FIG. 2 shows one particular system that utilizes the steps described above for treating a feedstock and then using the treated feedstock in a fermentation process to produce an alcohol. System 100 includes a module 102 in which a feedstock is initially mechanically treated (step 12, above), a module 104 in which the mechanically treated feedstock is structurally modified (step 14, above), e.g., by irradiation, and a module 106 in which the structurally modified feedstock is subjected to further mechanical treatment (step 16, above). As discussed above, the module 106 may be of the same type as the module 102, or a different type. In some implementations the structurally modified feedstock can be returned to module 102 for further mechanical treatment rather than being further mechanically treated in a separate module 106.

As described herein, many variations of system 100 can be utilized.

After these treatments, which may be repeated as many times as required to obtain desired feedstock properties, the treated feedstock is delivered to a fermentation system 108. Mixing may be performed during fermentation, in which case the mixing is preferably relatively gentle (low shear) so as to minimize damage to shear sensitive ingredients such as enzymes and other microorganisms. In some embodiments, jet mixing is used, as described in U.S. Serial No. 12/782,694, 13/293,977 and 13/293,985~~, the complete disclosures of which are incorporated herein by reference~~.

Referring again to FIG. 2, fermentation produces a crude ethanol mixture, which flows into a holding tank 110. Water or other solvent, and other non-ethanol components, are stripped from the crude ethanol mixture using a stripping column 112, and the ethanol is then distilled using a distillation unit 114, e.g., a rectifier. Distillation may be by vacuum distillation. Finally, the ethanol can be dried using a molecular sieve 116 and/or denatured, if necessary, and output to a desired shipping method.

In some cases, the systems described herein, or components thereof, may be portable, so that the system can be transported (e.g., by rail, truck, or marine vessel) from one location to another. The method steps described herein can be performed at one or more locations, and in some cases one or more of the steps can be performed in transit. Such mobile processing is described in U.S. Serial No. 12/374,549 and International Application No. WO 2008/011598~~, the full disclosures of which are incorporated herein by reference~~.

Any or all of the method steps described herein can be performed at ambient temperature. If desired, cooling and/or heating may be employed during certain steps. For example, the feedstock may be cooled during mechanical treatment to increase its brittleness. In some embodiments, cooling is employed before, during or after the initial mechanical treatment and/or the subsequent mechanical treatment. Cooling may be performed as described in U.S. Serial No. 12/502,629, now U.S. Patent No. 7,900,857 ~~the full disclosure of which is incorporated herein by reference~~. Moreover, the temperature in the fermentation system 108 may be controlled to enhance saccharification and/or fermentation.

The individual steps of the methods described above, as well as the materials used, will now be described in further detail.

PHYSICAL TREATMENT

Physical treatment processes can include one or more of any of those described herein, such as mechanical treatment, chemical treatment, irradiation, sonication, oxidation, pyrolysis or steam explosion. Treatment methods can be used in combinations of two, three, four, or even all of these technologies (in any order). When more than one treatment method is used, the methods can be applied at the same time or at different times. Other processes that change a molecular structure of a feedstock may also be used, alone or in combination with the processes disclosed herein.

Mechanical Treatments

In some cases, methods can include mechanically treating the feedstock. Mechanical treatments include, for example, cutting, milling, pressing, grinding, shearing and chopping. Milling may include, for example, ball milling, hammer milling, rotor/stator dry or wet milling, freezer milling, blade milling, knife milling, disk milling, roller milling or other types of milling. Other mechanical treatments include, e.g., stone grinding, cracking, mechanical ripping or tearing, pin grinding or air attrition milling.

Mechanical treatment can be advantageous for "opening up," "stressing," breaking and shattering cellulosic or lignocellulosic materials in the feedstock, making the cellulose of the materials more susceptible to chain scission and/or reduction of

Serial No. 12/429, 045 now U.S. Patent No. 7,932,065 and WO 2008/073186, ~~the full disclosures of which are incorporated herein by reference~~.

Radiation Treatment

One or more radiation processing sequences can be used to process the feedstock, and to provide a structurally modified material which functions as input to further processing steps and/or sequences. Irradiation can, for example, reduce the molecular weight and/or crystallinity of feedstock. Radiation can also sterilize the materials, or any media needed to bioprocess the material.

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 another approach, electromagnetic radiation (e.g., produced using electron beam emitters) can be used to irradiate the feedstock. In some embodiments, any combination in any order or concurrently of (1) through (3) may be utilized. The doses applied depend on the desired effect and the particular feedstock.

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 maximum oxidation is desired, oxygen ions can be utilized, and when maximum nitration is desired, nitrogen ions can be utilized. The use of heavy particles and positively charged particles is described in U.S. Serial No. 12/417,699, now U.S. Patent No. 7,931,784, ~~the full disclosure of which is incorporated herein by reference~~.

In one method, a first material that is or includes cellulose having a first number average molecular weight (M_{N1}) is irradiated, e.g., by treatment with ionizing radiation

reduced to the same extent as discussed above with respect to the other physical treatments.

In some embodiments, the second oxygen content is at least about five percent higher than the first oxygen content, e.g., 7.5 percent higher, 10.0 percent higher, 12.5 percent higher, 15.0 percent higher or 17.5 percent higher. In some preferred embodiments, the second oxygen content is at least about 20.0 percent higher than the first oxygen content of the first material. Oxygen content is measured by elemental analysis by pyrolyzing a sample in a furnace operating at 1300 °C or higher. A suitable elemental analyzer is the LECO CHNS-932 analyzer with a VTF-900 high temperature pyrolysis furnace.

Generally, oxidation of a material occurs in an oxidizing environment. For example, the oxidation can be effected or aided by pyrolysis in an oxidizing environment, such as in air or argon enriched in air. To aid in the oxidation, various chemical agents, such as oxidants, acids or bases can be added to the material prior to or during oxidation. For example, a peroxide (e.g., benzoyl peroxide) can be added prior to oxidation.

Some oxidative methods of reducing recalcitrance in a biomass feedstock employ Fenton-type chemistry. Such methods are disclosed, for example, in U.S. Serial No. 12/639,289, ~~the complete disclosure of which is incorporated herein by reference~~.

Exemplary oxidants include peroxides, such as hydrogen peroxide and benzoyl peroxide, persulfates, such as ammonium persulfate, activated forms of oxygen, such as ozone, permanganates, such as potassium permanganate, perchlorates, such as sodium perchlorate, and hypochlorites, such as sodium hypochlorite (household bleach).

In some situations, pH is maintained at or below about 5.5 during contact, such as between 1 and 5, between 2 and 5, between 2.5 and 5 or between about 3 and 5. Oxidation conditions can also include a contact period of between 2 and 12 hours, e.g., between 4 and 10 hours or between 5 and 8 hours. In some instances, temperature is maintained at or below 300 °C, e.g., at or below 250, 200, 150, 100 or 50 °C. In some instances, the temperature remains substantially ambient, e.g., at or about 20-25 °C.

In some embodiments, the one or more oxidants are applied as a gas, such as by generating ozone *in-situ* by irradiating the material through air with a beam of particles, such as electrons.

Yeast and *Zymomonas* bacteria, for example, can be used for fermentation or conversion. Other microorganisms are discussed in the Materials section, below. The optimum pH for fermentations is about pH 4 to 7. 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 168 (e.g., 24-96 hrs) hours with temperatures in the range of 20 °C to 40 °C (e.g., 26 °C to 40 °C), however thermophilic microorganisms prefer higher temperatures.

In some embodiments e.g., when anaerobic organisms are used, at least a portion of the fermentation is conducted in the absence of oxygen e.g., under a blanket of an inert gas such as N_2, Ar, He, CO_2 or mixtures thereof. Additionally, the mixture may have a constant purge of an inert gas flowing through the tank during part of or all of the fermentation. In some cases, anaerobic condition can be achieved or maintained by carbon dioxide production during the fermentation and no additional inert gas is needed.

In some embodiments, all or a portion of the fermentation process can be interrupted before the low molecular weight sugar is completely converted to a product (e.g. ethanol). The intermediate fermentation products include high concentrations of sugar and carbohydrates. The sugars and carbohydrates can be isolated as discussed below. These intermediate fermentation products can be used in preparation of food for human or animal consumption. Additionally or alternatively, the intermediate fermentation products can be ground to a fine particle size in a stainless-steel laboratory mill to produce a flour-like substance.

The fermentations include the methods and products that are disclosed in U.S. Provisional Application Serial No. 61/579,559, filed December, 2011 and U.S. Provisional Application Serial No. 61/579,576, filed December, 2011 ~~incorporated herein by reference~~.

Mobile fermentors can be utilized, as described in U.S. Provisional Patent Application Serial No. 60/832,735, now Published International Application No. WO 2008/011598. Similarly, the saccharification equipment can be mobile. Further, saccharification and/or fermentation may be performed in part or entirely during transit.

Fuel Cells

Where the methods described herein produce a sugar solution or suspension, this solution or suspension can subsequently be used in a fuel cell. For example, fuel cells utilizing sugars derived from cellulosic or lignocellulosic materials are disclosed in U.S. Provisional Application Serial No. 61/579,568, filed December 22, 2011~~, the complete disclosure of which is incorporated herein by reference~~.

Thermochemical Conversion

Thermochemical conversion can be performed on the treated feedstock to produce one or more desired intermediates and/or products. A thermochemical conversion process includes changing molecular structures of carbon-containing material at elevated temperatures. Specific examples include gasification, pyrolysis, reformation, partial oxidation and mixtures of these (in any order).

Gasification converts carbon-containing materials into a synthesis gas (syngas), which can include methanol, carbon monoxide, carbon dioxide and hydrogen. Many microorganisms, such as acetogens or homoacetogens are capable of utilizing a syngas from the thermochemical conversion of biomass, to produce a product that includes an alcohol, a carboxylic acid, a salt of a carboxylic acid, a carboxylic acid ester or a mixture of any of these. Gasification of biomass (e.g., cellulosic or lignocellulosic materials), can be accomplished by a variety of techniques. For example, gasification can be accomplished utilizing staged steam reformation with a fluidized-bed reactor in which the carbonaceous material is first pyrolyzed in the absence of oxygen and then the pyrolysis vapors are reformed to synthesis gas with steam providing added hydrogen and oxygen. In such a technique, process heat comes from burning char. Another technique utilizes a screw auger reactor in which moisture and oxygen are introduced at the pyrolysis stage and the process heat is generated from burning some of the gas produced in the latter stage. Another technique utilizes entrained flow reformation in which both external steam and air are introduced in a single-stage gasification reactor. In partial oxidation gasification, pure oxygen is utilized with no steam.

POST-PROCESSING

Distillation

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.

Other Possible Processing of Sugars

Processing during or after saccharification can include isolation and/or concentration of sugars by chromatography e.g., simulated moving bed chromatography, precipitation, centrifugation, crystallization, solvent evaporation and combinations thereof. In addition, or optionally, processing can include isomerization of one or more of the sugars in the sugar solution or suspension. Additionally, or optionally, the sugar solution or suspension can be chemically processed e.g., glucose and xylose can be hydrogenated to sorbitol and xylitol respectively. Hydrogenation can be accomplished by use of a catalyst e.g., Pt/γ-Al_2O_3, Ru/C, Raney Nickel in combination with H_2 under high pressure e.g., 10 to 12000 psi.

Some possible processing steps are disclosed in in U.S. Provisional Application Serial No. 61/579,552, filed December 22, 2011, and in U.S. Provisional Application Serial No. 61/579,576 , filed December 22, 2011~~, incorporated by reference above~~.

valuable starting materials for lubricants and agricultural chemicals, and Isobutene can be polymerized to butyl rubber, methyl tert-butyl ether and isooctane. In addition, synthetic petroleum kerosene can be synthesized by oligomerization of butenes. Other intermediates and products, including food and pharmaceutical products, for example edible materials selected from the group consisting of pharmaceuticals, nutriceuticals, proteins, fats, vitamins, oils, fiber, minerals, sugars, carbohydrates and alcohols, are described in U.S. Serial No. 12/417,900~~, the full disclosure of which is hereby incorporated by reference herein~~.

MATERIALS

Modified Plant Materials

The plant feedstock is obtained at least in part from one or more types of modified plants, as discussed herein. In some cases, the feedstock includes more than one type of plant, and/or more than one portion of the plant, e.g., the stalk, fruit, and cob of a corn plant. The plant may be, for example, a corn, soybean, beet, cotton, rapeseed, potato, rice, alfalfa, or sugarcane plant. The plant may also be any of the many types of genetically modified plants that are grown. The feedstock may contain a mixture of different types of plants, different parts of a particular plant, and/or mixtures of plant materials with other materials e.g., biomass materials.

In some cases the entire plant can be used. For example, in cases where a crop is ruined by adverse growing conditions (e.g., drought, frost, flooding, pest infestation) the ruined crop can be useful in the methods and processes described herein.

Other Feedstock Materials

In addition or as an alternative to the modified plant materials discussed above, the feedstock can include other materials e.g., biomass materials, that may or may not be genetically modified. The biomass can be, e.g., a cellulosic or lignocellulosic material. Such materials include paper and paper products (e.g., polycoated paper and Kraft paper), wood, wood-related materials, e.g., particle board, grasses, rice hulls, bagasse, jute, hemp, flax, bamboo, sisal, abaca, straw, switchgrass, alfalfa, hay, corn cobs, corn stover,

yeast species of genera *Zygosaccharomyces, Debaryomyces, Hansenula and Pichia*, and fungi of the dematioid genus *Torula*.

Commercially available yeasts 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, now Lalemand), GERT STRAND® (available from Gert Strand AB, Sweden) and FERMOL® (available from DSM Specialties).

OTHER EMBODIMENTS

A number of embodiments of the invention have been described. Nevertheless, it will be understood that various modifications may be made without departing from the spirit and scope of the invention.

For example, the process parameters of any of the processing steps discussed herein can be adjusted based on the lignin content of the feedstock, for example as disclosed in U.S. Serial No. 12/704,519~~, the full disclosure of which is incorporated herein by reference.~~

The process may include any of the features described in U.S. Application Serial No. 13/276,192~~, the full disclosure of which is incorporated herein by reference~~, including treating a cellulosic or lignocellulosic material to alter the structure of the material by irradiating the material with relatively low voltage, high power electron beam radiation, boiling or steeping the feedstock prior to saccharification, and irradiating a cellulosic or lignocellulosic material with an electron beam at a dose rate of at least 0.5 Mrad/sec.

While it is possible to perform all the processes described herein at one physical location, in some embodiments, the processes are completed at multiple sites, and/or may be performed during transport.

Lignin liberated in any process described herein can be captured and utilized. For example, the lignin can be used as captured as a plastic, or it can be synthetically upgraded to other plastics. In some instances, it can be utilized as an energy source, e.g., burned to provide heat. In some instances, it can also be converted to lignosulfonates, which can be utilized as binders, dispersants, emulsifiers or as sequestrants.

WHAT IS CLAIMED IS:

1. A method of making a product comprising:

~~physically treating a feedstock~~ providing a saccharified feedstock obtained at least in part from a plant that has been genetically modified to produce nutrients which enhance fermentation with respect to a wild type variety of the plant, the feedstock having been irradiated by an electron beam; and

contacting the saccharified feedstock with a microorganism to ferment the saccharified feedstock, wherein the saccharified genetically modified feedstock enhances fermentation by the microorganism.

2. The method of claim 1 wherein the feedstock comprises lignocellulosic or cellulosic material.

3. The method of claim 1 or 2 wherein the ~~plant has been genetically modified~~ enhanced fermentation is the result of the production of nutrients selected from the group consisting of fatty acids, glycerol, polyhydroxyalkanoate, amino acids, proteins, vitamins, and mixtures thereof.

4. The method of any of the above claims wherein the plant comprises recombinant DNA.

5. The method of any of the above claims wherein the plant comprises one or more recombinant genes.

6. The method of any of the above claims wherein the plant expresses a recombinant protein.

7. The method of any of the above claims wherein the plant expresses one or more recombinant materials.

8. The method of claim 7 wherein the recombinant material is a polymer or a macromolecule.

9. The method of any of the above claims further comprising obtaining from the feedstock a material selected from the group consisting of pharmaceuticals, nutriceuticals, proteins, fats, vitamins, oils, fiber, minerals, sugars, carbohydrates and alcohols.

10. The method of any of the above claims further comprising treating the feedstock with an ~~organism and/or~~ enzyme to produce a product.

11. The method of claim 10 wherein the product comprises a sugar.

~~12. The method of any of the above claims wherein the physical treatment comprises irradiation of the feedstock.~~

~~13. The method of claim 12 further comprising utilizing the irradiated feedstock as an animal feed.~~

~~14. The method of claim 12 wherein irradiating is performed using one or more electron beam devices.~~

~~15. The method of claim 12 wherein irradiating comprises applying a total dose of from about 5 Mrad to about 50 Mrad of radiation to the feedstock.~~

~~16~~12. The method of any of the above claims wherein the feedstock comprises a crop residue.

~~17~~13. The method of claim ~~16~~ 12 wherein the feedstock comprises corn cobs and/or corn stover.

~~18~~14. The method of claim ~~16~~ 12 wherein the feedstock comprises wheat straw.

~~19~~15. The method according to any one of the above claims wherein the plant comprises a genetically modified corn or soybean plant.

~~20~~16. The method of claim 11, further comprising fermenting the sugar.

~~21. The method of any one of the above claims wherein the plant has been modified with a modification selected from the group consisting of enhancement of resistance to insects, fungal diseases, and other pests and disease-causing agents; increased tolerance to herbicides; increased drought resistance; extended temperature range; enhanced tolerance to poor soil; enhanced stability or shelf-life; greater yield; larger fruit size; stronger stalks; enhanced shatter resistance; reduced time to crop maturity; more uniform germination times; higher or modified starch production; enhanced nutrient production,; modified lignin content; enhanced cellulose, hemicellulose and/or lignin degradation; reduced recalcitrance and enhanced phytate metabolism.~~

~~22~~17. The method of any one of the above claims wherein the plant is a genetically modified alfalfa, potato, corn, wheat, beet, cotton, rapeseed, rice, or sugarcane plant.

~~23. A product comprising sugar from a feedstock obtained at least in part from a plant that has been modified with respect to a wild type variety of the plant.~~

~~24~~18. A product comprising an irradiated feedstock ~~obtained~~ derived by a process comprising providing a saccharified feedstock obtained at least in part from a plant that has been genetically modified to produce nutrients which enhance fermentation with respect to a wild type variety of the plant, the feedstock having been irradiated by an electron beam.

~~25~~19. The product of claim ~~24~~ 18 further comprising a microorganism and/or an enzyme.

~~26~~20. The product of claim ~~24~~ 18 or ~~25~~ 19 further comprising a liquid medium.

~~27~~21. ~~A~~ The product of claim 18, wherein the irradiated feedstock is ~~comprising a physically treated~~ cellulosic or lignocellulosic feedstock ~~obtained at least in part from a plant that has been modified with respect to a wild type variety of the plant~~.