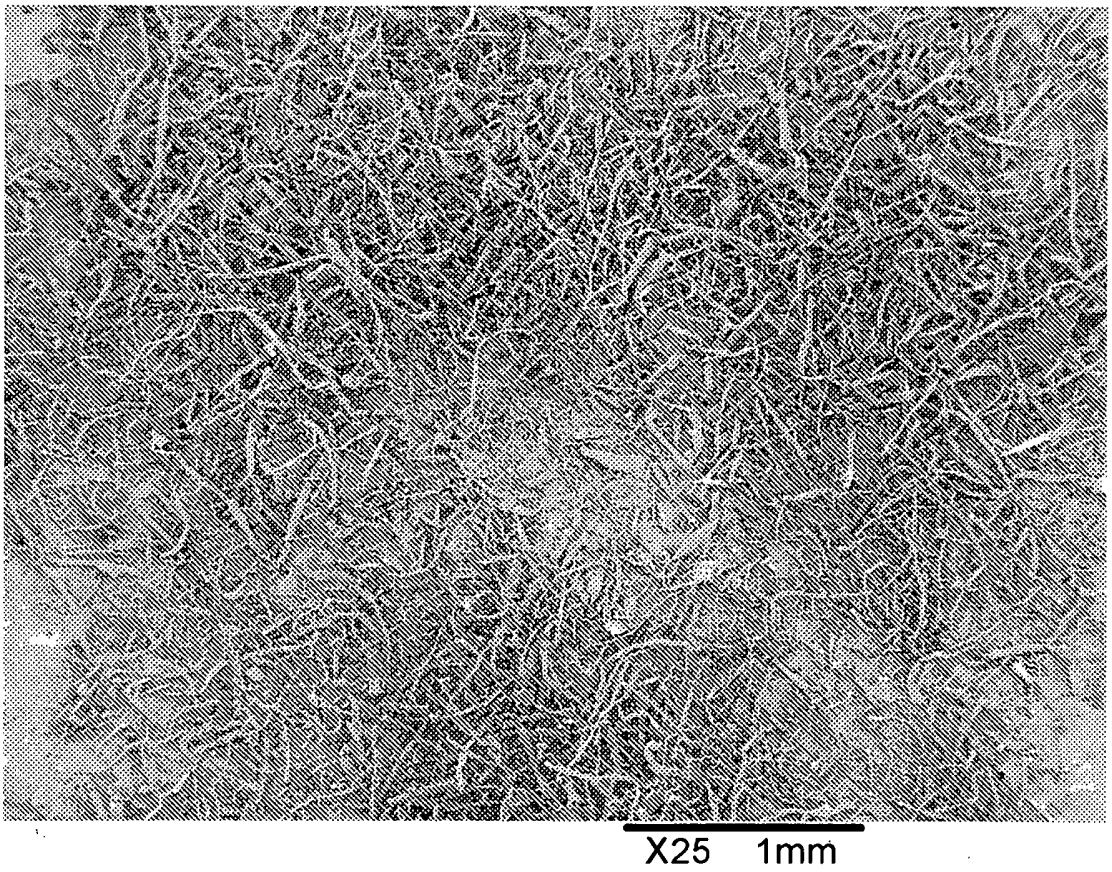


FIG. 29

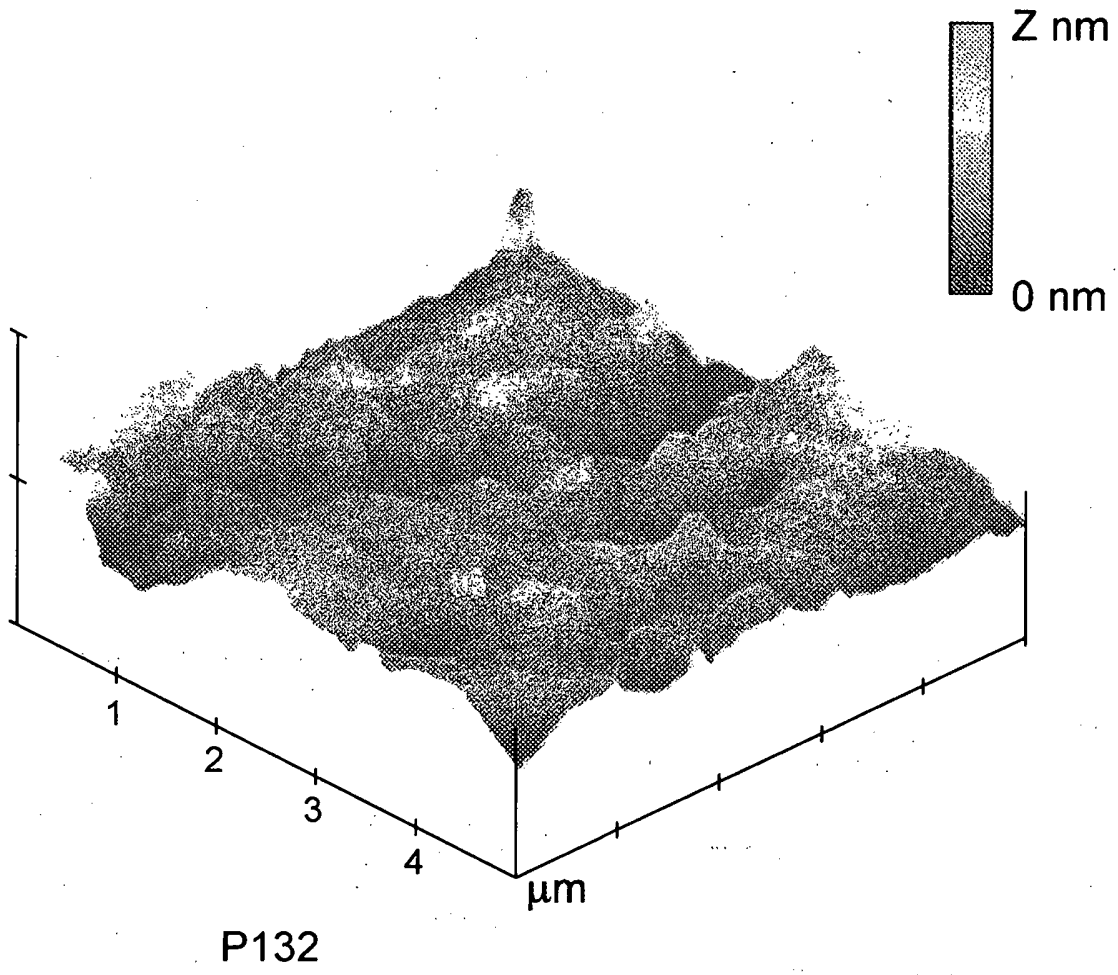


FIG. 29A

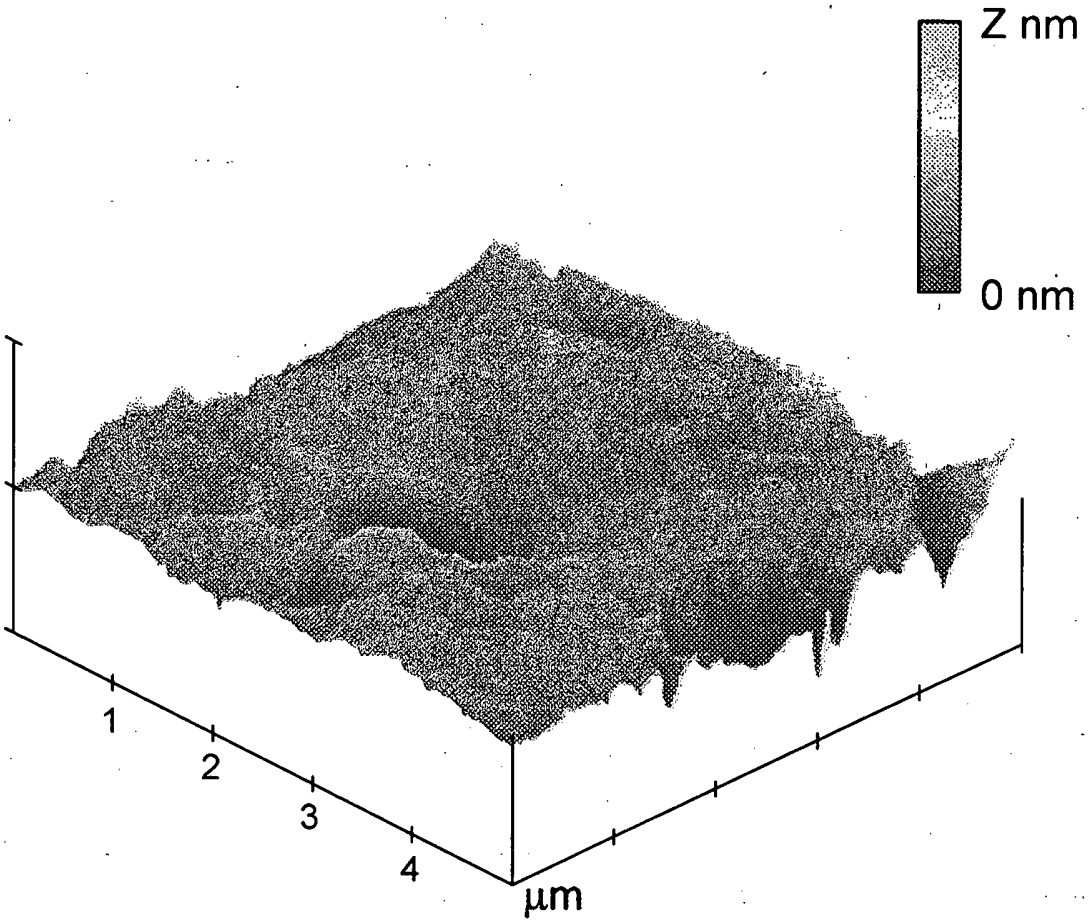


FIG. 29B

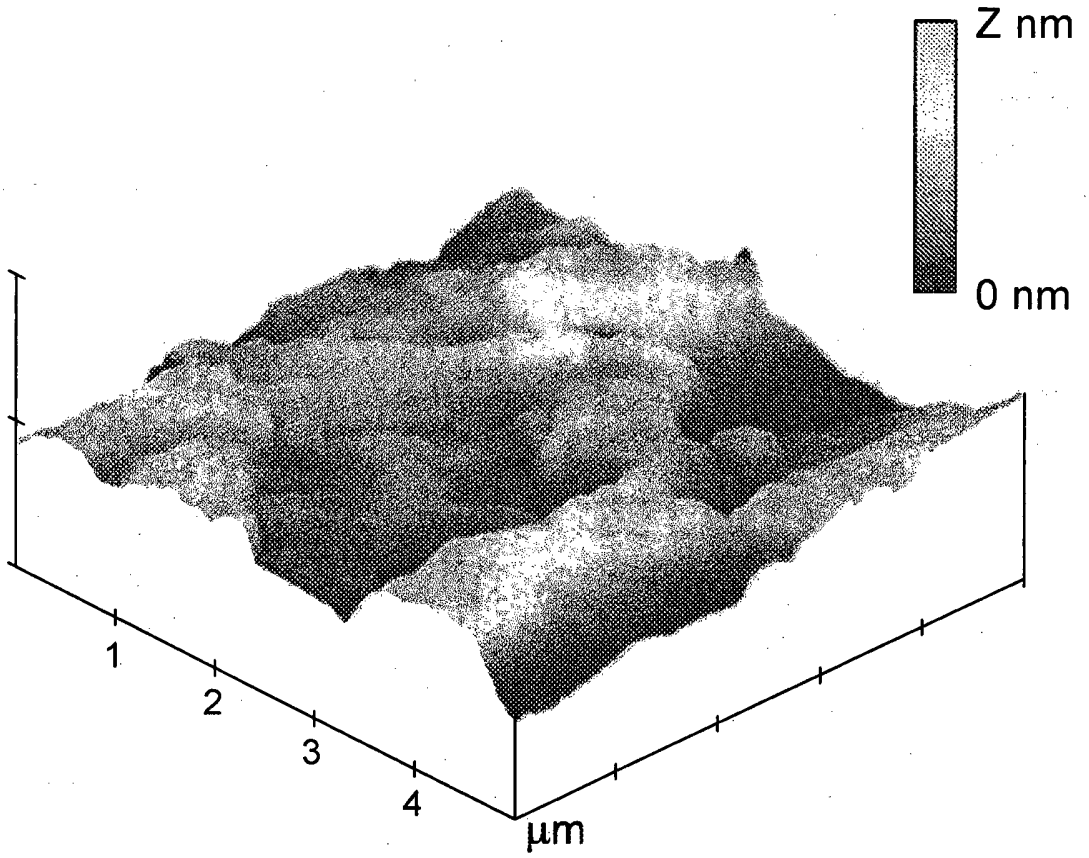


FIG. 29C

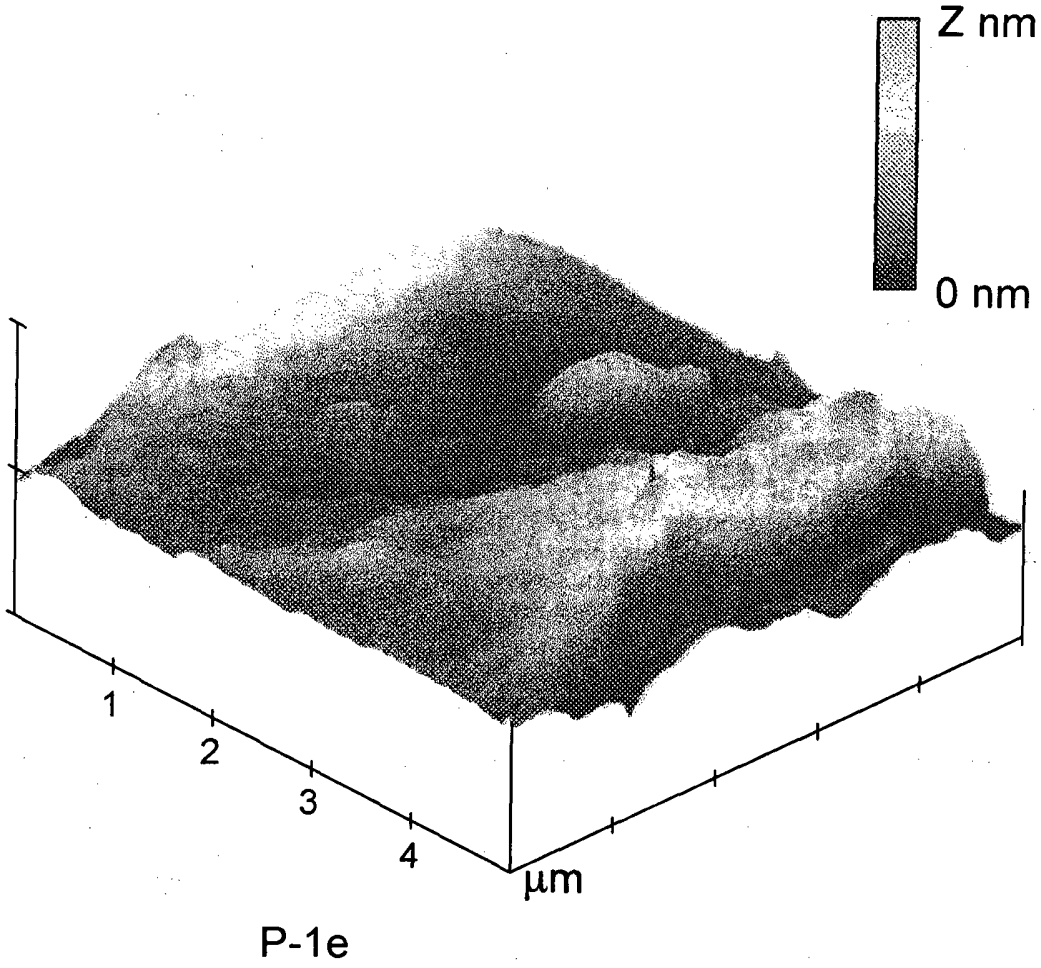


FIG. 29D

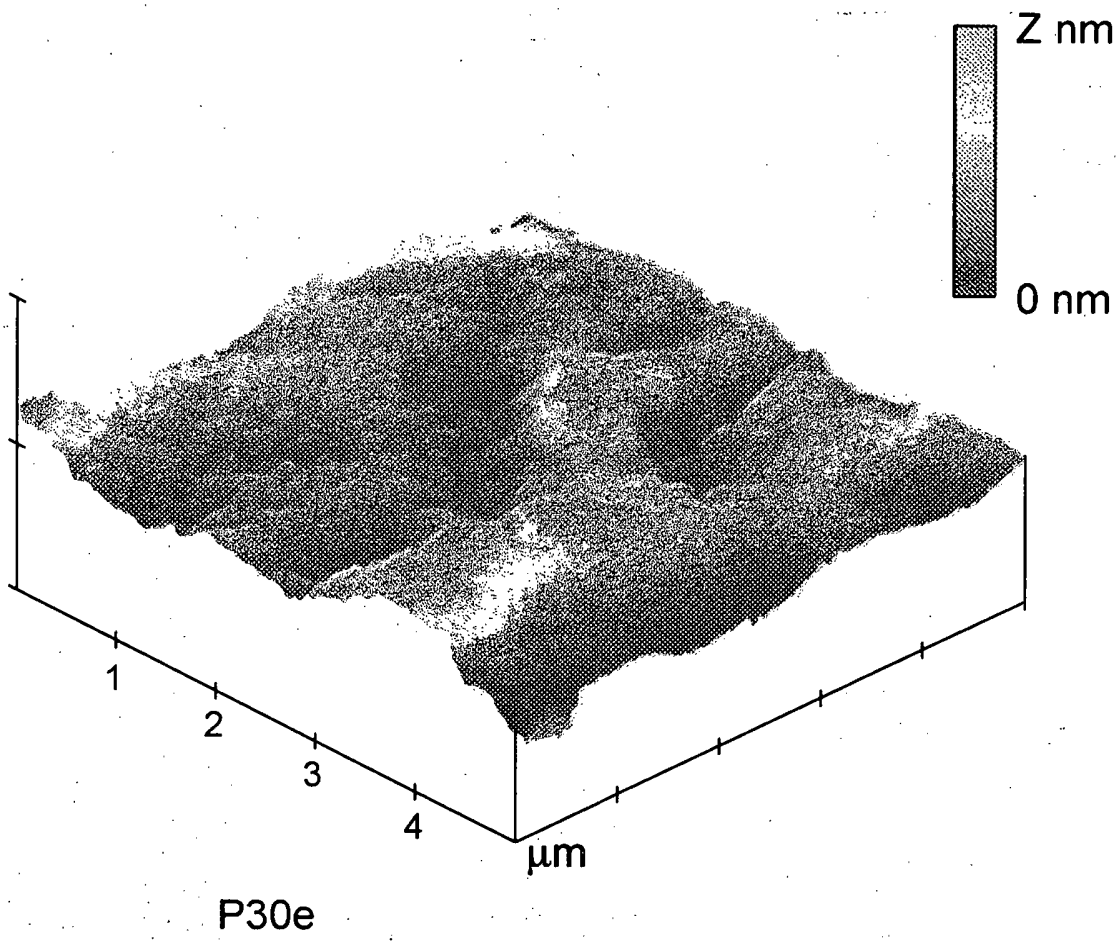


FIG. 29E

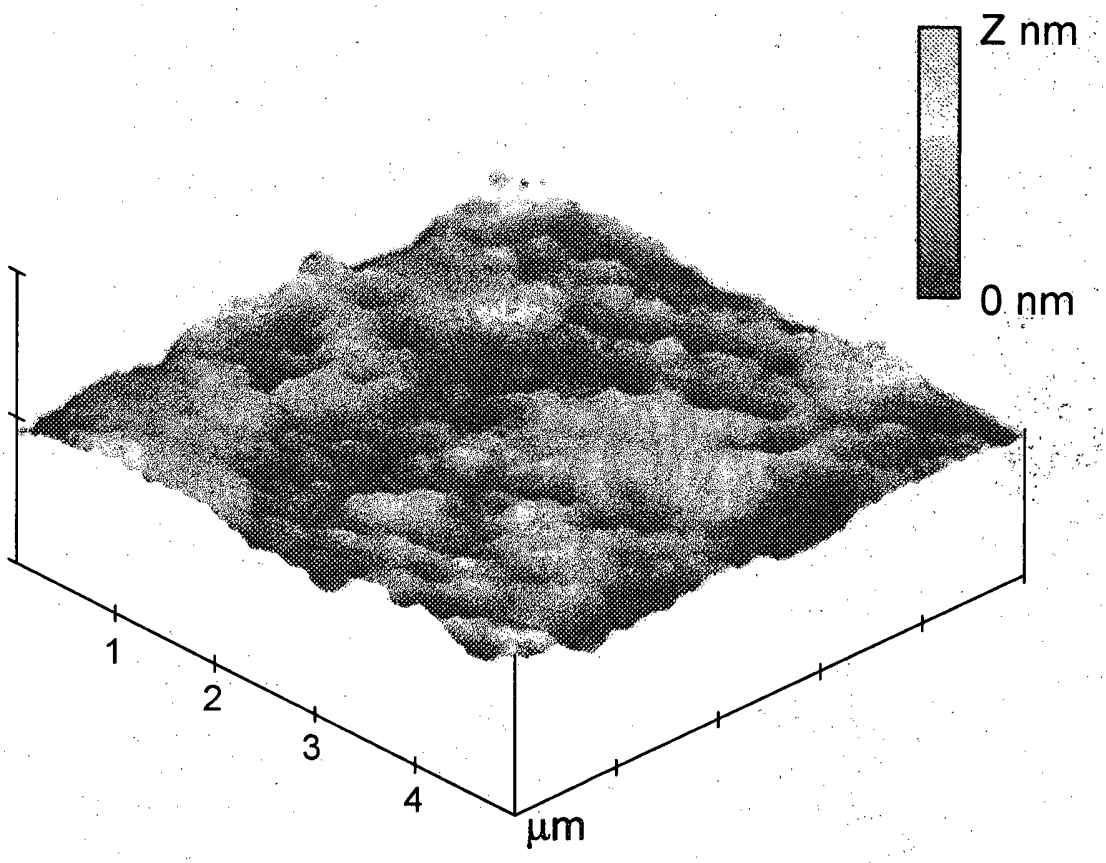


FIG. 29F

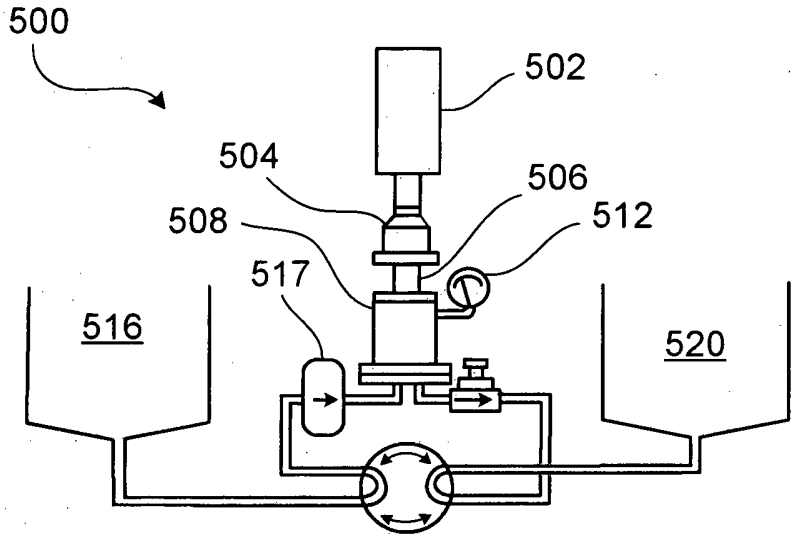


FIG. 30

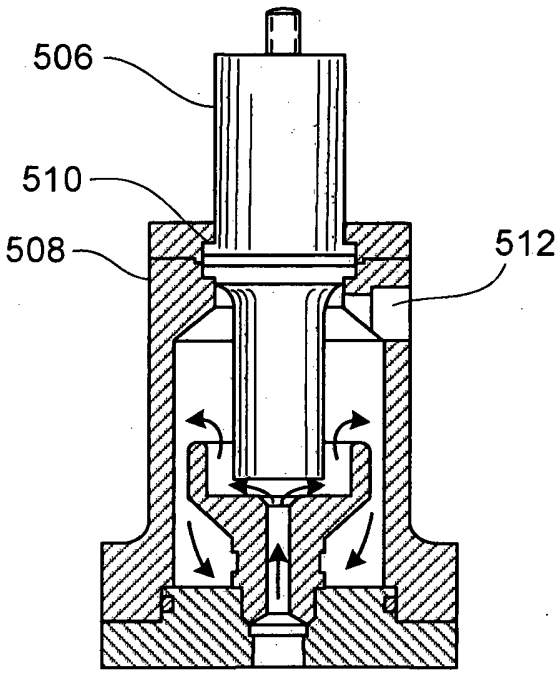


FIG. 31

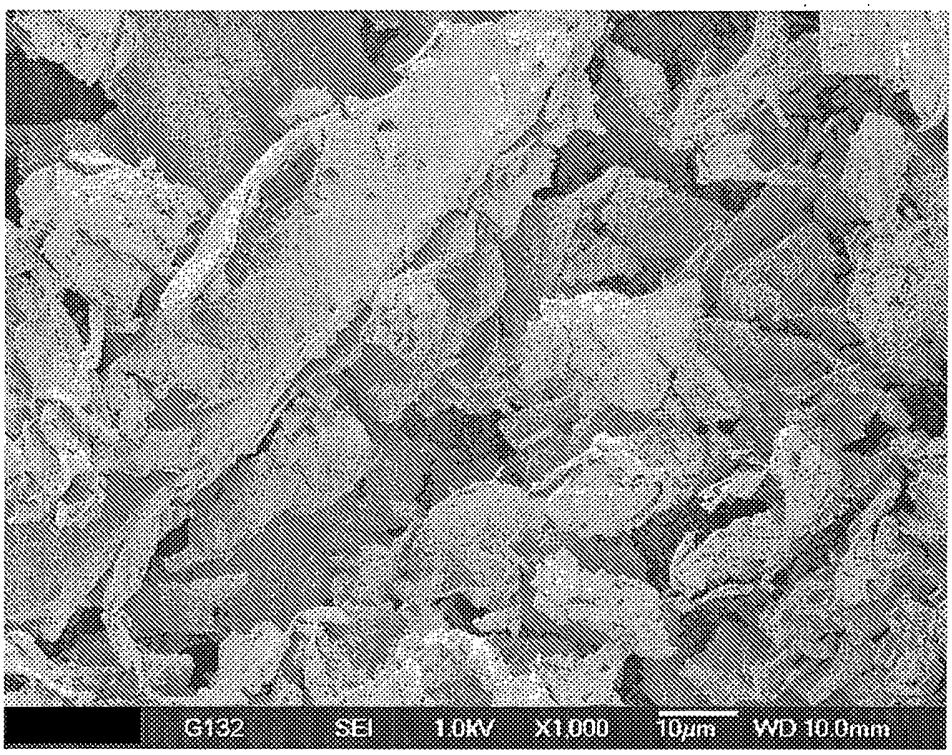

G132 SEI 1.0kV X1,000 10μm WD 10.0mm

FIG. 32

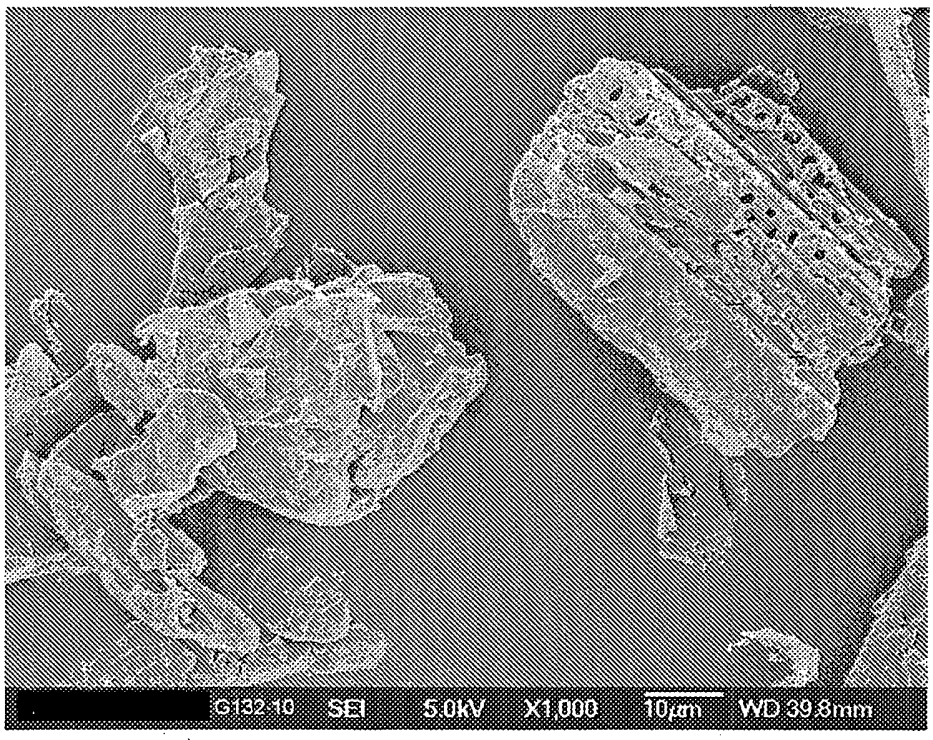

G132-10 SEI 5.0kV X1,000 10μm WD 39.8mm

FIG. 33

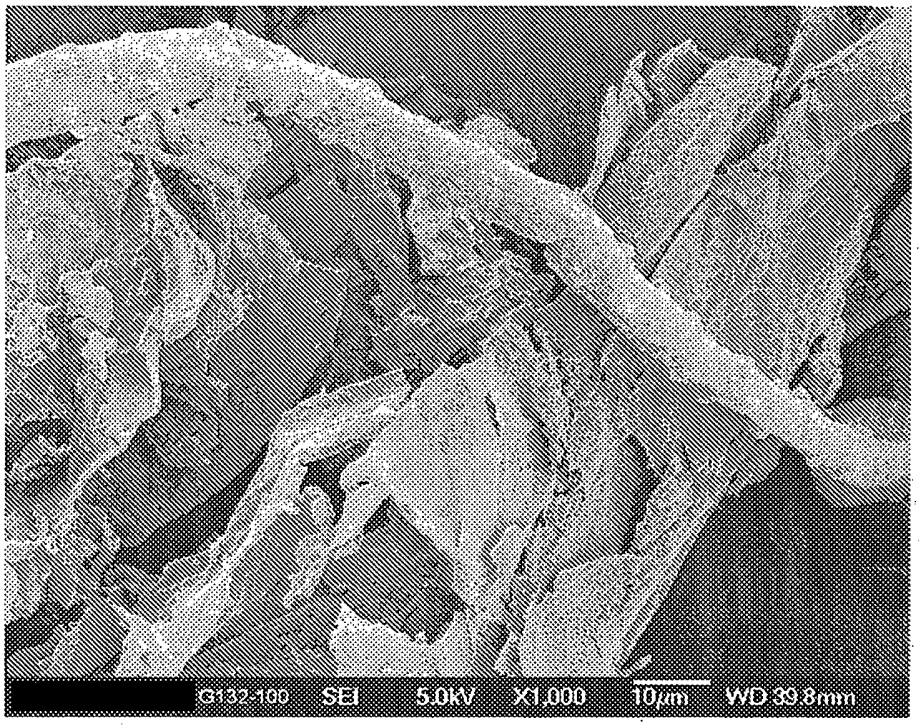

G132-100 SEI 5.0kV X1,000 10μm WD 39.8mm

FIG. 34

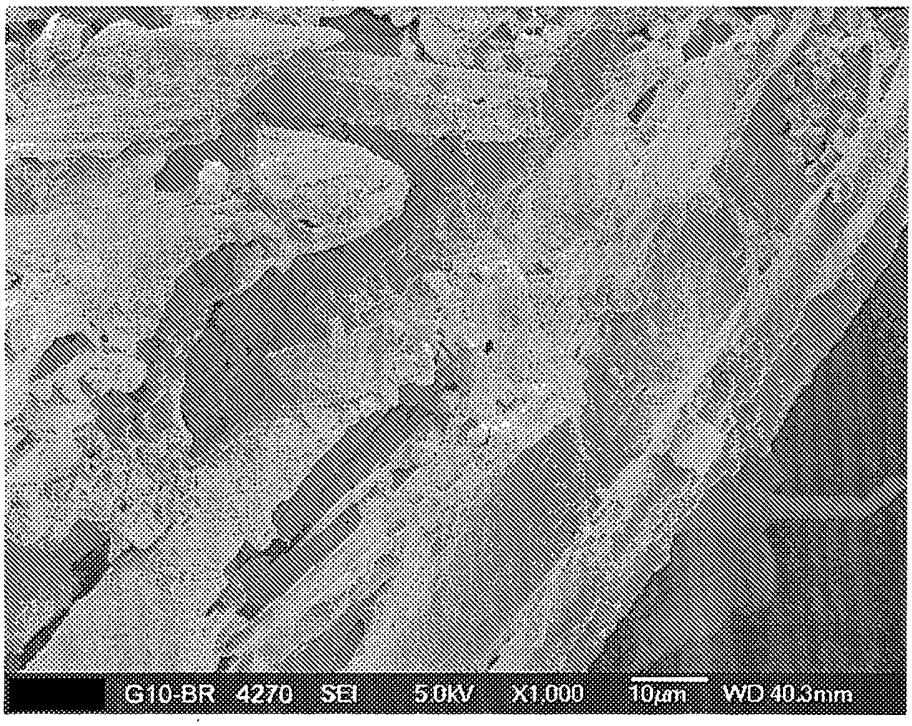

G10-BR 4270 SEI 5.0kV X1,000 10μm WD 40.3mm

FIG. 35

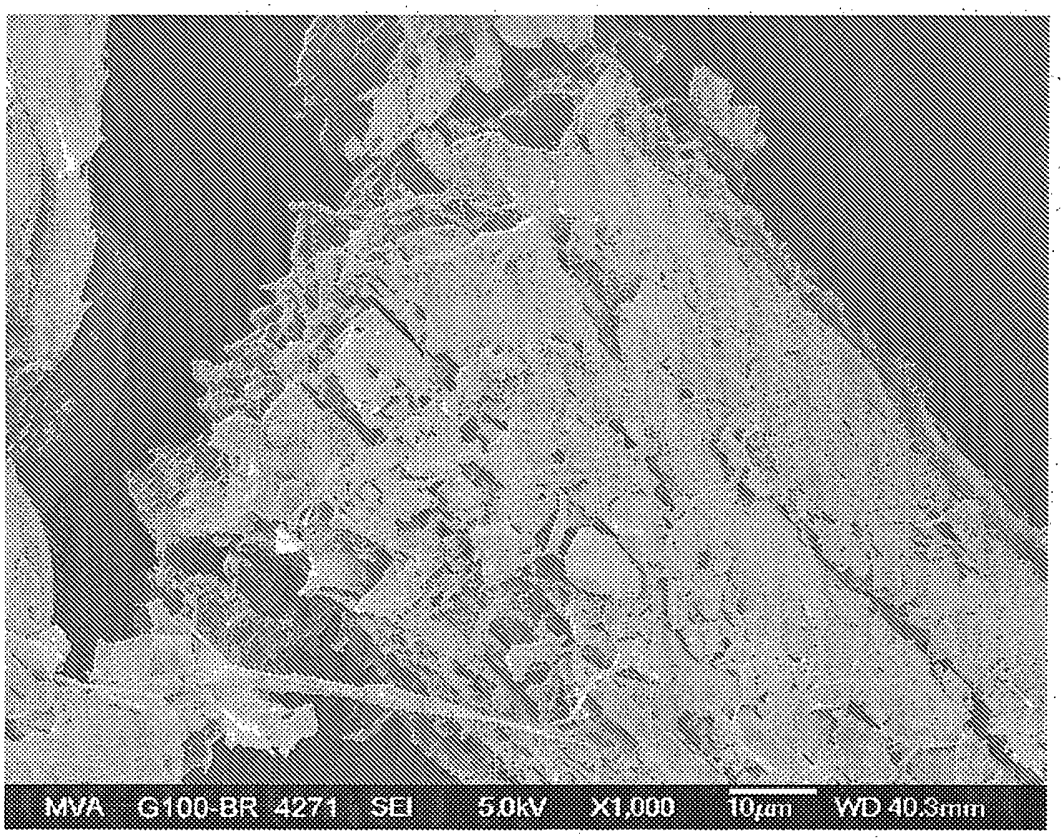


FIG. 36

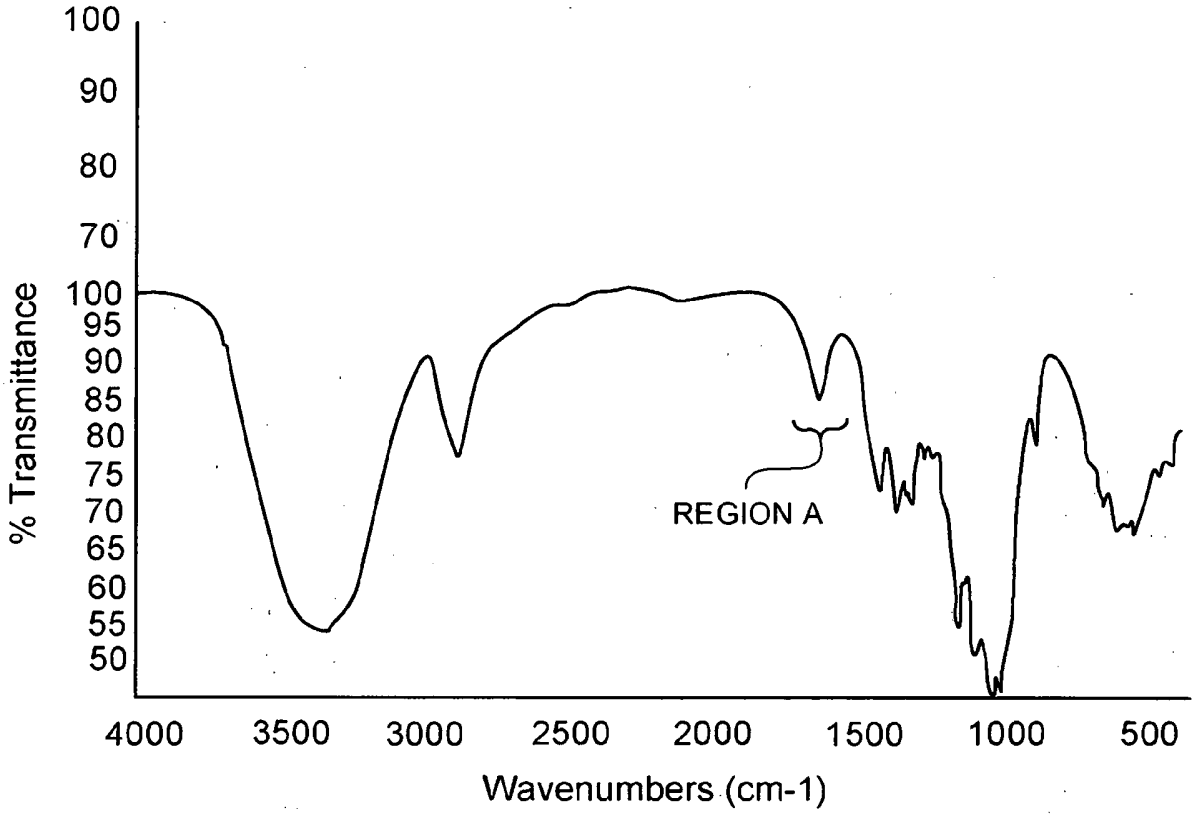

100
90
80
70
100
95
90
85
80
75
70
65
60
55
50
% Transmittance
REGION A
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

FIG. 37

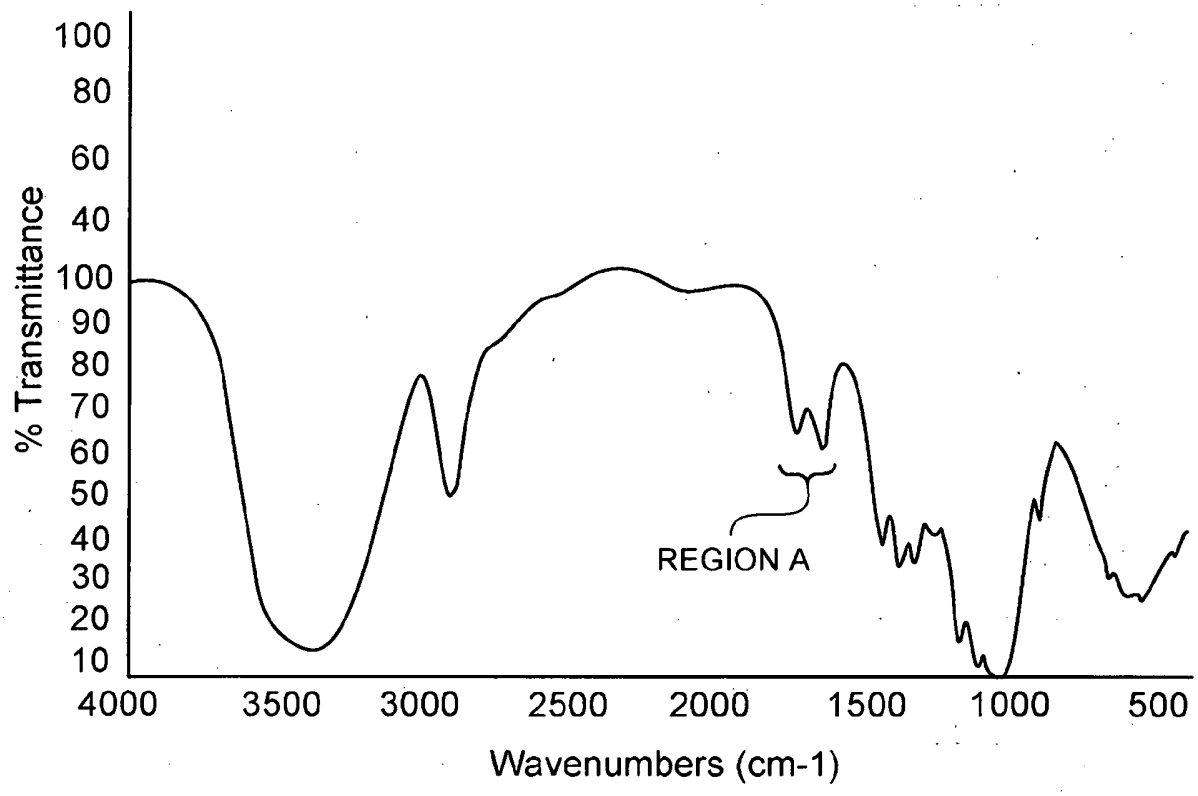

100
80
60
40
100
90
80
70
60
50
40
30
20
10
% Transmittance
REGION A
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers (cm-1)

FIG. 38

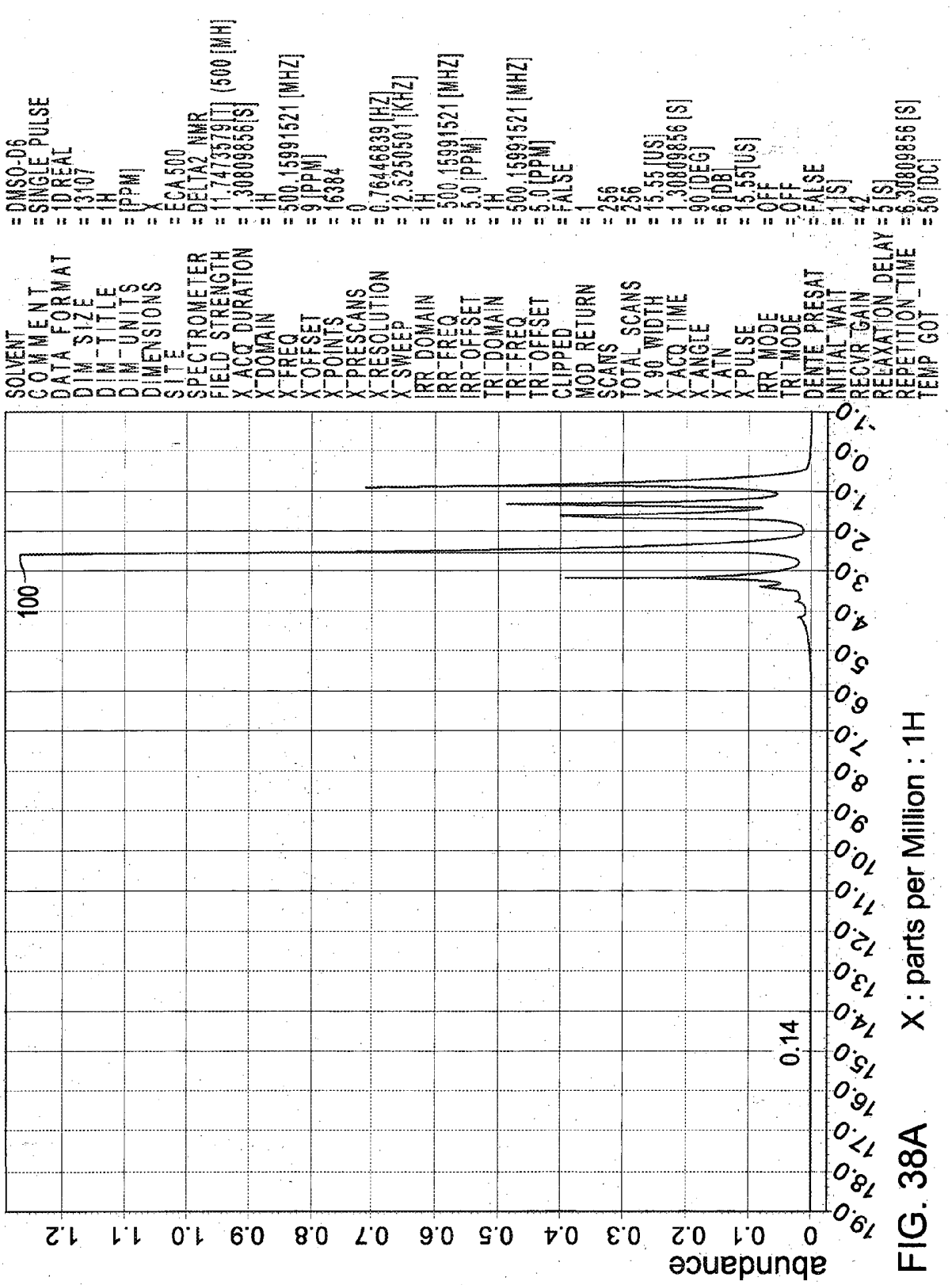

SOLVENT = DMSO-D6
COMMENT = SINGLE PULSE
DATA FORMAT = 1D REAL
DIM_SIZE = 13107
DIM_TITLE = 1H
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD STRENGTH = 11.747357[T] (500 [MH
X_ACQ_DURATION = 1.30809856[S]
X_DOMAIN = 1H
X_FREQ = 500.15991521 [MHZ]
X_OFFSET = 9 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 0.76446839[HZ]
X_SWEEP = 12.5250501[KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
TRI_DOMAIN = 1H
TRI_FREQ = 500.15991521 [MHZ]
TRI_OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD RETURN = 1
SCANS = 256
TOTAL_SCANS = 256
X_90_WIDTH = 15.55 [US]
X_ACQ_TIME = 1.30809856 [S]
X_ANGLE = 90 [DEG]
X_ATN = 6 [DB]
X_PULSE = 15.55 [US]
IRR_MODE = OFF
TRI_MODE = OFF
DENTE_PRESAT = FALSE
INITIAL_WAIT = 1 [S]
RECVR_GAIN = 42
RELAXATION_DELAY = 5 [S]
REPETITION_TIME = 6.30809856 [S]
TEMP GOT = 50 [DC]
100
0.14
abundance
X : parts per Million : 1H

FIG. 38A

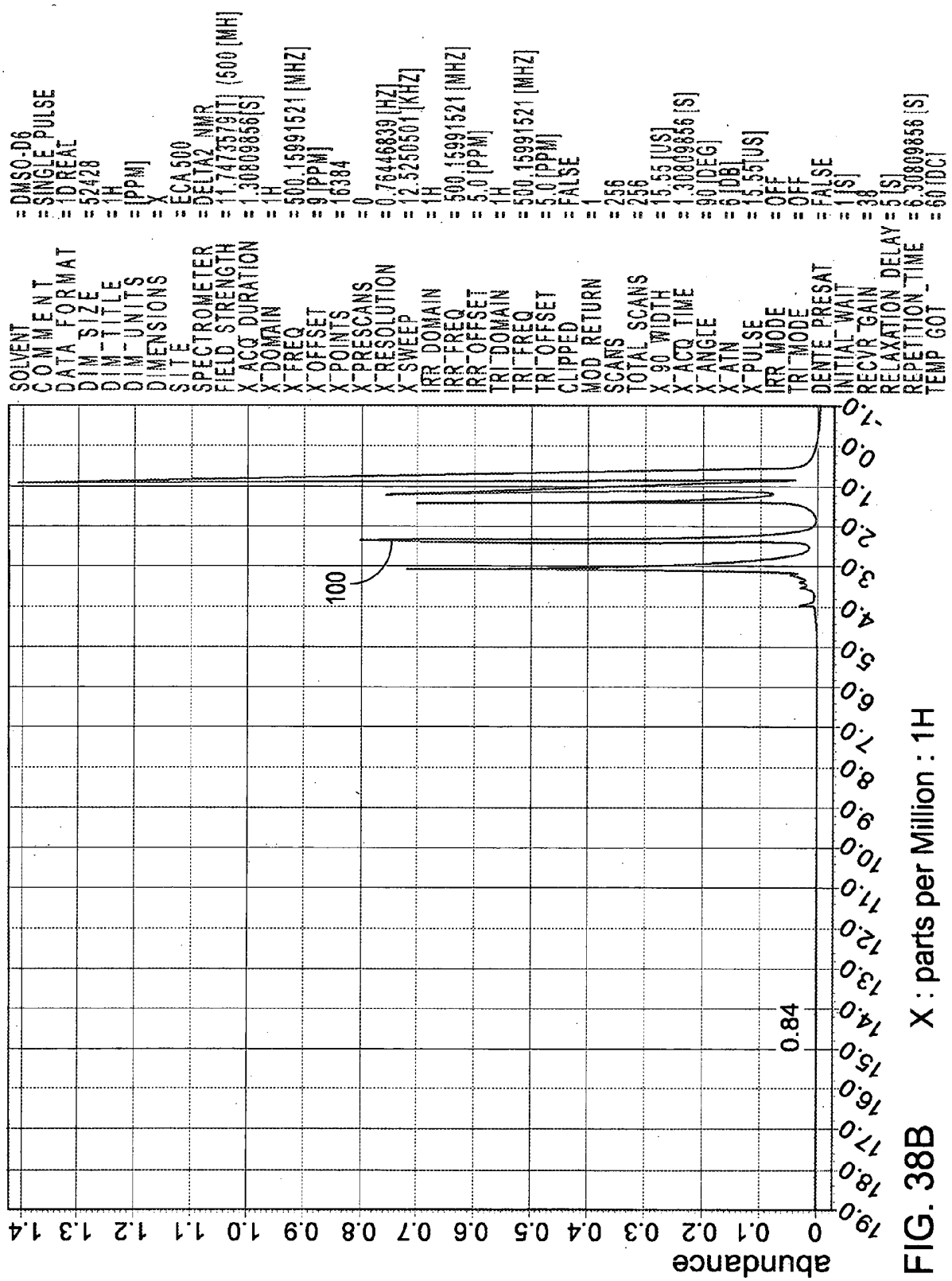


FIG. 38B X : parts per Million : 1H

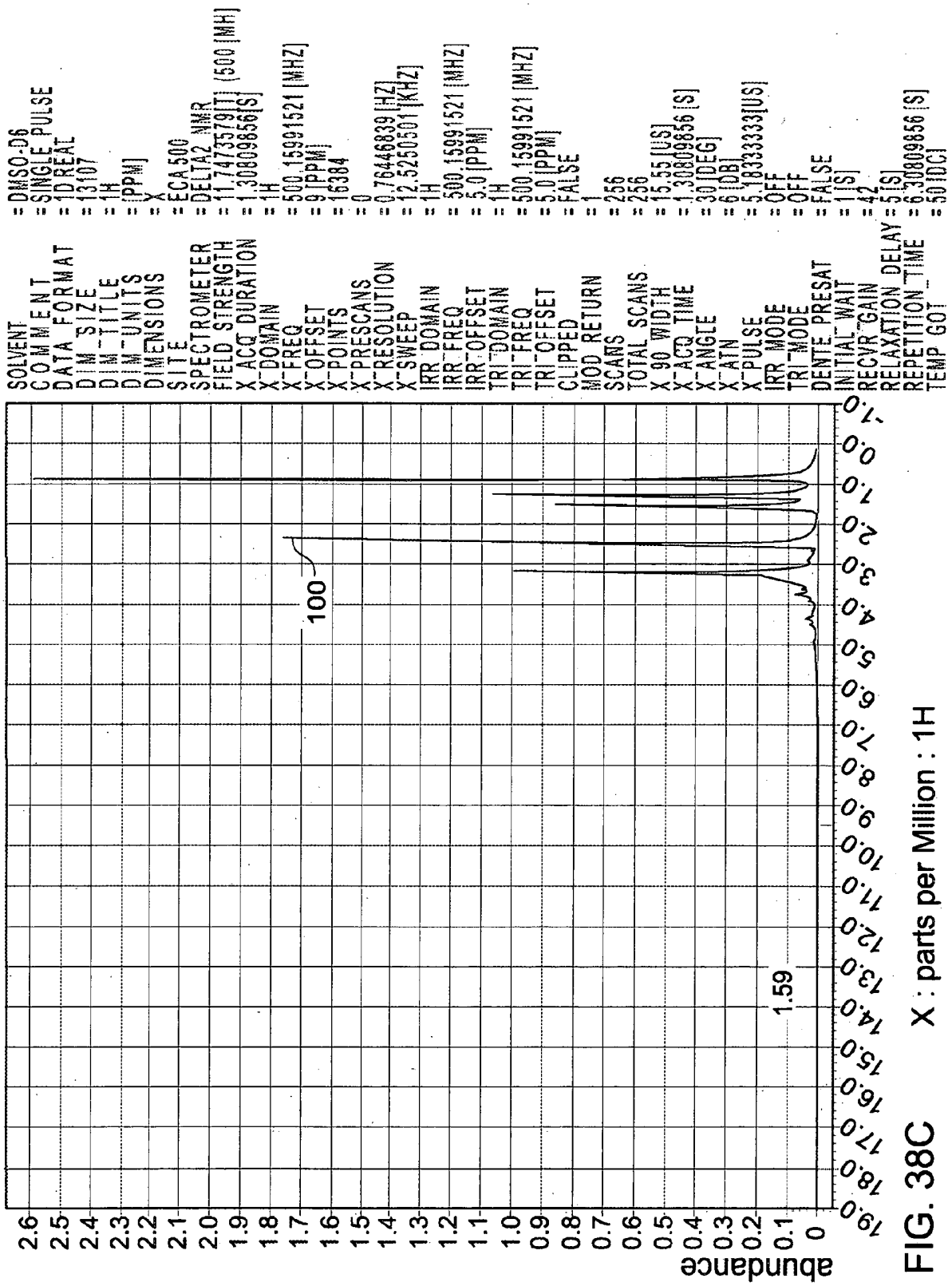

SOLVENT = DMSO-D6
COMMENT = SINGLE_PULSE
DATA_FORMAT = 1D_REAL
DIM_SIZE = 13107
DIM_TITLE = 1H
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD_STRENGTH = 11.7473579[T] (500 [MH
X_ACQ_DURATION = 1.30809856[S]
X_DOMAIN = 1H
X_FREQ = 500.15991521 [MHZ]
X_OFFSET = 9 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 0.76446839 [HZ]
X_SWEEP = 12.5250501 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
TRI_DOMAIN = 1H
TRI_FREQ = 500.15991521 [MHZ]
TRI_OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD_RETURN = 1
SCANS = 256
TOTAL_SCANS = 256
X_90_WIDTH = 15.55 [US]
X_ACQ_TIME = 1.30809856 [S]
X_ANGLE = 30 [DEG]
X_ATN = 6 [DB]
X_PULSE = 5.18333333[US]
IRR_MODE = OFF
TRI_MODE = OFF
DENTE_PRESAT = FALSE
INITIAL_WAIT = 1 [S]
RECVR_GAIN = 42
RELAXATION_DELAY = 5 [S]
REPETITION_TIME = 6.30809856 [S]
TEMP_GOT = 50 [DC]
100
1.59
abundance
X : parts per Million : 1H

FIG. 38C

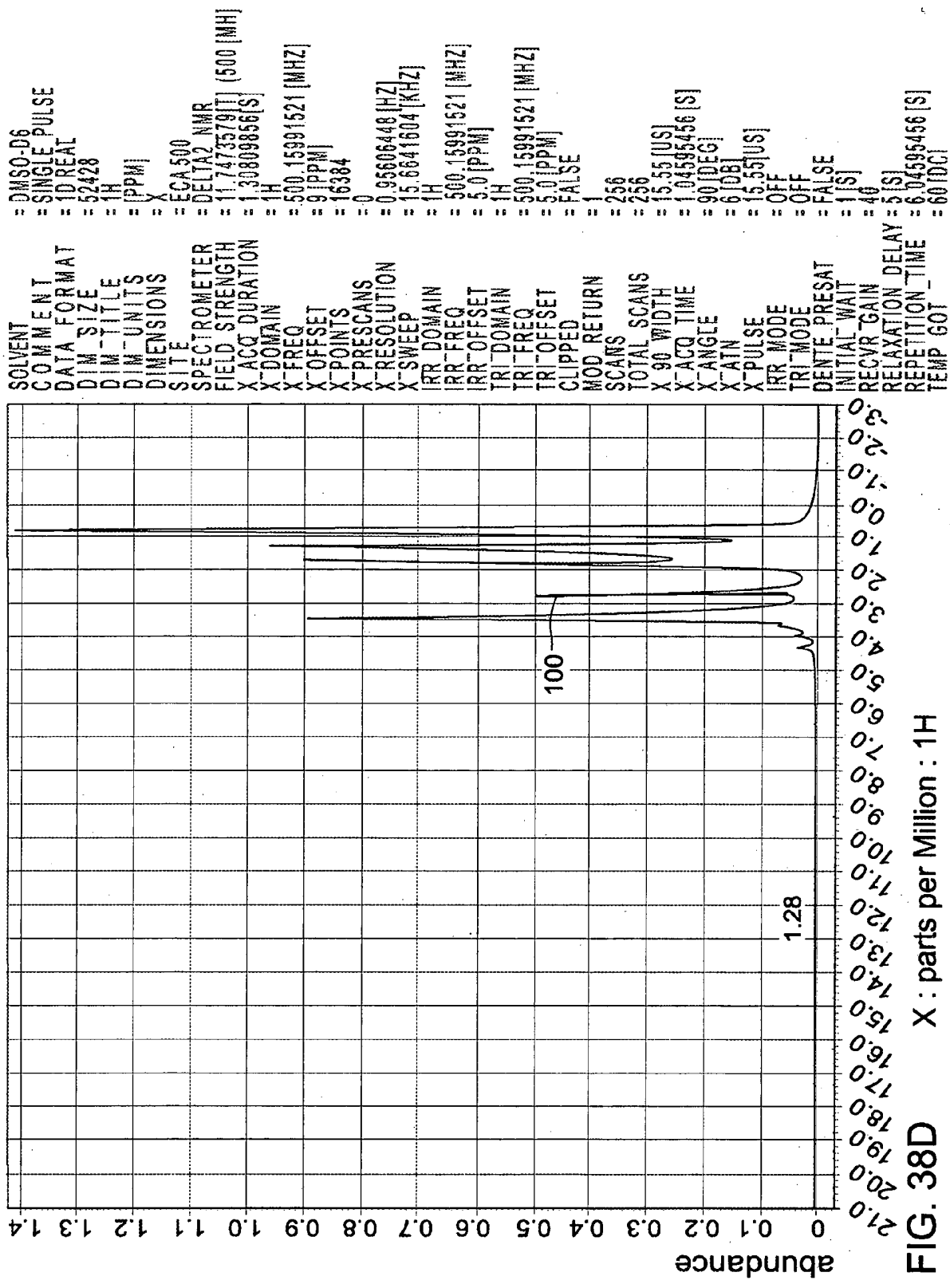

SOLVENT = DMSO-D6
COMMENT = SINGLE_PULSE
DATA_FORMAT = 1D_REAL
DIM_SIZE = 52428
DIM_TITLE = 1H
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD_STRENGTH = 11.74735791[T] (500 [MH]
X_ACQ_DURATION = 1.31809856[S]
X_DOMAIN = 1H
X_FREQ = 500.15991521 [MHZ]
X_OFFSET = 9 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 0.95606448 [HZ]
X_SWEEP = 15.66416047 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
TRI_DOMAIN = 1H
TRI_FREQ = 500.15991521 [MHZ]
TRI_OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD_RETURN = 1
SCANS = 256
TOTAL_SCANS = 256
X_90_WIDTH = 15.55 [US]
X_ACQ_TIME = 1.04595456 [S]
X_ANGLE = 90 [DEG]
X_ATN = 6 [DB]
X_PULSE = 15.55 [US]
IRR_MODE = OFF
TRI_MODE = OFF
DENTE_PRESAT = FALSE
INITIAL_WAIT = 1 [S]
RECVR_GAIN = 40
RELAXATION_DELAY = 5 [S]
REPETITION_TIME = 6.04595456 [S]
TEMP_GOT = 60 [DC]
100
1.28
abundance
X : parts per Million : 1H

FIG. 38D

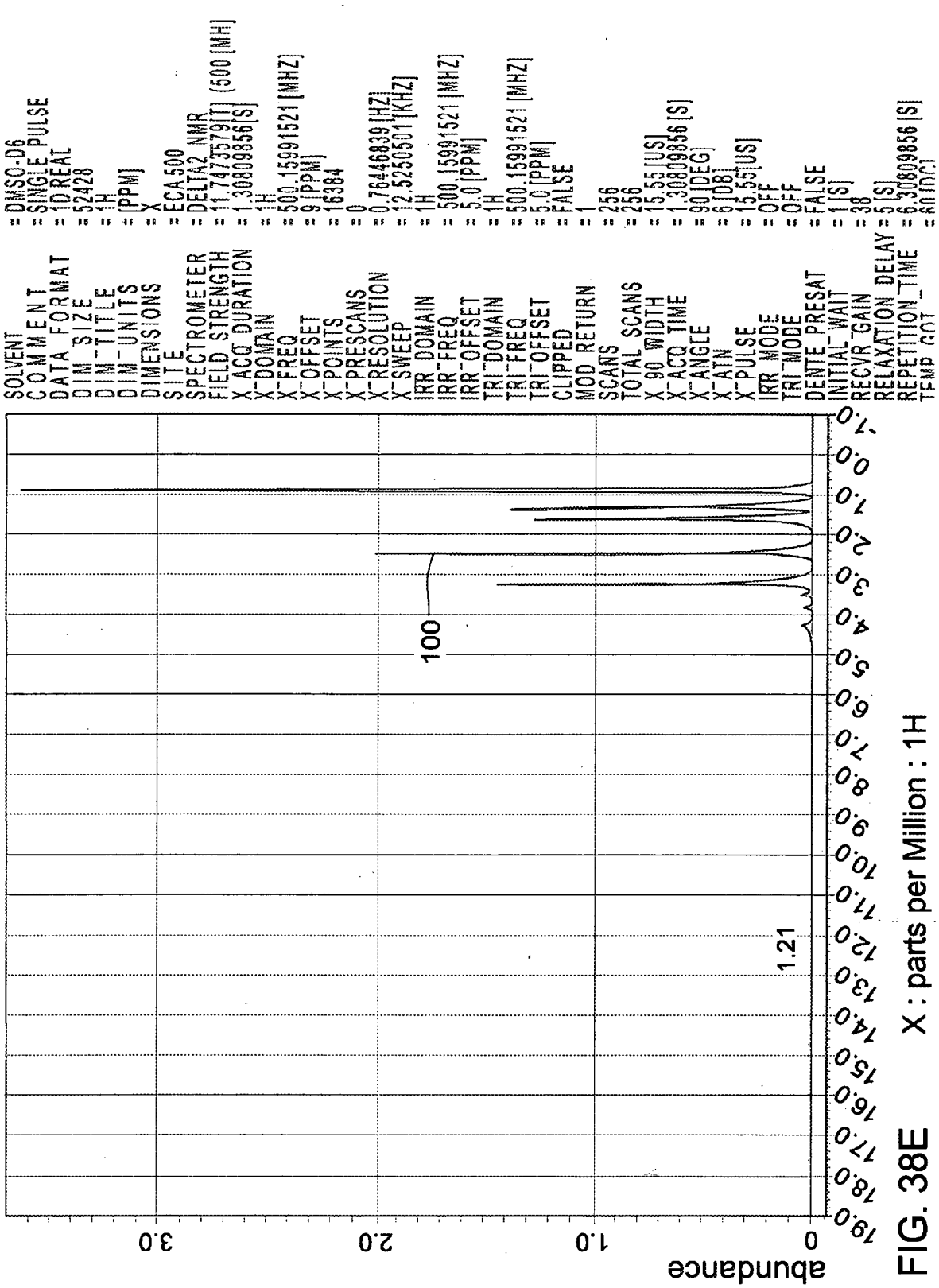

SOLVENT = DMSO-D6
COMMENT = SINGLE PULSE
DATA FORMAT = 1D REAL
DIM SIZE = 52428
DIM-TITLE = 1H
DIM-UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2 NMR
FIELD STRENGTH = 11.747579[T] (500 [MH
X ACQ DURATION = 1.30809856[S]
X-DOMAIN = 1H
X-FREQ = 500.15991521 [MHZ]
X-OFFSET = 9 [PPM]
X-POINTS = 16384
X-PRESCANS = 0
X-RESOLUTION = 0.76446839 [HZ]
X-SWEEP = 12.5250501 [KHZ]
IRR DOMAIN = 1H
IRR FREQ = 500.15991521 [MHZ]
IRR OFFSET = 5.0 [PPM]
TRI DOMAIN = 1H
TRI FREQ = 500.15991521 [MHZ]
TRI OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD RETURN = 1
SCANS = 256
TOTAL SCANS = 256
X 90 WIDTH = 15.55 [US]
X ACQ TIME = 1.30809856 [S]
X ANGLE = 90 [DEG]
X ATN = 6 [DB]
X PULSE = 15.55 [US]
IRR MODE = OFF
TRI MODE = OFF
DENTE PRESAT = FALSE
INITIAL WAIT = 1 [S]
RECVR GAIN = 38
RELAXATION DELAY = 5 [S]
REPETITION TIME = 6.30809856 [S]
TEMP GOT = 60 [DC]
100
1.21
abundance
3.0
2.0
1.0
0
X : parts per Million : 1H

FIG. 38E

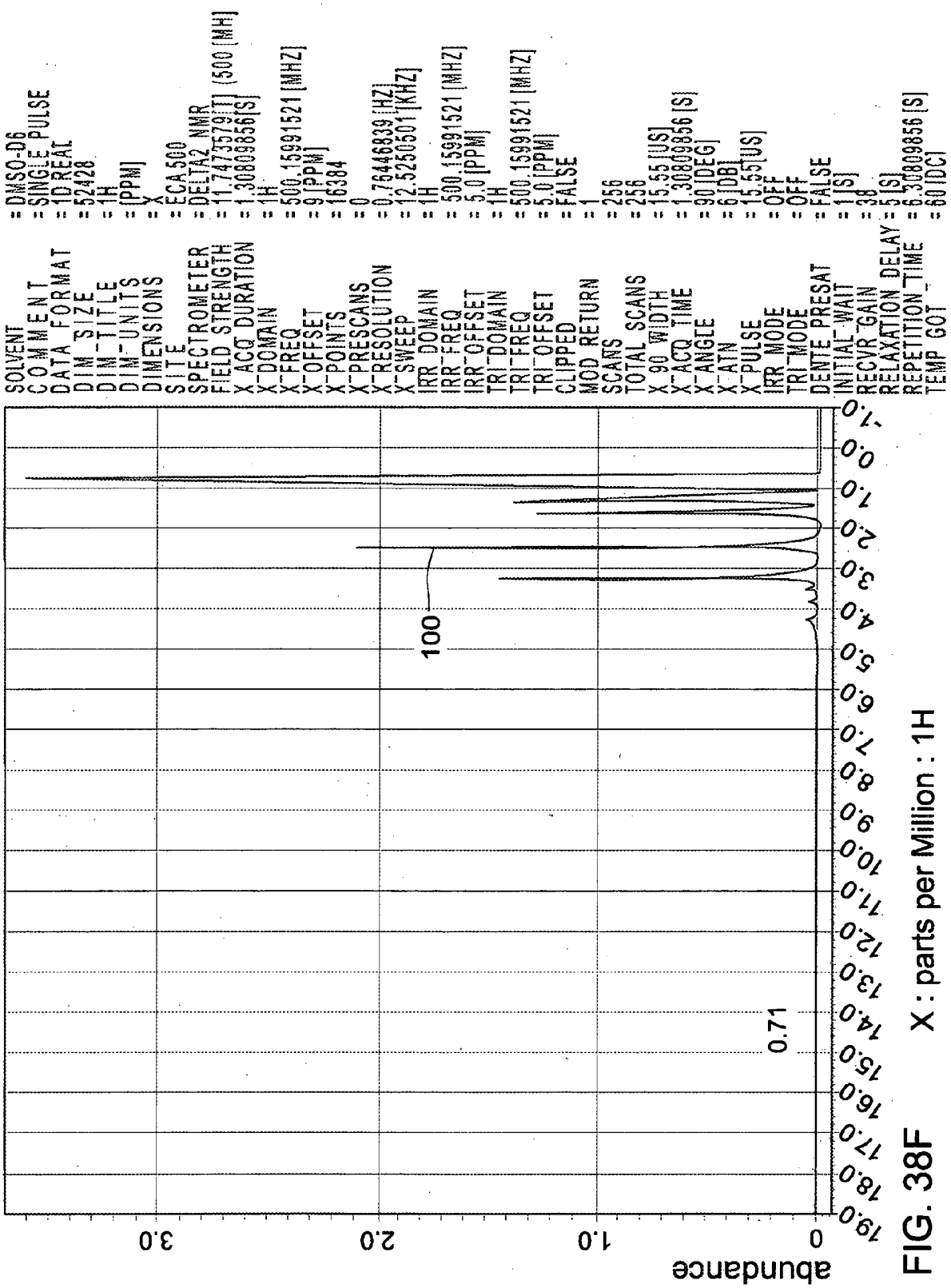

SOLVENT = DMSO-D6
COMMENT = SINGLE_PULSE
DATA_FORMAT = 1D REAL
DIM_SIZE = 52428
DIM_TITLE = 1H
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD_STRENGTH = 11.7473579[T] (500 [MH
X_ACQ_DURATION = 1.30809856[S]
X_DOMAIN = 1H
X_FREQ = 500.15991521 [MHZ]
X_OFFSET = 9 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 0.76446839 [HZ]
X_SWEEP = 12.5250501 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
TRI_DOMAIN = 1H
TRI_FREQ = 500.15991521 [MHZ]
TRI_OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD_RETURN = 1
SCANS = 256
TOTAL_SCANS = 256
X_90_WIDTH = 15.55 [US]
X_ACQ_TIME = 1.30809856 [S]
X_ANGLE = 90 [DEG]
X_ATN = 6 [DB]
X_PULSE = 15.55 [US]
IRR_MODE = OFF
TRI_MODE = OFF
DENTE PRESAT = FALSE
INITIAL_WAIT = 1 [S]
RECVR_GAIN = 38
RELAXATION_DELAY = 5 [S]
REPETITION_TIME = 6.30809856 [S]
TEMP GOT = 60 [DC]
100
0.71
abundance
3.0
2.0
1.0
0
X : parts per Million : 1H

FIG. 38F

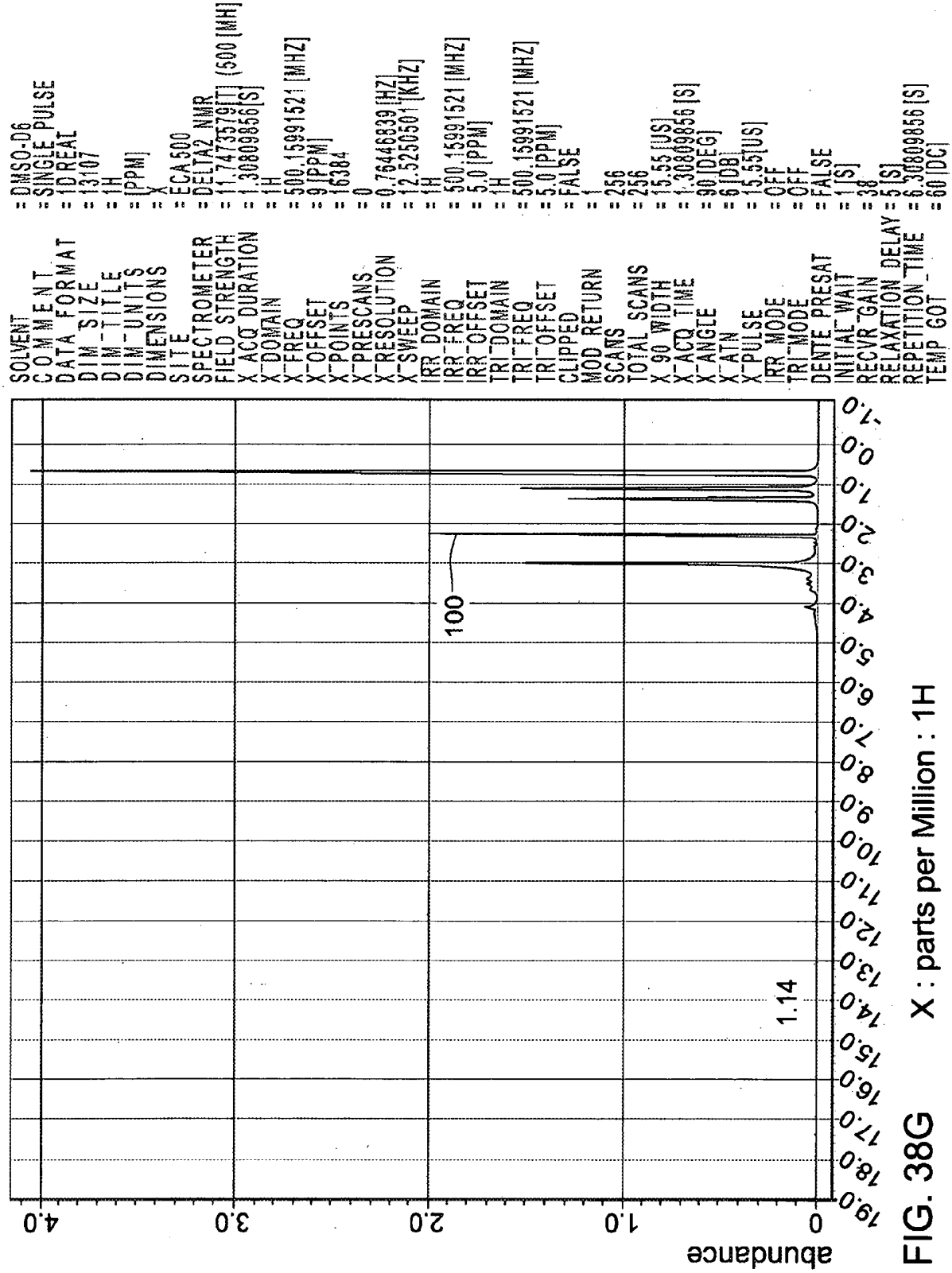

SOLVENT = DMSO-D6
COMMENT = SINGLE PULSE
DATA FORMAT = 1D REAL
DIM_SIZE = 13107
DIM_TITLE = 1H
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD STRENGTH = 11.747357971 (500 [MH]
X_ACQ_DURATION = 1.30809856[S]
X_DOMAIN = 1H
X_FREQ = 500.15991521 [MHZ]
X_OFFSET = 9 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 0.76446839 [HZ]
X_SWEEP = 12.5256501 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
TRI_DOMAIN = 1H
TRI_FREQ = 500.15991521 [MHZ]
TRI_OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD_RETURN = 1
SCANS = 256
TOTAL_SCANS = 256
X_90_WIDTH = 15.55 [US]
X_ACQ_TIME = 1.30809856 [S]
X_ANGLE = 90 [DEG]
X_ATN = 6 [DB]
X_PULSE = 15.55[US]
IRR_MODE = OFF
TRI_MODE = OFF
DENTE PRESAT = FALSE
INITIAL_WAIT = 1 [S]
RECVR_GAIN = 38
RELAXATION_DELAY = 5 [S]
REPETITION_TIME = 6.30809856 [S]
TEMP GOT = 60 [DC]
100
1.14
abundance
4.0
3.0
2.0
1.0
0
X : parts per Million : 1H

FIG. 38G

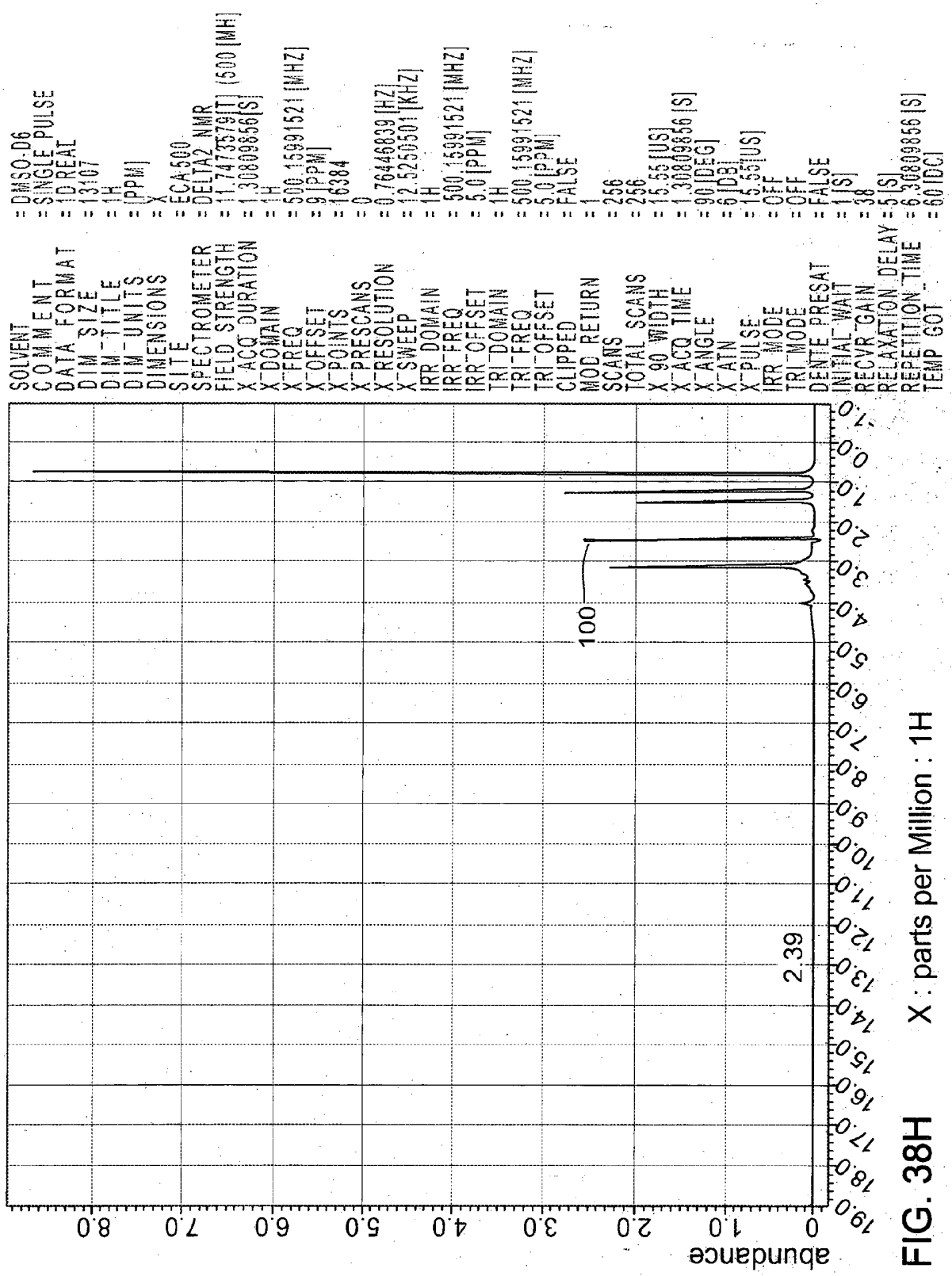

SOLVENT = DMSO-D6
COMMENT = SINGLE_PULSE
DATA_FORMAT = 1D_REAL
DIM_SIZE = 13107
DIM_TITLE = 1H
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD_STRENGTH = 11.747357971[T] (500 [MH]
X_ACQ_DURATION = 1.30809856[S]
X_DOMAIN = 1H
X_FREQ = 500.15991521 [MHZ]
X_OFFSET = 9 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 0.76446839 [HZ]
X_SWEEP = 12.5253501 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
TRI_DOMAIN = 1H
TRI_FREQ = 500.15991521 [MHZ]
TRI_OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD_RETURN = 1
SCANS = 256
TOTAL_SCANS = 256
X_90_WIDTH = 15.55 [US]
X_ACQ_TIME = 1.30809856 [S]
X_ANGLE = 90 [DEG]
X_ATN = 6 [DB]
X_PULSE = 15.55 [US]
IRR_MODE = OFF
TRI_MODE = OFF
DENTE_PRESAT = FALSE
INITIAL_WAIT = 1 [S]
RECVR_GAIN = 38
RELAXATION_DELAY = 5 [S]
REPETITION_TIME = 6.30809856 [S]
TEMP_GOT = 60 [DC]
100
2.39
abundance
X : parts per Million : 1H

FIG. 38H

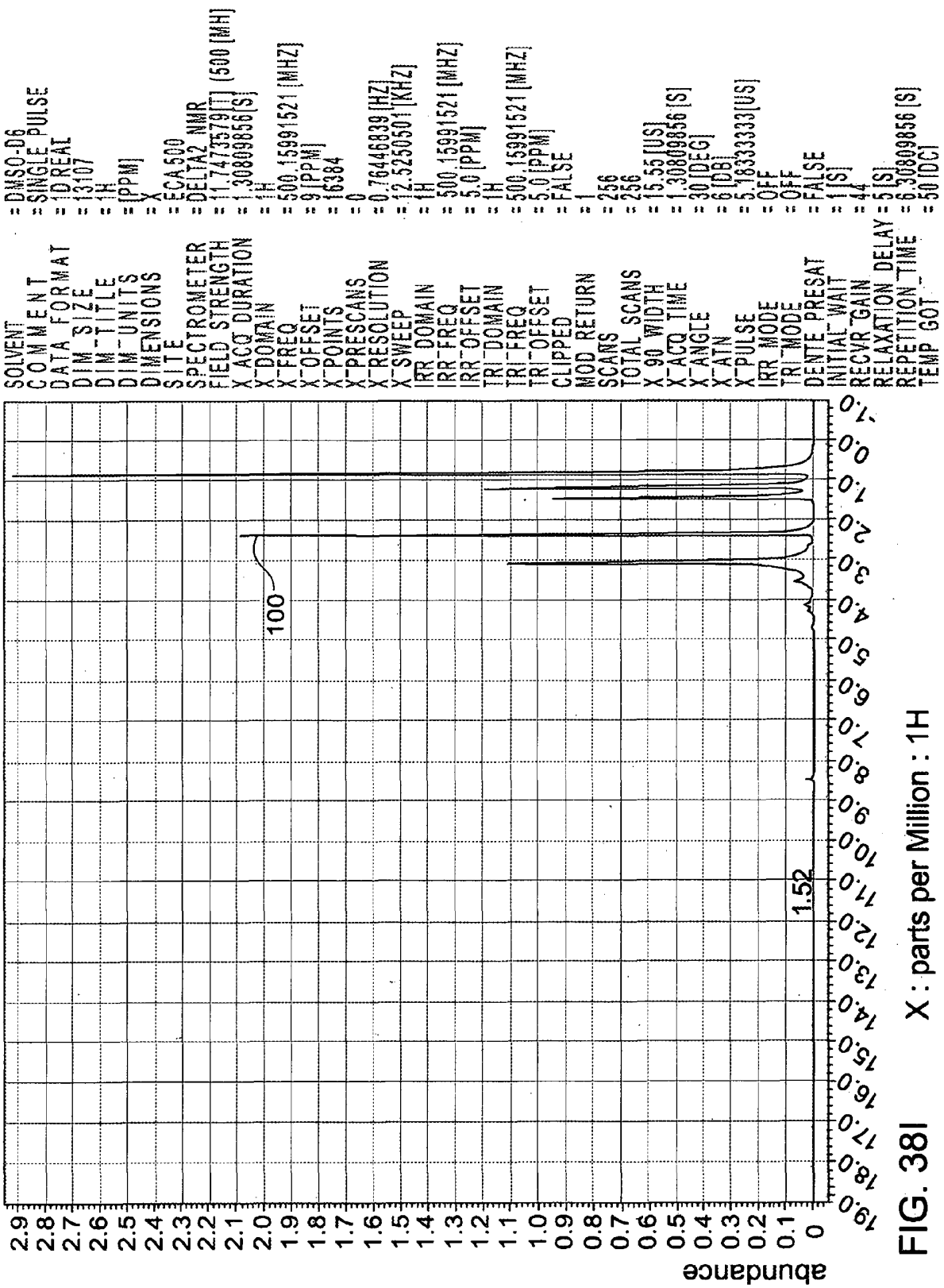

SOLVENT = DMSO-D6
COMMENT = SINGLE_PULSE
DATA_FORMAT = 1D REAL
DIM_SIZE = 13107
DIM_TITLE = 1H
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2 NMR
FIELD_STRENGTH = 11.747357917[T] [500 [MH]
X_ACQ_DURATION = 1.30809856[S]
X_DOMAIN = 1H
X_FREQ = 500.15991521 [MHZ]
X_OFFSET = 9 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 0.76446839 [HZ]
X_SWEEP = 12.525051 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
TRI_DOMAIN = 1H
TRI_FREQ = 500.15991521 [MHZ]
TRI_OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD_RETURN = 1
SCANS = 256
TOTAL_SCANS = 256
X_90_WIDTH = 15.55 [US]
X_ACQ_TIME = 1.30809856 [S]
X_ANGLE = 30 [DEG]
X_ATN = 6 [DB]
X_PULSE = 5.18333333[US]
IRR_MODE = OFF
TRI_MODE = OFF
DENTE_PRESAT = FALSE
INITIAL_WAIT = 1 [S]
RECVR_GAIN = 44
RELAXATION_DELAY = 5 [S]
REPETITION_TIME = 6.30809856 [S]
TEMP_GOT = 50 [DC]
100
1.52
abundance
X : parts per Million : 1H

FIG. 38I

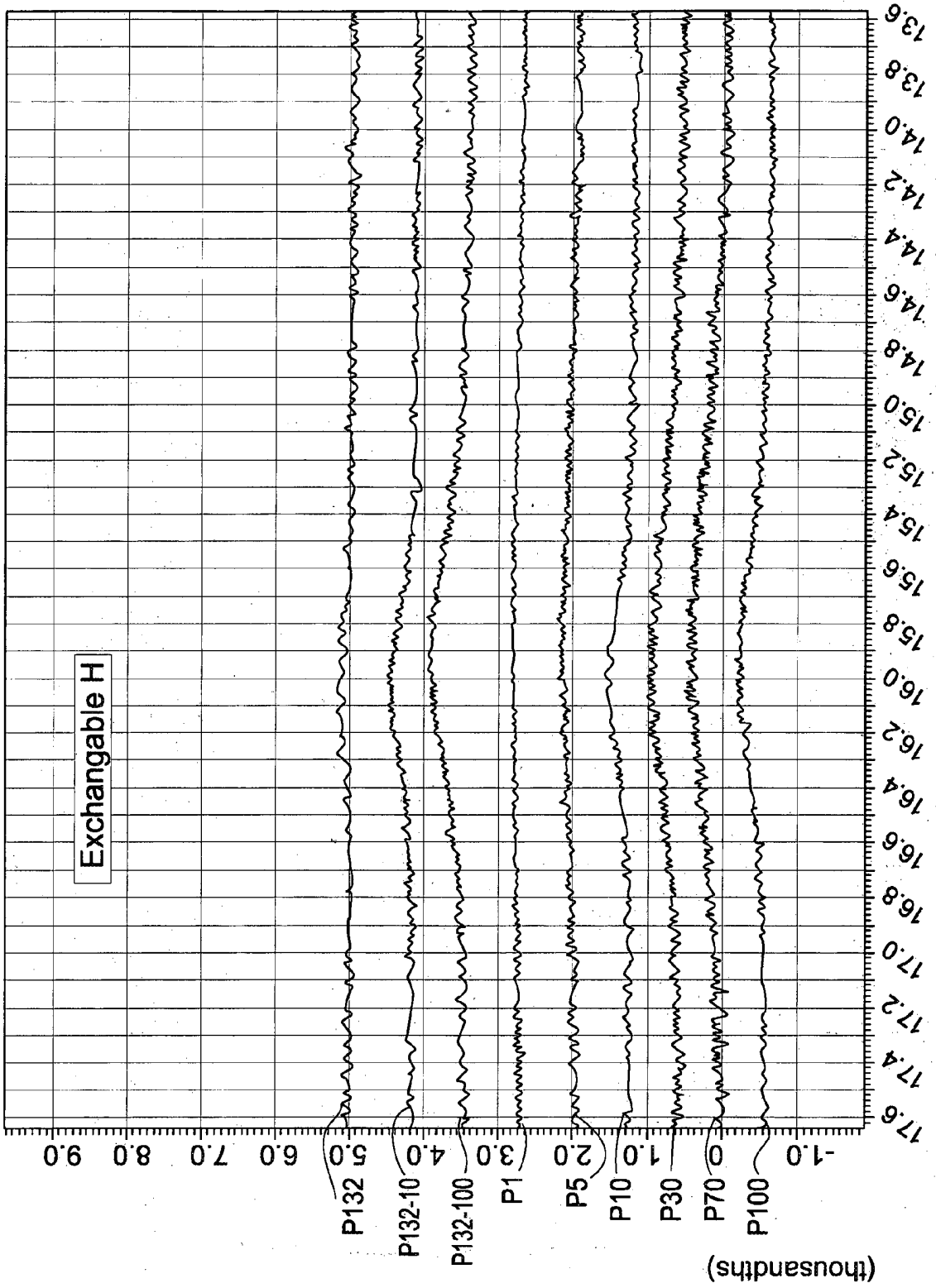

Exchangable H
P132
P132-10
P132-100
P1
P5
P10
P30
P70
P100
(thousandths)
X : parts per Million : 1H

FIG. 38J

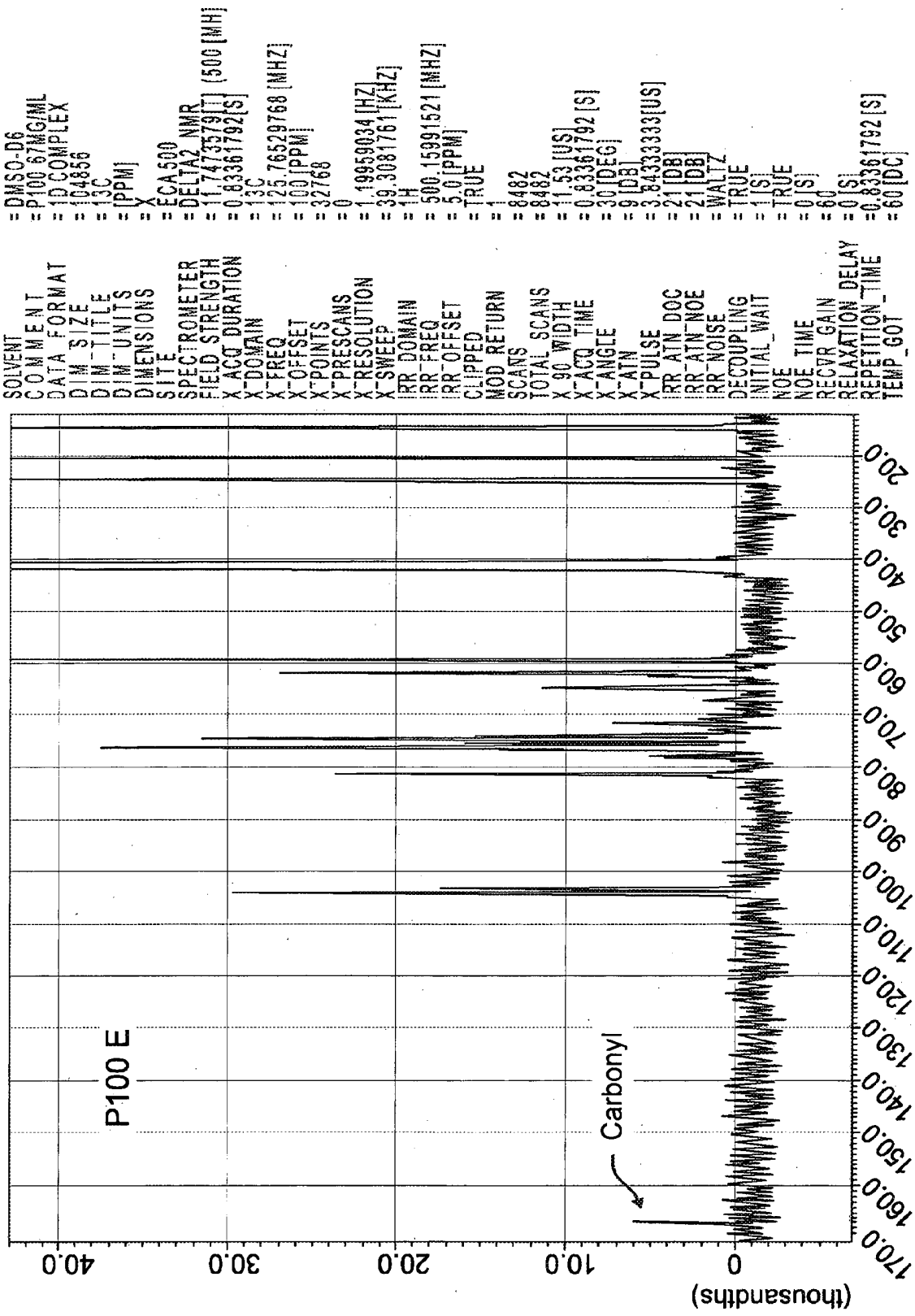

SOLVENT = DMSO-D6
COMMENT = P100 67MG/ML
DATA_FORMAT = 1D COMPLEX
DIM_SIZE = 104856
DIM_TITLE = 13C
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD_STRENGTH = 11.747357[T] (500 [MH]
X_ACQ_DURATION = 0.83361792[S]
X_DOMAIN = 13C
X_FREQ = 125.76529768 [MHZ]
X_OFFSET = 100 [PPM]
X_POINTS = 32768
X_PRESCANS = 0
X_RESOLUTION = 1.19959034 [HZ]
X_SWEEP = 39.30861761 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
CLIPPED = TRUE
MOD_RETURN = 1
SCANS = 8482
TOTAL_SCANS = 8482
X_90_WIDTH = 11.53 [US]
X_ACQ_TIME = 0.83361792 [S]
X_ANGLE = 30 [DEG]
X_ATN = 9 [DB]
X_PULSE = 3.84333333[US]
IRR_ATN_DOC = 21 [DB]
IRR_ATN_NOE = 21 [DB]
IRR_NOISE = WALTZ
DECOUPLING = TRUE
INITIAL_WAIT = 1 [S]
NOE = TRUE
NOE_TIME = 0 [S]
RECVR_GAIN = 60
RELAXATION_DELAY = 0 [S]
REPETITION_TIME =0.83361792 [S]
TEMP_GOT = 60 [DC]
P100 E
Carbonyl
40.0
30.0
20.0
10.0
0
(thousandths)
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
X : parts per Million : 13C

FIG. 38K

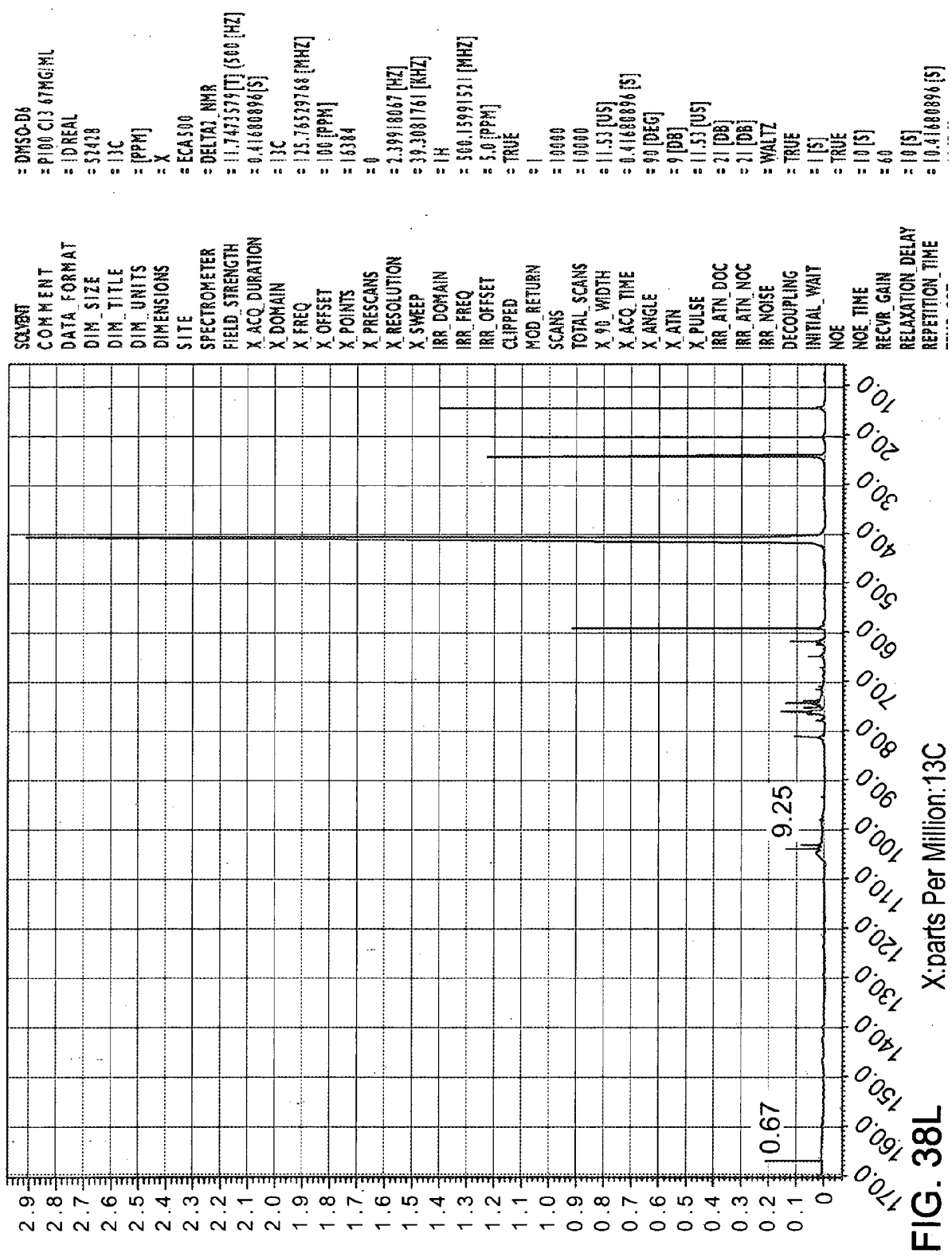

SOLVENT = DMSO-D6
COMMENT = P100 C13 67MG/ML
DATA_FORMAT = 1D REAL
DIM_SIZE = 32428
DIM_TITLE = 13C
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD_STRENGTH = 11.7473579[T] (500 [HZ]
X_ACQ_DURATION = 0.41680896[S]
X_DOMAIN = 13C
X_FREQ = 125.76529768 [MHZ]
X_OFFSET = 100 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 2.39918067 [HZ]
X_SWEEP = 39.3081761 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.13991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
CLIPPED = TRUE
MOD_RETURN = 1
SCANS = 10000
TOTAL_SCANS = 10000
X_90_WIDTH = 11.53 [US]
X_ACQ_TIME = 0.41680896 [S]
X_ANGLE = 90 [DEG]
X_ATN = 9 [DB]
X_PULSE = 11.53 [US]
IRR_ATN_DOC = 21 [DB]
IRR_ATN_NOC = 21 [DB]
IRR_NOISE = WALTZ
DECOUPLING = TRUE
INITIAL_WAIT = 1 [S]
NOE = TRUE
NOE_TIME = 10 [S]
RECVR_GAIN = 60
RELAXATION_DELAY = 10 [S]
REPETITION_TIME = 10.41680896 [S]
10.67
9.25
X:parts Per Million:13C

FIG. 38L

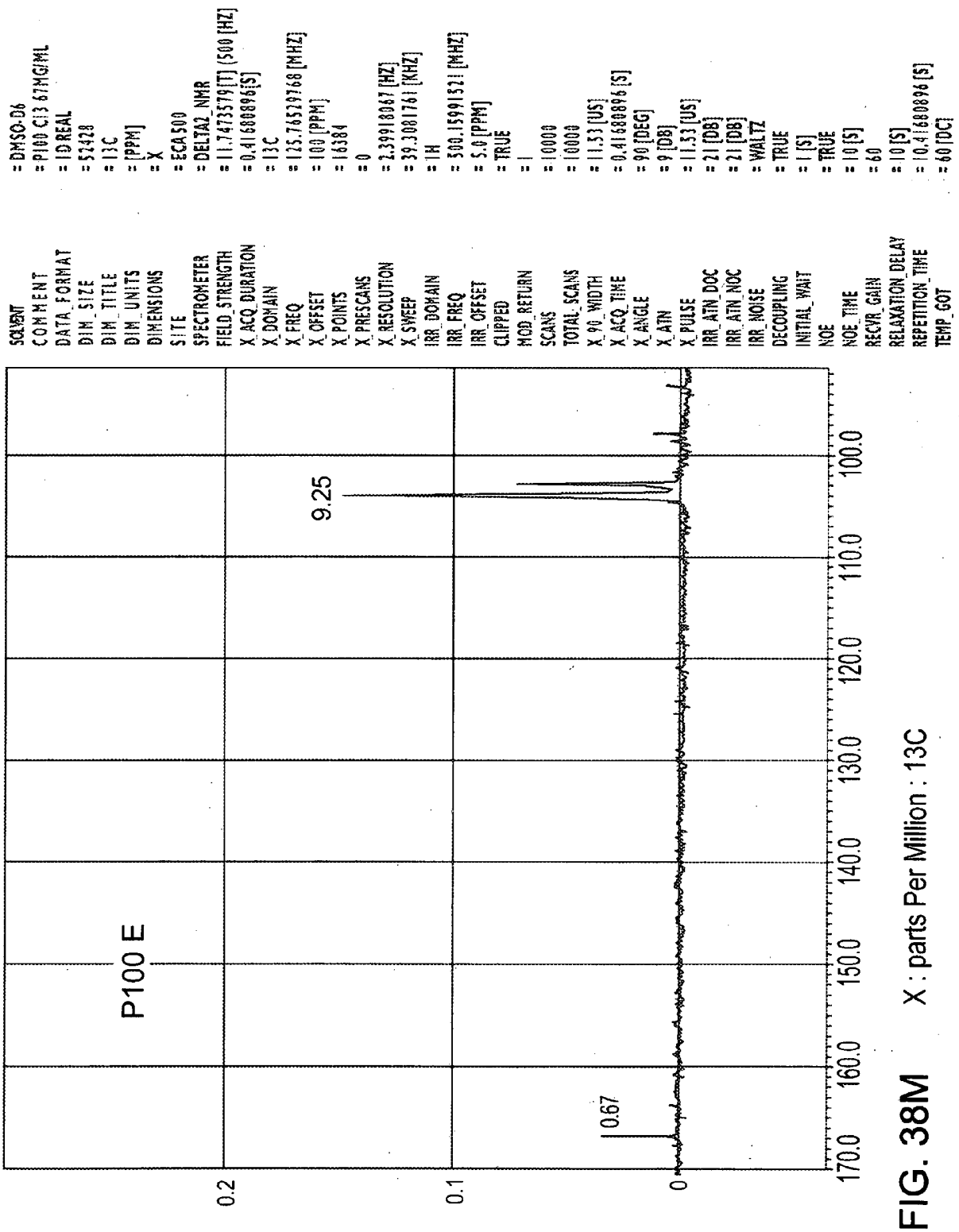

P100 E
9.25
0.67
0.2
0.1
0
170.0
160.0
150.0
140.0
130.0
120.0
110.0
100.0
SOLVENT = DMSO-D6
COMMENT = P100 C13 67MG/ML
DATA_FORMAT = 1D REAL
DIM_SIZE = 52428
DIM_TITLE = 13C
DIM_UNITS = [PPM]
DIMENSIONS = X
SITE = ECA500
SPECTROMETER = DELTA2_NMR
FIELD_STRENGTH = 11.7473579[T] (500 [MZ]
X_ACQ_DURATION = 0.41680896[S]
X_DOMAIN = 13C
X_FREQ = 125.76529768 [MHZ]
X_OFFSET = 100 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 2.39918067 [HZ]
X_SWEEP = 39.3081761 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
CLIPPED = TRUE
MOD_RETURN = 1
SCANS = 10000
TOTAL_SCANS = 10000
X_90_WIDTH = 11.33 [US]
X_ACQ_TIME = 0.41680896 [S]
X_ANGLE = 90 [DEG]
X_ATN = 9 [DB]
X_PULSE = 11.33 [US]
IRR_ATN_DOC = 21 [DB]
IRR_ATN_NOC = 21 [DB]
IRR_NOISE = WALTZ
DECOUPLING = TRUE
INITIAL_WAIT = 1 [S]
NOE = TRUE
NOE_TIME = 10 [S]
RECVR_GAIN = 60
RELAXATION_DELAY = 10 [S]
REPETITION_TIME = 10.41680896 [S]
TEMP_GOT = 60 [DC]
X : parts Per Million : 13C

FIG. 38M

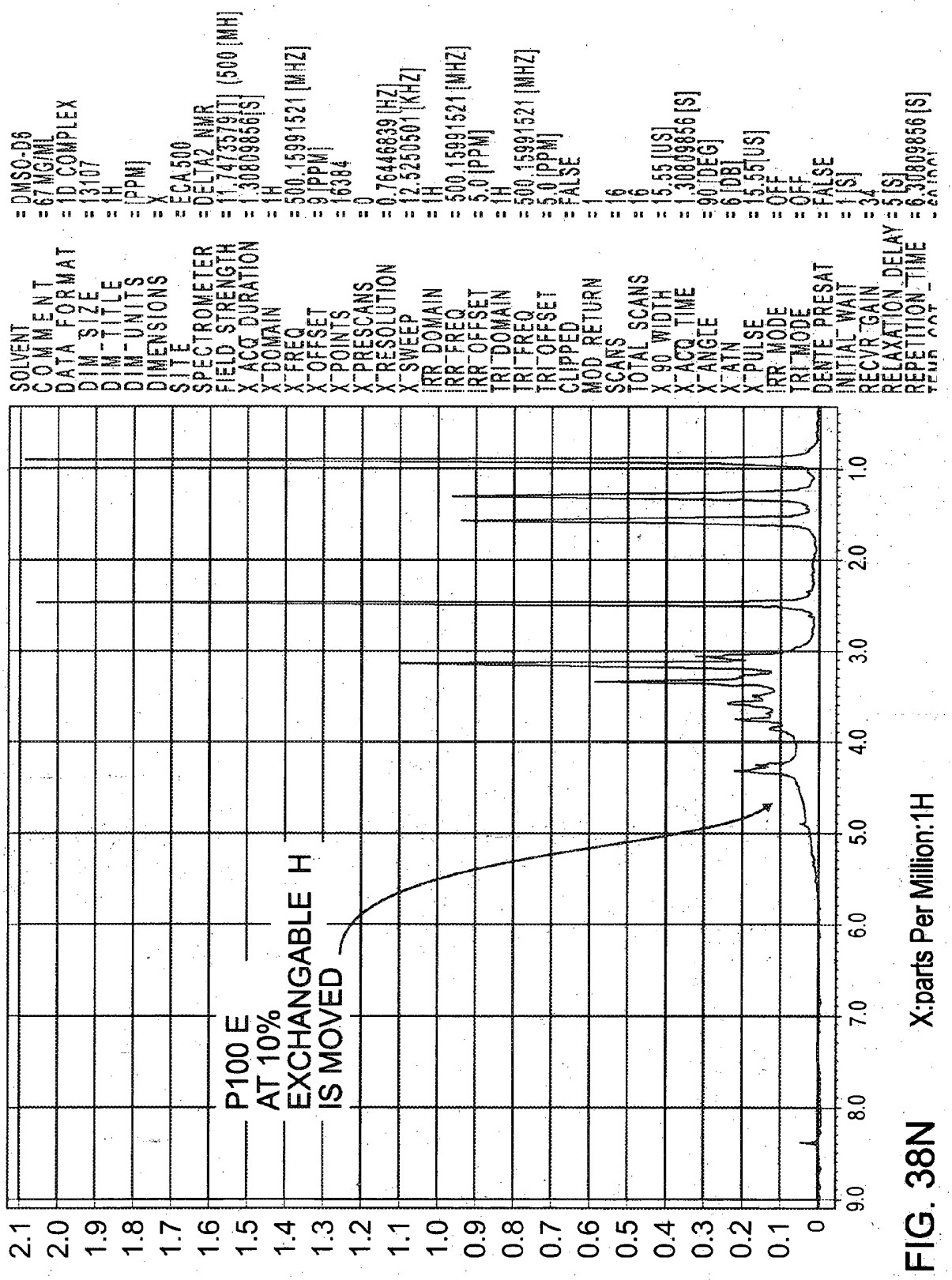

SOLVENT = DMSO-D6
COMMENT = 67 MG/ML
DATA_FORMAT = 1D COMPLEX
DIM_SIZE = 13107
DIM-TITLE = 1H
DIM-UNITS = [PPM]
DIMENSIONS = X
SITE = EGA500
SPECTROMETER = DELTA2 NMR
FIELD STRENGTH = 11.747357971 (500 [MH
X_ACQ_DURATION = 1.308098565[S]
X-DOMAIN = 1H
X_FREQ = 500.15991521 [MHZ]
X-OFFSET = 9 [PPM]
X_POINTS = 16384
X_PRESCANS = 0
X_RESOLUTION = 0.76446839 [HZ]
X_SWEEP = 12.525050 1 [KHZ]
IRR_DOMAIN = 1H
IRR_FREQ = 500.15991521 [MHZ]
IRR_OFFSET = 5.0 [PPM]
TRI_DOMAIN = 1H
TRI-FREQ = 500.15991521 [MHZ]
TRI_OFFSET = 5.0 [PPM]
CLIPPED = FALSE
MOD_RETURN = 1
SCANS = 16
TOTAL_SCANS = 16
X_90_WIDTH = 15.55 [US]
X-ACQ_TIME = 1.308098565 [S]
X_ANGLE = 90 [DEG]
X_ATN = 6 [DB]
X_PULSE = 15.55 [US]
IRR_MODE = OFF
TRI_MODE = OFF
DENTE PRESAT = FALSE
INITIAL_WAIT = 1 [S]
RECVR_GAIN = 34
RELAXATION_DELAY = 5 [S]
REPETITION_TIME = 6.308098565 [S]
P100 E
AT 10%
EXCHANGABLE H
IS MOVED
2.1 2.0 1.9 1.8 1.7 1.6 1.5 1.4 1.3 1.2 1.1 1.0 0.9 0.8 0.7 0.6 0.5 0.4 0.3 0.2 0.1 0
9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0
X:parts Per Million:1H

FIG. 38N

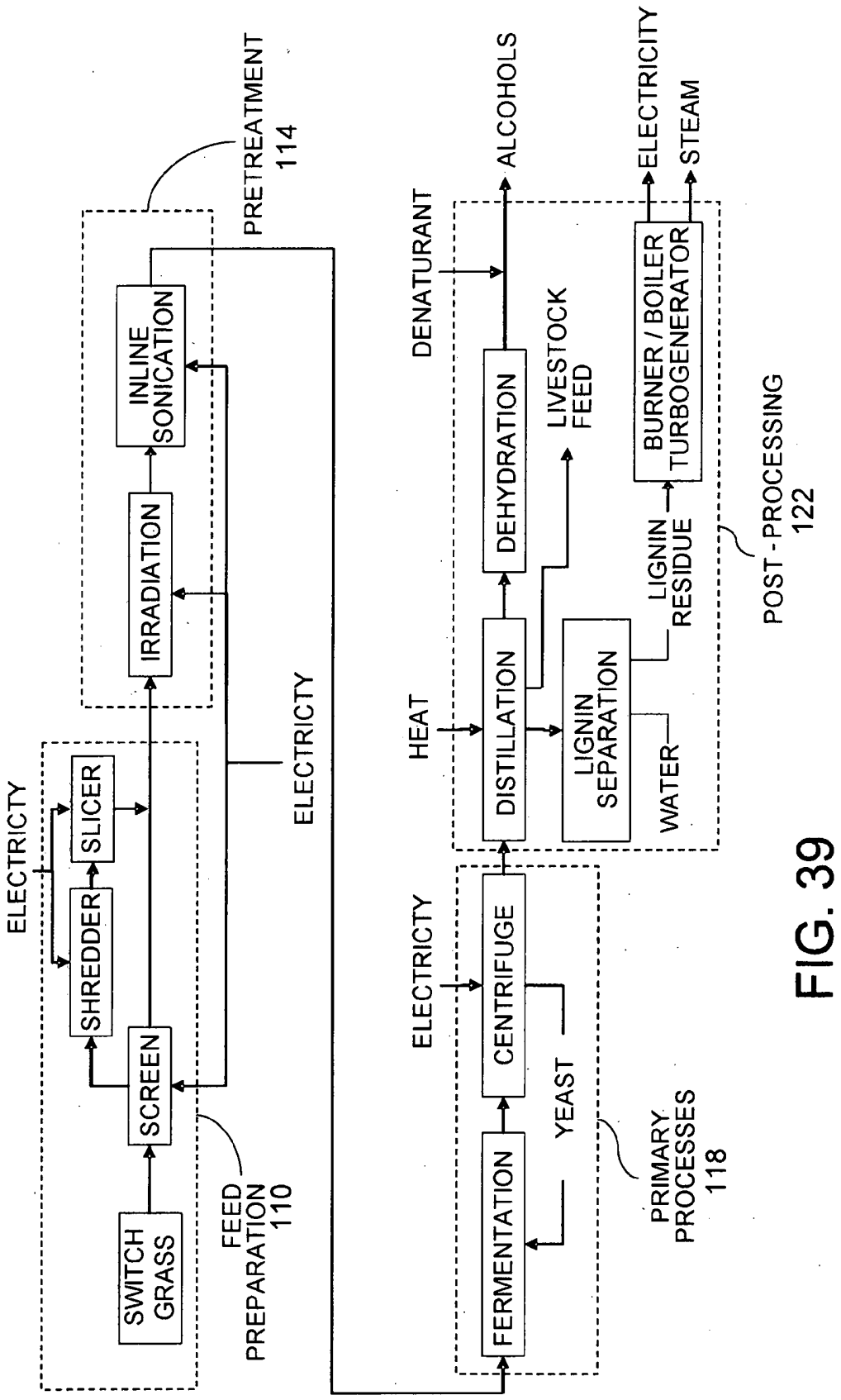

SWITCH GRASS
SCREEN
SHREDDER
SLICER
ELECTRICTY
FEED PREPARATION 110
IRRADIATION
INLINE SONICATION
PRETREATMENT 114
ELECTRICTY
FERMENTATION
CENTRIFUGE
YEAST
ELECTRICTY
PRIMARY PROCESSES 118
DISTILLATION
HEAT
LIGNIN SEPARATION
WATER
DEHYDRATION
DENATURANT
ALCOHOLS
LIVESTOCK FEED
LIGNIN RESIDUE
BURNER / BOILER TURBOGENERATOR
ELECTRICITY
STEAM
POST - PROCESSING 122

FIG. 39

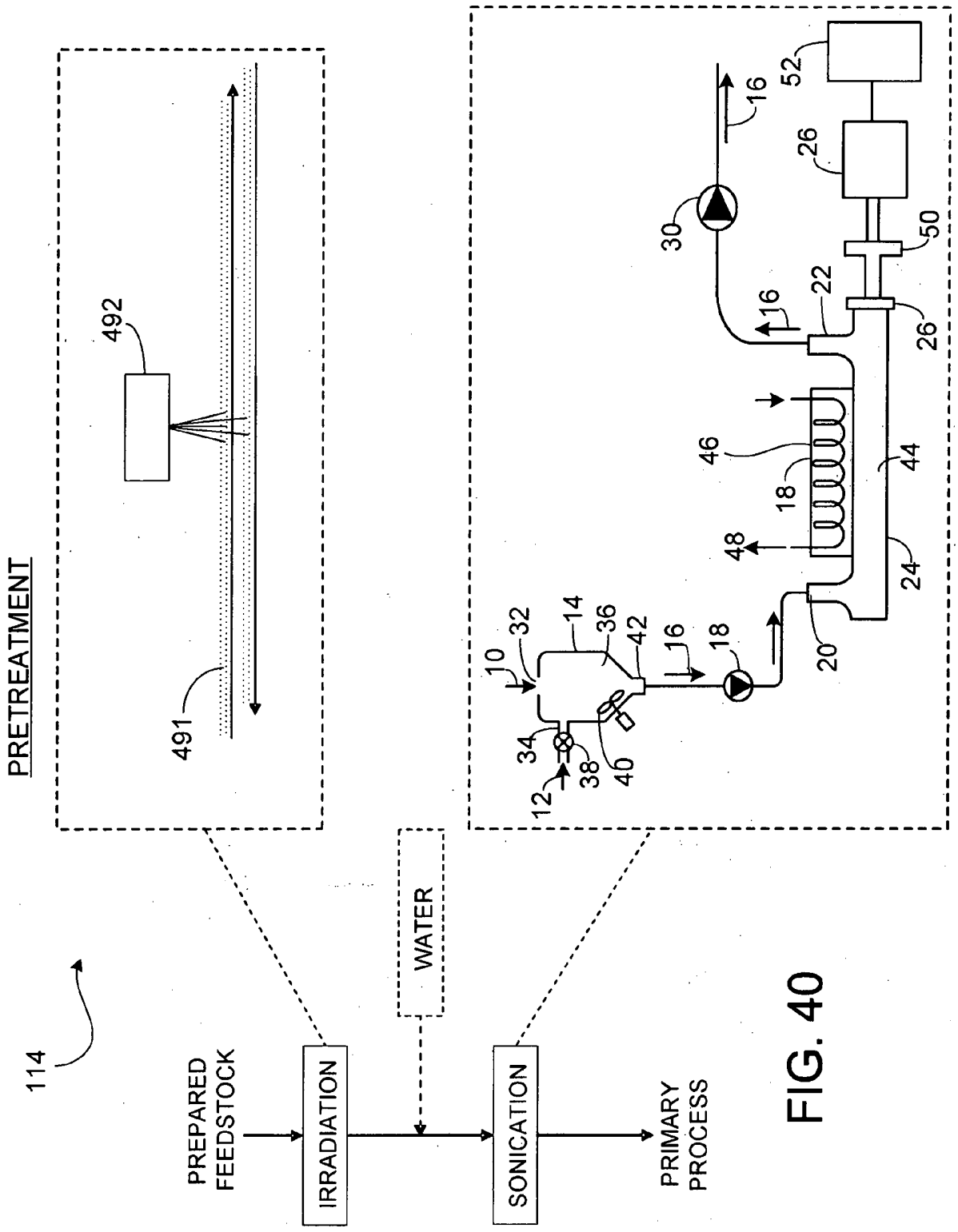

PRETREATMENT
492
491
114
PREPARED FEEDSTOCK
IRRADIATION
WATER
SONICATION
PRIMARY PROCESS
10
12
14
16
18
20
22
24
26
30
32
34
36
38
40
42
44
46
48
50
52

FIG. 40

REFERENCES CITED IN THE DESCRIPTION

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SZABADALMI IGÉNYPONTOK

1. Módszer, amely a következő lépésekből áll:

egy kis molekulasúlyú cukor éghető üzemanyaggá való konvertálása biomasszát, mikroorganizmust és oldószert vagy oldószerrendszert tartalmazó keverékben, továbbá a keverés előtt a biomassza besugárzása legalább 5 Mrad teljes dózisú ionizáló sugárzással, ahol a konvertálás fermentálási lépést foglal magában, ahol a biomassza nem emésztődik fel a fermentálás alatt és ahol a biomassza egy cellulóz- vagy lignocellulóz-tartalmú anyagot tartalmaz.

2. Az 1. igénypont szerinti módszer, ahol a konvertálási lépés során hagyjuk, hogy a mikroorganizmus a kis molekulasúlyú cukornak legalább egy részét etanollá konvertálja.

3. Az 1. vagy a 2. igénypont szerinti módszer, ahol a mikroorganizmusok tartalmaznak egy élesztőgombát, elsősorban az alábbi csoportból választva: S cerevisiae és P. stipitis, vagy ahol a mikroorganizmusok egy baktériumot tartalmaznak, például Zymomonas mobilis-t.

4. Az 1. igénypont szerinti módszer, ahol a besugárzás részecskesugárzással, például elektronnyalábbal történik.

5. A fenti igénypontok bármelyike szerinti módszer, ahol a biomassza térfogatsűrűsége a keverékhez való hozzáadást megelőzően körülbelül 0,5 g/cm^3-nél kisebb, és ahol előnyösen a biomassza rostos.

6. A fenti igénypontok bármelyike szerinti módszer, amely tartalmazza még a biomassza fizikai előkészítését is, például nyeséssel vagy a biomassza méretének kő-őrléssel, mechanikai tépéssel vagy szakítással, tüskés darálóval, zúzással vagy ultrafinom darálóval való csökkentésével.

7. A fenti igénypontok bármelyike szerinti módszer, ahol a biomassza BET-féle fajlagos felülete 0,25 m^2/g-nál nagyobb.

8. A fenti igénypontok bármelyike szerinti módszer, ahol a biomassza hosszúságának az átmérőhöz viszonyított aránya legalább 5.

9. A fenti igénypontok bármelyike szerinti módszer, ahol a biomassza az alábbi csoportból választott: papír, papírtermékek, papírhulladék, fa, forgácslemez, fűrészpor, mezőgazdasági hulladék, szennyvíz, siló, füvélék, rizskorpa, kipréselt cukornád, gyapot, juta, kender, len,

bambusz, szizálrost, abaka, szalma, kukoricacsutka, kukoricaszár, köles, lucerna, széna, kókuszhaj, gyapot, tengeri hinár, alga és ezek keverékei.

10. A fenti igénypontok bármelyike szerinti módszer, ahol a biomasszának belső rostjai vannak, és ahol az alapanyag betáplálás előtt olyan mértékű nyesésen esett át, amely a belső rostokat alaposan feltárta.

11. A fenti igénypontok bármelyike szerinti módszer, ahol a biomassza porozitása meghaladja az 50%-ot, például 70%-nál nagyobb.

12. A fenti igénypontok bármelyike szerinti módszer, ahol a konvertálási lépés százalékos teljesítménye legalább 140%-ot mutat, például legalább 170%-ot.

13. A fenti igénypontok bármelyike szerinti módszer, ahol az üzemanyag alkoholt tartalmaz.

14. A 13. igénypont szerinti módszer, ahol az alkohol etanolból áll.