

PROCESSING BIOMASS

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application incorporates by reference the full disclosure of the following co-pending provisional applications: the co-pending provisionals filed March 8, 2013: USSN 61/774,684; USSN 61/774,773; USSN 61/774,731; USSN 61/774,735; USSN 61/774,740; USSN 61/774,744; USSN 61/774,746; USSN 61/774,750; USSN 61/774,752; USSN 61/774,754; USSN 61/774,775; USSN 61/774,780; USSN 61/774,761; USSN 61/774,723; and USSN 61/793,336, filed March 15, 2013.

BACKGROUND OF THE INVENTION

[0002] Many potential lignocellulosic feedstocks are available today, including agricultural residues, woody biomass, municipal waste, oilseeds/cakes and seaweed, to name a few. At present, these materials are often under-utilized, being used, for example, as animal feed, biocompost materials, burned in a co-generation facility or even landfilled.

[0003] Lignocellulosic biomass includes crystalline cellulose fibrils embedded in a hemicellulose matrix, surrounded by lignin. This produces a compact matrix that is difficult to access by enzymes and other chemical, biochemical and/or biological processes. Cellulosic biomass materials (*e.g.*, biomass material from which the lignin has been removed) is more accessible to enzymes and other conversion processes, but even so, naturally-occurring cellulosic materials often have low yields (relative to theoretical yields) when contacted with hydrolyzing enzymes. Lignocellulosic biomass is even more recalcitrant to enzyme attack. Furthermore, each type of lignocellulosic biomass has its own specific composition of cellulose, hemicellulose and lignin.

SUMMARY

[0004] Generally, this invention relates to methods and processes for converting a material, such as a biomass feedstock, *e.g.*, cellulosic, starchy or lignocellulosic materials, to useful products, for example, alcohols (*e.g.*, ethanol and butanol), acids (*e.g.* acetic, propionic, butyric, succinic, D- and L-lactic, pyruvic acid) and sugars (*e.g.*, glucose and xylose). The invention also relates *e.g.*, to methods equipment and systems

for the separation of products (e.g., purification, isolation or concentration) from the converted biomass. For example, a mixture of two or more sugars can be fermented to leave one or more sugars in the mixture.

[0005] In one aspect the invention relates to a method of making a product. The method includes saccharifying, such as by using one or more enzymes, cellulosic or lignocellulosic material, e. g. a reduced recalcitrance cellulosic or lignocellulosic material, in a liquid, such as water, to form a mixture comprising two or more sugars, such as two or more monosaccharides. The method further includes contacting the saccharified material with an organism, wherein the organism selectively ferments a sugar released during the saccharification (e.g., including glucose and/or xylose) to provide one or more unfermented sugars (e.g., including glucose or xylose), fermentation solids and a fermentation product. Optionally, the fermentation product (e. g. an alcohol or an organic acid) can be isolated from one or more of the unfermented sugars and fermentation solids, or the fermentation product and one or more of the unfermented sugars can be isolated from the fermentation solids, or the fermentation product and fermentation solids can be isolated from one or more of the unfermented sugars. Optionally the methods of isolating, e.g., the fermentation product, includes filtering including ultrafiltration, centrifuging, evaporation, distillation, crystallization, precipitation, extraction, chromatography including simulated moving bed chromatography, electrodialysis including bipolar electrodialysis and combinations of these. Optionally, the methods also include isolating the one or more unfermented sugars from the fermentation solids, for example, by filtering, centrifuging, evaporation, distillation, crystallization, precipitation, extraction, chromatography and combinations of these (e.g., in any order). Optionally, the method includes isolating lignin-derived compounds, such as soluble lignin-derived compounds, from the saccharified material prior to contacting the saccharified material with the fermenting organism.

[0006] In some implementations the fermentation solids can be utilized as a nutrient source, for example as animal feed, for human consumption or for the growth of organisms (such as bacteria and yeasts). Optionally, the fermentation solids (e.g., that can contain living organisms and/or remnants of living organisms), can be utilized for a second fermentation of a saccharified lignocellulosic material.

[0007] In some other implementations, the methods further include converting the one or more unfermented sugars to another product, such as when the one or more sugars comprise xylose and the other product comprises xylitol. The fermentation product can

comprise an alcohol (e.g., ethanol). The fermenting organism can include a yeast, bacteria, fungi, or a mixture of organisms, such as a yeast and a bacterium.

[0008] In some implementations, the recalcitrance of the biomass material is reduced by irradiation with ionizing radiation, for example including accelerated electrons from an electron beam. Optionally, a total dose of radiation applied to the cellulose or lignocellulosic material is between about 10 Mrad and about 200 Mrad, such as between about 15 Mrad and about 75 Mrad or between about 20 Mrad and about 50 Mrad.

[0009] In implementation of the methods wherein the saccharified material includes two monosaccharides (e.g., glucose and xylose) dissolved in the liquids, the monosaccharides can include at least 50 wt.% of total carbohydrates available in the reduced recalcitrance cellulosic or lignocellulosic material, e.g., 60 wt.%, 70 wt.%, 80 wt.%, 90 wt.%. Optionally, the glucose can include least 10 wt.% of the monosaccharides present in the saccharified material, e.g., at least 20 wt.%, 30 wt.%, 40 wt.%, 50 wt.%, 60 wt.%, 70 wt.%, 80 wt.% or 90 wt.%.

[0010] In another aspect, the invention includes a method of making a product including producing a slurry including a liquid, a first sugar, a second sugar, and a saccharified cellulosic or lignocellulosic residue material produced by saccharification of an irradiated cellulosic or lignocellulosic material, such as by utilizing one or more enzymes. The method further includes fermenting the first sugar to produce a product, such as an alcohol. Optionally, the second sugar is produced at a concentration of at least about 20 g/L e.g., at least about 30 g/L, at least about 40 g/L, at least about 50 g/L, at least about 60 g/L, at least about 70 g/L, at least about 80 g/L, at least about 90 g/L, at least about 100 g/L. Optionally, the method further includes filtering the slurry to provide a filtrate comprising a liquid solution, such as an aqueous solution, of the second sugar and the residue. The method can further include isolating the product, such as an alcohol, from the second sugar by distilling the product and producing a distillate bottom comprising the second sugar.

[0011] Saccharified biomass can produce a mixture of products after saccharification that can be difficult to separate. For example, mono-saccharides, e.g., glucose and xylose, are often difficult to separate from each other by conventional means due to their chemical and physical similarities. For example, in many chromatography techniques, glucose and xylose elute at similar times. The selective fermentation of a sugar from a mixture of sugars can provide a product that is useful. In addition, the fermented product can have sufficiently different chemical and physical differences from the unfermented sugars that separation can be efficiently accomplished. For example, inoculating a

saccharified biomass with an organism that produces D- or L-lactic acid or their salt from the glucose sugar which results in a slurry including xylose and lactic acid, which can be isolated from each other in a straightforward manner.

[0012] Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

DESCRIPTION OF THE DRAWING

[0013] The foregoing will be apparent from the following more particular description of example embodiments of the invention, as illustrated in the accompanying. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments of the present invention.

[0014] FIG. 1 is a diagram illustrating exemplary enzymatic hydrolysis of biomass.

[0015] FIG. 2 is a flow diagram showing processes for manufacturing sugar solutions from a feedstock.

[0016] FIG. 3 is a flow diagram showing processes for manufacturing sugar solutions from a feedstock showing a second fermentation.

[0017] FIG. 4 is a flow diagram that shows conversion of biomass to xylose.

[0018] FIG. 5 is a flow diagram that shows a purification scheme for xylose.

[0019] FIG. 6 is a flow diagram that shows a purification scheme for xylose and an organic acid by two stages of electrodialysis treatment.

DETAILED DESCRIPTION

[0020] Using the equipment, methods and systems described herein, cellulosic and lignocellulosic feedstock materials, for example that can be sourced from biomass (e.g., plant biomass, animal biomass, paper, and municipal waste biomass) and that are often readily available but difficult to process, can be turned into useful products (e.g., sugars such as the mono saccharides xylose and glucose, and alcohols such as ethanol and butanol). Included are equipment, methods and systems to selectively remove one of the biomass-derived sugars from a mixture of sugars by fermenting the sugar and separating the fermentation product from the rest of the biomass-derived sugars. The methods and systems are therefore useful for producing pure or substantially pure (e.g., at least 90, 91, 92, 93, 94 or 95% by weight) biomass-derived products from a biomass feedstock.

[0021] Biomass is a complex feedstock. For example, lignocellulosic materials include different combinations of cellulose, hemicellulose and lignin. Cellulose is a linear polymer of glucose. Hemicellulose is any of several heteropolymers, such as xylan, glucuronoxytan, arabinoxytan and xyloglucan. The primary sugar monomer present (e.g., present in the largest concentration) in hemicellulose is xylose, although other monomers such as mannose, galactose, rhamnose, arabinose and glucose are present. Although all lignins show variation in their composition, they have been described as an amorphous dendritic network polymer of phenyl propene units. The amounts of cellulose, hemicellulose and lignin in a specific biomass material depends on the source of the biomass material. For example, wood-derived biomass can be about 38-49% cellulose, 7-26% hemicellulose and 23-34% lignin depending on the type. Grasses typically are 33-38% cellulose, 24-32% hemicellulose and 17-22% lignin. Clearly, lignocellulosic biomass constitutes a large class of substrates.

[0022] Enzymes and biomass-destroying organisms that break down biomass, such as the cellulose, hemicellulose and/or the lignin portions of the biomass as described above, contain or manufacture various cellulolytic enzymes (cellulases), ligninases, xylanases, hemicellulases or various small molecule biomass-destroying metabolites. FIG. 1 provides some examples of these biomass-destroying processes. A cellulosic substrate is initially hydrolyzed by endoglucanases at random locations producing oligomeric intermediates. These intermediates are then substrates for exo-splitting glucanases such as cellobiohydrolase to produce cellobiose from the ends of the cellulose polymer. Cellobiose is a water-soluble 1,4-linked dimer of glucose. Finally, cellobiase cleaves cellobiose to yield glucose. In the case of hemicellulose, a xylanase (e.g., hemicellulase) acts on this biopolymer and releases xylose as one of the possible products.

[0023] The enzymes as described above act on biomass in aqueous solutions, releasing the sugars which can dissolve in the solution. Due to the complex and diverse sources of the biomass, a varied mixture of sugars is often produced as a difficult-to-separate mixture.

[0024] FIG. 2 shows processes for manufacturing sugars and fermentation products from a feedstock (e.g., cellulosic or lignocellulosic materials). In an initial step (210) the method includes optionally mechanically treating a cellulosic and/or lignocellulosic feedstock. Before and/or after this treatment, the feedstock can be treated with another physical treatment (212) to reduce its recalcitrance, for example irradiation, sonication, steam explosion, oxidation, pyrolysis, various heat treatments, such as heated water

under pressure, or combinations of these, to reduce or further reduce its recalcitrance. A mixed sugar solution e.g., including glucose and xylose, is formed by saccharifying the feedstock (**214**). The saccharification can be, for example, accomplished efficiently by the addition of one or more enzymes, e.g., cellulases and/or xylanases (**211**). A product or several products can be derived from the sugar solution, for example, by fermentation to an alcohol (**216**). In particular, the product (or products) can be derived by the fermentation by one or more organisms that selectively ferment(s) only one sugar in the sugar solution. Following fermentation, the fermentation product (e.g., or products, or a subset of the fermentation products) can be isolated (**224**). One optional method of isolating the fermentation product, for example if the product is an alcohol, is by distillation. Optionally, after the fermentation product has been isolated, the materials (e.g., solution, mixture, slurry, solids) containing the unfermented sugars can be further processed (**226**), for example to isolate and/or purify one or more of the unfermented sugars. If desired, the steps of measuring lignin content (**218**) and setting or adjusting process parameters based on this measurement (**220**) can be performed at various stages of the process, for example, as described in U.S. Application Number 12/704,519, filed on February 11, 2011, the complete disclosure of which is incorporated herein by reference.

[0025] In some embodiments, it can be desirable to isolate the unfermented sugars from the solution. For example, one or more unfermented sugars can be removed from the fermentation product at step **224**. The fermentation product can be subsequently removed from the solution. For example, the fermentation product can be distilled and the unfermented sugars remain in the distillate bottom for optional further processing.

[0026] In some embodiments one or more of the unfermented sugars can be contacted with an organism or combination of organisms that ferments the unfermented sugar(s) to a product, e.g., a product disclosed herein. The unfermented sugar(s) can be fermented prior to isolation from the fermentation product of the first sugar, for example, between steps **216** and **224**. In one embodiment, the unfermented sugar(s) can be fermented after isolating the product of fermenting the first sugar, for example, after step **224**. In another embodiment, the unfermented sugar(s) can be isolated after isolation of the fermentation product of the first sugar, for example, after step **224**, and then the isolated unfermented sugar can be fermented with one or more organisms.

[0027] Referring to Fig. 3 after saccharification the mixture is fermented at step **217** such that only one of the sugars is fermented to form a first product within a mixture of at least a second (unfermented) sugar, and fermentation solids. The first product at step

225 is isolated by any of the isolation technique described herein. Optionally, the fermentation solids may be separated from at least the second (unfermented) sugar at step **232**. A second fermentation process at step **227** will convert the second sugar to a second product which can be isolated by any of the isolation techniques described herein at step **230**. Examples of the first and second sugar can be glucose and xylose, respectively, with the glucose being converted in the first fermentation step. Alternately, the first sugar can be xylose and the second sugar can be glucose. In this case, the xylose fermentation product is the first product.

[0028] In an additional embodiment, FIG 4 shows the steps of physically treating a biomass (**410**); treating the feedstock to reduce recalcitrance(**412**), mixing in an enzyme (**411**) and saccharifying the material to form a mixture that includes sugars, for example, glucose and xylose(**414**); inoculating with a microorganism (**428**) which selectively converts one sugar e.g. to an organic acid, while retaining the other sugars (**428**) leading to fermentation (**416**), which leads to a mixture of a retained sugars and a desired product (**424**) and then removing the product mixture (**426**) to obtain a mixture of sugars and the desired product.

[0029] FIG. 5 shows steps to separate the organic acid from a sugar, in this case xylose (**510**). The purification means (**520**) can be a simulated moving bed chromatography or other purification means that can separate sugars from other substrates.

[0030] Pertaining to FIG 6 two electrodialysis steps are shown as a purification strategy. To the fermentation product liquid mixture which has had solids removed from it (**610**), is added a base if needed to convert the organic acid to its salt form (**620**) and electrodialysis processing is done to separate the nonionic sugars from the salts (including the organic acid salts). Then the salt is processed in the bipolar membrane electrodialysis unit (**630**) in which the organic acid salt is converted to its neutralized form and isolated from the salts.

[0031] The selective fermentations as mentioned above can selectively convert to a fermentation product most or even all of one of the sugars from available sugars derived from the biomass. For example, the selective fermentation can remove at least 60% (e.g., at least 70%, at least 80%, at least 90%, at least 95%, at least 99%, or even 100%) of one of the sugars, or between 60 and 99% (e.g., between 70 and 99%, between 80 and 99%, between 90 and 99%, between 60 and 70%, between 70 and 80%, between 80 and 90%, or between 70 and 90%) of one of the sugars. The sugar can be fermented in stages with different conditions, for example different nutrients added, different temperatures,

different pH values (e.g., with average values differing by at least 8 units, at least 5 units, at least 3 units, at least 1 unit), different concentrations of organism (e.g., differences in cell counts of more than about 10 fold, more than about 50 fold, more than about 100 fold, more than about 500 fold, more than about 1000 fold), different agitation rates (e.g., for mixers differences of at least 2 rpm, at least 5 rpm, at least 10 rpm, at least 50 rpm, at least 100 rpm, at least 500 rpm), different oxygenation rates (e.g., aerobic, anaerobic) and combinations of these. The organisms can be in various fermentation stages, for example producing different products (e.g., hydrogen, carbon dioxide, acids, ketones, alcohols or combinations thereof). There can be more than one organism producing the same or different fermentation products. The organisms can work synergistically, for example, a first organism can directly ferment the sugar, for example, to produce an acid, and then another organism can ferment the product of the fermentation by the first organism, for example, to a hydrocarbon. In some embodiments, enzymes can be utilized, for example, a glucose isomerase can be used to isomerize glucose to fructose and then an organism can be used to remove fructose and/or glucose. Some relevant uses of isomerase are discussed in PCT Application No. PCT/US13/71093, filed on December 20, 2012, the entire disclosure of which is incorporated herein by reference.

[0032] In some embodiments the liquids after saccharification and/or fermentation can be treated to remove solids, for example, by centrifugation, filtration, screening, or rotary vacuum filtration. For example, some methods and equipment that can be used during or after saccharification are disclosed PCT Application No. PCT/US13/48963, filed on July 01, 2013, and U.S. Provisional Application Serial No. 61/774,684, filed on March 8, 2013, the entire disclosures of which are incorporated herein by reference. In addition other separation techniques can be used on the liquids, for example to remove ions, de-colorize. For example, chromatography, simulated moving bed chromatograph and electrodialysis may be especially useful to isolate the products and the intermediate mixtures. Some of these methods are discussed in U.S. Provisional Application No. 61/774,775, filed on March 8, 2013 and U.S. Provisional Application No. 61/774,780, filed on March 8, 2013, the entire disclosures of which are incorporated herein by reference. Solids that are removed during the processing can be utilized for energy co-generation, for example as discussed in U.S. Provisional Application No. 61/774,773, filed on March 8, 2013, the entire disclosure of which is incorporated herein by reference.

[0033] In some cases filtration after fermentation (e.g., fermentation of a first sugar, a second sugar or even a third or fourth sugar derived from the biomass) can provide a

nutrient rich solid (e.g., solid, semi-solid, and filter cake, particulate, extract) material. For example, the nutrient rich material can include cellular material from the organism as well as some unused nutrients added from the fermentations. The nutrient rich material can be further processed and/or can be sold as a product. In addition, the nutrient rich material can be used, directly or with further processing (e.g., sterilization, filtered, washed, diluted, pH adjusted) in the process, for example, as a nutrient during the fermentation. In some cases filtration or other means of separation (e.g., membrane filtration) can recover the fermentation organism in a viable (e.g., living) form. The recovered fermentation organism can be used to inoculate subsequent fermentations and/or sold.

[0034] In some embodiments the carbohydrates in the lignocellulosic material include at least two different sugars, for example, glucose and xylose. The sugars can be bound as part of a polymer or an oligomer. The sugars can also be present as monomers, dimers and/or trimers). For example, the lignocellulosic material can include cellulose, starch, hemicellulose, pectin and other heteropolysaccharides, oligomers of glucose, oligomers of xylose, dimers and trimers of glucose, dimers and trimers of xylose, glucose, xylose and combinations of these. The total concentration of these carbohydrates can be between about 10 wt.% and 90 wt.% of the dry weight biomass, wherein dry biomass has less than about 5 wt.% water (e.g. the total concentration of sugars is between about 10 wt.% and 80 wt.%, between about 10 wt.% and 60 wt.%, between about 10 wt.% and 50 wt.%, between about 10 wt.% and 40 wt.%, between about 20 wt.% and 90 wt.%, between about 20 wt.% and 80 wt.%, between about 20 wt.% and 70 wt.%, between about 20 wt.% and 60 wt.%, between about 20 wt.% and 50 wt.%, between about 30 wt.% and 90 wt.%, between about 30 wt.% and 80 wt.%, between about 30 wt.% and 70 wt.%, between about 30 wt.% and 60 wt.%, between about 30 wt.% and 50 wt.%, between about 40 wt.% and 90 wt.%, between about 40 wt.% and 80 wt.%, between about 40 wt.% and 70 wt.%, between about 40 wt.% and 60 wt.%, between about 50 wt.% and 100 wt.%, between about 50 wt.% and 90 wt.%, between about 50 wt.% and 80 wt.%, between about 50 wt.% and 70 wt.%, between about 60 wt.% and 100 wt.%, between about 60 wt.% and 90 wt.%, between about 60 wt.% and 80 wt.%, between about 70 wt.% and 90 wt.%). After saccharification the percent of these carbohydrates in monomeric form (e.g., not as part of a polymer or oligomer) can be, for example, at least 50 wt. % of the total available concentration of the carbohydrates in the dry biomass prior to saccharification. For example, if the total biomass comprises 70 wt. % carbohydrates, after saccharification the monomeric sugars

will comprise 35 wt. % of the unsaccharified biomass (50 % of the available 70 wt. % carbohydrates). In some implementations, after saccharification the percentage of these carbohydrates that are in monomeric form could be at least 60 wt. % of the total available concentration of the carbohydrates in the dry biomass (e.g., at least 70 wt. %, at least 80 wt. %, at least 90 wt. %). After saccharification, glucose (e.g., monomers) can be present as at least 10 wt.% of the total available concentration of the carbohydrates in the dry biomass (e.g., at least 10 wt.%, at least 30 wt.%, at least 40 wt.%, at least 50 wt.%, at least 60 wt.%, at least 70 wt.%, at least 80 wt.%, at least 90 wt.%). After saccharification, xylose (e.g., monomers) can be present in at least 5 wt.% of the total available concentration of the carbohydrates in the dry biomass (e.g., at least 10 wt.%, at least 20 wt.%, at least 30 wt.%, at least 40 wt.%, at least 50 wt.%, at least 60 wt.%, at least 70 wt.%, at least 80 wt.%, at least 90 wt.%). After saccharification the combined wt.% of the total available concentration of the carbohydrates in the dry biomass, of other sugars, for example, arabinose can be less than about 10 wt.% (e.g., less than 5 wt.%, less than 1 wt.%). After saccharification one or more of the sugars can be present in a concentration of at least 10 g/L (e.g., at least 20 g/L, at least 30 g/L, at least 40 g/L, at least 50 g/L, at least 60 g/L, at least, 70 g/L, at least 80 g/L at least 90 g/L, at least 100 g/L) without concentrating the solution. The solution can be concentrated after saccharification to values at least 10% higher (e.g., at least 20%, at least 30%, at least 50%, at least 100%, at least 200%, at least 500%, at least 1000%). The solution can even be concentrated to dryness (e.g., less than about 5 wt. % water). The solution after saccharification can also be diluted, for example, by at least 10% (e.g., at least 20%, at least 30%, at least 50%, at least 100%, at least 200%, at least 500%, at least 1000%).

[0035] After fermentation of the saccharified material a sugar (e.g., glucose or xylose) can be present in solution at a concentration of at least 10 g/L (e.g., at least 20 g/L, at least 30 g/L, at least 40 g/L, at least 50 g/L, at least 60 g/L, at least, 70 g/L, at least 80 g/L at least 90 g/L, at least 100 g/L) without concentrating the solution. The solution can be concentrated or diluted similarly to the saccharified material as previously discussed. The solution can be further processed, for example, purified and/or converted to other products (e.g., by hydrogenation) as discussed below.

[0036] In some embodiments the methods can produce a composition that includes lignin-derived products between about 1 and 30 wt. %, (e.g., between about 5 and 25%, between about 5 and 20 wt. %), a fermentation product from a first sugar of between about 5 and 20% (e.g., between about 10 wt. % and 20 wt. %) and an unfermented second sugar of between about 1 and 10 wt. %. The composition can include at least

about 40 wt.% water (e.g., 50 wt.% water, 60 wt.% water, 70 wt.% water, 80 wt.% water). The water can be evaporated from the composition, producing a material with less than about 50 wt.% water (e.g. less than about 40wt.% water, less than about 30 wt.% water, less than about 20 wt.% water, less than about 10 wt.% water, less than about 5 wt.% water).

BIOMASS MATERIALS

[0037] Lignocellulosic materials include, but are not limited to, wood, particle board, forestry wastes (e.g., sawdust, aspen wood, wood chips), grasses, (e.g., switchgrass, miscanthus, cord grass, reed canary grass), grain residues, (e.g., rice hulls, oat hulls, wheat chaff, barley hulls), agricultural waste (e.g., silage, canola straw, wheat straw, barley straw, oat straw, rice straw, jute, hemp, flax, bamboo, sisal, abaca, corn cobs, corn stover, soybean stover, corn fiber, alfalfa, hay, coconut hair), sugar processing residues (e.g., bagasse, beet pulp, agave bagasse), algae, seaweed, manure, sewage, and mixtures of any of these.

[0038] In some cases, the lignocellulosic material includes corncobs. Ground or hammer milled corncobs can be spread in a layer of relatively uniform thickness for irradiation, and after irradiation are easy to disperse in the medium for further processing. To facilitate harvest and collection, in some cases the entire corn plant is used, including the corn stalk, corn kernels, and in some cases even the root system of the plant.

[0039] Advantageously, no additional nutrients (other than a nitrogen source, e.g., urea or ammonia) are required during fermentation of corncobs or cellulosic or lignocellulosic materials containing significant amounts of corncobs.

[0040] Corncobs, before and after comminution, are also easier to convey and disperse, and have a lesser tendency to form explosive mixtures in air than other cellulosic or lignocellulosic materials such as hay and grasses.

[0041] Cellulosic materials include, for example, paper, paper products, paper waste, paper pulp, pigmented papers, loaded papers, coated papers, filled papers, magazines, printed matter (e.g., books, catalogs, manuals, labels, calendars, greeting cards, brochures, prospectuses, newsprint), printer paper, polycoated paper, card stock, cardboard, paperboard, materials having a high α -cellulose content such as cotton, and mixtures of any of these. For example paper products as described in U.S. App. No. 13/396,365 ("Magazine Feedstocks" by Medoff *et al.*, filed February 14, 2012), the full disclosure of which is incorporated herein by reference.

[0042] Cellulosic materials can also include lignocellulosic materials which have been partially or fully de-lignified.

[0043] In some instances other biomass materials can be utilized, for example starchy materials. Starchy materials include starch itself, *e.g.*, corn starch, wheat starch, potato starch or rice starch, a derivative of starch, or a material that includes starch, such as an edible food product or a crop. For example, the starchy material can be arracacha, buckwheat, banana, barley, cassava, kudzu, oca, sago, sorghum, regular household potatoes, sweet potato, taro, yams, or one or more beans, such as favas, lentils or peas. Blends of any two or more starchy materials are also starchy materials. Mixtures of starchy, cellulosic and or lignocellulosic materials can also be used. For example, a biomass can be an entire plant, a part of a plant or different parts of a plant, *e.g.*, a wheat plant, cotton plant, a corn plant, rice plant or a tree. The starchy materials can be treated by any of the methods described herein.

[0044] Microbial materials include, but are not limited to, any naturally occurring or genetically modified microorganism or organism that contains or is capable of providing a source of carbohydrates (*e.g.*, cellulose), for example, protists, *e.g.*, animal protists (*e.g.*, protozoa such as flagellates, amoeboids, ciliates, and sporozoa) and plant protists (*e.g.*, algae such as alveolates, chlorarachniophytes, cryptomonads, euglenids, glaucophytes, haptophytes, red algae, stramenopiles, and viridaplantae). Other examples include seaweed, plankton (*e.g.*, macroplankton, mesoplankton, microplankton, nanoplankton, picoplankton, and femtoplankton), phytoplankton, bacteria (*e.g.*, gram positive bacteria, gram negative bacteria, and extremophiles), yeast and/or mixtures of these. In some instances, microbial biomass can be obtained from natural sources, *e.g.*, the ocean, lakes, bodies of water, *e.g.*, salt water or fresh water, or on land. Alternatively or in addition, microbial biomass can be obtained from culture systems, *e.g.*, large scale dry and wet culture and fermentation systems.

In other embodiments, the biomass materials, such as cellulosic, starchy and lignocellulosic feedstock materials, can be obtained from transgenic microorganisms and plants that have been modified with respect to a wild type variety. Such modifications may be, for example, through the iterative steps of selection and breeding to obtain desired traits in a plant. Furthermore, the plants can have had genetic material removed, modified, silenced and/or added with respect to the wild type variety. For example, genetically modified plants can be produced by recombinant DNA methods, where genetic modifications include introducing or modifying specific genes from parental varieties, or, for example, by using transgenic breeding wherein a specific gene or genes

are introduced to a plant from a different species of plant and/or bacteria. Another way to create genetic variation is through mutation breeding wherein new alleles are artificially created from endogenous genes. The artificial genes can be created by a variety of ways including treating the plant or seeds with, for example, chemical mutagens (*e.g.*, using alkylating agents, epoxides, alkaloids, peroxides, formaldehyde), irradiation (*e.g.*, X-rays, gamma rays, neutrons, beta particles, alpha particles, protons, deuterons, UV radiation) and temperature shocking or other external stressing and subsequent selection techniques. Other methods of providing modified genes is through error prone PCR and DNA shuffling followed by insertion of the desired modified DNA into the desired plant or seed. Methods of introducing the desired genetic variation in the seed or plant include, for example, the use of a bacterial carrier, biolistics, calcium phosphate precipitation, electroporation, gene splicing, gene silencing, lipofection, microinjection and viral carriers. Additional genetically modified materials have been described in U.S. Application Serial No 13/396,369 filed February 14, 2012, the full disclosure of which is incorporated herein by reference. Any of the methods described herein can be practiced with mixtures of any biomass materials described herein.

BIOMASS MATERIAL PREPARATION – MECHANICAL TREATMENTS

[0045] The biomass can be in a dry form, for example with less than about 35% moisture content (*e.g.*, less than about 20 %, less than about 15 %, less than about 10 % less than about 5 %, less than about 4%, less than about 3 %, less than about 2 % or even less than about 1 %). The biomass can also be delivered in a wet state, for example as a wet solid, a slurry or a suspension with at least about 10 wt.% solids (*e.g.*, at least about 20 wt.%, at least about 30 wt. %, at least about 40 wt.%, at least about 50 wt.%, at least about 60 wt.%, at least about 70 wt.%).

[0046] The processes disclosed herein can utilize low bulk density materials, for example cellulosic or lignocellulosic feedstocks that have been physically pretreated to have a bulk density of less than about 0.75 g/cm³, *e.g.*, less than about 0.7, 0.65, 0.60, 0.50, 0.35, 0.25, 0.20, 0.15, 0.10, 0.05 or less, *e.g.*, less than about 0.025 g/cm³. 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. If desired, low bulk density materials can be densified, for example, by methods described in U.S. Pat. No. 7,971,809 to Medoff, the full disclosure of which is hereby incorporated by reference.

[0047] In some cases, the pre-treatment processing includes screening of the biomass material. Screening can be through a mesh or perforated plate with a desired opening size, for example, less than about 6.35 mm (1/4 inch, 0.25 inch), (*e.g.*, less than about 3.18 mm (1/8 inch, 0.125 inch), less than about 1.59 mm (1/16 inch, 0.0625 inch), is less than about 0.79 mm (1/32 inch, 0.03125 inch), *e.g.*, less than about 0.51 mm (1/50 inch, 0.02000 inch), less than about 0.40 mm (1/64 inch, 0.015625 inch), less than about 0.23 mm (0.009 inch), less than about 0.20 mm (1/128 inch, 0.0078125 inch), less than about 0.18 mm (0.007 inch), less than about 0.13 mm (0.005 inch), or even less than about 0.10 mm (1/256 inch, 0.00390625 inch)). In one configuration the desired biomass falls through the perforations or screen and thus biomass larger than the perforations or screen are not irradiated. These larger materials can be re-processed, for example, by comminuting, or they can simply be removed from processing. In another configuration material that is larger than the perforations is irradiated and the smaller material is removed by the screening process or recycled. In this kind of a configuration, the conveyor itself (for example a part of the conveyor) can be perforated or made with a mesh. For example, in one particular embodiment the biomass material may be wet and the perforations or mesh allow water to drain away from the biomass before irradiation.

[0048] Screening of material can also be by a manual method, for example, by an operator or mechanoid (*e.g.*, a robot equipped with a color, reflectivity or other sensor) that removes unwanted material. Screening can also be by magnetic screening wherein a magnet is disposed near the conveyed material and the magnetic material is removed magnetically.

[0049] For example, a portion of the conveying trough can be heated by use of a heating jacket. Heating can be, for example, for the purpose of drying the material. In the case of drying the material, this can also be facilitated, with or without heating, by the movement of a gas (*e.g.*, air, oxygen, nitrogen, He, CO₂, Argon) over and/or through the biomass as it is being conveyed. Optionally, pre-treatment processing can include cooling the material. Cooling material is described in US Pat. No. 7,900,857 to Medoff, the disclosure of which is incorporated herein by reference. For example, cooling can be by supplying a cooling fluid, for example water (*e.g.*, with glycerol), or nitrogen (*e.g.*, liquid nitrogen) to the bottom of the conveying trough. Alternatively, a cooling gas, for example, chilled nitrogen can be blown over the biomass materials or under the conveying system.

[0050] Another optional pre-treatment processing method can include adding a material to the biomass. The additional material can be added by, for example, by

showering, sprinkling and or pouring the material onto the biomass as it is conveyed. Materials that can be added include, for example, metals, ceramics and/or ions as described in U.S. Pat. App. Pub. 2010/0105119 A1 (filed October 26, 2009) and U.S. Pat. App. Pub. 2010/0159569 A1 (filed December 16, 2009), the entire disclosures of which are incorporated herein by reference. Optional materials that can be added include acids and bases. Other materials that can be added are oxidants (*e.g.*, peroxides, chlorates), polymers, polymerizable monomers (*e.g.*, containing unsaturated bonds), water, catalysts, enzymes and/or organisms. Materials can be added, for example, in pure form, as a solution in a solvent (*e.g.*, water or an organic solvent) and/or as a solution. In some cases the solvent is volatile and can be made to evaporate *e.g.*, by heating and/or blowing gas as previously described. The added material may form a uniform coating on the biomass or be a homogeneous mixture of different components (*e.g.*, biomass and additional material). The added material can modulate the optional subsequent irradiation step by increasing the efficiency of the irradiation, damping the irradiation or changing the effect of the irradiation (*e.g.*, from electron beams to X-rays or heat). The method may have no impact on the irradiation but may be useful for further downstream processing. The added material may help in conveying the material, for example, by lowering dust levels.

[0051] Biomass can be delivered to the conveyor (*e.g.*, the vibratory conveyors used in the vaults herein described) by a belt conveyor, a pneumatic conveyor, a screw conveyor, a hopper, a pipe, manually or by a combination of these. The biomass can, for example, be dropped, poured and/or placed onto the conveyor by any of these methods. In some embodiments the material is delivered to the conveyor using an enclosed material distribution system to help maintain a low oxygen atmosphere and/or control dust and fines. Lofted or air suspended biomass fines and dust are undesirable because these can form an explosion hazard or damage the window foils of an electron gun (if such a device is used for treating the material).

[0052] The material can be leveled to form a uniform thickness between about 0.0312 and 5 inches (*e.g.*, between about 0.0625 and 2.000 inches, between about 0.125 and 1 inches, between about 0.125 and 0.5 inches, between about 0.3 and 0.9 inches, between about 0.2 and 0.5 inches between about 0.25 and 1.0 inches, between about 0.25 and 0.5 inches).

[0053] In some cases, the mechanical treatment may include an initial preparation of the feedstock as received, *e.g.*, size reduction of materials, such as by comminution, *e.g.*, cutting, grinding, shearing, pulverizing or chopping. For example, in some cases, loose

feedstock (*e.g.*, recycled paper, starchy materials, or switchgrass) is prepared by shearing or shredding. Mechanical treatment may reduce the bulk density of the carbohydrate-containing material, increase the surface area of the carbohydrate-containing material and/or decrease one or more dimensions of the carbohydrate-containing material.

[0054] Alternatively, or in addition, the feedstock material can be treated with another treatment, for example chemical treatments, such as with an acid (HCl, H₂SO₄, H₃PO₄), a base (*e.g.*, KOH and NaOH), a chemical oxidant (*e.g.*, peroxides, chlorates, ozone), irradiation, steam explosion, pyrolysis, sonication, oxidation, chemical treatment. The treatments can be in any order and in any sequence and combinations. For example, the feedstock material can first be physically treated by one or more treatment methods, *e.g.*, chemical treatment including and in combination with acid hydrolysis (*e.g.*, utilizing HCl, H₂SO₄, H₃PO₄), radiation, sonication, oxidation, pyrolysis or steam explosion, and then mechanically treated. This sequence can be advantageous since materials treated by one or more of the other treatments, *e.g.*, irradiation or pyrolysis, tend to be more brittle and, therefore, it may be easier to further change the structure of the material by mechanical treatment. As another example, a feedstock material can be conveyed through ionizing radiation using a conveyor as described herein and then mechanically treated. Chemical treatment can remove some or all of the lignin (for example chemical pulping) and can partially or completely hydrolyze the material. The methods also can be used with pre-hydrolyzed material. The methods also can be used with material that has not been pre hydrolyzed. The methods can be used with mixtures of hydrolyzed and non-hydrolyzed materials, for example, with about 50% or more non-hydrolyzed material, with about 60% or more non- hydrolyzed material, with about 70% or more non-hydrolyzed material, with about 80% or more non-hydrolyzed material or even with 90% or more non-hydrolyzed material.

[0055] In addition to size reduction, which can be performed initially and/or later in processing, mechanical treatment can also be advantageous for “opening up,” “stressing,” breaking or shattering the carbohydrate-containing materials, making the cellulose of the materials more susceptible to chain scission and/or disruption of crystalline structure during the physical treatment.

[0056] Methods of mechanically treating the carbohydrate-containing material include, for example, milling or grinding. Milling may be performed using, for example, a hammer mill, ball mill, colloid mill, conical or cone mill, disk mill, edge mill, Wiley mill, grist mill or other mill. Grinding may be performed using, for example, a cutting/impact type grinder. Some exemplary grinders include stone grinders, pin

grinders, coffee grinders, and burr grinders. Grinding or milling may be provided, for example, by a reciprocating pin or other element, as is the case in a pin mill. Other mechanical treatment methods include mechanical ripping or tearing, other methods that apply pressure to the fibers, and air attrition milling. Suitable mechanical treatments further include any other technique that continues the disruption of the internal structure of the material that was initiated by the previous processing steps.

[0057] Mechanical feed preparation systems can be configured to produce streams with specific characteristics such as, for example, specific maximum sizes, specific length-to-width, or specific surface areas ratios. Physical preparation can increase the rate of reactions, improve the movement of material on a conveyor, improve the irradiation profile of the material, improve the radiation uniformity of the material, or reduce the processing time required by opening up the materials and making them more accessible to processes and/or reagents, such as reagents in a solution.

[0058] The bulk density of feedstocks can be controlled (*e.g.*, increased). In some situations, it can be desirable to prepare a low bulk density material, *e.g.*, by densifying the material (*e.g.*, densification can make it easier and less costly to transport to another site) and then reverting the material to a lower bulk density state (*e.g.*, after transport). The material can be densified, for example, from less than about 0.2 g/cc to more than about 0.9 g/cc (*e.g.*, less than about 0.3 to more than about 0.5 g/cc, less than about 0.3 to more than about 0.9 g/cc, less than about 0.5 to more than about 0.9 g/cc, less than about 0.3 to more than about 0.8 g/cc, less than about 0.2 to more than about 0.5 g/cc). For example, the material can be densified by the methods and equipment disclosed in U.S. Pat. No. 7,932,065 to Medoff and International Publication No. WO 2008/073186 (which was filed October 26, 2007, was published in English, and which designated the United States), the full disclosures of which are incorporated herein by reference. Densified materials can be processed by any of the methods described herein, or any material processed by any of the methods described herein can be subsequently densified.

[0059] 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.

[0060] For example, a fiber source, *e.g.*, that is recalcitrant or that has had its recalcitrance level reduced, can be sheared, *e.g.*, in a rotary knife cutter, to provide a first fibrous material. The first fibrous material is passed through a first screen, *e.g.*, having an average opening size of 1.59 mm or less (1/16 inch, 0.0625 inch), provide a second

fibrous material. If desired, the 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.

[0061] In some embodiments, the shearing of the fiber source and the passing of the resulting first fibrous material through a first screen are performed concurrently. The shearing and the passing can also be performed in a batch-type process.

[0062] For example, a rotary knife cutter can be used to concurrently shear the fiber source and screen the first fibrous material. A rotary knife cutter includes a hopper that can be loaded with a shredded fiber source prepared by shredding a fiber source.

[0063] In some implementations, the feedstock is physically treated prior to saccharification and/or fermentation. 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 biomass feedstock may also be used, alone or in combination with the processes disclosed herein.

[0064] Mechanical treatments that may be used, and the characteristics of the mechanically treated carbohydrate-containing materials, are described in further detail in U.S. Pat. App. Pub. 2012/0100577 A1, filed October 18, 2011, the full disclosure of which is hereby incorporated herein by reference.

RADIATION TREATMENT

[0065] The feedstock can be treated with radiation to modify its structure to reduce its recalcitrance. Such treatment can, for example, reduce the average molecular weight of the feedstock, change the crystalline structure of the feedstock, and/or increase the surface area and/or porosity of the feedstock. Radiation can be by, for example electron beam, ion beam, 100 nm to 28 nm ultraviolet (UV) light, gamma or X-ray radiation. Radiation treatments and systems for treatments are discussed in US. Patent 8,142,620

and US. Patent Application Series No. 12/417, 731, the entire disclosures of which are incorporated herein by reference.

[0066] 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. Electrons interact via Coulomb scattering and bremsstrahlung radiation produced by changes in the velocity of electrons.

[0067] 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 to change the molecular structure of the carbohydrate containing material, 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 atomic units.

[0068] Gamma radiation has the advantage of a significant penetration depth into a variety of material in the sample.

[0069] 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 102 eV, e.g., greater than 103, 104, 105, 106, or even greater than 107 eV. In some embodiments, the electromagnetic radiation has energy per photon of between 104 and 107, e.g., between 105 and 106 eV. The electromagnetic radiation can have a frequency of, e.g., greater than 1016 Hz, greater than 1017 Hz, 1018, 1019, 1020, or even greater than 1021 Hz. In some embodiments, the electromagnetic radiation has a frequency of between 1018 and 1022 Hz, e.g., between 1019 to 1021 Hz.

[0070] Electron bombardment may be performed using an electron beam device that has a nominal energy of less than 10 MeV, e.g., less than 7 MeV, less than 5 MeV, or less than 2 MeV, e.g., from about 0.5 to 1.5 MeV, from about 0.8 to 1.8 MeV, or from

about 0.7 to 1 MeV. In some implementations the nominal energy is about 500 to 800 keV.

[0071] The electron beam may have a relatively high total beam power (the combined beam power of all accelerating heads, or, if multiple accelerators are used, of all accelerators and all heads), e.g., at least 25 kW, e.g., at least 30, 40, 50, 60, 65, 70, 80, 100, 125, or 150 kW. In some cases, the power is even as high as 500 kW, 750 kW, or even 1000 kW or more. In some cases the electron beam has a beam power of 1200 kW or more, e.g., 1400, 1600, 1800, or even 3000 kW.

[0072] This high total beam power is usually achieved by utilizing multiple accelerating heads. For example, the electron beam device may include two, four, or more accelerating heads. The use of multiple heads, each of which has a relatively low beam power, prevents excessive temperature rise in the material, thereby preventing burning of the material, and also increases the uniformity of the dose through the thickness of the layer of material.

[0073] It is generally preferred that the bed of biomass material has a relatively uniform thickness. In some embodiments the thickness is less than about 1 inch (e.g., less than about 0.75 inches, less than about 0.5 inches, less than about 0.25 inches, less than about 0.1 inches, between about 0.1 and 1 inch, between about 0.2 and 0.3 inches).

[0074] It is desirable to treat the material as quickly as possible. In general, it is preferred that treatment be performed at a dose rate of greater than about 0.25 Mrad per second, e.g., greater than about 0.5, 0.75, 1, 1.5, 2, 5, 7, 10, 12, 15, or even greater than about 20 Mrad per second, e.g., about 0.25 to 2 Mrad per second. Higher dose rates allow a higher throughput for a target (e.g., the desired) dose. Higher dose rates generally require higher line speeds, to avoid thermal decomposition of the material. In one implementation, the accelerator is set for 3 MeV, 50 mA beam current, and the line speed is 24 feet/minute, for a sample thickness of about 20 mm (e.g., comminuted corn cob material with a bulk density of 0.5 g/cm³).

[0075] In some embodiments, electron bombardment is performed until the material receives a total dose of at least 0.1 Mrad, 0.25 Mrad, 1 Mrad, 5 Mrad, e.g., at least 10, 20, 30 or at least 40 Mrad. In some embodiments, the treatment is performed until the material receives a dose of from about 10 Mrad to about 50 Mrad, e.g., from about 20 Mrad to about 40 Mrad, or from about 25 Mrad to about 30 Mrad. In some implementations, a total dose of 25 to 35 Mrad is preferred, applied ideally over a couple of passes, e.g., at 5 Mrad/pass with each pass being applied for about one second.

Cooling methods, systems and equipment can be utilized before, after, during and/or between irradiations (e.g., cooled screw conveyors and cooled vibratory conveyors).

[0076] Using multiple heads as discussed above, the material can be treated in multiple passes, for example, two passes at 10 to 20 Mrad/pass, e.g., 12 to 18 Mrad/pass, separated by a few seconds of cool-down, or three passes of 7 to 12 Mrad/pass, e.g., 5 to 20 Mrad/pass, 10 to 40 Mrad/pass, 9 to 11 Mrad/pass. As discussed herein, treating the material with several relatively low doses, rather than one high dose, tends to prevent overheating of the material and also increases dose uniformity through the thickness of the material. In some implementations, the material is stirred or otherwise mixed during or after each pass and then smoothed into a uniform layer again before the next pass, to further enhance treatment uniformity.

[0077] In some embodiments, electrons are accelerated to, for example, a speed of greater than 75 percent of the speed of light, e.g., greater than 85, 90, 95, or 99 percent of the speed of light.

[0078] In some embodiments, any processing described herein occurs on lignocellulosic material that remains dry as acquired or that 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 25 wt. % retained water, measured at 25 °C and at fifty percent relative humidity (e.g., less than about 20 wt.%, less than about 15 wt.%, less than about 14 wt.%, less than about 13 wt.%, less than about 12 wt.%, less than about 10 wt.%, less than about 9 wt.%, less than about 8 wt.%, less than about 7 wt.%, less than about 6 wt.%, less than about 5 wt.%, less than about 4 wt.%, less than about 3 wt.%, less than about 2 wt.%, less than about 1 wt.%, or less than about 0.5 wt.%).

[0079] In some embodiments, two or more ionizing sources can be used, such as two or more electron sources. 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. The biomass is conveyed through the treatment zone where it can be bombarded with electrons.

[0080] It may be advantageous to repeat the treatment to more thoroughly reduce the recalcitrance of the biomass and/or further modify the biomass. In particular the process parameters can be adjusted after a first (e.g., second, third, fourth or more) pass depending on the recalcitrance of the material. In some embodiments, a conveyor can be

used which includes a circular system where the biomass is conveyed multiple times through the various processes described above. In some other embodiments multiple treatment devices (e.g., electron beam generators) are used to treat the biomass multiple (e.g., 2, 3, 4 or more) times. In yet other embodiments, a single electron beam generator may be the source of multiple beams (e.g., 2, 3, 4 or more beams) that can be used for treatment of the biomass.

[0081] The effectiveness in changing the molecular/supramolecular structure and/or reducing the recalcitrance of the carbohydrate-containing biomass depends on the electron energy used and the dose applied, while exposure time depends on the power and dose. In some embodiments, the dose rate and total dose are adjusted so as not to destroy (e.g., char or burn) the biomass material. For example, the carbohydrates should not be damaged in the processing so that they can be released from the biomass intact, e.g. as monomeric sugars.

[0082] In some embodiments, the treatment (with any electron source or a combination of sources) is performed until the material receives a dose of at least about 0.05 Mrad, e.g., at least about 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5.0, 7.5, 10.0, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, or 200 Mrad. In some embodiments, the treatment is performed until the material receives a dose of between 0.1-100 Mrad, 1-200, 5-200, 10-200, 5-150, 50-150 Mrad, 5-100, 5-50, 5-40, 10-50, 10-75, 15-50, 20-35 Mrad.

[0083] In some embodiments, relatively low doses of radiation are utilized, e.g., to increase the molecular weight of a cellulosic or lignocellulosic material (with any radiation source or a combination of sources described herein). For example, a dose of at least about 0.05 Mrad, e.g., at least about 0.1 Mrad or at least about 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, or at least about 5.0 Mrad. In some embodiments, the irradiation is performed until the material receives a dose of between 0.1Mrad and 2.0 Mrad, e.g., between 0.5Mrad and 4.0 Mrad or between 1.0 Mrad and 3.0 Mrad. It also can be desirable to irradiate from multiple directions, simultaneously or sequentially, in order to achieve a desired degree of penetration of radiation into the material. For example, depending on the density and moisture content of the material, such as wood, and the type of radiation source used (e.g., gamma or electron beam), the maximum penetration of radiation into the material may be only about 0.75 inch. In such instances, a thicker section (up to 1.5 inch) can be irradiated by first irradiating the material from one side, and then turning the material over and irradiating from the other side. Irradiation from multiple directions can be particularly useful with electron beam

radiation, which irradiates faster than gamma radiation but typically does not achieve as great a penetration depth.

RADIATION OPAQUE MATERIALS

[0084] The invention can include processing the material (e.g., for some of the processing steps) in a vault and/or bunker that is constructed using radiation opaque materials. In some implementations, the radiation opaque materials are selected to be capable of shielding the components from X-rays with high energy (short wavelength), which can penetrate many materials. One important factor in designing a radiation shielding enclosure is the attenuation length of the materials used, which will determine the required thickness for a particular material, blend of materials, or layered structure. The attenuation length is the penetration distance at which the radiation is reduced to approximately $1/e$ (e = Euler's number) times that of the incident radiation. Although virtually all materials are radiation opaque if thick enough, materials containing a high compositional percentage (e.g., density) of elements that have a high Z value (atomic number) have a shorter radiation attenuation length and thus if such materials are used a thinner, lighter shielding can be provided. Examples of high Z value materials that are used in radiation shielding are tantalum and lead. Another important parameter in radiation shielding is the halving distance, which is the thickness of a particular material that will reduce gamma ray intensity by 50%. As an example for X-ray radiation with an energy of 0.1 MeV the halving thickness is about 15.1 mm for concrete and about 2.7 mm for lead, while with an X-ray energy of 1 MeV the halving thickness for concrete is about 44.45 mm and for lead is about 7.9 mm. Radiation opaque materials can be materials that are thick or thin so long as they can reduce the radiation that passes through to the other side. Thus, if it is desired that a particular enclosure have a low wall thickness, e.g., for light weight or due to size constraints, the material chosen should have a sufficient Z value and/or attenuation length so that its halving length is less than or equal to the desired wall thickness of the enclosure.

[0085] In some cases, the radiation opaque material may be a layered material, for example having a layer of a higher Z value material, to provide good shielding, and a layer of a lower Z value material to provide other properties (e.g., structural integrity, impact resistance, etc.). In some cases, the layered material may be a "graded- Z " laminate, e.g., including a laminate in which the layers provide a gradient from high- Z through successively lower- Z elements. In some cases the radiation opaque materials can be interlocking blocks, for example, lead and/or concrete blocks can be supplied by NELCO Worldwide (Burlington, MA), and reconfigurable vaults can be utilized.

[0086] A radiation opaque material can reduce the radiation passing through a structure (e.g., a wall, door, ceiling, enclosure, a series of these or combinations of these) formed of the material by about at least about 10 %, (e.g., at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, at least about 99.9%, at least about 99.99%, at least about 99.999%) as compared to the incident radiation. Therefore, an enclosure made of a radiation opaque material can reduce the exposure of equipment/system/components by the same amount. Radiation opaque materials can include stainless steel, metals with Z values above 25 (e.g., lead, iron), concrete, dirt, and combinations thereof. Radiation opaque materials can include a barrier in the direction of the incident radiation of at least about 1mm (e.g., 5 mm, 10mm, 5 cm, 10 cm, 100cm, 1m or even about 10 m).

RADIATION SOURCES

[0087] The type of radiation determines the kinds of radiation sources used as well as the radiation devices and associated equipment. The methods, systems and equipment described herein, for example for treating materials with radiation, can utilized sources as described herein as well as any other useful source.

[0088] Sources of gamma rays include radioactive nuclei, such as isotopes of cobalt, calcium, technetium, chromium, gallium, indium, iodine, iron, krypton, samarium, selenium, sodium, thallium, and xenon.

[0089] 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.

[0090] 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.

[0091] Sources for ultraviolet radiation include deuterium or cadmium lamps.

[0092] Sources for infrared radiation include sapphire, zinc, or selenide window ceramic lamps.

[0093] Sources for microwaves include klystrons, Slevin type RF sources, or atom beam sources that employ hydrogen, oxygen, or nitrogen gases.

[0094] Accelerators used to accelerate the particles (e.g., electrons or ions) can be DC (e.g., electrostatic DC or electrodynamic DC), RF linear, magnetic induction linear

or continuous wave. For example, various 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, Cockroft Walton accelerators (e.g., PELLETRON® accelerators), LINACS, Dynamitrons (e.g., DYNAMITRON® accelerators), cyclotrons, synchrotrons, betatrons, transformer-type accelerators, microtrons, plasma generators, cascade accelerators, and folded tandem accelerators. 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®. Other suitable accelerator systems include, for example: DC insulated core transformer (ICT) type systems, available from Nissin High Voltage, Japan; S-band LINACs, available from L3-PSD (USA), Linac Systems (France), Mevex (Canada), and Mitsubishi Heavy Industries (Japan); L-band LINACs, available from Iotron Industries (Canada); and ILU-based accelerators, available from Budker Laboratories (Russia). 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. Some particle accelerators and their uses are disclosed, for example, in U.S. Pat. No. 7,931,784 to Medoff, the complete disclosure of which is incorporated herein by reference.

[0095] 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 and accelerated through an accelerating potential. An electron gun generates electrons, which are then accelerated through a large potential (e.g., greater than about 500 thousand, greater than about 1million, greater than about 2 million, greater than about 5 million, greater than about 6 million, greater than about 7 million, greater than about 8 million, greater than about 9 million, or even greater than 10 million volts) and then scanned magnetically in the x-y plane, where the electrons are initially accelerated in the z direction down the accelerator tube and extracted through a foil window. Scanning the electron beams is useful for increasing the irradiation surface when irradiating materials, e.g., a biomass,

that is conveyed through the scanned beam. Scanning the electron beam also distributes the thermal load homogenously on the window and helps reduce the foil window rupture due to local heating by the electron beam. Window foil rupture is a cause of significant down-time due to subsequent necessary repairs and re-starting the electron gun.

[0096] 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. Pat. No. 7,931,784 to Medoff, the complete disclosure of which is incorporated herein by reference.

[0097] A beam of electrons can be 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. Electron beams can also have high electrical efficiency (e.g., 80%), allowing for lower energy usage relative to other radiation methods, which can translate into a lower cost of operation and lower greenhouse gas emissions corresponding to the smaller amount of energy used. 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.

[0098] Electrons can also be more efficient at causing changes in the molecular structure of carbohydrate-containing materials, for example, by the mechanism of chain scission. In addition, electrons having energies of 0.5-10 MeV can penetrate low density materials, such as the biomass materials described herein, e.g., materials having a bulk density of less than 0.5 g/cm³, and a depth of 0.3-10 cm. Electrons as an ionizing radiation source can be useful, e.g., for relatively thin piles, layers or beds of materials, e.g., less than about 0.5 inch, e.g., less than about 0.4 inch, 0.3 inch, 0.25 inch, or less than about 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. Methods of irradiating materials are discussed in U.S. Pat. App. Pub. 2012/0100577 A1, filed October 18, 2011, the entire disclosure of which is herein incorporated by reference.

[0099] 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. Typically, generators

are housed in a vault, e.g., of lead or concrete, especially for production from X-rays that are generated in the process. Tradeoffs in considering electron energies include energy costs.

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, 1 m, 2 m or more are available. The scanning beam is preferred in most embodiments described herein because of the larger scan width and reduced possibility of local heating and failure of the windows.

ELECTRON GUNS - WINDOWS

[00100] The extraction system for an electron accelerator can include two window foils. The cooling gas in the two foil window extraction system can be a purge gas or a mixture, for example air, or a pure gas. In one embodiment the gas is an inert gas such as nitrogen, argon, helium and or carbon dioxide. It is preferred to use a gas rather than a liquid since energy losses to the electron beam are minimized. Mixtures of pure gas can also be used, either pre-mixed or mixed in line prior to impinging on the windows or in the space between the windows. The cooling gas can be cooled, for example, by using a heat exchange system (e.g., a chiller) and/or by using boil off from a condensed gas (e.g., liquid nitrogen, liquid helium). Window foils are described in PCT/US2013/64332 filed October 10, 2013 the full disclosure of which is incorporated by reference herein

[00101] When using an enclosure, the enclosed conveyor can also be purged with an inert gas so as to maintain an atmosphere at a reduced oxygen level. Keeping oxygen levels low avoids the formation of ozone which in some instances is undesirable due to its reactive and toxic nature. For example the oxygen can be less than about 20% (e.g., less than about 10%, less than about 1%, less than about 0.1%, less than about 0.01%, or even less than about 0.001% oxygen). Purging can be done with an inert gas including, but not limited to, nitrogen, argon, helium or carbon dioxide. This can be supplied, for example, from a boil off of a liquid source (e.g., liquid nitrogen or helium), generated or separated from air *in situ*, or supplied from tanks. The inert gas can be recirculated and any residual oxygen can be removed using a catalyst, such as a copper catalyst bed. Alternatively, combinations of purging, recirculating and oxygen removal can be done to keep the oxygen levels low.

[00102] The enclosure can also be purged with a reactive gas that can react with the biomass. This can be done before, during or after the irradiation process. The reactive

gas can be, but is not limited to, nitrous oxide, ammonia, oxygen, ozone, hydrocarbons, aromatic compounds, amides, peroxides, azides, halides, oxyhalides, phosphides, phosphines, arsines, sulfides, thiols, boranes and/or hydrides. The reactive gas can be activated in the enclosure, *e.g.*, by irradiation (*e.g.*, electron beam, UV irradiation, microwave irradiation, heating, IR radiation), so that it reacts with the biomass. The biomass itself can be activated, for example by irradiation. Preferably the biomass is activated by the electron beam, to produce radicals which then react with the activated or unactivated reactive gas, *e.g.*, by radical coupling or quenching.

[00103] Purging gases supplied to an enclosed conveyor can also be cooled, for example below about 25°C, below about 0°C, below about -40°C, below about -80°C, below about -120°C. For example, the gas can be boiled off from a compressed gas such as liquid nitrogen or sublimed from solid carbon dioxide. As an alternative example, the gas can be cooled by a chiller or part of or the entire conveyor can be cooled.

HEATING AND THROUGHPUT DURING RADIATION TREATMENT

[00104] Several processes can occur in biomass when electrons from an electron beam interact with matter in inelastic collisions. For example, ionization of the material, chain scission of polymers in the material, cross linking of polymers in the material, oxidation of the material, generation of X-rays (“Bremsstrahlung”) and vibrational excitation of molecules (*e.g.* phonon generation). Without being bound to a particular mechanism, the reduction in recalcitrance can be due to several of these inelastic collision effects, for example ionization, chain scission of polymers, oxidation and phonon generation. Some of the effects (*e.g.*, especially X-ray generation), necessitate shielding and engineering barriers, for example, enclosing the irradiation processes in a concrete (or other radiation opaque material) vault. Another effect of irradiation, vibrational excitation, is equivalent to heating up the sample. Heating the sample by irradiation can help in recalcitrance reduction, but excessive heating can destroy the material, as will be explained below.

[00105] The adiabatic temperature rise (ΔT) from adsorption of ionizing radiation is given by the equation: $\Delta T = D/C_p$: where D is the average dose in kGy, C_p is the heat capacity in J/g °C, and ΔT is the change in temperature in °C. A typical dry biomass material will have a heat capacity close to 2. Wet biomass will have a higher heat capacity dependent on the amount of water since the heat capacity of water is very high (4.19 J/g °C). Metals have much lower heat capacities, for example 304 stainless steel has a heat capacity of 0.5 J/g °C. The temperature change due to the instant adsorption of

radiation in a biomass and stainless steel for various doses of radiation is shown in Table 1.

[00106]

Table 1: Calculated Temperature increase for biomass and stainless steel.

Dose (Mrad)	Estimated Biomass ΔT ($^{\circ}\text{C}$)	Steel ΔT ($^{\circ}\text{C}$)
10	50	200
50	250, Decomposition	1000
100	500, Decomposition	2000
150	750, Decomposition	3000
200	1000, Decomposition	4000

[00107] High temperatures can destroy and or modify the biopolymers in biomass so that the polymers (e.g., cellulose) are unsuitable for further processing. A biomass subjected to high temperatures can become dark, sticky and give off odors indicating decomposition. The stickiness can even make the material hard to convey. The odors can be unpleasant and be a safety issue. In fact, keeping the biomass below about 200°C has been found to be beneficial in the processes described herein (e.g., below about 190°C , below about 180°C , below about 170°C , below about 160°C , below about 150°C , below about 140°C , below about 130°C , below about 120°C , below about 110°C , between about 60°C and 180°C , between about 60°C and 160°C , between about 60°C and 150°C , between about 60°C and 140°C , between about 60°C and 130°C , between about 60°C and 120°C , between about 80°C and 180°C , between about 100°C and 180°C , between about 120°C and 180°C , between about 140°C and 180°C , between about 160°C and 180°C , between about 100°C and 140°C , between about 80°C and 120°C).

[00108] It has been found that irradiation above about 10 Mrad is desirable for the processes described herein (e.g., reduction of recalcitrance). A high throughput is also desirable so that the irradiation does not become a bottle neck in processing the biomass. The treatment is governed by a dose rate equation: $M = FP/D * \text{time}$, where M is the mass of irradiated material (Kg), F is the fraction of power that is adsorbed (unit less), P is the emitted power ($\text{KW} = \text{Voltage in MeV} * \text{Current in mA}$), time is the treatment time (sec) and D is the adsorbed dose (KGy). In an exemplary process where the fraction of adsorbed power is fixed, the Power emitted is constant and a set dosage is desired, the throughput (e.g., M, the biomass processed) can be increased by increasing the irradiation time. However, increasing the irradiation time without allowing the material

to cool, can excessively heat the material as exemplified by the calculations shown above. Since biomass has a low thermal conductivity (less than about $0.1 \text{ Wm}^{-1}\text{K}^{-1}$), heat dissipation is slow, unlike, for example metals (greater than about $10 \text{ Wm}^{-1}\text{K}^{-1}$) which can dissipate energy quickly as long as there is a heat sink to transfer the energy to.

ELECTRON GUNS – BEAM STOPS

[00109] In some embodiments the systems and methods include a beam stop (*e.g.*, a shutter). For example, the beam stop can be used to quickly stop or reduce the irradiation of material without powering down the electron beam device. Alternatively the beam stop can be used while powering up the electron beam, *e.g.*, the beam stop can stop the electron beam until a beam current of a desired level is achieved. The beam stop can be placed between the primary foil window and a secondary foil window. For example the beam stop can be mounted so that it is movable, that is, so that it can be moved into and out of the beam path. Even partial coverage of the beam can be used, for example, to control the dose of irradiation. The beam stop can be mounted to the floor, to a conveyor for the biomass, to a wall, to the radiation device (*e.g.*, at the scan horn), or to any structural support. Preferably the beam stop is fixed in relation to the scan horn so that the beam can be effectively controlled by the beam stop. The beam stop can incorporate a hinge, a rail, wheels, slots, or other means allowing for its operation in moving into and out of the beam. The beam stop can be made of any material that will stop at least 5% of the electrons, *e.g.*, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or even about 100% of the electrons.

[00110] The beam stop can be made of a metal including, but not limited to, stainless steel, lead, iron, molybdenum, silver, gold, titanium, aluminum, tin, or alloys of these, or laminates (layered materials) made with such metals (*e.g.*, metal-coated ceramic, metal-coated polymer, metal-coated composite, multilayered metal materials).

[00111] The beam stop can be cooled, for example, with a cooling fluid such as an aqueous solution or a gas. The beam stop can be partially or completely hollow, for example with cavities. Interior spaces of the beam stop can be used for cooling fluids and gases. The beam stop can be of any shape, including flat, curved, round, oval, square, rectangular, beveled and wedged shapes.

[00112] The beam stop can have perforations so as to allow some electrons through, thus controlling (*e.g.*, reducing) the levels of radiation across the whole area of the window, or in specific regions of the window. The beam stop can be a mesh formed, for

example, from fibers or wires. Multiple beam stops can be used, together or independently, to control the irradiation. The beam stop can be remotely controlled, *e.g.*, by radio signal or hard wired to a motor for moving the beam into or out of position.

SONICATION, PYROLYSIS, OXIDATION, STEAM EXPLOSION

[00113] If desired, one or more sonication, pyrolysis, oxidative, or steam explosion processes can be used instead of or in addition to irradiation to reduce or further reduce the recalcitrance of the carbohydrate-containing material. For example, these processes can be applied before, during and or after irradiation. These processes are described in detail in U.S. Pat. No. 7,932,065 to Medoff, the full disclosure of which is incorporated herein by reference.

USE OF TREATED BIOMASS MATERIAL

[00114] Using the methods described herein, a starting biomass material (*e.g.*, plant biomass, animal biomass, paper, and municipal waste biomass) can be used as feedstock to produce useful intermediates and products such as organic acids, salts of organic acids, anhydrides, esters of organic acids and fuels, *e.g.*, fuels for internal combustion engines or feedstocks for fuel cells. Systems and processes are described herein that can use as feedstock cellulosic and/or lignocellulosic materials that are readily available, but often can be difficult to process, *e.g.*, municipal waste streams and waste paper streams, such as streams that include newspaper, kraft paper, corrugated paper or mixtures of these.

[00115] In order to convert the feedstock to a form that can be readily processed, the glucan- or xylan-containing cellulose in the feedstock can be hydrolyzed to low molecular weight carbohydrates, such as sugars, by a saccharifying agent, *e.g.*, an enzyme or acid, a process referred to as saccharification. The low molecular weight carbohydrates can then be used, for example, in an existing manufacturing plant, such as a single cell protein plant, an enzyme manufacturing plant, or a fuel plant, *e.g.*, an ethanol manufacturing facility.

[00116] The feedstock can be hydrolyzed using an enzyme, *e.g.*, by combining the materials and the enzyme in a solvent, *e.g.*, in an aqueous solution.

[00117] Alternatively, the enzymes can be supplied by organisms that break down biomass, such as the cellulose and/or the lignin portions of the biomass, contain or manufacture various cellulolytic enzymes (cellulases), ligninases or various small

molecule biomass-degrading metabolites. These enzymes may be a complex of enzymes that act synergistically to degrade crystalline cellulose or the lignin portions of biomass. Examples of cellulolytic enzymes include: endoglucanases, cellobiohydrolases, and cellobiases (beta-glucosidases).

[00118] During saccharification a cellulosic substrate can be initially hydrolyzed by endoglucanases at random locations producing oligomeric intermediates. These intermediates are then substrates for exo-splitting glucanases such as cellobiohydrolase to produce cellobiose from the ends of the cellulose polymer. Cellobiose is a water-soluble 1,4-linked dimer of glucose. Finally, cellobiase cleaves cellobiose to yield glucose. The efficiency (*e.g.*, time to hydrolyze and/or completeness of hydrolysis) of this process depends on the recalcitrance of the cellulosic material.

INTERMEDIATES AND PRODUCTS

[00119] Using the processes described herein, the biomass material can be converted to one or more products, such as energy, fuels, foods and materials. Specific examples of products include, but are not limited to, hydrogen, sugars (*e.g.*, glucose, xylose, arabinose, mannose, galactose, fructose, disaccharides, oligosaccharides and polysaccharides), alcohols (*e.g.*, monohydric alcohols or dihydric alcohols, such as ethanol, n-propanol, isobutanol, *sec*-butanol, tert-butanol or n-butanol), hydrated or hydrous alcohols (*e.g.*, containing greater than 10%, 20%, 30% or even greater than 40% water), biodiesel, organic acids (see below), hydrocarbons (*e.g.*, methane, ethane, propane, isobutene, pentane, n-hexane, biodiesel, bio-gasoline and mixtures thereof), co-products (*e.g.*, proteins, such as cellulolytic proteins (enzymes) or single cell proteins), and mixtures of any of these in any combination or relative concentration, and optionally in combination with any additives (*e.g.*, fuel additives). Other examples include carboxylic acids, salts of a carboxylic acid, a mixture of carboxylic acids and salts of carboxylic acids and esters of carboxylic acids (*e.g.*, methyl, ethyl and n-propyl esters), ketones (*e.g.*, acetone), aldehydes (*e.g.*, acetaldehyde), alpha and beta unsaturated acids (*e.g.*, acrylic acid) and olefins (*e.g.*, ethylene). Other alcohols and alcohol derivatives include propanol, propylene glycol, 1,4-butanediol, 1,3-propanediol, sugar alcohols (*e.g.*, erythritol, glycol, glycerol, sorbitol, threitol, arabitol, ribitol, mannitol, dulcitol, fucitol, iditol, isomalt, maltitol, lactitol, xylitol and other polyols), and methyl or ethyl esters of any of these alcohols. Other products include methyl acrylate, methyl methacrylate, lactic acid, citric acid, formic acid, acetic acid, propionic acid, lactic acid, tartaric acid, butyric acid, succinic acid, valeric acid, caproic acid, 3-hydroxypropionic acid, palmitic

acid, stearic acid, oxalic acid, malonic acid, glutaric acid, oleic acid, linoleic acid, glycolic acid, gamma-hydroxybutyric acid, and mixtures thereof, salts of any of these acids, mixtures of any of the acids and their respective salts. These acids include isomers of the acids and where stereochemical isomers are possible are also included (e. g. D- and L-lactic acid, D-, L-, and meso tartaric acid

[00120] Any combination of the above products with each other, and/or of the above products with other products, which other products may be made by the processes described herein or otherwise, may be packaged together and sold as products. The products may be combined, *e.g.*, mixed, blended or co-dissolved, or may simply be packaged or sold together.

[00121] Any of the products or combinations of products described herein may be sanitized or sterilized prior to selling the products, *e.g.*, after purification or isolation or even after packaging, to neutralize one or more potentially undesirable contaminants that could be present in the product(s). Such sanitation can be done with electron bombardment, for example, be at a dosage of less than about 20 Mrad, *e.g.*, from about 0.1 to 15 Mrad, from about 0.5 to 7 Mrad, or from about 1 to 3 Mrad.

[00122] The processes described herein can produce various by-product streams useful for generating steam and electricity to be used in other parts of the plant (co-generation) or sold on the open market. For example, steam generated from burning by-product streams can be used in a distillation process. As another example, electricity generated from burning by-product streams can be used to power electron beam generators used in pretreatment.

[00123] The by-products used to generate steam and electricity are derived from a number of sources throughout the process. For example, anaerobic digestion of wastewater can produce a biogas high in methane and a small amount of waste biomass (sludge). As another example, post-saccharification and/or post-distillate solids (*e.g.*, unconverted lignin, cellulose, and hemicellulose remaining from the pretreatment and primary processes) can be used, *e.g.*, burned, as a fuel.

[00124] Other intermediates and products, including food and pharmaceutical products, are described in U.S. Pat. App. Pub. 2010/0124583 A1, published May 20, 2010, to Medoff, the full disclosure of which is hereby incorporated by reference herein.

BIOMASS PROCESSING AFTER IRRADIATION

[00125] After irradiation the biomass may be transferred to a vessel for saccharification. Alternately, the biomass can be heated after the biomass is irradiated

prior to the saccharification step. The heated means can be created, for example, by IR radiation, microwaves, combustion (e.g., gas, coal, oil, biomass), resistive heating and/or inductive coils. The heat can be applied from at least one side or more than one side, can be continuous or periodic and can be for only a portion of the material or all the material. The biomass may be heated to temperatures above 90°C in an aqueous liquid that may have an acid or a base present. For example, the aqueous biomass slurry may be heated to 90 to 150°C, alternatively, 105 to 145°C, optionally 110 to 140°C or further optionally from 115 to 135°C. The time that the aqueous biomass mixture is held at the peak temperature is 1 to 12 hours, alternately, 1 to 6 hours, optionally 1 to 4 hours at the peak temperature. In some instances, the aqueous biomass mixture is acidic, and the pH is between 1 and 5, optionally 1 to 4, or alternately, 2 to 3. In other instances, the aqueous biomass mixture is alkaline and the pH is between 6 and 13, alternately, 8 to 12, or optionally, 8 to 11.

SACCHARIFICATION

[00126] The treated biomass materials can be saccharified, generally by combining the material and a cellulase enzyme in a fluid or liquid medium, *e.g.*, an aqueous solution. In some cases, the material is boiled, steeped, or cooked in hot water prior to saccharification, as described in U.S. Pat. App. Pub. 2012/0100577 A1 by Medoff and Masterman, published on April 26, 2012, the entire contents of which are incorporated herein.

[00127] The saccharification process can be partially or completely performed in a tank (*e.g.*, a tank having a volume of at least 4000, 40,000, or 500,000 L) in a manufacturing plant, and/or can be partially or completely performed in transit, *e.g.*, in a rail car, tanker truck, or in a supertanker or the hold of a ship. The time required for complete saccharification will depend on the process conditions and the carbohydrate-containing material and enzyme used. If saccharification is performed in a manufacturing plant under controlled conditions, the cellulose may be substantially entirely converted to sugar, *e.g.*, glucose in about 12-96 hours. If saccharification is performed partially or completely in transit, saccharification may take longer.

[00128] It is generally preferred that the tank contents be mixed during saccharification, *e.g.*, using jet mixing as described in International App. No. PCT/US2010/035331, filed May 18, 2010, which was published in English as WO 2010/135380 and designated the United States, the full disclosure of which is incorporated by reference herein.

[00129] The addition of surfactants can enhance the rate of saccharification. Examples of surfactants include non-ionic surfactants, such as a Tween® 20 or Tween® 80 polyethylene glycol surfactants, ionic surfactants, or amphoteric surfactants.

[00130] It is generally preferred that the concentration of the sugar solution resulting from saccharification be relatively high, *e.g.*, greater than 40%, or greater than 50, 60, 70, 80, 90 or even greater than 95% by dry weight basis. Water may be removed, *e.g.*, by evaporation, to increase the concentration of the sugar solution. This reduces the volume to be shipped, and also inhibits microbial growth in the solution.

[00131] Alternatively, sugar solutions of lower concentrations may be used, in which case it may be desirable to add an antimicrobial additive, *e.g.*, a broad spectrum antibiotic, in a low concentration, *e.g.*, 50 to 150 ppm. Other suitable antibiotics include amphotericin B, ampicillin, chloramphenicol, ciprofloxacin, gentamicin, hygromycin B, kanamycin, neomycin, penicillin, puromycin, streptomycin. Antibiotics will inhibit growth of microorganisms during transport and storage, and can be used at appropriate concentrations, *e.g.*, between 15 and 1000 ppm by weight, *e.g.*, between 25 and 500 ppm, or between 50 and 150 ppm. If desired, an antibiotic can be included even if the sugar concentration is relatively high. Alternatively, other additives with anti-microbial or preservative properties may be used. Preferably the antimicrobial additive(s) are food-grade.

[00132] A relatively high concentration solution can be obtained by limiting the amount of water added to the carbohydrate-containing material with the enzyme. The concentration can be controlled, *e.g.*, by controlling how much saccharification takes place. For example, concentration can be increased by adding more carbohydrate-containing material to the solution. In order to keep the sugar that is being produced in solution, a surfactant can be added, *e.g.*, one of those discussed above. Solubility can also be increased by increasing the temperature of the solution. For example, the solution can be maintained at a temperature of 40-50°C, 60-80°C, or even higher.

SACCHARIFYING AGENTS

[00133] Suitable cellulolytic enzymes include cellulases from species in the genera *Bacillus*, *Coprinus*, *Myceliophthora*, *Cephalosporium*, *Scytalidium*, *Penicillium*, *Aspergillus*, *Pseudomonas*, *Humicola*, *Fusarium*, *Thielavia*, *Acremonium*, *Chrysosporium* and *Trichoderma*, especially those produced by a strain selected from the species *Aspergillus* (see, *e.g.*, EP Pub. No. 0 458 162), *Humicola insolens* (reclassified as *Scytalidium thermophilum*, see, *e.g.*, U.S. Pat. No. 4,435,307), *Coprinus cinereus*,

Fusarium oxysporum, *Myceliophthora thermophila*, *Meripilus giganteus*, *Thielavia terrestris*, *Acremonium* sp. (including, but not limited to, *A. persicinum*, *A. acremonium*, *A. brachyphenium*, *A. dichromosporum*, *A. obclavatum*, *A. pinkertoniae*, *A. roseogriseum*, *A. incoloratum*, and *A. furatum*). Preferred strains include *Humicola insolens* DSM 1800, *Fusarium oxysporum* DSM 2672, *Myceliophthora thermophila* CBS 117.65, *Cephalosporium* sp. RYM-202, *Acremonium* sp. CBS 478.94, *Acremonium* sp. CBS 265.95, *Acremonium persicinum* CBS 169.65, *Acremonium acremonium* AHU 9519, *Cephalosporium* sp. CBS 535.71, *Acremonium brachyphenium* CBS 866.73, *Acremonium dichromosporum* CBS 683.73, *Acremonium obclavatum* CBS 311.74, *Acremonium pinkertoniae* CBS 157.70, *Acremonium roseogriseum* CBS 134.56, *Acremonium incoloratum* CBS 146.62, and *Acremonium furatum* CBS 299.70H. Cellulolytic enzymes may also be obtained from *Chrysosporium*, preferably a strain of *Chrysosporium lucknowense*. Additional strains that can be used include, but are not limited to, *Trichoderma* (particularly *T. viride*, *T. reesei*, and *T. koningii*), alkalophilic *Bacillus* (see, for example, U.S. Pat. No. 3,844,890 and EP Pub. No. 0 458 162), and *Streptomyces* (see, e.g., EP Pub. No. 0 458 162).

[00134] In addition to or in combination to enzymes, acids, bases and other chemicals (e.g., oxidants) can be utilized to saccharify lignocellulosic and cellulosic materials. These can be used in any combination or sequence (e.g., before, after and/or during addition of an enzyme). For example strong mineral acids can be utilized (e.g. HCl, H₂SO₄, H₃PO₄) and strong bases (e.g., NaOH, KOH).

SUGARS

[00135] In the processes described herein, for example after saccharification, sugars (e.g., glucose and xylose) can be isolated. For example sugars can be isolated by precipitation, crystallization, chromatography (e.g., simulated moving bed chromatography, high pressure chromatography), centrifugation, extraction, any other isolation method known in the art, and combinations thereof.

FERMENTATION

[00136] Yeast and *Zymomonas* bacteria, for example, can be used for fermentation or conversion of sugar(s) to alcohol(s). Other microorganisms are discussed below. The optimum pH for fermentations is about pH 4 to 7. For example, 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 hours (e.g., 24 to 96 hrs.) with

temperatures in the range of 20°C to 40°C (*e.g.*, 26°C to 40°C), however thermophilic microorganisms prefer higher temperatures.

[00137] 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 N₂, Ar, He, CO₂ 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.

[00138] 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 sugar and carbohydrates in high concentrations. The sugars and carbohydrates can be isolated via any means known in the art. 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. Jet mixing may be used during fermentation, and in some cases saccharification and fermentation are performed in the same tank.

[00139] Nutrients for the microorganisms may be added during saccharification and/or fermentation, for example the food-based nutrient packages described in U.S. Pat. App. Pub. 2012/0052536, filed July 15, 2011, the complete disclosure of which is incorporated herein by reference. In some cases, the food-based nutrient source is selected from the group consisting of grains, vegetables, residues of grains, residues of vegetables, residues of meat (*e.g.*, stock, extract, bouillon or renderings), and mixtures thereof. For example, the nutrient source may be selected from the group consisting of wheat, oats, barley, soybeans, peas, legumes, potatoes, corn, rice bran, corn meal, wheat bran, meat product residues, and mixtures thereof.

[00140] “Fermentation” includes the methods and products that are disclosed in International App. No. PCT/US2012/071097 (which was filed December 20, 2012, was published in English as WO 2013/096700 and designated the United States) and International App. No. PCT/US2012/071083 (which was filed December 20, 2012, was published in English as WO 2013/096693 and designated the United States) the contents of both of which are incorporated by reference herein in their entirety.

[00141] Mobile fermenters can be utilized, as described in International App. No. PCT/US2007/074028 (which was filed July 20, 2007, was published in English as WO

2008/011598 and designated the United States) and has a US issued Patent No. 8,318,453, the contents of which are incorporated herein in its entirety. Similarly, the saccharification equipment can be mobile. Further, saccharification and/or fermentation may be performed in part or entirely during transit.

FERMENTATION AGENTS

[00142] The microorganism(s) used in fermentation can be naturally-occurring microorganisms and/or engineered microorganisms. For example, the microorganism can be a bacterium (including, but not limited to, *e.g.*, a cellulolytic bacterium), a fungus, (including, but not limited to, *e.g.*, a yeast), a plant, a protist, *e.g.*, a protozoa or a fungus-like protest (including, but not limited to, *e.g.*, a slime mold), or an alga. When the organisms are compatible, mixtures of organisms can be utilized.

[00143] Suitable fermenting microorganisms have the ability to convert carbohydrates, such as glucose, fructose, xylose, arabinose, mannose, galactose, oligosaccharides or polysaccharides into fermentation products. Fermenting microorganisms include strains of the genus *Sacchromyces spp.* (including, but not limited to, *S. cerevisiae* (baker's yeast), *S. distaticus*, *S. uvarum*), the genus *Kluyveromyces*, (including, but not limited to, *K. marxianus*, *K. fragilis*), the genus *Candida* (including, but not limited to, *C. pseudotropicalis*, and *C. brassicae*), *Pichia stipitis* (a relative of *Candida shehatae*), the genus *Clavispora* (including, but not limited to, *C. lusitaniae* and *C. opuntiae*), the genus *Pachysolen* (including, but not limited to, *P. tannophilus*), the genus *Bretannomyces* (including, but not limited to, *e.g.*, *B. 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)). Other suitable microorganisms include, for example, *Zymomonas mobilis*, *Clostridium spp.* (including, but not limited to, *C. thermocellum* (Philippidis, 1996, *supra*), *C. saccharobutylacetonicum*, *C. tyrobutyricum* *C. saccharobutylicum*, *C. Puniceum*, *C. beijernckii*, and *C. acetobutylicum*), *Moniliella spp.* (including but not limited to *M. pollinis*, *M. tomentosa*, *M. madida*, *M. nigrescens*, *M. oedocephali*, *M. megachiliensis*), *Yarrowia lipolytica*, *Aureobasidium sp.*, *Trichosporonoides sp.*, *Trigonopsis variabilis*, *Trichosporon sp.*, *Moniliellaacetobutans sp.*, *Typhula variabilis*, *Candida magnoliae*, *Ustilaginomycetes sp.*, *Pseudozyma tsukubaensis*, yeast species of genera *Zygosaccharomyces*, *Debaryomyces*, *Hansenula* and *Pichia*, and fungi of the dematioid genus *Torula* (*e.g.*, *T.corallina*).

[00144] Additional microorganisms include the *Lactobacillus* group. Examples include *Lactobacillus casei*, *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactobacillus coryniformis*, e.g., *Lactobacillus coryniformis subspecies torquens*, *Lactobacillus pentosus*, *Lactobacillus brevis*. Other microorganisms include *Pediococcus penosaceus*, *Rhizopus oryzae*.

[00145] Several organisms, such as bacteria, yeasts and fungi, can be utilized to ferment biomass derived products such as sugars and alcohols to succinic acid and similar products. For example, organisms can be selected from; *Actinobacillus succinogenes*, *Anaerobiospirillum succiniciproducens*, *Mannheimia succiniciproducens*, *Ruminococcus flaverfaciens*, *Ruminococcus albus*, *Fibrobacter succinogenes*, *Bacteroides fragilis*, *Bacteroides ruminicola*, *Bacteroides amylophilus*, *Bacteriodes succinogenes*, *Mannheimia succiniciproducens*, *Corynebacterium glutamicum*, *Aspergillus niger*, *Aspergillus fumigatus*, *Byssoschlamys nivea*, *Lentinus degener*, *Paecilomyces varioti*, *Penicillium viniferum*, *Saccharomyces cerevisiae*, *Enterococcus faecali*, *Prevotella ruminicolas*, *Debaryomyces hansenii*, *Candida catenulata* VKM Y-5, *C. mycoderma* VKM Y-240, *C. rugosa* VKM Y-67, *C. paludigena* VKM Y-2443, *C. utilis* VKM Y-74, *C. utilis* 766, *C. zeylanoides* VKM Y-6, *C. zeylanoides* VKM Y-14, *C. zeylanoides* VKM Y-2324, *C. zeylanoides* VKM Y-1543, *C. zeylanoides* VKM Y-2595, *C. valida* VKM Y-934, *Kluyveromyces wickerhamii* VKM Y-589, *Pichia anomala* VKM Y-118, *P. besseyi* VKM Y-2084, *P. media* VKM Y-1381, *P. guilliermondii* H-P-4, *P. guilliermondii* 916, *P. inositovora* VKM Y-2494, *Saccharomyces cerevisiae* VKM Y-381, *Torulopsis candida* 127, *T. candida* 420, *Yarrowia lipolytica* 12a, *Y. lipolytica* VKM Y-47, *Y. lipolytica* 69, *Y. lipolytica* VKM Y-57, *Y. lipolytica* 212, *Y. lipolytica* 374/4, *Y. lipolytica* 585, *Y. lipolytica* 695, *Y. lipolytica* 704, and mixtures of these organisms.

[00146] Many such microbial strains are publicly available, either commercially or from depositories such as the ATCC (American Type Culture Collection, Manassas, Virginia, USA), the NRRL (Agricultural Research Service Culture Collection, Peoria, Illinois, USA), or the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany), to name a few.

[00147] 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® (Lallemand Biofuels and Distilled Spirits, Canada), EAGLE C6 FUEL™ or C6 FUEL™ (available from Lallemand Biofuels and Distilled Spirits, Canada), GERT STRAND®

(available from Gert Strand AB, Sweden) and FERMOL[®] (available from DSM Specialties).

[00148]

DISTILLATION

[00149] 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.

HYDROCARBON-CONTAINING MATERIALS

[00150] In other embodiments utilizing the methods and systems described herein, hydrocarbon-containing materials can be processed. Any process described herein can be used to treat any hydrocarbon-containing material herein described. “Hydrocarbon-containing materials,” as used herein, is meant to include oil sands, oil shale, tar sands, coal dust, coal slurry, bitumen, various types of coal, and other naturally-occurring and synthetic materials that include both hydrocarbon components and solid matter. The solid matter can include rock, sand, clay, stone, silt, drilling slurry, or other solid organic and/or inorganic matter. The term can also include waste products such as drilling waste and by-products, refining waste and by-products, or other waste products containing hydrocarbon components, such as asphalt shingling and covering, asphalt pavement, etc.

CONVEYING SYSTEMS

[00151] Various conveying systems can be used to convey the biomass material, for example, to a vault and under an electron beam in a vault. Exemplary conveyors are belt conveyors, pneumatic conveyors, screw conveyors, carts, trains, trains or carts on rails,

elevators, front loaders, backhoes, cranes, various scrapers and shovels, trucks, and throwing devices can be used. For example, vibratory conveyors can be used in various processes described herein, for example, as disclosed in US. Provisional Application 61/711,801 filed Oct. 10, 2012, the entire disclosure of which is herein incorporated by reference.

HYDROGENATION AND OTHER CHEMICAL TRANSFORMATIONS

[00152] The processes described herein can include hydrogenation. For example glucose and xylose can be hydrogenated to sorbitol and xylitol respectively. Hydrogenation can be accomplished by use of a catalyst (*e.g.*, Pt/gamma-Al₂O₃, Ru/C, Raney Nickel, or other catalysts known in the art) in combination with H₂ under high pressure (*e.g.*, 10 to 12000 psi). Other types of chemical transformation of the products from the processes described herein can be used, for example production of organic sugar derived products such (*e.g.*, furfural and furfural-derived products). Chemical transformations of sugar derived products are described in US Prov. App. No. 61/667,481, filed July 3, 2012, the disclosure of which is incorporated herein by reference in its entirety.

LIGNIN DERIVED PRODUCTS

[00153] The spent biomass (*e.g.*, spent lignocellulosic material) from lignocellulosic processing by the methods described are expected to have a high lignin content and in addition to being useful for producing energy through combustion in a Co-Generation plant, may have uses as other valuable products. 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 also be converted to lignosulfonates, which can be utilized as binders, dispersants, emulsifiers or as sequestrants.

[00154] When used as a binder, the lignin or a lignosulfonate can, *e.g.*, be utilized in coal briquettes, in ceramics, for binding carbon black, for binding fertilizers and herbicides, as a dust suppressant, in the making of plywood and particle board, for binding animal feeds, as a binder for fiberglass, as a binder in linoleum paste and as a soil stabilizer.

[00155] As a dispersant, the lignin or lignosulfonates can be used, *e.g.*, concrete mixes, clay and ceramics, dyes and pigments, leather tanning and in gypsum board.

[00156] As an emulsifier, the lignin or lignosulfonates can be used, e.g., in asphalt, pigments and dyes, pesticides and wax emulsions.

[00157] As a sequestrant, the lignin or lignosulfonates can be used, e.g., in micro-nutrient systems, cleaning compounds and water treatment systems, e.g., for boiler and cooling systems.

[00158] For energy production lignin generally has a higher energy content than holocellulose (cellulose and hemicellulose) since it contains more carbon than holocellulose. For example, dry lignin can have an energy content of between about 11,000 and 12,500 BTU per pound, compared to 7,000 and 8,000 BTU per pound of holocellulose. As such, lignin can be densified and converted into briquettes and pellets for burning. For example, the lignin can be converted into pellets by any method described herein. For a slower burning pellet or briquette, the lignin can be crosslinked, such as applying a radiation dose of between about 0.5 Mrad and 5 Mrad. Crosslinking can make a slower burning form factor. The form factor, such as a pellet or briquette, can be converted to a “synthetic coal” or charcoal by pyrolyzing in the absence of air, e.g., at between 400 and 950 °C. Prior to pyrolyzing, it can be desirable to crosslink the lignin to maintain structural integrity.

[00159] Co-generation using spent biomass is described in U.S. Provisional Application No. 61/774,773, filed March 8, 2013, the entire disclosure therein is herein incorporated by reference. The spent biomass may be the lignin byproducts described above and/or the fermentation solids from the first and/or the second fermentation.

OTHER EMBODIMENTS

[00160] Any material, processes or processed materials described herein can be used to make products and/or intermediates such as composites, fillers, binders, plastic additives, adsorbents and controlled release agents. The methods can include densification, for example, by applying pressure and heat to the materials. For example composites can be made by combining fibrous materials with a resin or polymer. For example radiation cross-linkable resin, e.g., a thermoplastic resin can be combined with a fibrous material to provide a fibrous material/cross-linkable resin combination. Such materials can be, for example, useful as building materials, protective sheets, containers and other structural materials (e.g., molded and/or extruded products). Absorbents can be, for example, in the form of pellets, chips, fibers and/or sheets. Adsorbents can be used, for example, as pet bedding, packaging material or in pollution control systems. Controlled release matrices can also be the form of, for example, pellets, chips, fibers

and or sheets. The controlled release matrices can, for example, be used to release drugs, biocides, fragrances. For example, composites, absorbents and control release agents and their uses are described in U.S. Serial No. PCT/US2006/010648, filed March 23, 2006, and US Patent No. 8,074,910 filed November 22, 2011, the entire disclosures of which are herein incorporated by reference.

[00161] In some instances the biomass material is treated at a first level to reduce recalcitrance, e.g., utilizing accelerated electrons, to selectively release one or more sugars (e.g., xylose). The biomass can then be treated to a second level to release one or more other sugars (e.g., glucose). Optionally the biomass can be dried between treatments. The treatments can include applying chemical and biochemical treatments to release the sugars. For example, a biomass material can be treated to a level of less than about 20 Mrad (e.g., less than about 15 Mrad, less than about 10 Mrad, less than about 5 Mrad, less than about 2 Mrad) and then treated with a solution of sulfuric acid, containing less than 10% sulfuric acid (e.g., less than about 9%, less than about 8%, less than about 7%, less than about 6%, less than about 5%, less than about 4%, less than about 3%, less than about 2%, less than about 1%, less than about 0.75%, less than about 0.50 %, less than about 0.25%) to release xylose. Xylose, for example that is released into solution, can be separated from solids and optionally the solids washed with a solvent/solution (e.g., with water and/or acidified water). Optionally, the solids can be dried, for example in air and/or under vacuum optionally with heating (e.g., below about 150 deg C, below about 120 deg C) to a water content below about 25 wt. % (below about 20 wt. %, below about 15 wt. %, below about 10 wt. %, below about 5 wt. %). The solids can then be treated with a level of less than about 30 Mrad (e.g., less than about 25 Mrad, less than about 20 Mrad, less than about 15 Mrad, less than about 10 Mrad, less than about 5 Mrad, less than about 1 Mrad or even not at all) and then treated with an enzyme (e.g., a cellulase) to release glucose. The glucose (e.g., glucose in solution) can be separated from the remaining solids. The solids can then be further processed, for example utilized to make energy or other products (e.g., lignin derived products).

[00162] Other than in the examples herein, or unless otherwise expressly specified, all of the numerical ranges, amounts, values and percentages, such as those for amounts of materials, elemental contents, times and temperatures of reaction, ratios of amounts, and others, in the following portion of the specification and attached claims may be read as if prefaced by the word “about” even though the term “about” may not expressly appear with the value, amount, or range. Accordingly, unless indicated to the contrary, the

numerical parameters set forth in the following specification and attached claims are approximations that may vary depending upon the desired properties sought to be obtained by the present invention. At the very least, and not as an attempt to limit the application of the doctrine of equivalents to the scope of the claims, each numerical parameter should at least be construed in light of the number of reported significant digits and by applying ordinary rounding techniques.

FLAVORS, FRAGRANCES AND COLORANTS

[00163] Any of the products and/or intermediates described herein, for example, produced by the processes, systems and/or equipment described herein, can be combined with flavors, fragrances, colorants and/or mixtures of these. For example, any one or more of (optionally along with flavors, fragrances and/or colorants) sugars, organic acids, fuels, polyols, such as sugar alcohols, biomass, fibers and composites can be combined with (e.g., formulated, mixed or reacted) or used to make other products. For example, one or more such product can be used to make soaps, detergents, candies, drinks (e.g., cola, wine, beer, liquors such as gin or vodka, sports drinks, coffees, teas), pharmaceuticals, adhesives, sheets (e.g., woven, none woven, filters, tissues) and/or composites (e.g., boards). For example, one or more such product can be combined with herbs, flowers, petals, spices, vitamins, potpourri, or candles. For example, the formulated, mixed or reacted combinations can have flavors/fragrances of grapefruit, orange, apple, raspberry, banana, lettuce, celery, cinnamon, chocolate, vanilla, peppermint, mint, onion, garlic, pepper, saffron, ginger, milk, wine, beer, tea, lean beef, fish, clams, olive oil, coconut fat, pork fat, butter fat, beef bouillon, legume, potatoes, marmalade, ham, coffee and cheeses.

[00164] Flavors, fragrances and colorants can be added in any amount, such as between about 0.001 wt.% to about 30 wt.%, e.g., between about 0.01 to about 20, between about 0.05 to about 10, or between about 0.1 wt.% to about 5 wt.%. These can be formulated, mixed and or reacted (e.g., with any one of more product or intermediate described herein) by any means and in any order or sequence (e.g., agitated, mixed, emulsified, gelled, infused, heated, sonicated, and/or suspended). Fillers, binders, emulsifier, antioxidants can also be utilized, for example protein gels, starches and silica.

[00165] In one embodiment the flavors, fragrances and colorants can be added to the biomass immediately after the biomass is irradiated such that the reactive sites created by

the irradiation may react with reactive compatible sites of the flavors, fragrances, and colorants.

[00166] The flavors, fragrances and colorants can be natural and/or synthetic materials. These materials can be one or more of a compound, a composition or mixtures of these (e.g., a formulated or natural composition of several compounds). Optionally the flavors, fragrances, antioxidants and colorants can be derived biologically, for example, from a fermentation process (e.g., fermentation of saccharified materials as described herein). Alternatively, or additionally these flavors, fragrances and colorants can be harvested from a whole organism (e.g., plant, fungus, animal, bacteria or yeast) or a part of an organism. The organism can be collected and or extracted to provide color, flavors, fragrances and/or antioxidant by any means including utilizing the methods, systems and equipment described herein, hot water extraction, supercritical fluid extraction, chemical extraction (e.g., solvent or reactive extraction including acids and bases), mechanical extraction (e.g., pressing, comminuting, filtering), utilizing an enzyme, utilizing a bacteria such as to break down a starting material, and combinations of these methods. The compounds can be derived by a chemical reaction, for example, the combination of a sugar (e.g., as produced as described herein) with an amino acid (Maillard reaction). The flavor, fragrance, antioxidant and/or colorant can be an intermediate and or product produced by the methods, equipment or systems described herein, for example and ester and a lignin derived product.

[00167] Some examples of flavor, fragrances or colorants are polyphenols. Polyphenols are pigments responsible for the red, purple and blue colorants of many fruits, vegetables, cereal grains, and flowers. Polyphenols also can have antioxidant properties and often have a bitter taste. The antioxidant properties make these important preservatives. One class of polyphenols are the flavonoids, such as Anthocyanidines, flavanones, flavan-3-ols, s, flavanones and flavanones. Other phenolic compounds that can be used include phenolic acids and their esters, such as chlorogenic acid and polymeric tannins.

[00168] Among the colorants inorganic compounds, minerals or organic compounds can be used, for example titanium dioxide, zinc oxide, aluminum oxide, cadmium yellow (E.g., CdS), cadmium orange (e.g., CdS with some Se), alizarin crimson (e.g., synthetic or non-synthetic rose madder), ultramarine (e.g., synthetic ultramarine, natural ultramarine, synthetic ultramarine violet), cobalt blue, cobalt yellow, cobalt green, viridian (e.g., hydrated chromium(III)oxide), chalcophylite, conichalcite, cornubite,

cornwallite and lroconite. Black pigments such as carbon black and self-dispersed blacks may be used.

[00169] Some flavors and fragrances that can be utilized include ACALEA TBHQ, ACET C-6, ALLYL AMYL GLYCOLATE, ALPHA TERPINEOL, AMBRETTOLIDE, AMBRINOL 95, ANDRANE, APHERMATE, APPLELIDE, BACDANOL®, BERGAMAL, BETA IONONE EPOXIDE, BETA NAPHTHYL ISO-BUTYL ETHER, BICYCLONONALACTONE, BORNAFIX®, CANTHOXAL, CASHMERAN®, CASHMERAN® VELVET, CASSIFFIX®, CEDRAFIX, CEDRAMBER®, CEDRYL ACETATE, CELESTOLIDE, CINNAMALVA, CITRAL DIMETHYL ACETATE, CITROLATE™, CITRONELLOL 700, CITRONELLOL 950, CITRONELLOL COEUR, CITRONELLYL ACETATE, CITRONELLYL ACETATE PURE, CITRONELLYL FORMATE, CLARYCET, CLONAL, CONIFERAN, CONIFERAN PURE, CORTEX ALDEHYDE 50% PEOMOSA, CYCLABUTE, CYCLACET®, CYCLAPROP®, CYCLEMAX™, CYCLOHEXYL ETHYL ACETATE, DAMASCOL, DELTA DAMASCONE, DIHYDRO CYCLACET, DIHYDRO MYRCENOL, DIHYDRO TERPINEOL, DIHYDRO TERPINYL ACETATE, DIMETHYL CYCLORMOL, DIMETHYL OCTANOL PQ, DIMYRCETOL, DIOLA, DIPENTENE, DULCINYL® RECRYSTALLIZED, ETHYL-3-PHENYL GLYCIDATE, FLEURAMONE, FLEURANIL, FLORAL SUPER, FLORALOZONE, FLORIFFOL, FRAISTONE, FRUCTONE, GALAXOLIDE® 50, GALAXOLIDE® 50 BB, GALAXOLIDE® 50 IPM, GALAXOLIDE® UNDILUTED, GALBASCON, GERALDEHYDE, GERANIOL 5020, GERANIOL 600 TYPE, GERANIOL 950, GERANIOL 980 (PURE), GERANIOL CFT COEUR, GERANIOL COEUR, GERANYL ACETATE COEUR, GERANYL ACETATE, PURE, GERANYL FORMATE, GRISALVA, GUAIYL ACETATE, HELIONAL™, HERBAC, HERBALIME™, HEXADECANOLIDE, HEXALON, HEXENYL SALICYLATE CIS 3-, HYACINTH BODY, HYACINTH BODY NO. 3, HYDRATROPIC ALDEHYDE.DMA, HYDROXYOL, INDOLAROME, INTRELEVEN ALDEHYDE, INTRELEVEN ALDEHYDE SPECIAL, IONONE ALPHA, IONONE BETA, ISO CYCLO CITRAL, ISO CYCLO GERANIOL, ISO E SUPER®, ISOBUTYL QUINOLINE, JASMAL., JESSEMAL®, KHARISMAL®, KHARISMAL® SUPER, KHUSINIL, KOAVONE®, KOHINOOL®, LIFFAROME™, LIMOXAL, LINDENOL™, LYRAL®, LYRAME SUPER, MANDARIN ALD 10% TRI ETH, CITR, MARITIMA, MCK CHINESE, MEIJIFF™, MELAFLEUR, MELOZONE, METHYL ANTHRANILATE, METHYL IONONE ALPHA EXTRA, METHYL

IONONE GAMMA A, METHYL IONONE GAMMA COEUR, METHYL IONONE GAMMA PURE, METHYL LAVENDER KETONE, MONTAVERDI®, MUGUESIA, MUGUET ALDEHYDE 50, MUSK Z4, MYRAC ALDEHYDE, MYRCENYL ACETATE, NECTARATE™, NEROL 900, NERYL ACETATE, OCIMENE, OCTACETAL, ORANGE FLOWER ETHER, ORIVONE., ORRINIFF 25%, OXASPIRANE, OZOFLEUR, PAMPLEFLEUR®, PEOMOSA, PHENOXANOL®, PICONIA, PRECYCLEMONE B, PRENYL ACETATE, PRISMANTOL, RESEDA BODY, ROSALVA, ROSAMUSK, SANJINOL, SANTALIFF™, SYVERTAL, TERPINEOL, TERPINOLENE 20, TERPINOLENE 90 PQ, TERPINOLENE RECT., TERPINYL ACETATE, TERPINYL ACETATE JAX, TETRAHYDRO, MUGUOL®, TETRAHYDRO MYRCENOL, TETRAMERAN, TIMBERSILK™, TOBACAROL, TRIMOFIX® O TT, TRIPLAL®, TRISAMBER®, VANORIS, VERDOX™, VERDOX™ HC, VERTENEX®, VERTENEX® HC, VERTOPIX® COEUR, VERTOLIFF, VERTOLIFF ISO, VIOLIFF, VIVALDIE, ZENOLIDE, ABS INDIA 75 PCT MIGLYOL, ABS MOROCCO 50 PCT DPG, ABS MOROCCO 50 PCT TEC, ABSOLUTE FRENCH, ABSOLUTE INDIA, ABSOLUTE MD 50 PCT BB, ABSOLUTE MOROCCO, CONCENTRATE PG, TINCTURE 20 PCT, AMBERGRIS, AMBRETTE ABSOLUTE, AMBRETTE SEED OIL, ARMOISE OIL 70 PCT THUYONE, BASIL ABSOLUTE GRAND VERT, BASIL GRAND VERT ABS MD, BASIL OIL GRAND VERT, BASIL OIL VERVEINA, BASIL OIL VIETNAM, BAY OIL TERPENELESS, BEESWAX ABS N G, BEESWAX ABSOLUTE, BENZOIN RESINOID SIAM, BENZOIN RESINOID SIAM 50 PCT DPG, BENZOIN RESINOID SIAM 50 PCT PG, BENZOIN RESINOID SIAM 70.5 PCT TEC, BLACKCURRANT BUD ABS 65 PCT PG, BLACKCURRANT BUD ABS MD 37 PCT TEC, BLACKCURRANT BUD ABS MIGLYOL, BLACKCURRANT BUD ABSOLUTE BURGUNDY, BOIS DE ROSE OIL, BRAN ABSOLUTE, BRAN RESINOID, BROOM ABSOLUTE ITALY, CARDAMOM GUATEMALA CO2 EXTRACT, CARDAMOM OIL GUATEMALA, CARDAMOM OIL INDIA, CARROT HEART, CASSIE ABSOLUTE EGYPT, CASSIE ABSOLUTE MD 50 PCT IPM, CASTOREUM ABS 90 PCT TEC, CASTOREUM ABS C 50 PCT MIGLYOL, CASTOREUM ABSOLUTE, CASTOREUM RESINOID, CASTOREUM RESINOID 50 PCT DPG, CEDROL CEDRENE, CEDRUS ATLANTICA OIL REDIST, CHAMOMILE OIL ROMAN, CHAMOMILE OIL WILD, CHAMOMILE OIL WILD LOW LIMONENE, CINNAMON BARK OIL CEYLAN, CISTE ABSOLUTE, CISTE ABSOLUTE COLORLESS, CITRONELLA OIL ASIA IRON FREE, CIVET ABS 75 PCT PG,

CIVET ABSOLUTE, CIVET TINCTURE 10 PCT, CLARY SAGE ABS FRENCH
DECOL, CLARY SAGE ABSOLUTE FRENCH, CLARY SAGE C'LESS 50 PCT PG,
CLARY SAGE OIL FRENCH, COPAIBA BALSAM, COPAIBA BALSAM OIL,
CORIANDER SEED OIL, CYPRESS OIL, CYPRESS OIL ORGANIC, DAVANA OIL,
GALBANOL, GALBANUM ABSOLUTE COLORLESS, GALBANUM OIL,
GALBANUM RESINOID, GALBANUM RESINOID 50 PCT DPG, GALBANUM
RESINOID HERCOLYN BHT, GALBANUM RESINOID TEC BHT, GENTIANE
ABSOLUTE MD 20 PCT BB, GENTIANE CONCRETE, GERANIUM ABS EGYPT
MD, GERANIUM ABSOLUTE EGYPT, GERANIUM OIL CHINA, GERANIUM OIL
EGYPT, GINGER OIL 624, GINGER OIL RECTIFIED SOLUBLE, GUAIAACWOOD
HEART, HAY ABS MD 50 PCT BB, HAY ABSOLUTE, HAY ABSOLUTE MD 50
PCT TEC, HEALINGWOOD, HYSSOP OIL ORGANIC, IMMORTELLE ABS YUGO
MD 50 PCT TEC, IMMORTELLE ABSOLUTE SPAIN, IMMORTELLE ABSOLUTE
YUGO, JASMIN ABS INDIA MD, JASMIN ABSOLUTE EGYPT, JASMIN
ABSOLUTE INDIA, ASMIN ABSOLUTE MOROCCO, JASMIN ABSOLUTE
SAMBAC, JONQUILLE ABS MD 20 PCT BB, JONQUILLE ABSOLUTE France,
JUNIPER BERRY OIL FLG, JUNIPER BERRY OIL RECTIFIED SOLUBLE,
LABDANUM RESINOID 50 PCT TEC, LABDANUM RESINOID BB, LABDANUM
RESINOID MD, LABDANUM RESINOID MD 50 PCT BB, LAVANDIN ABSOLUTE
H, LAVANDIN ABSOLUTE MD, LAVANDIN OIL ABRIAL ORGANIC,
LAVANDIN OIL GROSSO ORGANIC, LAVANDIN OIL SUPER, LAVENDER
ABSOLUTE H, LAVENDER ABSOLUTE MD, LAVENDER OIL COUMARIN
FREE, LAVENDER OIL COUMARIN FREE ORGANIC, LAVENDER OIL
MAILLETTE ORGANIC, LAVENDER OIL MT, MACE ABSOLUTE BB,
MAGNOLIA FLOWER OIL LOW METHYL EUGENOL, MAGNOLIA FLOWER
OIL, MAGNOLIA FLOWER OIL MD, MAGNOLIA LEAF OIL, MANDARIN OIL
MD, MANDARIN OIL MD BHT, MATE ABSOLUTE BB, MOSS TREE ABSOLUTE
MD TEX IFRA 43, MOSS-OAK ABS MD TEC IFRA 43, MOSS-OAK ABSOLUTE
IFRA 43, MOSS-TREE ABSOLUTE MD IPM IFRA 43, MYRRH RESINOID BB,
MYRRH RESINOID MD, MYRRH RESINOID TEC, MYRTLE OIL IRON FREE,
MYRTLE OIL TUNISIA RECTIFIED, NARCISSE ABS MD 20 PCT BB, NARCISSE
ABSOLUTE FRENCH, NEROLI OIL TUNISIA, NUTMEG OIL TERPENELESS,
OEILLET ABSOLUTE, OLIBANUM RESINOID, OLIBANUM RESINOID BB,
OLIBANUM RESINOID DPG, OLIBANUM RESINOID EXTRA 50 PCT DPG,
OLIBANUM RESINOID MD, OLIBANUM RESINOID MD 50 PCT DPG,

OLIBANUM RESINOID TEC, OPOPONAX RESINOID TEC, ORANGE BIGARADE OIL MD BHT, ORANGE BIGARADE OIL MD SCFC, ORANGE FLOWER ABSOLUTE TUNISIA, ORANGE FLOWER WATER ABSOLUTE TUNISIA, ORANGE LEAF ABSOLUTE, ORANGE LEAF WATER ABSOLUTE TUNISIA, ORRIS ABSOLUTE ITALY, ORRIS CONCRETE 15 PCT IRONE, ORRIS CONCRETE 8 PCT IRONE, ORRIS NATURAL 15 PCT IRONE 4095C, ORRIS NATURAL 8 PCT IRONE 2942C, ORRIS RESINOID, OSMANTHUS ABSOLUTE, OSMANTHUS ABSOLUTE MD 50 PCT BB, PATCHOULI HEART N°3, PATCHOULI OIL INDONESIA, PATCHOULI OIL INDONESIA IRON FREE, PATCHOULI OIL INDONESIA MD, PATCHOULI OIL REDIST, PENNYROYAL HEART, PEPPERMINT ABSOLUTE MD, PETITGRAIN BIGARADE OIL TUNISIA, PETITGRAIN CITRONNIER OIL, PETITGRAIN OIL PARAGUAY TERPENELESS, PETITGRAIN OIL TERPENELESS STAB, PIMENTO BERRY OIL, PIMENTO LEAF OIL, RHODINOL EX GERANIUM CHINA, ROSE ABS BULGARIAN LOW METHYL EUGENOL, ROSE ABS MOROCCO LOW METHYL EUGENOL, ROSE ABS TURKISH LOW METHYL EUGENOL, ROSE ABSOLUTE, ROSE ABSOLUTE BULGARIAN, ROSE ABSOLUTE DAMASCENA, ROSE ABSOLUTE MD, ROSE ABSOLUTE MOROCCO, ROSE ABSOLUTE TURKISH, ROSE OIL BULGARIAN, ROSE OIL DAMASCENA LOW METHYL EUGENOL, ROSE OIL TURKISH, ROSEMARY OIL CAMPHOR ORGANIC, ROSEMARY OIL TUNISIA, SANDALWOOD OIL INDIA, SANDALWOOD OIL INDIA RECTIFIED, SANTALOL, SCHINUS MOLLE OIL, ST JOHN BREAD TINCTURE 10 PCT, STYRAX RESINOID, STYRAX RESINOID, TAGETE OIL, TEA TREE HEART, TONKA BEAN ABS 50 PCT SOLVENTS, TONKA BEAN ABSOLUTE, TUBEROSE ABSOLUTE INDIA, VETIVER HEART EXTRA, VETIVER OIL HAITI, VETIVER OIL HAITI MD, VETIVER OIL JAVA, VETIVER OIL JAVA MD, VIOLET LEAF ABSOLUTE EGYPT, VIOLET LEAF ABSOLUTE EGYPT DECOL, VIOLET LEAF ABSOLUTE FRENCH, VIOLET LEAF ABSOLUTE MD 50 PCT BB, WORMWOOD OIL TERPENELESS, YLANG EXTRA OIL, YLANG III OIL and combinations of these.

[00170] The colorants can be among those listed in the Colour Index International by the Society of Dyers and Colourists. Colorants include dyes and pigments and include those commonly used for coloring textiles, paints, inks and inkjet inks. Some colorants that can be utilized include carotenoids, arylyde yellows, diarylyde yellows, β -naphthols, naphthols, benzimidazolones, disazo condensation pigments, pyrazolones, nickel azo

yellow, phthalocyanines, quinacridones, perylenes and perinones, isoindolinone and isoindoline pigments, triarylcarbonium pigments, diketopyrrolo-pyrrole pigments, thioindigoids. Carotenoids include.g., alpha-carotene, beta-carotene, gamma-carotene, lycopene, lutein and astaxanthin Annatto extract, Dehydrated beets (beet powder), Canthaxanthin, Caramel, β -Apo-8'-carotenal, Cochineal extract, Carmine, Sodium copper chlorophyllin, Toasted partially defatted cooked cottonseed flour, Ferrous gluconate, Ferrous lactate, Grape color extract, Grape skin extract (enocianina), Carrot oil, Paprika, Paprika oleoresin, Mica-based pearlescent pigments, Riboflavin, Saffron, Titanium dioxide, Tomato lycopene extract; tomato lycopene concentrate, Turmeric, Turmeric oleoresin, FD&C Blue No. 1, FD&C Blue No. 2, FD&C Green No. 3, Orange B, Citrus Red No. 2, FD&C Red No. 3, FD&C Red No. 40, FD&C Yellow No. 5, FD&C Yellow No. 6, Alumina (dried aluminum hydroxide), Calcium carbonate, Potassium sodium copper chlorophyllin (chlorophyllin-copper complex), Dihydroxyacetone, Bismuth oxychloride, Ferric ammonium ferrocyanide, Ferric ferrocyanide, Chromium hydroxide green, Chromium oxide greens, Guanine, Pyrophyllite, Talc, Aluminum powder, Bronze powder, Copper powder, Zinc oxide, D&C Blue No. 4, D&C Green No. 5, D&C Green No. 6, D&C Green No. 8, D&C Orange No. 4, D&C Orange No. 5, D&C Orange No. 10, D&C Orange No. 11, FD&C Red No. 4, D&C Red No. 6, D&C Red No. 7, D&C Red No. 17, D&C Red No. 21, D&C Red No. 22, D&C Red No. 27, D&C Red No. 28, D&C Red No. 30, D&C Red No. 31, D&C Red No. 33, D&C Red No. 34, D&C Red No. 36, D&C Red No. 39, D&C Violet No. 2, D&C Yellow No. 7, Ext. D&C Yellow No. 7, D&C Yellow No. 8, D&C Yellow No. 10, D&C Yellow No. 11, D&C Black No. 2, D&C Black No. 3 (3), D&C Brown No. 1, Ext. D&C, Chromium-cobalt-aluminum oxide, Ferric ammonium citrate, Pyrogallol, Logwood extract, 1,4-Bis[(2-hydroxy-ethyl)amino]-9,10-anthracenedione bis(2-propenoic)ester copolymers, 1,4-Bis[(2-methylphenyl)amino]-9,10-anthracenedione, 1,4-Bis[4-(2-methacryloxyethyl)phenylamino] anthraquinone copolymers, Carbazole violet, Chlorophyllin-copper complex, Chromium-cobalt-aluminum oxide,, C.I. Vat Orange 1, 2-[[2,5-Diethoxy-4-[(4-methylphenyl)thiol] phenyl]azo]-1,3,5-benzenetriol, 16,23-Dihydrodinaphtho [2,3-a:2',3'-i] naphth [2',3':6,7] indolo [2,3-c] carbazole- 5,10,15,17,22,24-hexone, N,N'-(9,10-Dihydro- 9,10-dioxo- 1,5-anthracenediyl) bisbenzamide, 7,16-Dichloro- 6,15-dihydro- 5,9,14,18-anthrazinetetrone, 16,17-Dimethoxydinaphtho (1,2,3-cd:3',2',1'-lm) perylene- 5,10-dione, Poly(hydroxyethyl methacrylate) -dye copolymers(3), Reactive Black 5, Reactive Blue 21, Reactive Orange 78, Reactive Yellow 15, Reactive Blue No. 19, Reactive Blue No. 4, C.I. Reactive Red 11, C.I. Reactive Yellow 86, C.I. Reactive Blue

163, C.I. Reactive Red 180, 4-[(2,4-dimethylphenyl)azo]-2,4-dihydro-5-methyl-2-phenyl-3H-pyrazol-3-one (solvent Yellow 18), 6-Ethoxy-2-(6-ethoxy-3-oxobenzo[b]thien-2(3H)-ylidene)benzo[b]thiophen-3(2H)-one, Phthalocyanine green, Vinyl alcohol/methyl methacrylate-dye reaction products, C.I. Reactive Red 180, C.I. Reactive Black 5, C.I. Reactive Orange 78, C.I. Reactive Yellow 15, C.I. Reactive Blue 21, Disodium 1-amino-4-[[4-[(2-bromo-1-oxoallyl)amino]-2-sulphonatophenyl]amino]-9,10-dihydro-9,10-dioxoanthracene-2-sulphonate (Reactive Blue 69), D&C Blue No. 9, [Phthalocyaninato(2-)]copper and mixtures of these.

EXAMPLES

[00171] Concentrations were determined by HPLC in aqueous diluted and filtered solutions with appropriate standards. Unless otherwise noted the reactants were obtained from Sigma/Aldrich, St. Louis MO, Fisher Scientific, Waltham MA or equivalent reactant supply house.

Saccharification

[00172] A cylindrical tank with a diameter of 32 Inches, 64 Inches in height and fit with ASME dished heads (top and bottom) was used in the saccharification. The tank was also equipped with a hydrofoil mixing blade 16" wide. Heating was provided by flowing hot water through a half pipe jacket surrounding the tank.

[00173] The tank was charged with 200 kg water, 80 kg of biomass, and 18 kg of Duet™ Cellulase enzyme available from Genencor, Palo Alto, CA. Biomass was corn cob that had been hammer milled and screened to a size of between 10 and 40 mesh. The biomass was irradiated with an electron beam to a total dosage of 35 Mrad. The pH of the mixture was adjusted and maintained automatically throughout the saccharification at 4.8 using Ca(OH)₂. This combination was heated to 53 °C, stirred at 180 rpm for about 24 hours after which the saccharification was considered completed.

[00174] A portion of this material was screened through a 20 mesh screen and the solution stored in an 8 gal carboy at 4 °C.

Fermentation of Glucose to Ethanol

[00175] About 400mL of the saccharified material was decanted into a 1L New Brunswick BioFlow 115 Bioreactor. The material was aerated and heated to 30 °C prior to inoculation. Stirring was set at 50 rpm. The pH was measured at 5.2, which is acceptable for fermentation so it was not adjusted. Aeration was discontinued and the contents of the bioreactor were inoculated with 5 mg of Thermosacc Dry Yeast

(Lallemand, Inc., Memphis TN) (*Saccharomyces cerevisiae*). Fermentation was allowed to proceed for about 24 hours.

[00176] After fermentation the glucose concentration was below the detection limit, the ethanol concentration was about 25 g/L, and the xylose concentration was about 30g/L.

Preparation of Distillate Bottoms

[00177] Distillate bottoms were prepared by distilling the ethanol from fermented material as described above. In addition, solids were removed by centrifugation. The final amount of dissolved solids was 5 to 10 wt. There also were fines in the suspended solid. After the distillation the xylose concentration was about 40 g/L. These bottoms were designated as Distillate Bottoms Lot A. A similarly prepared batch was designated as Lot R.

Fermentation of Xylose to Butyric Acid:

Distillate Bottoms Experiment (A)

[00178] Seven 1L New Brunswick BioFlow 115 Bioreactor were utilized in the experiment. All seven reactors were initially filled with 200 mL of 3x concentrate of P2 media (described below) and of 72 g Xylose (Danisco, Copenhagen, DE). Two of the reactors (BR2 and BR4) were charged with 120 mL of distillate bottom prepared as described above (Lot A). Two reactors (BR6 and BR8) were charged with 240 mL of distillate bottom (Lot A). Two (BR18 and BR20) were charged with 360mL of distillate bottom (Lot A). One reactor (BR22) was charged with 240 mL of distillate bottom (Lot R). All the bioreactors were brought to total volume of 600mL with DI water. For example, BR2 had 200 mL of P2 media, 120 mL of Distillate Bottoms Lot A, ~72 grams of xylose and DI water to make up to 600 mL. The Xylose concentration was 72 grams plus ~ 4.8 g from the Distillate Bottoms for a concentration of about 128 g/L. The reactors were sparged with N₂ gas and inoculated with 7% (45mL of *C.tyrobutyricum* (ATCC 25755). The seed was grown overnight at 37 ° C in 300mL of reinforced clostridia media from 1 mL freezer stocks. The bioreactors were sampled periodically submitted for GC and HPLC analysis. The fermentations were maintained above 6.0 using 3.7N ammonium hydroxide. Table 1 shows data collected for these experiments.

[00179] P2 based medium was made as described in US 6,358,717 but as a 3 fold concentrate (3X), that is only 1/3 of the water was used to make the solutions. P2 medium is made as follows. The medium is composed of the following separately prepared solutions (in grams per 100 ml of distilled water, unless indicated otherwise): 790 ml of distilled water (solution I), 0.5 g of K₂HPO₄, 0.5 g of KH₂PO₄, 2.2 g of

CH₃COONH₄ (solution II), 2.0 g of MgSO₄·7H₂O, 0.1 g of MnSO₄·H₂O, 0.1 g of NaCl, 0.1 g of FeSO₄·7H₂O (solution III), and 100 mg of p-aminobenzoic acid, 100 mg of thiamine, 1 mg of biotin (solution IV). Solutions I and II were filter sterilized and subsequently mixed to form a buffer solution. Solutions III and IV were filter sterilized. Portions (10 and 1 ml) of solutions III and IV, respectively, were added aseptically to the buffer solution. The final pH of the P2 medium was 6.6.

Table 1

Sample	Time (hr.)	Distillate bottom	Butyric Acid (g/L)	Xylose (g/L)
A-BR2	17	20% Lot A	8.5	95.1
A-BR4	17	20% Lot A	9.7	93.4
A-BR6	17	40% Lot A	7.9	90.4
A-BR8	17	40% Lot A	4.9	106.4
A-BR18	17	60% Lot A	4.8	94.8
A-BR20	17	60% Lot A	5.5	94.6
A-BR22	17	60% Lot R	7.8	115.4
A-BR2	24	20% Lot A	15.6	81.4
A-BR4	24	20% Lot A	16.3	79.1
A-BR6	24	40% Lot A	16.3	78.9
A-BR8	24	40% Lot A	8	91.5
A-BR18	24	60% Lot A	9.5	83.8
A-BR20	24	60% Lot A	11.2	81.9
A-BR22	24	60% Lot R	12.7	102
A-BR2	41	20% Lot A	29.6	40.4
A-BR4	41	20% Lot A	30.8	35.1
A-BR6	41	40% Lot A	31.3	44.4
A-BR8	41	40% Lot A	20.9	55.8
A-BR18	41	60% Lot A	27	54.5
A-BR20	41	60% Lot A	27.6	52.2
A-BR22	41	60% Lot R	28.7	60.9
A-BR2	48	20% Lot A	34	28.5
A-BR4	48	20% Lot A	36	22.4
A-BR6	48	40% Lot A	34.7	35.7
A-BR8	48	40% Lot A	27.5	44.8

A-BR18	48	60% Lot A	32.7	46.9
A-BR20	48	60% Lot A	33.2	44.6
A-BR22	48	60% Lot R	30.8	48.7
A-BR2	66	20% Lot A	48	5.6
A-BR4	66	20% Lot A	48.1	0.5
A-BR6	66	40% Lot A	39.1	19.1
A-BR8	66	40% Lot A	36.4	23.1
A-BR18	66	60% Lot A	38.1	28.7
A-BR20	66	60% Lot A	36.1	27.7
A-BR22	66	60% Lot R	38.1	25.2
A-BR2	72	20% Lot A	43.5	1.8
A-BR4	72	20% Lot A	42.8	NF
A-BR6	72	40% Lot A	41.3	14.9
A-BR8	72	40% Lot A	41	16.4
A-BR18	72	60% Lot A	39.3	23.6
A-BR20	72	60% Lot A	39	23.1
A-BR22	72	60% Lot R	48.9	18.2
A-BR2	138	20% Lot A	47.4	NF
A-BR4	138	20% Lot A	43.2	NF
A-BR6	138	40% Lot A	49	2.1
A-BR8	138	40% Lot A	46.1	0.5
A-BR18	138	60% Lot A	48.4	3
A-BR20	138	60% Lot A	47.5	3.9
A-BR22	138	60% Lot R	47.9	0.7
NF: not found, below detection limit				

Distillate Bottoms Experiment (B)

[00180] Six bioreactors were used in this experiment. For a 600 mL reactor charge, two reactors (B-BR2 and B-BR4) were filled with 72 g of xylose, 5ppm $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, and 6g/L Fluka brand yeast extract and DI water added to obtain 600 mL. Two other reactors (B-BR6 and B-BR8) were filled with 72 g of xylose, 5ppm $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 40% 240 mL distillate bottom and DI water added to obtain 600 mL. One reactor (B-BR18) was filled with 72g xylose. 200 mL of modified P2 supplemented with 240 mL distillate bottom and DI water added to obtain 600 mL. Another reactor (B-BR20) was

filled with 72 g of xylose, 200 mL of modified P2 supplemented (as described above, but not as the 3X concentrate) with 60g/L yeast extract and DI water added to obtain 600 mL. All six reactors were sparged with N₂ gas and then inoculated with 5% (30ml) of *C. tyrobutyricum* (ATCC 25755)). Table 2 shows this data.

[00181] The seed was grown overnight in a modified reinforced clostridia media consisting per liter of 10g peptone, 10g beef extract, 5g NaCl, .5g of L cysteine, 3g of sodium acetate, .5g of anhydrous agar and 5g of xylose. The media was made up in 900ml of di water without xylose; 270ml was aliquoted into 500ml bottles. The bottles were sparged, autoclaved, and 30ml of 50g/L xylose was injected through a .22 micron filter into each bottle. The xylose solution was sparged with N₂ gas prior to injection. A 1ml freezer stock was used per 300ml bottle.

[00182] The pH of the fermentation was maintained above 6.0 using 3.7N NH₄OH. Samples were taken periodically and analyzed with GC and HPLC.

Table 2

Sample	Time (hr.)	Media	Butyric Acid (g/L)	Xylose (g/L)
B-BR2	17	6g/L YE + 5mg/L FeSO ₄	NF	94
B-BR4	17	6g/L YE + 5mg/L FeSO ₄	NF	95.4
B-BR6	17	40% DB + 5mg/L FeSO ₄	NF	117.8
B-BR8	17	40% DB+ 5mg/L FeSO ₄	NF	119.2
B-BR18	17	P2 + 40% DB	0.3	104.4
B-BR20	17	P2 + 60g/L YE	NF	98.2
B-BR2	24	6g/L YE + 5mg/L FeSO ₄	0.8	84.4
B-BR4	24	6g/L YE + 5mg/L FeSO ₄	1.2	83.1
B-BR6	24	40% DB + 5mg/L FeSO ₄	0.8	113.2
B-BR8	24	40% DB+ 5mg/L FeSO ₄	0.7	113
B-BR18	24	P2 + 40% DB	0.9	101
B-BR20	24	P2 + 60g/L YE	1.7	99
B-BR2	41	6g/L YE + 5mg/L FeSO ₄	5.5	51.9
B-BR4	41	6g/L YE + 5mg/L FeSO ₄	9.3	48.5

B-BR6	41	40% DB + 5mg/L FeSO ₄	14.8	88
B-BR8	41	40% DB+ 5mg/L FeSO ₄	14	91.4
B-BR18	41	P2 + 40% DB	7.9	73.7
B-BR20	41	P2 + 60g/L YE	32.5	3.8
B-BR2	48	6g/L YE + 5mg/L FeSO ₄	7.1	44.1
B-BR4	48	6g/L YE + 5mg/L FeSO ₄	11	41.9
B-BR6	48	40% DB + 5mg/L FeSO ₄	18.8	77.3
B-BR8	48	40% DB+ 5mg/L FeSO ₄	19.2	81.9
B-BR18	48	P2 + 40% DB	16.8	66.2
B-BR20	48	P2 + 60g/L YE	37.1	NF
B-BR2	66	6g/L YE + 5mg/L FeSO ₄	9.5	31.5
B-BR4	66	6g/L YE + 5mg/L FeSO ₄	15.2	30.1
B-BR6	66	40% DB + 5mg/L FeSO ₄	27.4	53.6
B-BR8	66	40% DB+ 5mg/L FeSO ₄	25.2	61
B-BR18	66	P2 + 40% DB	28.7	43.9
B-BR20	66	P2 + 60g/L YE	41.3	NF
B-BR2	72	6g/L YE + 5mg/L FeSO ₄	9.5	29
B-BR4	72	6g/L YE + 5mg/L FeSO ₄	17.8	27.8
B-BR6	72	40% DB + 5mg/L FeSO ₄	27.6	47.8
B-BR8	72	40% DB+ 5mg/L FeSO ₄	26.7	52.4
B-BR18	72	P2 + 40% DB	30.6	36.5
B-BR20	72	P2 + 60g/L YE	36.2	NF
B-BR2	137	6g/L YE + 5mg/L FeSO ₄	9.8	19.4
B-BR4	137	6g/L YE + 5mg/L FeSO ₄	20.6	16.6
B-BR6	137	40% DB + 5mg/L FeSO ₄	41.9	24.1
B-BR8	137	40% DB+ 5mg/L FeSO ₄	42.6	16.6

B-BR18	137	P2 + 40% DB	40.2	4.3
B-BR20	137	P2 + 60g/L YE	36.3	NF
NF: not found, below detection limit				
YE: yeast extract				
DB: distillate bottom				
P2: modified P2 media				

Isolation of Butyrate Using an Acidic Resin

[00183] Amberlite™ IRA 400 resin (500 g) was washed with water (2 x 500 mL) in a 5 L round bottom flask. Excess water was removed carefully with a pipette before adding a fermentation broth to the wet resin. Fermentation broth (2 L) containing 44.7g/L butyric acid was added and the resulting mixture was stirred using a magnetic stirrer for 1.5 h. A small analytical sample was removed and was found to contain 32.5 g/L butyric acid (27 % loss) by GC head space analysis. This indicated that 24.5 g of butyric acid was adsorbed onto the resin.

[00184] The supernatant solution was poured off and the wet resin was loaded onto a glass column with a wire sieve at the bottom to prevent clogging. Fermentation broth was rinsed off the resin with a flow of water (2 L) until the eluent was clear. The resin was then transferred to a 2 L round bottom flask containing a magnetic stirring bar and then treated with 100 mL of 1 N HCl followed by 8 mL of 6 N HCl. The resulting mixture was stirred for 5 minutes and the pH was found to be 2.5, which was then subjected to distillation. A total of five bulb to bulb distillations gave 150-250 mL fractions. In between distillations more water and 1 N HCl was added to the resin. Fractions were made basic with 20 % aqueous NaOH and concentrated by rotary evaporation. Drying *in vacuo* at 120 ° C overnight gave 16.23 g as a combined crude solid or 14.13 g of sodium butyrate in the sample. This amounts to a 57.7 % recovery for the five distillations. Additional distillations would lead to a higher recovery.

Isolation of Butyrate using a Basic Resin

[00185] To 400 mL of butyric acid fermentation broth (44.58 g/L) in a 1 L round bottom flask 100 mL of Amberlite™ IRN 150 (basic component) wet resin was added. The resulting mixture was stirred at room temperature for 2 hours and then allowed to stand for 10 minutes. A small analytical sample (1/2 mL) was removed and placed in a

vial. This was found to have 24.29 g/L butyric acid (54.49 % reduction) by GC head space analysis. This indicated that 9.72 g was adsorbed onto the resin.

[00186] The supernatant solution was poured off and the remaining broth was removed with a 50 mL pipette. The resin was rinsed with water (8 x 25 mL) and then treated with a 10 % solution of H₂SO₄ in EtOH (50 mL). The resulting mixture was stirred at room temperature for 5 minutes and then the ethanolic solution was removed by pipette. The resin was then rinsed with EtOH (10 x 25 mL), followed by water (10 x 25 mL). The EtOH rinse solutions were combined and basified with 20 % NaOH (pH 11) and then concentrated by rotary evaporation. The water rinse solutions were treated similarly and both solids were dried further *in vacuo* at 120 ° C to give 6.74 g (72.57 % sodium butyrate by LC analysis) from ethanol and 1.90 g (80.44 % sodium butyrate by LC analysis) from water. The total recovery from the resin was 66.1 %.

Conversion of Butyrate to Ethyl Butyrate

[00187] A crude mixture of solids containing a total of 8.9 g of sodium butyrate was treated with 50 mL of ethanol in a 250 mL round bottom flask and the resulting mixture was cooled in a water bath and slowly treated with concentrated sulfuric acid (16 g) while stirring with a magnetic stirring bar. The round bottom flask was fitted with a reflux condenser and the reaction mixture was boiled for 4 hours under N₂. After cooling to room temperature the reaction mixture was poured into a separatory funnel containing a 150 mL aqueous solution of Na₂HPO₄ (40 g). The final pH of the solution after mixing was 7. The top layer was separated out and filtered through glass wool to remove sludge giving 4.5 mL of ethyl butyrate. This sample was combined with other similar samples to give about 29 g of a crude liquid that was distilled to give 23.6 g (88 % purity by LC analysis) ethyl butyrate. The impurities were mostly ethanol (9.2 %) and ethyl acetate (2 %).

Hydrogenolysis of Ethyl Butyrate

Ethyl butyrate (20.8 g, 0.176 mol) in 225 mL of dry ethanol was added to 0.5 % Re on alumina (8.1 g, reduced) in a 1 L stainless steel autoclave. After purging with N₂ and evacuating, the resulting mixture was filled with 116 psi H₂ and then stirred at 600 rpm and heated at 270 °C for a total of 25.5 h over a 4 day period. The autoclave was depressurized to room temperature each morning and then more H₂ was added (108-112 psi). Pressures ranging from 1400-1500 psi were used for the hydrogenation. Gas chromatography head space analysis indicated a greater than 65 % molar conversion of ethyl butyrate with a greater than 90 % selectivity to n-butanol.

Biomass Produced L-lactic acid and Xylose Stream with *Lactobacillus rhamnosus*

[00188] Saccharified biomass made utilizing similar steps as described above was used as the sugar source to produce an L-lactic acid/xylose stream.

[00189] The glucose to L-Lactic acid fermenting organism *Lactobacillus rhamnosus* NRRL B-445 was grown in 25 mL of MRS medium (BD Diagnostic Systems No.: 288130) from 250 uL freezer stocks. The culture was incubated overnight in a shaker incubator at 37 °C and 150-200rpm.

[00190] Fermentation to produce the lactic acid was conducted in a bioreactor equipped with stirring paddle, heating mantel, stirring impellers, pH monitoring probes and temperature monitoring thermocouples.

[00191] The production medium for an experiment used 11 L of saccharified biomass, 22g of yeast extract, 1.6 mL of antifoam AFE-0010. The media was heated to 70 °C for 1 hour and then cooled to 37 °C. The pH of the media was raised to 6.5 using 12.5N NaOH solution. The media was then inoculated with 1% (110 mL) of the *Lactobacillus rhamnosus*. Fermentation was allowed to proceed at 37 °C while the solution was stirred at 200 rpm and the pH maintained above 6.5. Glucose was completely consumed by 48 hours. The product is L-lactic acid as the sodium salt. The xylose is essentially unconverted during the biomass conversion.

Biomass Produced D-lactic acid and Xylose Stream with *Lactobacillus coryniformis*

[00192] Saccharified biomass made utilizing similar steps as described above was used as the sugar source to produce an L-lactic acid xylose stream.

[00193] The glucose to D-Lactic acid fermenting organism *Lactobacillus coryniformis* subspecies torquens B-4390 was grown in 25 mL of MRS medium (BD Diagnostic Systems No.: 288130) from 250 µL freezer stocks. The culture was incubated overnight at 37 °C without agitation.

[00194] The production medium for an experiment used 644 mL of saccharified biomass, 5 g/L of tryptone, and 100 µL of antifoam AFE-0010. The media was heated to 70 °C for 1 hour and then cooled to 37 °C. The pH was raised to 6.5 using 12.5N NaOH solution and maintained thereafter using the same base solution. The media was inoculated with 1% of the B-4390 and the fermentation wall allowed to proceed at 37 °C while the media was stirred at 200 rpm and the pH maintained at about 6.5. Glucose consumption was complete in 144 hours. The product is D-lactic acid as the sodium salt. The xylose is essentially unconverted during the biomass conversion.

Processing of Sodium Lactate solution

[00195] Both the D-lactic acid and L-lactic acid derived sodium lactate were decolorized as described here. Fermentations were run repeatedly to provide larger quantities of material and facilitate the decolorization.

[00196] Thirty liters of fermentation medium containing sodium lactate prepared by fermentation as described above were centrifuged at 4200 rpm for 60 minutes. The supernatant was filtered through a 0.22 micron cartridge filter producing 26.5 L of filtrate. Nineteen liters of the filtrate were percolated through a column containing 2.7 L of a highly porous styrenic polymeric bead type resin, Mitsubishi Diaion SP-700, at a flow rate of 1.5 BV/h. The first 1.5 L of eluate are discarded and the rest of the medium and an additional 1.5 L of water are eluted and pooled. The remaining portion of the medium was decolorized in a similar manner resulting in 7.5 L of pale colored solution. The two batches of decolorized material were pooled and stored in the cold if not used immediately.

Desalination Electro Dialysis of decolorized Lactate solution

[00197] The decolorized medium prepared as described above was subjected to electro dialysis using a desalination membrane.

[00198] The a reservoir of the Electrodialysis apparatus was charged with the decolorized sodium lactate medium and the Concentrate reservoir of the apparatus was charged with 4 L of deionized water. Electrodialysis was continued for 5 hours using a voltage of 40 V and a maximum current of 5 A.

[00199] This procedure produced a concentrated lactate stream with a typical concentration of around 66 g/L (starting at 38 g/L) and a concentrated xylose stream with a typical conductivity of 5 μ S/cm (starting 34 μ S/cm).

Bipolar Electrodialysis

[00200] The liquid in the stream in sodium lactate produced as described above can be subjected to a second electro dialysis using a bipolar membrane to produce a lactic acid solution and a sodium hydroxide solution. The procedure that can be followed is described here.

[00201] Sodium lactate (1.6 L) solution prepared by desalination electrodialysis is added to the Diluate reservoir. Deionized water (1 L) is added to each reservoir for the lactic acid and sodium hydroxide streams. The electrodialysis is carried out using a 4-chamber electro dialysis cell fitted with a bipolar membrane stack. The voltage is set to 23 V and the maximum current is set to 6.7 A. The dialysis can be carried out for 5 hours or until the conductivity of the dilute stream is < 5 % of its starting value.

[00202] This procedure produced a concentrated lactate stream with a typical concentration of around 66 g/L (starting at 38 g/L) and a xylose stream with a typical conductivity of 5 μ S/cm (starting 34 μ S/cm) and concentration of 30g/L . The lactate stream is typically 96% lactic acid to 4 % xylose after the bipolar membrane dialysis. The xylose stream is typically 93 % xylose to 7 % lactic acid after the bipolar membrane dialysis.

[00203] Notwithstanding that the numerical ranges and parameters setting forth the broad scope of the invention are approximations, the numerical values set forth in the specific examples are reported as precisely as possible. Any numerical value, however, inherently contains error necessarily resulting from the standard deviation found in its underlying respective testing measurements. Furthermore, when numerical ranges are set forth herein, these ranges are inclusive of the recited range end points (i.e., end points may be used). When percentages by weight are used herein, the numerical values reported are relative to the total weight.

[00204] Also, it should be understood that any numerical range recited herein is intended to include all sub-ranges subsumed therein. For example, a range of “1 to 10” is intended to include all sub-ranges between (and including) the recited minimum value of 1 and the recited maximum value of 10, that is, having a minimum value equal to or greater than 1 and a maximum value of equal to or less than 10. The terms “one,” “a,” or “an” as used herein are intended to include “at least one” or “one or more,” unless otherwise indicated.

[00205] Any patent, publication, or other disclosure material, in whole or in part, that is said to be incorporated by reference herein is incorporated herein only to the extent that the incorporated material does not conflict with existing definitions, statements, or other disclosure material set forth in this disclosure. As such, and to the extent necessary, the disclosure as explicitly set forth herein supersedes any conflicting material incorporated herein by reference. Any material, or portion thereof, that is said to be incorporated by reference herein, but which conflicts with existing definitions, statements, or other disclosure material set forth herein will only be incorporated to the extent that no conflict arises between that incorporated material and the existing disclosure material.

[00206] While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

WHAT IS CLAIMED IS:

1. A method of making a product, the method comprising:
saccharifying a reduced recalcitrance cellulosic and/or reduced recalcitrance lignocellulosic material, such as by using one or more enzymes, in a liquid, to form a mixture comprising two or more sugars, and
contacting saccharified material with an organism, wherein the organism selectively ferments a sugar released during saccharification of the reduced recalcitrance cellulosic or lignocellulosic material to provide one or more unfermented sugars, fermentation solids and a first fermentation product.
2. The method of claim 1 further comprising isolating the first fermentation product from unfermented sugars and fermentation solids.
3. The method of claims 1 or 2 further comprising isolating the first fermentation product by a method selected from the group consisting of filtering, centrifuging, evaporation, distillation, crystallization, precipitation, extraction, chromatography, electrodialysis, and combinations thereof.
4. The method of any one of the above claims further comprising distilling the fermentation product from the one or more unfermented sugars and fermentation solids.
5. The method of any one of the above claims further comprising isolating the one or more unfermented sugars from the fermentation solids.
6. The method of any one of the above claims further comprising isolating the one or more unfermented sugars by a method selected from the group consisting of filtering, centrifuging, evaporation, distillation, crystallization, precipitation, extraction, chromatography, electrodialysis, and combinations thereof.
7. The method of any one of the above claims further comprising utilizing the fermentation solids as a nutrient source.

8. The method of claim 5 further comprising utilizing the fermentation solids for a second fermentation.

9. The method of any one of the above claims further comprising utilizing the fermentation solids to assist in a second fermentation, wherein the fermentation solids contain living organisms or remnants of living organisms.

10. The method of any one of the above claims further comprising converting the one or more unfermented sugars to another product, such as when the one or more sugars comprise xylose.

11. The method of any one of the above claims wherein one of the two or more sugars is glucose, and wherein the organism selectively ferments glucose.

12. The method of claim 11 wherein a product of the fermentation comprises an alcohol.

13. The method of claim 11 wherein a product of the fermentation comprises ethanol.

14. The method of claim 1 or 11 wherein the organism comprises a yeast, or a mixture of organisms, such as a yeast and a bacterium.

15. The method of claim 10 where the another product is xylitol.

16. The method of any one of the above claims wherein the recalcitrance of the biomass material is reduced by irradiation with ionizing radiation.

17. The method of claim 16 wherein the ionizing radiation comprises accelerated electrons from an electron beam.

18. The method of claims 16 or 17 wherein a total dose of radiation applied to the cellulose or lignocellulosic material is between about 10 Mrad and about 200 Mrad, such as between about 15 Mrad and about 75 Mrad or between about 20 Mrad and about 50 Mrad.

19. The method of any one of the above claims further comprising isolating lignin-derived compounds, such as soluble lignin-derived compounds, from the saccharified material prior to contacting the saccharified material with the fermenting organism.

20. The method of any one of the above claims wherein the saccharified material comprises at least two monosaccharides dissolved in the liquid.

21. The method of claim 20 wherein the monosaccharides comprise at least 50 wt. % of total carbohydrates available in the reduced recalcitrance cellulosic or lignocellulosic material.

22. The method of claims 20 or 21 wherein the two of the monosaccharides are glucose and xylose.

23. The method of any one of claims 20 through 22 wherein glucose comprises at least 10 wt. % of the monosaccharides present in the saccharified material.

24. A method of making a first product, the method comprising, producing a mixture comprising a liquid, a first sugar, a second sugar, and a saccharified cellulosic or lignocellulosic residue material produced by saccharification of an irradiated cellulosic or lignocellulosic material, such as by utilizing one or more enzymes, and fermenting the first sugar to produce the first product.

25. The method of claim 24 wherein the second sugar is produced at a concentration of at least about 20 g/L.

26. The method of claims 24 or 25 further comprising, filtering the slurry to provide a filtrate comprising a liquid solution of the second sugar and the residue.

27. The method of any one of claims 24 through 26 further comprising isolating the product, from the second sugar by distilling the product.

28. A method of making a second fermentation product, the method comprising,

producing a mixture comprising a liquid, a first sugar, a second sugar, and a saccharified cellulosic or lignocellulosic residue material produced by saccharification of an irradiated cellulosic or lignocellulosic material, such as by utilizing one or more enzymes, and fermenting the first sugar to produce a first product, isolating the first product leaving at least a second sugar and fermentation byproducts, and fermenting the second sugar to produce a second product.

29. The method of claim 28 further comprising isolating the second fermentation product from unfermented sugars and fermentation solids.

30. The method of claims 28 or 29 further comprising isolating the second fermentation product by a method selected from the group consisting of filtering, centrifuging, evaporation, distillation, crystallization, precipitation, extraction, chromatography, electrodialysis, and combinations thereof.

31. The method of claims 1, 2, 3, 28, 29, 30 where the first or second product is selected from the group consisting of sugars, sugar alcohols, alcohols, organic acids, unsaturated acids, carboxylic esters, unsaturated esters, anhydrides, aldehydes, ketones, hydrogen, carbon dioxide, fuels, biodiesel and combinations thereof.

32. The method of claim 1 or claim 24 where the liquid is aqueous.

33. The method of claim 7 where the fermentation solids are used as nutrient source for mammals.

34. The method of claim 4 where the fermentation solids are isolated as distillation bottoms.

35. The method of claim 34 where the second fermentation uses distillation bottoms as a nutrient source for the second fermentation.

36. The method of claim 31 where the product of the fermentation is an organic acid.

37. The method of claim 36 where the product is acetic or butyric acid.